Nutrición Hospitalaria

Nutr Hosp. 2015;31(1):278-285
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original / *Síndrome metabólico*

Relationships between serum calcium and magnesium levels and lipoproteins, homocysteine and insulin resistance/sensitivity markers at birth

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Abstract

Background: **The relationship between serum minerals and homocysteine, lipoprotein and glucose homeostasis markers at birth has been scarcely reported. This study aims to determine a) the relationship between calcium, magnesium, cardiovascular disease (CVD) markers (e.g. lipids, lipoproteins, homocysteine) and insulin sensitivity/resistance markers (e.g. glucose, insulin, HOMA) in cord serum; and b) to find out the possible influence of reduced or increased levels of serum calcium and magnesium on those markers.**

Subjects and Methods: **Forty-eight eutocic, normoweight and appropriated-for-gestational age, full-term, singleton without foetal distress newborns from the Mérida Study were studied. Parameter percentiles for serum calcium and magnesium as well as for the Ca/Mg ratio were stated. CVD and insulin sensitivity/resistance markers in neonates within the first quartile for calcium, magnesium and their ratio were compared with those of neonates within the forth quartile for these minerals.**

Results: **Serum calcium negative correlated with HDL-c ($p<0.05$), arylesterase (AE) ($p<0.01$), the Apo A1/Apo B ($p<0.05$) and AE/HDL-c ($p<0.05$) ratios. Also, negative and significant correlations were found between the Ca/Mg ratio and AE ($p<0.01$), and AE/HDL-c ($p<0.05$). Neonates within the highest quartile for Mg displayed significantly higher levels of LDL-c and homocysteine ($p<0.05$). Newborns within the Ca/Mg ratio first quartile presented higher activities of AE while those of with high Ca/Mg ratio showed low levels of insulin.**

Conclusions: **Calcium and magnesium levels appear related to CVD and insulin sensitivity/resistance markers at birth. Future follow-up studies, mostly in neonates, with high magnesemia and/or high Ca/Mg ratio at birth are recommended.**

(*Nutr Hosp*. 2015;31:278-285)

DOI:10.3305/nh.2015.31.1.8007

Keywords: *Calcium. Magnesium. Lipids. Lipoproteins. Insulin. HOMA. Neonates.*

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Recibido: 4-IX-2014.
Aceptado: 5-X-2014.

RELACIONES DE LOS NIVELES SÉRICOS DE CALCIO Y MAGNESIO CON LOS DE HOMOCISTEÍNA, LIPOPROTEÍNAS, Y MARCADORES DE SENSIBILIDAD/ RESISTENCIA A LA INSULINA AL NACIMIENTO

Resumen

Introducción **La relación entre los niveles de minerales en suero con los de homocisteína, lipoproteínas y marcadores homeostáticos de glucosa al nacimiento es poco conocida. El objetivo del presente estudio fue doble: a) determinar la relación entre calcio, magnesio, marcadores de riesgo de enfermedad cardiovascular (CVD) (p.e. lípidos, lipoproteínas, homocisteína) y marcadores de sensibilidad/resistencia a la insulina (p.e. glucosa, insulina, HOMA) en sangre de cordón; b) encontrar la posible influencia de los niveles elevados o reducidos de calcio y magnesio en suero sobre tales marcadores.**

Sujetos y Métodos **Se testaron 48 recién nacidos a término, de parto eutócico, normopeso, peso adecuado para su edad gestacional, sin distrés fetal del Estudio Mérida. Se obtuvieron percentiles para calcio, magnesio y la relación Ca/Mg y se compararon los niveles de marcadores CVD y sensibilidad/resistencia a la insulina de los neonatos clasificados en el primer cuartil para calcio, magnesio y cociente Ca/Mg con aquellos del cuarto cuartil.**

Resultados **El calcio sérico se correlacionó negativamente con HDL-c ($p<0,05$), arilesterasa (AE) ($p<0,01$), cocientes Apo A1/Apo B ($p<0,05$) y AE/HDL-c ($p<0,05$). El cociente Ca/Mg correlacionó negativa y significativamente con AE ($p<0,01$) y AE/HDL-c ($p<0,05$). Los neonatos del cuarto cuartil para Mg presentaron niveles más elevados de LDL-c y homocisteína ($p<0,05$). Aquellos del primer cuartil para Ca/Mg presentaron actividades de AE más elevadas, mientras que los del cuarto cuartil para Ca/Mg, menores insulinemias.**

Conclusiones **Calcemia y magnesemia aparecen relacionadas al nacimiento con marcadores CVD y de sensibilidad/resistencia a la insulina. Se recomiendan estudios de seguimiento, particularmente en aquellos niños con niveles elevados de magnesio y/o cocientes Ca/Mg elevados al nacimiento.**

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Palabras Clave: *Calcio. Magnesio. Lípidos. Lipoproteínas. Homocisteína. Insulina. HOMA. Neonatos.*

Abbreviations

AE: Arylesterase.
Apo: Apolipoprotein.
BMI: Body mass index.
TC: Total cholesterol.
CVD: Cardiovascular disease.
HDL-c: Cholesterol transported by high density lipoproteins.
HOMA: Homeostatic model assessment.
HOMA-IR: HOMA indicator of insulin resistance.
HOMA-S: HOMA indicator of insulin sensitivity.
Lp(a): Lipoprotein (a).
LDL-c Cholesterol transported by low density lipoproteins.
oxLDL: oxidized LDL.
QUICKI: Quantitative Insulin Sensitivity Check Index.
TG: Triglycerides.
tHcyt: total homocysteine.

Introduction

Many degenerative diseases have a paediatric and very likely embryonic and foetal origin[1,2]. It can be caused by various factors such as limited nutrient support during pregnancy[1,2]. In fact, our group has been studied the importance of determination of biomarkers for cardiovascular disease (CVD)[3,4] or metabolic syndrome at birth[2,5]. Thus, the levels of some risk markers for CVD at the age of 4 (e.g. TC/HDL-c, Apo A1/Apo B) can be predicted by considering the concentration of these factors at birth and those of their respective parents[6]. Due to the central role of minerals in lipoprotein and glucose metabolisms, altered concentrations of those micronutrients in plasma or serum from umbilical cord blood may be useful markers. Thus, calcium ions present in the intracellular and in the extracellular fluids play a role in various biochemical processes, including cell communication, muscle contraction and blood clotting[7]. Ca^{2+} is required for activation of arylesterase (AE), an enzyme present in the HDL[8] with pleiotropic antioxidant effects but of particular importance under CVD point of view[9,10]. Ca deficiencies are rare in neonates due to the homeostatic control of calciotropic hormones, which allows little variation in Ca serum levels[11].

Magnesium is essential for the synthesis of fatty acids and proteins, and it is critical in metabolic processes that require energy derived from ATP[12]. The study of Bastida et al.[13] suggests that Mg is an important factor in the metabolism of lipoproteins at birth. Hypomagnesaemia affects insulin resistance and is a risk factor for type 2 and gestational diabetes mellitus. Magnesium is involved in multiple steps of signal transduction pathways of insulin, such as secretion of the hormone, binding and receptor activity, which suggests that the reduction of intracellular Mg^{2+} decreases the activity of the insulin receptor, inhibiting the post-receptor action, which would result in an increase in insulin resistance[14].

Because of the antagonistic role of calcium and magnesium, the Ca/Mg ratio is considered more informative than that of both ions separately[15]. The Ca/Mg ratio was inversely correlated with levels of TC in contrast to that reported in adults[16].

The concurrence of altered lipids and markers of insulin sensitivity/resistance at birth has been scarcely investigated. The recent study of Gesteiro et al.[17] has demonstrated that concurrence of dyslipaemia and dysglycaemia causes an increase in insulin resistance markers and negatively alters the lipoprotein profile at birth. However, as far as we know, no studies exist linking mineral levels and insulin sensitivity/resistance markers at birth. On the other hand there is considerable disparity about the cord serum mineral levels in different studies[18,19]. In addition, mineral levels and neonatal CVD and insulin sensitivity/resistance markers are easily influenced by maternal characteristics such as age, pregnancy diet quality[2,20], pregnancies complicated by pre-eclampsia and intrauterine growth restriction[21], Diabetes mellitus[22], and the environment in which they live[23]. Taking into account previous premises, the present study hypothesized that at birth the serum calcium, magnesium and their ratio are associated with some CVD and insulin sensitivity/resistance markers. The aim of the present paper was to study possible relationships between calcium, magnesium, and their ratio with some CVD and insulin sensitivity/resistance markers at birth in strictly selected neonates.

Materials and methods

Subjects and samples

The participant subjects of the present study were women who gave birth at the Mérida Hospital (Mérida, Badajoz, Spain) and participated in a cross-sectional study aimed to search the relationship at birth between serum minerals and different CVD and glucose homeostasis biomarkers. The Merida Hospital is a regional hospital of 400 beds, placed in a town of approximately 54,000 inhabitants, giving medical support to 150,000 people. This study was performed in accordance with the Helsinki Declaration and approved by the Mérida Hospital's Direction and this Hospital Ethics Committee. All participant mothers gave their written informed consent after receiving verbal and written information about the study. Since there is a well-established relationship between some metals and foetal development[24], and because previous reports confirm an association between perinatal conditions (such as gestational age, perinatal distress, mode of delivery) and lipoprotein profile[25]

and between birth weight and insulin levels[2,26], newborns were selected in accordance with the following criteria: (i) Caucasian race; (ii) singleton live birth; (iii) eutocic delivery with cephalic presentation and without instrumental help; (iv) full-term, between the beginning of the 37th wk and the end of the 41st wk; (v) normoweight, between 2,500 and 3,999 kg, and appropriate-for-gestational-age; and (vi) without perinatal distress (Apgar scores of ≥7 at the 1st min and ≥9 at the 5th min).

Due to cord blood had been used for other analysis and, a minimum volume of 0.5 mL of serum was demanded for the determination of calcium and magnesium only 48 samples fulfilling the above-mentioned criteria were included and studied. The participation rate was about 23% of participating women in the Mérida Cohort study.

Analytical procedure

Immediately after delivery, about 3 mL mixed umbilical cord blood samples were collected in disposable plastic tubes. Blood was centrifuged (3,500 rpm for 5 min) to obtain serum. Aliquots were frozen at -18ºC until processed. Duplicate serum samples were analysed for calcium and magnesium by flame atomic absorption spectrometry with previous acid digestion[27]. Lantanum chloride (Merck, Darmstadt, Germany) was added to samples and standards (final concentration 0.5% Lantanum) to avoid interferences. Aliquots of human serum were used as an internal control to assess precision in the mineral determinations. The interassay relative standard deviation was <10% for both minerals. Certified reference materials (CRM 63, CRM 185, Community Bureau of Reference, Brussels, Belgium) were used to estimate accuracy. All laboratory equipment employed in the mineral analysis was washed with 10N nitric acid to avoid contamination. Distilled-deionized water (Milli Q plus, Millipore) was used for the preparation of dilutions and standards of the mineral analysis.

Serum TC concentrations were measured by the colorimetric enzymatic method (CHOD-PAP, Roche Diagnostics). HDL-c was tested using the HDL-cholesterol plus 2nd generation homogeneous enzymatic test (Roche Diagnostics). Triglycerides (TG) were determined by the colorimetric enzymatic method (GPO-PAP, Roche Diagnostics) and LDL-c was calculated using the formula of Friedewald et al. as validated in neonates by Glueck et al.[28] Apo A1 and Apo B and Lipoprotein (a) [Lp(a)] were determined by immunoturbidimetric methods (Tina-quant®, Roche Diagnostics). All these determinations were processed in a Roche/Hitachi Modular P (Roche Diagnostics, Basel, Switzerland) analyser. Total homocysteine (tHcyt) was measured by fluorescence polarization immunoassay supplied by Abbott in an IMX® System analyser (Abbott Diagnostics, IL, USA). AE activity was measured according to Nus et al. method[10] at 37 ºC. One unit of AE was defined as the mmol of phenol formed from phenyl acetate per minute monitorized using a thermostated T80+ spectrophotometer (PG Instruments® Ltd. Wibtoft, Leics, UK). OxLDL was determined using an ELISA test kit from Mercodia Laboratories (Upsala, Sweden). The colorimetric end point was measured at 450 nm using a model ELx808 BioTek® spectrophotometer (BioTek Instruments, Winoosky, VT., U.S.A.).

Serum glucose and insulin were measured by the glucose hexokinase method and electrochemiluminescence immunoassay (ECLIA) both supplied by Roche Diagnostics (Basel, Switzerland). HOMA-IR[29] and the quantitative insulin sensitivity check index (QUICKI) were also calculated[30].

Our laboratory participates in the Spanish Clinical Chemistry Society (SEQC) External Quality Evaluation Program, which follows UNE-EN-ISO 9001:2000 standards and is certified by AENOR. All assays were properly calibrated and performed under internal and external quality controls provided by the manufacturers and SEQC, respectively. Intra-assay and inter-assay variation coefficients were 0.8% and 1.7% for TC; 1.5% and 1.8% for TG; 0.9% and 1.85% for HDL-c; 1% and 2.4% for Apo A1; 1.5% and 2.5% for Apo B; 2.3% and 2.8% for tHcyt; 8% and 8.9% for AE, and 4.5% and 5.0% for oxLDL, 1.0 and 1.7% for glucose; and 1.5 and 4.9 for insulin respectively.

Statistical studies

The Kolmogorov-Smirnov test was used to analyse the normal distribution of data. TG, HOMA-IR and HOMA-S indices were normalized by natural log transformation. Pearson product-moment correlations were applied to correlate serum mineral levels and the other analysed markers. The homogeneity of variances was assessed by Levene test (independent samples). Neonate distributions were made into quartiles (Q1 or percentiles 25 and Q4 or percentile 75, respectively). To ascertain the effect of the serum levels of minerals on different parameters, CVD and insulin sensitivity/resistance markers of neonates within the lowest quartile were compared with those of neonates within the highest quartile for calcium, magnesium and their ratio by the Student´s *t*-test. Statistical significance was set at $p < 0.05$ while marginally significant at $p < 0.1$ using SPSS (version 19) statistical software package.

Results

Table I shows means, standard errors, and the 25th, 50th, and 75th percentiles of the different anthropometric, lipid, lipoprotein, and insulin sensitivity/resistance markers tested.

Table I
Anthropometric characteristics, calcaemia, magnesaemia, the Ca/Mg ratio, lipids, homocysteine, glucose, insulin and insulin sensitivity/resistance markers of the Mérida study newborns

	Mean	*SE*	*Percentile*		
			25th	*50th*	*75th*
Birth weight (kg)	3299	42.76	3065	3305	3510
BMI (kg/m^2)	13.20	0.16	12.26	13.24	13.83
Gestational age (wks)	39.74	0.18	38.86	39.93	40.71
Calcium (μg/mL)	84.77	3.52	63.08	86.9	104.25
Magnesium (μg/mL)	24.23	1.12	20.43	23.8	26.6
Ca/Mg	3.63	0.16	2.84	3.41	4.45
Total cholesterol (TC) (mg/dL)	60.83	3.66	48.0	56.0	66.75
Triglycerides (mg/dL)	31.5	3.54	17.25	27.0	39.0
LDL-c (mg/dL)	27.1	2.14	20.05	24.7	30.7
HDL-c (mg/dL)	27.44	1.46	20.25	26.5	32.5
Apo A1 (mg/dL)	72.63	2.9	62.0	69.65	78.98
Apo B (mg/dL)	32.56	4.3	17.13	22.35	32.93
oxLDL (U/L)	46.45	5.61	15.59	41.94	71.0
Arylesterase (AE) (U/L)	29.75	3.94	7.32	21.42	42.84
Homocysteine (μmol/L)	5.66	0.26	4.37	5.44	6.51
TC/HDL-c	2.26	0.06	1.97	2.18	2.53
ApoA1/ApoB	3.12	0.21	2.18	3.04	3.84
AE/HDL-c (U/mg)	0.11	0.02	0.03	0.07	0.17
oxLDL/LDL-c (U/mg)	0.19	0.03	0.08	0.17	0.25
Glucose (mg/dL)	69.8	4.9	48.8	66.0	79.8
Insulin (mUI/mL)	5.07	0.61	2.08	4.05	6.42
Glucose/Insulin	23.55	4.19	10.69	17.41	25.96
HOMA-IR	1.05	0.20	0.25	0.6	1.16
HOMA-S	3.51	0.83	0.86	1.66	4.06
QUICKI	0.44	0.02	0.37	0.42	0.5

Data obtained from 48 strictly selected neonates. For more details see text.

Table II shows significant and marginally significant correlations between Ca, Mg and the Ca/Mg ratio and CVD and insulin sensitivity/resistance markers. At birth serum calcium negative correlated with HDL-c ($p<0.05$), AE ($p<0.01$), the Apo A1/Apo B ($p<0.05$) and AE/HDL-c ($p<0.05$) ratios. Also, negative and significant correlations were found between the Ca/Mg ratio and AE ($p<0.01$), and AE/HDL-c ($p<0.05$). Mg shows negative and marginally significant ($p<0.1$) correlations with HDL-c/LDL-c. The Ca/Mg ratio also showed correlations that were marginally significant ($p<0.1$) with TC, HDL-c, Apo A1, insulin and HOMA-IR.

Significant or marginally significant comparisons between levels of CVD or insulin sensitivity/resistance markers of neonates within the first and forth quartiles for Ca, Mg and the Ca/Mg ratio are shown in Table III. No significant differences were found for TG, TC, Apo A1, Apo B, Lp(a), LDL-c/Apo B, oxLDL/LDL-c ratios. With regard to Ca, neonates within the forth quartile showed lower AE ($p<0.01$) and tended to present lower ($p<0.1$) levels of HDL-c, HDL-c/Apo A1 and Apo A1/ApoB ratios than those of neonates within the lowest quartile. LDL-c and tHcyt were significantly higher (both $p<0.05$) while the ApoA1/ApoB ratio lower ($p<0.05$) in neonates within the highest quartiles of Mg. Neonates within the forth quartile of Mg tended to have reduced levels of the HDL-c/LDL-c but higher of the oxLDL/LDL-c ratios (both $p<0.1$) than those of within the lower Mg quartile. Neonates wi-

Table II
Pearson product–moment correlations between cardiovascular and insulin resistance biomarkers and calcium, magnesium and the Ca/Mg ratio levels in cord serum of newborns from Mérida Study

	Ca	*Mg*	*Ca/Mg*
TC	NS	NS	-0.268^{+}
HDL-c	-0.287*	NS	-0.270^{+}
LDL-c	NS	NS	NS
Arylesterase (AE)	-0.411**	NS	-0.375**
Apo A1	NS	NS	-0.271^{+}
Apo B	NS	NS	NS
TC/HDL-c	NS	NS	NS
HDL-c/LDL-c	-0.305*	-0.267^{+}	NS
LDL-c/Apo B	NS	NS	NS
Apo A1/Apo B	-0.365*	NS	NS
AE/HDL-c	-0.339*	NS	-0.321*
Glucose	NS	NS	NS
Insulin	NS	NS	-0.260^{+}
HOMA-IR	NS	NS	-0.269^{+}
QUICKI	NS	NS	NS
Glucose/insulin	NS	NS	NS

***$p < 0.001$; **$p < 0.01$; *$p \leq 0.05$; $^{+}p < 0.1$, borderline for statistical significance; NS, not significant. For units and abbreviations, see Table I and general abbreviations.

thin the highest quartile for the Ca/Mg ratio showed reduced activities of AE (p<0.01), lower insulin levels (both p<0.05) and AE/HDL-c ratio (p<0.01) but higher glucose/insulin ratio (p<0.05) in neonates within the forth quartile of the Ca/Mg ratio.

Discussion

Information about the possible role of minerals on lipoprotein metabolism at birth is very scarce[13,31]. Even less data exist about the possible role of minerals on insulin sensitivity/resistance at birth or in the association of dysglycemia-dyslipemia also at birth. Therefore, this report should be considered as a pioneering research in those topics.

Studied newborns had a bodyweight and body mass index (BMI) that did not significantly differ from the whole population of the Merida Study[5,17,20] and were also similar to those other studies in Spanish neonates[4]. The concentrations of lipid, lipoproteins, tHcyt, glucose, insulin and other studied markers were quite similar to those of found by our group in the whole neonatal population,[5,17] indicating that the selected neonates were representative from those of the Merida Study. Birth concentrations of minerals, as well as quartiles, suggest large distribution amplitude for some minerals. International bibliography shows that there is considerable disparity in the cord blood-serum mineral levels[18,19]. We do not know the involved reasons, but differences in mothers' diets, or in some methodological issues may explain, at least partially, differences in neonatal mineral concentrations between studies.

The negative correlations found between Ca and many CVD risk factors are interesting although, a possible CVD protective role of calcium-rich diets has been reported in adults[32]. Due to the known anti-oxidant role of HDL[8,33] and that of its AE related enzyme[8,9], it could be hypothesized that less HDL-c and AE are demanded in those neonates within the first quartile for Ca. When comparing neonates classified according the Ca percentiles we found that, although there was no significant effect of this mineral on insulin sensitivity/resistance markers, those newborns within the highest quartile for Ca had lower AE activities than those from the lowest quartile. As the AE enzyme shows active centre for Ca and this ion has been known to clearly enhance AE activity[10], it can be hypothesized that less AE activity is demanded when higher Ca levels are available. Results also suggest some decrease in the level of HDL-c and ApoA1, probably related to the AE decrease as AE is a HDL enzyme with important CVD role and pleiotropic antioxidant effects[7,8].

Magnesaemia seems to be an important factor affecting CVD markers at birth as LDL-c and homocysteine was higher while the ApoA1/ApoB ratio appeared lower in neonates within the highest Mg quartile. This fact is even more relevant considering the increase tendency of LDL oxidation (given by the oxLDL/LDL-c ratio). LDL oxidation has been defined as an important factor for atherosclerosis initiation and development[33].

No clear hypothesis is available to explain this potential negative association considering the neonatal strict selection performed. However, no significant association was found between serum Mg and any insulin sensitivity/resistance markers at birth. Mg is essential element for the synthesis of fatty acids and protein, and participates in metabolic processes that require energy derived from ATP[12]. It is involved in multiple steps of signal transduction pathways of insulin, such as secretion of the hormone, binding and receptor activity, which suggests that the reduction of intracellular Mg^{2+} decreases the activity of the insulin receptor which would result in an increase in insulin resistance[14].

Differing from the current study, previous results of our group suggested that the serum Ca/Mg ratio affected neonatal TC[13]. Differences can be attributed to stricter neonatal selection performed in the present study where large- or small-for-gestational-age children were not included.

Table III
Lipoprotein, arylesterase (AE) and homocysteine, insulin, and glucose levels in neonates from the Mérida Study classified according to quartiles of serum calcium, magnesium or the Ca/Mg ratio

		Ca	*Mg*	*Ca/Mg*
HDL-c (mg/dL)	P ≤ 25	30.73 ± 3.87^{+}	NS	NS
	P > 75	22.75 ± 1.94		
LDL-c (mg/dL)	P ≤ 25	NS	21.7 ± 2.73*	NS
	P > 75		28.43 ± 1.69	
oxLDL (U/L)	P ≤ 25	NS	29.56 ± 9.69^{+}	NS
	P > 75		62.16 ± 15.86	
AE (U/L)	P ≤ 25	39.66 ± 8.86*	NS	49.38 ± 9.50**
	P > 75	18.64 ± 5.19		14.12 ± 3.94
Homocysteine (μmol/L)	P ≤ 25	NS	4.77 ± 0,27*	NS
	P > 75		6.06 ± 0.48	
TC/HDL-c	P ≤ 25	NS	NS	NS
	P > 75			
HDL-c/LDL-c	P ≤ 25	NS	1.57 ± 0.38^{+}	NS
	P > 75		0.91 ± 0.05	
HDL-c/Apo A1	P ≤ 25	0.38 ± 0.02^{+}	NS	NS
	P > 75	0.34 ± 0.01		
Apo A1/Apo B	P ≤ 25	3.6 ± 0.47^{+}	3.87 ± 0.43*	NS
	P > 75	2.47 ± 0.44	2.55 ± 0.34	
AE/HDL-c (U/mg)	P ≤ 25	NS	NS	0.21 ± 0.05**
	P > 75			0.07 ± 0.02
Insulin (mUI/mL)	P ≤ 25	NS	NS	6.35 ± 1.26*
	P > 75			3.38 ± 0.42
Glucose/insulin	P ≤ 25	NS	NS	14.22 ± 2.27*
	P > 75			20.55 ± 2.02

Data are the mean ± standard error. Only parameters showing significant differences or at the borderline for the statistical significance are shown. P, percentile for the concentrations of the different minerals. Significant differences from: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; $^{+}p < 0.1$ differences in borderline for statistical significance, NS: Not significant. For abbreviations, see general abbreviations.

Similarly to that already commented for Ca, the Ca/Mg ratio appears relevant affecting AE activity and probably related to the role of Ca on the AE activation[7,9]. The effect of the Ca/Mg ratio on many insulin sensitivity/resistance markers seems remarkable. None of the neonates within the highest quartile of the Ca/Mg ratio had insulin levels > 6 μUI/mL, cut-off point suggested by Gesteiro et al.[5], while over 60% of newborns within the lowest quartile had insulin levels > 6 μUI/mL. Thus, present results suggest that neonates having a higher Ca/Mg ratio showed a lower insulin levels, glucose/insulin ratio, and thus higher insulin sensitivity. Mg ion is an important enzyme co-factor that is associated with carbohydrate metabolism because it plays a role in the regulation and inhibition of insulin.[14] Moreover, insulin is an important hormone that regulates the balance of Mg[14]. Further, as insulin is secreted by a Ca-dependent process, dysfunction of Ca channel is associated with diabetes[34]. Ca and Mg, which are associated with the role of insulin, play an antagonistic role in the cells[35]. Thus, it was suggested that the Ca/Mg ratio may be more important in the determination of the activity of cells than the absolute concentration of both ions[15], and concluded that glucose increases the influx of calcium and the release of insulin at birth. However, glucose blood concentration seems to be independent of the extracellular Ca^{2+} concentration when the ratio is not altered.

Since only one neonate presented concurrence of high TG and elevated glucose, and another neonate had low HDL-c and elevated serum glucose, the

effect of minerals on the concurrence of dyslipemia and dysglycemia in newborns was not tested nor discussed. Nonetheless, six neonates showed the concurrence of low TG and high HDL-c levels while high TG and low HDL-c levels concurred in just two newborns. Calcium (p=0.034) and the Ca/Mg ratio (p=0.016) were significantly lower in those neonates presenting the concurrence of two metabolic syndrome components, suggesting a role of Ca and the Ca/Mg ratio in that concurrence. However, due to the small number of neonates showing those concurrences, results have to be carefully considered and demand ampler studies.

Although the study was performed in neonates selected according to strict criteria (singletons, normoweight, appropriate-for-gestational-age, full term, with foetal distress), it has to be pointed out that the present study has limitations as a) the reduced number of neonate studies and b) clinical determination of some minerals could have low specificity and sensitivity in the general population[36]. Moreover, the concentration in cord serum is the resultant of interactions between maternal factors (e.g. diet) and organ uptakes and efflux.

Conclusion

The current study results suggest the existence of multiple relationships between calcium and CVD and insulin sensitivity/resistance markers at birth. Particularly, outstanding effects on the markers were observed in cases of Mg on lipoproteins and tHcys, and the Ca/Mg ratio on insulin sensitivity/resistance markers. Therefore, it seems recommendable to perform future follow-up studies in a larger number of infants to improve knowledge in the relationship between serum mineral concentrations and metabolic syndrome markers at birth.

Acknowledgements

The authors are indebted to neonates and their families participating in the Mérida Study, to the Services of Biochemistry and Obstetrics and Gynaecology of Hospital of Mérida. The study was partially supported by the Project AGL-2008 04892-C03-02.

Disclosure statement

The authors have nothing to disclose

References

1. Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995; 311: 171-4.
2. Sánchez-Muniz FJ, Gesterio E, Espárrago Rodilla M, Rodríguez-Bernal B, Bastida S. La alimentación de la madre durante el embarazo condiciona el desarrollo pancreático, el estatus hormonal del feto y la concentración de biomarcadores al nacimiento de Diabetes mellitus y síndrome metabólico. *Nutr Hosp* 2013; 28: 250-74.
3. Bastida S, Sánchez-Muniz FJ, Cuesta C, Perea S, Ureta A. Serum apolipoproteins A-I and B in male and female full-term newborns of the Toledo-Study (Spain). *Acta Paediatr* 1996; 85: 750-2.
4. Bastida S, Sánchez-Muniz FJ, Cuesta C, Perea S, Aragonés A. Male and female cord blood lipoprotein profile differences throughout the term-period. *J Perin Med* 1997; 27: 184-91.
5. Gesteiro E, Bastida S, Sánchez-Muniz FJ. Insulin resistance markers in term normoweight neonates. The Mérida Cohort. *Eur J Ped* 2009; 168: 281-8.
6. Bastida S, Sánchez-Muniz FJ, Cuena R, Aragonés A, Bravo C. Lipid and lipoprotein concentrations at age 4. Association with neonatal and parental levels. *Med Clin (Barc)* 2007; 128: 521–8.
7. Narbona Lopéz E, Contreras Chova F, Pérez Iañez R, García Iglesias F, Miras Baldo MJ. Metabolismo fosfocálcico en el periodo neonatal. Protocolos de Neonatología de la Asociación Española de Pediatría, 2008; 169-76.
8. Canales A, Sánchez-Muniz FJ. Paraoxonase, anything else than one enzyme? *Med Clin (Barc)* 2003; 12: 537-48.
9. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high density lipoproteins oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest* 1998; 101:1581–90.
10. Nus M, Sánchez-Muniz FJ, Sánchez-Montero JM. A new method for the determination of arylesterase activity in human serum using simulated fluid. *Atherosclerosis* 2006; 188: 155-9.
11. Minagawa M, Yasuda T, Kobayashi Y, Niimi H. Transient pseudohypoparathyroidism of the neonate. *Eur J Endocrinol* 1995; 133: 151–5.
12. Al-Ghadi SMG, Cameron EC, Sutton RAL. Magnesium deficiency: pathophysiologic and clinical overview. *Am J Kidney Dis* 1994; 24: 737–52.
13. Bastida S, Vaquero MP, Veldhuizen M, Sánchez-Muniz FJ. Selected trace elements and minerals in cord blood: association with lipids and lipoproteins at birth. *Acta Paediatr 2000*; 89: 1201-6.
14. Naira AV, Hocherb B. Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. *PNAS* 2012; 109: 11324–9.
15. Atwater I, Beigelman PM. Dynamic characteristics of electrical activity in pancreatic beta-cells. I. - Effects of calcium and magnesium removal. *J Physiol (Paris)* 1976; 72: 769-86.
16. Speich MS, Gelot S, Arnaud P, Van Goc N, Robinet N, Pineu A. Multiple and simple correlations between magnesium, calcium, zinc, potassium, total and HDL-cholesterol in 111 reference subjects. *Magn Bull* 1984; 4: 137–41.
17. Gesteiro E, Bastida S, Sánchez-Muniz FJ. Cord-blood lipoproteins, homocysteine, insulin sensitivity/resistance marker profile, and concurrence of dysglycaemia and dyslipaemia in full-term neonates of the Mérida Study. *Eur J Pediatr* 2013; 172: 883-94.
18. Speich M, Bousquet B, Auget JL, Gelot S, Laborde O. Association between magnesium, calcium, phosphorus, copper, and zinc in umbilical cord plasma and erythrocytes, and the gestational age and growth variables of full-term newborns. *Clin Chem* 1992; 38: 141-3.
19. Negi R, Pande D, Karki K, Kumar A, Khanna RS, Khanna HD. Trace elements and antioxidant enzymes associated with oxidative stress in the pre-eclamptic/eclamptic mothers during fetal circulation. *Clin Nutr* 2012; 31: 946-50.
20. Gesteiro E, Bastida S, Sánchez-Muniz FJ. Effects of maternal glucose tolerance, pregnancy diet quality and neonatal insulinemia upon insulin resistance/sensitivity biomarkers in normoweight neonates. *Nutr Hosp* 2011; 26: 1447-55.
21. Rodie VA, Caslake MJ, Stewart F. Fetal cord plasma lipoprotein status in uncomplicated human pregnancies and in preg-

nancies complicated by pre-eclampsia and intrauterine growth restriction. *Atherosclerosis* 2004; 176: 181–7.
22. Al-Saleh E, Nandakumaran M, Al-Shammari M, Makhseed M, Sadan T, Harouny A. Maternal-fetal status of copper, iron, molybdenum, selenium and zinc in insulin-dependent diabetic pregnancies. *Arch Gynecol Obstet* 2005; 271: 212–7.
23. Lin YY, Hwang YH, Chen PC, Chen BY, Wen HJ, Liu JH, et al. Contribution of gestational exposure to ambient traffic air pollutants to fetal cord blood manganese. *Environ Res* 2012; 112: 1-7.
24. Ghebremeskel K, Burns L, Burden TJ, Harbige L, Costeloe K, Powell JJ, et al. Vitamin A and related essential nutrients in cord blood; relationships with anthropometric measurements at birth. *Early Hum Dev* 1994; 39: 177–88.
25. Lane DM, McConathy WJ. Factors affecting the lipid and apolipoprotein levels of cord sera. *Pediatr Res* 1983; 17: 83–91.
26. Yada KK, Gupta R, Gupta A, Gupta M. Insulin levels in low birth weight neonates. *Indian J Med Res* 2003; 118: 197-203.
27. Ball DW. Field Guide to Spectroscopy. Spie Press, USA 2006.
28. Glueck CJ, Steiner P, Leuba U Cord blood low-density lipoprotein-cholesterol estimation versus measurement with the preparative ultracentrifuge. *J Lab Clin Med* 1973; 82: 467-72.
29. Livesey G. A systematic review of the glycaemic response to foods and health. ILSI Europe workshop. Glycaemic response on health. International Life Sciences Institute, Brussels, 2006; 82-127.
30. Katz A, Nambi SS, Mather K. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85: 2402–10.
31. Yount NY, McNamara DJ, Al-Ohtman AA, Lei KY. The effect of copper deficiency on rat hepatic 3-hydroxy-3-methylglutaryl co-enzyme A reductase activity. *J Nutr Biochem* 1990; 1: 27–33.
32. Kromhout D. Diet and cardiovascular diseases. *J Nutr Health Aging* 2001; 5: 144-9.
33. Ross R. Atherosclerosis an inflammatory disease. *New Engl J Med* 1999; 340:115-26.
34. Ojuka EO. Role of calcium and AMP kinase in the regulation of mitochondrial biogenesis and GLUT4 levels in muscle. *Proc Nutr Soc* 2004; 63: 275–8.
35. Iseri LT, French JH. Magnesium: nature's physiological calcium blocker. *Am Heart J* 1984; 108: 188–94.
36. Mataix Verdú J (ed.). Tratado de Nutrición y Alimentación. Ergón, Majadahonda (Madrid), 2009.