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Cord-blood lipoproteins, homocysteine, insulin sensitivity/resistance marker profile, and concurrence of dysglycaemia and dyslipaemia in full-term neonates of the Mérida Study

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Abstract Early alterations in glucose homeostasis increase the risk of developing insulin resistance and obesity later in life. The concurrence of altered lipids and insulin sensitivity/resistance markers at birth has been scarcely investigated. The study aimed to ascertain level ranges of homocysteine (tHcyt), arylesterase (AE), lipids/lipoproteins, and insulin resistance/sensitivity markers in full-term neonates and to determine the concurrence effect of dyslipaemia and dysglycaemia on those parameters at birth. Participants were 197 full-term, 2.5 to <4.0 kg, without foetal distress Spanish newborns from the Mérida Study. Parameter percentiles for males and females were stated. The effect of the concurrence high glucose/high triglycerides (high glucose/high TG) or high glucose/low cholesterol transported by HDL (HDL-c) on tHcyt, LDL-c, HDL-c, lipoprotein (a) (Lp(a)), oxidised LDL (oxLDL), AE, glucose, insulin sensitivity (QUICKI) and insulin resistance index (HOMA-IR) was studied. Females had higher total cholesterol (TC), HDL-c, Apo A1, Lp(a) and HDL-c/Apo A1, but lower relative transport of TC (%TC) by the very low lipoprotein fraction than males. No gender differences were found for glucose, HOMA-IR and QUICKI. Neonates at the 2.5- to 2.999-kg range display more adequate HOMA-IR and QUICKI levels that their >3.0 kg counterparts. The concurrence of high glucose/high TG or high glucose/low HDL-c increased TC/HDL-c and HOMA-IR, but decreased, oxLDL, oxLDL/LDL-c and QUICKI with respect to that of low glucose/low TG or glucose/high HDL-c. The concurrence glucose/TG has predictive value for low QUICKI, whilst that of glucose/HDL-c for low QUICKI and high HOMA-IR, suggesting the importance of routine TG, HDL-c and glucose screening at birth as it would identify candidates for insulin resistance.

Keywords Neonates · Homocysteine · Lipoproteins · Insulin sensitivity/resistance makers · Concurrence of risk factors

Abbreviations

AE	Arylesterase
Apo	Apolipoproteins
AUC	Area under the curve
BMI	Body mass index
CVD	Cardiovascular disease
DM	Diabetes mellitus
DMT2	Type 2 diabetes mellitus
HDL-c	Cholesterol transported by HDL
HOMA-IR	Homeostatic model assessment—insulin resistance
tHcyt	Total homocysteine
LDL-c	Cholesterol transported by LDL
Lp(a)	Lipoprotein (a)
MS	Metabolic syndrome
oxLDL	Oxidised LDL
PON1	Paraoxonase-1
QUICKI	Quantitative Insulin Sensitivity Check Index
TC	Total cholesterol
TG	Triglycerides
VLDL	Very low-density lipoproteins
%TC	Relative transport of TC

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Introduction

The perinatal period is very sensitive to several disorders which can increase the risk of metabolic diseases later in life

[5, 9, 18]. The detection of high total cholesterol (TC), apolipoprotein (Apo) B, and the low Apo A1 and Apo A1/Apo B ratio in umbilical cord blood from term newborns could identify higher risk for cardiovascular disease (CVD) neonates [1, 10, 30, 34]. Our group has shown that the level of some CVD risk markers (e.g. TC, TC/cholesterol transported by HDL (TC/HDL-c), Apo A1/Apo B) at age 4 can be predicted considering these factors at birth and those of their respective parents [8]. Elevated serum lipoprotein (a) (Lp(a)) [24] and total homocysteine (tHcyt) [32] have been suggested to be independent risk factors for atherosclerosis and CVD. Delivery represents a significant stress to both mother and child. The dramatic decrease of foetal antioxidant capacity at the end of labour may induce lipoprotein oxidation increase. It has been suggested that paraoxonase (PON 1) and the arylesterase (AE)-PON 1 activity protects low- and high-density lipoproteins (LDL and HDL, respectively) from lipid peroxidation [3]. Our group has determined the AE activity in neonates carrying APOA5 S19W gene polymorphisms [14]. However, few studies have defined the normality ranges for oxidised LDL (oxLDL) and AE in full-term neonates.

Early alterations in main hormones controlling glucose homeostasis increase the risk of developing insulin resistance and obesity later in life [17]. Thus, elevated insulin levels during perinatal life may predispose for the development of diabetes mellitus (DM) in children and adults [28]. A relatively high prevalence of increased Homeostatic Model Assessment—Insulin Resistance (HOMA-IR) values has been found in Spanish neonates [12, 13]. Early identification of candidates to develop the metabolic syndrome (MS) and, thus, DMT2 and/or CVD should be demanded as these conditions are becoming more and more frequent in children and adolescents [22, 34].

Taking previous premises into account, the aims of the present study were to ascertain in umbilical cord blood of full-term newborns the influence of gender, gestational age and body weight on (a) tHcyt, lipids, lipoproteins, Apos, as well as the atherogenic indices and (b) glycaemia, insulinaemia, and HOMA-IR indices and the Quantitative Insulin Sensitivity Check Index (QUICKI). In addition, this paper studies the possible relationships between lipoprotein and glucose metabolism markers and the effect of the concurrence of two major components of MS (high glucose/high triglycerides (TG) and high glucose/low HDL-c) on tHcyt, lipoproteins and insulin sensitivity/resistance markers at birth.

Materials and methods

Sample characteristics

This study of 197 neonates from the Hospital of Mérida (Badajoz, Spain), a regional hospital of 400 beds giving medical support to 150,000 people, was performed in accordance with the Helsinki Declaration of 1975 as revised in 1983, following approval by the Management and Ethical Committee of the hospital. All selected neonates were Caucasian, singleton, full-term (between 37 and <42 weeks), normoweight (between 2.5 and <4.0 kg) infants born without foetal distress (Apgar scores ≥7 and ≥9 at the first and fifth minutes, respectively).

Protocol

Data concerning delivery (type, primiparity/multiparity), mothers (age and gestational weight gain) and neonates (birthweight, length, gender, gestational age, and Apgar score at the first and fifth minutes) were obtained from hospital records. Anthropometrical measurements were taken by the Obstetric Department-trained personnel following routine Mérida Hospital protocols. After delivery, the umbilical cord was cut and blood was obtained by arterial puncture and collected in BD Vacutainer® SST II tubes with a separation gel (Becton Dickinson, Plymouth, UK). Blood was centrifuged (3,500 rpm for 5 min) to obtain serum. Aliquots were frozen at −18 °C until processed. Samples for AE and oxLDL determination were frozen at −80 °C.

Assays

Serum glucose was measured using the glucose hexokinase method (Gluco-quant®, Roche Diagnostics) and TC concentrations using the colorimetric enzymatic method (CHOD-PAP, Roche Diagnostics). HDL-c was tested using the HDL-cholesterol plus second-generation homogeneous enzymatic test (Roche Diagnostics). TG were determined using 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. [15]. Apo A1, Apo B and Lp(a) were determined using immunoturbidimetric methods (Tina-quant®, Roche Diagnostics). All these determinations were processed in a Roche/Hitachi Modular P (Roche Diagnostics, Basel, Switzerland) analyser. Insulin concentrations were determined using the electrochemiluminescence immunoassay in a Roche/Hitachi Modular Analytics E 170 analyser (Roche Diagnostics). tHcyt was measured using the fluorescence polarization immunoassay supplied by Abbott in an IMX® System analyser (Abbott Diagnostics, Abbott Park, IL, USA). AE activity was measured according to the method of Nus et al. [25] at 37 °C. One unit of AE was defined as millimoles of phenol formed from phenyl acetate per minute monitored using a thermostated T80+ spectrophotometer (PG Instruments® Ltd. Wibtoft, Leics, UK). OxLDL was determined using an ELISA test kit from Mercodia Laboratories (Uppsala, Sweden). The colorimetric end point was measured at 450 nm using a model ELx808 BioTek® spectrophotometer (BioTek Instruments, Winoosky, VT, USA).

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. The intra- and inter-assay variation coefficients were 1 and 1.7 % for glucose, 1.5 and 4.9 % for insulin, 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, respectively.

Insulin resistance or sensitivity was tested using the QUICKI [19], calculated as $1/[(\text{logInsulin}) \ (\text{mIU/L}) + (\text{logGlucose}) \ (\text{mg/dL})]$ and HOMA-IR [23] as Glucose $(\text{mmol/L}) \times \text{Insulin (mIU/L)}/22.5$. In addition, the glucose/insulin ratio was calculated.

Dysglycaemia and dyslipaemia concurrences

The prevalence of high-glucose/high-TG and high-glucose/low-HDL-c levels in the present neonatal population was tested. Data of neonates showing those concurrences were compared with those presenting concurrency of low-glucose/low-TG and low-glucose/high-HDL-c values, respectively.

As neonates were singleton, normoweight, full-term and did not have foetal distress at birth, percentiles 25 and 75 of the current study were selected for defining low-/high-TG and HDL-c cutoff points. As cutoff points 81.3 mg/dL in males and 82.5 mg/dL in females for high serum glucose and 53.5 mg/dL in males and 56.0 mg/dL in females for low glucose were selected as reported in a previous paper performed im control male and female full-term neonates whose mothers were negative at the O'Sullivan test [12].

Statistical studies

The Kolmogorov–Smirnov test was used to analyse the normal distribution of data. TG and Insulin sensitivity/resistance markers were normalised by natural log transformation. Student's t test was employed to compare male and female neonates. This study was designed to have a power at least of 90 % (nominal $\alpha = 0.05$) to detect mean differences of 7 mg/dL for TC, 0.25 for TC/HDL-c, 4.0 mUI/L for insulin and 1.5 for HOMA-IR between male and female neonates. One-way ANOVA followed by the Bonferroni post hoc study was used for multiple comparisons. The sample size was also adequate to test absolute differences of 9 mg/dL for TC, 0.30 for TC/HDL-c, 4.5 mUI/L for insulin and 2.5 for HOMA-IR with

Table 1 Anthropometrical data, gestational age and Apgar indices of full-term neonates from the Mérida Study

		N	Mean±SD (95%CI)	Significance	P25	P50	P75
Bodyweight (g)	All	197	3,301±331 (3,255–3,347)		3,050	3,315	3,548
	Females	110	3,263±335 (3,200–3,326)	0.069	3,020	3,280	3,500
	Males	87	3,348±320 (3,281–3,416)		3,080	3,340	3,615
Height (cm)	All	197	50.0±1.4 (49.8–50.2)		49.5	50.0	50.9
	Females	110	49.9±1.4 (49.7–50.2)	0.556	49.0	50.0	50.5
	Males	87	50.1±1.3 (49.8–50.3))		49.5	50.0	51.0
Body mass index (kg/m^2)	All	197	13.2±1.1 (13.0–13.4)		12.5	13.2	13.9
	Females	110	13.1±1.2 (12.9–13.3)	0.050	12.2	13.2	13.8
	Males	87	13.4±1.1 (13.1–13.6)		12.6	13.3	14.2
Ponderal index (kg/m^3)	All	197	26.4±2.4 (26.1–26.7)		24.9	26.2	27.9
	Females	110	26.2±2.5 (25.7–26.7)	0.145	24.8	26.1	27.7
	Males	87	26.7±2.3 (26.2–27.2)		25.1	26.3	28.5
Apgar 1st minute	All	197	9.0±0.7 (8.9–9.1)		9.0	9.0	9.0
	Females	110	9.0±0.6 (8.9–9.1)	0.860	9.0	9.0	9.0
	Males	87	9.0±0.8 (8.8–9.2)		9.0	9.0	9.0
Apgar 5th minute	All	197	10.0±0.3 (9.9–10.0)		10.0	10.0	10.0
	Females	110	10.0±0.2 (9.9–10.0)	0.585	10.0	10.0	10.0
	Males	87	9.9±0.3 (9.9–10.0)		10.0	10.0	10.0
Gestational age (weeks)	All	197	40±1 (39.7–40.0)		39	40	41
	Females	110	40±1 (39.8–40.2)	0.098	39	40	41
	Males	87	40±1 (39.4–39.9)		39	40	40

Data are the mean±SD (95%CI) of the indicated number of neonates. Differences between females and males were significant at $p \leq 0.05$. P25, P50 and P75 are the 25th, 50th and 75th percentiles, respectively

at least a power of 72 and 60 %, between neonates with high-glucose/high-TG vs. low-glucose/low-TG levels and high-glucose/low-HDL-c vs. low-glucose/high-HDL-c levels, respectively. Spearman correlations between neonatal anthropometrical and clinical data were tested. A contingency table was used to determine the sensitivity and predictive value of the combined use of glucose and TG or glucose and HDL-c as previously indicated on the concentration of insulin sensitivity/resistance markers and lipoprotein levels at different cutoff points. To compare the method predictability of glucose/HDL-c or glucose/TG combinations, a receiver operating characteristic (ROC) curve was constructed in which the sensitivity/resistance markers or the lipoprotein levels and the prognostic probability group were analysed as predictive variables. The area under the curve (AUC) represents the discriminative test power. Values are expected to be between 0.5 (indicating no discriminative ability) and 1.0 (indicating the highest detection accuracy). Statistical significance was set at $p \leq 0.05$ using the SPSS (version 15.0) and the SAS (version 9.2) statistical software packages.

Results

Anthropometrical data (mean, SD, 95%CI, percentiles) are shown in Table 1. No significant differences for anthropometric values, except for body mass index (BMI; $p = 0.050$), were found between male and female neonates.

Data related to lipids, lipoproteins, Apos and tHcyt are shown in Table 2. TC, HDL-c, Apo A1 and Lp(a) were

Table 2 Lipid, lipoprotein and apolipoprotein concentrations in cord blood of full-term neonates from the Mérida Study

		N	Mean±SD (95%CI)	Significance	P25	P50	P75
Total cholesterol (TC) (mg/dL)	All	197	59.8±18.3 (57.3–62.4)		48.0	58.0	69.5
	Females	110	62.1±15.5 (59.1–65.0)	0.045	50.0	64.5	73.0
	Males	87	57.0±21.0 (52.5–61.5)		47.0	56.0	65.0
HDL cholesterol (HDL-c) (mg/dL)	All	197	27.5±9.5 (26.1–28.8)		21.0	27.0	33.0
	Females	110	29.0±8.6 (27.4–30.6)	0.012	22.5	29.0	34.0
	Males	87	25.6±10.3 (23.4,27.8)		19.0	24.0	29.0
LDL cholesterol (LDL-c) (mg/dL)	All	197	25.4±10.7 (23.9–26.9)		18.7	24.4	31.0
	Females	110	26.2±9.6 (24.4–28.1)	0.227	19.7	25.6	32.2
	Males	87	24.4±11.9 (21.8–26.9)		17.6	23.4	28.2
Oxidised LDL (oxLDL) (U/L)	All	197	36.3±35.9 (30.6–41.9)		6.2	26.1	53.4
	Females	110	34.0±35.2 (26.6–41.4)	0.381	4.6	23.4	51.1
	Males	87	39.0±36.9 (30.3–47.8)		8.6	33.1	59.6
Apolipoprotein A1 (mg/dL)	All	197	72.6±16.3 (70.3–74.9)		63.5	72.2	82.5
	Females	110	74.9±13.1 (72.4–77.4)	0.025	66.9	74.8	83.9
	Males	87	69.7±19.3 (65.5–73.8)		59.7	68.4	77.8
Apolipoprotein B (mg/dL)	All	197	32.0±31.3 (27.7–36.4)		17.0	21.6	31.1
	Females	110	32.4±30.2 (26.7–38.1)	0.866	18.0	23.0	30.7
	Males	87	31.6±32.8 (24.6–38.6)		16.1	20.4	31.2
Lp(a) (mg/dL)	All	197	4.2±4.9 (3.5–4.9)		1.0	2.7	6.3
	Females	110	5.1±5.3 (4.0–6.1)	0.003	1.6	3.9	7.5
	Males	87	3.1±4.1 (2.2–4.0)		0.5	1.9	4.6
Triglycerides (TG) (mg/dL)	All	197	34.3±17.9 (31.8–36.8)		22.5	31.0	41.0
	Females	110	33.5±15.4 (30.6–36.4)	0.806	23.0	31.0	40.0
	Males	87	35.3±20.6 (30.9–39.7)		22.0	33.0	44.0
Arylesterase (AE) (U/L)	All	197	30.6±27.0 (26.8–34.5)		9.8	21.4	42.8
	Females	110	29.8±26.3 (24.7–34.9)	0.635	9.3	21.4	42.8
	Males	87	31.7±27.8 (25.6–37.7)		10.4	21.4	42.8
Homocysteine (μmol/L)	All	197	5.4±1.9 (5.1–5.7)		4.2	5.1	6.2
	Females	110	5.3±1.8 (5.0–5.6)	0.378	4.1	5.1	6.1
	Males	87	5.5±2.0 (5.1–6.0)		4.3	5.2	6.4

Data are the mean±SD (95%CI) of the indicated number of neonates. Differences between females and males were significant at $p \leq 0.05$. P25, P50 and P75 are percentiles 25, 50 and 75, respectively

Table 3 Cardiovascular disease ratios and cholesterol relative transport by lipoproteins in cord blood of full-term neonates from the Mérida Study

		N	Mean±SD (95% CI]	Significance	P25	P50	P75
TC/HDL-c	All	197	2.3±0.5 (2.2–2.3)		1.9	2.2	2.6
	Females	110	2.2±0.5 (2.1–2.3)	0.077	1.9	2.1	2.4
	Males	87	2.3±0.5 (2.2–2.5)		1.9	2.3	2.6
HDL-c/Apo A1	All	197	0.37±0.07 (0.36–0.38)		0.32	0.36	0.42
	Females	110	0.38±0.07 (0.37–0.40)	0.033	0.34	0.38	0.43
	Males	87	0.36±0.08 (0.34–0.38)		0.30	0.35	0.40
LDL-c/Apo B	All	197	1.1±0.4 (1.0–1.1)		0.83	1.1	1.3
	Females	110	1.1±0.4 (1.0–1.2)	0.664	0.89	1.1	1.4
	Males	87	1.1±0.4 (1.0–1.1)		0.81	1.1	1.3
Apo A1/Apo B	All	197	3.3±1.7 (3.1–3.5)		2.3	3.3	4.0
	Females	110	3.4±1.8 (3.0–3.7)	0.434	2.3	3.3	4.0
	Males	87	3.2±1.5 (2.9–3.5)		2.3	3.1	3.9
HDL-c/LDL-c	All	197	1.2±0.7 (1.1–1.3)		0.82	1.1	1.5
	Females	110	1.3±0.7 (1.1–1.4)	0.317	0.85	1.1	1.5
	Males	87	1.2±0.6 (1.0–1.3)		0.78	1.0	1.4
AE/HDL-c (U/mg)	All	197	12.2±11.7 (10.5–13.9)		3.7	8.4	17.9
	Females	110	10.9±10.6 (8.0–13.0)	0.086	3.3	7.9	17.2
	Males	87	13.8±12.7 (11.0–16.6)		4.5	9.2	19.5
oxLDL/LDL-c (U/mg)	All	197	0.15±0.15 (0.12–0.17)		0.02	0.11	0.21
	Females	110	0.13±0.14 (0.10–0.16)	0.101	0.02	0.10	0.18
	Males	87	0.17±0.16 (0.13–0.21)		0.04	0.12	0.24
%TC of HDL-c	All	197	46.2±10.1 (44.8–47.6)		39.2	46.0	52.1
	Females	110	47.4±9.8 (45.5–49.2)	0.072	41.2	46.9	53.1
	Males	87	44.8±10.2 (42.6–47.0)		37.9	43.8	51.5
%TC of LDL-c	All	197	42.2±9.4 (40.8–43.5)		35.3	43.1	49.0
	Females	110	41.8±9.2 (40.0–43.5)	0.506	35.7	42.6	48.3
	Males	87	42.7±9.5 (40.6–44.7)		35.0	43.5	49.5
%TC of VLDL-c	All	197	11.6±4.9 (11.0–12.3)		7.7	11.2	14.4
	Females	110	10.9±4.2 (10.1–11.7)	0.019	7.7	10.8	13.0
	Males	87	12.6±5.5 (11.4–13.7)		7.9	11.6	16.2

Data are the mean±SD (95%CI) of the indicated number of neonates. Differences between females and males were significant at $p \leq 0.05$. For abbreviations, see Table 2. P25, P50 and P75 are percentiles 25, 50 and 75, respectively

Table 4 Glucose, insulin and insulin sensitivity/resistance biomarker concentrations in cord blood of full-term neonates from the Mérida Study

		N	Mean±SD (95% CI)	Significance	P25	P50	P75
Glucose (mg/dL)	All	197	76.8±38.2 (71.4–82.2)		55.0	71.0	84.0
	Females	110	76.8±35.6 (70.1–83.5)	0.993	58.5	72.0	86.3
	Males	87	76.8±41.0 (67.9–85.6)		54.0	66.0	81.0
Insulin (mUI/L)	All	197	6.4±5.4 (5.6–7.1)		2.1	3.8	6.9
	Females	110	7.0±5.9 (6.0–8.0)	0.026	2.6	4.2	7.2
	Males	87	5.5±6.0 (4.5–6.5)		1.6	3.0	6.6
HOMA-IR	All	197	1.5±2.8 (1.1–1.9)		0.30	0.59	1.3
	Females	110	1.8±3.1 (1.2–2.4)	0.059	0.41	0.66	1.3
	Males	87	1.2±2.3 (0.73–1.7)		0.24	0.46	1.2
QUICKI	All	197	0.44±0.12 (0.42–0.45)		0.37	0.42	0.48
	Females	110	0.42±0.12 (0.40–0.45)	0.121	0.37	0.41	0.45
	Males	87	0.44±0.12 (0.42–0.48)		0.36	0.43	0.50
Glucose/insulin	All	197	29.3±43.9 (23.1–37.5)		10.1	18.1	31.3
	Females	110	25.5±41.8 (17.6–33.5)	0.188	8.8	15.9	26.5
	Males	87	33.9±46.2 (24.0–43.7)		12.1	33.6	40.4

Data are the mean±SD (95%CI) of the indicated number of neonates. Differences between females and males were significant at $p \leq 0.05$. P25, P50 and P75 are percentiles 25, 50 and 75, respectively

HOMA-IR Homeostatic Model Assessment—Insulin Resistance, *QUICKI* Quantitative Insulin Sensitivity Check Index

Table 5 Spearman correlations between selected anthropometrical parameters, lipids, lipoproteins, Apo and insulin sensitivity/resistance markers in full-term neonates from the Mérida Study

	Gestational age	BMI	TG	Lp(a)	oxLDL	tHcyt	AE	Glucose	Insulin	HOMA-IR	QUICKI
Bodyweight	0.269**	ND							0.184*	0.210*	
BMI	0.189*								0.197*	0.199*	
TC			0.351**	0.201*	0.242**			0.215*			
LDL-c			0.279**		0.242**						
HDL-c								0.267**			
TG									−0.196*		
Apo A1								0.296**			−0.188*
Apo B			0.247**		0.245*	0.199*	−0.226*	0.189**			
Glucose									0.364**	ND	ND
HOMA-IR											ND

Only correlations $p<0.01$ have been included. For parameter units, see Tables 1 and 2

AE arylesterase, *BMI* body mass index, *HDL-c* HDL cholesterol, *HOMA-IR* Homeostatic Model Assessment—Insulin Resistance, *tHyct* total homocysteine, *LDL-c*, LDL cholesterol, *oxLDL* oxidized LDL, *QUICKI* Quantitative Insulin Sensitivity Check Index, *TG* triglycerides, *TC* total cholesterol, *ND* not determined as one of the two parameters correlated would be part of the calculation

*$*p<0.01$; **$p<0.001$

significantly higher (at least $p<0.05$) in females. The CVD ratios, the oxLDL/LDL-c and the AE/HDL-c ratios and the relative transport of TC (%TC) by very low-density lipoproteins (VLDL), LDL and HDL are shown in Table 3. The HDL-c/Apo A1 ratio was higher ($p=0.033$), but the relative transport of TC (%TC) by the VLDL fraction was significantly lower ($p=0.019$) in females. Table 4 shows that significant gender differences were found for insulin ($p=0.026$).

Several significant correlations ($p<0.05$) were found. However, gestational age did not significantly correlate with any biochemical (tHcyt, lipoprotein and/or insulin resistance/sensitivity) markers. To emphasize relationships between anthropometrical, lipoprotein and insulin sensitivity/resistance markers, Table 5 reports only correlations at the level of $p<0.01$. Among them, it can be remarked that bodyweight significantly correlated with insulin and HOMA-IR. Glucose correlated positively with HDL-c and insulin. TG were positively correlated with LDL-c and Apo B and inversely with insulin. Insulin positively correlated significantly with glucose, but negatively with TG. tHcyt was

Fig. 1 Association of 500-g scale birthweight at birth and the arylesterase (*AE*), oxidised LDL (*oxLDL*), HOMA-IR and QUICKI levels. All parameter results were significant (ANOVA, $p<0.05$). *Bars bearing a different letter* were significantly different (Bonferroni post hoc test)
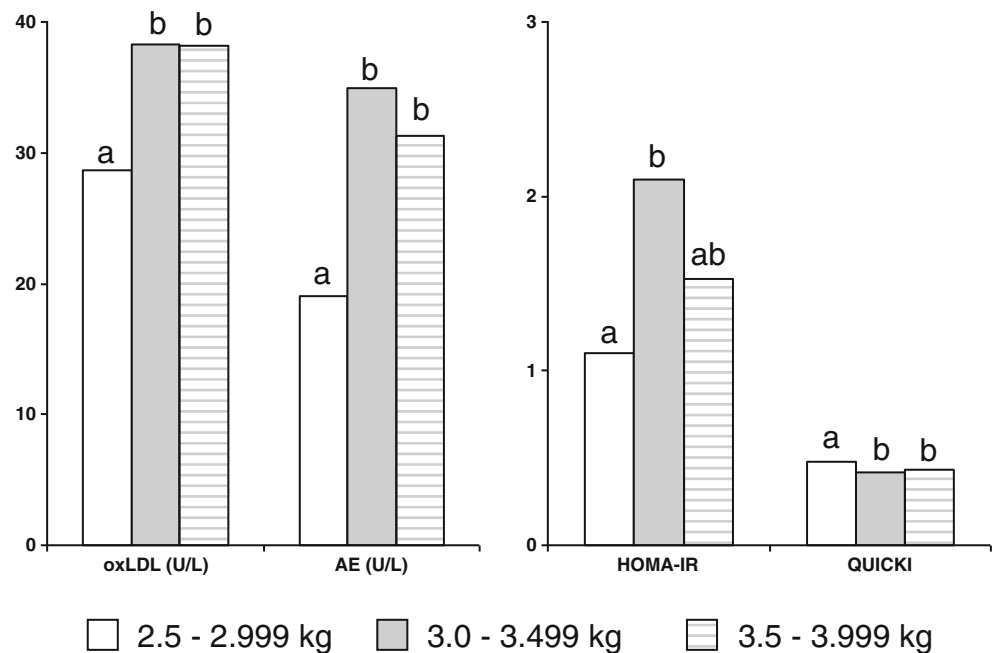
significantly correlated with Apo B. AE correlated negatively with Apo B. oxLDL correlated positively with Apo B and LDL-c. Lp(a) correlated positively with TC.

When neonates were classified according to a 500-g scale in 2,500–2,999 g, 3,000–3,499 g and 3,500–3,999 g, oxLDL, HOMA-IR and QUICKI appeared associated with bodyweight (at least $p<0.05$). Neonates with 2,500–2,999 g bodyweight showed the lowest oxLDL, AE and HOMA-IR and the highest QUICKI levels (Fig. 1). These 2,500 to 2,999-g neonates also showed reduced BMI, ponderal index and cephalic and thoracic perimeters (data not shown).

Table 6 shows the anthropometrical and analytical data of term neonates in which concurrences of high glucose/high TG and high glucose/low HDL-c were present. The prevalence of neonates with high glucose/high TG was 6.6 %, whilst that of neonates with high glucose/low HDL-c was 2.5 %. Neonates where the concurrence of high glucose/high TG exists present higher TC ($p<0.001$), LDL-c ($p<0.05$), TC/HDL-c ($p<0.05$),

%TC by VLDL ($p<0.001$), Apo A1 ($p<0.001$), glucose/insulin ratio ($p<0.05$) and HOMA-IR ($p<0.05$), but lower oxLDL ($p<0.05$), oxLDL/LDL-c ($p<0.01$) and QUICKI ($p<0.05$) with respect to their low-glucose/low-TG counterparts. Neonates with the concurrence of high glucose/low HDL-c vs. low glucose/high HDL-c presented higher CT/HDL-c ratio ($p<0.01$), insulin ($p<0.01$), HOMA-IR ($p<0.001$), %TC by LDL ($p<0.05$) and %TC by VLDL ($p<0.001$), but lower TC ($p<0.001$), oxLDL ($p<0.01$), Apo A1 ($p<0.001$), HDL-c/Apo A1 ($p<0.001$), oxLDL/LDL-c ($p<0.05$), QUICKI ($p<0.05$) and %TC by HDL ($p<0.01$).

Table 7 shows data about the sensitivity, specificity and positive predictive values of the combined use of glucose and TG or glucose and HDL-c. The concurrence of glucose/TG displayed significant contingence ($p=0.003$) for the distribution of neonates at the lower/higher quartile for QUICKI values. The concurrence of glucose/HDL-c displayed significant values for the distribution of neonates

Table 6 Concurrence of high glucose with high TG or with low HDL-c in cord blood and their effects on lipids, lipoproteins, Apo, CVD ratios and insulin sensitivity/resistance biomarkers in full-term neonates from the Mérida Study

	Glucose and TG		Glucose and HDLc	
	Low glucose/lowTG	high glucose/high TG	Low glucose/high HDL-c	High glucose/low HDL-c
No. of neonates (prevalence, %)	19 (9.6)	13 (6.6)	8 (4.1)	5 (2.5)
Total cholesterol (TC) (mg/dL)	44.2±14.5 (37.2–51.2)	64.4±12.4 (56.9–71.9)***	75.8±7.2 (69.7–81.8)	49.4±5.7 (42.3–56.5)***
HDL-c (mg/dL)			NT	NT
LDL-c (mg/dL)	20.9±8.2 (17.0–24.9)	27.0±6.8 (22.7–31.3)*		
oxLDL (U/L)	40.7±29.6 (24.3–57.1)	18.7±25.4 (1.6–35.8)*	52.4±37.0 (18.1–86.7)	12.2±11.5 (0.0–24.4)*
Apo A1 (mg/dL)	56.2±14.1 (49.4–63.0)	75.1±10.9 (68.0–81.6)***	88.9±6.8 (81.2–92.6)	59.7±5.5 (52.8–66.6)***
TG (mg/dL)	NT	NT		
TC/HDL-c	2.3±0.5 (2.1–2.5)	2.6±0.5 (2.3–3.0)*	2.0±0.22 (1.8–2.2)	3.0±0.66 (2.2–3.8)**
HDL-c/LDL-c			1.3±0.33 (0.98–1.5)	0.77 ±0.42 (0.25–1.3)*
HDL-c/Apo A1			0.44±0.04 (0.40–0.47)	0.28±0.06 (0.21–0.36)***
oxLDL/LDL-c (U/mg)	0.22±0.17 (0.12–0.31)	0.05±0.07 (0.0–0.11)**	0.17±0.13 (0.05–0.29)	0.07±0.05 (0.0–0.12)*
Glucose (mg/dL)	NT	NT	NT	NT
Insulin (mUI/L)			3.7±2.2 (1.9–5.6)	13.1±8.2 (3.0–23.2)**
HOMA-IR	0.96±1.9 (0.03–1.9)	4.1±5.4 (0.71–5.7)*	0.39±0.31 (0.11–0.68)	3.8±2.6 (0.58–7.0)***
QUICKI	0.47±0.09 (0.43–0.52)	0.39±0.11 (0.33–0.46)*	0.50±0.07 (0.43–0.57)	0.39±0.05 (0.27–0.45)*
Glucose/insulin	14.2±11.5 (8.6–19.7)	41.9±53.9 (11.5–78.0)*		
%TC of HDL-c	45.4±9.3 (41.0–49.9)	39.0±7.4 (34.3–43.7)*	50.3±5.9 (45.3–55.2)	34.6±9.6 (22.8–46.7)**
%TC of LDL-c			41.1±6.1 (36.0–46.3)	48.7±6.2 (41.2–56.2)*
%TC of VLDL-c	7.5±2.5 (6.3–8.7)	18.1±3.7 (15.8–20.3)***	8.6±2.6 (6.4–10.8)	16.7±2.6 (13.4–20.0)***

Data are the mean±SD (95% CI) of the indicated number of neonates. Only significant data are shown. Non-significant differences were found between group comparison for any of the anthropometrical parameter tested: tHyct, Apo B, Lp(a), ApoA1/ApoB, AE, AE/HDL-c and LDL-c/Apo B ratio. For abbreviations, see footnote of Table 3

NT not tested

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 7 Sensitivity, specificity and positive predictive values of the concurrence of high glucose with high TG or with low HDL-c in cord blood and for high and low levels of resistance/sensitive biomarkers of insulin resistance in full-term neonates from the Mérida Study

	Low glucose/ low TG ($n=13$)	High glucose/ high TG ($n=19$)	Statistical variables	Low glucose/ high HDL-c ($n=8$)	High glucose/ low HDL-c ($n=5$)	Statistical variables
High insulin level (>Q4 for males and females)	False positive: $n=5$	True positive: $n=6$	$p=0.463$; sensitivity: 54.5 %; specificity : 80 %; positive predictive value: 64.6 %	False positive: $n=1$	True positive: $n=4$	$p=0.016$; sensitivity: 80 %; specificity : 80 %; positive predictive value: 100 %
Low insulin level (<Q1 for males and females)	True negative: $n=5$	False negative: $n=5$		True negative: $n=4$	False negative: $n=0$	
High HOMA-IR index (>Q4 for males and females)	False positive: $n=1$	True positive: $n=7$	$p=0.106$; sensitivity: 87.5 % specificity: 80 %; positive predictive value: 63.6 %	False positive: $n=0$	True positive: $n=5$	$p=0.005$; sensitivity: 100 %; specificity: 100 %; Positive predictive value: 100 %
Low HOMA-IR index (<Q1 for males and females)	True negative: $n=4$	False negative: $n=4$		True negative: $n=3$	False negative: $n=0$	
Low QUICKI index (<Q1 for males and females)	False positive: $n=2$	True positive: $n=6$	$p=0.003$; sensitivity: 75 %; specificity: 80 %; positive predictive value: 63.6 %	False positive: $n=1$	True positive: $n=3$	$p=0.047$; sensitivity: 75 %; specificity: 75 %; Positive predictive value: 100 %
High QUICKI index (>Q4 for males and females)	True negative: $n=10$	False negative: $n=1$		True negative: $n=3$	False negative: $n=0$	

n=number. Contingences were significant at $p \leq 0.05$. Q1 and Q4 are the 1st and 4th quartiles, respectively

HOMA-IR Homeostatic Model Assessment—Insulin Resistance, *QUICKI* Quantitative Insulin Sensitivity Check Index

at the lower/higher quartile for insulin ($p=0.016$), HOMA-IR ($p=0.005$) and QUICKI ($p=0.047$).

Figure 2 presents the method predictability by the combination of quartile distributions of glucose and TG or glucose and HDL-c. In the case of QUICKI and HOMA-IR, the combination glucose/TG was associated with large AUC with QUICKI quartile distribution (AUC=830, 95%CI=0.621–1.038, $p=0.017$) and HOMA-IR quartile distribution (AUC=813, 95%CI=0.513–1.032, $p=0.023$), whilst the concurrence of glucose/HDL-c was associated with a large AUC with HOMA-IR quartile distribution (AUC=1,000, 95%CI=1.000–1.000, $p=0.036$).

Discussion

To the best of our knowledge, this is the first study relating at birth tHcyt, lipoprotein and Apo values with insulin sensitivity/resistance markers in an ample sample of full-term, 2.5- to <4.0-kg birthweight, and without distress neonates. The results suggest that the concurrence of dyslipaemia and dysglycaemia negatively affected some neonatal CVD risk markers and the insulin resistance/sensitivity indexes.

The anthropometrical characteristics were similar to those of other studies as males tended to have had higher bodyweight and presented higher BMI than females [6, 7, 10, 16, 29]. Our results on lipids, lipoproteins and Apo were consistent with those of different studies [6, 7, 10, 11, 16, 27, 29] which found that neonatal data (e.g. lipids and lipoprotein lipids) are quite similar in newborns from different countries and racial and ethnic origins. However, in previous studies of our group, 5 % of neonates were hypercholesterolaemics [7, 29], whilst in the present study only four neonates (approx. 2 %) showed TC≥100 mg/dL, a marker of neonatal hypercholesterolaemia [16].
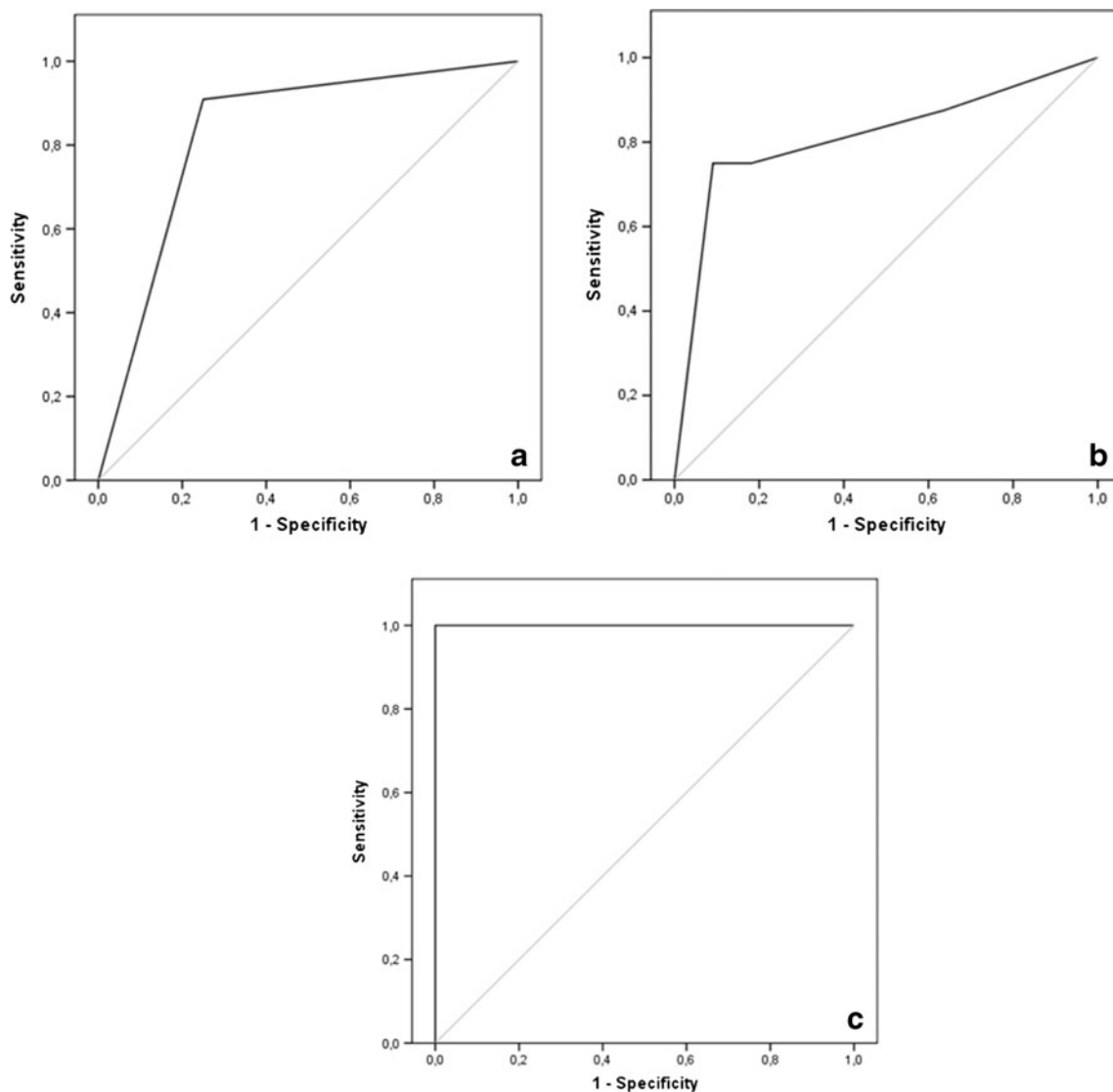
Fig. 2 ROC curve of probabilities obtained from the concurrence of glucose/TG or glucose/HDL-c as dependent variables and quartiles for insulin, HOMA-IR and QUICKI as independent variables. Larger AUC indicates a higher prognostic value. *HOMA-IR* Homeostatic Model Assessment—Insulin Resistance, *QUICKI* Quantitative Insulin Sensitivity Check Index. **a** ROC curve for QUICKI quartiles at the glucose/TG concurrence (AUC=830, 95%CI=0.621–1.038, p=0.017). **b** ROC curve for HOMA-IR quartiles at the glucose/TG concurrence (AUC=813, 95%CI=0.593–1.032, p=0.023). **c** ROC curve for HOMA-IR quartiles at the glucose/HDL-c concurrence (AUC=1,000, 95%CI=1.000–1.000, p=0.036

The OxLDL levels were lower than those found in control or in intrauterine growth restriction neonates. However, their average gestational age was 35.7 weeks and most of them were delivered by caesarean section [21]. Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of oxidised lipoproteins and apoptotic cells. Among the several suggested immunogens present in the atherosclerotic plaque, oxidation-specific epitopes, as occur in oxLDL, are immunodominant. The oxLDL/LDL-c ratio has been used as a marker of LDL particles being oxidised [31]. This ratio and the AE were also lower (five to seven times) than those found in young Spanish adults [31], suggesting that lipoprotein oxidation significantly increases after birth. The AE is one of the major enzyme activities of PON1 and one of the first enzymes to act in cases of lipid peroxidation [3]. The low AE levels found in neonates suggest that this enzyme was used to maintain oxLDL levels as low as possible due to the high metabolic demands at late pregnancy and delivery. The Lp(a) levels were similar to those of several studies [10, 20, 26], but much lower than those described in a neonatal Iranian population [4]. tHcyt has received interest during the last decades because hyperhomocysteinaemia has been associated with an increased risk of CVD [32]. The present tHcyt data are lower than those found by Couto et al. [11] in Brazilian neonates.

Although several factors would be engaged, a high intake of folates during pregnancy (unpublished data) would be involved.

In agreement with other studies, female neonates presented higher levels of TC, HDL-c [4, 27, 29] and Apo A1 [6, 10]. Lp(a) appeared higher in females, suggesting a central role of foetal sexual hormones on lipoprotein metabolism. Other studies did not show any significant gender differences in the mean and median Lp(a) concentrations [4, 10]. However, the number of neonates tested in those studies was lower than that of the current study.

Neonatal glucose, insulin, HOMA-IR and the QUICKI levels concur with others [12, 14, 20, 26]. In addition, the HOMA-IR values were positively correlated with birthweight, and neonates weighing 2.5–2.999 kg showed higher QUICKI values, suggesting that insulin resistance decreases and insulin sensitivity increases in full-term neonates with lower birthweight in order to improve their glucose cell uptake and growth. Increases in insulin sensitivity have been found in undernourished animal models [33] and intrauterine growth restriction neonates [21, 33, 34].

Gestational age did not correlate significantly with any lipoprotein and insulin resistance/sensitivity markers; thus, it did not influence, in general terms, the lipoprotein and insulin resistance/sensitivity markers as only the HDL-c/LDL-c, the Apo B and the glucose/insulin ratio appeared correlated ($p < 0.05$, but $p > 0.01$). Our group found in an ample neonatal population that TG [7], Apo B and Apo A1 [6] increased slightly through the term period.

When neonates were classified according to a birthweight scale (2,500–2,999 g, 3,000–3,499 g and 3,500–3999 g), non-significant effects on most lipids and lipoprotein levels, but on AE and oxLDL, were observed due to birthweight, suggesting that in full terms, bodyweight affected more the antioxidant status than the lipoprotein profile. However, HOMA-IR and QUICKI were associated with bodyweight. Hardell [16] reported that birthweight affected TG, but has minimal influence on newborns' TC. Boyne et al. [9] found that weight was inversely related to HDL-c, but did not significantly correlate with plasma cholesterol, TG and HOMA-IR at birth.

The concurrence of at least three of the following factors is required for MS diagnosis: abdominal obesity, hypertriglyceridaemia, low levels of HDL-c, high blood pressure and hyperglycaemia [2, 35]. One central aspect of MS is the presence of hyperinsulinaemia and/or insulin resistance. Full-term neonates presenting concurrence of high glucose/high TG or high glucose/low HDL-c displayed higher levels of HOMA-IR, but lower QUICKI, oxLDL and oxLDL/LDL-c with respect to their corresponding counterparts. The lower oxLDL levels and oxLDL/LDL-c ratio suggest low lipoprotein oxidation as a consequence of an intra-utero metabolism reduction in those neonates.

The altered HOMA-IR and QUICKI levels suggest insulin resistance and reduced insulin sensitivity, respectively, in those MS-risk neonates. Of the neonates with concurrence of high glucose/low HDL-c, 83 % showed insulin levels >6 mUI/L, which is the considered cutoff point for high insulin levels at birth [13]. In fact, this concurrence showed high sensitivity, specificity and positive predictive values (100 %) for HOMA-IR. Moreover, 33 % of them displayed insulinaemia >15 mUI/L. These results are relevant. As was previously stated, high insulinaemia during perinatal life may cause predispositions for the development of DM in later life [28]. In addition, the relative transport of TC by the VLDL and by the HDL fractions was higher and lower, respectively, suggesting a more atherogenic lipoprotein profile in those neonates. However, other lipoprotein markers were distinctly affected when TG or HDL-c was selected as the MS factor. Thus, the concurrence of high glucose/high TG induced higher TC, LDL-c and Apo A1 levels, whilst the concurrence of high glucose/low HDL-c decreased TC and Apo A1 and increased the TC/HDL-c ratio and %TC by the LDL fraction, suggesting that the last concurrence was most deleterious for the lipoprotein metabolism than that of high glucose/high TG in term neonates. Moreover, the concurrence of high glucose/low HDL-c also showed positive predictive values for high insulin, high HOMA-IR and low QUICKI values whilst that of high glucose/high TG for low QUICKI values. However, the relatively low number of neonates presenting high-glucose/low-HDL-c concurrence demands future studies in more ample newborn populations.

In conclusion, this study has allowed us to establish the ranges for cord serum lipoproteins (LDL-c, HDL-c, Lp(a), oxLDL), AE and tHcyt in an ample sample of Spanish Caucasian, normoweight, full-term non-distressed neonates. The neonatal concurrence of dysglycaemia and dyslipaemia determines increased insulin resistance and negatively modifies the lipoprotein profile metabolism at birth. In addition, the benefit of glucose, triglyceride and HDL-c screening at birth seems evident as it may discover candidates for T2DM and MS, permitting their early follow-up and adequate prevention and/or treatment. However, more studies are needed to assess the importance of these neonatal findings in MS development and diagnosis later in life.

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