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S. Bastida · S. Perea · F. J. Sánchez-Muniz

Do neonates with high serum cholesterol levels have a different high density lipoprotein composition?

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Abstract In order to ascertain whether the high density lipoprotein (HDL) composition of neonates with high serum cholesterol levels (≥2.59 mmol/l or ≥100 mg/dl) differs from that of neonates with normal serum cholesterol levels (<2.59 mmol/l), 548 cord blood samples were examined from full-term newborns of the Toledo Study (Spain) of whom no perinatal factors were known which could alter cord blood lipid levels. Newborns were selected according to the following criteria: single and eutocic delivery with cephalic presentation, gestational age between the beginning of the 37th week and the end of the 41st week, body weight between 2.5 kg and 3.999 kg and an Apgar score of ≥7 and ≥9 at 1 min and 5 min, respectively. The prevalence of high serum total cholesterol (TC) level was greater ($P < 0.02$) in females than in males. Newborns with high TC levels had higher triglyceride ($P < 0.01$), HDL-cholesterol ($P < 0.001$) and apoprotein (Apo) A-I ($P < 0.001$) levels, and a higher TC/HDL-cholesterol ratio ($P < 0.05$), but a lower HDL-cholesterol/Apo A-I ratio ($P < 0.05$). ANOVA two-way analysis showed a significant effect of gender and serum cholesterol level and a statistical interaction of these two factors upon triglycerides, Apo A-I, and the HDL-cholesterol/Apo A-I ratio. However, HDL-cholesterol and the TC/HDL-cholesterol ratio were higher in neonates (males plus females) with high serum TC but they were not affected by sex. The larger HDL particles in males with high TC levels (HM) should be associated with the higher triglyceride level found in those individuals.

Conclusion The composition of high density lipoproteins in newborns is influenced by the serum cholesterol level and by gender. Neonates with high total cholesterol have larger average high density lipoprotein (HDL) particles. If total cholesterol is elevated, HDL from males carries more cholesterol than HDL from females.

Key words Apoprotein A-I · Full-term neonates · Gender-differences · HDL-cholesterol · Serum lipids

Abbreviations *Apo* apoprotein · *HDL* high density lipoproteins · *HF* female neonates with TC levels ≥2.59 mmol/l · *HM* male neonates with TC levels ≥2.59 mmol/l · *LDL* low density lipoproteins · *NF* female neonates with TC levels <2.59 mmol/l · *NM* male neonates with TC levels <2.59 mmol/l · *TC* total serum cholesterol · *VLDL* very low density lipoproteins

S. Bastida · F. J. Sánchez-Muniz (✉)
Departamento de Nutrición y Bromatología (I),
Nutrición, Facultad de Farmacia,
Universidad Complutense, E-28040 Madrid, Spain,
Tel.: 34-1-394 18 28, Fax: 34-1-394 17 32,
E-mail: frasan@eucmax.sim.ucm.es

S. Perea
Sevicio de Farmacia,
Hospital Virgen de la Salud,
E-45006-Toledo, Spain

Introduction

Metabolic studies of apoprotein (Apo) in high density lipoproteins (HDL) in human volunteers have revealed that variations in HDL levels are partially due to differences in fractional catabolic rates. Large HDL particles with increased ratios of HDL-cholesterol/Apo A-I plus Apo A-II exhibit decreased fractional catabolic rates [5, 6]. These results suggest that HDL composition might be important in controlling HDL levels [5] and in turn, in processes that influence the development of atherosclerosis [15]. However, newborn HDL particles appear to be the smallest in the HDL profile [4]. Few studies have indicated lipoprotein level differences between male and female neonates [1, 3, 21]. Moreover, few data related to lipoprotein differences between male and female newborns with normal and high total serum cholesterol (TC) levels are available. The aim of the present study was to ascertain the differences in the HDL composition (HDL-cholesterol and Apo A-I levels) of neonates with normal and high TC levels. Since in previous studies differences in lipoprotein levels between male and female newborns were found [3, 21], the possible effect of gender on HDL composition was also studied.

Subjects and methods

Subjects and samples

All participating mothers were Caucasians born in Spain. Mothers whose pregnancies were complicated by hypertension or diabetes, or who had received drugs or consumed excessive alcohol during pregnancy were excluded from the study. The study was performed in accordance with the Helsinki Declaration of 1964 (as amended in 1983 and 1989) and approved by the Consejería de la Salud de la Comunidad Autónoma Castilla-La Mancha, Spain. Throughout a period of 11 months, 705 cord serum samples were collected at the "Virgen de la Salud" Hospital, Toledo (Spain), under this hospital's direction and mothers' consent. Because earlier reports [10, 13, 18, 19] established an association between perinatal conditions and cord serum lipid, lipoprotein and/or Apo levels, only, full-term newborns were selected according to the following criteria: (1) singleton live birth; (2) eutocic delivery with cephalic presentation; (3) gestational age between the beginning of the 37th week and the end of the 41st week; (4) body weight between 2.500 and 3.999 kg; and (5) Apgar scores of ≥7 at the 1st min and ≥9 at the 5th min. Of the original 705 cord blood samples, 548 (307 from males and 241 from females) fulfilled the above-mentioned criteria and were studied. The cut off point for defining high cord serum cholesterol levels was 100 mg/dl (2.59 mmol/l), as suggested by Glueck et al. [11]. Data concerning the mothers, their pregnancies and deliveries were obtained from the notes in the records made by obstetricians and paediatricians, according to the strict routine practice of the Department of Obstetrics and Gynaecology of the Virgen de la Salud Hospital, Toledo. Gestational age was determined based on menstrual history, obstetrical findings and the Dubowitz score of newborns.

Analytical procedures

Immediately after delivery, about 3 ml of umbilical cord blood were collected and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation (30 min, 2500 rpm) and kept at 4°C until analysis within a maximum of 72 h. Serum TC

Table 1 Anthropometric characteristics of male and female full-term neonates with normal (<2.59 mmol/l or <100 mg/dl) and high serum total cholesterol (≥2.59 mmol/l or ≥100 mg/dl) (*NS* not significant)

	Normal serum total cholesterol (<2.59 mmol/l)						High serum total cholesterol (≥2.59 mmol/l)						Gender effect	Cholesterol level effect	Gender × cholesterol level interaction
	All		Male		Female		All		Male		Female				
Number of neonates	493		282		211		31		11		20				
Gestational age (weeks)	39.6	± 1.0	39.7	± 1.1	39.6	± 1.0	39.6	± 0.9	39.4	± 0.9	39.6	± 0.9	NS	NS	NS
Body weight (kg)	3.265	± 0.340	3.309	± 0.304	3.207	± 0.330	3.190	± 0.330	3.182	± 0.327	3.248	± 0.329	NS	NS	NS
Body mass index (kg/m^2)	12.7	± 1.1	12.8	± 1.1	12.6	± 1.0	12.5	± 0.9	12.3	± 0.8	12.7	± 0.9	NS	NS	NS
Cephalic perimeter (cm)	35.2	± 1.6	35.4	± 1.5	34.9	± 1.7	35.2	± 1.1	35.4	± 1.4	35.2	± 0.9	NS	NS	NS
Thoracic perimeter (cm)	33.6	± 1.4	33.7	± 1.4	33.5	± 1.4	33.7	± 1.0	33.6	± 0.8	33.8	± 1.2	NS	NS	NS

Values are mean ± standard deviation. Comparisons between all children with normal serum total cholesterol and all children with high serum total cholesterol were not significant

was measured by the enzymatic cholesterol esterase-cholesterol oxidase method (Boehringer Mannheim, Germany). HDL-cholesterol was measured by the same method after precipitation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) with dextran sulphate-Mg^{2+} [23]. Triglycerides were assayed by the enzymatic glycerol phosphate oxidase method proposed by Boehringer Mannheim. Apo A-I was determined by kinetic immunoturbidimetry following the method and indications of the Behring Institute. Lipid internal quality control was carried out according to the laboratory manual of the Lipid Research Clinics Program. External quality control was provided by a quality control laboratory (Wellcome, Sociedad Española de Química Clínica, Barcelona). Apo A-I was standardized against the IUIS-NHLBI-CDS 1883 control sample assayed in the International Collaborative Study Centers for Disease Control for Apolipoproteins Standardization. More details related to Apo A-I quality control have been published [3]. Sometimes not enough serum was available; measurements that could not be done within 72 h of collection were excluded. This explains the discrepancy in the number of sample determinations.

Statistical analysis

The influence of TC on the different parameters tested was studied by means of the unpaired Student's-*t*-test. The prevalence of high serum TC levels amongst male and female newborns was compared by the chi-square test. The effects of gender and cholesterol level were studied by two-way ANOVA. Kormogorov, and Shapiro and Wick normality tests were applied. When data were not normally distributed they were analysed by logarithmic or arc sine transformation. All calculations were performed using the S.A.S. 5.18 statistical packet in an IBM 4321, 22 model with VM/SP operating system. Statistical significance was stated at $P < 0.05$.

Results

Using the criteria of Glueck et al. [11], 20 females and 11 males (5.9%) had high TC levels. The percentage of HF was higher than that of HM: 8.66% versus 3.75% ($P < 0.02$). Table 1 shows that height, body weight, body mass index and cephalic and thoracic perimeters were similar in neonates with normal and high TC levels. This table indicates the absence of a significant effect of either gender or TC levels on height, body weight, body mass index and cephalic and thoracic perimeters, however, the gender-cholesterol level interaction was on the borderline of statistical significance ($P < 0.08$) for body weight. Table 2 shows that neonates with high TC levels presented higher triglyceride ($P < 0.01$), HDL-cholesterol ($P < 0.001$), Apo A-I ($P < 0.001$) levels, along with a higher TC/HDL-cholesterol ratio ($P < 0.05$) and a higher HDL-cholesterol/Apo A-I ratio ($P < 0.05$) than the newborns with TC < 2.59 mmol/l. Table 3 indicates a significant effect of both gender and TC levels on triglycerides, and Apo A-I levels. HDL-cholesterol levels and the HDL-cholesterol/Apo A-I ratio were significantly influenced by TC levels. A significant interaction ($P < 0.05$) between gender and cholesterol level was found for triglycerides and Apo A-I values and for the HDL-cholesterol/Apo A-I ratio. The HDL fraction transports about 36% of the TC in HF, but about 45% in HM. Differences in the relative contribution of HDL-cholesterol to TC are shown in Fig. 1.

Discussion

Factors during pregnancy and delivery, as well as certain diseases, can influence fetal lipid metabolism, thus both primary and secondary hyperlipidaemia may be present at birth [17]. Serum cholesterol [8, 10] also seems related to gestational age and birth weight. Taking into account these influences, newborns were selected according to strict criteria (see Methods). No differences in age, body weight and blood pressure were observed in mothers of male and female newborns with high TC (data not shown). Thus the differences found in the lipoprotein parameters of newborns do not seem to be related to any of the maternal characteristics studied. Mean TC levels in newborn infants are similar throughout the world, regardless of race or sex, and this lipid is evenly distributed between low- and high-density lipoproteins [1, 13, 21]. However, previous studies have proposed different cut off points for the definition of hypercholesterolaemia in newborns. Glueck et al. [11] suggested a serum cholesterol level at birth above 100 mg/dl as cut off. Higher TC levels were found in females both within the population with normal cholesterol levels, as well as in the whole full-term newborn population of the Toledo Study [2, 21]. The higher percentage of HF than HM should first be attributed to this fact. Averna et al. [1] have found a very significant higher TC in females than in males.

Table 2 Lipids, HDL-cholesterol and apoprotein A-I levels, and total cholesterol/HDL-cholesterol and HDL-cholesterol/apoprotein A-I ratios in full-term neonates with normal (< 2.59 mmol/l or <100 mg/dl) and high serum total cholesterol (≥2.59 mmol/l or ≥100 mg/dl) (*ND* not determined)

	Normal serum total cholesterol (< 2.59 mmol/l)	High serum total cholesterol (≥2.59 mmol/l)	Statistical significance
Triglycerides (mmol/l)	0.43 ± 0.20 (493)	0.67 ± 0.38 (31)	< 0.01
Total cholesterol (mmol/l)	1.71 ± 0.36 (493)	2.99 ± 0.47 (31)	ND
HDL-cholesterol (mmol/l)	0.78 ± 0.23 (468)	1.16 ± 0.47 (31)	< 0.001
Total cholesterol/HDL-cholesterol ratio	2.42 ± 0.75 (468)	2.92 ± 1.13 (31)	< 0.05
Apoprotein A-I (g/l)	0.73 ± 0.14 (488)	0.88 ± 0.18 (31)	< 0.001
HDL-cholesterol/apoprotein A-I ratio[a]	0.39 ± 0.11 (468)	0.45 ± 0.15 (31)	< 0.05

[a] Calculated from data in g/l

Values are mean ± standard deviation. Number of analyses in parentheses

Table 3 Lipids, HDL-cholesterol and apoprotein A-I levels, and total cholesterol/HDL-cholesterol and HDL-cholesterol/apoprotein A-I ratios in male and female full-term neonates with normal (< 2.59 mmol/l or < 100 mg/dl) and high serum total cholesterol (≥2.59 mmol/l or ≥100 mg/dl), (*NS* not significant, *ND* not determined)

	Normal serum total cholesterol (< 2.59 mmol/l)		High serum total cholesterol (≥2.59 mmol/l)		Gender effect	Cholesterol level effect	Gender × cholesterol level interaction
	Male	Female	Male	Female			
Triglycerides (mmol/l)	0.44 ± 0.21 (282)	0.41 ± 0.19 (211)	0.81 ± 0.34 (11)	0.59 ± 0.39 (20)	< 0.01	< 0.0001	< 0.05
Total cholesterol (mmol/l)	1.68 ± 0.36 (282)	1.78 ± 0.37 (211)	2.87 ± 0.28 (11)	3.09 ± 0.56 (20)	< 0.0001	ND	ND
HDL-cholesterol (mmol/l)	0.77 ± 0.34 (266)	0.80 ± 0.24 (202)	1.28 ± 0.48 (11)	1.12 ± 0.48 (20)	NS	< 0.001	NS
Total cholesterol/ HDL-cholesterol ratio	2.40 ± 0.80 (266)	2.44 ± 0.68 (202)	2.64 ± 1.36 (11)	3.08 ± 1.04 (20)	NS	< 0.02	NS
Apoprotein A-I (g/l)	0.72 ± 0.16 (280)	0.75 ± 0.15 (208)	0.77 ± 0.15 (11)	0.92 ± 0.18 (20)	< 0.01	< 0.001	< 0.05
HDL-cholesterol/apoprotein A-I ratio[a]	0.38 ± 0.11 (266)	0.39 ± 0.10 (202)	0.57 ± 0.18 (11)	0.42 ± 0.13 (20)	< 0.05	< 0.01	< 0.05

[a] Calculated from data in g/l
Values are mean ± standard deviation. Number of analyses in parentheses

Fig. 1 HDL-cholesterol contribution (percentage) to total serum cholesterol (TC) in male neonates with TC < 2.59 mmol/l (NM), female neonates with TC < 2.59 mmol/l (NF), male neonates with TC ≥ 2.59 mmol/l (HM), and female neonates with TC ≥ 2.59 mmol/l (HF)

Glueck et al. [11] compared cholesterol levels in α-lipoproteins (HDL) and β-lipoproteins (LDL) of normo- and hypercholesterolaemic newborns and found that changes in TC were related to changes in β-lipoproteins. However, in the present study neonates with high TC levels presented 49% higher HDL-cholesterol levels than infants with TC < 2.59 mmol/l. In newborns, before the onset of enteral feeding, the supply of free cholesterol for LCAT mediated esterification and subsequently HDL incorporation, should be sparce. Consequently, their reverse cholesterol transport would be depressed and the high concentration of the smallest HDL (HDL_{3c}) particles hence could reflect a transport potential not utilized [4, 17], however, an increase in the proportion of larger HDL particles ($HDL_{2a,2b}$) has been related to high levels of TC in neonates [17]. Neonates with high TC displayed, according to the HDL-cholesterol/Apo A-I ratio, larger average HDL particles than neonates with TC < 2.59 mmol/l. According to Brinton et al. [5, 6] larger HDL particles displayed a decreased fractional catabolic rate and are more enriched in cholesterol than in Apo A-I. A main gender difference amongst neonates with high TC in the current study is the relatively lower transport of cholesterol by the HDL fraction in HF than in HM. A related study [2] demonstrated that the LDL fraction carried more cholesterol in HF than in HM. These results suggest that differences in the production and/or clearance of lipoproteins between male and female neonates with high TC levels take place. In the current study, HM presented higher triglyceride levels with respect to HF. A detailed picture of HM indicates that a large percentage of them presented, despite the absence of neonatal distress, triglyceride levels ≥70 mg/dl, a cut off point used for hypertriglyceridaemia in other newborn studies [22]. It has been indicated that

the hypertriglyceridaemic state induces the substitution of cholesterol by triglycerides in HDL_2 particles transforming them into high triglyceride content-HDL_{2b} particles. These HDL_{2b} particles have been considered to be rather less effective in the reverse cholesterol transport [14, 17]. Concomitant with the lecithin-cholesterol acyltransferase reaction, another pathway involves removal of cholesterol ester via cholesterol ester/triglyceride exchange between HDL and the triglyceride-rich lipoproteins (mainly VLDL), followed by HDL triglyceride hydrolysis. However, significant changes in the core and surface domains of both LDL and HDL have been found in relation to increasing plasma triglyceride levels [7, 16]. As is well established, Apo A-I is the major protein of HDL [9], and is present in no other lipoprotein in neonates. The HDL-cholesterol/Apo A-I ratio, therefore, gives an idea of HDL transport of both components, as well as of the average structural characteristics of the HDL fraction. This ratio suggests that HM presented 36% more cholesterol-enriched HDL than HF. Considering a MW of 28 kd for Apo A-I [12], it is possible to calculate the number of cholesterol molecules per Apo A-I molecule in an average male or female neonate HDL particle. An average NM or NF HDL particle presented about 30 molecules of cholesterol per Apo A-I molecule, while that of HM and HF exhibited about 47 and 34 molecules of cholesterol per Apo A-I molecule, respectively. The higher HDL-cholesterol/HDL-Apo A-I ratio also suggests the presence of higher levels of HDL_2 in HM. When the effect of sex and cholesterol level were separately studied it was found that HDL-cholesterol levels were about 65% higher and the HDL-cholesterol/Apo A-I ratio was 50% higher in HM than NM. However, the Apo A-I levels were found to be similar in HM and NM. HF presented 40% higher HDL-cholesterol and 23% higher Apo A-I levels than NF, but the HDL-cholesterol/Apo A-I ratio in HF did not change with respect to that of NF. Thus, the present data suggest a decreased fractional catabolic rate of HM HDL particles in comparison to HF HDL. In short, our study demonstrates that gender and serum cholesterol levels are key factors influencing the HDL composition at birth.

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