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# Lipid and lipoprotein changes throughout the term-period in neonates from the Toledo Study

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Serum lipids and lipoproteins were studied in 548 newborns from the Toledo Study (Spain) selected according to the following criteria: single delivery alive childbirth, eutocic delivery with cephalic presentation, gestational age between 37.0 and 41.86 wk, body weight between 2.500 and 3.999 kg and Apgar score of > 7 at 1 min and > 9 at 5 min. Mean cord total cholesterol (TC) value (1.80 ± 0.48 mmol/l) was in agreement with those of many studies. However, more than 5 % of newborns had more than 2.60 mmol/l (100 mg/dl). TC was equally carried by LDL and HDL (0.81 ± 0.40 vs 0.80±0.27 mmol/l, respectively). Triglyceride (Tg) values were 0.44 ± 0.23 mmol/l the cut-point being (95th percentile) for future studies at 0.87 mmol/l. With the exception of Tg, serum and lipoprotein lipids were kept rather constant between wks 38 and 42. However, TC resulted significantly higher ($p < 0.05$) at wk 38 than at wk 37. Tg were positive and significantly correlated with gestational age ($p < 0.001$), showing a negative but significant correlation with the body mass index ($p < 0.05$) and with the Apgar score ($p < 0.001$) of term-newborns.

Key words: Newborns, Term-period, Cholesterol, Triglycerides, Lipoproteins, Percentile distribution.

The pathologic precursors of ischemic coronary disease (ICD) are now recognised as originating in childhood (3, 20, 23), possibly induced by the same atherogenic factors as those found in adults. However, years of research will be needed to ascertain the actual role these factors play, when present in children, in provoking early lesions (2).

Serum lipoproteins are considered important risk factor variables of ICD, with low density lipoproteins (LDL) and

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high density lipoproteins (HDL) showing positive and inverse risk associations, respectively (14, 20, 32).

Levels of lipids and lipoproteins in cord sera should be a reflection of the status of plasma lipid metabolism in the infant at birth. Earlier reports have shown an association between perinatal conditions and levels of cord serum lipids. Most of these studies have shown that lipid levels are altered under conditions related to perinatal distress (6, 15, 19).

WHO published a number of definitions and recommendations related to the perinatal period (33). Between those referable to gestational age, term period was defined as the period from 37 to less than 42 completed weeks (259 to 293 days).

Although maternal lipids cross the placenta with difficulty, fetal lipid metabolism during gestation is very active. Lipogenesis from glucose is high and essential fatty acids are available to the fetus by means of their direct or lipase-mediated transfer through the placenta (16).

Because the deposition of triglycerides continues up to 40 wk gestation as weight rapidly increases (26), the rate of lipid metabolism must increase significantly. At the same time factors that normally modify lipid metabolism might have the same effects *in utero* and they are expressed by changes in the serum lipids and lipoproteins (19).

However, despite the recognized importance of lipids, e.g. LDL-cholesterol and HDL-cholesterol, little is established concerning variations throughout the term period.

The purposes of the present work are to study the lipoprotein changes occurring throughout the term period in a selected sample of neonates from the Toledo Study; to determine their normal values according to the gestational age; and to evaluate the possible influence of some neonatal variables such as gestational age, body weight, body mass index and Apgar score on lipid and lipoprotein concentration of selected neonates.

## Materials and Methods

The Toledo Study must be defined as a neonatal screening programme to detect infants with abnormal levels of some ICD risk factors. Throughout a period of 11 months, 705 cord sera samples were collected at the "Virgen de la Salud" Hospital, Toledo (Spain). With the aim of obtaining reference data from the newborns, only healthy full-term newborns were selected according to the following criteria: single delivery alive childbirth, eutocic delivery with cephalic presentation; gestational age between 37.00 and 41.86 wk; body weight between 2.500 and 3.999 kg and Apgar scores of > 7 at 1 min, and > 9 at 5 min. After these selections 548 neonate cord blood was studied.

Data concerning the mothers, their pregnancies and deliveries were obtained from notes in the records made by obstetricians and pediatricians, according to the strict routine practice in the "Departamento de Obstetricia y Ginecología" of the Hospital. Gestational age was determined according to menstrual history and the results of the pediatric examination of newborns, and expressed in weeks as from the first day of the last menstrual period.

The study was performed in accordance with the Helsinki Declaration of 1964 (as amended in 1983 and 1989). The investigation was approved by the Hospital's Direction and the "Consejería de Salud de la Comunidad Autónoma Castilla-La Mancha" (Spain).

Immediately after delivery, mixed (arterial and venous) umbilical cord blood was collected in chilled tubes and allowed to clot at room temperature for 30-60 min. Serum was obtained after centrifugation and immediately refrigerated at 4 °C.

Serum total cholesterol (TC) was measured by the enzymatic cholesterol esterase-cholesterol-oxidase method (Boehringer Mannheim, Germany). HDL-cholesterol was measured by the same method after precipitation of VLDL and LDL using the dextran sulphate $Mg^{2+}$ procedure (31). Triglycerides (Tg) were assayed by the enzymatic glycerol-phosphate-oxidase method (GPO-PAP) using a commercial test (Boehringer Mannheim). Cholesterol in LDL was estimated by the FRIEDEWALD *et al.* (11) equation.

Lipids internal quality control was carried out according to the laboratory manual of the Lipid Research Clinics Program (22). External quality control was provided by a quality control laboratory (Wellcome, S. E. de Química Clínica).

On the other hand, serum aliquots were on a few occasions accidentally mislaid which meant that some of the results were lost. Therefore, sometimes not enough serum was available, which explains the discrepancy in the number of observations.

The various groups were compared using ANOVA one-way and Duncan tests. Kormogorov, and Shapiro and Wick normality tests were applied. When data were not normally distributed, they were analysed with logarithmic transformation. The relationship between lipids, lipoprotein lipids and some neonatal variables within the selected neonates was study using the Pearson product-moment correlations.

## Results

Tables I, II and III show the concentrations of lipids and lipoproteins and the ratio TC/HDL-cholesterol in the newborns. Percentile distributions and changes of lipids and lipoproteins throughout the term period are also included. Only Tg concentrations were significantly affected by the gestational age. However, all lipids and lipoproteins tended to increase between wk 37 and 38, the increase in TC being significant.

Pearson product-moment correlations show only significant correlations between Tg levels and gestational age ($p < 0.001$) or body mass index ($p < 0.05$). Tg levels show negative and significant correlations with Apgar scores at 1 min ($p < 0.001$) and at 5 min ($p < 0.001$).

## Discussion

Factors during pregnancy and delivery, as well as certain diseases, can influence the foetal lipid metabolism, thus both primary and secondary hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia, may be present at birth (15). Serum cholesterol (4, 9, 10) also seems to be related to gestational age and birth weight. Taking into account these influences, newborns were selected according to strict criteria related to the kind of delivery, gestational age, body weight and foetal distress.

Table I.- *Lipid and lipoprotein levels and percentile distribution of full-term newborns.*

| | n | Mean ± SD | Range | Percentile 5th | 10th | 90th | 95th |
|---|---|---|---|---|---|---|---|
| Triglycerides (mmol/l) | 533 | 0.44 ± 0.23 | 0.08-1.81 | 0.18 | 0.22 | 0.71 | 0.87 |
| Total cholesterol (mmol/l) | 524 | 1.80 ± 0.48 | 0.75-4.18 | 1.15 | 1.27 | 2.39 | 2.67 |
| LDL-cholesterol (mmol/l) | 470 | 0.81 ± 0.40 | 0.06-2.88 | 0.19 | 0.36 | 1.31 | 1.52 |
| HDL-cholesterol (mmol/l) | 494 | 0.81 ± 0.27 | 0.26-2.67 | 0.48 | 0.52 | 1.14 | 1.30 |
| Total Cholesterol HDL-cholesterol | 472 | 2.36 ± 0.78 | 1.2-5.9 | 1.5 | 1.6 | 3.5 | 3.9 |

n = number of neonates.

Table II.- *Gestational age effect upon lipid levels (mmol/l) of full-term newborns in the Toledo Study.*

| Weeks: | 37 | | 38 | | 39 | | 40 | | 41 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD |
| Triglycerides[1] | (17) | 0.35 ± 0.15[a] | (93) | 0.38 ± 0.21[a] | (181) | 0.41 ± 0.22[a] | (166) | 0.50 ± 0.23[b] | (76) | 0.50 ± 0.23[b] |
| Total cholesterol[2] | (16) | 1.60 ± 0.30[a] | (91) | 1.84 ± 0.54[b] | (177) | 1.77 ± 0.45[ab] | (165) | 1.83 ± 0.49[b] | (75) | 1.80 ± 0.46[ab] |

In parentheses the number of subjects analysed. ANOVA one-way and Duncan test. [1]$p < 0.001$; [2]b borderline for statistical significance ($p < 0.06$). Values bearing a common letter are not significantly different.

Table III.- *Percentile values (mmol/l) for lipids and lipoproteins of full term newborns in the Toledy Study.*
Data of wk 37 are not presented because of the relatively small number of neonates born at this wk.

| | Week 38 | | | | Week 39 | | | | Week 40 | | | | Week 41 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5th | 10th | 90th | 95th | 5th | 10th | 90th | 95th | 5th | 10th | 90th | 95th | 5th | 10th | 90th | 95th |
| Triglycerides | 0.15 | 0.18 | 0.62 | 0.84 | 0.17 | 0.20 | 0.64 | 0.80 | 0.22 | 0.27 | 0.77 | 1.03 | 0.20 | 0.26 | 0.76 | 0.89 |
| Total cholesterol | 1.22 | 1.25 | 2.48 | 2.82 | 1.14 | 1.27 | 2.29 | 2.65 | 1.16 | 1.27 | 2.5 | 2.75 | 1.13 | 1.18 | 2.27 | 2.53 |
| LDL-cholesterol | 0.26 | 0.45 | 1.28 | 1.54 | 0.19 | 0.34 | 1.37 | 1.52 | 0.16 | 0.31 | 1.33 | 1.56 | 0.17 | 0.37 | 1.22 | 1.44 |
| HDL-cholesterol | 0.48 | 0.56 | 1.23 | 1.37 | 0.46 | 0.52 | 1.11 | 1.25 | 0.49 | 0.51 | 1.19 | 1.32 | 0.41 | 0.53 | 1.04 | 1.18 |
| Total cholesterol/ HDL-cholesterol | 1.50 | 1.60 | 3.30 | 3.60 | 1.40 | 1.60 | 3.50 | 3.80 | 1.50 | 1.60 | 3.40 | 4.10 | 1.50 | 1.60 | 3.50 | 4.40 |

Mean cord TC values are in agreement with those of many studies (2, 5, 12, 18, 19, 25). However, a large number of neonates (more than 5 %) have TC levels higher than 100 mg/dl, cut-point utilized by GLUECK *et al.* (12) for newborn hypercholesterolemia. This cut-point was rather close to the 95th percentile found in the current study and might be taken as a statistically valid normal limit.

According to our data, cholesterol was equivalently distributed among LDL and HDL (both about 45 % of TC). However, AVERNA *et al.* (1) found that HDL-cholesterol was lower than LDL-cholesterol and about 37 % of TC. VALDIVIESO *et al.* (29) found higher values in LDL than in HDL in Hispanic and Arab newborns, whereas DIAZ *et al.* (9) indicated that HDL is the major cholesterol carrier in neonates. BELLÚ *et al.* (2) found that HDL was the major carrier in male and female neonates without a family history of ICD.

Percentile 95th for LDL-cholesterol in the present study was higher than 1.5 mmol/l (~58 mg/dl). KWITEROVICH *et al.* (17) found that the concentration of LDL-cholesterol in cord blood permits the ascertainment of the affected child of a parent with the form of type-II hyperlipoproteinaemia, having approximately half of the offsprings at risk LDL-cholesterol above 41 mg/dl. This conclusion was based on the use of cut-off limits derived experimentally but selected arbitrarily (as the upper 5 % of the normal distribution).

TC/HDL-cholesterol values were in agreement with those derived from some studies (25) but lower than those described by others (1). In young populations a TC/HDL-cholesterol ratio higher than 4 has been suggested as a risk level for future ICD. According to the TC/HDL-cholesterol ratio found, less than 5 % of newborns has a ratio higher than 4.

Data of Tg were similar to those of others (2, 19, 29). The increase of Tg with gestational age probably reflects the progressive fatty deposition occurring during the latter stages of gestation. As might be expected with metabolically active compounds, Tg showed much greater variation than TC or lipoprotein-cholesterol at the time of the term period. THIBAULT *et al.* (28) studying the ontogeny of intestinal lipid and lipoprotein synthesis observed a progressive increase in the lipoprotein fractions produced by the foetal intestine (such as chylomicrons, VLDL and HDL) during development.

According to VIDEIRA-AMARAL *et al.* (30) term neonates have higher Tg levels than pre-terms. Moreover, HARDELL (15) showed that Tg distribution was displaced to the left in pre-terms and to the right in post-terms. Although Tg level is not considered in many studies as a primary risk for future ICD, others (30) have selected the level of 70 mg/dl as a cut-point. Less than 10 % of the studied neonates presented Tg values higher than 70 mg/dl (0.79 mmol/l).

Contrasting with Tg, results in TC, HDL-cholesterol and LDL-cholesterol suggest that throughout wks 38-42 minor changes may be produced in cholesterol metabolism. However, current results show significant variations in TC between wks 37 and 38.

Foetal plasma LDL-cholesterol levels have been inversely related to the plasma concentrations of the major foetal adrenal secretory product, i.e. dehydroepiandrosterone sulphate (DHEAS). PARKER *et al.* (24) have postulated that the observed fall in plasma LDL-cholesterol levels is caused by an increased rate of uptake and use of LDL-cholesterol pool for steroid hormone production by the foetal adrenals near term, but this finding came from a relatively small number of neonates and from a pair of weeks, i.e. 36-37. Moreover,

no significant relationship was found between serum hormone concentrations (i.e. DHEAS) and gestational age at term delivery (27). These findings would explain the steady-state of TC and cholesterol-lipoproteins throughout a large part of the term period.

Linear correlations were applied in order to evaluate the possible influence of some neonatal variables on the lipoprotein concentration of neonates. Apart from the positive and significant relationship found between gestational age and Tg levels, present data indicate a weak negative but significant correlation between Tg and body mass index (BMI). HARDELL (15) found a negative correlation between birth weight and Tg.

Increases in serum Tg has often been related to age and obesity (7, 8), however the extent to which such index actually represents assessment of adiposity is nonetheless controversial (21). In a previous work (13) a moderate positive correlation between serum Tg and the fat percentage or the body fat content was found in boys but not in girls.

Although Apgar scores of selected newborns were into a narrow range and indicate absence of perinatal stress, a negative and significant relationship was found between Apgar scores and Tg levels. According to HARDELL (15) newborns with Apgar score below 7 show a distribution of Tg displaced towards higher concentrations.

In conclusion, cord blood results for a large series of newborns indicate that lipids, with the exception of Tg, are kept rather constant between wks 38 and 42. More studies should be performed in order to confirm the TC changes found between weeks 37 and 38.

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Se estudian los lípidos y las lipoproteínas séricas en 548 neonatos del Estudio Toledo (España) seleccionados según las siguientes premisas: Niños nacidos vivos de parto único, eutócicos y con presentación cefálica, con edad gestacional entre 37,0 y 41,86 semanas, con peso al nacer de 2,5 a 3,999 kg y con índices de Apgar de > 7 al min y > 9 a los 5 min. La concentración media de colesterol total (CT) (1,80 ± 0,48 mmol/l) es similar a la de otros estudios. Sin embargo, más de un 5 % de neonatos tienen más de 2,60 mmol/l de CT (100 mg/dl). El CT está igualmente distribuido en LDL y HDL (0,81 ± 0,40 y 0,80 ± 0,27 mmol/l, respectivamente). La concentración de los triglicéridos (Tg) es de 0,44 ± 0,23 mmol/l, estando el nivel de corte para futuros estudios (percentil 95) en 0,87 mmol/l. Con la excepción de los Tg, los lípidos y lipoproteínas séricas se mantienen constantes entre la semana 38 y la 42, sin embargo el CT es más elevado en la semana 38 que en la 37 ($p < 0,05$). Los Tg correlacionan de forma positiva con la edad gestacional ($p < 0,001$) y muestran una correlación negativa y significativa con el índice de masa corporal ($p < 0,05$) y con los índices de Apgar ($p < 0,001$).

Palabras clave: Neonatos, Período a término, Colesterol, Triglicéridos, Lipoproteínas, Distribución en percentiles.

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