Journal of Developmental Origins of Health and Disease (2016), **7**(6), 672–677.

doi:10.1017/S2040174416000362

ORIGINAL ARTICLE

Lipids and leukocytes in newborn umbilical vein blood, birth weight and maternal body mass index

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Maternal obesity during pregnancy may influence fetal development and possibly predispose offspring to cardiovascular disease. The aim of the present study was to evaluate the relationship between maternal pre-pregnancy body mass index (BMI) and weight gain during pregnancy, and newborn birth weight, with lipid profile, high-sensitivity C-reactive protein (hs-CRP) and leukocyte in newborns. We performed a cross-sectional study of 245 mothers and their children. Blood was collected from the umbilical vein and assayed for lipid profile, hs-CRP and leukocyte count. Newborns average weight was 3241 g, total cholesterol 53.9 mg/dl, high-density lipoprotein cholesterol (HDL-c) 21.9 mg/dl, low-density lipoprotein cholesterol (LDL-c) 26.2 mg/dl, triglyceride 29.5 mg/dl and leukocytes 13,777/mm^3. There was a direct correlation of pre-pregnancy BMI of overweight mothers with total cholesterol ($r = 0.220$, $P = 0.037$) and LDL-c ($r = 0.268$, $P = 0.011$) of newborns. Total cholesterol, LDL-c and HDL-c were higher in pre-term newborns (66.3 ± 19.7, 35.9 ± 14.6 and 25.2 ± 7.7 mg/dl, respectively) that in full-term (52.4 ± 13.1, 25.0 ± 8.7 and 21.5 ± 6.0 mg/dl), with $P = 0.001$, 0.001 and 0.003, respectively. Leukocyte counts were higher in full-term newborns (14,268 ± 3982/mm^3) compared with pre-term (9792 ± 2836/mm^3, $P < 0.0001$). There was a direct correlation between birth weight and leukocyte counts of newborns ($r = 0.282$, $P < 0.0001$). These results suggest the possible interaction of maternal weight and fetal growth with lipid metabolism and leukocyte count in the newborn, which may be linked to programming of the immune system.

Received 25 August 2015; Revised 2 June 2016; Accepted 17 June 2016; First published online 30 August 2016

Key words: cord blood, high-sensitivity C-reactive protein, leukocytes, lipid profile, newborn

Introduction

Cardiovascular diseases (CVD) account for 30% of all deaths worldwide, according to the World Health Organization; cardiovascular deaths are projected to increase from 17.1 million in 2004 to 23.4 million in 2030. CVD remains as one of the most important cause of death worldwide. The percentage of premature deaths from CVDs ranges from 4% in high-income countries to 42% in low-income countries. Atherosclerosis, the leading cause of coronary artery disease,[1,2] is an inflammatory disease in which the immune system interacts with metabolic risk factors to initiate, activate and propagate arterial lesions.[3]

Fatty streaks, which are seen as atherosclerotic precursor lesions, may be formed as early as during fetal development.[4] Maternal cholesterol is actively transmitted from the placenta to the fetus.[5] Therefore, the possibility of maternal clinical conditions influencing the lipid profile of the newborn should be better exploited.

This suggests that humans may be 'programmed' in early prenatal life by adverse environmental circumstances such as maternal malnutrition, hypercholesterolemia, smoking and placental insufficiency, resulting in long-term CVD.[5–7] The prevalence of fatty streaks is increasing in children and young adults, and in some cases they may progress to more advanced stages of atherosclerotic lesions in adult life.[8–10]

Early life events may result in a hyper-responsive innate immune system. Programming of the immune system could result in a longstanding up regulation of pro-inflammatory gene expression. If this activation occurs during a critical period for gene expression, it might produce a long-lasting or even permanent tendency for an increased pro-inflammatory state. Undernutrition and other stressors during fetal development cause alterations of gene expression, which lead to a chronic, low-grade state of inflammation that could predispose to the development of the metabolic syndrome, diabetes and coronary heart disease. Such a pro-inflammatory tendency could help explain the association of low birth weight with elements of the metabolic syndrome and ischemic heart disease.[7]

The increasing prevalence of maternal obesity, gestational diabetes (GD) and other metabolic abnormalities has been associated with obesity and diabetes in the offspring, and animal models have established a causal relationship for atherogenic programming caused by specific maternal factors, consistent with the epidemiological findings in humans.[4,11–17] Studies have shown that obesity is an inflammatory state, with higher levels of C-reactive protein and leukocytes in adults.[3,7]

Thus, the main objective of this study was to evaluate the lipid profile and levels of hs-CRP and leukocytes in newborns, and its relationship with pre-pregnancy body mass index (BMI), diabetes and excessive weight gain during pregnancy, and birth weight of

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the newborn. The main hypotheses were that lipid levels, hs-CRP and leukocytes would be increased in the cord blood of low birth weight newborns, and in newborns of overweight and obese mothers or mothers with excessive weight gain during pregnancy.

Methods

This cross-sectional study included newborns and their mothers recruited from a maternity hospital in southern Brazil. The hospital is a local reference center and receives ~130 pregnant women per month. The institutional Research Ethics Committee approved the study.

On admission to the obstetric ward, mothers were informed about the study and signed the consent form. One member of the research team (T.B.) performed data collection everyday, including weekends, during the period of study. Singleton pregnancies with complete prenatal evaluation in the institution were included. Cases in which fetuses had congenital anomalies were excluded.

Physical examination included measurements of weight in kilograms, height in meters and blood pressure in millimetres of mercury. A questionnaire was applied, inquiring age, ethnicity, number of previous pregnancies, number of children, presence of smoking, thyroid disease, diabetes mellitus, hypercholesterolemia, high blood pressure and/or family history of these conditions. The pre-pregnancy BMI was calculated by dividing the body weight (last measurement before pregnancy) by the square of height in meters (kg/m^2).

Weight gain during pregnancy was calculated by subtracting the pre-pregnancy weight from the last measured weight before delivery. This value was classified according to Institute of Medicine[18] recommendations of pre-pregnancy BMI values and ranges of total weight gain during pregnancy that defines as underweight, a pre-gestational BMI of $<18.5\,kg/m^2$; normal weight, a pre-gestational BMI of 18.5–24.9 kg/m^2; overweight, a pre-gestational BMI of 25–29.9 kg/m^2; and obesity, a pre-gestational BMI of $\geqslant 30\,kg/m^2$, being expected a total weight gain in kilograms of 12.5–18, 11.5–16, 7–11.5 and 5–9 kg, respectively, for each of these BMI categories. Mothers who exceeded the range of total weight gain in relation to their pre-pregnancy BMI were classified as having excessive weight gain. GD was assessed according to the Brazilian Society of Diabetes.[19]

Data on gestational age and birth weight were obtained from the standard institutional charts. Neonates were considered premature when born before 37 weeks of gestation, and at term if born between 37 and 42 weeks of gestation. Medical records were reviewed to assess maternal diseases, such as human immunodeficiency virus antibody testing (anti-HIV), hepatitis B surface antigen (HBsAg), venereal disease research laboratory (VDRL) test, toxoplasmosis immunoglobulin M (IgM) and urine cultures. The presence of hypertensive disorders of pregnancy was assessed according to the guidelines of the National High Blood Pressure Education Program, reviewed in 2009 and published in the *Journal of Prenatal Medicine*, which identified four specific hypertensive disorders of pregnancy.

Newborn blood was collected by puncturing the umbilical vein immediately after cord clamping and removal of 6 ml of blood at the proximal end. Biochemical analysis of total cholesterol, high-density lipoprotein cholesterol (HDL-c) and triglycerides were determined in serum obtained by centrifugation of blood samples, through enzymatic method on an automated analyzer (Selectra E; Vital Scientific, USA), using reagent kits and protocols according to instructions of the manufacturer. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation. Levels of hs-CRP were determined in serum by nephelometry, using a Behring Nephelomefer 100 Analyzer (Dade Behring, USA). The number of leukocytes was determined using whole blood collected with ethylenediaminetetraacetic acid, in an automated analyzer (Coulter Act; Coulter, USA). For biochemical analysis (cholesterol, HDL-c, triglycerides and ultra-sensitive CRP), blood was stored for a maximum of 48 h. For total leukocytes count, blood was processed immediately.

Statistical analysis

The sample size was calculated from data obtained by Kelishadi *et al.*[28] for cholesterol. Considering a difference of 2 mg/dl, S.D. of 8.0, power of 0.8 and α of 0.05, the sample size was determined as 245 mothers.

Data were analyzed using the Statistical Package for Social Sciences 17.0 software. Numerical variables are presented as means and standard deviations, and categorical variables are described as proportions. We performed analysis for normality, through histograms, central tendencies, dispersion measures and Kolmogoroff–Smirnoff and Shapiro–Wilk tests.

The correlation of lipid profile and leukocyte numbers with maternal pre-pregnancy BMI and weight of newborns was investigated with the Pearson's correlation coefficient. We performed stratified analysis to examine the effects of confounding factors such as birth weight and gestational age. The Student's *t*-test was used to compare the lipid profile and leukocyte counts with GD, excessive maternal weight gain, gestational age, presence of urinary infection during pregnancy and hypertensive disorders of pregnancy. Association between categorical variables was determined using the χ^2 test. In all analysis, we considered an α P value of 0.05.

Results

A total of 520 mothers were admitted from October 2009 to January 2010. A total of 245 mothers with their newborns fulfilled the inclusion criteria and signed informed consent. Of those not included, 25 were twin pregnancies, 34 did not agree to participate and 227 had prenatal evaluation elsewhere. These mothers were not significantly different regarding age, but had less prenatal visits than the mother who were included.

Among the newborns, 135 (55.1%) were girls and 110 (44.9%) were boys. The clinical characteristics of mothers,

https://doi.org/10.1017/S2040174416000362 Published online by Cambridge University Press

Table 1. *Clinical characteristics of mothers and newborns*

Characteristics of mothers ($n = 245$)	
Age (years) [mean (S.D.)]	25.8 (6.8)
Pre-pregnancy BMI (kg/m^2) [mean (S.D.)]	24.2 (4.8)
Underweight <18.5 (kg/m^2)	4.9%
Normal weight 18.5–24.9 (kg/m^2)	58.5%
Overweight 25.0–29.9 (kg/m^2)	24.8%
Obesity ⩾30 (kg/m^2)	11.8%
Weight gain (kg) [mean (S.D.)]	14.6 (5.7)
Gestational diabetes	6.9%
Elevated blood pressure levels during pregnancy	10.2%
Smoking before pregnancy	40.2%
Smoking during pregnancy	24%
Type of delivery	
Vaginal	59.59%
Cesarean section	40.41%
Characteristics of newborns ($n = 245$)	
Birth weight (g) [mean (S.D.)]	3241 (549)
Length (cm) [mean (S.D.)]	48.5 (2.6)
Gestational age	
Pre-term (<37 weeks)	27 (11%)
Term (⩾37 weeks)	218 (89%)
Total cholesterol (mg/dl)	53.9 (14.6)
HDL cholesterol (mg/dl)	21.9 (6.3)
LDL cholesterol (mg/dl)	26.2 (10.1)
Triglycerides (mg/dl)	29.5 (16.0)
Leukocytes (mm^3)	13,777 (4115)

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

newborns, cord blood lipid and leukocytes are presented in Table 1. Diabetes was observed in 17 (6.9%) of mothers. Mean pre-pregnancy BMI was below 18.5 in 4.9% of the mothers, between 18.5 and 24.9 in 58.5% of them, between 25.0 and 29.9 in 24.8%, and ⩾30 in 11.8% of the mothers.

Table 2 shows the biochemical profile and leukocyte count of newborns according to pre-pregnancy maternal weight status. Total cholesterol and LDL-c were significantly lower in overweight or obese mothers, when compared with those with normal pre-pregnancy BMI.

Among the 245 pregnant women evaluated, 116 (47.4%) showed excessive weight gain during late gestation. No significant differences in biochemical profile and leukocyte count of newborns were observed among the categories of weight gain, but absolute values of total cholesterol and LDL-c were lower in mothers with higher weight gain, while total leukocyte counts were higher (Table 3).

Two mothers (16.7%) with pre-pregnancy BMI <18.5 kg/m^2 showed excessive weight gain during gestation. Of the mothers with pre-pregnancy BMI between 18.5 and 24.9, 25.0 and 29.9 or ⩾ 30 kg/m^2, 59 (41.9%), 36 (59.0%) and 18 (62.1%) showed excessive weight gain, respectively.

No differences were found in lipid profile and leukocyte counts of newborns according to maternal conditions including

Table 2. *Biochemical characteristics and leukocyte count of newborns according to pre-pregnancy maternal weight status*

Lipid profile and leukocytes [mean (S.D.)]	Normal weight/ underweight (mothers) ($n = 156$)	Overweight/ obese (mothers) ($n = 90$)	P
Total cholesterol (mg/dl)	54.58 (14.14)	53.10 (15.45)	0.038
HDL cholesterol (mg/dl)	22.12 (5.85)	21.32 (7.02)	0.230
LDL cholesterol (mg/dl)	26.41 (9.83)	25.99 (10.60)	0.011
Triglycerides (mg/dl)	29.79 (15.82)	29.12 (16.51)	0.725
Leukocytes (mm^3)	13,699 (4027)	13,911 (4282)	0.479

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

diabetes, excessive weight gain during pregnancy, elevated blood pressure levels and urinary tract infection in pregnancy.

There was an inverse correlation of birth weight with total cholesterol ($r = -0.160$, $P = 0.012$) and with LDL-c ($r = -0.175$, $P = 0.006$). Birth weight was directly correlated with leukocyte counts of newborn infants ($r = 0.282$, $P < 0.0001$) (Table 4).

Levels of hs-CRP were above the minimum detection limit in only three of the 245 newborns evaluated. There were no positive results for HBsAg, and only one sample was positive for anti-HIV, VDRL and toxoplasmosis IgM.

Totally, 27 (11%) of the newborns were pre-term and 218 (89%) were at term. The levels of total cholesterol, low-density lipoprotein and high-density lipoprotein were higher in pre-term newborns compared with full-term infants. Leukocyte counts were higher in full-term newborns as compared with pre-term infants. Triglyceride levels were also higher in full-term infants, but the difference was not statistically significant (Table 5).

The analysis of biochemical markers according to the mother's nutritional status, but excluding pre-term babies, yielded similar results, but loosing significance for total cholesterol and LDL-c ($P = 0.063$ and 0.048, respectively). Stratified analysis considering birth weight, type of delivery and gestational age did not differ significantly from the correlation analysis performed with the total sample.

Discussion

In this study of mothers and newborns, we observed that total cholesterol and LDL-c were significantly higher in newborns whose mothers were underweight or had normal weight before pregnancy, as compared with mothers who were overweight or obese. Total cholesterol and LDL-c were also inversely correlated with newborn birth weight. There was no difference in lipids according to the mother's weight gain during pregnancy, although absolute numbers were lower in newborns whose mothers showed higher weight gains.

https://doi.org/10.1017/S2040174416000362 Published online by Cambridge University Press

Table 3. *Biochemical characteristics and leukocyte count of newborns according to maternal weight gain*

Lipid profile and leukocytes [mean (S.D.)]	Weight gain below recommended ($n = 51$)	Recommended weight gain ($n = 76$)	Weight gain above recommended ($n = 119$)	*P*
Total cholesterol (mg/dl)	54.76 (16.307)	56.01 (14.646)	52.47 (13.759)	0.237
HDL cholesterol (mg/dl)	22.33 (7.522)	22.41 (5.813)	21.24 (6.023)	0.370
LDL cholesterol (mg/dl)	26.61 (11.123)	27.33 (10.168)	25.42 (9.599)	0.422
Triglycerides (mg/dl)	29.39 (17.349)	30.08 (13.582)	29.27 (17.013)	0.940
Leukocytes (mm^3)	12,897.45 (3381.451)	13,974.16 (4178.549)	14,028.55 (4335.351)	0.230

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 4. *Correlation between birth weight and lipid profile and leukocytes in newborns*

Variable	*r*	*P*
Total cholesterol	−0.160[a]	0.012
HDL cholesterol	−0.049	0.443
LDL cholesterol	−0.175[a]	0.006
Triglycerides	−0.058	0.369
Leukocytes	0.282[a]	<0.0001

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a] $P < 0.05$ – Pearson's correlation test.

Table 5. *Comparison of total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and leukocytes from pre-term and full-term newborns*

Variable [mean (S.D.)]	Pre-term	Full-term	*P*
Total cholesterol (mg/dl)	66.3 ± 19.7	52.4 ± 13.1	0.001
LDL-c (mg/dl)	35.9 ± 14.6	25.0 ± 8.7	0.001
HDL-c (mg/dl)	25.2 ± 7.7	21.5 ± 6.0	0.003
Leukocytes (mm^3)	9792 ± 2836	14,268 ± 3982	<0.0001

Although our initial hypothesis was that obese mothers would have higher lipid levels, there are many possible explanations for our findings. Many of the associations observed regarding metabolic imprinting and birth weight are nonlinear, U-shaped relations. This means that both underweight and overweight may play a role in determining future disease. Moreover, life course epidemiology implies in complex relations between intrauterine factors and later behavioral and environmental conditions. For example, maternal obesity may influence the child during fetal life, through intrauterine programming, but also later, through family behaviors and feeding practices.[20]

Obesity and overweight may be a metabolic burden to the mother, but they may also represent a protection for the fetus regarding intrauterine programming related to nutrient restriction. An adequate supply of nutrients to the fetus is fundamental for its growth and development. During pregnancy, maternal metabolism is increased, and dietary energy and nutrient requirements generally increase to adapt to these requirements, plus the delivery of nutrients to the fetus.[21] Our results showed that mothers with excessive weight gain had children with higher birth weight. This situation may mirror, in some aspects, what recent literature has been calling the 'obesity paradox': although obesity may be an important risk factor for various diseases, including metabolic disturbances in the fetus, it may also represent a positive prognostic factor.[22] The impaired fetal growth indicated by low birth weight is associated with increased risk for coronary disease in future life.[23] In addition, lower birth weight was also associated with type 2 diabetes,[24] which may contribute to increased risk of atherosclerotic disease.[25] In a multivariate analysis controlling weight for gestational age, we found an inverse correlation between birth weight with total cholesterol and LDL-c, suggesting, thus, an unfavorable lipid profile with lower newborn weight. A longitudinal study showed evidence that the association between low birth weight and the risk of future ischemic heart disease was entirely mediated by fetal growth restriction, and not by gestational age.[26]

It is also important to take into account that BMI is a crude measure of body mass that is useful in clinical research settings, but may not reflect body composition or metabolic profile. In addition, maternal nutrient deficiencies can occur in the presence of normal weight, excess weight or underweight.[20]

In a randomized controlled trial of Danish women that used dietary advice and physical activity to prevent weight gain, intervention resulted in a small, but significant difference in gestational weight gain compared with the control group. However, no significant differences were observed between randomized groups regarding offspring body composition or metabolic risk factors at 2.8 years. In addition, when the authors compared offspring of obese women with offspring of normal weight mothers all outcomes were similar.[20,27]

The lipid profile of newborns is unique with respect to concentration and composition.[20] Consistent with previous reports,[20–22] the present work showed HDL-c levels slightly below the levels of LDL-c, and lipid and lipoprotein levels markedly below those of children and adolescents.[27,28] Similarly, Pardo *et al.* observed higher levels of ApoB/ApoA-I in pre-term infants compared with full-term newborns.[29] Our

https://doi.org/10.1017/S2040174416000362 Published online by Cambridge University Press

data showed higher triglyceride levels in newborns at term. Although the difference was not statistically significant, these results are similar to those reported by other studies.[28,30] The higher triglyceride levels seen in full-term newborns may reflect the increase in total body fat which occurs during the last weeks of pregnancy.

We observed a positive correlation between birth weight and leukocyte count. Leukocyte counts were similar in newborns of mothers with different categories of pre-pregnancy BMI, and in mothers with or without excessive weight gain during pregnancy.

The studies that have investigated the association between low birth weight and subsequent levels of inflammation markers had contradictory findings. In a study which assessed the relationship of birth weight and the 1st year of life with total leukocyte count in adulthood, Canoy *et al.*[26] observed a lower score in the categories of higher birth weight. The inverse association was more pronounced for newborns with lower birth weight and the lowest tertile of weight achieved in 1 year, suggesting a role for a mechanism of inflammation, linking poor early growth with risk of coronary disease[31] as seen in another recent study[32] on the association between birth weight and leukocyte count demonstrates the inverse association of the white blood cell count with birth weight in children and adults. Our results, however, showed a direct correlation between birth weight and leukocyte count. Leukocyte counts were higher in full-term than in pre-term infants. McElrath *et al.*[32] showed that compared with appropriate for gestational age infants, growth-restricted infants tend to have higher circulating concentrations of inflammation-related proteins 1–2 weeks after birth, but not at the childbirth.

Some limitations of our study merit discussion. As this was an observational study, it is important to consider the possibility of residual confounding in the relationships studied, specially as effect sizes are small. Maternal lipid profiles before or during pregnancy, and detailed sonographic fetal measurements were not available. Mothers included in the study did not differ different regarding age from those not included, but had less prenatal visits. This could have resulted in selection bias, as mother with incomplete prenatal evaluation may also have a lower socio-economic position and a higher proportion of undernutrition.

In conclusion, there were significant differences in total cholesterol and LDL-c according to pre-pregnancy BMI, birth weight and gestational age. There was also a positive correlation between birth weight and leukocyte count. As inflammatory markers and lipid alterations are described to track in childhood and to predict later metabolic and vascular disease, these findings may have future implications for a more effective planning of preventive measures.

Acknowledgments

The authors wish to thank the nurses and the laboratory staff working on the project, and Professor Sergio Kakuta Kato for his valuable statistical guidance.

Financial Support

This study was funded by Fundo de Apoio do Instituto de Cardiologia de Porto Alegre à Ciência e Cultura.

Conflicts of Interest

None.

Ethical Standards

The institutional Research Ethics Comittee approved the study. All mothers were informed about the study and signed the consent form.

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