Associations of Apgar score and size at birth with lipoprotein subclasses in juvenile obesity

Osama H. BEKHET[1], Jelena VEKIC[1,]*, Aleksandra ZELJKOVIC[1], Dusan PARIPOVIC[2], Tamara GOJKOVIC[1],
Jelena JANAC[1], Vesna SPASOJEVIC-KALIMANOVSKA[1], Amira PECO-ANTIC[2,3],
Gordana MILOSEVSKI-LOMIC[2], Zorana JELIC-IVANOVIC[1], Aleksandra STEFANOVIC[1]

[1]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[2]Department of Nephrology, University Children's Hospital, Belgrade, Serbia
[3]School of Medicine, University of Belgrade, Belgrade, Serbia

Background/aim: Juvenile obesity is associated with several metabolic abnormalities, one of them being atherogenic dyslipidemia. Suboptimal fetal growth is associated with obesity risk in childhood, but also with increased rate of metabolic diseases in later life. This study investigated associations of neonatal data (Apgar score, birth weight and birth length) with low-density lipoprotein and high-density lipoprotein (LDL and HDL) subclasses in a group of obese children, as well as a possible impact of breastfeeding duration on obesity-associated lipoprotein subclasses distributions.

Materials and methods: We included 42 obese children, aged 14.2 ± 2.1 years. LDL and HDL subfractions were separated by gradient gel electrophoresis and biochemical parameters were assessed by routine methods.

Results: Compared with obese children with Apgar ≥ 9, the group with Apgar < 9 had significantly higher percentages of small, dense LDL particles (P < 0.05), due to reduced LDL I (P < 0.01) and increased LDL III subclasses (P < 0.05). Birth weight was positively associated with the proportions of LDL I particles (P < 0.001), whereas birth height positively correlated with the amount of HDL 2b subclasses (P < 0.05). The group of never or less than 3 months breastfed children had significantly smaller LDL size (P < 0.01) and lower proportion of HDL 2a particles (P < 0.05) than their ≥3 months breastfed peers.

Conclusion: The results showed significant associations of neonatal characteristics with LDL and HDL particle distributions in obese children. In addition, our results point toward positive aspects of longer breastfeeding duration on lipoprotein particle distributions in obese children.

Key words: Childhood obesity, small dense LDL, HDL subclasses, Apgar, breastfeeding

1. Introduction

The growing trend of childhood obesity represents a major health concern worldwide (1). It is now well documented that obesity is linked to increased cardiovascular disease (CVD) risk in adults (2). However, even if CVD complications usually become manifested in adulthood, the process of atherogenesis might start much earlier. To date, a body of evidence has been gathered implying that atherosclerosis may begin in childhood (3). Taken all together, childhood obesity seems to be significant risk factor for the onset of CVD (4).

Although there is no doubt that any strategy for prevention of atherosclerosis should include children and adolescents, there is no consensus regarding independent association of childhood obesity with CVD risk factors in later life (5). However, it has been demonstrated that

overweight and obese children have a greater likelihood of developing hypertension and type-2 diabetes, as well as ischemic heart and brain diseases (6). In general, obesity is associated with numerous metabolic abnormalities (3), which may magnify cardiovascular risk. Excessive adipose tissue accumulation results in the release of several adipokines that contribute to the pathogenesis of inflammation and hypertension (7). Apart from inflammation, abnormalities in serum lipid levels are also typically seen in obesity. Although increased low-density lipoprotein (LDL)-cholesterol (LDL-C) and low high-density lipoprotein (HDL)-cholesterol (HDL-C) concentrations are well-known as major atherosclerosis risk factors, it is now recognized that certain lipoprotein subclasses, particularly small, dense LDL and HDL, may further distinguish an individual's CVD risk (8). In

accordance, atherogenic dyslipidemia has emerged as an increasingly prevalent risk factor in children, concomitant with obesity (9).

It is well known that predisposition to obesity is determined by genetic and environmental risk factors. On the other hand, the concept that fetal life is a critical time for lifelong cardiovascular consequences is also well supported (10). According to the available evidence, gestational obesity is able to predispose the offspring to the development of CVD in adulthood (11). We have recently demonstrated that higher proportions of small, dense LDL particles in maternal plasma before delivery were independently associated with smaller weight and length of newborns (12). A low size at birth, related to suboptimal fetal environment, has been associated with obesity risk in childhood (13) but also with increased rate of metabolic diseases in later life (14). In addition, Apgar score, an indicator of a newborn's vitality, can also offer additional prognostic information on future morbidity and mortality (15). So far, there have been no studies on the association between neonatal data and lipoprotein subclasses profile in obese children.

Another important aspect related to further cardiovascular health of the child is dietary pattern during the early postnatal period. Nowadays, exclusive breastfeeding is considered the optimal source of nutrition in early life (16). Moreover, breastfeeding has been found to be protective against development of atherosclerosis in terms of reducing CVD risk factors (17). In the reviewed literature, there is no study investigating the impact of breastfeeding on lipoprotein subclasses distribution in obese children.

In this study we aimed to evaluate possible associations of neonatal data (Apgar score, birth weight, and birth length) with LDL and HDL subclasses in a group of obese children. In addition, we examined whether breastfeeding duration had an impact on obesity-associated lipoprotein subclass distributions.

2. Materials and methods

2.1. Patients

The study included obese children who attended medical check-ups at the University Children's Hospital. The inclusion criteria were absence of secondary hypertension and obesity associated with any medical conditions, as well as use of any medication. As a result, a total group of 42 subjects, aged 10–18 years, who had completed all required data and measurements were included in the study. Neonatal data were gathered from medical records and included Apgar score, birth weight, and birth height. Breastfeeding was considered as any kind of breastfeeding, i.e. exclusive and partial breastfeeding. Duration of breastfeeding was self-reported by parents.

The study was executed following the ethical guidelines of the Declaration of Helsinki. The study protocol was approved by the institutional review committee. All patients and their parents were completely informed and gave written consent for participation.

2.2. Anthropometric measurements

Each patient's weight, height, waist, and hip circumferences were measured. Body-mass index (BMI) and BMI z-score values were calculated according to a previously proposed model (18). Childhood obesity was considered if BMI was ≥95th percentile.

2.3. Laboratory analyses

Blood samples were collected into evacuated tubes containing EDTA and serum sample tubes after a 12-h fasting period. Plasma and serum were separated by immediate centrifugation at $1500 \times g$ for 10 min at 4 °C. Aliquots of each sample were stored at −80 °C and thawed immediately before analyses.

Concentrations of glucose, total cholesterol (TC), triglycerides (TG), LDL-C, and HDL-C were measured by routine laboratory methods (Olympus Diagnostica GmbH, Hamburg, Germany). LDL and HDL subclasses were measured by the modified method of Rainwater et al. (19), as previously published in detail (20). We determined migration distances for absorbance peaks and used them for assessment of the particle diameter. Diameters of the most prominent peaks in the LDL and HDL regions of each scan were labelled as dominant particle sizes. The areas under the peaks corresponding to each LDL and HDL subclass were also determined and referred to as relative proportions of particular subfractions. The proportion of small, dense LDL was also ascertained as the area at or below 25.5 nm in the densitometric scan. Likewise, percentage of small, dense HDL particles was determined as the area of the densitometric scan at or below 8.8 nm (20). Increased small, dense LDL particle proportion was considered if the absorbance in the region corresponding to LDL size ≤ 25.5 nm was higher than 50% (19). All assays were performed blindly.

2.4. Statistical analysis

Normally distributed continuous variables were shown as mean ± standard deviation (SD). Variables that did not fit into normal distribution were presented as median (interquartile range), and categorical variables as relative or absolute frequencies. Group differences were analyzed by Student's t-test (if the data were normally distributed) or by Mann–Whitney test (if the data were asymmetrical). Categorical data were analyzed by chi-square test. We employed Spearman's correlation analysis for assessment of significant associations. Minimal statistical significance was set at $P < 0.05$ and the statistical software MedCalc for Windows version 9.6.3. (Mariakerke, Belgium) was used for analysis.

3. Results

Table 1 represents the demographic and laboratory data of 42 obese children. The study participants were 14.2 ± 2.1 years old and 37 of them (88.1%) were pubertal. All children had BMI above the 95th percentile and were characterized by central obesity, as indicated by high waist-to-hip ratios (range: 0.82–1.04). Regarding neonatal data, all children were born at term. Normal birth weight had 35 (83.3%) participants, 6 (14.3%) had high birth weight (>4000 g), and one child had low birth weight (<2500 g). According to Apgar rating, 10 infants (23.8%) had a score less than 9, including three subjects with a score less than 7. Data on breastfeeding were available for 40 children. Among them, 85% were breastfed for at least 1 month (median: 7.5 months).

The study participants were initially classified according to the Apgar score (Table 2). The two groups were homogeneous in terms of age and sex. Obese children with Apgar < 9 had significantly lower birth weight than the group with Apgar ≥ 9. On the other hand, anthropometric variables (weight, BMI, waist and hip circumferences) in childhood did not differ between the groups. Similarly, no differences in lipid status parameters were detected. Compared with the children with Apgar ≥ 9, the group with Apgar < 9 showed a tendency toward smaller LDL and HDL particles sizes (P = 0.098 and P = 0.087, respectively). Furthermore, the children with Apgar < 9 had significantly increased proportion of LDL III (P < 0.05) with parallel reduction in LDL I subclasses (P < 0.01) than those with Apgar ≥ 9. As a result, the proportion of small, dense LDL particles was significantly higher in the children with Apgar < 9 (P < 0.05). HDL subclasses distribution did not differ between the groups.

Next, we performed Spearman's correlation analysis (Table 3) and found that Apgar score was in positive correlation with LDL size and relative proportions of LDL I subclasses, and in inverse correlation with the percentage of small, dense LDL particles. Moreover, Apgar score was positively associated with the proportions of HDL 3a subclasses.

With regard to the previous findings, we additionally analyzed potential associations of lipoprotein heterogeneity with birth size (Table 3). We found that birth weight was positively related to the proportions of LDL I and inversely associated with the proportions of HDL 3c subclasses (Table 3). No significant correlations were found between birth height and LDL subclasses. On the other hand, HDL size and relative proportions of HDL 2b particles were positively associated, while the proportions of HDL 3c were in negative correlation with birth height.

In further analysis, we examined the impact of breastfeeding on clinical and laboratory parameters in childhood (Table 4). Eight children did not breastfeed at

Table 1. Clinical and laboratory parameters of study participants.

	Obese children (n = 42)
Age, years	14.2 ± 2.1
Sex, boys/girls	30/12
Prepubertal/pubertal	5/37
Apgar score	8.7 ± 1.3
Birth weight, g	3392.6 ± 587.4
Birth length, cm	52.4 ± 3.5
Breastfeeding, %	85
Breastfeeding duration, months [#]	7.5 ± 3.13
Weight, kg	88 ± 20.0
Height, m	170 ± 12.8
BMI, kg/m²	30.2 ± 3.9
BMI percentile	97.2 ± 1.2
BMI z-score	3.14 ± 0.99
Waist circumference, cm	97 ± 9.4
Hip circumference, cm	103 ± 11.9
Waist-to-hip ratio	0.94 ± 0.06
Glucose, mmol/L	4.79 ± 0.57
TC, mmol/L	4.25 ± 1.14
LDL-C, mmol/L	2.74 ± 1.16
HDL-C, mmol/L	1.06 ± 0.24
TG, mmol/L [#]	0.88 (0.69–1.22)
LDL size, nm	27 ± 1.29
LDL I, %	21 ± 6.7
LDL II, %	24 ± 4.0
LDL III, %	20 ± 4.6
LDL IV, %	35 ± 8.4
Small, dense LDL, %	55 ± 9.4
HDL size, nm	9 ± 1.17
HDL 2b, %	40 ± 7.9
HDL 2a, %	19 ± 4.9
HDL 3a, %	15 ± 2.8
HDL 3b, %	9 ± 3.3
HDL 3c, %	17 ± 7.5
Small, dense HDL, %	41 ± 9.9

Data are presented as mean ± SD, and absolute or relative frequencies for categorical variables
[#] Data are presented as median (IQR).
BMI, body-mass index; TC, total cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol ; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Table 2. Clinical and laboratory parameters according to Apgar score.

	Apgar < 9 (n = 10)	Apgar ≥ 9 (n = 9)	P
Age, years	14.7 ± 2.1	14.1 ± 2.2	0.369
Sex, boys/girls	9/1	20/11	0.231
Apgar score	7.0 ± 1.5	9.3 ± 0.5	<0.001
Birth weight, g	2944 ± 667.4	3523 ± 496.5	<0.05
Birth length, cm	50 ± 4.5	53 ± 2.8	0.085
Weight, kg	96 ± 25.5	85 ± 17.8	0.149
Height, m	175 ± 12.8	168 ± 12.4	0.136
BMI, kg/m^2	30.7 ± 5.4	30.0 ± 3.4	0.652
BMI z-score	3.22 ± 1.52	3.12 ± 0.81	0.807
Waist circumference, cm	98 ± 12.3	97 ± 8.5	0.870
Hip circumference, cm	106 ± 17.6	103 ± 9.7	0.383
Waist-to-hip ratio	0.93 ± 0.07	0.95 ± 0.05	0.286
Glucose, mmol/L	4.65 ± 0.49	4.85 ± 0.59	0.349
TC, mmol/L	4.09 ± 0.69	4.33 ± 1.26	0.582
LDL-C, mmol/L	2.53 ± 0.52	2.84 ± 1.31	0.476
HDL-C, mmol/L	1.04 ± 0.22	1.07 ± 0.25	0.731
TG, mmol/L [#]	1.02 (0.64–1.20)	0.86 (0.70–1.23)	0.870
LDL size, nm	26 ± 1.4	27 ± 1.2	0.098
LDL I, %	17 ± 3.1	22 ± 7.3	<0.01
LDL II, %	23 ± 3.8	25 ± 4.1	0.207
LDL III, %	22 ± 2.1	19 ± 4.9	<0.05
LDL IV, %	38 ± 7.2	34 ± 8.7	0.190
Small, dense LDL, %	60 ± 6.3	53 ± 9.9	<0.05
HDL size, nm	9 ± 0.8	10 ± 1.2	0.087
HDL 2b, %	41 ± 7.4	40 ± 8.1	0.770
HDL 2a, %	20 ± 6.6	18 ± 4.1	0.248
HDL 3a, %	14 ± 2.3	15 ± 2.9	0.146
HDL 3b, %	8 ± 3.3	10 ± 3.2	0.207
HDL 3c, %	17 ± 9.2	17 ± 6.8	0.970
Small, dense HDL, %	39 ± 10.2	42 ± 9.7	0.421

Data are presented as mean ± standard deviation, and absolute or relative frequencies for categorical variables. Continuous variables were analyzed by Student's t-test. Categorical variables were analyzed by chi-square test.
[#] - Data are presented as median (IQR) and analyzed by Mann–Whitney U test.
BMI, body-mass index; TC, total cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol ; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

all, while seven children were breastfed for 1 or 2 months solely. On the other hand, 19 children were breastfed ≥ 6 months (min. 6 months; max. 24 months) and six children were breastfed 3, 4, or 5 months. Due to unequal distribution of children, the study participants were classified according to breastfeeding duration into the group of never or less than 3 months breastfed children and the group of children who were breastfed for 3 months or more. The newly formed groups were even in term of age and sex. Analysis of neonatal data showed that children

Table 3. Correlations of lipoprotein subclasses with Apgar score, birth weight, and birth height.

Lipoprotein subclasses	Apgar score	Birth weight, g	Birth height, cm
LDL size, nm	0.365 *	0.161	0.146
LDL I, %	0.386 *	0.517 **	0.164
LDL II, %	0.172	0.173	−0.013
LDL III, %	−0.238	−0.186	−0.115
LDL IV, %	−0.271	−0.020	−0.020
Small, dense LDL, %	−0.306 *	−0.047	−0.047
HDL size, nm	0.248	0.224	0.350 *
HDL 2b, %	0.002	0.165	0.357 *
HDL 2a, %	−0.036	0.057	−0.033
HDL 3a, %	0.363 *	0.260	−0.002
HDL 3b, %	0.175	0.115	−0.157
HDL 3c, %	−0.182	−0.348 *	−0.322 *
Small, dense HDL, %	−0.009	−0.139	−0.267

** $P < 0.001$; * $P < 0.05$

in the group breastfed ≥ 3 months had significantly higher Apgar score than those who were not breastfed at all or were breastfed for less than 3 months. Anthropometric variables did not differ between the two groups. Even if we did not find differences in lipid status parameters, further insight into the characteristics of lipoprotein particles showed that the group of never or less than 3 months breastfed children had significantly smaller LDL size (P < 0.01) and reduced proportion of HDL 2a particles (P < 0.05) than their ≥3 months breastfed peers.

4. Discussion
Over the past decades the prevalence of obesity among children and adolescent has considerably increased (1). Hence, in view of the growing trends of childhood obesity, primary prevention in youths seems to be the main approach to combat the development of numerous metabolic consequences, particularly CVD (6). Although the concept of obesity-related dyslipidemia is well understood in adults, quantitative evaluation in pediatric population is more complex. Namely, even if serum lipid parameters in obese children and adults have similar levels, in terms of higher TG and reduced HDL-C, younger populations usually have desirable or slightly elevated LDL-C concentrations (21). In line with previous research, obese children in the current study showed no dramatic abnormalities in serum lipid parameters (Table 1). However, our more in-depth analysis of lipid status revealed high proportions of small, dense LDL and HDL particles (Table 1), in spite of relatively optimal serum lipid parameters. Recently, LDL and HDL subclasses

characteristics in a group of healthy children of the same origin were published by Zeljkovic et al. (22). Both small, dense LDL and HDL particles in the current study of obese children were higher when compared to the data from healthy children in the study by Zeljkovic et al. (22). A prevalence of small, dense LDL particles in obese children has been previously reported, although the results were not entirely consistent (9). In contrast, a relationship of small HDL particles with childhood obesity has been firmly established (23). To date, numerous studies provided solid evidence that small, dense LDL particles have the greatest atherogenic potential, due to easier penetration into arterial intima, longer retention in subendothelium, and higher oxidative susceptibility (8). It is well known that high HDL-cholesterol levels are associated with decreased cardiovascular risk. By reverse cholesterol transport HDL particles accept cholesterol from the periphery, such as arterial wall cells, and deliver it to the liver. Reverse cholesterol transport is the major mechanism of antiatherogenic effects of HDL (24). In addition, a hypothesis that smaller HDL particles have diminished anti-atherogenic capacity in the conditions associated with dyslipidemia, inflammation, and enhanced oxidative stress was also confirmed (24). Therefore, a finding of pro-atherogenic small, dense LDL and HDL particles in obese children could indicate higher cardiovascular risk than anticipated by standard lipid measurement.

So far, numerous risk factors in children have been evaluated in terms of possible association with increased CVD risk in later life. Recently reviewed data suggest that cardiovascular risk in adulthood is more likely associated

Table 4. Clinical and laboratory parameters according to breastfeeding duration.

	Breastfeeding		
	<3 months and none (n = 15)	≥3 months (n = 25)	P
Age, years	14.0 ± 2.1	14.4 ± 2.2	0.573
Sex, boys/girls	10/5	19/6	0.716
Apgar score	8.2 ± 1.7	9.0 ± 0.9	<0.05
Birth weight, g	3256 ± 638.5	3456 ± 571.9	0.312
Birth length, cm	52 ± 4.5	52 ± 2.7	0.653
Breastfeeding duration, months [#]	0 (0–1)	12 (5–13.5)	<0.001
Weight, kg	87.1 ± 20.6	88.8 ± 20.6	0.801
Height, m	167 ± 12.6	171 ± 13.1	0.322
BMI, kg/m^2	30.7 ± 4.1	29.9 ± 3.9	0.545
BMI z-score	3.32 ± 0.95	3.06 ± 1.06	0.427
Waist circumference, cm	97 ± 10.7	97 ± 8.9	0.987
Hip circumference, cm	103 ± 12.7	104 ± 11.9	0.764
Waist-to-hip ratio	0.95 ± 0.06	0.94 ± 0.06	0.608
Glucose, mmol/L	4.65 ± 0.52	4.84 ± 0.56	0.294
TC, mmol/L	4.15 ± 1.64	4.38 ± 0.76	0.546
LDL-C, mmol/L	2.82 ± 1.67	2.76 ± 0.78	0.612
HDL-C, mmol/L	1.09 ± 0.24	1.04 ± 0.25	0.868
TG, mmol/L [#]	0.83 (0.60–1.10)	1.02 (0.78–1.27)	0.125
LDL size, nm	26 ± 1.19	27 ± 1.32	<0.01
LDL I, %	19 ± 4.4	22 ± 7.9	0.176
LDL II, %	25 ± 3.1	24 ± 4.6	0.404
LDL III, %	20 ± 4.6	20 ± 4.6	0.887
LDL IV, %	36 ± 6.2	34 ± 9.8	0.529
Small, dense LDL, %	56 ± 6.9	54 ± 11.0	0.621
HDL size, nm	9 ± 1.36	9 ± 1.09	0.845
HDL 2b, %	41 ± 7.6	40± 8.3	0.684
HDL 2a, %	17 ± 5.0	20 ± 4.1	<0.05
HDL 3a, %	14 ± 3.1	15 ± 2.7	0.304
HDL 3b, %	10 ± 3.8	9 ± 2.9	0.443
HDL 3c, %	18 ± 8.2	16 ± 6.8	0.341
Small, dense HDL, %	42 ± 10.9	40 ± 9.3	0.502

Data are presented as mean ± standard deviation, and absolute or relative frequencies for categorical variables. Continuous variables were analyzed by Student's t-test. Categorical variables were analyzed by chi-square test.
[#] - Data are presented as median (IQR) and analyzed by Mann–Whitney U test.
BMI, body-mass index; TC, total cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol ; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

with tracking of overweight and dietary habits while growing up, rather than with obesity in childhood (5). On the other hand, prospective data indicate an inverse association of smaller neonatal body size with the onset of CVD in adulthood (25). In recent years, great attention has been paid to analyzing the link between different biomarkers in maternal plasma and smaller birth size, as well as their consequences on further morbidity

and mortality risk of infants. In our previous study, we demonstrated independent associations between smaller LDL and HDL particles in maternal plasma before delivery and lower newborn's size (12), indicating that adverse lipoprotein subclasses profile in pregnancy affects fetal growth. The current study extends our previous observation, showing that size at birth might be associated with lipoprotein subclasses distribution in childhood. Our finding of inverse associations of birth weight and length with relative proportions of the largest LDL and HDL particles (Table 3) suggests that regular fetal growth may have a positive influence on lipid profile in childhood, even in the presence of juvenile obesity. According to the "fetal programming" concept, a growth restriction in utero has an important impact on CVD risk in adulthood. The hypothesis has been supported by an inverse association between birth weight and subsequent blood cholesterol concentration (26), although some authors dispute this theory (27). In our obese children, both smaller birth length and height were related to increased proportion of small, dense HDL particles (Table 3). It has been established that obesity is associated not only with increased prevalence of small, dense HDL particles, but also with changes in their composition, which further affects their functionality (28). Bearing in mind that these particles have compromised atheroprotective potential (24), the observed inverse association of smaller birth size and increased smaller HDL could be another piece of the puzzle linking restricted fetal growth with future cardiovascular complications. It should also be stressed that, following systematic analysis of available data, Huxley et al. (27) concluded that the effect of impaired fetal growth on cholesterol level, and consequently on CVD risk, should be considered marginal. This is not surprising giving the fact that plasma cholesterol concentration per se has low predictive value for CVD. Moreover, this conclusion further supports the concept of advanced lipoprotein testing for cardiovascular prevention (29), starting from infancy onward.

Besides newborn size, optimal fetal environment is reflected by Apgar score. A lower Apgar score at birth is an indicator of poorer vitality of the newborn and it has been connected to later developmental disorders and risk of cancer (15). Yet, a link between Apgar score and CVD risk factors in childhood has not been investigated so far. According to our results, childhood obesity is unrelated to Apgar score, since the majority of the study participants had desirable Apgar values (Table 2). However, in our obese children lower Apgar score was also associated with lower birth weight (Table 2), suggesting that this specific scenario might further affect CVD risk. Indeed, this group of obese children was characterized by significant redistribution of LDL subclasses towards small, dense particles (Table 2). A possible explanation of our results could lie in the fact that our obese children with lower Apgar score have comparable present values of anthropometric variables as their higher Apgar score counterparts (Table 2), suggesting that these children experienced accelerated growth. It has been demonstrated that rapid growth during infancy is associated with longer-term problems of obesity (30). Hence, subsequent accelerated weight gain could also be responsible for the observed accumulation of small, dense LDL particles in the group with Apgar < 9. Additionally, our for-the-first time documented inverse association between Apgar score and small, dense LDL particles (Table 3) forms the basis for exploring this link in future investigations.

Nutrition in infancy seems to have important effect on both weight gain and lipoprotein metabolism. There is a consensus that breast milk and prolonged breastfeeding prevent long-term overweight and obesity (31). To the best of our knowledge, this study is the first to describe an impact of breastfeeding duration on lipoprotein subclasses distribution in obese children. Our analysis showed that never or less than 3 months breastfed children have smaller LDL size and less HDL 2 particles than children who were breastfed for 3 months or more (Table 4). The results of the studies investigating the impact of breastfeeding on serum lipid profile are inconclusive. Some authors reported that breastfeeding duration was inversely associated with LDL-C levels (32), while others found no significant association (33). On the other hand, use of nutrient enriched formula, as an alternative or supplement to breastfeeding, was associated with alteration in serum lipid profile of infants (34). In addition, Ravelli et al. (35) reported that adults who were exclusively breastfed had lower LDL-C and apolipoprotein B, as well as lower LDL/HDL ratio than their formula-fed peers. Our present findings extend previous observations (35) and provide additional evidence that consumption of breast milk is an important strategy to prevent CVD development from a young age.

In conclusion, the results of the current study have shown significant associations of neonatal characteristics with LDL and HDL particle distributions in obese children. In particular, we have demonstrated that lower Apgar score (<9) and smaller newborn's size are associated with pro-atherogenic LDL and HDL particles in childhood. In addition, our results point toward positive aspects of longer breastfeeding duration on lipoprotein particle distributions in obese children.

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