

CARDIOVASCULAR RISK FACTORS IN 7–13 YEARS OLD CHILDREN FROM VOJVODINA (SERBIA)

FAKTORI KARDIOVASKULARNOG RIZIKA KOD DECE
UZRASTA 7–13 GODINA IZ VOJVODINE (SRBIJA)

Darko D. Dželajlija¹, Slavica S. Spasić², Jelena M. Kotur-Stevuljević², Nataša B. Bogavac-Stanojević²

¹College of Technical Sciences, Pančevo, Serbia

²Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Summary

Background: Atherosclerosis is a chronic inflammatory disease which starts early in life and depends on many factors, an important one being dyslipoproteinemia. According to several studies, atherosclerotic plaques or their precursors could be seen in children younger than 10 years. During later life, interaction with a sedentary way of life, as well as unhealthy nutrition, smoking, alcohol consumption, obesity and family history of cardiovascular disease cause the burden of atherosclerotic disease.

Methods: Study included 624 children (316 boys, 308 girls), aged from 7–13 years. We analysed socio-demographic data (BMI, blood pressure, cardiovascular family history, smoking status), as well as lipid status with lipoprotein little a–Lp(a), and apolipoproteins: Apo AI, Apo B-100 for all children. This enabled us to calculate new atherogenic indices Tg/HDL-c, lipid tetrad index (LTI) and lipid pentad index (LPI). Cardiovascular risk for later life was estimated by using modified Risk Score for Young Individuals (RS), which divided the subjects according to the score level: low, medium and higher risk.

Results: The older children (13 y) had better lipid status than the younger children, i.e. significantly lower total cholesterol, LDL-C, triglycerides and non-HDL-C concentration and significantly higher HDL-C concentration than the younger children and this was in accordance with the RS level. Children with a positive family history of CV disease had significantly higher Lp(a) concentration and blood pressure. LPI was significantly higher in children with a higher RS.

Conclusions: The results of our work could be used for cardiovascular risk assessment in apparently healthy children

Kratak sadržaj

Uvod: Ateroskleroza je hronična inflamatorna bolest koja počinje rano u detinjstvu i zavisi od mnogo faktora; jedan od najvažnijih je dislipoproteinemija. Razvoj aterosklerotskog plaka počinje pre desete godine života. Interakcija sa sedentarnim načinom života, nezdravom ishranom, pušenjem, unošenjem alkohola, gojaznošću i pozitivnom porodičnom anamnezom za kardiovaskularne bolesti uzrokuje razvoj aterosklerotske bolesti u kasnijem dobu. Cilj ove studije je bio da se kod zdrave dece izmere i procene pomenuti faktori rizika i kvantifikuje rizik za razvoj ateroskleroze kasnije u životu.

Metode: U studiju je uključeno 624 deteta (316 dečaka i 308 devojčica), uzrasta 7–13 godina. Analizirali smo indeks telesne mase – ITM, krvni pritisak, porodičnu anamnezu, status pušenja, osnovni lipidni status sa dodatnim parametrima: koncentracija lipoproteina a–Lp(a) i apolipoproteina: Apo AI, Apo B-100. Računali smo nove aterogene indekse: odnos koncentracija Tg i HDL-c, lipid tetrad (LTI) i lipid pentad indeks (LPI). Rizik za razvoj kardiovaskularnih bolesti u kasnijem životu je procenjen upotrebom Skora rizika za mlade osobe (SR) na osnovu koga su ispitanici podeľjeni na one sa niskim, srednjim i višim rizikom.

Rezultati: Starija deca (13 g.) imaju bolji lipidni status od mlađe dece, tj. niže koncentracije LDL-h, triglicerida i non-HDL-h i više koncentracije HDL-h, što je bilo u skladu sa nivoom SR. Deca sa pozitivnom porodičnom anamnezom imala su više koncentracije Lp(a) i viši krvni pritisak. LPI je bio značajno viši kod dece sa višim SR.

Zaključak: Rezultati ove studije bi se mogli koristiti za procenu kardiovaskularnog rizika kod zdrave dece, da bi se sprečio nastanak nekih od faktora rizika.

Address for correspondence:

Dr. Jelena Kotur-Stevuljević, Assoc. Prof.
Faculty of Pharmacy, University of Belgrade
Department of Medical Biochemistry
Vojvode Stepe 450, Belgrade
Phone: +381 11 3951 272
e-mail: jkotur@pharmacy.bg.ac.rs

to provide preventive measures which could control the changeable risk factors.

Keywords: children, obesity, lipids, family history of CVD, risk score

Introduction

Atherosclerosis is a chronic inflammatory disease which starts early in life (1) and depends on many factors; the most important one is dyslipoproteinemia i.e. lipid metabolism disturbance. According to several studies, atherosclerotic plaques or their precursors could be seen in children younger than 10 years (2–5). During later life, interaction with sedentary lifestyle, coupled with unhealthy nutrition, smoking, alcohol consumption, obesity and family history of cardiovascular disease, causes the burden of atherosclerotic disease (2). Atherosclerosis is a prerequisite for different diseases development: acute myocardial infarction, stroke, peripheral vascular disease and many others. All of the abovementioned diseases are characterized with high mortality rate, and increased rate of patients' disability, promoting atherosclerosis as an important economic and social problem of the modern societies, complex but preventable to a certain extent (3–5).

Increased values of total cholesterol (T-C), triglycerides (TG), LDL-cholesterol (LDL-C), non-HDL-cholesterol (non-HDL-C), and decreased HDL-C values are all together important risk factors for atherosclerotic changes in arteries in children (2). Moreover, increased Apo B and Lp(a) and decreased Apo AI also contribute to atherosclerosis risk (2). Lipid status parameters, as well as Apo AI and Apo B are dietary intervention modifiable parameters, and Lp(a) is an independent risk factor which is genetically determined and nonmodifiable during life (6, 7).

Obesity is also an independent risk factor for the development of dyslipidemia, hypertension, and thus cardiovascular disease in later life (8–10). According to the data of the Institute of Public Health of Serbia (11), people from Vojvodina (northern part of Serbia) have the highest acute coronary syndrome mortality rates per 100.000 subjects.

The aim of the present study was to explore cardiovascular risk in apparently healthy children regarding their general lipid status, Lp(a), apolipoproteins AI and B-100 concentration, obesity and cardiovascular related family history.

Materials and Methods

This study included 624 elementary school children (316 boys, 308 girls), divided in three groups according to their age: 7 years (190 subjects), 10

Ključne reči: deca, gojaznost, lipidi, pozitivna porodična anamneza kardiovaskularnih bolesti, skor rizika

years (212 subjects) and 13 years (222 subjects). The study group was selected in South Banat (Vojvodina, Republic of Serbia) during regular medical check-up. All the children and parents read and signed their informed consent and all procedures were planned according to the ethical guidelines of Helsinki Declaration. The institutional ethical and review committee of the University of Belgrade Faculty of Pharmacy approved our study protocol. Data about family history regarding acute myocardial infarction and/or stroke were collected from parents by the interviewer during the same medical appointment.

Venous blood was drawn from the antecubital vein into evacuated, serum separator tubes, after 12 hours of overnight fasting. T-C and TG were assayed immediately by routine enzymatic methods using an Artax analyzer (Menarini, Florence, Italy) and Menarini reagents (Florence, Italy). HDL-C was isolated by using the precipitating method with phosphotungstic acid in the presence of Mg^{2+} ions. LDL-C concentration was calculated using the Friedewald formula (12).

Serum aliquots were preserved at $-80^{\circ}C$ until analysis of apolipoproteins concentration (Apo AI and Apo B-100), and lipoprotein little a – Lp(a). Apo AI, Apo B and Lp (a) were assayed by routine immunoturbidimetric methods using the nephelometric analyser BN proSpec and spectrophotometric analyser Rxl Max. The instruments were purchased from the same manufacturer, DADA Behring (Margburg, Germany) with DADA Behring reagents (Margburg, Germany).

Non-HDL concentration is calculated as the difference between T-C and HDL-C, and atherogenic index as the ratio between TG and HDL-C concentrations. New atherogenic indices which involved several lipid parameters were calculated as lipid tetrad index (LTI) and lipid pentad index (LPI). LTI was calculated using the formula $[Tc \times Tg \times Lp(a)] / HDL-c$, and LPI was calculated using the formula: $[Tc \times Tg \times Lp(a) \times apoB] / apoA-I$ (13).

Body mass was measured using a portable electronic scale (Tanita, Amsterdam, Netherlands) to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a portable wall-mounted stadiometer. Waist (just above the iliac crest) and hip (the widest part of the hips at the middle of the pelvis) circumferences were measured to the nearest 0.1 cm. Body mass index was calculated as body weight (kg) divided by height squared (m). Overweight was defined as a BMI $\geq 85^{th}$ – $< 95^{th}$ percentile and obesity was defined as a BMI $\geq 95^{th}$ percentile for children of the

same age and sex. Blood pressure was measured by a mercury sphygmomanometer (Quicksilver-sphygmomanometer Riester, Jungingen, Germany).

Limits for prehypertension and hypertension status are defined according to the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (13).

The risk for CVD was estimated using LTI and LPI (14) and modified Risk Score for identifying young individuals with a high probability of having advanced atherosclerotic lesions, reported by McMahan et al. (15). According to risk score values, we have additionally divided the children into Low risk group (score lower or equal to 4 points), Medium risk group (5–8 points) and Higher risk group (above 8 points).

Statistical Methods

Parameters with a normal, Gaussian type of distribution are presented as means and standard deviations, but triglycerides, Lp(a), LTI, LPI deviated from normal distribution and are presented as geometrical mean and 95% confidence interval. For inter-group comparison, we have used ANOVA or Kruskal-Wallis

test, with the distinct *post hoc* test where appropriate and chi-square test for frequency data comparison. ROC analysis was used to test the discriminatory capability of the measured and/or calculated parameters regarding low and higher risk status towards cardiovascular disease.

Results

We have compared basic lipid status parameters and parameters connected with lipid metabolism in children divided in groups according to their age, by using ANOVA and *post hoc* Tukey test for data with Gaussian distribution. Data which deviated from Gaussian distribution were compared with nonparametric methods (Kruskal-Wallis ANOVA followed by Mann-Whitney U test). The results are presented in Table I.

The older children (13 y.) had better lipid status than younger children, i.e. significantly lower total cholesterol, LDL-C, triglycerides and non-HDL-C concentrations and significantly higher HDL-C concentrations than the younger children. Moreover, 13 y. old children had lower Lp(a), and higher Apo AI than the two younger groups. The oldest children also had the

Table I Basic anthropometric, lipid status, and inflammation parameters in healthy children according to the age subgroups.

Parameter	Age subgroups			P
	7 y. (n=190)	10 y. (n=212)	13 y. (N=222)	
Sex, boy/girl, n (%)	99/91 (52/48)	101/111 (48/52)	116/106 (52/48)	$\chi^2=1.15$, P=0.560
BMI, kg/m ²	19.97±3.10	21.77±3.89 ^{aaa}	19.84±3.51 ^{bbb}	<0.001
Systolic BP, mm Hg	110.53±10.38	114.03±13.77 ^a	111.02±13.46	<0.01
Diastolic BP, mm Hg	72.24±10.50	75.78±12.00 ^a	72.64±11.79 ^b	<0.003
Positive family history for CVD, n (%)	10 (5.3%)	17 (8%)	25 (11%)	$\chi^2=4.86$, P=0.088
Glucose, mmol/L	4.62±0.53	4.68±0.47	4.70±0.53	0,158
TC, mmol/L	4.56±0.79	4.58±0.71	4.12±0.66 ^{aaa,bbb}	<0.001
TG, mmol/L [#]	1.19 (1.12–1.27)	0.94 (0.89–0.99) ^{aaa}	0.87 (0.82–0.91) ^{aaa}	<0.001
LDL-C, mmol/L	2.44±0.93	2.69±0.70 ^{aa}	2.14±0.82 ^{aa,bbb}	<0.001
HDL-C, mmol/L	1.51±0.43	1.43±0.34	1.58±0.61 ^{bb}	<0.003
Non-HDL-C, mmol/L	3.04±0.88	3.16±0.74	2.53±0.75 ^{aaa,bbb}	<0.001
Apo AI, g/L	1.34±0.38	1.37±0.29	1.45±0.40 ^{aa, b}	0.003
Apo B-100, g/L	0.80±0.32	0.91±0.28 ^{aaa}	0.80±0.31 ^{bbb}	<0.001
Lp(a), mg/L [#]	222 (204–242)	171 (157–187) ^{aaa}	168 (143–197) ^{aaa}	<0.001
TG/HDL-C [#]	0.838 (0.555–1.203)	0.639 (0.480–0.876) ^{aaa}	0.538 (0.409–0.868) ^{aaa,bb}	<0.001
LTI [#]	722 (450–1695)	474 (271–757) ^{aaa}	280 (199–716) ^{aaa}	<0.001
LPI [#]	695 (373–1308)	417 (244–790) ^{aaa}	235 (126–600) ^{aaa,bbb}	<0.001
Fibrinogen, g/L	3.1 (2.6–3.6)	3.0 (2.6–3.5)	3.0 (2.7–3.3)	0.220

a, ^{aaa}P<0.05, 0.001, compared to 7 y. group; b,^{bbb}P<0.05, 0.001 compared to 10 y. group

– parameters with non-Gaussian distribution presented as 50th percentile (25th – 75th percentile); compared by nonparametric Kruskal-Wallis ANOVA and subsequent Mann-Whitney U test

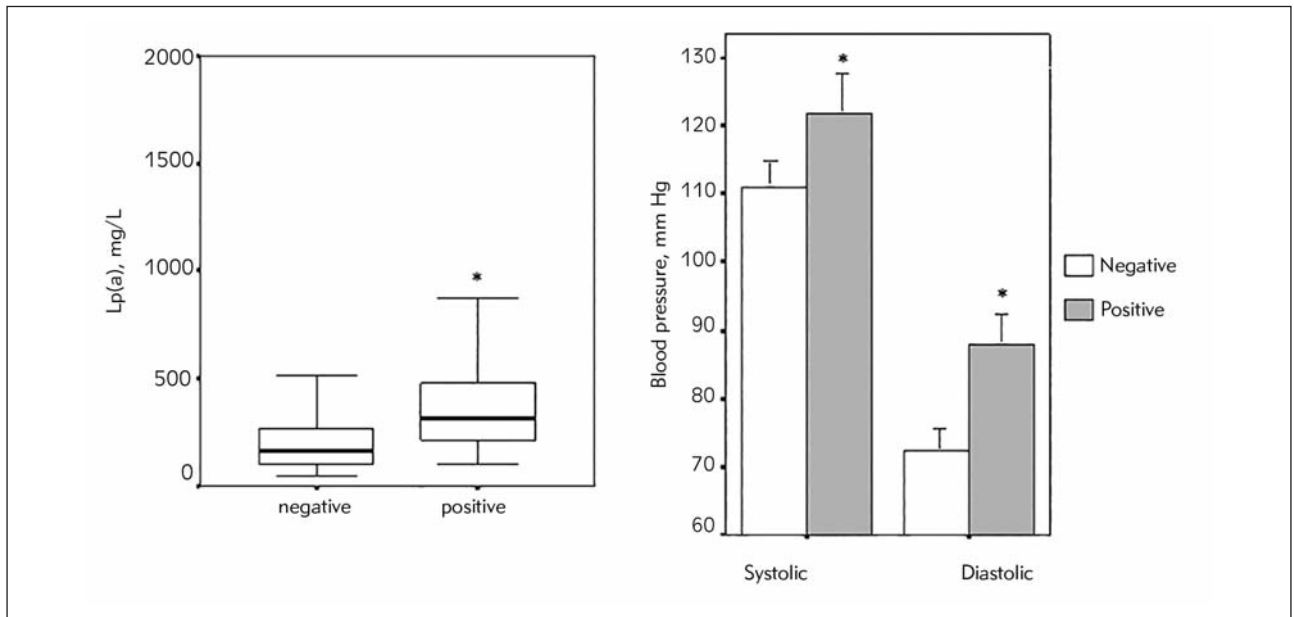


Figure 1 Differences in Lp(a) concentration and blood pressure values in two categories related with presence of family history of cardiovascular disease in apparently healthy children.

* $P < 0.05$ vs. Negative family history children (Lp(a) compared by Mann-Whitney U test; blood pressure compared with Student's t test)

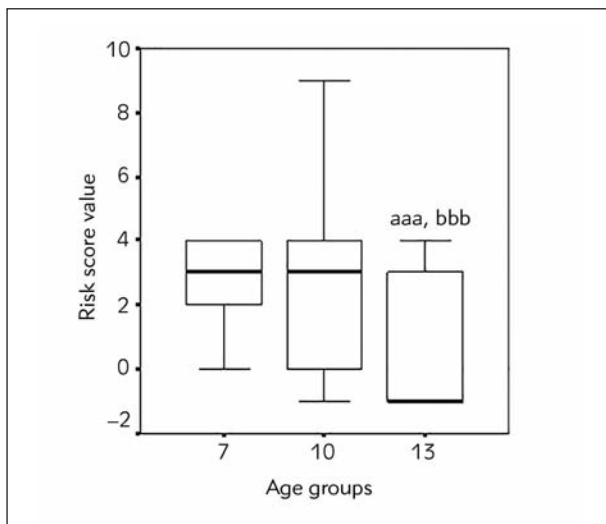


Figure 2 Risk score value in different age groups. aaa, bbb $P < 0.001$ vs. 7 y. and 13 y. respectively according to Kruskal-Wallis and then Mann-Whitney U test

lowest LTI and LPI risk scores values. The number of children with positive family history for coronary heart disease increased with age, but the difference was not statistically significant. Afterward, we divided the children according to their family history for CV disease status and compared the other measured parameters. Our results showed that children with a positive family history of CV disease had significantly higher Lp(a) concentration, and blood pressure (Figure 1).

In order to estimate possible risk for cardiovascular disease development in apparently healthy children, we used the Risk Score Calculator proposed by McMahan et al. (15). This calculator uses non-HDL-C, HDL-C, smoking status, hypertension, hyperglycemia, and sex parameters for the risk score calculation.

Figure 2 shows risk score values in the age subgroups. Younger age groups of children have significantly higher risk score values compared to 13 y. old children (data are presented in Figure 2 as box plots of median values and interquartile range).

Then, we divided the group of healthy children into three subgroups stratified by the calculated risk score level as Low, Medium and Higher risk groups. The results of this part of analysis are shown in Table II. Unexpectedly, the youngest children had the highest level of risk for CVD development in later life when compared to the older children subgroups. These children had significantly higher BMI, and worse lipid status than the children with lower risk and also higher blood pressure. The number of positive family history for CVD children did not differ among the risk level subgroups. Regarding LTI and LPI values, children with higher risk score for CVD value had the latter index significantly higher compared to the same parameter in both lower risk groups (Figure 3).

We also wanted to test the lipid status, inflammation and BMI parameters' capability to discriminate between Low Risk and Higher Risk status, so we performed the receiver operating characteristics curve (ROC) analysis. The parameters analysed were those which

Table II Biochemical parameters in healthy children (7–13 y.) according to risk score level subgroups.

Parameter Points (number of children)	Risk score value			P
	Low ≤ 4 (506)	Medium 5–7 (92)	Higher ≥ 8 (26)	
Age	10.4±2.46	9.4±2.06 ^{aa}	8.5±1.94 ^{aaa}	<0.001
BMI, kg/m ²	20.3±3.39	21.1±4.43	22.6±4.19 ^{aa}	<0.01
TC, mmol/L	4.25±0.661	4.90±0.683 ^{aaa}	5.78±0.350 ^{aaa, bbb}	<0.001
TG, mmol/L	1.06±0.503	1.08±0.461	1.52±0.881 ^{aaa, bbb}	<0.001
LDL-C, mmol/L	2.21±0.739	3.15±0.624 ^{aaa}	3.91±0.497 ^{aaa, bbb}	<0.001
TG/HDL [#]	0.615 (0.445–0.909)	0.811(0.580–1.153)	1.194 (0.714–1.536) ^a	0.011
Lp(a), mg/L [#]	179 (100–280)	170 (100–270)	240 (146–382)	ns
Apo AI, g/L	1.40±0.356	1.34±0.362	1.44±0.363	ns
Apo B-100, g/L	0.829±0.289	0.860±0.365	0.889±0.400	ns
Fibrinogen, g/L	3.04±0.551	3.00±0.589	3.02±0.550	ns
Systolic BP, mm Hg	110±12	117±12 ^{aaa}	123±12 ^{aaa}	<0.001
Diastolic BP, mm Hg	72±11	78±12 ^{aaa}	82±16 ^{aaa}	<0.001
Risk score value (points) [#]	2.0 (–1.0–3.0)	6.0 (5.0–6.0) ^{aaa}	8.0 (8.0–9.0) ^{aaa, bbb}	<0.001
Positive family history for CVD n (%)	41 (8%)	8 (9%)	3 (12%)	$\chi^2=0.4$, P=0.818

^{a,aa,aaa}P<0.05, 0.01, 0.001, compared to Low Risk group; ^{bbb}P< 0.001 compared to Medium Risk group with ANOVA and Tukey test
ns-non-significant; # – parameters with non-Gaussian distribution presented as 50th (25th – 75th percentile); compared by non-parametric Kruskal-Wallis ANOVA and subsequent Mann-Whitney U test

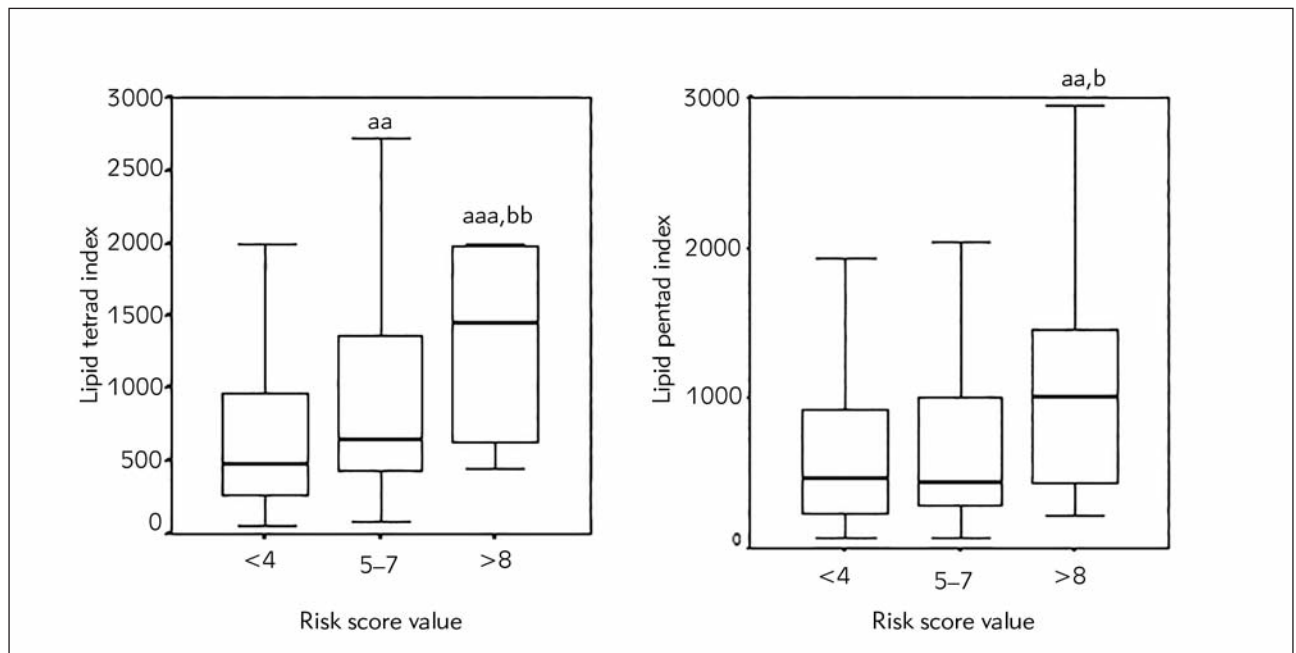


Figure 3 Lipid tetrad (LTI) and lipid pentad index (LPI) according to risk score values subgroups.
(^{aa}P<0.01, ^{aaa}P<0.001 vs. Low risk group, ^bP<0.05, ^{bb}P<0.01 vs. Medium risk group)

were not used for Risk Score calculation. The results of this analysis are shown in *Table III* and *Figure 4*.

Total cholesterol and LDL-C had the best discriminatory potential, which was not unexpected because non-HDL cholesterol was used for the Risk Score calculation. LPI and BMI had significantly lower AUCs and thus lower discriminating power, but still the AUCs were significantly higher than 0.5.

Table III The results of ROC analysis for discriminating two risk levels i.e. risk score below 4 and risk score ≥ 8 .

Variable	AreaC	95% Confidence Interval	Std. Error
TC	0.969***	0.949–0.989	0.010
LDL-C	0.968***	0.941–0.995	0.014
Tg	0.663**	0.551–0.776	0.057
Lp(a)	0.601	0.495–0.707	0.054
Apo AI	0.566	0.452–0.679	0.058
Apo B	0.469	0.352–0.587	0.060
LTI	0.562	0.447–0.677	0.059
LPI	0.680**	0.584–0.776	0.049
BMI	0.662**	0.544–0.780	0.060
Fibrinogen	0.499	0.378–0.620	0.062

** , *** $P < 0.01$ and 0.001 respectively for AUC values compared to 0.5

Discussion

Many studies, among them YUSAD study (16) and Bogalusa study (17, 18) indicate a close relationship between lipid and nonlipid atherogenic risk factors in early childhood and atherosclerosis development in later life. Identification of the risk factors and their limitation lead to significant decrease of the possibility of cardiovascular disease development (19).

BMI values among the age groups increased with age when we compared the 7 y. and 10 y. subgroups (19.97 ± 3.10 kg/m² vs. 21.77 ± 3.89 kg/m², $P < 0.001$) and decreases in 13 y subgroup (19.84 ± 3.51 , $P < 0.001$ compared to 10 y. subgroup). This BMI changing with the children's development is the same as in Ninic et al. (20), who assessed 7–15 y. old children from Vojvodina. Systolic and diastolic pressure values are comparable with Lurbe et al. data (21) for healthy children and adolescents aged 1–17 y. There was no difference in blood pressure values among different age groups (*Table I*).

Triglycerides, non-HDL-C and Tg/HDL-C ratio (*Table I*) are higher than Ninic et al. (20) reported. Older children in our current study had better lipid status than the younger ones; significantly lower total cholesterol values, LDL-C, triglycerides, non-HDL-C and Lp(a), and also significantly higher HDL-C and Apo-AI. Lipid parameter values in children from this study are within the recommended boundaries given by Serbian National Guide for Good Clinical Practice (22–24). According to this recommendation for lipid disorder diagnosis and treatment, for children, borderline LDL-C concentrations are considered those higher than 2.85 mmol/L and high are above 3.36 mmol/L; borderline high total cholesterol value is above 4.40 mmol/L and T-C values higher than 5.17 mmol/L are considered high. Schulps et al. (25, 26) have investigated lipid parameters' distribution in

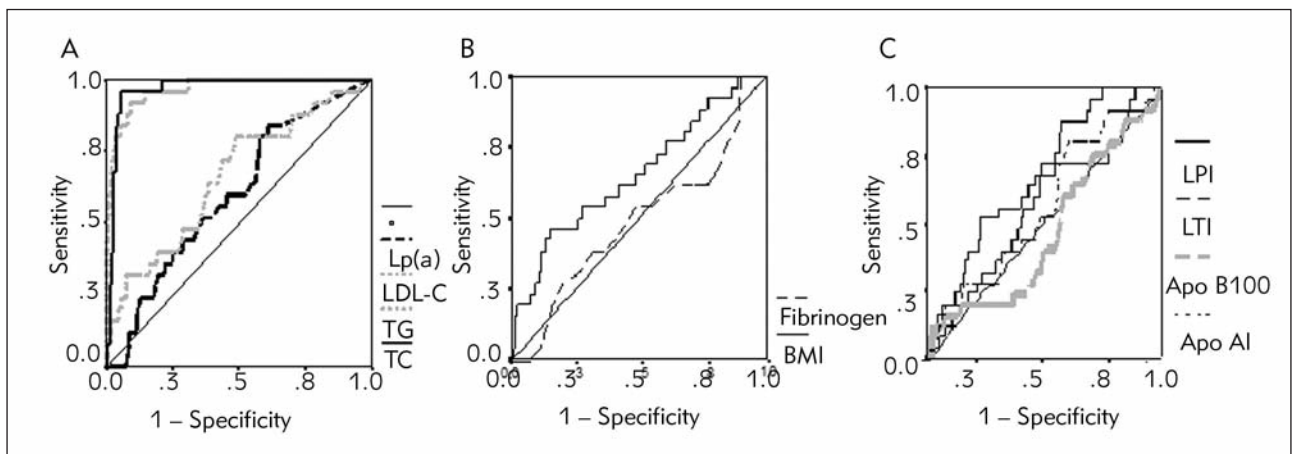


Figure 4 ROC curves for lipids, lipoproteins, apolipoproteins, BMI and fibrinogen and their ratios as markers of risk of cardiovascular disease development in later life. Panel A) HDL-c, TC, LDL-C, TG, Lp(a); panel B) BMI, fibrinogen; panel C) Apo AI, Apo B-100, LTI and LPI.

Greek children and their results showed slightly higher levels of serum HDL-C and lower levels of TG, LDL-C and Lp(a) than in our children of similar age. Better lipid status of the Greek schoolchildren could be explained with the genetic factors and by the nutritional habits of the Greek population, regarding consumption of olive oil and other components of the Mediterranean diet. This finding compared to our results could imply that healthier nutritional habits could be protective concerning the risk for CVD development in later life.

LTI and LPI indices are calculated in order to assess more precisely the cardiovascular risk coming from a detailed lipid status. It is evident that older children had lower LTI and LPI compared to younger children (Table I). The LTI and LPI indices have recently been described as a new form of assessment of lipid profiles, which have been analysed in some populations (27). A characteristic of these new indices is the broad approach to atherogenic and nonatherogenic lipid particles, resulting in a single value. Based on the conventional lipid profile and the emerging risk factors such as Lp(a), apolipoproteins AI and B-100, the LTI and LPI appear as models in global risk assessment, reflecting the multifactorial nature of CVD. Bogalusa (17) and YUSAD (16) studies analyzed the relation between lipid status and age of children. The YUSAD study (16) demonstrated that over a 5 and 10-year period (10 y. children compared to 15 y. and 20 y. children), BMI and waist circumference values showed significant increase. Such an observation is in correlation with the physiological changes (growth) during this period. However, the proportion of the waist circumference increase was higher in girls between 10 and 15 years of age than between 15 and 19–20 years of age, while there was less discrepancy in the difference between the proportions of the two 5-year age groups in boys' population. Moreover, the authors of the Bogalusa study (17, 18) showed a connection between obesity and lipid status, and suggested a relation between high insulin concentration and cardiovascular risk. Children from our study were all normoglycemic, so we supposed that their insulin concentrations were among reference values (28), and did not contribute to increased CV risk.

BMI and lipid status values are comparable with values reported by Costa et al. (29), who found a negative correlation between BMI and HDL-C values, and a positive correlation between BMI and triglyceride concentration in a group of children younger than 12 y. Dai et al. (30) and Jolliffe and Janssen (31) have similar results and have commented about lipid status dependence on age. The age and gender specific threshold values reflect the natural fluctuation in lipoprotein concentration that occurs with growth and maturation. Difference in lipid status between 13 y old children and the younger ones could be explained by their puberty status and the intensive growth period of older children. Namely, the metabolism of

puberty period children is devoted to rapid growth and protein synthesis (16, 21, 32). The decrease in serum lipids levels during puberty is also related to an increase in the plasma testosterone concentration in boys or estradiol concentration in girls (32).

We included 624 children in total, among them 52 (8.3%) with a positive family history regarding cardiovascular disease (presence of hypertension, acute myocardial infarction or stroke in first or second line relatives). As expected, the number of positive family history cases increased with increasing age (5.3%, vs. 8.0% vs. 11%, with a marginally statistically significant difference $P=0.088$), because in younger children relatives are also younger and with smaller chances of having some unwanted cardiovascular related condition. Our results showed significantly higher Lp(a) and blood pressure values in children with positive family history compared to children without family history of CVD (Figure 1). Still, Lp(a) values in our group of children with CVD family history were lower than in Italian children of similar age who also had CVD family history (19), but higher compared with healthy 14 y. old Swedish children from Bergström's study (22). It is well-known that Lp(a) is an independent CV risk factor (6, 7) and increased values of this parameter could predict early cardiovascular disturbance development, even if other risk factors are within a tolerable range (18). On the contrary, Guardamagna et al. (19) considered that the mutual involvement of increased Lp(a) values with positive family history was directly connected with increased risk for cardiovascular development. Similar findings have been presented by others (29, 31), regarding positive correlation between increased Lp(a) values and LDL-c concentrations, as well as Lp(a) and total cholesterol values and Apo B-100 (19), especially in children with positive family history. According to our results, significantly higher Lp(a) concentration and blood pressure values reflected additional risk factor existence such as positive family history of CVD.

In order to assess the real cardiovascular risk level, we implemented the scale for Cardiovascular Risk Score (CRS) calculation from McMahan's study (15). That study correlated the cumulative effects of modifiable risk factors (non-HDL-cholesterol, HDL-cholesterol, smoking, hypertension, obesity and hyperglycemia) and the level of atherosclerotic deposits on coronary arteries. Scoring system given by McMahan et al. (15) gave the ratio between RS level and probabilities of having significant stenosis on coronary arteries, where score 10 gives the probability of 10%, score 20 – 25% and score 30 – almost 60% for having significant atherosclerotic lesions. In our current study, apparently healthy children aged 7–13 y. had RS levels from –1 to 11, which means that at the moment, children had about 10% probability of having significant atherosclerosis on coronary arteries. However, in order to grade the risk level, we divided the children according to tertile values for this group; i.e. Low CRS

≤ 4 (N=506), Medium CRS 5–7 (N=92) and Higher CRS ≥ 8 (N=26) (Table II). Along with better lipid status, older children had significantly lower CRS values than younger children (Figure 2). Nonetheless, children with the highest CRS values had also significantly higher values of total cholesterol, triglycerides, LDL-c, Tg/HDL-c ratio, Lp(a) concentration and systolic and diastolic blood pressure (Table II). These findings are in accord with other studies (32–35) which assessed risk factors in adolescents or young adults and then measured carotid artery intima-media thickness (IMT) in the same individuals, 12 to 22 years later. In all of these studies, one or more of the risk factors were associated with the carotid IMT of individuals. The study of Bild et al. (36) found a similar association between coronary arteries calcification and risk factors measured 10 years earlier. Oren et al. (37) found strong associations between current risk factors and carotid IMT in young adults. LTI and LPI values are also higher in higher CRS values groups, and this finding is in agreement with Morais, McMahan and Das' work (14, 15, 27). These findings reinforce the idea that the conventional lipid profile in many cases may not offer an actual estimation of coronary risk. When this profile is under reference limits, it does not necessarily rule out the risk due to specific forms of dyslipidemia. Consequently, the introduction of laboratory tests related to an unconventional lipid profile would be extremely desirable in search of the real coronary risk estimate, as an addition to all the other traditionally investigated risk factors.

We wanted to estimate which of the measured parameters could predict CRS value, so we implemented a ROC analysis. The result of ROC analysis showed that, besides total cholesterol and LDL-C which are directly connected with non-HDL-C, as one of the points for the CRS calculation, LPI and BMI had

good predictive potential (Table III, Figure 4). This suggested that the values of the comprehensive parameter LPI, which included several lipid parameters, and which is the most important Lp(a), could be a reliable sign of growing cardiovascular risk. Our results are in agreement with Bogavac-Stanojevic et al. study (38). We have considered only lipid, anthropometric and family history related risk, as some kind of traditional cardiovascular risk factors. Karikas et al. (39) proposed assessment of several other new, emerging risk factors in early age. Those parameters were homocysteine and paraoxonase activity, as already established cardiovascular risk factors in the adult population. According to this, the additional work in atherosclerosis related risk factors recognition in children is certainly warranted in the future.

We believe that the results of our current work could be used by clinicians in order to arrange preventive measures to control changeable risk factors in children.

Acknowledgements

This work was financially supported by grants from the Serbian Ministry of Education, Science and Technological Development (Project number 175035). The study funder had no role in study design, in the collection, analysis, and interpretation of data; nor in the writing of the report and the decision to submit the manuscript for publication.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

- Mellies M, Glueck CJ. Lipids and the development of atherosclerosis in schoolchildren. *J Paediatr Gastroenterol Nutr* 1983; 2: 5298–03.
- da Luz GM, Villela Barocini LA, Champoski AF, Carla A, Biazon B. Household cardiovascular screening in adolescents from high-risk families. *Atherosclerosis* 2013; 226 (1): 286–90.
- Chavers BM, Li S, Collins AJ, Herzog CA. Cardiovascular disease in paediatric chronic dialysis patients. *Kidney Int* 2002; 62: 648–53.
- Litwin M, Grenda R, Prokurat S, Abuauba M, Latoszyńska J, Jobs K, et al. Survival and causes of death in children on haemodialysis and continuous ambulatory peritoneal dialysis. *Pediatr Nephrol* 2001; 16: 996–01.
- McDonald SP, Craig JC. Long-term survival of children with end stage renal disease. *N Engl J Med* 2004; 350: 2654–62.
- Sandkamp M, Kunke H. Lipoprotein (a) is an independent risk factor for myocardial infarction in young age. *Clin Chem* 1990; 36: 20–3.
- Krempler F, Kostner G, Bolzano K, Sandhofer F. Lipoprotein (a) is not a metabolic product of other lipoproteins constraining apolipoprotein B. *Biochim Biophys Acta* 1979; 575: 63–70.
- Must A, Strauss RS. Risk and consequences of childhood and adolescent obesity. *Int J Obes Relat Metab Disord* 1999; 23: 2–11.
- Kocova M, Sukarova-Angelovska E, Tanaskoska M, Palcevska-Kocevska S, Krstevska M. Metabolic setup and risks in obese children. *J Med Biochem* 2015; 34: 31–7.
- Zdravković V, Sajić S, Mitrović J, Stefanović I, Pavićević P, Nikolić D, Dimić J, Lalić MN. The diagnosis of prediabetes in adolescents. *J Med Biochem* 2015; 34: 38–45.

11. Incidence and mortality of acute coronary syndrome in Serbia, Institute of Public Health of Serbia »Dr Milan Jovanović Batut« Belgrade 2013.
12. Friedewald WT, Levy RI, Fredricson DS estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499–507.
13. Age specific recommendations for blood pressure (BP) measurement and diagnosis of Hypertension 27–37. In: Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents. US Department of Health and Human Services, National Health, Lung and Blood Institute. 12-7486A October 2012.
14. Morais CAS, Oliviera SHV, Lima LM. Lipid tetrad index (LTI) and lipid pentad index (LPI) in healthy subjects. *Arg Bras Cardiol* 2013; 100: 322–7.
15. McMahan CA, Gidding SS, Fayad ZA, Zieske AW, Malcom GT, Tracy RE, et al. Risk scores predict atherosclerotic lesions in young people. *Arch Intern Med* 2005; 165: 883–90.
16. Simeunović S, Nedeljković S, Milinčić Z, Vukotić M, Novaković I, Nikolić D, et al. Anthropometric and lipid parameters trends in school children: one decade of YUSAD Study. *Srp Arh Celok Lek* 2011; 139: 465–9.
17. Berenson GS, Srinivasan SR, Bao W, Newman WR, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults: Bogalusa Heart Study. *N Engl J Med* 1998; 338: 1650–56.
18. Freedman DS, Serdula MK, Srinivasan SR, Berenson GS. Relation of circumferences and skinfold thicknesses to lipid and insulin concentrations in children and adolescents: the Bogalusa Hearth study. *Am J Clin Nutr* 1999; 69: 308–17.
19. Guardamagna O, Abello F, Anfossi G, Piro M. Lipoprotein (a) and family history of cardiovascular disease in children with family dyslipidemias. *J Pediatr* 2011; 159: 314–9.
20. Ninić A, Spasojević-Kalimanovska V, Kotur- Stevuljević J, Bogavac-Stanojević N, Stefanović A, Jelić-Ivanović Z, et al. Associations between anthropometric parameters and serum lipids in preadolescent and adolescent girls and boys. *Clin Lipidol* 2015; 10: 2.
21. Lurbe E, Cifkova R, Cruickshank K. Management of high blood pressure in children and adolescents: recommendations of the European Society of Hypertension. *J Hypertens* 2009; 27: 1719–42.
22. Karbasy K, Ariadne P, Gaglione S, Nieuwesteeg M, Adeli K. Advances in Pediatric Reference Intervals for Biochemical Markers: Establishment of the CALIPER Database in Healthy Children and Adolescents. *J Med Biochem* 2015; 34: 23–30.
23. National Cholesterol Education Program (NCEP): highlights of the report of the expert panel on blood cholesterol levels in children and adolescents. *Pediatrics* 1992; 89: 495–501.
24. Milašinović G. Lipids disorder in: National guide of good clinical practice for lipid disorder diagnosis and treatment. Agency for accreditation of health care institutions in Serbia, Belgrade, Serbia 2012; 18, www.zdravlje.gov.
25. Schulpis KH, Karikas GA, Gavrili S, Georgala S. Evaluation of serum lipoprotein (a) levels in Greek schoolchildren. *Acta Paediatr* 2001; 90: 225–6.
26. Schulpis K, Karikas GA. Serum cholesterol and triglyceride distribution in 7767 school-aged Greek children. *Pediatrics* 1998; 101: 5.
27. Das B, Daga MK, Gupta SK. Lipid pentad index: a novel bioindex for evaluation of lipid risk factors for atherosclerosis in young adolescents and children of premature coronary artery disease patients in India. *Clin Biochem* 2007; 40: 18–24.
28. Blake DR, Meigs JB, Muller DC, Najjar SS, Andres R, Nathan DM. Impaired glucose tolerance, but not impaired fasting glucose, is associated with increased level of coronary heart disease risk factors. Results from the Baltimore Longitudinal Study on Aging. *Diabetes* 2004; 53: 2095–2100.
29. Costa GB, Horta N, Resende ZF. Body mass index has a good correlation with proatherosclerotic profile in children and adolescents. *Arg Cardiol* 2009; 93: 261–7.
30. Dai S, Fulton JE, Harrist RB, Grunbaum JA, Steffen LM, Labrthe DR. Blood lipids in children: age-related patterns and association with body-fat incidences: Project heart-beat! *Am J Prev Med* 2009 (1); 37: 56–64.
31. Jolliffe CJ, Jansen I. Distribution of lipoproteins by age and gender in adolescents. *Circulation* 2006; 114: 1056–62.
32. Stožický F, Slaby P, Volenikova L. Longitudinal study of serum cholesterol, apolipoproteins and sex hormones during puberty. *Acta Paediatr Scand* 1991; 80: 1139–44.
33. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intima-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study. *Circulation* 2001; 104: 2815–19.
34. Li S, Chen W, Srinivasan SR. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMMA* 2003; 290: 2271–6.
35. Raitakari OT, Jounala M, Kahonen M. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular in Young Finns Study. *JAMMA* 2003; 290: 2277–83.
36. Bild DE, Folsom AR, Lowe LP. Prevalence and correlates of coronary calcification in black and white young adults: the coronary artery risk development in young adults (CARDIA) Study. *Arterioscler Thromb Vasc Biol* 2001; 21: 852–7.
37. Oren A, Vos LE, Uiterwaal CS, Grobbee, DE, Bots ML. Cardiovascular risk factors and increased carotid intima-media thickness in healthy young adults: the atherosclerosis risk in young adults (ARYA) study. *Arch Intern Med* 2003; 163: 1787–92.
38. Bogavac-Stanojević N, Jelić-Ivanović Z, Spasojević-Kalimanovska V, Spasić S, Kalimanovska-Oštrić D. Lipid and inflammatory markers for the prediction of coronary artery disease: A multi-marker approach. *Clin Biochem* 2007; 40: 1000–06.
39. Karikas GA, Kriebardis A, Samara I, Schulpis K, Papachristodoulou K, Fytou-Pallikari A. Serum homocysteine levels and paraoxonase 1 activity in preschool aged children in Greece. *Clin Chem Lab Med* 2006; 44 (5): 623–7.

Received: November 26, 2015

Accepted: December 24, 2015