

Lipoprotein (a) Concentrations in School Children and Adolescents in Croatia

D. Vrhovski-Hebrang, Z. Flegar-Meštrić, T. Bobetić-Vranić and B. Šurina

ABSTRACT

Lipoprotein (a) concentrations in sera were determined on 536 healthy reference children and adolescents aged 8-19 years from Zagreb, Croatia. The frequency distribution showed that 20.4% boys and girls had lipoprotein (a) concentrations above 0.3 g/L what is considered as a cut-off value for the increased risk of early atherosclerosis. The results of correlation studies and factor analysis support the concept that the concentrations of lipoprotein (a) could be an independent risk factor and therefore may have a great value in the prediction of atherosclerosis early in life.

Introduction

Elevated plasma concentrations of lipoprotein (a) (Lp(a)) are associated with an increased incidence of coronary artery disease, unrelated to the rest of the lipoproteins. The serum concentration of Lp(a) may be genetically determined¹ and remain constant even after metabolic perturbations that markedly change the amounts of the other lipoproteins present². Therefore, plasma Lp(a) is considered as an independent risk factor of coronary artery disease¹⁻³. The initial stages of atherosclerosis are strongly related to childhood concentrations of serum lipids and lipoproteins⁴. A number of epidemiological studies in children popu-

lations (French⁴, Korean⁵, Italian⁶, Belgian⁷, Spanish^{8,9}, Japanese¹⁰, German¹¹, American¹², Israeli¹³) have shown that when present in high levels in the plasma, Lp (a) is recognized as a marker for premature development of coronary artery disease. A concentration of Lp(a) greater than 0,30 g/L in white populations is considered to double the risk of developing coronary artery disease^{14,15}. There are no such data for pediatric population in our country.

As there are a wide interindividual¹⁶⁻¹⁸ and intraindividual¹⁷⁻¹⁹ variability in plasma concentration of Lp(a), and considering that reference values well differentiated according to age and sex, are a prerequisite for early detection of car-

diovascular risk, the aim of our study was to determine the distribution of Lp(a) concentrations in the healthy reference population of school children and adolescents from Zagreb, Croatia and to evaluate the frequency of increased Lp(a) concentrations considered to be of risk for premature development of coronary artery disease.

Patients and Methods

Lipoprotein (a) concentrations were determined in 269 boys and 265 girls, aged 8–19 years. This group of children was the part of the previously selected group of 998 reference children, according to the IFCC recommendations^{20–22}, from the territory of Zagreb, Croatia²³.

The venous blood samples have been collected from the children according to the standardised procedure recommended by the Scandinavian Committee²². Lipid and lipoprotein constituents of sera were determined on fresh sera, on the day of blood collection. Sera for determination of Lp(a) concentrations were frozen at -70°C , analysed within six months and thawed just before the analysis, as the storage of serum at this conditions did not change the apolipoprotein and lipoprotein concentrations^{17,24}. Lp(a) was quantified with an enzyme-linked immunosorbent assay (ELISA) plaque technique (Apo-Tek Lp(a)TM, PerImmune Inc. USA). The method employs an immobilized murine monoclonal antibody that is specific for Apo(a) and a horseradish peroxidase-labeled polyclonal antibody to Apo B (Conjugate). This assay design facilitates accurate quantitation of Lp(a) in serum regardless of isoform^{25,26}. One lot of reagents was used throughout the study to obtain the consistency of results. The limit of detection for Lp(a) was 0,01 g/L. Samples were assayed in duplicates. Imprecision was assessed by using the control sera for normal and pathologi-

cal concentrations (PerImmune Inc. USA). Coefficient of variation was 7.6% and 4.7% respectively.

Standard automated enzymatic methods were applied to measure total cholesterol (TC) and triacylglycerols (TG), (CHOD-PAP and GPO-PAP methods, Boehringer-Mannheim, Germany). HDL cholesterol (HDL-C) was determined enzymatically after precipitation of the sera samples with freshly prepared magnesium chloride-phosphotungstic acid reagent (quantitative precipitation of chylomicrons, VLDL cholesterol, LDL cholesterol (LDL-C) and Lp(a)) and subsequent determination of cholesterol in the supernatant after centrifugation. LDL cholesterol was calculated using Friedewald-Fredrikson formula: $\text{LDL-C} = \text{TC} - (\text{TG}/2,2 + \text{HDL-C})$. Because of that this value represents the cholesterol contained in both LDL and Lp (a) lipoproteins²⁷. In addition, lipid ratios were calculated as the ratio of LDL cholesterol and HDL cholesterol and the ratio of total cholesterol and HDL cholesterol²⁸.

For all examined lipid and lipoprotein quantities the imprecision expressed as coefficient of variation was between 1.3 and 3.3% and inaccuracy expressed as bias % was between 1.7 and 3.2% assessed using the control sera for normal and pathological concentrations (Preci-norm »L« and Precipath »L«, Boehringer Mannheim, Germany).

Non-parametric statistics has been used throughout since the data were not normally distributed (Kolmogorov Smirnov test). The Mann-Whitney U-test was used for the comparison of two independent variables, the Kruskal-Wallis one way analysis variance to detect differences between any two groups. The associations of serum Lp(a) with age, body mass index, lipid and lipoprotein analytes were tested by the Spearman's rank correlation. The two-tailed error probability of $p < 0.05$ was considered signifi-

cant. In addition, factor analysis was done where factors were rotated according to varimax criteria.

Results

Mann-Whitney U-test and Kruskal-Wallis test showed no statistically significant differences for Lp(a) concentrations between either sex or age in the examined children population ($p > 0.05$).

The investigation of the frequency distributions (Figure 1) showed that in 52.4% of Lp (a) concentrations were below 0.1 g/L. It is important to stress that 20,4 % boys ($n = 55$) and 20.4% girls ($n = 54$), had Lp(a) concentration above 0,3 g/L. Median plasma concentrations of Lp(a) were 0,09 g/L for boys and girls with the 97.5th upper reference value of 0,69 g/L. Table 1 and Figure 2 show distributions (percentiles) of serum Lp(a) concentrations in the examined pediatric population according to age groups.

The Spearman's correlation coefficients presented in Table 4, show no statistically significant correlation of Lp(a) with age, body mass index, HDL-cholesterol and triacylglycerol while it was statistically significant ($p < 0,01$) for total cholesterol ($r = 0.139$) and LDL-cholesterol ($r = 0.190$). This correlation disappeared when adjusting total cholesterol and LDL cholesterol concentrations in accordance with the cholesterol amounts present in Lp(a) particles²⁷. The corrected total cholesterol and LDL cholesterol concentrations in relations to Lp(a) variations considering ranges of 0.1 g/L are presented in Table 2.

In addition to correlation studies factor analysis was performed (Table 3). The first four axes defined by principal-components analyses explain in total 84.2% of variance among individuals described with the original set of seven variables (age, body mass index and five lipid analytes). The first factor defined with to-

TABLE 1
PERCENTILES OF SERUM LIPOPROTEIN (a) CONCENTRATIONS IN THE POPULATION OF SCHOOL CHILDREN AND ADOLESCENTS ACCORDING TO AGE GROUPS

Age, years (boys and girls)	n	Lipoprotein (a), g/L - percentiles	
		50.0th	97.5th
8	36	0.06	0.59
9	84	0.08	0.57
10	80	0.09	0.73
11	120	0.11	0.75
12	48	0.12	0.63
13	28	0.07	0.65
14	18	0.05	0.33
15	21	0.12	0.51
16	32	0.05	0.42
17	31	0.11	0.50
18	23	0.08	0.84
19	13	0.03	0.19
Total	534	0.09	0.69

tal cholesterol and LDL cholesterol explain 29.9% of the total variance; the second factor defined with age and body mass index explain 26.0%, the third is bipolar defined with HDL cholesterol (-) and triacylglycerol (+) and explained 14.5% and the fourth factor defined only with lipoprotein (a) explained 13.8% of the total variance.

Discussion

According to the anthropometric studies Croatian children population showed no significant changes in Lp(a) concentrations with sex, age and body mass index. Previously published data for Belgian⁷ and Spanish⁸ schoolchildren show the lack of association between serum Lp(a) and body mass index. In Spanish^{8,9} children populations there were no significant difference with age and sex. Japanese children¹⁰ showed no sex relations while in American children¹² small but significant sex difference was found and no age related trend. In Korean children population aged 6-11 years⁵ and in

RELATIVE FREQUENCY (%)

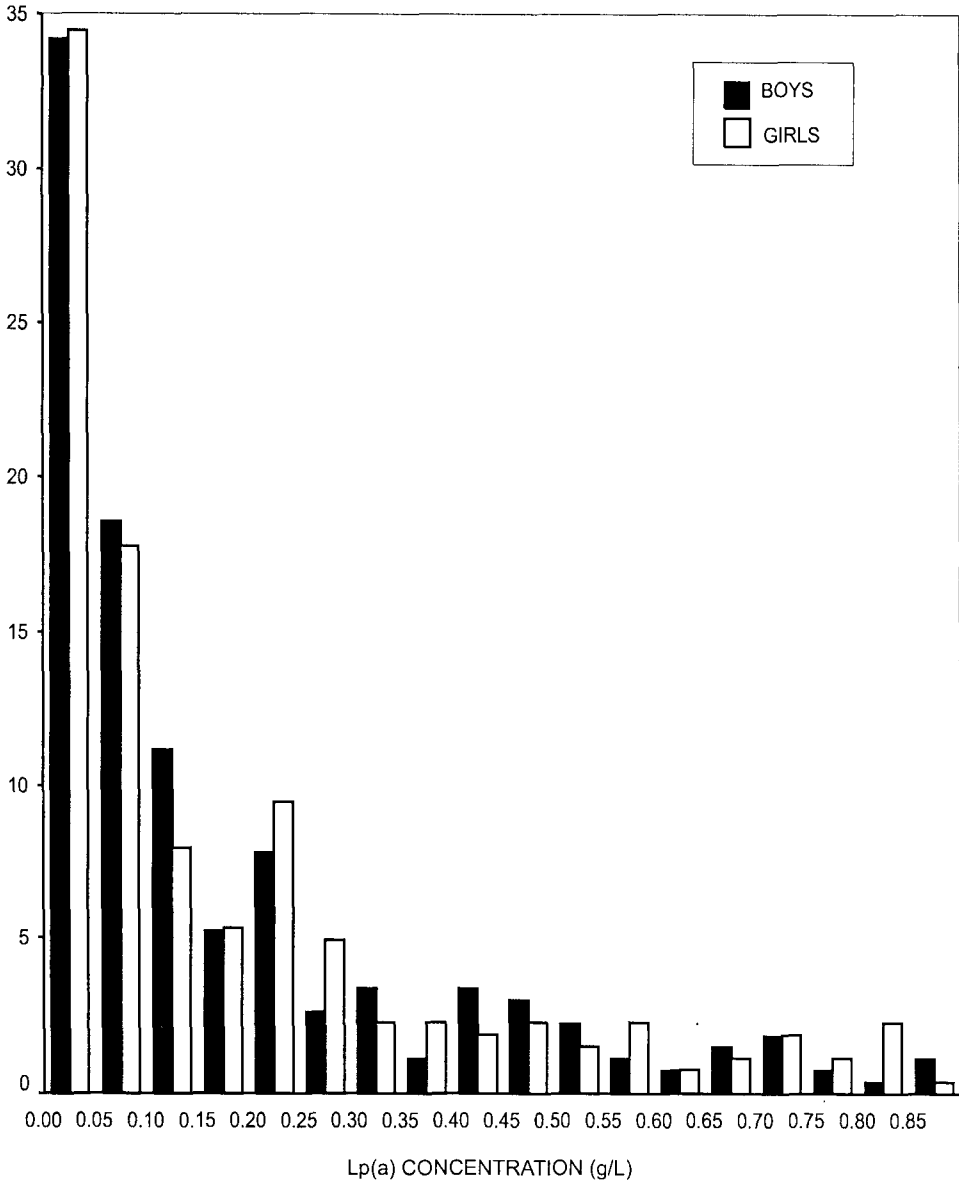


Fig. 1. Frequency distribution of Lp(a) concentrations in 269 boys and 265 girls aged between 8 and 19 years.

Italian pediatric population⁶, Lp(a) concentrations were not influenced by sex

but the data indicated a progressive increase with age. Gozlan found for Israeli

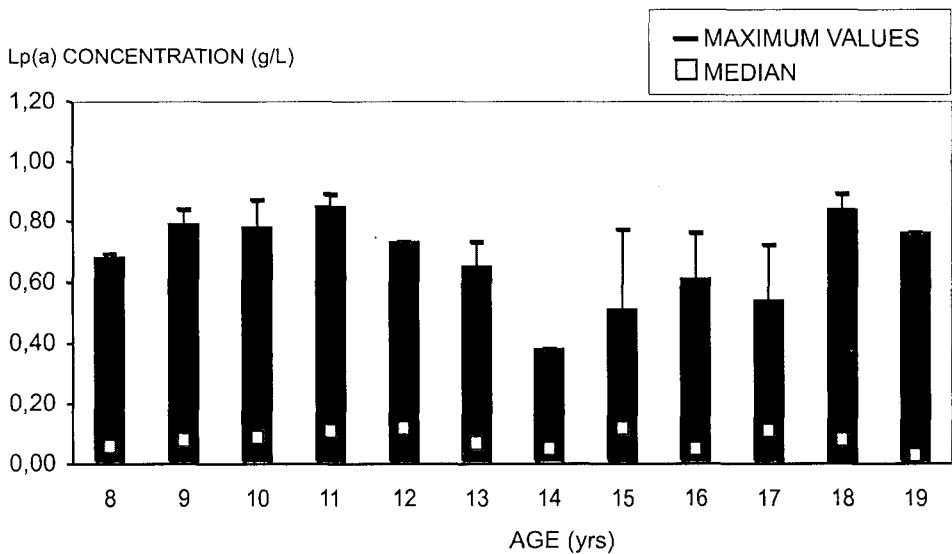


Fig. 2. Boxplots for Lp(a) concentrations according to age groups. The box contain the results within 2.5 and 97.5 percentiles.

TABLE 2
SERUM LIPIDS LEVELS ACCORDING TO LIPOPROTEIN (a) CONCENTRATIONS AND CORRECTED TOTAL CHOLESTEROL AND LDL-CHOLESTEROL CONCENTRATIONS

n	Lp(a) g/L	Total chol. mmol/L	LDL chol. mmol/L	HDL chol. mmol/L	Triacyl- glycerol mmol/L	Total chol. corrected mmol/L	LDL chol. corrected mmol/L
280	0.01–0.09	4.43 ± 0.77	2.58 ± 0.69	1.49 ± 0.33	0.82 ± 0.39	4.39 ± 0.77	2.53 ± 0.69
79	0.10–0.19	4.61 ± 0.69	2.79 ± 0.61	1.48 ± 0.32	0.79 ± 0.31	4.44 ± 0.69	2.62 ± 0.60
63	0.20–0.29	4.53 ± 0.87	2.74 ± 0.75	1.46 ± 0.30	0.76 ± 0.35	4.27 ± 0.86	2.48 ± 0.75
27	0.30–0.39	4.60 ± 0.53	2.70 ± 0.46	1.55 ± 0.27	0.75 ± 0.35	4.20 ± 0.52	2.30 ± 0.46
27	0.40–0.49	4.65 ± 0.72	2.81 ± 0.65	1.47 ± 0.27	0.82 ± 0.27	4.13 ± 0.72	2.29 ± 0.65
19	0.50–0.59	4.76 ± 0.82	2.95 ± 0.67	1.47 ± 0.34	0.77 ± 0.27	4.14 ± 0.83	2.32 ± 0.68
12	0.60–0.69	4.80 ± 0.77	2.99 ± 0.70	1.48 ± 0.29	0.72 ± 0.30	4.05 ± 0.76	2.23 ± 0.71
15	0.70–0.79	4.94 ± 0.72	3.14 ± 0.65	1.41 ± 0.27	0.85 ± 0.34	4.07 ± 0.72	2.28 ± 0.65
13	0.80–0.89	4.98 ± 1.05	3.19 ± 1.07	1.42 ± 0.15	0.80 ± 0.31	3.97 ± 1.02	2.18 ± 1.03

Lp(a) = lipoprotein (a); LDL = low density lipoprotein;
HDL = high density lipoprotein; chol. = cholesterol.

children population¹³ the steepest Lp(a) increase with age (about seven times between 0–18 years).

In Table 4 we compare the levels of Lp(a) concentrations obtained for Croatian children with other white children populations because Lp(a) concentrations

are markedly higher in black than in white children¹⁸. The frequency distribution of Lp(a) concentrations in our study is markedly skewed with higher frequency at low values. According to the median values the majority of children have Lp(a) levels about 0.1 g/L what is similar with other children popula-

TABLE 3
RESULTS OF FACTOR ANALYSIS OF AGE, BODY MASS INDEX AND LIPID ANALYTES
IN BOYS AND GIRLS (N=534)

Lipid analyte	Factor 1	Factor 2	Factor 3	Factor 4
Age	-0.1287	0.8535	0.1134	-0.0281
Body mass index	0.1144	0.8576	0.1107	0.0198
Total cholesterol	0.9931	-0.0247	-0.0860	0.0305
HDL - cholesterol	0.2754	-0.1478	-0.8062	-0.1915
LDL - cholesterol	0.9278	0.0108	0.0797	0.1584
Triacylglycerol	0.2436	0.1102	0.8071	-0.1802
Lp(a)	0.1479	-0.0081	-0.0051	0.9601
Communality	0.7586	0.7631	0.9952	0.7784
Eigenvalue	2.0942	1.8191	1.0135	0.9644
% of variance	29.9	26.0	14.5	13.8

Lp(a) = lipoprotein (a); LDL = low density lipoprotein; HDL = high density lipoprotein.

TABLE 4
SERUM LEVELS OF LIPOPROTEIN (a) CONCENTRATIONS (g/L) IN CHILDREN
POPULATIONS ACCORDING TO LITERATURE DATA

Children population (reference)	n	Sex	Age (yrs)	Mean \pm SD	Median
Croatian-Zagreb region	534	boys and girls	8-19	0.182 \pm 0.216	0.090
Korean (5)	269	boys and girls	6-11	0.094*	
Italian (6)	220	boys and girls	2-10	0.064 \pm 0.057	0.040
Belgian (7)	136	boys	9-16	0.172 \pm 0.262	0.082
	130	girls		0.193 \pm 0.218	0.094
Spain-Burgos region (8)	279	boys and girls	11-19	0.19 \pm 0.15	0.120
Spain-Madrid region (9)	965	boys	4-18	0.141 \pm 0.151	0.080
	1005	girls		0.160 \pm 0.179	0.090
Japanese (10)	269	boys and girls	8-13	0.155 \pm 0.180	0.011
German (11)	1336	boys	1-17	0.167	0.067
	1218	girls		0.183	0.068
American (12)	767	boys	8-17	0.164 \pm 0.168	0.092
	786	girls		0.179 \pm 0.184	0.095
Israel (13)	197	boys and girls	13-18	0.085 \pm 0.076	

*Geometric mean; SD = standard deviation.

tion⁴⁻¹³. Concentrations above 0.3 g/L are considered as a cut-off value for the increased risk of coronary artery disease and early atherosclerosis²⁹⁻³¹. They were found in relatively high percent (20.4%) of our children population. Similar incidence of the risk Lp(a) values were found in French and Belgian children population⁴ where it was about 20%, Spain^{8,9} 15-19% and in Japanese children¹⁰ where it was 18.8%. The reference values determined for other lipid and lipopro-

teins constituents of sera in Croatian children population²³, presented in Table 5, also exceed desirable concentrations considering increase in the premature development of coronary heart disease^{32,33}. It is considered that 90% of serum concentrations of Lp (a) are genetically determined, and only the rest could be due to environmental factors or to pathological conditions. For this reason family history and genetic differences rather than the acquired risk factors could be one of the

TABLE 5
 REFERENCE INTERVALS (2.5th-97.5th PERCENTILES) FOR SERUM LIPID AND LIPO-
 PROTEINS ANALYTES OF CHILDREN AGED 8-18 YEARS, ZAGREB, CROATIA (21)

Analytes	Units	School children and adolescents				Reference intervals (2.5th -97.5th)
		n	Sex	Age (yrs)		
Total cholesterol	mmol/L	540	boys and girls	8-12	3.3-6.1	
		206	boys	13-18	2.8-5.6	
		250	girls	13-18	3.3-6.2	
HDL cholesterol	mmol/L	693	boys and girls	8-14	0.9-2.2	
		137	boys	15-18	0.9-1.8	
		166	girls	15-18	0.9-2.0	
LDL cholesterol	mmol/L	458	boys	8-18	1.5-4.1	
		537	girls	8-18	1.6-4.3	
Triacylglycerols	mmol/L	540	boys and girls	8-12	0.3-1.6	
		206	boys	13-18	0.4-2.1	
		249	girls	13-18	0.4-1.9	
LDL cholesterol/ HDL cholesterol	-	458	boys	8-18	1.0-3.3	
		537	girls	8-18	1.0-3.4	
Total cholesterol/ HDL cholesterol	-	458	boys	8-18	2.1-4.8	
		537	girls	8-18	2.2-4.9	

LDL = low density lipoprotein; HDL = high density lipoprotein.

major contributor to high serum Lp(a) concentrations¹⁻⁴. Our results show significant correlation of Lp(a) concentrations only with total and LDL cholesterol concentrations, probably due to the amount of cholesterol within the Lp(a) particles. The lack of correlation when Lp(a) cholesterol is subtracting from the total and LDL cholesterol concentrations and the results of factor analysis support the concept that Lp(a) could be an independent risk factor for coronary artery disease.

Conclusion

The obtained results are in accordance with other epidemiological studies of children populations indicating that the pathological precursors of coronary artery disease begin in childhood. As the detection of high concentrations of serum

Lp(a), during childhood signals the requirement to manage the other atherosclerotic risk factors that can be reduced (lowering of LDL cholesterol, diet, cessation of drugs and smoking, lifestyle), the estimation of Lp(a) concentrations for children population in addition to the lipid profile may have a great value in the prediction of coronary artery disease risk early in life.

Acknowledgements

This study is the part of a scientific project that was financially supported by Ministry of Science and Technology, Zagreb, Croatia. We express our gratitude to Prof. J. Božikov for the statistical analysis of the data, and Dr. N. Jagarinec for her help and useful suggestions in the preparation of the manuscript.

REFERENCES

1. SCANU, A. M., *Clin. Chem.*, 41 (1995) 170. —
2. KREMLER, F., G. M. KOSTNER, K. BOLZANO, F. SANDHOFER, *Biochim. Biophys. Acta.*, 575 (1979)
63. — 3. SANDKAMP, M., H. FUNKE, H. SCHULTE, E. KÖHLER, G. ASSMANN, *Clin. Chem.*, 36/1 (1990)
20. — 4. BAILLEUL, S., R. COUDERC, C. ROSSIG-

- NOL, J. FERMANIAN, F. BOUTOUCHENT, M.-A. FARNIER, J. ETIENNE, Clin. Chem., 41/2 (1995) 241. — 5. CHOE, Y. H., Y. CHOI, J. I. Q. KIM, Ann. Clin. Biochem., 34 (1997) 179. — 6. BARONI, S., D. SCRIBANO, P. VALENTINI, C. ZUPPI, O. RANNO, B. GIARDINA, Clin. Biochem., 29/6 (1996) 603. — 7. COBBAERT, C., L. DEPROST, P. MULDER, K. ROMBAUT, G. GIJSELS, H. KESTELOOT, Int. J. Epidemiol., 24/1 (1995) 78. — 8. VELLA, J. C., E. JOVER, Clin. Chem., 39/3 (1993) 477. — 9. GOMEZ GERIQUE, J. A., A. PORRES, D. LOPEZ MARTINEZ, L. A. ALVAREZ SALA, E. BLAZQUEZ, M. T. MONTOYA, M. De OYA, Acta Paediatr., 85 (1996) 38. — 10. ARISAKA, O., S. FUJIWARA, N. MIYAKE, H. MOKUNO, K. YABUTA, J. Pediatric Gastroenterology and Nutrition, 24 (1997) 533. — 11. SCHUMACHER, M., S. WEIGERT, W. G. WOOD, Eur. J. Clin. Chem. Clin. Biochem., 34 (1996) 909. — 12. SRINIVASAN, S. R., G. H. DAHLEN, R. A. JARPA, L. S. WEBBER, G. S. BERENSON, Circulation, 84/1 (1991) 160. — 13. GOZLAN, O., D. GROSS, N. GRUENER, Clin. Biochem., 27 (1994) 305. — 14. KOSTNER, G. M., P. AVOGARO, G. CAZZOLATO, E. MARTH, G. BITOLO-BON, G. B. QUINCI, Atherosclerosis, 38 (1981) 51. — 15. DAHLÉN, G. H., J. R. GUYTON, M. ATTAR, J. A. FARMER, J. A. KAUTZ, A. M. J. R. GOTTO, Circulation, 74 (1986) 758. — 16. MARCOVINA, S. M., V. P. GAUR, J. J. ALBERS, Clin. Chem., 40/4 (1994) 574. — 17. PANTEGHINI, M., F. PAGANI, Eur. J. Clin. Chem. Clin. Biochem., 31 (1993) 23. — 18. SANDHOLZER, C., D. M. HALLMAN, N. SAHA, G. SIGURDSSON, C. LACKNER, A. CSÁSZÁR, et al., Hum. Genet., 86 (1991) 607. — 19. NAZIR, D. J., M. J. MCQUEEN, Clin. Biochem., 30/2 (1997) 163. — 20. PETITCLERC, C., H. E. SOLBERG, J. Clin. Chem. Clin. Biochem., 26 (1987) 639. — 21. SASSE, E. A., Arch. Pathol. Lab. Med., 116 (1992) 710. — 22. LINDBALD, B., T. ALSTRÖM, A. BO HANSEN, R. GRÅSBECK, H. HERTZ, C. HOLMBERG, et al., Scand. J. Clin. Lab. Invest., 50 (1990) 99. — 23. JAGARINEC N., Z. FLEGAR-MEŠTRIĆ, B. ŠURINA, D. VRHOVSKI-HEBRANG, V. PREDENKEREKOVIĆ, Clin. Chem. Lab. Med., 36/5 (1998) 327. — 24. EVANS, K., J. MITCHESON, M. F. LAKER, Clin. Chim. Acta, 258 (1997) 219. — 25. TADDEI-PETERS, W. C., B. T. BUTMAN, G. R. JONES, T. M. VENETIA, P. F. MACOMBER, J. H. RANSON, Clin. Chem., 39 (1993) 1382. — 26. TATE, J. R., N. RIFAL, K. BERG, R. COUDERS, F. DATI, G. M. KOSTNER, I. SAKURABAYASHI, A. STEINMETZ, Clin. Chem., 44/8 (1998) 1629. — 27. LI, K. M., D.E.L. WILCKEN, N.P.B. DUDMAN, Clin. Chem. 40/4 (1994) 571. — 28. ORTOLÁ, J., M. J. CASTIÑEIRAS, X. FUENTES-ARDERIU, Clin. Chem., 38/1 (1992) 56. — 29. JIALAL, I., Clin. Chem., 44/8 (1998) 1827. — 30. KRONENBERG, F., A. STEINMETZ, G. M. KOSTNER, H. DIEPLINGER, CRC 33/6 (1996) 495. — 31. RADER, D. J., H. B. BREWER, JAMA, 267/8 (1992) 1109. — 32. CONSENSUS CONFERENCE 1985, J. Am. Med. Ass., 253 (1985) 2080. — 33. KWITEROVITCH, P. O., Pediatrics, 78 (1986) 349.

D. Vrhovski-Hebrang

*Institute of Clinical Chemistry, University Hospital »Merkur«,
I. Zajca 19, 10000 Zagreb, Croatia*

KONCENTRACIJE LIPOPROTEINA (a) KOD ŠKOLSKE DJECE I MLADEŽI U HRVATSKOJ

S A Ž E T A K

Koncentracije lipoproteina (a) u serumu određeni su kod 536 zdrave djece i mladeži starosti između 18 i 19 godina iz Zagreba, Hrvatska. Razdioba učestalosti pokazala je kako je 20,4% dječaka i djevojčica imalo koncentracije lipoproteina (a) iznad 0,3 g/L – što se smatra graničnom vrijednošću povećanog rizika rane arteroskleroze. Rezultati korelacijske i faktorske analize u skladu su s pretpostavkom da je koncentracija lipoproteina (a) neovisan čimbenik rizika koji ima veliku vrijednost pri predviđanju arteroskleroze u ranoj životnoj dobi.