CLINICAL STUDY

Lipoprotein (a) and apolipoprotein (a) phenotypes in healthy Macedonian children

Tosheska K[1], Labudovic D[1], Alabakovska S[1], Handziski Z[2], Spiroski M[3], Todorova B[1]

Department of Medical and Experimental Biochemistry, Medical Faculty, Skopje, Republic of Macedonia.
tosheskatrajkovska@gmail.com

Abstract

Background: **Plasma levels of lipoprotein(a) [Lp(a)] are determined largely by genetic variation in the gene encoding apolipoprotein(a) [apo(a)], the unique protein component of Lp(a).**
High plasma levels of Lp(a) increase the risk of premature atherosclerosis. However, the association of apo(a) phenotypes with the development of these diseases remains largely unexplored.
Objectives: **Determination of Lp(a) levels and apo(a) isoforms (phenotypes) in 100 (51 boys, 49 girls) Macedonian healthy children aged 9–18.**
Methods: **We used 3–15 % gradient SDS-PAGE for separation of apo(a) isoforms. According to the different apo(a) electrophoretic mobilities, Apo(a) was classified into five single and respective double-band phenotypes.**
Results: **Each individual expressed a single (homozygotic), double band (heterozygotic) or no band (null phenotype). The apo(a) phenotype frequencies revealed that the frequency of single-band phenotype expression (64 %) was higher than that of double bands (32 %) and that the frequency of phenotypes representative of low molecular weight was very low (4 %). The most frequent phenotype was S4 (42.65 %). The distribution of plasma Lp(a) levels was skewed, with the highest frequencies at low levels. The mean Lp(a) concentration was 11.95 (SD of 5.98 and median of 9.62 mg/dL). We did not find differences in the mean and median plasma Lp(a) levels between boys and girls ($p>0.05$).**
A strong inverse relationship was found between the apparent molecular weight of apo(a) phenotypes and plasma Lp(a) concentration ($r= -0.4257$).
Conclusions: **Determination of Lp(a) levels and apo(a) phenotypes in children, may help in preventing and reducing the risk of atherosclerotic development *(Tab. 6, Ref. 32)*. Full Text (Free, PDF) *www.bmj.sk*.**
Key words: lipoprotein(a), apolipoprotein(a), phenotypes.

Cardiovascular disease remains the number one killer of men and women around the world despite advances in diagnosis and treatment. Coronary artery disease (CAD) is almost always the result of atherosclerosis. Most authorities believe that the process of atherosclerosis is lifelong, beginning in childhood or early adulthood (1, 2).

The interest in lipoprotein (a) [Lp(a)] has arisen considerably since several case-control studies (3, 4, 5, 6) have demonstrated that increased plasma Lp(a) levels are linked to a high risk of premature CAD unrelated to other lipoproteins.

Plasma levels of Lp(a) are determined largely by genetic variation in the LPA gene encoding apolipoprotein(a) [apo(a)], the specific protein component of Lp(a) (7, 8).

Apo(a) contains a 5' signal sequence, a 3' plasminogen (PLG)-like protease domain and ten different types of kringle IV domains (KIV-1 to KIV-10) (9). The number of KIV-2 is variable, ranging from 1–42 copies in individual LPA alleles and apo(a) isoforms (apo(a) size polymorphism = KIV-2 VNTR) (10, 11).

[1]Department of Medical and Experimental Biochemistry, Medical Faculty, Skopje, Republic of Macedonia
[2]Department of Physiology and Anthropology, Medical Faculty, Skopje, Republic of Macedonia
[3]Department of Immunobiology and Human Genetics, Medical Faculty, Skopje, Republic of Macedonia

Address for correspondence: K. Tosheska, MD, MSc, Dept of Medical and Experimental Biochemistry, Medical Faculty, 50 Divizija No. 6, 1000 Skopje, Republic of Macedonia.
Phone: +389.2.3217303, Fax: +389.2.3230431

Acknowledgment: This work was supported by the Ministry of Education and Science of the Republic of Macedonia, Research Grant No. 08-2901/1, 2001.

The genetically determined variation in the number of kringle IV repeats leads to the synthesis of apo(a) isoforms (phenotypes) whose molecular weights varies between 200 and 800 kDa (11, 12, 13).

The size polymorphism and additional genetic variation of the LPA gene (14, 15, 16) largely control Lp(a) levels so that Lp(a) concentrations have interindividual and interethnic variability, with plasma levels ranging between 0 and 300 mg/dL (11, 17). It seems that the Lp(a) values in childhood remain unaltered up to adulthood (18, 19).

There is an inverse trend between Lp(a) concentration and the size of its apo(a) component: low Mr phenotypes (B, S1, S2) are associated with high plasma Lp(a) concentrations and high Mr phenotypes (S3, S4) with low Lp(a) concentrations. In the "null" phenotype no apo(a) band is detected during immunoblotting and the Lp(a) level is low or absent (12, 20). It has been speculated that apo(a) phenotypes contribute to the risk of atherosclerosis independently of plasma levels of Lp(a) (21). If apo(a) phenotypes can predict the risk of future cardiovascular disease from childhood, it could be clinically valuable, and it may be possible to develop targeted intervention strategies to reduce the risk in genetically susceptible children. Although it is now accepted that the natural history of atherosclerosis begins in youth (1, 22), its origin may exist even in fetal life (2).

However, the association of apo(a) phenotypes with the risk of premature development of atherosclerosis remains largely unexplored.

The firs step toward elucidating these issues is to establish the apo(a) phenotype variations in populations. Data on children are particularly important from the standpoints discussed above.

Plasma Lp(a) concentration and apo(a) phenotypes in the age group of young children and adolescents have not been previously studied in the Macedonian population.

In the present study we used the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blott technique (21, 23) to identify apo(a) phenotypes among Macedonian children and evaluated the relation of apo(a) phenotypes to Lp(a) levels.

Material and methods

Subjects. One hundred (100) healthy children aged between 9 and 18 years (51 boys and 49 girls), recruited from the Department of Physiology and Anthropology, Medical faculty, Skopje, Republic of Macedonia, were included in the study. Written informed consent was obtained from parents of all included children before their enrolment into the study. Blood samples were obtained after agreement of the children.

The K3EDTA plasma was obtained through venipuncture after 14-hours of overnight fasting. Plasma was prepared by low speed centrifugation at 1800 rpm/m for 15 min and stored at -80 °C as aliquots in segments of plastic tubes until assayed (24).

Electrophoresis. Denaturing 3–15 % gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a vertical Bio-Rad Mini Protean II System (Bio-Rad Laboratories, Hercules, CA, USA) was used for Apo(a) isoforms separation. Two peristaltic pumps (Masterflex L/S, Cole Parmer Instrument Co., Vernon Hills, IL, USA) that were controlled by Cole Parmer software, were used to convey the gradient gel characteristics. Sample preparation, electrophoresis and immunoblotting have been presented in previous publications (21, 23).

Twenty μL of plasma was mixed with 80 μL of reducing buffer (containing ß-mercapthoethanol, bromphenol blue in glycerol, SDS) to give a total volume of 100 μL and boiled in a water bath for 5 min. For the stacking gel, 3.3 % polyacrylamide gel [0.5 M Tris-HCl buffer pH 6.8, 10 % SDS, TEMED and 10 % APS (Bio-Rad Laboratories)] was prepared. A commercial standard, purchased from Immuno Ag, Vienna, Austria, that contained the B, S1, S3, S4, >S4 isoforms was used. Ten μL of treated plasma samples and 5 μL of the standard were resolved in 3–15 % gradient gels. A maximum of eight samples per gel was applied to avoid edge effects. Electrophoresis was performed in Tris-glycine buffer, for 90 min (until the dye front was just out of the gels), at 100 V, at room temperature.

Immunochemistry. The proteins separated on polyacrylamide gels were transferred to nitrocellulose membranes (BA 83, 0.2 μm, NC.; S & S Protran, Dassel, Germany) by electroblotting using a Hoefer TE22 Transfer Unit (Amersham Pharmacia Biotech, Vienna, Austria), in Tris-glycine methanol buffer, for 17 hours at 50 V. The apo(a) isoforms were visualized immunochemically with an Lp(a) phenotyping kit (Immuno Ag, Vienna, Austria) containing primary antibody [a polyclonal antihuman Lp(a) (sheep)] and secondary antibody [an alkaline phosphatase-conjugated antisheep IgG (rabbit)] that was diluted with 5 % Blotto solution (non fat dry milk, Tween 20, 10X PBS and antifoam A (Sigma Chemical Co., St Louis, MO, USA)].

Five apo(a) isoforms were designated according to their relative electrophoretic mobility compared with apo B-100, using the terminology proposed in (12). B is a band with the same mobility as apo B-100 and S1, S3, S4, >S4 denoting larger isoforms with progressively lower mobility than apo B-100. The Mr of each apo(a) band was calculated by comparison with the standards located in the adjacent lane (21). Gels were scanned with a Pharmacia LKB Ultra Scan XL laser (Pharmacia LKB Biotechnology, Uppsala, Sweden) controlled from a computer running the Image Master Software (Pharmacia Biotechnology, Uppsala, Sweden).

Measurment of lipids, lipoproteins and apolipoproteins. Total cholesterol (TC), triglycerides (TG) and HDL cholesterol (HDL-C) were measured with the enzymatic assays and (for HDL cholesterol) a precipitation method. LDL cholesterol (LDL-C) was calculated using the Friedewald formula. Apolipoproteins (Apo A-1 and ApoB) were determined by nephelometric method (Dade Behring Marburg GmbH, Marburg, Germany).

Lipoprotein(a) assay. The Lp(a) concentration was measured at the Department of Immunobiology and Human Genetics, Medical Faculty, Skopje, Republic of Macedonia, on a Behring Nephelometer Analyzer using immunonephelometrical kits (Dade Behring Marburg GmbH, Marburg, Germany).

Statistical analysis. All statistical procedures were carried out using the STATWIN statistical software package (version 5.0

Tab. 1. BMI, plasma lipids, glucose and apoliopoprotein levels in children, mean±SD.

Parameter	Boys (n=51)	Girls (n=49)	Total (n=100)
BMI (kg/m^2)	19.25±3.10	20.49±3.40	20.07±3.23
Total cholesterol	3.92±0.69	3.99±0.64	3.95±0.66
HDL cholesterol (mmol/L)	1.41±0.31	1.30±0.24	1.35±0.28
LDL cholesterol (mmol/L)	2.13±0-56	2.30±0.57	2.21±0.57
Triglyceride (mmol/L)	0.72±0.35	0.80±0.33	0.76±0.34
Glucose (mmol/L)	4.75±0.56	4.70±0.57	4.73±0.57
Apo A-1 (mg/dL)	136.05±17.23	133.55±15.46	134.71±16.25
Apo B (mg/dL)	95.70±16.82	94.35±16.94	95.04±16.81

n – number of individuals; BMI – body mass index; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B.

Tab. 2. Frequency of apo(a) phenotypes in 100 healthy children.

Isoform	Boys		Girls		Total	
	n	%	n	%	n	%
Single	35	68.63	29	59.18	64	64.00
Double	14	27.45	18	36.73	32	32.00
Null	2	3.92	2	4.08	4	4.00
Total	51	100.00	49	100.00	100	100.00

n – number of individuals

Tab. 3. Apo(a) phenotype frequencies in children.

Isoform		
Single	n	%
>S4	22	32.35
S4	29	42.65
S3	13	19.12
S1	0	0
B	0	0
Null	4	5.88
Total	68	100
Double	n	%
>S4/S4	8	25
>S4/S3	9	28.13
>S4/S1	1	3.13
S4/S3	11	34.38
S4/S1	1	3.13
>S4/B	0	0
S4/B	1	3.13
S3/S1	1	3.13
S3/B	0	0
S1/B	0	0
Total	32	100

n – number of individuals

Tab. 4. Distribution of Lp(a) concentration (%) in healthy children.

Lp(a) concentration	Frequency	%
0.00<x<=9.99	77	77
10.00<x<=19.99	12	12
20.00<x<=29.99	9	9
30.00<x<=39.99	1	1
40.00<x<=49.99	1	1
50.00<x<=59.99	0	0
60.00<x<=69.99	0	0
70.00<x<=79.99	0	0
80.00<x<=89.99	0	0
90.00<x<=100.00	0	0

n – number of individuals

A, Statsoft Inc.1984–95; Tulsa, Oklahoma,USA). Mean values and SDs were compared by Student's t test. Because of their non-Gaussian frequency distributions, data on Lp(a) are also presented as medians. Correlations between apo(a) size and Lp(a) levels were calculated by the Spearman rank test. P values <0.05 were considered significant.

Results

Descriptive statistics are provided in Table 1. There were no significant differences between two gender groups in lipids, apolipoproteins, glucose and BMI.

The apo(a) isoforms were classified according to the system of Utermann et al (12). Using SDS-PAGE and immunoblotting, we were able to resolve five apo(a) isoforms (B, S1, S3, S4 and >S4). In each plasma, one of these five bands was generally present, either alone (single-banded phenotype) or in combination with another band (double-banded phenotype). The phenotype was defined as "null" when there were no detectable apo (a) bands.

The apo(a) phenotype frequencies in the children are given in Table 2. The prevalence of single banded phenotype was higher (64 %) than double-banded phenotype (32 %). Since there was no statistically significant difference in the apo(a) frequency distribution between gender groups for single-banded (chi-square=2.82, p=0.42) and double-banded phenotype (chi-

Tab. 5. Plasma level of Lp(a) in boys and girls (n=100).

	Lp(a) (mg/dL) (mean+SD)	Lp(a) (mg/dL) (median)	Min–Max (mg/dL)
Boys	11.65±5.91	9.62	9.62–41.4
Girls	12.29±6.10	9.62	9.62–30.2
Total	11.95±5.98	9.62	9.62–41.4

Tab. 6. Relationship between the Mr of apo(a) isoforms and Lp(a) concentrations.

Isoform	Mr (mean±SD)	Lp(a) (mg/dL) (mean±SD)
>S4	784.06±16.87	10.25±4.21
S4	714.04±30.03	10.78±2.19
S3	585.92±20.25	14.77±9.09
S1	–	–
B	–	–

square=5.55, p=0.47), distribution of apo(a) phenotypes in all children is presented in Table 3. In most children (42.65 %), dominant phenotype was S4 which belong to HMW phenotypes. In our study group, we did not detect carriers of LMW phenotypes which are associated with high values of Lp(a). The S4S3 phenotype (34.38 %) was the most frequent among the double-banded phenotypes.

As presented in Table 4, the distribution of Lp(a) concentration is highly skewed towards lower concentrations. Most of the subjects, (98 %) had an Lp(a) level beneath the cut-off point of 30 mg/dL, with 77 % of children showing levels of 10 mg/dL or less. The plasma level of Lp(a) in two gender groups is presented in Table 5. There was no difference in plasma Lp(a) levels in boys vs girls (t-test=0.800, p>0.05). In this sample (n=100), mean (±SD) and median Lp(a) values were 11.95±mg/dL and 9.62 mg/dL, respectively.

The relationship between the Mr of apo(a) isoforms and Lp(a) levels are presented in Table 6. We found a significant inverse correlation (r)=-0.4257, p<0.001 between the Mr of apo(a) isoforms and plasma levels of Lp(a).

Discussion

Concentrations of Lp(a) have been widely described in children but little is known about apo(a) phenotypic expression in this population. The data presented here, supported by studies of Utermann et al (12) and Gaubatz et al (25), suggest that modified Western blot technique is highly sensitive method for identification of apo(a) isoforms.

This is to our knowledge the first systematic study on apo(a) phenotypes and Lp(a) concentration in Macedonian children.

The frequency of single banded expression in Macedonian children was 64 %, which is in accordance with the same expression in Macedonian adults (59.44 %) (26) and the reports of Gaubatz et al (25) (59.5 %) and Akanji (70 %) (27).

Because population distribution of apo(a) isoforms may vary racially, the apo(a) isoform distributions in Macedonian children were found to be different from Japanese children (22), being characterized by higher frequencies of double band isoforms (55 %) compared to single band isoforms (44 %).

In the present study, among the single band expressing children, the most frequent isoforms were S4 (42, 65 %), followed by >S4 (32, 35 %).

These results are consistent with study of Min et al (28) who observed that representatives of HMW isoforms were the most frequent among 103 healthy Koreans.

The Mr of the apo(a) isoforms observed ranged from 565,000 to 800,000. This is in agreement with our previous study on healthy adults where the Mr of the observed apo(a) phenotypes ranged from 417,000–785,000 (26) and the data of Gaubatz et al (25), who resolved 11 apo(a) isoforms using SDS-PAGE with Mr from 419,000 to 838,000.

The pattern of distribution of Lp(a) levels found in our sample, markedly skewed, was similar to that found in previous studies performed in children from Europe (29), Australia (30) and Japan (31). In our study, no differences were found between boys and girls in Lp(a) levels. Although Srinivasan et al (32) described a small but significant sex difference in Lp(a) levels, this finding has not been described in other studies (29, 31).

In agreement with the results of Utermann et al (12) and Gaubatz et al (25), we observed an inverse correlation between the molecular size of the apo(a) isoform and the respective plasma Lp(a) concentration.

These results obtained in healthy children indicate that apo(a) phenotype is primarily genetically determined, and is not affected by plasma lipid and apoprotein concentration in childhood.

In evaluating children's lipid profiles, measurement of Lp(a) and identification of apo(a) phenotypes may help to identify children and their families at increased risk for premature development of CAD and this may facilitate the targeting of preventive measures.

References

1. Newman WP 3rd, Freedman DS, Voors AW et al. Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis: the Bogalusa Heart Study. New Engl J Med 1986; 314 (3): 138—144.

2. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet 1993; 341 (8850): 938—941.

3. Sandkamp M, Funke H, Schulte H, Kohler E, Assmann G. Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. Clin Chem 1990; 36 (1): 20—23.

4. Kostner KM, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Qunici GB. Lipoprotein (a) and the risk for myocardial infarction. Atherosclerosis 1981; 38 (1—2): 51—61.

5. Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg AL. Lp(a) lipoprotein as a risk factor for myocardial infarction. J Amer Med Ass 1986; 256 (18): 2540—2544.

6. Kronenberg F, Steinmetz A, Kostner GM, Dieplinger H. Lipoprotein (a) in health and disease. Crit Rev Clin Lab Sci 1996; 33 (6): 495—543.

7. Harvie NR, Schultz JS. Studies of Lp-lipoprotein as a quantitative genetic trait. Proc Natl Acad Sci USA 1970; 66 (1): 99—103.

8. Kraft HG, Köchl S, Menzel HJ, Sandholzer C, Utermann G. The apolipoprotein (a) gene: a transcribed hypervariable locus controlling plasma lipoprotein (a) concentration. Hum Genet 1992; 90 (3): 220—230.

9. McLean JW, Tomlinson JE, Kuang W et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 1987; 330 (6144): 132—137.

10. Koschinsky ML, Beisiegel U, Henne-Bruns D, Eaton DL, Lawn RM. Apolipoprotein (a) size heterogeneity is related to variable number of repeat sequences in its mRNA. Biochemistry 1990; 29 (3): 640-644.

11. Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein (a) gene accounts for greater than 90 % of the variation in plasma lipoprotein (a) concentrations. J Clin Invest 1992; 90 (1): 52—60.

12. Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes. J Clin Invest 1987; 80 (2): 458—465.

13. Sandholzer C, Hallman DM, Saha N et al. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum Genet 1991; 86 (6): 607—614.

14. Mooser V, Mancini FP, Bopp S et al. Sequence polymorphism in the apo(a) gene associated with specific levels of Lp (a) in plasma. Hum Mol Genet 1995; 4 (2): 173—181.

15. Trommsdorff M, Köchl S, Lingenhel A et al. A pentanucleotide repeat polymorphism in the 5' control region of the apolipoprotein (a) gene is associated with lipoprotein(a) plasma concentrations in Caucasians. J Clin Invest 1995; 96: 150—157.

16. Zysow BR, Lindahl GE, Wade DP, Knight Bl, Lawn RM. C/T polymorphism in the 5' untranslated region of the apolipoprotein (a) gene introduces an upstream ATG and reduces in-vitro translation. Arterioscler Thromb 1995; 15: 58—64.

17. Utermann G. The mysteries of lipoprotein (a). Science 1989; 246 (4932): 904—910.

18. Wang XL, Wang J. Lipoprotein (a) in children and adolescence. Pediatr Endocrinol Rev 2003; 1(2): 109—119.

19. Kurvinen E, Aasvee K, Zordania R, Jauhiainen M, Sundvall J. Serum lipid and apolipoprotein profiles in newborns and six-year-old children: the Tallinn Young Family Study. Scand J Clin Lab Invest 2005; 65 (7): 541—550.

20. Gavish D, Azrolan N, Breslow JL. Plasma Lp(a) concentration is inversely correlated with the ratio of kringle IV/kringle V encoding domains in the apo(a) gene. J Clin Invest 1989; 84 (6): 2021—2027.

21. Rainwater DL. Genetic basis for multimodal relationship between apolipoprotein(a) size and lipoprotein(a) concentration in Mexican-Americans. Atherosclerosis 1995; 115 (2): 165—171.

22. Arisaka O, Fujiwara S, Miyake N, Mokuno H, Yabuta K. Lipoprotein (a) phenotypes in Japanese children: a cohort study. J Pediatr Gastroenterol Nutr 1997; 24 (5): 533—536.

23. Labudovic D, Todorova B, Rainwater D, Alabakovska S, Tosheska K. Apoprotein(a) isoform separation by 3—15 % gradient SDS denaturating polyacrylamide gel electrophoresis. Maked Med Preg 1999; 53 (3—4): 126—131.

24. Parent X, Hmad Z, Aberer P. Effect of storage of samples at -80°C on the assay of lipoprotein(a) by rate nephelometry (Beckman 360 Array). Ann Biol Clin 1995; 53 (1—2): 1—3.

25. Gaubatz JW, Ghanem KI, Guevara J Jr, Nava ML, Patsch W, Morrisett JD. Polymorphic forms of human apolipoprotein (a): inheritance and relationship of their molecular weights to plasma levels of lipoprotein (a). J Lipid Res 1990; 31 (4): 603—613.

26. **Tosheska K, Labudovic D, Alabakovska S, Todorova B.** Apoprotein(a) polymorphisms in a healthy Macedonian population. BJMG 2005; 8 (3—4): 49—55.

27. **Akanji AO**. Apo(a) isoforms do not predict risk for coronary heart disease in a Gulf Arab population. Ann Clin Biochem 2000; 37 (Pt 3): 360—366.

28. Min WK, Lee JO, Kim CH, Song J, Kim JQ. Identification of Apo(a) phenotypes in a Korean population using a standardized nomenclature system based on the number of kringle IV repeats. Ann Clin Biochem 1997; 34 (6): 681—687.

29. Taimela S, Viikari JS, Porkka KV, Dahlen GH. Lipoprotein(a) levels in children and young adults: the influence of physical activity. The cardiovascular Risk in young Finns study. Acta Paediatr 1994; 83 (12): 1258—1263.

30. Wilcken DE, Xing L Wang, Greenwood J, Lynch J. Lipoprotein (a) levels and apolipoproteins B and A-1 in children and coronary vascular events in their grandparents. J Pediatr 1993; 123 (4): 519—526.

31. Okada T, Sato Y, Yamakazi T et al. Lipoprotein (a) levels and apolipoprotein A-1 and B in schoolchildren whose grandparents had coronary and cerebrovascular events: a preliminary study of 12-13-year-old Japanese children. Acta Paediatr Jap 1995; 37 (5): 582—587.

32. Srinivasan SR, Dahlen GH, Jarpa RA, Webber LS, Berenson GS. Racial (black-white) differences in serum lipoprotein (a) distribution and its relation to parental myocardial infarction in children. Circulation 1991; 84 (1): 160—167.

Received September 11, 2006.
Accepted March 23, 2007.