RELATIONS FOR APO B/APO A-I RATIO WITH DIFFERENT CARDIOVASCULAR RISK FACTORS IN OBESE CHILDREN

ELENA DANIELA CĂŞĂRIU[*, **], *BOGDANA VÎRGOLICI*[**], *DANIELA LIXANDRU*[**], *MIHAELA STANCU*[***], *MARIA GREABU*[**], *ALEXANDRA TOTAN*[**], *DANIELA MIRICESCU* [**], *MARIA MOHORA*[**]

* S.C. CABINET DANAMED S.R.L.
**Department of Biochemistry, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, e-mail: mohoramaria@yahoo.com
*** National Sanitary Veterinary and Food Safety Agency

Abstract. Dyslipidemia in childhood predicts the development of atherosclerosis in young adults. The aim of this study is to investigate the relation of apoB/apoA-I ratio with different cardiovascular risk factors, in obese children. A total of 41 overweight obese children and 30 healthy children were enrolled. Correlations between apoB/apoA-I ratio with different cardiovascular risk factors: C reactive protein (CRP), blood pressure, dyslipidemia markers, gamma-glutamyl transferase activity were calculated. Ultrasounds were used for carotid intima-media thickness (IMT) and fatty liver estimation. All the studied parameters were higher in obese children *versus* normal subjects. ApoB/apoA-I ratio was correlated ($p < 0.05$) with γ-GT activity ($r = 0.35$), IMT ($r = 0.38$), CRP ($r = 0.59$), alanine aminotransferase activity ($r = 0.37$), waist circumference ($r = 0.44$) and diastolic blood pressure ($r = 0.33$), while apoA-I was inversely related to triglycerides ($r = –0.44$), CRP ($r = –0.39$) and waist circumference ($r = –0.42$). HDL-C was positively correlated with apoA-I ($r = 0.82$) and negatively with apoB/apoA-I ($r = –0.65$), while LDL-C was positively correlated with apoB ($r = 0.79$). The apoB/apo A-I ratio is higher in obese children than in normal children. The ratio is correlated with increased values of other cardiovascular risk factors and fatty liver markers.

Key words: obesity, children, apoB/apoA-I ratio, atherosclerosis, intima-media thickness, cardiovascular risk factors.

INTRODUCTION

Obesity itself appears to augment the incidence of cardiovascular events because it is associated with major risk factors for atherosclerosis including: hyperlipidemia, diabetes mellitus, hypertension, and metabolic syndrome [6, 11].

In order to evaluate the lipid-related risk factors for cardiovascular disease, high blood total cholesterol (TC), high triglycerides (TG), high low-density lipoprotein cholesterol (LDL-C) or low levels of high-density lipoprotein cholesterol (HDL-C) should be considered [10].

Received: September 2012;
in final form October 2012.

ROMANIAN J. BIOPHYS., Vol. 22, No. 2, P. 83–94, BUCHAREST 2012

The major lipoproteins have a variety of apoproteins associated with them, and these proteins have one or more functions. Each molecule of very low-density lipoprotein (VLDL), produced in the liver, contains only one molecule of apoB-100, and this apoprotein remains in the structure of lipoproteins during the transformation of VLDL to IDL and next to LDL [7]. ApoB is also a major component of Lipoprotein(a), (Lp(a)) that consists of an LDL molecule whose apoB-100 component is covalently bound by a disulfide bridge with apo(a), a glycoprotein homologous to plasminogen. The Lp(a) is an acute phase protein that can increase substantially after acute trauma or infection. ApoB-100 is formed in the liver and as it was mentioned, it is found in all potentially atherogenic lipoproteins (VLDL, VLDL remnants, IDL, Lp(a) and LDL)[15]. Hence, plasma concentration of apoB reflects the number of atherogenic particles [30] and its high value is associated with increased risk for cardiovascular diseases.

Apoprotein A-I (apoA-I) constitutes about 70% of the apolipoproteins of HDL and acts as the major antiatherogenic protein in the HDL particles. Plasma apoA-1 value is an alternative to HDL-C measurement in the blood [1].

More publications support the new concept that apoB and especially the apoB/apoA-I ratio may be superior to LDL concentration as predictors of cardiovascular disease risk [25, 27, 32]. Some data suggest that the apoB/apoA-I ratio reflects the balance of proatherogenic and antiatherogenic lipoproteins [28] and can be used as a target for lipid-lowering therapy [25].

Childhood levels of apoB/apoA-I ratio predict carotid artery intima-media thickness *(*IMT) and endothelial function in adulthood [14]. In middle-aged adults, apoB/apoA-I ratio is related to IMT value [22, 30] and is a strong predictor of coronary events [19, 29]. Thus, apoB/apoA-I ratio may be the best single lipoprotein variable related to coronary risk [19, 28].

The aim of this prospective study is to investigate the relation of apoB/apoA-I ratio with IMT and other cardiovascular risk factors, in obese children.

SUBJECTS AND METHODS

A total of 41 overweight children (22 boys and 19 girls) with a mean age of 13.32 ± 4.6 years and 30 healthy children and adolescents (16 boys and 14 girls) with normal weight were enrolled. Subjects were recruited from S.C. CABINET DANAMED S.R.L. Children under medications or those with chronic disease (endocrine disease, hereditary disease, or systemic inflammation) were excluded. All subjects were nonsmokers.

The study protocol was approved by the Ethical Commission of "Carol Davila" University of Medicine, Bucharest and a written informed consent was obtained from each parent.

CLINICAL CHARACTERISTICS

Anthropometric measurements: body mass index (BMI), waist/hip ratio (WHR), waist circumference (WC), hip circumference (HC) were assessed. BMI was calculated as body weight (kg) divided by square height (m^2). Overweight is defined as a BMI at or above the 85th percentile and under the 95th percentile. Obesity is defined as a BMI at or above the 95th percentile for children of the same age and gender [17]. Obese group was formed by overweight and obese children. Waist circumference (WC) was measured at the midway between the lower rib and the iliac crest and hip circumference was measured at the widest part at the gluteal region. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10-minute rest, in the supine position using an automated sphygmomanometer.

BIOCHEMICAL MEASUREMENTS

Blood samples were taken after an overnight fast. Metabolic markers: fasting serum glucose, serum lipids (total cholesterol, HDL-cholesterol, triglycerides), serum creatinine, urea, uric acid and enzymes activities for alanine aminotransferase (ALT) and for γ–Glutamyl transferase (γ-GT) were performed by standard methods using an automatic analyzer HITACHI and kits from Diasys (Germany). LDL-cholesterol was calculated according to the Friedewald equation [8].

Enzymes activities for alanine aminotransferase (ALT) and for γ-Glutamyl transferase (γ-GT) were performed by standard methods using an automatic analyzer HITACHI and kits from Diasys (Germany). C peptide was measured by using Human C-Peptide ELISA Kit (Diametra Italy). The ALT activity and liver ultrasounds were evaluated as indicators of fatty liver due to obesity.

ULTRASOUND MEASUREMENTS

Carotid intima media thickness (IMT) was measured by B-mode ultrasound using a 10 MHz linear transducer (echograph Chison 600 M). IMT was measured at the common carotid artery near the bifurcation, during end diastole. At least three measurements were taken approximately 10 mm proximal to the bifurcation and the mean value was used for statistical purposes [14].

STATISTICAL ANALYSIS

The source of variation between the control group and the obese subjects was assessed by the unpaired Student t-test. The associations of variables were examined by Pearson correlation.

RESULTS

The clinical characteristics and routine biochemistry of the healthy children and adolescents with normal weight and overweight children of school age are shown in Table 1. No differences in age and gender were noticed between the control and the obese groups. Higher values for anthropometric parameters (weight, BMI, WC, HC, WHR), for plasma triglycerides, uric acid, apoB, apoB/apoA ratio, total cholesterol, LDL-cholesterol, blood pressure, creatinine and ALT activity were measured in overweight children *versus* lean children.

Table 1

Clinical characteristics and biochemical important determinations in the studied groups

Parameters	**Control group (*n* = 30)**	**Obese group (*n* = 41)**	**Obese *versus* control *(p)***
Age (10–18 years)	14.03± 2.09	13.32 ± 4.6	Ns
Sex (F/M)	14/16	19/22	Ns
Weight (kg)	44.82 ± 6.14	73.55±19.7	<0.001
Height (m)	1.62± 0.07	1.52 ± 0.18	Ns
BMI (kg/m^2)	18.81 ± 1.7	30.2 ± 2.4	<0.001
WC (cm)	67.4 ± 6.6	91.75 ± 9.2	<0.001
HC (cm)	83.46 ± 7.1	99.26 ± 15.22	<0.001
WHR	0.8 ± 0.07	0.92 ± 0.05	<0.001
Uric Acid (mg/dL)	4.48 ± 0.5	5.8 ± 1.4	<0.04
γ-GT (UI/liter)	13.38 ± 1.3	16.48 ± 5.0	<0.05
Glycemia (mg/dL)	85.2 ± 3.4	86.25± 7.5	Ns
Total cholesterol (mg/dL)	134.46± 16,3	177.25± 34.37	<0.01
HDL-C (mg/dL)	50.55±12.7	53.4±9.7	Ns
LDL-C (mg/dL)	81.1± 6.8	104.23±21.7	<0.03
ALT (UI/liter)	13.13 ± 0.24	18.84± 7.57	<0.01
Triglycerides (mg/dL)	68.4±28.7	108.8±54.3	<0.001
SBP (mmHg)	97.3 ± 5.2	110.97 ± 7.08	<0.001
DBP (mmHg)	59.25 ± 2.6	72.58 ± 6.17	<0.001
IMT(mm)	0.41 ± 0.03	0.506 ± 0.06	<0.01
Albumin (g/dL)	4.16±0.35	3.93±0.31	Ns
Albumin/globulin ratio	1.47±0.19	1.26±0.15	<0.006
Creatinine (mg/dL)	0.87±0.15	0.75±0.16	<0.03
Bilirubin (mg/dL)	0.55±0.29	0.5±0.31	Ns
apoA (g/liter)	1.18±0.05	1.34±0.26	<0.03
apoB (g/liter)	0.52±0.03	0.74±0.15	<0.001

Table 1 (continued)

apoB/apoA	0.43±0.04	0.58±0.2	<0.01
Fibrinogen (g/liter)	3.23±0.12	3.28±0.16	Ns
CRP mg/dL	0.95±0.5	2.3±2.10	Ns
C peptide ng/mL	1.15±0.7	1.92±1.23	<0.01

Data are expressed as means ± SD. *BMI* = body mass index; *WC* = waist circumference; *HC*=hip circumference; *WHR* = waist to hip ratio; *ALT* = alanine aminotransferase; γ–Glutamyl transferase *(γ-GT*); *HDL-C* = high-density lipoprotein-cholesterol; *LDL-C* = low-density lipoprotein-cholesterol; *TG*-triglycerides; *SBP* = systolic blood pressure; *DBP* = diastolic blood pressure; *IMT* = carotid artery intima-media thickness; *CRP* = C reactive protein.

In order to determine the association of dyslipidemia with other cardiovascular risk factors, we performed correlations between apoB/apoA-I ratio with different markers. In our obese group, univariate analysis showed statistically significant ($p < 0.05$) positive correlations of apoB/apoA-I ratio with: ALT ($r = 0.37$), γ-GT($r = 0.35$), IMT ($r = 0.38$), CRP ($r = 0.59$), WC ($r = 0.45$), HC ($r = 0.42$), weight ($r = 0.45$) and a negative correlation with HDL-C ($r = –0.65$).

Positive correlations were observed between apoB and: total cholesterol ($r = 0.79$), LDL-C ($r = 0.80$), ALT ($r = 0.55$), IMT ($r = 0.45$), glycemia ($r = 0.43$), albumin ($r = 0.34$), α-1 globulins ($r = 0.39$), α-2 globulins ($r = 0.37$) and β-globulins ($r = 0.47$).

Negative correlations were observed between apoA with: apoB/apoA-I ratio ($r = –0.66$), CRP ($r = –0.39$), WC ($r = –0.43$), HC ($r = –0.46$), weight ($r = –0.48$), and positive correlations between apoA-I with α-1 globulins ($r = 0.40$), α-2 globulins ($r = 0.56$), total cholesterol ($r = 0.66$) and HDL-C ($r = 0.82$).

DISCUSSION

Excessive amount of adipose tissue in children and adolescents represents a growing health problem throughout the world. Obesity in childhood is associated with an increased mortality due to cardiovascular disease in adulthood [20].

Childhood obesity is associated with insulin resistance, inflammation, elevated blood pressure and higher levels for: total cholesterol, LDL-C, triglycerides and lower concentrations for HDL-C [9]. All these biochemical changes contribute to atherosclerosis which starts at an early age in obese children [2].

It is known that IMT value is a marker for atherosclerosis and predicts future cardiovascular disease [33]. In our study we measured IMT and other risk factors for cardiovascular disease and we demonstrated that their values have been already altered in obese children and adolescents. According to our results, the association of apoB/apoA ratio with waist circumference ($r = 0.44$), and with carotid IMT values ($r = 0.38$), may explain the link of dyslipidemia with visceral obesity and with early atherosclerosis (Fig. 1 and Fig. 2).

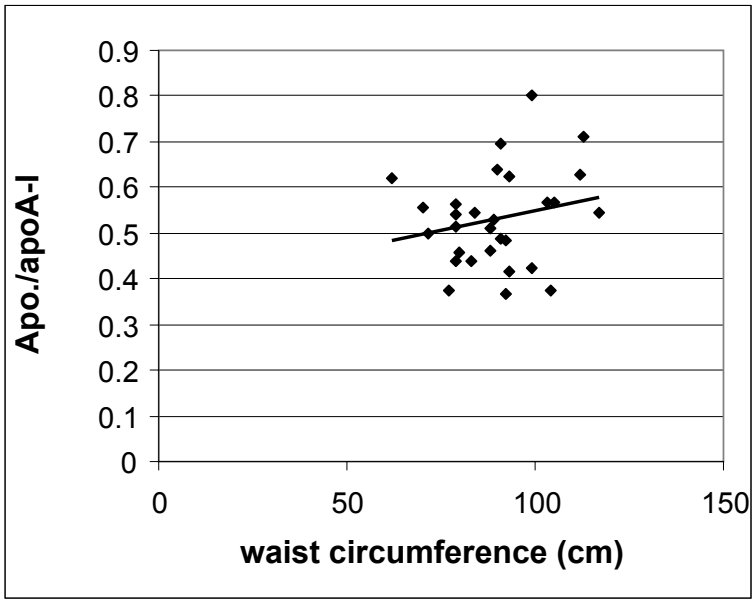

Fig. 1. Relation between apoB/apoA-1 and waist circumference.

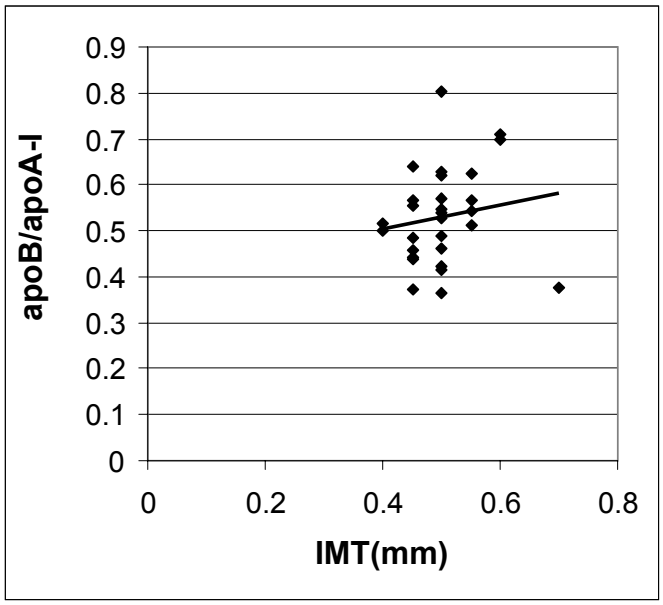

Fig. 2. Relation between apoB/apoA-I and IMT.

In Lee S. *et al*. study, waist circumference (which is strongly associated with visceral fat) was found to be an independent predictor of insulin resistance [16]. Visceral fat is associated with high plasma levels for inflammatory markers which determine triglycerides storage formation in non-adipose tissues (ectopic places). Also, visceral fat is more easily mobilized than subcutaneous fat and so, it is associated with higher plasmatic values for free fatty acids [18]. All these modifications have an important contribution for insulin resistance in obesity. In our study, plasma C peptide concentration (a marker for insulin resistance) was higher in the obese group and it was correlated with triglycerides level (r = 0.35), demonstrating the relation between dyslipidemia and insulin resistance.

Low-grade systemic inflammation and dyslipidemia may also explain the link between obesity and endothelial dysfunction. Adipose tissue, especially visceral fat, is known to produce several pro-inflammatory molecules, such as adipocytokines and acute-phase reactants. Concentrations of high-sensitive C-reactive protein (hs-CRP) and soluble adhesion molecules have been found to be higher in obese than in lean children [5]. In our study, univariate analysis showed a statistically significant positive correlation of apoB/apoA-I ratio with CRP (r = 0.59) (Fig. 3) and a negative correlation of apoA-I with CRP (r = –0.39). The predictive role of CRP and apoB/apoA-I ratio in the earliest stage of atherosclerosis remains to be studied in obese children.

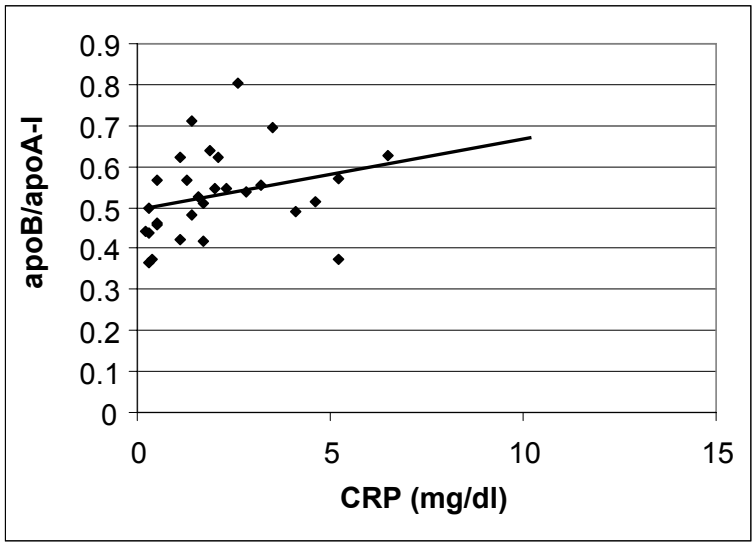

Fig. 3. Relation between apoB/apoA-I and CRP.

In the Insulin Resistance Atherosclerosis Study [31], the researchers compared the associations of apoB and LDL-C with a wide array of measures of cardiovascular risk factors and finally they suggested that apoB is a better predictor

of vascular risk than LDL-C [31]. In our study, plasma apoB was positively correlated with vascular risk factors such as: cholesterol (r = 0.79), LDL-C (r = 0.80) and IMT(r = 0.45), while apoB/apoA-I was negatively correlated with HDL (r = –0.65) (Fig. 4).

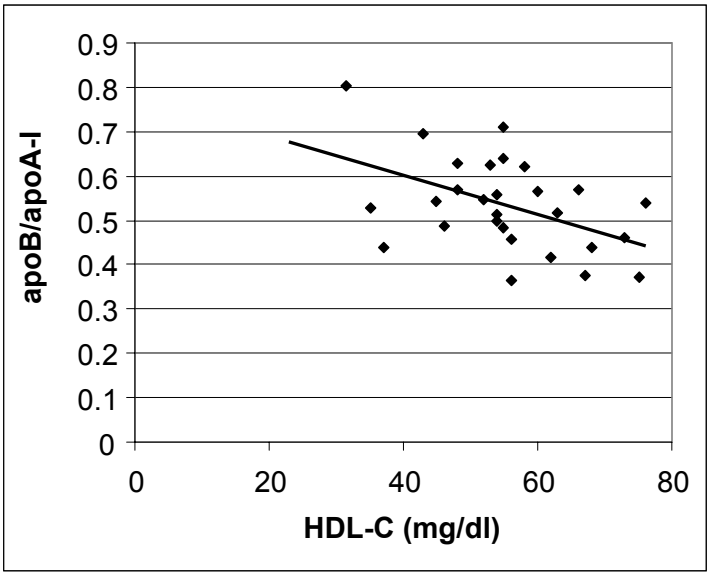

Fig. 4. Relation between apoB/apoA-I and HDL-C.

It was demonstrated that increased production of small dense LDL particles in obese subjects with metabolic syndrome is related to an increased influx of fatty acids to the liver, insulin resistance and hyperinsulinemia [3, 12]. It seems that insulin resistance precedes the development of the metabolic syndrome in obese children [4]. Dyslipidemia and insulin resistance have additive effects for cardiovascular disease risk. In this study we demonstrated that apoB was correlated with fasting glycaemia (r = 0.43).

Small dense LDL particles may have increased atherogenic potential because of their low affinity to LDL receptors, prolonged plasma half-life time and high penetration of the intima. γ-GT can catalyze the oxidation of low-density lipoprotein (LDL) augmenting the development of atherosclerosis [24]. γ-GT is present on the outer surface of plasma membrane of most cell types and in blood, where it has been shown to form complexes with several plasma components, in particular with albumin and lipoproteins [13]. The enzyme is expressed in the atheromatous core of coronary plaques, where it colocalizes with oxidized LDL and foam cells [23]. In our study γ-GT levels were positively correlated with apoB/apoA-I ratio (r = 0.35) (Fig. 5).

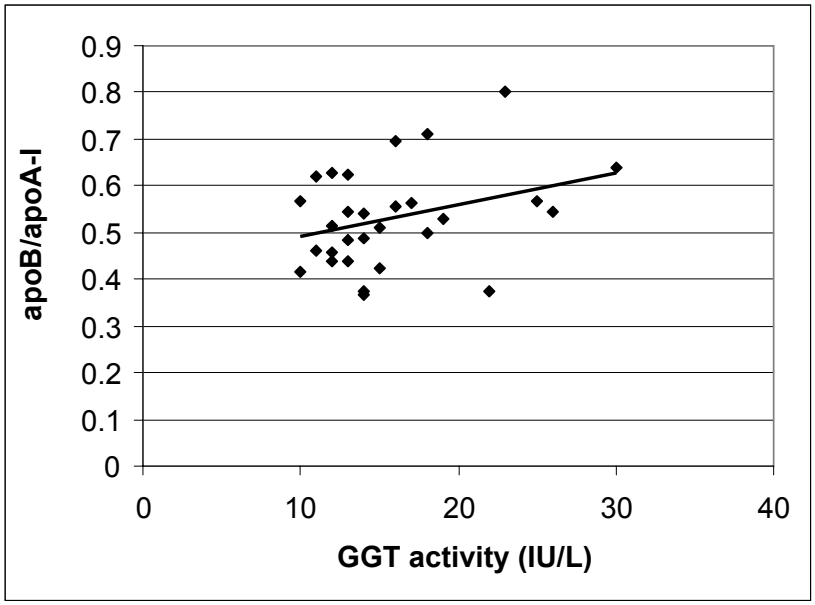

Fig. 5. Relation between apoB/apoA-I and GGT activity.

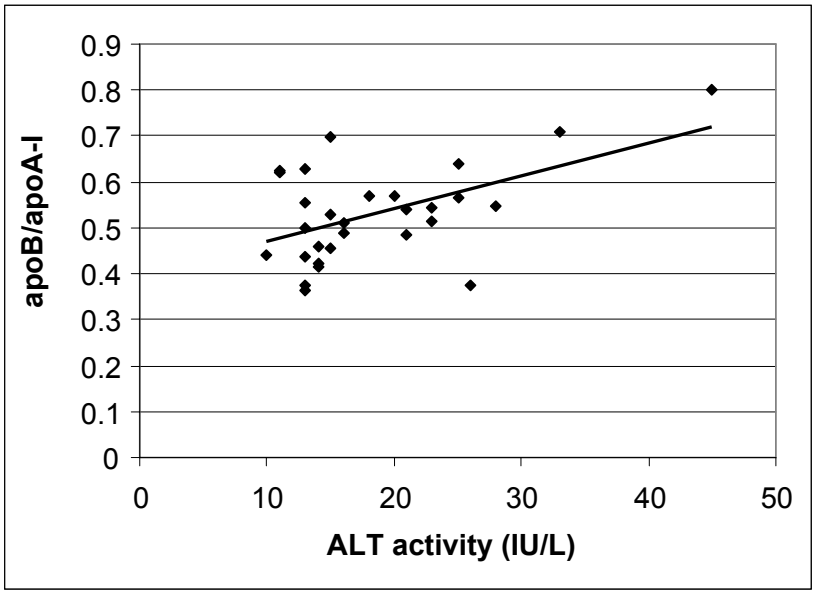

Fig. 6. Relation between apoB/apoA1 and ALT activity.

Non alcoholic fatty liver disease (NAFLD) is associated with increased prevalence of cardiovascular disease [21]. NAFLD should be suspected in overweight and obese children older than 3 years with increased waist circumference especially if there is a NAFLD history in relatives. Abdominal ultrasound and liver function tests (including ALT), followed by exclusion of other liver diseases, serve as a diagnostic tool for non alcoholic fatty liver disease [26]. The average ALT activity measured in our obese group was in normal range but much higher than in the lean group. Also, in the obese group, the ALT activity correlated with apoB/apoA-I ratio (r = 0.37) (Fig. 6) and ultrasounds images suggested fatty liver disease.

CONCLUSIONS

The apoB/apoA-I ratio is higher in obese children than in lean children and the ratio is correlated with increased values of other cardiovascular risk factors (IMT, CRP, waist circumference, γ-GT, diastolic blood pressure and NAFLD). These correlations may help physicians to understand and prevent comorbidities associated with childhood obesity.

Acknowledgements. Bogdana Vîrgolici and Daniela Lixandru acknowledge the postdoctoral program POSDRU/89/1.5/S/60746, from European Social Fund.

REFERENCES

1. BARTER, P.J., K.A. RYE, The rationale for using apoA1 as a clinical marker of cardiovascular risk, *J. Intern. Med.*, 2006, **259**, 447– 454.
2. BERENSON, G.S., S.R. SRINIVASAN, W. BAO, W.P. NEWMAN III, R.E. TRACY, W.A. WATTIGNEY, Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study, *N. Engl. J. Med.,* 1998, **338**, 1650–1656.
3. BLAHA, M.J., R.S. BLUMENTHAL, E.A. BRINTON, T.A. JACOBSON, The importance of non–HDL cholesterol reporting in lipid management, *J. Clin. Lipid.*, 2008, **2**, 267–273.
4. CRUZ, M.L., M.I. GORAN, The Metabolic Syndrome in Children and Adolescents, *Current Diabetes Reports,* 2004, **4**, 53–62.
5. DESIDERI, G., M. DE SIMONE, L. IUGHETTI, T. ROSATO, M.L. IEZZI, M.C. MARINUCCI, V. COFINI, G. CROCE, G. PASSACQUALE, S. NECOZIONE, C. FERRI, Early activation of vascular endothelial cells and platelets in obese children, *J. Clin. Endocrinol. Metab.,* 2005, **90**, 3145–3152.
6. ECKEL, R.H., W.W. BAROUCH, A.G. ERSHOW, Report of the National Heart, Lung, and Blood Institute – National Institute of Diabetes and Digestive and Kidney Diseases Working Group on the pathophysiology of obesity associated cardiovascular disease, *Circulation,* 2002, **105**, 2923–2928.
7. ELOVSON, J., J.E. CHATTERTON, G.T. BELL V.N. SCHUMAKER, M.A. REUBEN, D.L. PUPPIONE, J.R.JR. REEVE, N.L. YOUNG, Plasma very low density lipoproteins contain a single molecule of apolipoprotein B, *J. Lipid. Res*., 1998, **29**, 1461–73.

8. FRIEDEWALD, W.T., R.I. LEVY, D.S. FREDICKSON, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, *Clin. Chem.,* 1972, **18**, 499–502.
9. GRUNDY, S.M., J.I. CLEEMAN, S.R. DANIELS, K.A. DONATO, R.H. ECKEL, B.A. FRANKLIN, D.J. GORDON, R.M. KRAUSS, P.J. SAVAGE, S.C.JR. SMITH, J.A. SPERTUS, F. COSTA, Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statemen, *Circulation,* 2005, **112,** 2735–2752.
10. GRUNDY, S.M., Low-density lipoprotein, non-high-density lipoprotein, and apolipoprotein B as targets of lipid-lowering therapy, *Circulation*, 2002, **106**, 2526–2529.
11. GRUNDY, S.M., Obesity, metabolic syndrome and coronary atherosclerosis, *Circulation,* 2002, **105**, 2696–2698.
12. GUNGOR, N., R. SAAD, J. JANOSKY, S. ARSLANIAN, Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents, *J. Pediatr*., 2004, **144**, 47–55.
13. HUSEBY, N.E., Multiple forms of serum gamma-glutamyltransferase. Association of the enzyme with lipoproteins, *Clin. Chim. Acta,* 1982, **124**, 103–112.
14. JUONALA, M., J.S. VIIKARI, M. KÄHÖNEN, T. SOLAKIVI, H. HELENIUS, A. JULA, J. MARNIEMI, L. TAITTONEN, T. LAITINEN, T. NIKKARI, O.T. RAITAKARI, Childhood levels of serum apolipoproteins B and A-I predict carotid intima-media thickness and brachial endothelial function in adulthood: the cardiovascular risk in young Finns study, *J. Am. Coll. Cardiol.,* 2008, **52**, 293–299.
15. KWITEROVICH, P.O., *The Johns Hopkins Textbook of Dislipidemia*, Lippincott Williams@Wilkins, Philadelphia, PA 19106, USA, 2010, pp.1–20.
16. LEE, S., F. BACHA, N. GUNGOR, S.A. ARSLANIAN, Waist circumference is an independent predictor of insulin resistance in black and white youths, *J. Pediatr.,* 2006, **148**, 188–194.
17. MANCINI, M.C., Metabolic syndrome in children and adolescents-criteria for diagnosis, *Diabetol. Metab. Syndr.*, 2009, **1**, 20.
18. MARSHALL, W.J., S.K. BANGERT, *Clinical Biochemistry, Metabolic and clinical aspects*, second edition*,* Churchill Livingstone, Elsevier, 2008.
19. MCQUEEN, M.J., S. HAWKEN, X. WANG, S. OUNPUU, A. SNIDERMAN, J. PROBSTFIELD, K. STEYN, J.E. SANDERSON, M. HASANI, E. VOLKOVA, K. KAZMI, S. YUSUF, Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study, *Lancet*, 2008, **372**, 224–233.
20. MUST, A., P.F. JACQUES, G.E. DALLAL, C.J. BAJEMA, W.H. DIETZ, Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard Growth Study of 1922 to 1935, *N. Engl. J. Med.,* 1992, **327**, 1350–1355.
21. PACIFICO, L., V. NOBILI, C. ANANIA, P. VERDECCHIA, C, CHIESA, Pediatric nonalcoholic fatty liver disease, metabolic syndrome and cardiovascular risk, *World J. Gastroenterol.*, 2011, **14, 17(26)**,3082–3091.
22. PANAYIOTOU, A., M. GRIFFIN, N. GEORGIOU, D. BOND, T. TYLLIS, C. TZIAKOURI-SHIAKALLI, C. FESSAS, A. NICOLAIDES, ApoB/ApoA1 ratio and subclinical atherosclerosis, *Int. Angio.,* 2008, **27**, 74–80.
23. PAOLICCHI, A., M. EMDIN, E. GHLIOZENI, E. CIANCIA, C. PASSINO, G. POPOFF, A. POMPELLA, Images in cardiovascular medicine. Human atherosclerotic plaques contain gamma-glutamyl transpeptidase enzyme activity, *Circulation*, 2004, **109**, 1440.
24. PAOLICCHI, A., M. EMDIN, C. PASSINO, E. LORENZINI, F. TITTA, S. MARCHI, G. MALVALDI, A. POMPELLA, Beta-lipoprotein- and LDL-associated serum gamma-glutamyltransferase in patients with coronary atherosclerosis, *Atherosclerosis,* 2006, **186**, 80–85.
25. SNIDERMAN, A.D., C.D. FURBERG, A. KEECH, J.E. ROETERS VAN LENNEP, J. FROHLICH, I. JUNGNER, G. WALLDIUS, Apolipoproteins versus lipids as indices of coronary risk and as targets for statin therapy treatment, *Lancet,* 2003, **361**, 777–780.

26. VAJRO, P., S. LENTA, P. SOCHA, A. DHAWAN, P. MCKIERMAN, U. BAUMANN, O. DURMAZ, F. LACAILLE, V. MCLIN, V. NOBILI, Diagnosis of nonalcoholic fatty liver disease in children and adolescents:position paper of the ESPGNIAN hepatology committee, *J. Pediatr. Gastroenterol. Nutr*., 2012, **54**, 700–713.
27. WALLDIUS, G., A.H. AASTVEIT, I. JUNGNER, Stroke mortality and the apoB/apoA1 ratio: results of the AMORIS prospective study, *J. Intern Med*., 2006, **259**, 259–266.
28. WALLDIUS, G., I. JUNGNER, A.H. AASTVEIT, I. HOLME, C.D. FURBERG, A.D. SNIDERMAN, The apoB/apoA1 ratio is better than the cholesterol ratios to estimate the balance between plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk, *Clin. Chem. Lab. Med.,* 2004, **42**, 1355–1363.
29. WALLDIUS, G., I. JUNGNER, I. HOLME, A.H. AASTVEIT, W. KOLAR, E. STEINER, High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study, *Lancet*, 2001, **358**, 2026–2033.
30. WALLENFELDT, K., L. BOKEMARK, J. WIKSTRAND, J. HULTHE, B. FAGERBERG, Apolipoprotein B/apolipoprotein A-I in relation to the metabolic syndrome and change in carotid artery intima-media thickness during 3 years in middle-aged men, *Stroke*, 2004, **35**, 2248–2252.
31. WILLIAMS, K., A.D. SNIDERMAN, N. SATTAR, R.JR. D'AGOSTINO, L.E. WAGENKNECHT, S.M. HAFFNER, Comparison of the associations of apolipoprotein B and low-density lipoprotein cholesterol with other cardiovascular risk factors in the Insulin Resistance Atherosclerosis Study (IRAS), *Circulation,* 2003, **108**, 2312–2316.
32. YUSUF, S., S. HAWKEN, S. OUNPUU, *et al.*, Obesity and the risk of myocardial infarction in 52 countries a case-control study, *Lancet*, 2005, **366**, 1640–1649.
33. ZHU, W., X. HUANG, J. HE, M. LI, H. NEUBAUER, Arterial intima-media thickening and endothelial dysfunction in obese Chinese children, *Eur. J. Pediatr.,* 2005, ***164,*** 337–344.