Original article

New Criteria in Defining the Metabolic Syndrome in Children? – An Analysis of the Relationship Between the Hepatic Enzymes and the Insulin Resistance, HOMA-IR And Lipid Parameters in the Obese Children

Ana-Maria Pelin[1], Gabriela Balan[2], Cristina Stefanescu[3], Stefan Rosca[4], Camelia Busila[2]

[1] Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Department of Pharmaceutical Sciences, Galati, Romania; [2]Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Clinical Medical Department, Galati, Romania; [3] Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Departament of Dentistry, Galati, Romania; [4] Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Clinical Medical Department, Galati, Romania

Authorship: All authors have contributed equally to the study

Summary. *Background :* The purpose of the study was aimed towards ascertaining whether the fat non-alcoholic fatty liver disease (NAFLD) that manifested through raised ALT and AST values could be considered a hepatic manifestation of metabolic syndrome in children. *Methods:* A number of 108 children aged between 7 and 18 years old (12.89±2.93), with body mass index - BMI > 25 kg/m^2 were studied, who underwent metabolic syndrome parameters' assessments. Determinations of blood pressure, abdominal perimeter, fasting glucose, insulinemia were made, the HOMA-IR index (Homeostatic model assessment for insulin resistance) was calculated. The value of the HOMA-IR index between 2-4 was considered as glucose tolerance decrease and HOMA-IR>4 was considered diabetes for both sexes. We also made laboratory determinations of cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides. We correlated the identified groups with diabetes mellitus and the glucose tolerance decrease according to HOMA-IR with the values of liver enzymes AST (aspartataminotransferase) and ALT (alaninaminotransferase). *Results:* The calculated HOMA-IR showed, in 53.7% of the obese children, the glucose tolerance decrease, (HOMA-IR > 2). The individual level of the HOMA-IR index was significantly lower at older ages, the correlation was indirect, moderate in intensity (r = - 0.217; p=0.024), suggesting that approximately 22% of children have a lower HOMA-IR level at older ages. The following are confirmed as good predictors of diabetes: LDL cholesterol (AUC=0.632; IC95%: 0.526-0.738; p=0.018), ALT (AUC=0.645; IC95%: 0.541-0.750; p=0.009) and AST (AUC=0.617; IC95%: 0.510-0.724; p=0.036). The HDL cholesterol parameter proves to be a good predictor of decreased glucose tolerance (AUC=0.607; IC95%: 0.469-0.745). *Conclusions:* The increased liver enzymes (mainly AST, but also ALT) and LDL cholesterol are positively associated with the prevalence of the metabolic syndrome in paediatric populations and with diabetes mellitus. Although there is a correlation between AST, ALT and LDL-cholesterol and decreased glucose tolerance or diabetes mellitus, a larger batch analysis of obese children is required to determine whether liver enzymes and LDL-cholesterol can be introduced as diagnostic criteria of the metabolic syndrome in obese children.

Key words: obesity, child, glucose tolerance decrease, hepatic enzymes

Introduction

The liver is the main metabolic organ in the human body and its normal function is essential both for the children's development and to prevent the future risk of cardiovascular diseases and other metabolic disorders (1). Non-alcoholic fatty liver disease (NAFLD), is one of the most common liver diseases in the children with particular risk factors, including obesity, metabolic syndrome, sedentary lifestyle

and/or predisposing genetics (2). Insulin resistance, central adiposity, which is the accumulation of fat in the visceral organs, plays a specific contribution in determining the disease risk (3,4). We define the metabolic syndrome – MetS, by using the criteria of F.I.D. (International Diabetes Federation), W.H.O (World Health Organisation) and N.C.E.P. AT.P.III (National Cholesterol Education Program - Adult Treatement Panel lII), modified for children, but none of these criteria included the values of the liver enzymes (5). Several clinical and experimental studies suggested that the non-alcoholic fatty liver (NAFLD), which mainly varies from the pure fatty liver to non-alcoholic steatohepatitis (NASH) and cryptogenic cirrhosis, is simply the hepatic manifestation of the metabolic syndrome (6). The prevalence of MetS ranges from 6 to 39% depending on the applied definition criteria (7). The individual's risk for developing MetS is influenced by genetics and epigenetics (gestational programming and epigenetic inheritance) and is associated with the birth weight and early return of adiposity (8,9,10). Over the past decade, studies have shown that, in parallel with the increased prevalence of obesity in the paediatric population, NAFLD has become the most common form of liver disease during childhood (11). In fact, its prevalence has doubled in the last 20 years (12). The overall prevalence in children reached approximately 10%, growing up to 17% in adolescents and 40% -70% in obese children. (13). The development of NAFLD is strongly influenced by age, gender, race and ethnicity (11,12, 14). NAFLD and MetS are closely related, so NAFLD has been described as the hepatic expression of MetS (15,16,17), insulin resistance being the engine of pathogenesis. A recent study reported that 66% of investigated children with NAFLD, proven with biopsy, had MetS. Specifically, 63% had hypertriglyceridemia, 45% had low HDL cholesterol, 40% suffered from hypertension, and 10% had impaired glucose tolerance. In addition, an association between the disease's histological severity and some components of the MetS have been reported (18). Despite recent advances in understanding of paediatric NAFLD, the natural history and consequences of this condition are still unclear (19). MetS does not have very well established diagnostic criteria in children precisely because they can change depending on whether or not they maintain the status of obese with age. The pathogenic link between MetS and NAFLD represented by insulin resistance makes it necessary to evaluate liver function in obese children diagnosed with MetS.

Material and methods

The retrospective study was carried out on 108 obese children with ages ranging from 7 to 18 years old (12.89±2.93), with a body mass index BMI> 25 kg/m^2 (BMI= body weight in kg / height in square meters**,** overweight IMC > 25 kg/m^2 , obesity> 30 kg/m^2 . Only children with exogenous obesity were included in the study group and we excluded patients who were already diagnosed with liver disease, diabetes, or decreased glucose tolerance. The retrospective study was conducted according to the declaration of Helsinki II and approved by the Ethics Committee of the Emergency Hospital for Children "Sfantul Ion" Galati. Data related to weight and height were collected from the patients' files and the body mass index was calculated. Evaluation of the insulin resistance homeostasis (HOMA-IR) model was calculated using the formula: glucose (mg / dL) × insulinemia / 405. HOMA-IR limit values for insulin resistance were defined as <2 normal, 2 -5 decreased glucose tolerance and> 5 diabetes.

Metabolic syndrome (MetS) has been diagnosed according to the criteria of the International Diabetes Federation (IDF): • Abdominal obesity: PA (abdominal perimeter) white race: -> 94 cm men -> 80 cm women Plus two of the criteria: • BT> 1.7 mmol / L (≥150 mg / dl), or treatment of dyslipidemia • HDL <1.3 mmol / L in women (<45 mg / dl) • BP> 130/85 mmHg or medication • fasting blood glucose> 5.6 mmol / L (100 mg / dl) or diagnosis of diabetes Data were also collected from patient records: TGO, TGP, blood glucose, insulinemia, HDL-cholesterol, triglycerides, abdominal circumference and blood pressure. In data processing we used the Pearson correlation coefficient (r) with the significance level (p) and the Skewness test to determine whether the batch distributions meet the normal condition and can be extrapolated to the general population. The Skewness and Kurtosis tests (-2 <p <2) are tests that measure the normality of the value series, in order to be able to

determine whether or not the variables are continuous. If the values are homogeneous, tests of significance at mean ± standard deviation (SD) may be applied: - t-Student test - parametric test that compares the average values recorded in 2 groups with normal distributions; - Paired Samples T test used in which the average level is compared for 2 estimates of the same variable in groups with normal distributions.

Results

Study lot - features

A number of 108 children with BMI >25 kg/m^2, 19 overweight and 89 obese were introduced, the sex ratio being 1/1, with ages ranging from 7 to 18 years, the average level being of approximately 13 years. The initial evaluation of the study group shows a percentage of 7.4% of the children had an initial glycaemia over 100mg/dl, and 21.3% insulinemia over 24.9 μU/ml. Liver enzymes had above normal levels in 9.3% (ALT) and 13.9% (AST), 61,1% had HDL cholesterol > 45 md/dl, 73,1% had higher triglyceride levels, 50% had cholesterol higher 170mg/dl and 38% has LDL-cholesterol higher 110mg/dl.

Lipid and hepatic parameters in relation to the obese status

Higher triglyceride levels (RR=1.96; IC95%: 0.62-6.23; p=0.622104), total cholesterol (RR=1.71; IC95%: 0.73-4.02; p=0.204) and LDL cholesterol (RR=1.47; IC95%: 0.65-3.31; p=0.357) highlights a higher estimated risk in the overweight children. Lower HDL cholesterol levels (RR=3.39; IC95%: 0.65-3.31; p=0.357) highlights an estimated risk more than 3 times higher in the overweight children. ALT and AST had highest levels in obese children (table 1).

HOMA-IR Index

HOMA-IR (Homeostatic model assessment for insulin resistance) calculated using fasting insulin

Table 1. Correlation of lipid and hepatic markers with obesity

Parameters	Obese (n=89)	Overweight (n=19)	Chi2 test P	Rr	IR95%
Total cholesterol, mg/dl					
Increased	42 (47.2%)	12 (63.2%)	0.204	1.71_S	0.73-4.02
Normal	47 (52.8%)	18 (36.8%)			
LDL cholesterol, mg/dl					
Increased	32 (36.0%)	9 (47.4%)	0.357	1.47_S	0.65-3.31
Normal	57 (64.0%)	10 (52.6%)			
HDL cholesterol, mg/dl					
Low	50 (56.2%)	16 (84.2%)	**0.016**	3.39_S	1.05-10.94
Normal	39 (43.8%)	3 (15.7%)			
ALT, UI/l					
Increased	8 (9.0%)	2 (10.5%)	0.836	1.15_S	1.31-4.29
Normal	81 (91.0%)	17 (89.5%)			
AST, UI/l					
Increased	14 (15.7%)	1 (5.3%)	0.187	1.16_O	0.05-2.39
Normal	75 (84.3%)	18 (97.4%)			
Triglycerides, mg/dl					
Increased	63 (70.8%)	16 (84.2%)	0.210	1.96_S	0.62-6.23
Normal	26 (29.2%)	3 (15.8%)			

S – risk for the overweight O – risk for the obese

concentration (mg/dl) x fasting glucose concentration (mg/dl)/405 revealed in 21.3% of total diabetes study group (HOMA-IR > 5), and in 53.7% of children the decrease in glucose tolerance (HOMA-IR > 2) (Figure 1). The percentage distribution of cases with diabetes (15.8% *vs.* 22.5%) and insulin resistance (52.6% *vs* 53.9%) was slightly higher in obese children, but differences in weight status were not statistically significant (p=0.693) (Figure 2).

Cases with insulin resistance (59.3% *vs* 48.1%) predominate in both male and female patients, but it should be noted that in the female sex the share of normal cases was 31.5% vs. 18.5%, but the percentage differences were not statistically significant (p=0.286).

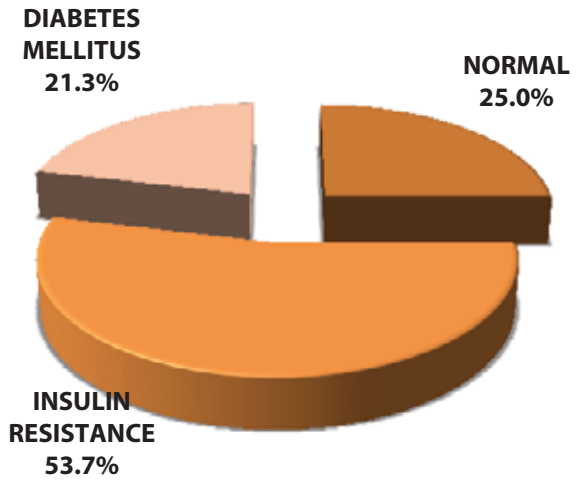

Figure 1. Case distribution depending on HOMA-IR level

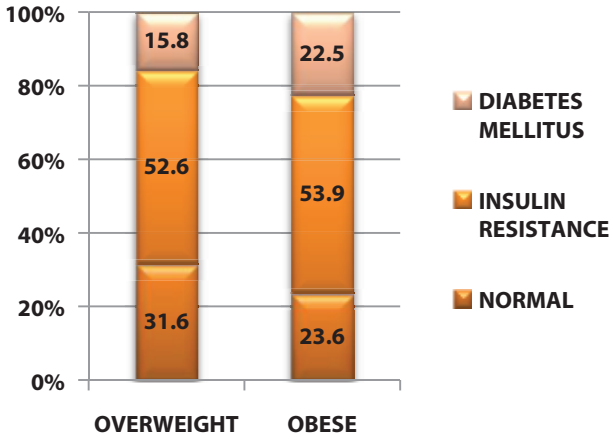

Figure 2. Case distribution depending on the HOMA-IR level and weight status

The individual level of the HOMA-IR index was significantly lower at older ages, the correlation was indirect, moderate in intensity (r = - 0.217; p=0.024), suggesting that approximately 22% of children had a lower HOMA-IR level at older ages (Figure 3)

In the patients with decreased glucose tolerance or diabetes, the average LDL cholesterol levels were significantly higher compared to the patients with normal HOMA-IR levels (93.14 *vs* 107.01 and 100.09 mg/dL respectively; p=0.05), and ALT levels were significantly lower in patients with diabetes than those with decreased or normal glucose tolerance (22.86 *vs* 28.39 and 25.15 mg/dL, respectively; p=0.036) (table 2).

The high level of total cholesterol (50% *vs* 43.5%; p=0.596), LDL (50% *vs* 34.8%; p=0.212) and HDL cholesterol (62.1% *vs* 47.8%; p=0.234), as well as AST (17.2% *vs* 8.7%; p=0.307) was not significantly different depending on the insulin resistance or the diabetes mellitus status (table V). Increased ALT was encountered only in 15.5% of the patients with insulin resistance (p=0.011) (table IV). Both in the patients with the glucose tolerance decrease and those with diabetes mellitus, the level of triglycerides over the reference limits was noticed in more than 70% of the children (74.1% vs 70.4%; p=0.709) (table 3).

By tracing the ROC curve, the following are confirmed as good predictors of diabetes mellitus: LDL cholesterol (AUC=0.632; IC95%: 0.526-0.738; p=0.018), ALT (AUC=0.645; IC95%: 0.541-0.750;

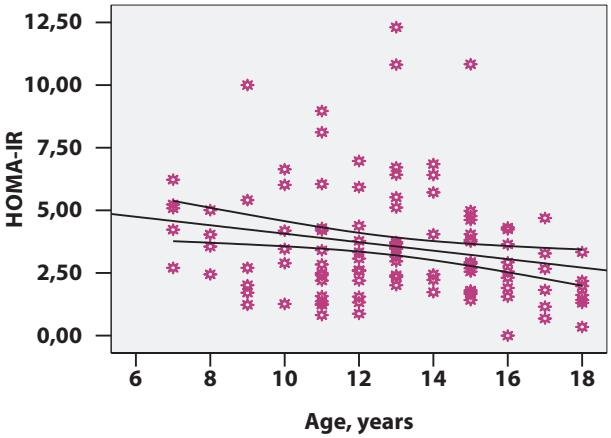

Figure 3. Correlation of HOMA-IR with age

Table 2. Average values of hepatic and lipidic markers depending on the HOMA-IR Level

Parameters	HOMA-IR			F ANOVA test p
	< 2	2 - 5	> 5	
Total cholesterol	172.56±28.46	169.40±29.13	167.90±30.18	0.840
LDL cholesterol	93.14±20.33	107.01±25.63	100.09±24.99	**0.050**
HDL cholesterol	41.07±10.16	43.24±11.58	46.29±12.47	0.278
ALT	25.15±9.13	28.39±10.06	22.86±5.49	**0.036**
AST	26.19±9.52	30.64±12.11	26.00±10.17	0.111
Triglycerides	107.26±45.70	112.67±45.90	99.26±36.92	0.464

Table 3. Correlation of hepatic markers with the glucose tolerance decrease and diabetes mellitus

Parameters	HOMA-IR > 2 (n=58)	HOMA-IR > 5 (n=23)	Chi2 test P	Rr	IR95%
Total cholesterol					
Increased	29 (50.0%)	10 (43.5%)	0.596	1.08	0.82-1.42
Normal	29 (50.0%)	13 (56.5%)			
LDL cholesterol					
Increased	29 (50.0%)	8 (34.8%)	0.212	1.19	0.91-1.56
Normal	29 (50.0%)	15 (65.2%)			
HDL cholesterol					
Low	36 (62.1%)	11 (47.8%)	0.243	1.18	0.88-1.59
Normal	22 (37.9%)	12 (52.2%)			
ALT					
Increased	9 (15.5%)	0 (0%)	**0.011**	1.47	1.25-1.72
Normal	49 (84.5%)	23 (100%)			
AST					
Increased	10 (17.2%)	2 (8.7%)	0.307	1.20	0.89-1.61
Normal	48 (82.2%)	21 (91.3%)			
Triglycerides					
Increased	42 (72.4%)	18 (78.3%)	0.584	0.92	0.54-2.97
Normal	16 (27.6%)	5 (21.4%)			

p=0.009) and AST (AUC=0.617; IC95%: 0.510-0.724; p=0.036). Only HDL cholesterol proves to be a good predictor of decreased glucose tolerance (AUC=0.607; IC95%: 0.469-0.745) (Fig. 4).

Discussion

Our study analyzes 108 overweight and obese children from the perspective of the diagnostic parameters of metabolic syndrome. We attempt to establish a correlation between the metabolic changes induced by obesity (decreased glucose tolerance, diabetes mellitus) and the lipid, but especially liver parameters. It is confirmed that HDL cholesterol is a useful parameter in the diagnosis of the metabolic syndrome in the obese children. Increases in the liver enzymes (AST, ALT) induced by metabolic steatohepatitis are a predictor for the occurrence of diabetes mellitus. Also, the increase in LDL-cholesterol levels is the predictor of diabetes

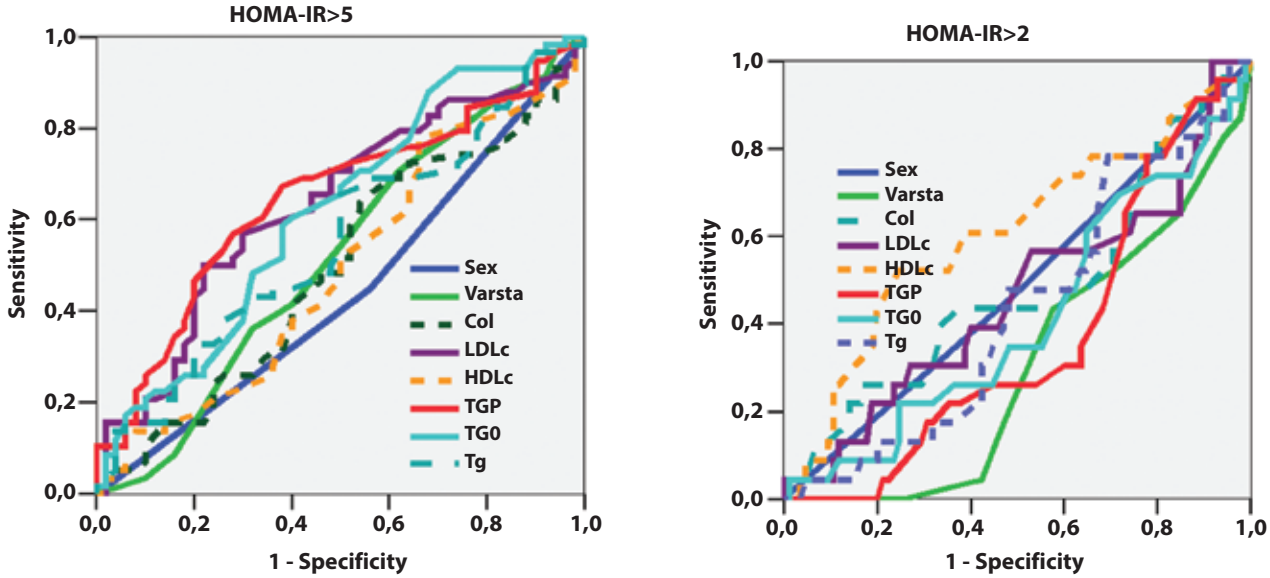

Figure 4. ROC Curve. Predictor markers of diabetes mellitus and glucose tolerance decrease

mellitus. The analysis showed that there are no statistically significant differences in metabolic changes between the status of obese and overweight. Early intervention by applying measures to correct metabolic disorders (exercise, lifestyle change, balanced diets, inducing normal weights) is essential for stopping the progression to diabetes mellitus. Monitoring of metabolic disorders, but especially their favorable evolution can be done by measuring lipid parameters (cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), glycemic (glycaemia, insulinemia), but especially liver ones (AST - aspartataminotransferase) and ALT –alaninaminotransferase), without the need for more laborious procedures such as the glucose tolerance test. Thus, it can be said that AST and ALT values can be included as a diagnosis parameter of the metabolic syndrome in obese children and teenagers.

In recent years, there has been an increase in the number of publications addressing the epidemiology and diagnostic criteria of MetS in children and adolescents. The definition of MetS in the child and its clinical usefulness remains a subject of continuous debate. The possibility of adding new MetS components (NAFLD, hyperuricemia, sleep disturbance, subclinical hypothyroidism) to the standard definition is one of the "hot subjects" in this field. It also discusses the possibility of using new biomarkers able to promptly identify the people affected by MetS (20). Modified levels of adipocytokine have been demonstrated as a common feature not only in obesity, but also in NAFLD and MetS in children and teenagers (21, 22).

Conflict of interests: The authors declared no conflict of interests regarding the publication of this manuscript

References

1. Duijts L, Vernooij MW, Gaillard R, Jaddoe VWV. Liver Fat and Cardiometabolic Risk Factors Among School-Age Children. Hepatology. 2020; 72: 119–129. http://dx.doi.org/10.1002/hep.31018
2. Mann JP, Valenti L, Scorletti E, Byrne CD, Nobili V. Non-alcoholic Fatty Liver Disease in Children. Semin Liver Dis. 2018 Feb;38(1):1-13. doi: 10.1055/s-0038-1627456. Epub 2018 Feb 22. PMID: 29471561.
3. Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients 2013;5(05): 1544–1560
4. Valenti L, Bugianesi E, Pajvani U, Targher G. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? Liver Int 2016;36(11):1563–1579
5. DeBoer MD. Assessing and Managing the Metabolic Syndrome in Children and Adolescents. Nutrients. 2019 Aug 2;11(8):1788. doi: 10.3390/nu11081788. PMID: 31382417; PMCID: PMC6723651.

6. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003; 37:917–923
7. Bussler S, Penke M, Flemming G, Elhassan YS, Kratzsch J, Sergeyev E, Lipek T, Vogel M, Spielau U, Körner A, de Giorgis T, Kiess W. Novel Insights in the Metabolic Syndrome in Childhood and Adolescence. Horm Res Paediatr. 2017;88(3-4):181-193. doi: 10.1159/000479510. Epub 2017 Aug 28. PMID: 28848168.
8. Neitzke U, Harder T, Plagemann A: Intrauterine growth restriction and developmental programming of the metabolic syndrome: a critical appraisal. Microcirculation 2011;18: 304–311.
9. Smith CJ, Ryckman KK: Epigenetic and developmental influences on the risk of obesity, diabetes, and metabolic syndrome. Diabetes Metab Syndr Obes 2015;8:295–302.
10. Boney CM, Verma A, Tucker R, Vohr BR: Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 2005; 115:e290–e296.
11. Marzuillo P, Miraglia del Giudice E, Santoro N: Pediatric fatty liver disease: role of ethnicity and genetics. World J Gastroenterol 2014; 20:7347–7355.
12. Welsh JA, Karpen S, Vos MB: Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988–1994 to 2007–2010. J Pediatr 2013;162:496.
13. Clemente MG, Mandato C, Poeta M, Vajro P: Pediatric non-alcoholic fatty liver disease: recent solutions, unresolved issues, and future research directions. World J Gastroenterol 2016;22:8078–8093.
14. Giorgio V, Prono F, Graziano F, Nobili V: Pediatric non alcoholic fatty liver disease: old and new concepts on development, progression, metabolic insight and potential treatment targets. BMC Pediatr 2013;13:40.
15. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH: Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988–1994. Arch Pediatr Adolesc Med 2003;157:821–827.
16. Bonci E, Chiesa C, Versacci P, Anania C, Silvestri L, Pacifico L: Association of nonalcoholic fatty liver disease with subclinical cardiovascular changes: a systematic review and meta-analysis. Biomed Res Int 2015;2015: 213737.
17. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P: Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. Dig Liver Dis 2015;47:181–190.
18. Sundaram SS, Zeitler P, Nadeau K: The metabolic syndrome and non-alcoholic fatty liver disease in children. Curr Opin Pediatr 2009; 21:529–535.
19. Penke M, Kiess W, Giorgis T: Non-alcoholic fatty liver disease in children and adolescents. J Pediatr Endocrinol Metab 2016;29:1329– 1330.
20. Zhang Y, Li C, Li H, Song Y, Zhao Y, Zhai L, Wang H, Zhong R, Tang H, Zhu D: miR-378 Activates the pyruvate-PEP futile cycle and enhances lipolysis to ameliorate obesity in mice. EBioMedicine 2016;5:93–104
21. Kotnik P, Fischer PP, Wabitsch M: Endocrine and metabolic effects of adipose tissue in children and adolescents. Zdr Varst 2015;54:131– 138.
22. Alterio A, Alisi A, Liccardo D, Nobili V: Nonalcoholic fatty liver and metabolic syndrome in children: a vicious circle. Horm Res Paediatr 2014;82:283–289

Correspondence
Ana Maria Pelin
Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Department of Pharmaceutical Sciences, 35 A.I.Cuza Str., 800010, Galati, Romania
E-mail anapelin@gmail.com