Research Article

Early Clinical Indicators of Metabolic Syndrome and Insulin Resistance in A Cohort of Greek Children with Obesity

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Abstract

Background: Prevalence of childhood obesity in Greece is reported to be among the highest worldwide.

Objective: To investigate whether anthropometric indices such as Body Mass Index (BMI), Waist to Height Ratio (WHtR) and Acanthosis nigricans can be useful early indicators of Metabolic Syndrome (MetS) and Insulin Resistance (IR) in Greek children affected by obesity. Furthermore, to estimate the prevalence of Mets in this population.

Method: Data from 189 pre-pubertal children with overweight and obesity (45% boys) with mean age 9.8 ± 2.3 years were analyzed.

Results: IR, as indicated by Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) of ≥ 3, was present in 33.3% of the children, while 12.7% of them met the criteria for Mets. The mean BMI was greater in children with IR ($p < 0.001$). Also, children with IR had greater Waist Circumference (WC) ($p < 0.001$). Acanthosis nigricans ($p = 0.041$) and increased fat percentage ($p = 0.003$) were associated with greater likelihood for IR. WHtR was associated with greater odds for IR ($p = 0.048$). Among children with MetS, 54.2% had IR and they were all affected by obesity. Increased WC was associated with the presence of MetS ($p = 0.046$), while for one unit increase in BMI the likelihood for MetS was found to increase about 14% ($p = 0.001$).

Conclusions: Increased WC, BMI, WHtR and Acanthosis nigricans are early clinical indicators for increased metabolic risk.

Keywords

Metabolic syndrome, Insulin resistance, Obesity, Children, Overweight

Introduction

The pandemic of obesity has become worldwide a very concerning public health issue [1]. The increasing prevalence of paediatric obesity constitutes a major health problem as it is estimated that in the United States one third of children and adolescents are overweight or obese [2]. Prevalence of childhood obesity and in particular abdominal obesity in Greece is reported to be among the highest worldwide (25.2% and 25.3% for boys and girls respectively) [3].

Increased weight is not synonymous with increased adiposity. For children older than two years of age, Body Mass Index (BMI) is proposed as the standard

measure to estimate the degree of obesity as it correlates well with the degree of adiposity [4, 5]. A number of other indices have been used as well, in order to assess adiposity and in particular central adiposity in children and adolescents such as Waist Circumference (WC), Waist-to-hip ratio, Waist to Height Ratio (WHtR) and skinfold thickness.

The rise of the prevalence of obesity in pediatric population, parallels the rise of the prevalence of comorbidities of obesity resulting in unfavorable cardio metabolic profile and higher risk for premature death during adulthood [6]. Obesity related complications include impaired glucose tolerance and Insulin Resistance (IR) which lead to increased risk for type II diabetes mellitus [7, 8]. Puberty is a physiologic inducer of insulin resistance. Insulin sensitivity decreases by 25% during adolescence [9]. Hyperinsulinemic euglycemic clamp is the gold standard technique in order to evaluate insulin resistance, however is expensive and difficult to perform. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [10] is used as a surrogate marker of IR and it has been validated as a tool for the estimation of insulin sensitivity [11]. The prevalence of IR differs among Caucasian, African, American, Hispanic, Pima Indian and Asian children [12-14]. Regarding Greek pediatric population, there are limited data for the prevalence of IR. One recent study demonstrated that IR, estimated by HOMA-IR, was observed in half of the 185 children and adolescent participants with overweight/obesity [15].

Metabolic syndrome (MetS) is a constellation of metabolic abnormalities such as central obesity, glucose intolerance, dyslipidemia and hypertension. However, there is no consensus for the definition [16, 17]. Pubertal age and growth are associated with metabolic changes which lead to instability of the diagnosis. Prevalence of MetS increases with the severity of obesity [18, 19]. MetS enhances the risk for incident Cardiovascular Disease (CVD) and all-cause mortality in adults [20, 21]. The concept of MetS means that the clustering of factors increases the risk for CVD beyond the risk of each individual factor [22]. The severity of MetS in children has been linked to long term cardiovascular morbidity [23]. Moreover, genetic background is an important factor that influences the presence of MetS. Early screening for MetS may be expensive and time consuming. Thus, it is very important to identify clinical factors that are associated with IR and MetS in order to recognize children at risk and implement preventive measures in childhood in order to eliminate long term consequences. To our knowledge there are no data regarding MetS in Greek pre-pubertal children.

This study aimed to clarify whether BMI, WC, WHtR and Acanthosis nigricans could be used as clinical markers for early detection of IR and MetS among pre-pubertal children with overweight and obesity, so as to plan further evaluation and aggressive life style changes to prevent progression of MetS components [24, 25].

Method

Participants

The study population consisted of 189 pre-pubertal children (45% boys), who were referred to our Department of Endocrinology-Growth and Development, in Athens, Greece, during the time period between 2013 to 2016, for investigation and management of increased body weight. Their mean age was 9.8 ± 2.3 years. The investigations were carried out under their routine care and there was given approval for the retrospective analysis of the medical record data by the Ethics Committee of "P&A Kyriakou" Children's Hospital. In the analysis we included all the children who were pre-pubertal and had all the under investigation parameters recorded.

Children were excluded from the analysis if they had obesity related genetic syndromes, hypothyroidism, type 1 or type 2 diabetes mellitus, long-term corticosteroid use, primary hyperlipidemia, or hypertension.

Participants' height, weight, WC, arterial Blood Pressure (BP) and body composition were measured, while BMI and WHtR were calculated. Participants' height was measured barefoot, using a wall mounted Harpenden Stadiometer Holtain Ltd. Their weight was measured with an electronic scale (SOEHNLE Professional 2755) to the nearest 0.1 kg, while they were barefoot and dressed only in light underwear and T-shirt. WC was measured twice, midway between the lowest border of rib cage and the upper border of iliac crest with the use of inextensible anthropometric tape while the child was standing with arms at the sides and feet closed together [26]. BMI was calculated as weight (in kilograms) divided by height (in meters) squared.

Arterial BP was measured twice with a calibrated G-Care SP-800 sphygmomanometer, with two minutes interval between the two of them. The mean value of the two measurements was used for the analysis. The presence of Acanthosis nigricans, and family history of obesity was recorded. Body composition was assessed by Tanita Body Composition Analyzer Type BC = 418 MA. Fat percentage was considered as high according to the 95th body fat reference curve for children [27].

The vast majority of the sample (97.4%) was affected by obesity (BMI ≥ 95th CDC percentile) while only 2.6 % of the children were classified as overweight (BMI ≥ 85th percentile but < 95th percentile for age and sex). All participants were pre-pubertal according to Tanner staging.

Obesity was defined as BMI equal to or greater than the sex- and age-specific 95th percentile of CDC Anthropometric Reference Data for Children and Adults, 2007-2010 [28]. Abdominal obesity was defined as WHtR ≥ 0.5 and WC equal to or greater than the sex- and age-specific 90th [29].

The diagnosis of MetS was established according to Cook et al. [30], in the presence of at least three of the following variables: increased WC for gender and age (≥ 90%), increased BP for gender, age and height (≥ 90%), fasting plasma glucose (FPG) ≥ 100 mg/dl, High-Density Lipoprotein (HDL) ≤ 40 mg/dl, triglycerides (Tg) ≥ 110 mg/dl. HOMA-IR was calculated as fasting plasma insulin (FPI U/l) × FPG mg/dl)/405 [10]. A cut-off value ≥ 3 was used for HOMA-IR [31, 32].

Assays

Laboratory investigations, following 12 h overnight fast, included the following: Venous samples were collected in BD Vacutainer® spray coated K2EDTA Tubes and in WEGO serum vacuum tubes. Plasma glucose levels and fasting serum lipids [Tg, total cholesterol, HDL and low-density lipoprotein (LDL) cholesterol], were measured within 10 minutes, by an enzymatic, colorimetric method in a Cobas c501 auto analyzer (Roche Laboratory Systems). The intra and inter-assay coefficient of variation with this method of glucose measurement in our laboratory is less than 3.2%. Serum insulin concentrations were measured using the immune-metric reaction, ECLIA, Elecsys 2010, Roche Diagnostics, Greece, all conducted in a CLIA approved laboratory.

Statistical analysis

Quantitative variables are presented with mean and standard deviation (SD) for continuous variables, as absolute numbers and percentages in parentheses for categorical variables. Qualitative variables are presented with absolute and relative frequencies. Chi-square test was used for the comparison of proportions. Logistic regression analysis was used in order to find factors associated with HOMA-IR ≥ 3 and MetS. All reported p values are two-tailed. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS statistical software (version 22.0).

Results

In the current study, 189 pre-pubertal children (45% boys) with mean age 9.8 years (SD ± 2.3) were analyzed with full data for HOMA-IR and MetS. Demographic and clinical characteristics are presented in table 1. The vast majority (97.4%) of the children were affected by obesity, with mean BMI = 27.3 (SD ± 3.6) and mean fat percentage 36.8 (SD ± 5.4). Most of the children had abdominal obesity as evidenced by WHtR ratio mean 0.62 (SD ± 0.05), and WC mean 89.0 (SD ± 10.4). Acanthosis nigricans was present in 19% of the children. Positive family history for obesity was reported in 57.1% of the participants. MetS was found in 12.7% of the children. HOMA-IR ≥ 3, consistent with IR, was observed in 33.3% of the children.

The prevalence of IR and the association of HOMA-IR with clinical characteristics of the sample are presented in Table 2. HOMA-IR ≥ 3 was found in 34.2% of the children with obesity and in none of those with overweight. Furthermore, percentage of Acanthosis nigricans among children with HOMA-IR ≥ 3 was statistically significantly higher (p = 0.041). In this cohort, positive family history of obesity did not prove to be a risk factor for insulin resistance.

Mean BMI was higher among children with HOMA-IR ≥ 3 (29.4, SD ± 3.7) as compared to the corresponding percentage among children with HOMA-IR< 3 (26.2, SD ± 3.1) which was statistically significant (p < 0.001) as it is shown in table 2 and figure 1. Moreover, HOMA-IR ≥ 3 was statistically significantly associated with elevated WHtR ratio

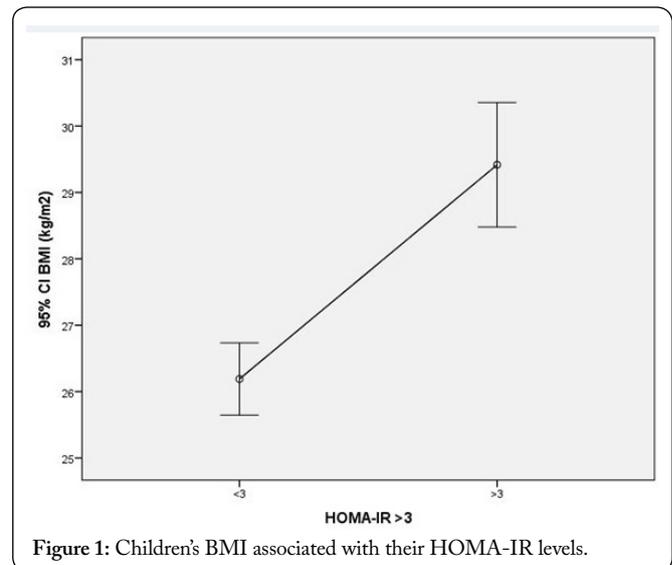(p = 0.048), fat percentage (p = 0.003) (Table 2) and WC (p < 0.001) (Table 2 and Figure 2).

Figure 1: Children's BMI associated with their HOMA-IR levels.

Table 1: Demographics and clinical characteristics.

Demographics	N (%)
Age (yrs.), mean (SD)	9.8 (2.3)
Sex	
Boys	85 (45.0)
Girls	104 (55.0)
Clinical characteristics	
BMI (kg/m²), mean (SD)	27.3 (3.6)
Overweight	5 (2.6)
Obese	184 (97.4)
WHtR ratio, mean (SD)	0.62 (0.05)
% Fat, mean (SD)	36.8 (5.4)
Waist circumference (cm), mean (SD)	89.0 (10.4)
Obesity family history	
No	78 (42.9)
Yes	104 (57.1)
Acanthosis nigricans	
No	149 (81.0)
Yes	35 (19.0)
HOMA-IR ≥ 3	
< 3	126 (66.7)
≥ 3	63 (33.3)
Metabolic syndrome	
No	165 (87.3)

SD- Standard Deviation, BMI- Body Mass Index, WHtR- Waist to Height Ratio, HOMA-IR- Homeostasis Model Assessment of Insulin Resistance

Correlation of Mets with clinical characteristics of our sample is presented in table 3. WC as well as mean BMI were

Table 2: Association of HOMA-IR with clinical characteristics.

	HOMA-IR		OR (95% CI)	p value
	< 3	≥ 3		
	N (%)	N (%)		
Sex				
Boys	56 (65.9)	29 (34.1)		
Girls	70 (67.3)	34 (32.7)	0.94 (0.51 - 1.72)	0.836
BMI (kg/m²), mean (SD)	26.2 (3.1)	29.4 (3.7)	1.32 (1.19 - 1.47)	< 0.001
BMI (kg/m²)				
Overweight	5 (100.0)	0 (0.0)		
Obese	121 (65.8)	63 (34.2)	-	-
WHtR ratio, mean (SD)	0.61 (0.06)	0.63 (0.04)	1.07 (1.00 - 1.15)	0.048
% Fat, mean (SD)	35.5 (4.8)	39.1 (5.8)	1.15 (1.05 - 1.26)	0.003
Waist Circumference (cm), mean (SD)	85.8 (10.5)	93.8 (8.1)	1.10 (1.05 - 1.15)	< 0.001
Obesity Family history				
No	46 (59.0)	32 (41.0)		
Yes	76 (73.1)	28 (26.9)	0.53 (0.28 - 0.99)	0.046
Acanthosis nigricans				
No	104 (69.8)	45 (30.2)		
Yes	18 (51.4)	17 (48.6)	2.18 (1.03 - 4.62)	0.041
WHtR ratio				
< 0.5	2 (100.0)	0 (0.0)		
≥ 0.5	68 (58.6)	48 (41.4)	-	-

HOMA-IR- Homeostasis Model Assessment of Insulin Resistance, OR- Odds Ratio, CI- Confidence Intervals, BMI- Body Mass Index, SD- Standard Deviation, WHtR- Waist to Height Ratio
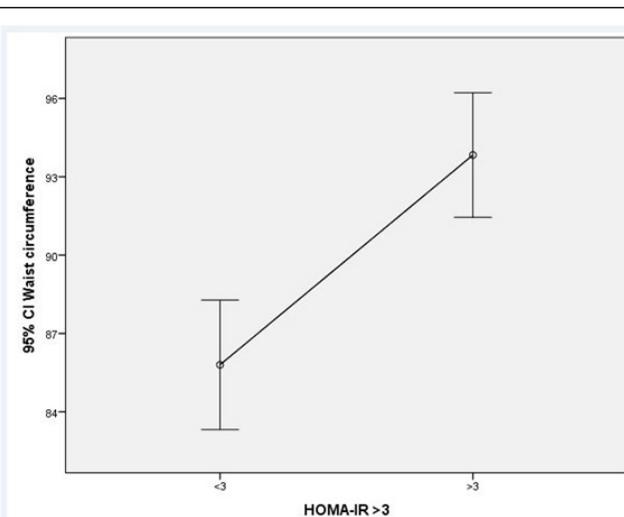

Figure 2: Children's waist circumference associated with their HOMA-IR levels.

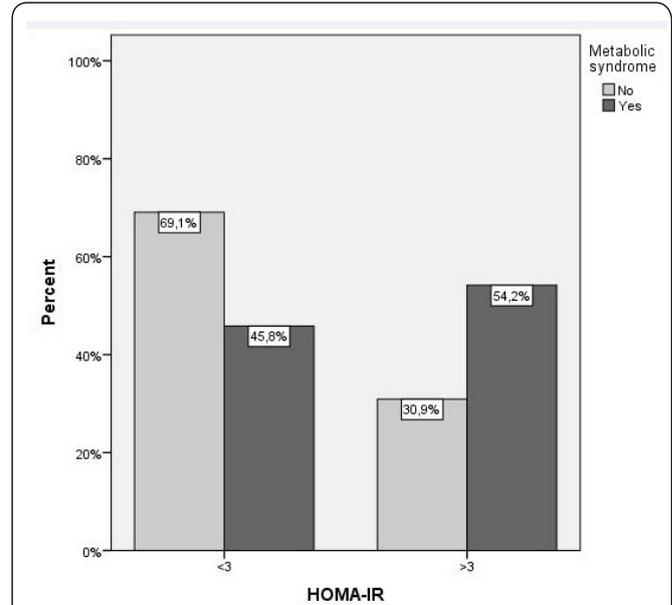significantly greater among children with MetS, p = 0.046 and p = 0.028 respectively.

Figure 3: Percentages of children with HOMA-IR ≥ 3 in those with and without MetS.

Table 3: Association of Metabolic Syndrome (MetS) with clinical characteristics.

	Metabolic syndrome		OR (95% CI)	p
	No	Yes		
	N (%)	N (%)		
Sex				
Boys	76 (89.4)	9 (10.6)		
Girls	89 (85.6)	15 (14.4)	1.42 (0.59 - 3.44)	0.433
BMI (kg/m²), mean (SD)	27 (3.5)	28.8 (4.1)	1.14 (1.01 - 1.27)	0.028
Overweight	5 (100.0)	0 (0.0)		
Obese	160 (87.0)	24 (13.0)	-	-
WHtR ratio, mean (SD)	0.62 (0.05)	0.63 (0.05)	1.04 (0.94 - 1.15)	0.43
% Fat, mean (SD)	36.7 (5.7)	37.4 (3.7)	1.03 (0.93 - 1.13)	0.611
Waist circumference, mean (SD)	88.3 (10.4)	91.9 (9.9)	1.04 (1.00 - 1.09)	0.046
Obesity Family history				
No	69 (88.5)	9 (11.5)		
Yes	89 (85.6)	15 (14.4)	1.29 (0.53 - 3.13)	0.57
Acanthosis nigricans				
No	133 (89.3)	16 (10.7)		
Yes	28 (80.0)	7 (20.0)	2.08 (0.78 - 5.52)	0.142
WHtR ratio				
< 0.5	1 (50.0)	1 (50.0)		
≥ 0.5	95 (81.9)	21 (18.1)	0.22 (0.01 - 3.68)	0.293

OR- Odds Ratio, CI- Confidence Intervals, BMI- Body Mass Index, SD- Standard Deviation, WHtR- Waist to Height Ratio

On figure 3 the differences and the frequency of HOMA-IR ≥ 3 and HOMA-IR < 3 between participants with and

without MetS are shown. The prevalence of IR (HOMA-IR ≥ 3) among children with MetS was almost double as compared to the prevalence of the children without MetS, 54.2% *vs* 30.9% respectively (p = 0.024).

Finally, of great clinical significance were the findings that for one unit increase in BMI the likelihood for MetS was found to increase by 14%, while for one unit increase in WC the likelihood for MetS was found to increase about 4% (Table 3).

Discussion

Increased adiposity induces the clustering of cardio metabolic risk factors. Detection of increased adiposity and in particular abdominal adiposity can predict the presence of MetS. The present study investigated whether anthropometric indices, such as BMI, WC, WHtR and Acanthosis nigricans, as well as positive family history for obesity, can be useful markers for early detection of MetS and IR in Greek children with overweight and obesity. IR alone was more prevalent than the presence of Mets. Mets was significantly correlated with increased HOMA-IR, BMI and WC, which proves that increased body weight combined with abdominal obesity, is a major risk factor for IR and MetS in this population.

Defining Mets in pediatric population is quite complex, as it is evident by 40 pediatric definitions described in the literature. Thus, there is no consensus regarding definition [33]. Given this lack of clarity of definition, the need for the development of simple, accessible, and reliable clinical methods for predicting IR and cardio metabolic risk factors in children is crucial. Recent evidence suggests that focusing on early recognition of cardio metabolic risk factors, which are components of MetS in children, can accurately prevent long term risk for atherosclerotic CVD and type 2 diabetes mellitus in adulthood [34].

Obesity has been strongly associated with IR in children [35] and both are known to represent elements of MetS [36]. The most commonly used surrogate markers to assess IR are FPI and HOMA-IR which have been validated in childhood with the hyperinsulinemic euglycemic clamp [37, 38]. IR leads to hyperinsulinemia which can progress to type 2 diabetes mellitus [39]. In addition, MetS and cardio metabolic risk factors are linked to IR with several proposed mechanisms such as endothelial dysfunction, inflammation and reduced brachial artery flow-mediated dilation [40].

Individuals with increased body weight do not always develop metabolic complications [41]. Furthermore, there is controversy in regards to whether childhood obesity predicts cardiovascular risk in adulthood [42]. There is recent evidence indicating that children and adolescents with severe obesity (BMI ≥ 120% of the 95th percentile or absolute BMI ≥ 35 kg/m²) are considered to be at higher risk because they manifest multiple CVD risk factors, such as endothelial dysfunction and subclinical atherosclerosis and they are more likely to evolve to adults with obesity [43, 44].

In the present cohort, 12.7% of the participants met the criteria of MetS according to Cook definition [30]. The prevalence of MetS varies widely among pediatric populations, ranging from 0.4% to 24.6% in different studies, depending on different definitions [45]. Prevalence of MetS in Italian children with obesity was reported to be 29.2% [46], while in a nationally representative sample of US adolescents, prevalence of MetS was estimated to be 10.1%, with Hispanic males and females having significantly greater odds for the presence of syndrome [47]. Gurka et al identified significant sex and racial differences in MetS components and proposed a novel sex and race/ethnicity-specific MetS risk score [48]. These data underline the necessity to have studies which assess MetS prevalence and components among different ethnicities.

One out of 3 of the children with obesity who participated in this Greek cohort, exhibited IR. To our knowledge reference ranges for HOMA-IR in pre-pubertal pediatric population with obesity have not yet been determined. Cut off values are related to the age and pubertal status of children as shown in the IDEFICS cohort [49]. The 97th percentile, in this study, corresponds to HOMA 3.0 for children with an average age of about 10 years, which is the mean age of our study participants. However, HOMA cut-off values in this study address to pre-pubertal normal weight European children. Furthermore, Shashaj et al used a cut-off point of 3.02 which represents the 75th percentile of HOMA-IR in the whole young Caucasian population of their study [50]. In another study, Marko Kostovski et al used HOMA-IR above 3.16 as a cut-off value for both genders [32]. Based on the above data, HOMA-IR cut-off point in the present study was 3.0. IR results in hyperinsulinemia and it is strongly correlated to type II diabetes mellitus. It is also associated with hypertriglyceridemia, hypertension, and low plasma HDL-cholesterol, which are well known risk factors for CVD [51].

Pre-pubertal children with obesity with HOMA-IR ≥ 3 had higher BMI, higher WHtR, fat percentage and WC as compared to the corresponding values among children with HOMA-IR < 3. Additionally, as it was expected, Acanthosis nigricans was present in higher percentage in children with IR. Among children with MetS, WC was significantly greater as well as mean BMI. Moreover, the percentage of children having HOMA-IR ≥ 3 was almost double in comparison with that of the children without MetS. Although positive family history for obesity was present in more than half of the children with obesity, it doesn't seem to increase the risk of insulin resistance.

These associations indicate that the higher the BMI, WC and WHtR, the higher the likelihood of the child having IR and the same holds true for Mets. Thus, they can be used as early clinical indicators for children with obesity at risk for IR and Mets.

The strengths of the present study include the following. To our knowledge, this is the first study to be conducted in Greek pediatric population evaluating MetS and IR among pre-pubertal children with overweight and obesity.

It is very important to have such studies in different ethnic populations, as the presence of obesity and IR differs among ethnicities. It is notable that different factors depending on the ethnic background contribute to the diagnosis of MetS syndrome and such studies could be helpful to establish

appropriate criteria which are not subject to ethnical differences. Furthermore, the study population consisted of pre-pubertal children only. Insulin sensitivity is well known to decline during puberty and improves at completion of puberty. In this study there are not confounding factors to be considered, regarding IR, such as puberty or presence of polycystic ovary syndrome.

There are some limitations in this study. All the participants included in this cohort originate from only one referral center. A larger number of participants would reflect more appropriately the general population. Furthermore, the level of physical activity was not assessed as a parameter which makes an impact on insulin sensitivity and body composition. Finally, the retrospective design of the study may be a limitation and therefore prospective, large population cohorts are necessary in order to clarify prevalence, diagnosis and prevention of MetS and IR in pediatric population.

Conclusion

In conclusion, high prevalence of MetS was found in Greek pre-pubertal children with obesity. Regardless of MetS definition, abdominal obesity and IR are the most common elements among children with MetS. The findings of this study implicate that increased WC and BMI, as well as the presence of Acanthosis nigricans are early clinical indicators for increased metabolic risk. Children with BMI at or greater than the 95th percentile, as well as abdominal obesity, should be screened regularly for MetS components, as anthropometric indices in this study were proved to have strong predictive capacity for the syndrome.

Conflict of Interest

The authors declare that the research was conducted in the absence of any interests mentioned.

Acknowledgements

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