ACTA MÉDICA PORTUGUESA
A Revista Científica da Ordem dos Médicos

Nonalcoholic Fatty Liver Disease and Continuous Metabolic Syndrome in Adolescents with Overweight/Obesity

Doença Hepática Não-Alcoólica e Síndrome Metabólica Contínua em Adolescentes com Excesso de Peso/Obesidade

Sofia FERREIRA✉[1,2], Joana MENDES[1], Daniela COUTO[1], Dário FERREIRA[3], Carla RÊGO[4,5,6]
Acta Med Port 2024 Mar;37(3):177-186 ▪ https://doi.org/10.20344/amp.19834

ABSTRACT

Introduction: Nonalcoholic fatty liver disease is the leading cause of pediatric chronic liver disease. Although nonalcoholic fatty liver disease is closely associated with obesity, its relationship with metabolic syndrome in children is not fully understood. The main aim of this study was to evaluate the association between nonalcoholic fatty liver disease and a combination of cardiometabolic risk factors in adolescents with overweight/obesity, using a pediatric metabolic syndrome score (PsiMS) to predict metabolic syndrome.

Methods: A retrospective cohort study was conducted. Subjects with overweight/obesity aged 10 to 17 followed at two clinical centers in Portugal (2018 - 2021) were enrolled. The independent association of nonalcoholic fatty liver disease with PsiMS, and of other potential predictors, was tested through multiple regression analyses. Receiver operator characteristic curve analysis was performed to estimate the optimal cutoff of PsiMS to discriminate metabolic syndrome.

Results: Eighty-four subjects were included (median age at baseline 11.5 years). The prevalence rate of nonalcoholic fatty liver disease was 51% and the prevalence rate of metabolic syndrome was 7%. The mean PsiMS was 2.05 ± 0.48 at the first evaluation, and 2.11 ± 0.52 at the last evaluation (mean follow-up time was 15 months). The nonalcoholic fatty liver disease group had significantly ($p < 0.05$) higher weight and body mass index z-scores, higher rate of severe obesity and higher waist circumference percentile. PsiMS was highly accurate in predicting metabolic syndrome (area under the curve = 0.96), with an optimal cutoff of 2.46 (sensitivity 100%, specificity 89%). In the univariate analysis, no statistically significant association was observed between nonalcoholic fatty liver disease and PsiMS. In the multiple regression analysis, female sex had a negative association with PsiMS (first and last evaluation). Independent predictors of a higher PsiMS at first evaluation were: ≥ 2 metabolic syndrome criteria, body mass index z-score, insulin resistance and dyslipidemia. At the last evaluation, independent predictors of a higher PsiMS were: nonalcoholic fatty liver disease, baseline PsiMS and body mass index increase from baseline.

Conclusion: The results suggest a good performance of the PsiMS to assess metabolic syndrome and that nonalcoholic fatty liver disease is associated with PsiMS at follow-up.

Keywords: Adolescent; Metabolic Syndrome; Non-alcoholic Fatty Liver Disease; Obesity; Overweight

RESUMO

Introdução: A doença hepática não alcoólica é a principal causa de doença hepática crónica pediátrica. Embora intimamente associada à obesidade, a relação desta patologia com a síndrome metabólica em idade pediátrica não está totalmente esclarecida. O principal objetivo deste estudo foi explorar a associação entre a doença hepática não alcoólica e uma agregação de fatores de risco cardiometabólicos em adolescentes com excesso de peso/obesidade, usando um *score* de síndrome metabólica pediátrica (PsiMS) para discriminar síndrome metabólica.

Métodos: Foi realizado um estudo de coorte retrospetivo, incluindo adolescentes (10 - 17 anos) com excesso de peso/obesidade, seguidos em dois centros clínicos em Portugal (2018 - 2021). A associação independente entre a doença hepática não alcoólica e o PsiMS, e de outros potenciais preditores, foi avaliada com análise de regressão linear múltipla. O ponto-de-corte ideal do PsiMS para prever síndrome metabólica foi estimado pela análise da curva de características receptor-operador.

Resultados: Foram incluídos 84 adolescentes (idade mediana no início do estudo 11,5 anos). A taxa de prevalência de doença hepática não alcoólica foi 51% e a taxa de prevalência de síndrome metabólica foi 7%. O PsiMS médio foi 2,05 ± 0,48 na primeira avaliação e 2,11 ± 0,52 na última avaliação (tempo médio de *follow-up* 15 meses). O grupo com doença hepática não alcoólica apresentava um peso, *z-score* do índice de massa corporal e percentil do perímetro abdominal significativamente ($p < 0,05$) superiores, e maior proporção de obesidade grave ($p < 0,05$). O PsiMS foi preciso na previsão de síndrome metabólica (área abaixo da curva = 0,96), com o ponto-de-corte de 2,46 (sensibilidade 100%, especificidade 89%). Na análise univariada, não se observou uma associação estatisticamente significativa entre a doença hepática não alcoólica e o PsiMS. Na regressão linear múltipla, o sexo feminino apresentou uma associação negativa com o PsiMS (primeira e última avaliação). Os preditores independentes de um PsiMS mais elevado na primeira avaliação foram: ≥ 2 critérios de síndrome metabólica, *z-score* do índice de massa corporal, insulinorresistência e dislipidemia. Na última avaliação a doença hepática não alcoólica, o PsiMS inicial e o aumento do e do índice de massa corporal associaram-se a um *score* mais elevado.

Conclusão: Os resultados sugerem um bom perfil do PsiMS para prever síndrome metabólica e que a doença hepática não alcoólica está associada com o PsiMS durante o seguimento.

Palavras-chave: Adolescente; Doença Hepática Não Alcoólica; Excesso de Peso; Obesidade; Síndrome Metabólica

1. Centro Hospitalar Universitário Cova da Beira. Covilhã. Portugal.
2. Faculty of Health Sciences. Universidade da Beira Interior. Covilhã. Portugal.
3. Department of Mathematics and Center of Mathematics and Applications. Universidade da Beira Interior. Covilhã. Portugal.
4. Hospital CUF Porto. Oporto. Portugal.
5. Center for Health Technology and Services Research (CINTESIS). Faculty of Medicine. Universidade do Porto. Oporto. Portugal.
6. Faculty of Biotechnology. Universidade Católica. Oporto. Portugal.

✉ **Autor correspondente:** Sofia Ferreira. asferreira@chcbeira.min-saude.pt

Recebido/*Received*: 07/03/2023 - **Aceite/*Accepted*:** 10/07/2023 - **Publicado *Online*/*Published Online*:** 08/02/2024 - **Publicado/*Published*:** 01/03/2024

INTRODUCTION

The prevalence of pediatric obesity and cardiometabolic comorbidities, which all cluster together under the umbrella of metabolic syndrome (MS), has reached epidemic proportions in the last few decades. These comorbidities include impaired glucose tolerance/insulin resistance (IR), dyslipidemia, and hypertension.[1,2]

The definition of MS remains a controversial topic in the pediatric population. In adults, MS is defined by a subset of at least three out of five risk factors: hyperglycemia, increased central adiposity, elevated triglycerides (TG), decreased high density lipoprotein cholesterol (HDL), and elevated blood pressure (BP).[2] In pediatrics, however, multiple definitions have been proposed with no clear consensus on which to use.[1,2] Besides the lack of consensus, the dichotomous nature of the current definitions might result in a loss of information for several reasons. First, the prevalence of MS is lower in the pediatric population. Second, minor changes of MS features in one subject or minimal differences between subjects could result in differing MS classifications. This might also limit the evaluation of treatment effect over time.[1,3,4]

Considering these limitations, continuous MS scores (cMS) have been developed for both adults and children, using standardized residuals in linear regression U (z-scores) or factor scores of principal component analysis.[3-11] As a continuous variable, the cMS has greater discriminant power when compared to a dichotomous classification, and allows assessment of cardiometabolic burden in MS-free individuals.[3,5] Lower values indicate a better MS profile and higher values a poorer MS profile, compared to the study sample.[3,5,7] These scores have some limitations that hamper their use in clinical practice, including the complexity of the required calculations and the sample specificity not allowing direct comparisons between different samples or populations.[3,5,7]

Recently, a simpler and more easily calculated score, PsiMS, was proposed by Vukovic *et al.*, in accordance with the current International Diabetes Federation (IDF) definition of pediatric MS.[4] The authors state that, besides being accurate and easy to calculate, this score can be used in clinical practice to compare values between different timepoints and different populations.[4,12]

Nonalcoholic fatty liver disease (NAFLD) is defined by excessive fat accumulation in the liver in the absence of excessive alcohol consumption or other known liver conditions.[13,14] Due to its close association with obesity, the prevalence rate of NAFLD has increased, and has become the most common cause of chronic liver disease in children.[13-16] Its true incidence and prevalence rates are unknown, but prevalence estimates in pediatric age range between 3% and 10%, rising up to between 34% and 74% in individuals with obesity.[13,15,17] Liver biopsy remains the gold standard for NAFLD diagnosis. Nevertheless, abdominal ultrasound (US) is widely used in clinical practice and clinical research, due to its accessibility and low cost.[13,18-20]

Nonalcoholic fatty liver disease includes a wide spectrum of severity, from simple steatosis and nonalcoholic steatohepatitis to progressive fibrosis and end-stage liver disease.[14] Besides, it is considered as a multisystem disease where the most common cause of mortality is cardiovascular disease, followed by liver-related complications and extra-hepatic malignancies.[19,21-23]

This disease is strongly associated with abdominal obesity, hypertension, atherogenic dyslipidemia, and impairment of glucose and insulin metabolism, all features of MS.[13,15,18,22] The association between NAFLD and MS has been confirmed in previous studies in adults and in children.[15,18,19,23-27]

Although previous studies assessed the association between NAFLD and cardiometabolic risk in pediatric patients, to date and to the best of our knowledge, none has used a continuous score in this analysis.

Therefore, the main aim of this study was to evaluate the association between NAFLD and a combination of cardiometabolic risk factors in adolescents with overweight/obesity, by assessing the impact of NAFLD on the PsiMS value at baseline and at follow-up. Other aims were: 1) to assess the validity of PsiMS to discriminate MS; 2) to estimate a cutoff value of PsiMS to discriminate MS; and 3) to explore associations between PsiMS and other cardiometabolic risk factors.

METHODS

Study design

We conducted a retrospective cohort study. The exposure/study group was NAFLD at baseline (a dichotomous variable). The primary outcome was PsiMS both at baseline and at follow-up. The secondary endpoints were the MS dichotomous classification and the number of MS criteria.

Study population

We included children and adolescents with a diagnosis of obesity or overweight, followed in outpatient visits at two clinical centers in Portugal between June 2018 and June 2021.

The following inclusion criteria were applied: 1) aged 10 to 17 years at the time of the last complete evaluation; 2) diagnosis of overweight or obesity according to the World Health Organization (WHO) criteria[28,29]; 3) at least one year of follow-up; 4) at least one abdominal ultrasound (US) at baseline; 5) at least two complete evaluations within a 12-month interval (± 4 months).

Exclusion criteria were: 1) subjects with secondary causes of obesity (i.e., hypothalamic, genetic, or endocrine causes); 2) subjects with liver disease or other possible causes other than NAFLD (viral hepatitis, alcohol consumption, use of steatogenic medications, or history of parenteral nutrition); 3) diagnosis of type 1 diabetes mellitus; 4) use of drugs that may affect cardiometabolic risk at baseline, such as oral antidiabetic drugs, antihypertensive drugs, or statins.

Data collection

Data were collected retrospectively through the assessment of the electronic health records.

Complete evaluations were considered when enough data for MS classification and body mass index (BMI) and PsiMS calculation was available, specifically: weight, height, waist circumference (WC), BP, glucose, HDL, and TG. Data from evaluations performed every 12 ± 4 months were collected.

The first or baseline evaluation was considered when subjects had a complete evaluation plus an abdominal US performed within a four month interval.

Regarding follow-up evaluations, we only considered the first available follow-up and the last available follow-up.

Body weight and height were measured and recorded to the nearest 0.1 kg and 0.1 cm, respectively, according to previously described methodology.[30]

Body mass index was calculated as the ratio between weight and squared height (kg/m^2). Z-scores of weight, height and BMI were defined in accordance with WHO, using the application AnthroPlus.[28,29]

Overweight was defined as a BMI z-score greater than 1 and less than or equal to 2, obesity was defined as a BMI z-score greater than 2 and less than or equal to 3, and severe obesity as a BMI z-score greater than 3.[28,29]

Waist circumference was measured to the nearest 0.1 cm, as recommended by the WHO, and percentiles were determined.[31]

Waist circumference-to-height ratio (WHR) was calculated as WC (cm) divided by height (cm), and 0.5 was considered the cutoff to represent cardiovascular risk.[31]

Blood pressure was measured and recorded in mmHg and centiles, according to the recommendations of the American Academy of Pediatrics.[32]

Laboratory assessments

Blood tests were routinely performed in these patients, after an overnight fast of 12 hours. Data from the blood tests at baseline and every 12 ± 4 months thereafter were collected. The following parameters were recorded: glucose, insulin, C-reactive protein (CRP), alanine aminotransferase (AST), aspartate aminotransferase (ALT), total cholesterol (TC), low density lipoprotein-cholesterol (LDL), HDL, TG, uric acid, 25 OH vitamin D.

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated by the formula: HOMA-IR = insulin [µU/mL] x glucose [mg/dL] / 405.[33]

Abdominal ultrasound

The ultrasonographic report was classified as either NAFLD present or absent. We considered NAFLD in the presence of a diagnosis made by the radiologist, or the description of increased echogenicity.

Pediatric metabolic syndrome score

Clustering of cardiometabolic risk factors was determined using PsiMS, calculated as previously reported, using WC, height, glucose (Gluc), TG, systolic blood pressure (SBP) and HDL, with the following formula:[4]

$$\text{PsiMS} = \frac{\frac{2 \times \text{WC (cm)}}{\text{Height (cm)}}}{} + \frac{\text{Gluc (mmol/L)}}{5.6} + \frac{\text{TG (mmol/L)}}{1.7} + \frac{\text{SBP (mmHg)}}{130} - \frac{\text{HDL (mmol/L)}}{1.02}$$

Metabolic syndrome

Along with the IDF definition, subjects under 16 years were classified as having MS in the presence of abdominal obesity (WC ≥ 90th percentile for age, or adult cutoff if lower) plus two or more of the other features: 1) TG ≥ 150 mg/dL, 2) HDL < 40 mg/ dL, 3) SBP ≥ 130 mmHg or diastolic BP (DBP) ≥ 85 mmHg, 4) Fasting glucose ≥ 100 mg/dL or known type 2 diabetes mellitus (T2DM).[2]

Adolescents aged ≥ 16 years were classified has having MS in the presence of abdominal obesity (WC ≥ 94 cm in male subjects, ≥ 80 cm in female subjects) plus two or more of the other parameters: 1) TG ≥ 150 mg/dL, 2) HDL < 50 mg/ dL in female subjects, < 40 mg/dL in male subjects, or specific treatment for dyslipidemia; 3) SBP ≥ 130 mmHg or DBP ≥ 85 mmHg, or treatment of previously diagnosed hypertension; and 4) Fasting glucose ≥100 mg/dL, or known T2DM.[2]

Statistical analysis

Normal distribution of continuous variables was assessed by using a Shapiro-Wilks test in combination with graphical methods. Continuous data with normal distribution were presented as mean ± standard deviation, and between-group comparisons were performed using Student's *t*-test. Continuous data with non-normal distribution were presented as median with interquartile ranges and compared by liver status (NAFLD) using the Wilcoxon rank-sum test. Non-continuous variables were presented as percentages and compared across liver status groups using a

Pearson chi-square test and Fisher's exact test when appropriate. Spearman correlation analysis was used to assess correlations between baseline and follow-up scores.

To estimate the optimal cutoff of PsiMS to discriminate MS, receiver operator characteristic (ROC) curve analysis was used with an estimation of the sensitivity and specificity. The cutoff was selected as the value which maximized the sum of sensitivity and specificity. This cutoff was used to assess the positive predictive value (PPV) and the negative predictive value (NPV). The same calculation was performed by applying the threshold previously proposed by Lee *et al* (2.6 in boys and 2.5 in girls).[12]

Multiple regression analyses were conducted to test the independent association of PsiMS with NAFLD and other potential predictors. The statistical models for baseline PsiMS were adjusted for age, sex, NAFLD and significant characteristics, considering a $p < 0.10$ in the univariate study. For the follow-up PsiMS regression models we also included baseline PsiMS and its independent predictors. The individual components of the PsiMS formula were not included in the regression models. Multiple regression assumptions were evaluated. *P* values less than 0.05 were considered statistically significant. Statistical analysis was conducted using the program Stata® v16.1.

Ethical considerations

This study protocol was submitted and approved by the local Ethics Committee and the Data Protection Officer, in accordance with the Declaration of Helsinki. Data were collected by the investigators and remained anonymous and confidential.

RESULTS

General characteristics of subjects

From the 264 children identified with abdominal US, 84 (75% female, n = 63) met the inclusion criteria. Median age at first evaluation was 11.5 years [IQR 9.9, 14.4], and at the last evaluation was 14.3 years [IQR 12.4, 16.2]. The ultrasound evaluation revealed NAFLD in 43 (51%). At baseline, the prevalence rate of obesity was 69% and the prevalence rate of overweight was 31%.

Table 1 presents the baseline characteristics of the study sample by liver status at baseline. The NAFLD group had higher weight and BMI z-scores (2.28 *vs* 1.79, $p = 0.004$; 2.46 *vs* 2.14, $p = 0.017$, respectively), higher rate of severe obesity (20.9 *vs* 2.4%, $p = 0.015$), and higher WC and WC percentile (91.3 cm *vs* 85.2 cm, $p = 0.012$; 96.4 *vs* 92.1, $p < 0.001$, respectively). The remaining baseline characteristics were similar between groups (Table 1). The mean follow-up time was 15 months for both groups (Table 1 in Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19834/15330).

Metabolic syndrome and PsiMS accuracy

The global prevalence rate of MS was 7% at baseline, and 8% at the last follow-up. The mean PsiMS score was 2.05 ± 0.48 at baseline, and 2.11 ± 0.52 at follow-up. As graphically represented in Fig. 1, the correlation coefficients of PsiMS at baseline were highly correlated with PsiMS at the first available follow-up (rho = +0.68) and at the last available follow-up (rho = +0.67).

Regarding the ability of PsiMS to predict MS, a significantly higher PsiMS was noted in individuals with MS at baseline compared with those without MS [2.81 (± 0.48) *vs* 1.99 (± 0.48), $p < 0.001$, data not shown]. Likewise, a significant association between PsiMS and the number of MS components was observed ($p < 0.001$) (Fig. 1 in Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19834/15330).

The pediatric metabolic syndrome score was highly accurate in predicting MS, as shown in the ROC curve and box plot in Fig. 2. The cutoff of PsiMS at baseline, representing the maximum sum of sensitivity and specificity to define MS, was ≥ 2.46 (sensitivity 100%, specificity 89%, data not shown). By applying this threshold, 14 (17%) of the subjects were considered in risk of MS (PPV = 36%, NPV = 99%, data not shown). Using the cutoff proposed by Lee *et al,*[12] 10 (12%) of the subjects were considered in risk of MS (PPV = 40%, NPV = 97%, data not shown).

Metabolic syndrome and PsiMS by liver status

In the univariate analysis, no statistically significant differences were observed between the NAFLD and non-NAFLD groups regarding PsiMS and dichotomous classification, as well as the number of MS criteria at baseline and at the last available follow-up (Table 2).

Multivariable regression – NAFLD and other potential predictors of PsiMS

The multivariable regression analysis for PsiMS at baseline revealed a protective effect of female sex (β -0.28, 95% CI -0.46 to -0.09, $p = 0.004$) (Table 3). A higher number of MS criteria (≥ 2 criteria: β 0.35, 95% CI 0.15 to 0.54, $p = 0.001$), BMI z-score (β 0.23, 95% CI 0.07 to 0.39 $p = 0.006$), as well as the presence of IR (β 0.19, 95% CI 0.02 to 0.36 $p = 0.032$) and dyslipidemia (β 0.27, 95% CI 0.10 to 0.44 $p = 0.002$) were associated with a higher PsiMS at baseline (Table 3).

The multivariable regression analysis for PsiMS at the last available follow-up retained the protective effect of female sex (β -0.17, 95% CI -0.33 to -0.01, $p = 0.045$). The following predictors of a higher PsiMS were identified: baseline presence of NAFLD (β 0.15, 95% CI 0.01 to 0.29, $p = 0.033$), higher baseline PsiMS (β 0.49, 95% CI 0.31 to 0.66, $p < 0.001$) and higher BMI z-score increase from baseline

Table 1 – Characteristics of study population by liver status at baseline (NAFLD *versus* non-NAFLD)*

Characteristics	NAFLD (n = 43)	Non-NAFLD (n = 41)	*p* value
Age -yr	11.4 [9.9, 14.0]	12.6 [10.2, 14.5]	0.485
Female sex	30 (70%)	33 (81%)	0.317
Birth weight – g (n = 52)	3293 (406)	3227 (296)	0.251
Age obesity starts – yr (n = 46)	4 [1, 7]	5 [4, 8]	0.262
Previous treatment (n = 82)	27 (64%)	21 (53%)	0.279
Sports practice (n = 80)	27 (66%)	24 (62%)	0.688
Weight - kg	63.5 (15.5)	60.8 (16.2)	0.435
Weight z-score	2.28 (0.80)	1.79 (0.71)	**0.004**
BMI - kg/m^2	26.4 [23.6, 29.6]	24.8 [23.3, 28.4]	0.190
BMI z-score	2.46 (0.6)	2.14 (0.6)	**0.017**
WC - cm	91.3 (10.8)	85.2 (10.8)	**0.012**
WC percentile	96.4 [94.4, 98.0]	92.1 [86.9, 96.0]	**<0.001**
Waist-for-height ratio	0.41 (0.08)	0.39 (0.08)	0.346
WFH ≥ 0.5	7 (16%)	5 (12%)	0.587
SBP - mmHg	117 (1.7)	115 (1.5)	0.352
SBP percentile	79.6 (22.1)	77.3 (19.7)	0.621
DBP - mmHg	62.4 (8.3)	65.7 (8.5)	0.081
DBP percentile	49.1 (24.5)	57 (24.3)	0.139
Glucose - mg/dL	86 [83, 93]	87 [81, 89]	0.294
Insulin - µUI/mL (n = 72)	12.4 [8.9, 15.1]	15.1 [9.3, 21.2]	0.408
HOMA-IR (n = 72)	2.7 [1.9, 3.2]	2.7 [1.9, 4.7]	0.449
ALT - U/l (n = 83)	22 [19, 30]	22 [16, 27]	0.288
AST - U/l (n = 83)	23 [18, 29]	19 [16, 24.5]	0.114
Vit D - ng/mL (n = 64)	24.1 (7.2)	25.3 (7.0)	0.522
CRP - mg/dL (n = 58)	0.13 [0.10, 0.22]	0.21 [0.11, 0.40]	0.144
Uric Acid - mg/dL (n = 68)	4.89 (0.84)	4.47 (0.92)	0.052
TC - mg/dL	159 (29)	163 (32)	0.519
LDL - mg/dL	93 (24)	98 (22)	0.325
HDL - mg/dL	53 (12)	53 (11)	0.817
TG - mg/dL	68 (35)	79 (34)	0.262
Obesity †	33 (77%)	25 (61%)	0.118
Severe obesity ‡	9 (21%)	1 (2%)	**0.015**
HTN §	15 (35%)	10 (24%)	0.293
Dyslipidemia ¶	13 (30%)	12 (29%)	0.923

* Values are no. (%), mean (SD), or median [25th, 75th percentile].
N (n =) is reported for variables with missing values.
NAFLD: nonalcoholic fatty liver disease; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostatic model assessment of insulin resistance; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CT: total cholesterol; LDL: LDL-cholesterol; HDL: HDL-cholesterol; TG: triglycerides; HTN: hypertension.
† Obesity was defined as a BMI z-score > 2.[28,29]
‡ Severe obesity was defined as a BMI z-score > 3.[28,29]
§ Hypertension was defined as per SBP ≥ 130 mmHg or ≥ 90th centile, or DBP ≥ 80 mmHg or ≥ 90th centile.[32]
¶ Dyslipidemia was considered when any of the following was present: 1) CT ≥ 200 mg/dL, 2) LDL ≥ 130 mg/dL, 3) HDL < 40 mg/dL, 4) TG ≥ 130 mg/dL if age ≥ 10, and ≥ 100 mg/dL if age < 10.[46]
To convert values for cholesterol (CT, HDL, and LDL) to mmol/L, multiply by 0.02586; for fasting plasma glucose (Gluc), multiply by 0.05551; for TG, multiply by 0.01129.

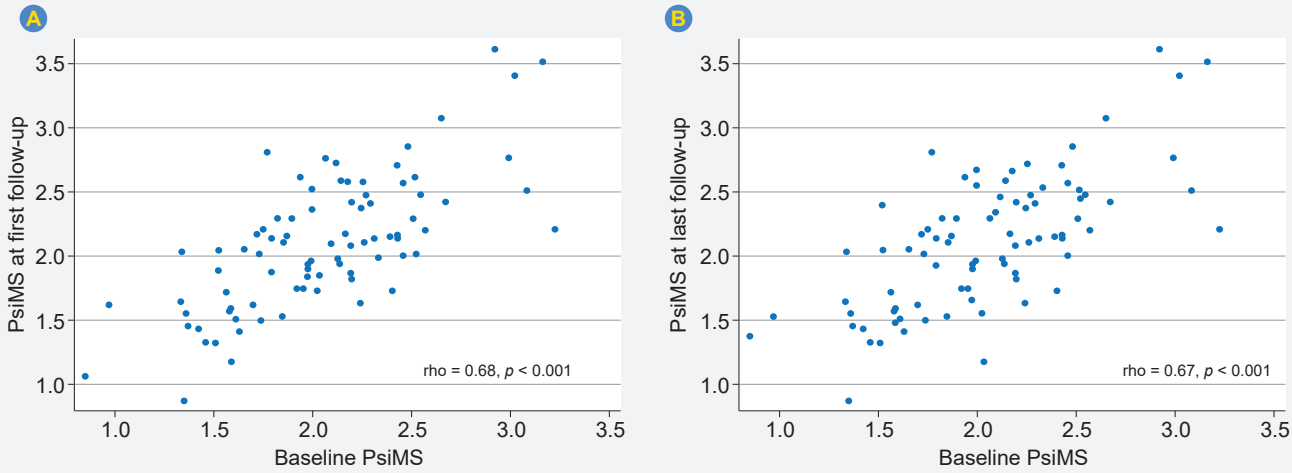

Figure 1 – Scatterplot showing the correlation of baseline PsiMS with PsiMS at the first available follow-up (A) and last available follow-up (B)

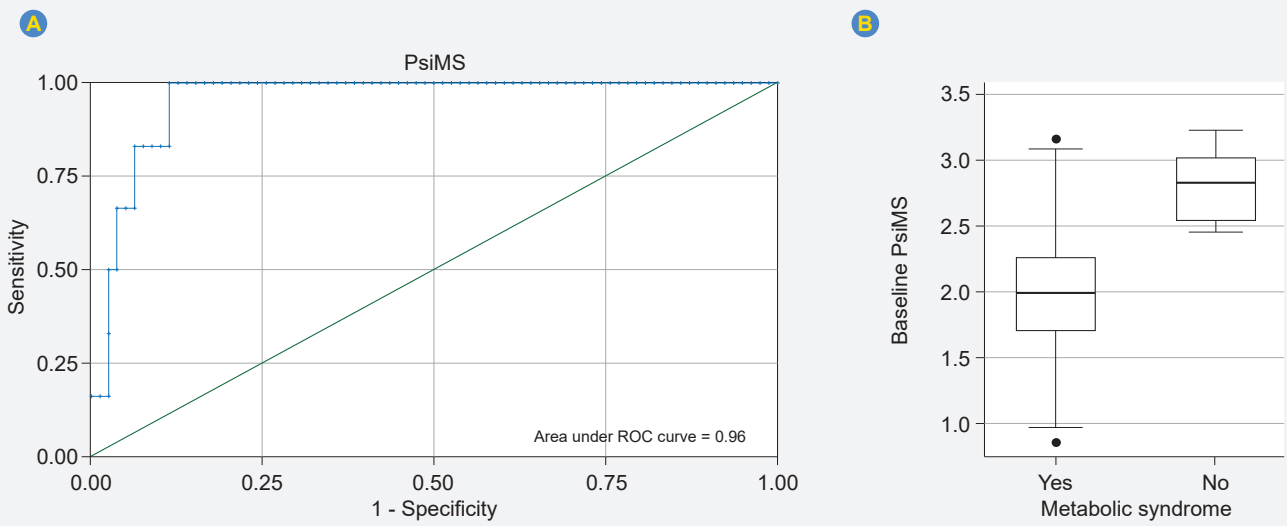

Figure 2 – Receiver operator characteristic curve of PsiMS to discriminate MS at baseline (A). Box plot of PsiMS by MS category (B).

(β 0.20, 95% CI 0.05 to 0.35, $p = 0.009$) (Table 3). The maximum variation inflation factor obtained was 1.738. Residual normality, homoscedasticity and autocorrelation were also evaluated, and it was verified that all regression assumptions were fulfilled.

DISCUSSION

Our study assessed the association between NAFLD and cardiometabolic risk factor aggregation in adolescents with overweight/obesity, using the PsiMS as a predictor of MS.

Our results point towards a NAFLD prevalence rate of 51% in children with overweight/obesity, which is a significant and worrying rate, although not unprecedented.[13,14,17] NAFLD was significantly associated with both obesity and central obesity, with the NAFLD group showing higher weight and BMI z-scores, higher rate of severe obesity, and higher WC and WC percentile. Several authors have reported an association between BMI and NAFLD.[17,19,20,24,25,34,35] In a meta-analysis by Andersen *et al*, the odds ratio for NAFLD in children with overweight/obesity *versus* normal weight children was 13.4 and for participants with obesity *versus* normal weight/overweight participants was 13.7.[17] WC as a marker of central obesity has also been previously associated with NAFLD.[19,20,24,26,34] In studies using liver histology, a higher BMI z-score and higher WC were further linked with

Table 2 – PsiMS and MS by liver status at baseline (NAFLD *versus* non-NAFLD)*

Outcomes	NAFLD (n = 43)	Non-NAFLD (n = 41)	*p* value
PsiMS at baseline	2.08 (0.48)	2.02 (0.48)	0.573
PsiMS at last follow-up	2.21 (0.53)	2.01 (0.5)	0.081
PsiMS delta	0.13 (0.40)	-0.01 (0.38)	0.102
MS at baseline	3 (7%)	3 (7%)	1.000
MS at last follow-up	6 (14%)	1 (2%)	0.110
Number of MS criteria at baseline			0.613
< 2	26 (61%)	27 (66%)	
≥ 2	17 (40%)	14 (34%)	
Number of MS criteria at last follow-up			0.337
< 2	25 (58%)	28 (68%)	
≥ 2	18 (42%)	13 (32%)	

* Values are no. (%) or mean (SD).
PsiMS: simplified continuous metabolic syndrome score; MS: dichotomous metabolic syndrome

Table 3 – Multivariable regression model on predictors of PsiMS at baseline and at last follow-up*

	Coefficient β	95% CI	*p* value	Adjusted R^2
Baseline PsiMS				0.52
NAFLD †	0.004	(-0.15, 0.16)	0.964	
Female sex	-0.28	(-0.46, -0.09)	**0.004**	
Age †	0.03	(0.00, 0.07)	**0.049**	
≥ 2 MS criteria †	0.35	(0.15, 0.54)	**0.001**	
Insulin Resistance †‡ (n = 72)	0.19	(0.02, 0.36)	**0.032**	
Dyslipidemia †§	0.27	(0.10, 0.44)	**0.002**	
BMI z-score	0.23	(0.07, 0.39)	**0.006**	
PsiMS at last follow-up				0.68
NAFLD †	0.15	(0.01, 0.29)	**0.033**	
Female sex	-0.17	(-0.33, -0.01)	**0.045**	
Age ¶	-0.03	(-0.06, 0.00)	0.064	
Baseline PsiMS	0.49	(0.31, 0.66)	**<0.001**	
≥ 2 MS criteria ¶	0.38	(0.22, 0.54)	**<0.001**	
Insulin Resistance †‡ (n = 72)	0.10	(-0.05, 0.25)	0.193	
Dyslipidemia †§	0.14	(-0.03, 0.30)	0.102	
Change in BMI z-score‖	0.20	(0.05, 0.35)	**0.009**	

* The variables above were included in the final regression model, as predictors of PsiMS at baseline and at the last available follow-up. Tested interactions were not shown as they were not significant. N (n =) is reported for variables with missing values.
† Variables at baseline evaluation.
‡ Insulin resistance defined as HOMA-IR ≥ 3.5 or insulin ≥ 15 μUI/mL.[33]
§ Dyslipidemia was considered when any of the following was present: 1) CT ≥ 200 mg/dL, 2) LDL ≥ 130 mg/dL, 3) HDL < 40 mg/dL, 4) TG ≥ 130 mg/dL if age ≥ 10, and ≥ 100 mg/dL if age < 10.[46]
¶ Variable at last available follow-up.
‖ Change of BMI z-score between the baseline evaluation and the last available follow-up.

NAFLD severity.[25,26]

The association between higher uricemia and NAFLD was marginally significant (p = 0.052) and this relationship was also shown in other studies.[35,36]

Associations between NAFLD and other variables were described by other authors in adult and pediatric populations, but were not significant in our analysis, perhaps attributable to the small sample size. These include male sex,[14,34,35] higher WFH ratio,[24] higher BP,[19,20,27,35] glucose/insulin metabolism disorders and IR,[19,20,24,25,27,35] lipid

disorders,[19,20,27,34,35] and higher CRP.[24,35]

Surprisingly, we found no association between ALT and AST levels and NAFLD. Aspartate aminotransferase has been proposed as a marker of NAFLD, and therefore we would expect an association with US diagnosis as previously reported.[34,35] Nevertheless, other studies have failed to find this association,[37] and it has been shown that a significant rate of children with obesity and fatty infiltration on US or histology may have low ALT values.[25,38]

The prevalence rate of MS in this study was 7%. A higher prevalence rate in children with obesity has been reported, ranging from 10% to 66%.[3,25,39] However, we should consider that our sample included patients with overweight, in whom MS is less frequent than in samples just including patients with obesity, and we used the IDF definition which is stricter and generally provides a lower prevalence rate than other definitions.[39] Furthermore, a similar prevalence rate was found in two studies including Portuguese pediatric patients.[40,41] Despite this relatively low prevalence rate, 37% had ≥ 2 MS criteria which represents a significant metabolic burden.

The pediatric metabolic syndrome score at baseline revealed a strong correlation with PsiMS at the first and at the last follow-up, suggesting good consistency over time. It was highly accurate in predicting patients with MS, with an AUC of 0.96. The score was also positively associated with the number of MS components. In a sample of 2983 adolescents aged 10 to 18, similar results were observed.[12] Applying our cutoff, or even the cutoff proposed by Lee *et al*, enabled the identification of a higher proportion of children with increased cardiometabolic risk compared to the binary classification of MS.[12]

Applying the binary definition, in which each component is either normal or abnormal, beneath or above the cutoff values, a subject can only be diagnosed as either having MS or not, losing track of the overall cardiometabolic risk profile. Using a continuous score like PsiMS may overcome this limitation and better reflect the pathophysiological process, as proposed first by the American Diabetes Association and the European Association for the Study of Diabetes, and thereafter stated by several authors in adult and pediatric patients.[3-12]

No statistically significant differences were observed in the NAFLD *versus* the non-NAFLD group, concerning PsiMS, dichotomous MS, as well as the number of MS criteria. On the other hand, in the multivariable analysis NAFLD emerged as a predictor of PsiMS at the last follow-up. Hence, after adjusting for the other variables in the model, the presence of NAFLD at baseline increased the follow-up PsiMS by 0.15 units.

Female sex revealed a protective effect, reducing the score by 0.28 at baseline and 0.17 at follow-up when adjusting for the other variables in the models. Having ≥ 2 MS criteria, higher BMI z-score, IR and dyslipidemia was associated with a higher PsiMS at baseline. For PsiMS at the last available follow-up besides NAFLD, baseline PsiMS and BMI change from baseline were potential predictors.

In a previous cross-sectional study including 313 adolescents with obesity aged 12 to 18, boys had a significantly worse cardiometabolic risk profile than girls.[42] An increased risk of MS related to the male sex was also found among extremely obese adolescents.[43] Likewise, mean PsiMS was significantly higher in boys in a national sample of Korean adolescents.[12] The association of IR and MS was previously reported,[25,44] as well as BMI[6] and dyslipidemia.[45]

Several authors have reported an association between NAFLD and MS in children.[15,25-27] In one study regarding children and adolescents (6 - 17 years old), MS was common amongst subjects with NAFLD and was associated with the severity of steatosis. Individual MS components, namely IR and central obesity, were also associated with the severity of NAFLD.[26] In a cohort of 120 children aged 3 - 18 with biopsy-proven NAFLD, the presence of MS seemed to be related to histological features of nonalcoholic steatohepatitis.[25] Schwimmer *et al* also showed that in children with overweight or obesity NAFLD was strongly associated with several cardiovascular risk factors.[27]

The pathophysiology of the clustering of metabolic risk factors termed MS is complex and probably does not have a single underlying cause, but apparently depends on the presence of two factors: excess body fat and genetic susceptibility, with interactions by multiple mediators. Ectopic fat deposition, such as NAFLD, is probably an important factor in the pathogenesis of MS and other obesity-related comorbidities.

In our sample no significant association between NAFLD and MS was observed in the univariate analysis and in the regression models to predict baseline PsiMS. However, we did observe a nonsignificant tendency of the NAFLD group towards a worse metabolic profile at the last follow-up with higher PsiMS, higher MS prevalence rate, and higher rate of individuals with ≥ 2 MS criteria. Additionally, in the last follow-up, the PsiMS regression model NAFLD did become a significant predictor, which may suggest that the potential impact of NAFLD on cardiometabolic risk increases over time.

The present study should be interpreted considering its acknowledged limitations. The main limitation is related to the retrospective nature of the study, which resulted in several missing data that hampered the inclusion of a large proportion of our patients, and subsequently a small sample size. Moreover, no subgroup analysis was performed. Several selection biases hinder the extension of the results to the general population. We analyzed a convenience

clinical sample, excluding patients without US and at least two complete evaluations. Hence, the proportion of overweight/obesity and female/male probably may not reflect the distribution on the target population.

These issues could be overcome with a prospective longitudinal study including other centers, allowing for a proper and robust longitudinal analysis.

Besides these limitations, some strengths should also be mentioned. To the best of our knowledge this is the first study addressing the relationship between NAFLD and MS in Portuguese pediatric patients. Additionally, it is the first study analyzing this issue using a continuous MS score. We used a recently proposed, simpler score that is potentially useful both in research and in clinical practice. Our project can be regarded as a pilot study for future and more robust research in this field.

CONCLUSION

The results indicate a good performance of the PsiMS to assess MS and suggest that NAFLD is associated with PsiMS at follow-up.

ACKNOWLEDGEMENTS

The authors acknowledge Brian Claggett for his kind help and guidance in the data analyses performed in this study.

AWARDS

This study was awarded the “Crescemos Consigo” Prize sponsored by Pfizer Vaccines, at the 22nd Pediatrics National Congress which was held in October 2022.

AUTHOR CONTRIBUTIONS

SF: Conception/design of the study; data collection, analysis, and interpretation; drafting and critical review of the manuscript.

JM, DC: Data collection and interpretation; critical review of the manuscript.

DF: Data analysis and interpretation; drafting of the manuscript.

CR: Conception/design of the study; data analysis and interpretation; critical review of the manuscript.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients’ data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

FUNDING SOURCES

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Magge SN, Goodman E, Armstrong SC, Daniels S, Corkins M, De Ferranti S, et al. The metabolic syndrome in children and adolescents: Shifting the focus to cardiometabolic risk factor clustering. Pediatrics. 2017;140:e20171603.
2. Zimmet P, Alberti GK, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatr Diabetes. 2007;8:299-306.
3. Eisenmann JC. On the use of a continuous metabolic syndrome score in pediatric research. Cardiovasc Diabetol. 2008;7:1-6.
4. Vukovic R, Milenkovic T, Stojan G, Vukovic A, Mitrovic K, Todorovic S, et al. Pediatric siMS score: a new, simple and accurate continuous metabolic syndrome score for everyday use in pediatrics. PLoS One. 2017;12:1-10.
5. Villa JK, E Silva AR, Santos TS, Ribeiro AQ, Sant’Ana LF. Metabolic syndrome risk assessment in children: use of a single score. Rev Paul Pediatr. 2015;33:187-93.
6. Peterson MD, Saltarelli WA, Visich PS, Gordon PM. Strength capacity and cardiometabolic risk clustering in adolescents. Pediatrics. 2014;133:e896-903.
7. Shafiee G, Kelishadi R, Heshmat R, Qorbani M, Motlagh ME, Aminaee T, et al. First report on the validity of a continuous Metabolic Syndrome score as an indicator for Metabolic Syndrome in a national sample of paediatric population - The CASPIAN-III study. Endokrynol Pol. 2013;64:278-84.
8. Peterson MD, Liu D, Iglay Reger HB, Saltarelli WA, Visich PS, Gordon PM. Principal component analysis reveals gender-specific predictors of cardiometabolic risk in 6th graders. Cardiovasc Diabetol. 2012;11:146.
9. Kelishadi R, Heshmat R, Mansourian M, Motlagh ME, Ziaodini H, Taheri M, et al. Association of dietary patterns with continuous metabolic syndrome in children and adolescents; A nationwide propensity score-matched analysis: The CASPIAN-V study. Diabetol Metab Syndr. 2018;10:52.
10. Heshmat R, Heidari M, Ejtahed HS, Motlagh ME, Mahdavi-Gorab A, Ziaodini H, et al. Validity of a continuous metabolic syndrome score as an index for modeling metabolic syndrome in children and adolescents: The CASPIAN-V study. Diabetol Metab Syndr. 2017;9:89.
11. Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2005;28:2289-304.
12. Lee YJ, Seo MY, Kim SH, Park MJ. Validity of the pediatric simple metabolic syndrome score. Obes Res Clin Pract. 2020;14:508-13.
13. Ko JS. New perspectives in pediatric nonalcoholic fatty liver disease: epidemiology, genetics, diagnosis, and natural history. Pediatr Gastroenterol Hepatol Nutr. 2019;22:501-10.
14. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children: recommendations from the expert committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). J Pediatr Gastroenterol Nutr. 2017;64:319-34.
15. Pacifico L, Nobili V, Anania C, Verdecchia P, Chiesa C. Pediatric

nonalcoholic fatty liver disease, metabolic syndrome and cardiovascular risk. World J Gastroenterol. 2011;17:3082-91.
16. Schwimmer J. Clinical advances in pediatric nonalcoholic fatty liver disease. Hepatology. 2016;63:1718-25.
17. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. PLoS One. 2015;10:e0140908.
18. Martins E, Oliveira A. NAFLD and cardiovascular disease. Porto Biomed J. 2018;3:e2.
19. Motamed N, Rabiee B, Poustchi H, Dehestani B, Hemasi GR, Khonsari MR, et al. Non-alcoholic fatty liver disease (NAFLD) and 10-year risk of cardiovascular diseases. Clin Res Hepatol Gastroenterol. 2017;41:31-8.
20. Motamed N, Ajdarkosh H, Ahmadi M, Perumal D, Ashrafi GH, Nikkhah M, et al. Non-alcoholic fatty liver disease is not independent risk factor for cardiovascular disease event: a cohort study. World J Hepatol. 2020;12:323–31.
21. Targher G. Non-alcoholic fatty liver disease, the metabolic syndrome and the risk of cardiovascular disease: the plot thickens. Diabet Med. 2007;24:1-6.
22. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med. 2010;363:1341-50.
23. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut. 2017;66:1138-53.
24. Loureiro C, Martínez-Aguayo A, Campino C, Carvajal C, Fardella C, García H. Esteatosis Hepática: ¿Preludio de diabetes tipo 2 en población pediátrica? Nutr Hosp. 2014;29:350-8.
25. Manco M, Marcellini M, DeVito R, Comparcola D, Sartorelli MR, Nobili V. Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. Int J Obes. 2008;32:381-7.
26. Patton HM, Yates K, Unalp-Arida A, Behling CA, Huang TT, Rosenthal P, et al. Association between metabolic syndrome and liver histology among children with nonalcoholic fatty liver disease. Am J Gastroenterol. 2010;105:2093-102.
27. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. Circulation. 2008;118:277-83.
28. Guerra A. As curvas de crescimento da Organização Mundial de Saúde. Acta Pediatr Port. 2009;40:41-5.
29. Onis M. World Health Organization Reference Curves. The free obesity ebook. 2014. [cited 2023 Feb 13]. Available from: https://ebook.ecog-obesity.eu/wp-content/uploads/2015/02/ECOG-Obesity-eBook-World-Health-Organization-Reference-Curves.pdf.
30. Green Corkins K, Teague EE. Pediatric nutrition assessment: anthropometrics to zinc. Nutr Clin Pract. 2017;32:40-51.
31. Sharma AK, Metzger DL, Daymont C, Hadjiyannakis S, Rodd CJ. LMS tables for waist-circumference and waist-height ratio z-scores in children aged 5-19 y in NHANES III: association with cardio-metabolic risks. Pediatr Res. 2015;78:723-9.
32. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics. 2017;140:e20171904.
33. Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. Diabetes Care. 2004;27:314-9.
34. Mohamed RZ, Jalaludin MY, Anuar Zaini A. Predictors of non-alcoholic fatty liver disease (NAFLD) among children with obesity. J Pediatr Endocrinol Metab. 2020;33:247-53.
35. Sartorio A, Del Col A, Agosti F, Mazzilli G, Bellentani S, Tiribelli C, et al. Predictors of non-alcoholic fatty liver disease in obese children. Eur J Clin Nutr. 2007;61:877-83.
36. Mosca A, Nobili V, De Vito R, Crudele A, Scorletti E, Villani A, et al. Serum uric acid concentrations and fructose consumption are independently associated with NASH in children and adolescents. J Hepatol. 2017;66:1031-6.
37. Shannon A, Alkhouri N, Carter-Kent C, Monti L, Devito R, Lopez R, et al. Ultrasonographic quantitative estimation of hepatic steatosis in children With NAFLD. J Pediatr Gastroenterol Nutr. 2011;53:190-5.
38. Ezaizi Y, Kabbany MN, Conjeevaram Selvakumar PK, Sarmini MT, Singh A, Lopez R, et al. Comparison between non-alcoholic fatty liver disease screening guidelines in children and adolescents. JHEP Rep. 2019;1:259-64.
39. Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome—a critical look on the discrepancies between definitions and its clinical importance. Int J Obes. 2021;45:12-24.
40. Moniz M, Marques T, Cabral M, Nizarali Z, Coelho R, Monteiro A, et al. Factores de risco cardiovascular e obesidade infantil. Acta Med Port. 2011;24:327-32.
41. Silva F, Ferreira E, Gonçalves R, Cavaco A. Obesidade pediátrica: a realidade de uma consulta. Acta Med Port. 2012;25:91-6.
42. Barstad LH, Júlíusson PB, Johnson LK, Hertel JK, Lekhal S, Hjelmesæth J. Gender-related differences in cardiometabolic risk factors and lifestyle behaviors in treatment-seeking adolescents with severe obesity. BMC Pediatr. 2018;18:1-8.
43. Lafortuna CL, Adorni F, Agosti F, De Col A, Sievert K, Siegfried W, et al. Prevalence of the metabolic syndrome among extremely obese adolescents in Italy and Germany. Diabetes Res Clin Pract. 2010;88:14-21.
44. Jankowska A, Brzeziński M, Romanowicz-Sołtyszewska A, Sidorkiewicz AS. Metabolic syndrome in obese children-clinical prevalence and risk factors. Int J Environ Res Public Health. 2021;18:1-11.
45. Thomas NE, Rowe DA, Murtagh EM, Stephens JW, Williams R. Associations between metabolic syndrome components and markers of inflammation in Welsh school children. Eur J Pediatr. 2018;177:409-17.
46. De Jesus JM. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: Summary report. Pediatrics. 2011;128:213-56.