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Alimentary Tract

Salivary markers of hepato-metabolic comorbidities in pediatric obesity

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ABSTRACT

Background: The pediatric obesity epidemic calls for the noninvasive detection of individuals at higher risk of complications.
Aims: To investigate the diagnostic role of combined salivary uric acid (UA), glucose and insulin levels to screen noninvasively for metabolic syndrome (MetS) and nonalcoholic fatty liver disease.
Methods: Medical history, clinical, anthropometric, and laboratory data including serum triglyceride, glucose, insulin, HOMA, HDL-cholesterol, and UA levels of 23 obese children (15 with [St+] and 8 without [St−] ultrasonographic hepatic steatosis) and 18 normal weight controls were considered.
Results: Serum and salivary UA ($p < 0.05$; $R^2 = 0.51$), insulin ($p < 0.0001$; $R^2 = 0.79$), and HOMA ($p < 0.0001$; $R^2 = 0.79$) levels were significantly correlated; however their values tended to be only slightly higher in the obese patients, predominately in [St+], than in the controls. Notably, UA and insulin levels in both fluids increased in parallel to the number of MetS components. After conversion of the z-logit function including salivary/anthropometric parameters in a stepwise logistic regression analysis, a factor of 0.5 allowed for predicting hepatic steatosis with high sensitivity, specificity, and total accuracy.
Conclusions: Salivary testing together with selected anthropometric parameters helps to identify noninvasively obese children with hepatic steatosis and/or having MetS components.

1. Introduction

Obesity has become a global and critical medical issue in both adults and children, and the provisions regarding the tracking of this phenomenon into adulthood are alarming [1].

A disquieting aspect is the parallel increase in this disease's comorbidities, such as hypertension, hyperglycemia, dyslipidemia [high triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-Col)] that, together with visceral obesity, contribute to the framework of metabolic syndrome (MetS), a condition whose exact definition is still debated regarding the pediatric population. Depending on the classification, this condition occurs in between 1 and 23% of the total pediatric population and in up to 60% in the obese and overweight [2].

Hepatic steatosis with/without inflammatory and fibro-cirrhotic changes in the wider context of non-alcoholic fatty liver disease (NAFLD) is now considered another feature of MetS. Its detection is of particular relevance because it appears able to detect/forecast a higher probability of cardiovascular (CV) disease [3]. Elevated values of Homeostatic Model Assessment-Insulin Resistance [HOMA-IR] [4] and of serum uric acid [UA] [5] have also proven to be useful and reliable markers of MetS and/or hepatic steatosis.

Based on these premises and given that (A) hepato-cardio-metabolic morbidity associated with obesity is not necessarily due to the degree of obesity *per se*, (B) blood tests and/or ultrasonography may still represent a financial/organizative barrier in so large populations at risk; (C) saliva testing has a high diagnostic potential, especially in children, as it is a noninvasive, painless and simple procedure [6–8], and (D) that evidence of a good posi-

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tive correlation exists between serum and salivary levels of several analytes/biomarkers, including glucose [7,9], insulin [6,10] and UA [11], we designed a pilot study aiming to further assess the possible diagnostic value of the combination of these three salivary markers for the noninvasive study of obesity-related metabolic abnormalities and the prediction of hepatic steatosis in the pediatric population.

2. Methods

2.1. Population and study design

Forty-one subjects (aged 10–15 years) with documented good oral *health* were enrolled after obtaining parental agreement and written informed consent. The cohort included 23 consecutive obese children (body mass index [BMI] >95th percentile) followed at our obesity clinic and 18 consecutive normal weight children (NW; BMI from 5th to less than 85th percentile) recruited at the Pediatric Surgery division, where they were listed for minor surgery.

Patients were characterized based on clinical/anthropometric data (blood pressure, BMI, waist circumference [WC] [12], laboratory parameters (serum alanine aminotransferase [ALT], aspartate aminotransferase [AST], total and HDL cholesterol, triglyceride [TG], UA, glucose, and insulin levels), and ultrasonography (US) [evaluating the presence (St+) or absence (St−) of hepatic steatosis]. US examination was performed using an US apparatus (Hitachi Aloka, Wallingford, Connecticut, USA) equipped with a convex pediatric probe. Liver steatosis (liver brightness *vs.* renal echogenicity) was categorized as absent, mild, moderate or severe according to the usual US criteria [12,13].

Blood tests were performed using a standard laboratory chemistry analyzer and an immunoassay for insulin (Abbott Diagnostics, Santa Clara, CA).

The cohort study was carried out in accordance with the ethical principles of the Declaration of Helsinki 2013 [14] and was approved by the local institutional ethics committee. Each subject underwent saliva sampling by means of a saliva swab collection device (Salivette®, Sarstedt, Nümbrecht, Germany), which was then centrifuged and frozen at −20 °C before testing. To enable the detection of small concentrations in small amounts of saliva, salivary tests were performed using gas chromatography–mass spectrometry (GC–MS-QP2010SE, Shimadzu, Kyoto, Japan) for UA and glucose levels measurements and using high-performance liquid chromatography–mass spectrometry (HPLC–MS/MS–LCMS 8050, Shimadzu, Kyoto, Japan) for insulin levels.

Although it is emerging that not even one individual criteria over pathological WC should be underestimated in pediatric MetS-related comorbidities risk development [15], MetS was defined according to the International Diabetes Foundation (IDF) as the presence of central obesity (*i.e.*, WC >95th percentile) in addition to 2 of the 4 remaining criteria: TG >150 mg/dl; blood glucose >100 mg/dl; systolic blood pressure (SBP) >95th percentile; and HDL cholesterol <40 mg/dl [16].

2.2. Hepatic steatosis predictor index

To obtain a relation among the salivary and anthropometric parameters that would allow us to predict hepatic involvement among the obese patients, we built a stepwise logistic regression analysis [17] model. In the stepwise variable selection, significant variables were inserted sequentially; after introducing a variable in the model, we checked and removed variables that had become insignificant. The criterion for the insertion of a variable was a p-value of <0.10 [17]; a variable was removed from the model if its associated significance level was >0.20 [17]. All variables, apart from gender, were evaluated as continuous.

The estimated logit function ($\mathcal{Z}$), which included the β_0 intercept parameter, was converted to the PEdiatric STeatosis Index (PESTI) using a classical sigmoidal function:

$$PESTI = \left(\frac{1}{1 + e^{\mathcal{Z}}} \right)$$

The usefulness of the PESTI parameter for discriminating liver steatosis was assessed by calculating the area under the receiver operating characteristic (ROC) curve (AUC). The standard error of the AUC was calculated according to Hanley and McNeil [18]. The sensitivity (S), specificity (SP), positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV), and accuracy (A) for the best cut-off value and their relative standard errors were calculated [19].

2.3. Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software Inc., CA, USA) and R [20]. Data were checked for normality using the Shapiro–Wilk test. Student's *t*-test was used for comparing means; one-way analysis of variance (ANOVA) was used to determine the significance of differences among the means of three or more independent groups; Pearson's linear regression/correlation was used to evaluate associations between two continuous variables. All statistical tests were two-tailed, and significance was assessed based on a p-value of <0.05.

3. Results

The clinical and laboratory characteristics of the study population are reported in Table 1. Liver involvement, defined by the finding of any grade of US liver brightness, enabled the allocation of obese patients into 2 groups: obese with ([St+], n = 15) and without ([St−], n = 8) hepatic steatosis. Table S1 shows that ten of the 23 obese patients had a WC >90th%le + 2 concurrent abnormal parameters (BP and Glucose in 4/10; BP + TG in 2/10; BP + HDL in 2/10; BP and Glucose and TG in 1/10; BP and Glucose and HDL in 1/10), fulfilling the IDF definition of MetS [16].

There were correlations between the salivary and blood levels of UA ($p < 0.05$; $R^2 = 0.51$), insulin ($p < 0.0001$; $R^2 = 0.79$), glucose ($p < 0.0001$; $R^2 = 0.62$), and HOMA index ($p < 0.0001$; $R^2 = 0.79$) (Fig. 1).

Serum UA levels tended to be slightly higher in the obese patients *vs.* controls, as well as in those with steatosis *vs.* those without steatosis (Fig. 2A). In addition, salivary UA levels were higher in the obese patients but did not differ among obese subjects with and without a fatty liver (p-value = 0.98). Although the single highest individual values were found in patients with a fatty liver (Fig. 2A), the predictive value was low (Table 2).

Both serum and saliva levels of insulin (Fig. 2B), HOMA (Fig. 2C) and glucose (Fig. 2D) tended to be slightly higher in the obese patients *vs.* the controls and in those with steatosis *vs.* those without steatosis, and the predictive performance remained unsatisfactory (Table 2)

Serum and salivary levels of UA and insulin, as well as the HOMA index, increased in parallel with the number of MetS components (Fig. 3). In both biological fluids, UA and insulin levels were linearly correlated with the WC measurements; the same correlation also existed for the serum and salivary UA levels as well as systolic blood pressure (Fig. S1).

Table 1
Characteristics of the study population.

Anthropometric and laboratory parameters	Controls (n = 18)	Obese with steatosis (n = 15)	Obese without steatosis (n = 8)	All obese (n = 23)
Gender (M/F)	13/5	10/5	4/4	14/9
Age (years)	10.53 ± 2.57	12.48 ± 2.77*	12.51 ± 2.79*	12.49 ± 2.71*
Weight (kg)	37.42 ± 11.26	79.99 ± 28.76*	71.9 ± 17.31*	77.18 ± 25.24*
Height (cm)	140.17 ± 15.17	153.41 ± 19.27*	157.45 ± 11.97*	154.52 ± 16.88*
BMI (kg/cm^2)	18.52 ± 2.92	32.80 ± 6.94*	28.93 ± 5.58*	31.45 ± 6.65*
BMI percentile	23.75 ± 34.25	95.14 ± 0.53*	95.67 ± 1.03*	95.40 ± 1.05*
BMI Z-score	0.43 ± 1.06	2.31 ± 0.40*	2.02 ± 0.44*	2.21 ± 0.43*
WC (cm)	61.14 ± 7.11	93.27 ± 12.68*	86.00 ± 14.53*	90.74 ± 13.49*
WC percentile	65.85 ± 24.58	94.98 ± 0.97*	94.38 ± 1.77*	94.78 ± 1.04*
WC centimeters >95th percentile	0	21.03 ± 10.57*	14.00 ± 10.99*	18.59 ± 11.01*
WtHR	0.43 ± 0.03	0.61 ± 0.05*	0.55 ± 0.08*	0.59 ± 0.07*
Neck circumference (cm)	27.67 ± 2.41	36.05 ± 4.33*	34.69 ± 4.08*	35.58 ± 4.20*
N C percentile	44.12 ± 33.22	95.57 ± 5.35*	92.61 ± 3.15	94.09 ± 4.26*
NC centimeters >95th percentile	0	3.71 ± 2.77*	2.41 ± 2.75*	3.26 ± 2.77*
SBP (mmHg)	95.98 ± 11.95	127.47 ± 8.95*	125.63 ± 20.23*	126.83 ± 13.49*
SBP percentile	50.00 ± 0	86.93 ± 19.36*	83.50 ± 20.96*	85.74 ± 19.52*
DBP (mmHg)	55.00 ± 10.77	61.53 ± 10.42*	60.75 ± 11.70*	61.26 ± 10.62*
DBP percentile	50.00 ± 0	56.00 ± 15.83*	55.00 ± 14.14*	55.65 ± 14.95*
ALT (U/l)	17.33 ± 4.31	50.17 ± 28.75*	34.50 ± 37.74*	44.72 ± 32.21*
AST (U/l)	24.72 ± 4.87	46.19 ± 28.58*	19.75 ± 5.85	37.00 ± 26.39*
Total cholesterol (mg/dl)	148.78 ± 16.38	158.17 ± 21.91*	162.00 ± 24.20*	159.50 ± 22.26*
HDL (mg/dl)	56.94 ± 14.45	45.07 ± 10.21*	48.00 ± 5.50*	46.09 ± 8.83*
Triglyceride (mg/dl)	Not available	90.59 ± 26.97	138.63 ± 91.90	107.30 ± 60.80
Blood glucose (mg/dl)	83.17 ± 6.61	88.59 ± 10.36*	90.00 ± 10.34*	89.08 ± 10.14*
Salivary glucose (μM)	3338.36 ± 1274.73	3167.86 ± 1192.75	2647.09 ± 1227.77	2986.70 ± 1203.86
Blood insulin (U/l)	10.27 ± 5.22	24.24 ± 10.95*	19.60 ± 6.63*	22.62 ± 9.77*
Salivary insulin (nM)	5.79 ± 2.85	20.89 ± 8.69*	17.26 ± 6.37*	19.60 ± 8.00*
Blood HOMA-IR	2.01 ± 1.16	5.34 ± 2.60*	4.11 ± 2.16*	4.91 ± 2.48*
Salivary HOMA-IR	119.7 ± 73.99	401.81 ± 231.17*	278.79 ± 162.48*	358.20 ± 215.35*
Blood uric acid (mg/dl)	4.04 ± 0.76	5.06 ± 1.23*	4.42 ± 0.92*	4.84 ± 1.15*
Salivary uric acid (μM)	143.46 ± 4.53	157.29 ± 13.04*	156.45 ± 15.31*	157.00 ± 13.53*

Abbreviations: ALT: alanine transaminase; AST: aspartate transaminase; BMI: body mass index; DBP: diastolic blood pressure; HDL: high density lipoproteins; HOMA-IR: Homeostasis Assessment Model-Insulin Resistance, WC: waist circumference; NC: neck circumference; SBP: systolic blood pressure; WtHR: waist to height ratio

* p Value <0.05 compared to controls.

Table 2
Predictive performance of the single features included in the PESTI index.

	Cut-off	Sensitivity		Specificity		PLR	NLR	PPV		NPV		AUC		Accuracy
		Value	SE	Value	SE	Value	Value	Value	SE	Value	SE	Value	SE	Value
Age	12.83	0.67	0.12	0.73	0.09	2.48	0.46	0.79	0.08	0.59	0.12	0.63	0.10	0.71
BMI	23.53	0.93	0.06	0.77	0.08	4.04	0.09	0.95	0.05	0.70	0.10	0.89	0.05	0.83
DBP	54	0.87	0.09	0.42	0.10	1.50	0.32	0.85	0.10	0.46	0.09	0.63	0.09	0.59
H	142	0.48	0.10	0.46	0.09	0.90	1.12	0.48	0.10	0.46	0.09	0.65	0.10	0.47
NCE	1.2	0.80	0.10	0.88	0.06	6.93	0.23	0.88	0.06	0.80	0.10	0.84	0.07	0.85
SG	11.23	0.81	0.10	0.44	0.10	1.45	0.43	0.79	0.11	0.48	0.10	0.59	0.09	0.59
SHM	0.07	0.87	0.09	0.81	0.08	4.51	0.17	0.91	0.06	0.72	0.11	0.86	0.06	0.83
SI	2.45	0.87	0.09	0.81	0.08	4.51	0.17	0.91	0.06	0.72	0.11	0.69	0.06	0.83
SBP	114	0.93	0.06	0.73	0.09	3.47	0.09	0.95	0.05	0.67	0.10	0.83	0.07	0.80
SUA	2.48	0.80	0.10	0.77	0.08	3.47	0.26	0.87	0.07	0.67	0.11	0.77	0.08	0.78
WCE	5	1.00	0.00	0.77	0.08	4.33	0.00	1.00	0.00	0.71	0.10	0.91	0.05	0.85
W	70	0.73	0.11	0.88	0.06	6.36	0.30	0.85	0.07	0.79	0.11	0.81	0.08	0.83
WtHR	0.74	1.00	0.00	0.69	0.09	3.25	0.00	1.00	0.00	0.65	0.10	0.77	0.08	0.80
PESTI	0.5	1.00	0.00	1.00	0.00	ND	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00

Abbreviations: AUC, area under the curve; BMI, body mass index; DBP, diastolic pressure; NCE, neck circumferences excess to 95th percentile; H, height; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, Positive likelihood ratio; PPV, positive predictive value; PESTI, pediatric steatosis index; SG, salivary glucose; SHM, salivary HOMA index; SI, salivary insulin; SBP, systolic pressure; SUA, salivary uric acid; W, weight; WCE, waist circumference excess to 95th percentile; WtHR, waist to height ratio.

3.1. *Hepatic steatosis predictor index*

For predicting hepatic steatosis noninvasively, we performed a stepwise logistic regression analysis. The $\mathcal{Z}$ logit function was estimated as follows:

$$\mathcal{Z} = 3605.37 + 53.75A + 16.30W - 21.81H - 53.16BMI$$
$$+14.79WCE - 16.24WtHR - 12.87NCE - 0.67SP - 6.63DP$$
$$-10.66SUA + 4.17SI - 27.88SG + 1308.74SHM$$

where A is age (years); W is weight (kg); H is height (cm); BMI is the body mass index (kg/m^2); WCE is the number of centimeters by which WC exceeds the 95th percentile; WtHR is the waist-to-height ratio; NCE is the number of centimeters by which neck circumference (NC) exceeds the 95th percentile; SP and DP are the systolic and diastolic blood pressure (mm Hg), respectively; SAU is the UA salivary content (mg/dl); SI is the salivary insulin content (U/L); SG is the salivary glucose content (mg/dl); and SHM is the salivary HOMA. All variables, apart from sex, were evaluated as continuous.

After conversion of the logit function in the PESTI parameter, a PESTI value of 0.5 was found to represent the cut-off value pro-

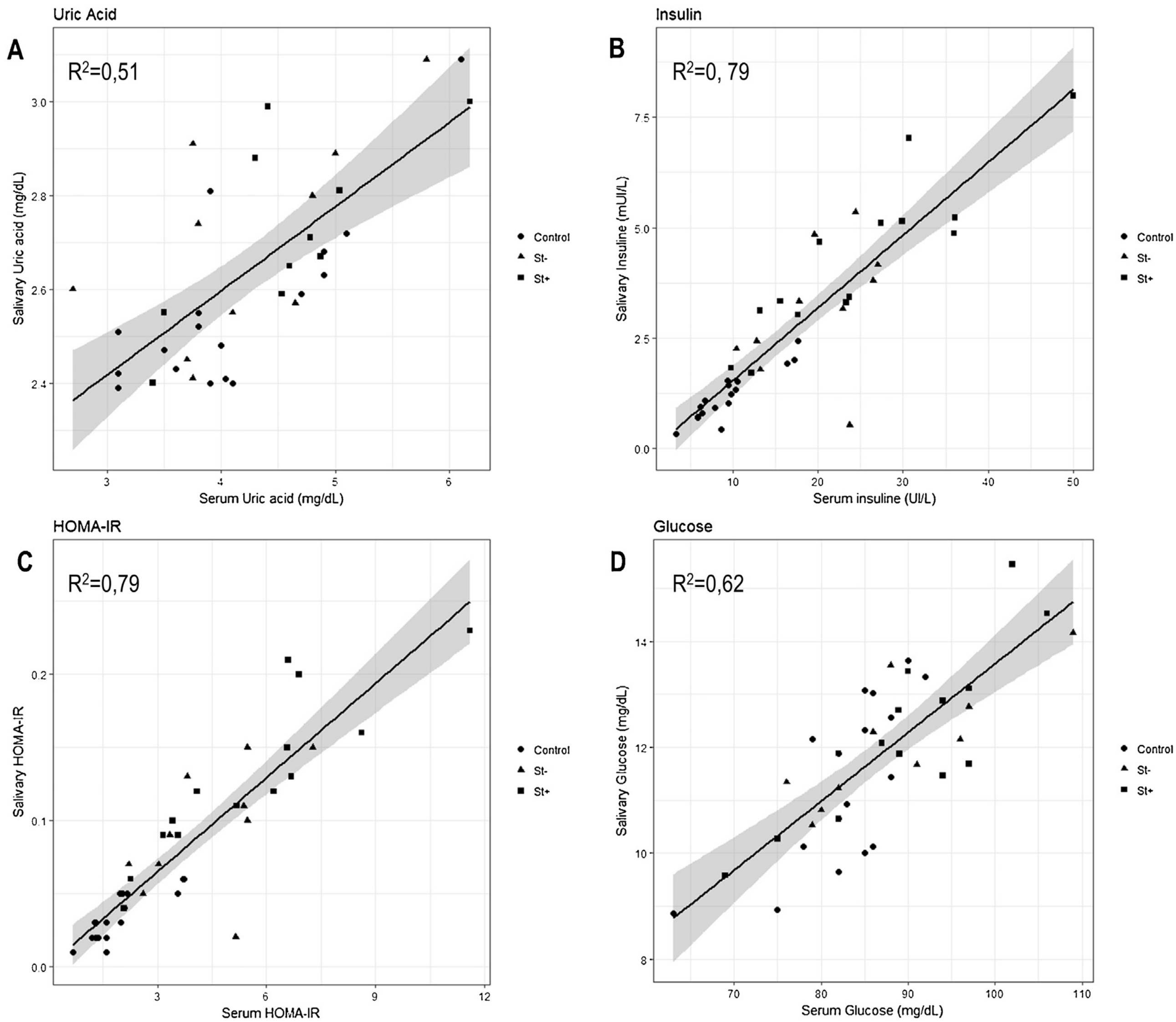

Fig. 1. Correlations between serum and salivary concentrations of uric acid (panel A), insulin (panel B), HOMA (panel C) and glucose (panel D) ($p < 0.05$ for each correlation). *Abbreviations*: HOMA-IR, homeostasis model assessment for insulin resistance.

ducing the highest AUC value (1.0 ± 0.0). This cut-off enabled the prediction of the presence of hepatic steatosis in our study population with a sensitivity of 1.0 ± 0.0, a specificity of 1.0 ± 0.0, an NPR = 0.0, an NPV = 1.0 ± 0.0, a PPV = 1.0 ± 0.0, and a total accuracy of 100% (Fig. S2)

4. Discussion

Although saliva is the most easily accessible and readily obtained biofluid, there has been some reluctance to use it for everyday laboratory evaluations. Some studies, however, have shown its suitability for investigations of some individual metabolites, not only in disorders of the oral cavity [21] but also in systemic conditions such as pediatric obesity and metabolic syndrome [6,7,9–11].

Here, we have presented data supporting and adding to the usefulness of saliva testing as a noninvasive and resourceful diagnostic method that may be used to investigate not only pediatric obesity-related MetS but also liver disease, *i.e.*, two of the main associated comorbidities of obesity that necessitate accelerated and more intensive medical intervention efforts in both adults and in children [15].

In agreement with some of the data reported in adults [6,22,23] and adolescents [8], we found a positive correlation between the serum and salivary levels of glucose and insulin, and the levels of these two molecules tended to be higher in the saliva of obese subjects with or without MetS than in healthy controls. That is, in our patients, insulin levels tended to be higher in the obese children than in the controls and higher in those with steatosis than in those without steatosis. Levels of salivary insulin increased in parallel with the number of MetS components, consistent with the dominant role of hyperinsulinemia in early MetS stages as a marker of disease [24]. Conversely, glucose levels in both fluids were not significantly different between the obese patients and the controls, possibly due to a variable response to hyperinsulinemia.

Insulin resistance is associated with the components of metabolic syndrome among obese children and adolescents [5,25] and appears to perform better than glucose alone in pediatric MetS identification [26]. Differently from the values of salivary glucose and insulin considered individually [27], to our knowledge, salivary HOMA-IR has never been previously studied. By assuming that the relationship between salivary glucose and insulin is the same as that for serum, the salivary HOMA index for our entire patients cohort was calculated by substituting the glucose-insulin serum

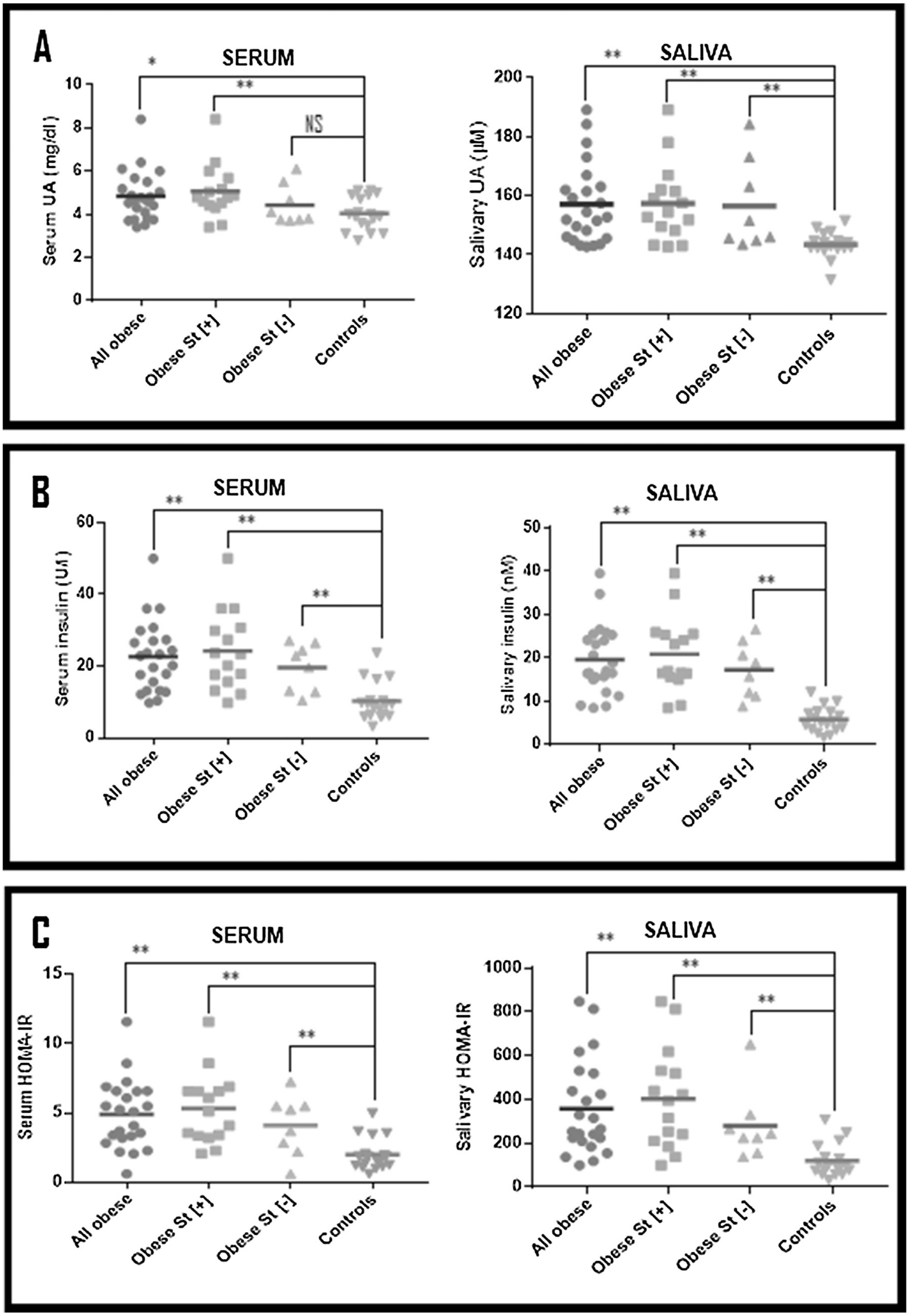

Fig. 2. Serum and salivary levels of uric acid (A), insulin (B) and HOMA index (C) in obese patients, with and without liver steatosis *vs.* controls.
Abbreviations: NS, not significant; HOMA, homeostasis model assessment; UA, uric acid; St[−], absence of hepatic steatosis; St[+], presence of hepatic steatosis.
*p < 0.05; **p < 0.01

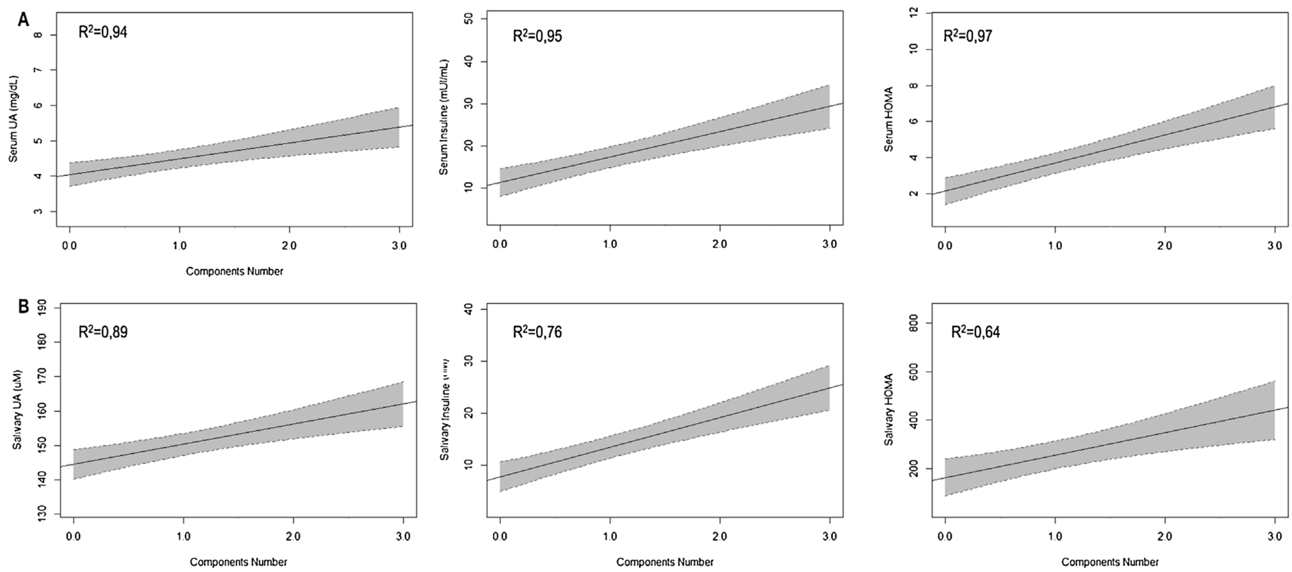

Fig. 3. Correlation between number of components of the metabolic syndrome and serum (panels A) and salivary (panels B) uric acid, insulin and HOMA levels ($p < 0.05$ for each correlation).
Abbreviations: HOMA, homeostasis model assessment; UA, uric acid.

values with the corresponding salivary values. Our data indicated a positive correlation between the HOMA indices in the 2 fluids, and the values for the obese subjects were higher than those for the controls, especially when the obesity was complicated by a fatty liver rather than by the number of MetS components.

Although hyperuricemia does not formally represent one of the components of metabolic syndrome, it has been proven that a close association exists between UA levels and MetS, and fatty liver [28,29], probably as a marker of elevated fructose/sucrose intake. Little evidence in the literature indicates that a link also exists for salivary UA levels [11,27]. Altogether, our data suggest the existence of a direct and proportional relationship between serum and salivary UA levels, with increased levels of both in obese children *vs.* NW controls, thus confirming the recent data of Martinez for Mexicans [11]. However, while blood levels tended to be higher in obese patients with steatosis, a result that confirms the data obtained by Mosca et al. [28], this distinction appeared less noticeable in saliva (Fig. 2). The possible gender effect on the interpretation of the results [30] does not appear likely to have played a role in our series due to the similar gender ratio between the controls and the obese patients.

Regarding MetS, consistent with the results of Nejatinamini et al. [5], we found that both serum and salivary UA levels tended to increase in parallel to the number of MetS components. Specifically, in both biological fluids, UA was linearly correlated with WC and systolic blood pressure, in good agreement with the results obtained in serum by others [24,28,31]. This relationship also exists between UA and other MetS components: in both biological fluids, UA was linearly correlated with TG and was inversely correlated with HDL cholesterol, confirming the data for serum [32].

Some inconsistencies may require a particular comment on MetS in children. Several attempts to unquestionably define MetS in the pediatric population have been inconclusive because the construct is difficult to delineate and continues to have unclear implications for clinical care. In a recent Clinical Report, the AAP Committee on Nutrition therefore focuses on the importance of screening for and treating each individual risk factor component of MetS and emphasizes that attention should be focused on children with cardiovascular metabolic risk factors clustering over the need to define a pediatric MetS [15].

The prevalence of MetS and NAFLD in obese adolescents has almost doubled in recent decades according to NHANES (National Health and Nutrition Examination Survey) reports [33–35]. Consistent with these trends, the World Health Organization (WHO) predicts that approximately 70 million infants and young children will be overweight or obese in 2025. The prevalence of MetS and NAFLD is strictly related to the degree of obesity [36]. Pediatric obesity is an early risk factor for adult morbidity and mortality [37,38], and up to 85% of obese children are reported to become obese adults [37,39]. The early detection of MetS and fatty liver in childhood can be a valuable tool to prevent further health complications in adulthood and to minimize the global socioeconomic burden of hepatic and cardiovascular obesity-associated complications. An IDF consensus report recently suggested that if important preventive actions are not taken, we might see the first generation of children with a shorter life expectancy than their parents [16].

None of the above individually considered clinical/anthropometric or salivary laboratory markers alone clearly differentiated subjects with or without the hepatic steatosis complication of obesity (Fig. 4). This posed a problem because also the finding of hepatic steatosis has been considered an important additional criterion in detecting a pediatric MetS phenotype in children at higher risk for long-term cardiovascular morbidity [3]. An essential novel aspect of our study *vs.* the previous literature is, therefore, the introduction of a function that can accurately and noninvasively assess the presence/absence of obesity comorbid conditions by combining several common clinical/anthropometric [13,32] and laboratory salivary values, which were not adequately predictive when considered individually. The availability of such an accurate noninvasive tool may prove useful in future studies, especially for evaluating specific populations at risk of obesity-related complications, such as schoolchildren and adolescents. Without resorting to blood sampling to decide who may need early further investigation [40], pediatricians will be able to start targeting their most intensive intervention efforts to those subsets of obese children and adolescents who appear at greatest need of risk reduction. As suggested by the AAP Committee on Nutrition, increasing awareness of comorbid conditions such as NAFLD will further enable pediatricians to address and refer to subspecialists as needed [15].

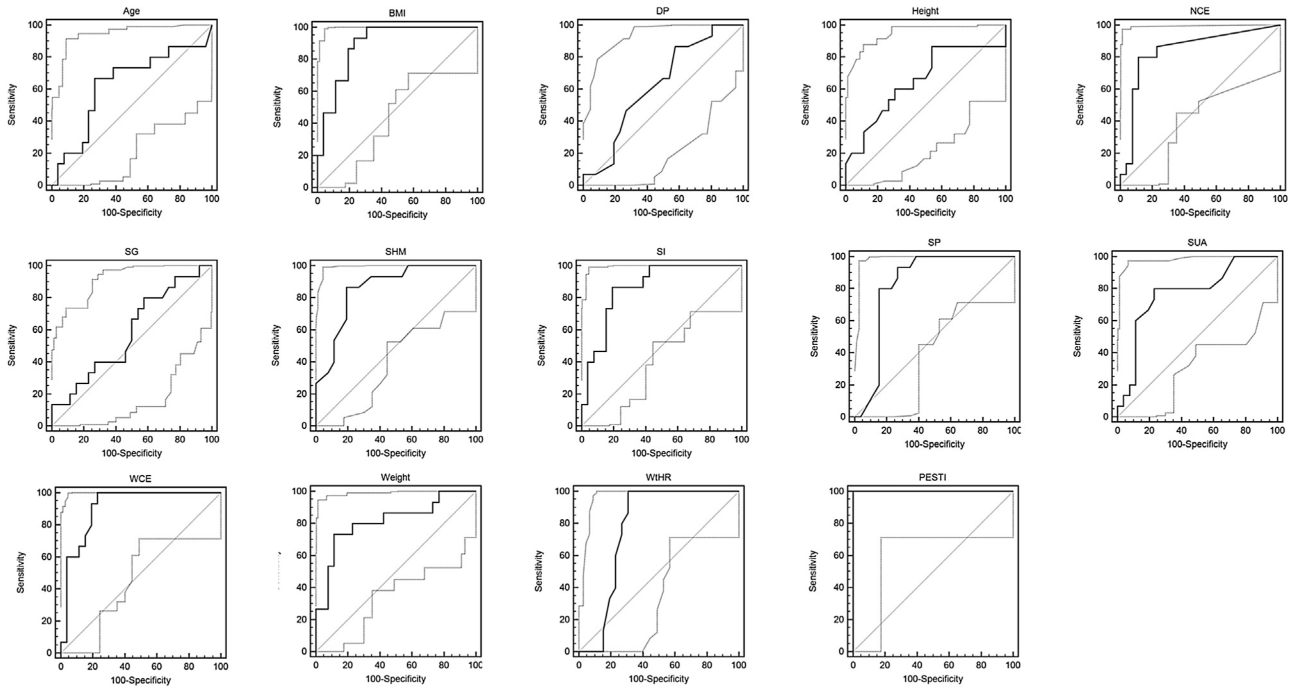

Fig. 4. Receiver operating characteristic curve of the features used for the PESTI index. vertical and horizontal axes = % sensitivity and specificity, respectively. *Abbreviations*: BMI, body mass index; DP, diastolic pressure; NCE, neck circumferences excess to 95th percentile; PESTI, pediatric steatosis index; SG, salivary glucose; SHM, salivary HOMA index; SI, salivary insulin; SP, systolic pressure; SUA, salivary uric acid; WCE, waist circumference excess to 95th percentile; WtHR, waist to height ratio.

4.1. Limitations and strengths of the study

First, a limitation of our preliminary study includes the relatively small sample of consecutively recruited patients, which might have reduced the interpretability of the individual tests. Second, although it is the reference test for use in everyday clinical practice, the diagnosis of NAFLD by ultrasound may be insensitive compared with biopsy or MRI. Even so, liver biopsies cannot be a screening procedure as they are expensive, invasive, not riskless, and not exempt from possible sampling errors as well. Among the less invasive imaging options to assess hepatic steatosis, ultrasound has the obvious advantages of being universally available, relatively inexpensive, and repeatable as it does not require sedation or delivery of ionizing radiation [12,41]. Although it is the less robust of the numerous imaging options [42] methodological progresses (*e.g.* by combining increased echogenicity and vascular attenuation) have been shown to allow a diagnostic specificity and sensitivity up to >90% when steatosis involves as little as 20% of the hepatocytes [43]. Third, as GC–MS laboratory instrumentation may not always be available for routine diagnostics and owing to the large number of children to be screened a centralized laboratory approach might probably overcome this potential obstacle with affordable costs (ranging from 20 to 50 Euros/test). Fourth, the simultaneous measurement also of salivary inflammatory/fibrogenetic markers (*e.g.* interleukins such as TNF-alpha, or matrix metalloproteinase-8 and its tissue inhibitor TIMP-1) should probably be tested in future studies to individuate those (more rare) obese children at an even higher hepato-metabolic risk [44]. Liver biopsy and/or fibroscan data for confirmation should however be part of these future confirmatory studies.

Yet our study has several strengths in that it clearly indicates that salivary UA and insulin appear to represent valuable surrogates for serum levels and can be used to screen for subjects who have one or more components of MetS, a subject of particular relevance in the pediatric population. Regarding hepatic steatosis prediction, the PESTI index formula will need to be further validated and likely recalculated in a bigger and independent cohort, possibly having also even more strict criteria for steatosis diagnosis.

Overall, our results indicate that the study of salivary molecules appears to represent a potentially useful tool for epidemiologic studies that can be applied to everyday preventive actions to differentiate children who are at higher risk for obesity-related systemic/hepato-metabolic complications, including NAFLD and MetS, thus overcoming the need for blood sampling, which is necessary when using previously proposed tools [45].

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.11.009.

References

[1] WHO C. Ending chilhood obesity: report of the Commission on Ending Childhood Obesity (ECHO). http://apps.who.int/iris/bitstream/10665/204176/1/9789241510066_eng.pdf?ua=1.

[2] Friend A, Craig L, Turner S. The prevalence of metabolic syndrome in children: a systematic review of the literature. Metab Syndr Relat Disord 2013;11:71–80, http://dx.doi.org/10.1089/met.2012.0122.

[3] Rutigliano I, Vinci R, De Filippo G, Mancini M, Stoppino L, d'Apolito M, et al. Metabolic syndrome, hepatic steatosis, and cardiovascular risk in children. Nutrition 2017;36:1–7, http://dx.doi.org/10.1016/j.nut.2016.10.017.

[4] Tresaco B, Bueno G, Pineda I, Moreno LA, Garagorri JM, Bueno M. Homeostatic model assessment (HOMA) index cut-off values to identify the metabolic syndrome in children. J Physiol Biochem 2005;61:381–8.

[5] Nejatinamini S, Ataie-Jafari A, Qorbani M, Nikoohemat S, Kelishadi R, Asayesh H, et al. Association between serum uric acid level and metabolic syndrome

components. J Diabetes Metab Disord 2015;14:70, http://dx.doi.org/10.1186/s40200-015-0200-z.

[6] Goodson JM, Kantarci A, Hartman M-L, Denis GV, Stephens D, Hasturk H, et al. Metabolic disease risk in children by salivary biomarker analysis. PLoS One 2014;9:e98799, http://dx.doi.org/10.1371/journal.pone.0098799.

[7] Hartman M-L, Goodson JM, Barake R, Alsmadi O, Al-Mutawa S, Ariga J, et al. Salivary glucose concentration exhibits threshold kinetics in normal-weight, overweight, and obese children. Diabetes Metab Syndr Obes 2015;8:9–15, http://dx.doi.org/10.2147/DMSO.S72744.

[8] Hartman M-L, Goodson JM, Barake R, Alsmadi O, Al-Mutawa S, Ariga J, et al. Salivary biomarkers in pediatric metabolic disease research. Pediatr Endocrinol Rev 2016;13:602–11.

[9] Shi P, Goodson JM, Hartman M-L, Hasturk H, Yaskell T, Vargas J, et al. Continuous metabolic syndrome scores for children using salivary biomarkers. PLoS One 2015;10:e0138979, http://dx.doi.org/10.1371/journal.pone.0138979.

[10] Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World J Diabetes 2014;5:730–8, http://dx.doi.org/10.4239/wjd.v5.i6.730.

[11] Martinez AD, Ruelas L, Granger DA. Association between body mass index and salivary uric acid among Mexican-origin infants, youth and adults: gender and developmental differences. Dev Psychobiol 2017;59:225–34, http://dx.doi.org/10.1002/dev.21492.

[12] Vajro P, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr 2012;54:700–13, http://dx.doi.org/10.1097/MPG.0b013e318252a13f.

[13] Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. J Hepatol 2009;51:433–45, http://dx.doi.org/10.1016/j.jhep.2009.05.023.

[14] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013;310:2191–4, http://dx.doi.org/10.1001/jama.2013.281053.

[15] Magge SN, Goodman E, Armstrong SC. The metabolic syndrome in children and adolescents: shifting the focus to cardiometabolic risk factor clustering. Pediatrics 2017, http://dx.doi.org/10.1542/peds.2017-1603.

[16] Zimmet P, Alberti KGM, 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, http://dx.doi.org/10.1111/j.1399-5448.2007.00271.x.

[17] Menard S. Applied logistic regression analysis, vol. 106. Sage; 2002.

[18] Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29–36, http://dx.doi.org/10.1148/radiology.143.1.7063747.

[19] Oken U, Okoro C. Evaluating measures of indicators of diagnostic test performance: fundamental meanings and formulars, vol. 03; 2012, http://dx.doi.org/10.4172/2155-6180.1000132.

[20] R Development CORE TEAM R. R: a language and environment for statistical computing. Vienna, Austria: R foundation for statistical computing; 2008.

[21] Rai V, Mukherjee R, Ghosh AK, Routray A, Chakraborty C. Omics in oral cancer: new approaches for biomarker discovery. Arch Oral Biol 2018;87:15–34, http://dx.doi.org/10.1016/j.archoralbio.2017.12.003.

[22] Mussavira S, Dharmalingam M, Omana Sukumaran B. Salivary glucose and antioxidant defense markers in type II diabetes mellitus. Turk J Med Sci 2015;45:141–7.

[23] Fekete Z, Korec R, Feketeova E, Murty VL, Piotrowski J, Slomiany A, et al. Salivary and plasma insulin levels in man. Biochem Mol Biol Int 1993;30:623–9.

[24] Pacifico L, Cantisani V, Anania C, Bonaiuto E, Martino F, Pascone R, et al. Serum uric acid and its association with metabolic syndrome and carotid atherosclerosis in obese children. Eur J Endocrinol 2009;160:45–52, http://dx.doi.org/10.1530/EJE-08-0618.

[25] Cho J, Hong H, Park S, Kim S, Kang H. Insulin resistance and its association with metabolic syndrome in Korean children. Biomed Res Int 2017;2017:7.

[26] Costa RF, Santos NS, Goldraich NP, Barski TF, Andrade KS, Kruel LF. Metabolic syndrome in obese adolescents: a comparison of three different diagnostic criteria. J Pediatr (Rio J) 2012;88:303–9, http://dx.doi.org/10.2223/JPED.2200.

[27] Fabre B, Maccallini G, Oneto A, Gonzalez D, Hirschler V, Aranda C, et al. Measurement of fasting salivary insulin and its relationship with serum insulin in children. Endocr Connect 2012;1:58–61, http://dx.doi.org/10.1530/EC-12-0024.

[28] 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, http://dx.doi.org/10.1016/j.jhep.2016.12.025.

[29] Yadav D, Lee ES, Kim HM, Lee EY, Choi E, Chung CH. Hyperuricemia as a potential determinant of metabolic syndrome. J Lifestyle Med 2013;3:98–106.

[30] 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:e0189232, http://dx.doi.org/10.1371/journal.pone.0189232.

[31] Cardoso AS, Gonzaga NC, Medeiros CCM, de Carvalho DF. Association of uric acid levels with components of metabolic syndrome and non-alcoholic fatty liver disease in overweight or obese children and adolescents. J Pediatr (Rio J) 2013;89:412–8, http://dx.doi.org/10.1016/j.jped.2012.12.008.

[32] Hatipoğlu N, Doğan S, Mazıcıoğlu MM, Kurtoğlu S. Relationship between neck circumference and non-alcoholic fatty liver disease in childhood obesity. J Clin Res Pediatr Endocrinol 2016;8:32–9, http://dx.doi.org/10.4274/jcrpe.2313.

[33] 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 Pediat Adolesc Med 2003;157:821–7.

[34] Cook S, Auinger P, Li C, Ford ES. Metabolic syndrome rates in United States adolescents, from the National Health and Nutrition Examination Survey, 1999–2002. J Pediatr 2008;152:165–70.

[35] 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;162:496–500.

[36] Weiss R, Bremer AA, Lustig RH. What is metabolic syndrome, and why are children getting it? Ann N Y Acad Sci 2013;1281:123–40, http://dx.doi.org/10.1111/nyas.12030.

[37] Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. Pediatrics 1998;101:518–25.

[38] Tirosh A, Shai I, Afek A, Dubnov-Raz G, Ayalon N, Gordon B, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. N Engl J Med 2011;364:1315–25.

[39] Must A, Strauss RS. Risks and consequences of childhood and adolescent obesity. Int J Obes 1999;23:S2.

[40] Eng K, Lopez R, Liccardo D, Nobili V, Alkhouri N. A non-invasive prediction model for non-alcoholic steatohepatitis in paediatric patients with non-alcoholic fatty liver disease. Dig Liver Dis 2014;46:1008–13.

[41] 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;22:8078–93, http://dx.doi.org/10.3748/wjg.v22.i36.8078.

[42] 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.

[43] Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 2009;51:1061–7.

[44] Rathnayake N, Åkerman S, Klinge B, Lundegren N, Jansson H, Tryselius Y, et al. Salivary biomarkers for detection of systemic diseases. PloS One 2013;8:e61356.

[45] Koot BG, van der Baan-Slootweg OH, Bohte AE, Nederveen AJ, van Werven JR, Tamminga-Smeulders CL, et al. Accuracy of prediction scores and novel biomarkers for predicting nonalcoholic fatty liver disease in obese children. Obesity 2013;1:583–90.