Association of fibroblast growth factor 21 with metabolic syndrome and endothelial function in children: a prospective cross-sectional study on novel biomarkers

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Running title: Biomarkers: metabolic syndrome & endothelial function in children

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Abstract

**Purpose:** Metabolic and cardiovascular disease prevention starting in childhood is critical for reducing morbidity in later life. This study aimed to investigate the association of novel biomarkers with metabolic syndrome and vascular function/structure indices of early atherosclerosis in children.

**Methods:** This is a prospective study of 78 children (8-16 years old) grouped by the presence or not of metabolic syndrome (MS). The serum biomarkers studied were fibroblast growth factor 21 (FGF21), leptin, adiponectin and insulin-like growth factor binding protein-1. Endothelial function and carotid atherosclerosis were assessed with brachial artery flow-mediated dilation and carotid intima-media thickness, respectively.

**Results:** Children with MS (n=12) had higher levels of FGF21 (median [interquartile range]: 128 [76-189] vs. 60 [20-98] pg/ml, p=0.003) and leptin (18.1 [11-34.8] vs. 7.5 [1.9-16.5] ng/ml, p=0.003), and lower levels of insulin-like growth factor binding protein-1 (1.5 [1.2-2.1] vs. 2.3 [1.5-6] ng/ml, p=0.028) compared to children without MS. Flow-mediated dilation was inversely correlated with FGF21 (Spearman’s rho: −0.24; p=0.035) and leptin (rho: −0.24; p=0.002) in all children. The best cut-off value of FGF21 levels for MS diagnosis was above 121.3 pg/ml (sensitivity/specificity: 58/86%). Only FGF21 was significantly associated with the presence of MS after adjustment for body-mass-index, age and gender (odds ratio per 10 pg/ml increase: 1.10 [95% CI: 1.01-1.22]; p=0.043).

**Conclusion:** Increased FGF21 levels were associated with the presence of MS and worse endothelial function in children. Larger studies are needed to evaluate the potential value of FGF21 as a biomarker that could predict future metabolic/cardiovascular disease at an early stage.

**Keywords:** Metabolism, Obesity, Fibroblast growth factor 21, Metabolic syndrome, Leptin, Flow-mediated dilation
Introduction

Cardiovascular diseases and metabolic disorders such as atherosclerosis, diabetes mellitus and metabolic syndrome (MS) are considered as the most frequent causes of morbidity and mortality worldwide. Diagnosis in very early stages (i.e. during childhood) by new easily quantifiable markers of atherosclerosis and metabolic disorders could be invaluable for the prevention of these diseases in adults.

A recently discovered molecule, fibroblast growth factor 21 (FGF21), has been proposed as a potent metabolic regulator. FGF21 is a signaling protein secreted in the blood stream, and it is an endocrine member of the 22 fibroblast growth factors family (1, 2). FGF21 is principally expressed in the liver, the brown and white adipose tissue in the pancreas and in multiple other tissues in lesser amounts. The hepatic tissue is principally responsible for the major part of the circulating FGF21 (3). Aiming for metabolic homeostasis, the actions of FGF21 are mediated through its receptor (FGFR) and a co-receptor, β-klotho in periphery but also in central nervous system (4). Through this heterodimer FGF21 acts in hepatic tissue to lower cholesterol proving its actions in multiple tissues and in different pathways of metabolism especially of lipids and glucose (5). Serum levels of FGF21 are influenced by various metabolic disorders, they are increased in obesity and correlate with fasting insulin, homeostasis model assessment-insulin resistance (HOMA-IR), waist circumference and body-mass-index (BMI) in adults (6-8). FGF21 is also increased in the serum of patients with coronary artery disease (8). A possible FGF21 resistance has been shown in states of obesity which suggests that supra-physiological doses are required to mediate the effects of this molecule in such states (9). Other biologic molecules such as leptin, adiponectin and insulin-like growth factor binding protein-1 (IGFBP1) have been studied in adults as potential markers of metabolic and cardiovascular diseases due to their action on metabolism and their response to different metabolic conditions (10-12). However, there are limited data on the
role of FGF21 and the other molecules in childhood.

The aim of the study was to investigate the potential value of the novel candidate markers FGF21, leptin, adiponectin and IGFBP1, of early cardiovascular and metabolic disorders in childhood. Additionally, we studied the association of these molecules with vascular function and structure indices of early endothelial dysfunction and atherosclerosis (i.e. ultrasound-based flow-mediated dilation [FMD] and carotid intima-media thickness [cIMT]) in a pediatric population.

Materials and methods

1. Population

We performed a prospective cross-sectional study in a pediatric population, in Northwestern Greece. Eligibility criteria were determined based on age (i.e., within 8-16 years) and good health conditions (i.e., absence of illness and no use of any medication). All children were free of any disease and visited the Pediatric Department of the University Hospital for routine medical examination prior to initiation of sport activities. We enrolled 100 children of which 22 were excluded due to incomplete/unavailable data. Written informed consent was signed by their parents/guardians. The study was conducted according to the Declaration of Helsinki and the study protocol was approved by the local Bioethics Committee.

2. Measures and definitions

Personal health history was recorded for all children. Standing height and body weight were measured in the morning, with light clothing and no shoes using a wall mounted stadiometer and an electronic scale respectively. MS and waist circumference were determined by the International Diabetes Federation (IDF) definition (13, 14). The BMI was calculated as weight/height$^2$ (kg/m$^2$), and BMI-SDS and BMI percentiles were derived from the World
Health Organization growth charts and they were used for defining normal weight, overweight or obesity for each child. Blood pressure and heart rate were assessed by electronic sphygmomanometer before any further procedures were initiated, in a quiet environment.

The metabolic cluster score was assessed since it has been suggested as a powerful predictor of cardiovascular risk that can be calculated at any age by using the following equation (15):

\[ \frac{1}{5} \times \left( \frac{\text{waist circumference} - 77.7}{11.4} - \frac{\text{high-density lipoprotein cholesterol} - 44.6}{10.2} + \frac{\text{triglycerides} - 90.5}{52.9} + \frac{\text{systolic blood pressure} - 107.6}{9.2} + \frac{\text{glucose} - 88.2}{7.4} \right) \]

Serum samples were collected by standard centrifuge procedure and for some parameters they were directly analyzed while for the rest they were stored at a −80°C laboratory freezer until assaying.

Liver function tests, i.e. aspartate aminotransferase and alanine aminotransferase, and lipid profile, i.e. total cholesterol, triglycerides and high density lipoprotein level cholesterol, were analyzed on Beckman Coulter AU5800. Low density lipoprotein cholesterol was derived from the Friedewald formula. Apolipoprotein A, apolipoprotein B and lipoprotein A were analyzed on the nephelometer Siemens BM ProSpec.

Glucose homeostasis was assessed by HOMA-IR \[ \frac{\text{fasting blood glucose} (\text{mmol/L}) \times \text{fasting blood insulin} (\text{\(\mu\text{U/mL}\})}}{22.5}. \] (16) Glucose was analyzed on the Beckman Coulter AU5800 and insulin was measured on Beckman Coulter Dx1800.

The measurements of the biomarkers fibroblast growth factor 21, adiponectin, leptin and insulin growth factor binding protein 1 (IGFBP1) were carried out by enzyme-linked immune-assays. The kit used for FGF21 was purchased from R&D Systems, MN, USA (Human FGF21 Quantikine ELISA kit). The sensitivity of the method was 4.67 pg/ml and the CVs were 3.9% and 10.9% for intra and inter assays, respectively. Leptin (Leptin Sandwich ELISA) was assessed by using the kit by DRG GmbH (Germany). The sensitivity of the
method was 1.0 ng/ml and the respective CVs were 6% and 9%. Adiponectin (Human adiponectin ELISA) was assessed using the kit by Biovendor (Research and Diagnostic Products, Brno Czech Republic). The sensitivity of the method was 26 ng/ml and the respective CVs were 5.9% and 6.3%. Insulin was measured by using the kit by DIA Source (IGFBP-1 ELISA KARME 01, Belgium). The sensitivity of the method was 0.02 ng/ml and the respective CVs were 5.2% and 5.9%.

3. Ultrasound vascular studies
The studies were performed after fasting for 6 hours. Measurements were taken in the supine position in a temperature-controlled room (~22ºC) in a quiet environment. An Echo-Doppler ultrasound (Ultrasound ATL, HDI 5000, Bothell WA, USA) and a 5-12 MHz transducer was used for optimal imaging.

1) Carotid artery intima-media thickness (cIMT)
Measurement in the common carotid artery was performed using a standardized protocol according to previously published recommendations for children and adolescents.(17, 18) Three consecutive longitudinal images of each common carotid artery 1–2 cm proximal to the bifurcation were acquired. The mean values of IMT for right and left common carotid artery were obtained by averaging three measurements at the far wall of each artery. Finally, the max carotid IMT of the two CCAs was determined for each child.

2) Brachial artery flow-mediated dilation (FMD)
Endothelial function was assessed by measurement of endothelium-dependent vasodilation in the right brachial artery in response to hand hyperemia, based on previously described methodology in children and adolescents.(17, 18) Images were acquired at baseline and every
30 seconds, from the first to the third minute after deflation of a wrist cuff inflated to 250 mmHg for 4 min for measurement of FMD (%increase in arterial diameter compared to baseline diameter). Brachial artery blood flow was measured by pulsed wave Doppler at baseline and 15 seconds after cuff release.

Determination of the relative vascular wall margins at end-diastolic brachial diameter and carotid IMT was performed by two blinded operators off-line using the software QLAB (Philips Ultrasound, Bothell, WA, USA).

3) Shear stimulus and normalized FMD
Imposed shear stress stimulus magnitude is related to FMD magnitude. To compare the FMD response between groups, the variation in the magnitude of the shear stress stimulus was taken into account and thus the FMD response was normalized to the magnitude of the shear stimulus (19). Shear rate, an estimate of shear stress without viscosity, was used to quantify the stimulus for FMD and it was calculated as mean blood flow velocity/vessel diameter (19).
As the peak of the shear stress stimulus induced by reactive hyperemia occurs within the first seconds post cuff-release, the brachial artery mean blood flow velocity at 15 sec post cuff-release was used to calculate the peak shear rate. The peak %FMD during the first 90 sec (i.e. within the time that the peak vessel diameter adaptation is typically observed) was divided by the peak shear rate resulting in the normalized FMD that was used for all comparisons.

4. Statistical analysis
The normality of the data was tested using the Kolmogorov-Smirnov test and most variables of interest (including FGF21, leptin, HOMA-IR, IGFBP1 and normalized FMD) were found to deviate from the normal distribution. Accordingly, continuous variables are presented as median (interquartile range [IQR]). Non-parametric analysis using the Wilcoxon-Mann-
Whitney test was employed to compare continuous variables between two groups. The correlation between continuous variables (e.g. FGF21 serum levels and normalized FMD) was studied using the Spearman’s rho (ρ) correlation coefficient. Receiver-operating characteristic curve analysis was performed to identify the best cut-off values (Youden index: max [Sensitivity and Specificity]) of biomarkers (FGF21 and leptin) for the diagnosis of MS. Logistic regression analysis was performed to investigate the association of biomarkers (FGF21 and leptin) with MS. The level of significance was set at p<0.05. The SPSS statistical software package (version 17.0 for Windows, SPSS Inc., USA) was used for the analysis.

**Results**

1. **Metabolic syndrome and obesity in childhood**

Twelve out of the 78 children studied (15%) were diagnosed with MS, all of whom were also obese. Of the 66 children without MS (nonMS), 37 had normal BMI (nonMS-N) and 29 had increased BMI, (i.e. obese/overweight; nonMS-O). The results on biochemistry tests, metabolic markers and vascular indices for all groups/subgroups are shown in Table 1 and Supplementary Table 1.

2. **Glucose homeostasis, lipid profile and liver function in children with and without MS**

HOMA-IR in children with MS was significantly higher compared to those without MS (Table 1). A significant increase was observed in apolipoprotein B (ApoB) and the ApoB/ApoA ratio whereas lipoprotein(a) was decreased in the MS compared to the nonMS group. Liver function assessment showed increased serum alanine aminotransferase values in the MS vs. nonMS group (Table 1).
3. Novel metabolic markers in children with and without MS

The MS group showed significantly augmented FGF21 and leptin serum levels compared to the nonMS group (Fig. 1 and Supplementary Table 1). Adiponectin levels were not significantly different, while IGFBP1 was significantly reduced in the MS versus the nonMS group.

The comparisons between the MS and the nonMS groups as well as between the MS and each of the nonMS-N and nonMS-O subgroups led to similar results except for leptin, leptin/adiponectin ratio and IGFBP1 that did not differ between the MS and the nonMS-O groups (Fig. 1 and Supplementary Table 1).

4. Metabolic syndrome cluster score

As expected, children in the MS group had higher cluster score compared to the nonMS ones (median: 1.20 [IQR: 0.73 to 1.62] vs. −0.03 [IQR: −0.50 to 0.51], p<0.001).

5. Vascular indices in children with and without MS

Normalized FMD was lower without reaching statistical significance in children with MS compared to the nonMS group (p=0.06), while it was significantly lower in children with MS vs. the nonMS-N subgroup (Supplementary Table 1 and Fig. 2). Normalized FMD did not differ between the MS group and the nonMS-O subgroup. There were no differences in cIMT measurements between groups and subgroups (Supplementary Table 1 and Fig. 2).

6. Correlations: Biomarkers, metabolic cluster score and normalized FMD

Supplementary Table 2 presents a list of the correlations. Normalized FMD showed a
significant negative correlation with FGF21, leptin (Fig. 3), HOMA-IR and leptin/adiponectin ratio. Carotid IMT did not correlate with any of the parameters studied. FGF21 correlated positively with leptin and HOMA-IR, and negatively with IGFBP1. Leptin had a positive correlation with HOMA-IR and a negative one with adiponectin and IGFBP1. IGFBP1 was also negatively correlated with HOMA-IR. The MS cluster score showed a positive correlation with FGF21, leptin and HOMA-IR.

7. FGF21 and leptin for the diagnosis of metabolic syndrome
The ROC analyses for the markers FGF21 and leptin to diagnose MS are demonstrated in Fig. 4. The areas under the curve showed similar discriminatory ability for FGF21 (0.78, 95% CI: 0.65-0.90; p=0.003) and leptin (0.77, 95% CI: 0.65-0.90; p=0.003), whereas IGFBP had weaker results (0.71, 95% CI: 0.56-0.85; p=0.028). Adiponectin was not included in the ROC analyses since it was previously shown that there was no significant difference in adiponectin levels between the MS and nonMS groups.

In our population, the best cut-off value of FGF21 levels to identify the presence of MS was above 121.3 pg/ml, with a sensitivity of 58.3% and a specificity of 86.4%. A high FGF21 value (>121.3 ng/L) was significantly associated with MS diagnosis (odds ratio: 8.87 [95% CI: 2.31-34.06], p=0.001). For every 10 pg/ml increase of FGF21 serum levels, the odds for presence of MS increased by 12% (odds ratio: 1.12 [95% CI: 1.03-1.22]; p=0.006). For leptin, the best cut-off value was above 5.85 ng/mL, with a sensitivity of 100% and a specificity of 47%. For every 1 ng/ml increase in leptin serum levels, the odds for presence of MS increased by 8% (odds ratio: 1.08 [95% CI: 1.01-2.09]; p=0.015).

After adjusting for BMI-SDS, age and gender, only FGF21 remained significantly associated with MS (odds ratio per 10 pg/ml increase: 1.10 [95% CI: 1.01-1.22]; p=0.043), whereas leptin was no longer significantly associated (odds ratio per 1 ng/ml increase: 1.01 [95% CI:
Therefore, after adjustment for BMI, age and gender, for every 10 pg/ml increase in FGF21 serum levels, the odds for presence of MS increased by 10%.

Discussion

The main findings of this study are the following: (i) serum levels of the novel metabolic biomarkers FGF21, leptin, and IGFBP1 were found to differ depending on the presence of MS or obesity in our pediatric population; (ii) FGF21 was the only biomarker that differentiated children with MS from those without, irrespective of BMI, age and gender, and thus, augmented FGF21 serum levels were an independent predictor for the presence of MS with a good diagnostic performance; (iii) FMD was found to be impaired in children with MS; and (iv) FGF21, leptin and HOMA-IR were inversely correlated to normalized FMD.

A novel finding of our study is the correlation of FGF21 with impaired vascular endothelial function in childhood. Also, the cut-off serum value for FGF21 (i.e. 121.3 ng/ml) was found to have good diagnostic accuracy for identifying the presence of MS in our population.

1. Effect of MS on metabolic parameters in children

In a school based cross-sectional study of 1513 black, white and Hispanic adolescents the prevalence of MS was determined using definitions from the National Cholesterol Education Program Adult Treatment Panel III and World Health Organization guidelines. Among the population studied, obesity prevailed as a risk factor for MS (20). Similarly, in a Mediterranean pediatric cohort, the prevalence of MS was assessed using the International Diabetes Federation criteria and a low prevalence of MS (0.7%) was shown among the children studied, all of whom were obese (21). In agreement to these studies, in our population, all children with MS were found to be overweight/obese. Further categorization based on the BMI of the population provided useful insight in revealing subtle differences
between children with MS and children who were obese but did not fulfill the diagnostic criteria for MS.

Liver function has been shown to be affected in states of obesity or MS, reflected by increased levels of serum transaminases (22-24). In our study, the up-regulation of alanine aminotransferase in the MS versus the nonMS subgroups confirms what was shown in these earlier studies. Central obesity has a well-known association with insulin resistance, lipid profile and increased blood pressure (25-27). This association is supported by our findings of increased serum insulin and HOMA-IR in our group of children with MS in comparison to the group of children without MS. Additionally, this association is supported by the fact that the difference in HOMA-IR between MS and normal weight nonMS children was larger than the comparison between MS and overweight/obese nonMS children.

Serum ApoB/ApoA and TChol/HDL ratios are considered strong predictors of atherosclerosis. Alemzadeh, et al. have studied the relationships between ApoB/ApoA-1 and TChol/HDL ratios with markers of inflammation and insulin resistance in obese adolescents; however, non-obese adolescents were not included in that study. They reported the presence of higher ApoB/ApoA-1 and TChol/HDL ratios in adolescents that met the criteria for MS (28). These findings are consistent with our results which also show a shift towards an atherogenic profile in the MS compared to the nonMS group.

2. Regulation of newer metabolic biomarkers in MS and obesity: the distinct role of FGF21

Leptin is known to be up-regulated in states of obesity (29) and this may explain the similar up-regulation of leptin serum levels in both our children with MS and those without MS but with obesity. In contrast to leptin, FGF21 levels did differ between MS and each one of the two nonMS subgroups, and thus, were significantly increased only in association with MS.
but not with obesity. FGF21 actions are involved in cross-talks with pathways of the MS as it has been shown to act on white adipose tissue lipolysis, increase insulin-dependent glucose uptake in adipocytes and revert insulin resistance (30). Moreover, FGF21 has been found to promote and support the metabolic homeostasis by controlling the underlying mechanisms of MS and nonalcoholic fatty liver disease (31). Increased FGF21 serum levels have been reported in obese adults, adults with Type 2 diabetes mellitus, with nonalcoholic fatty liver disease and with MS (32, 33). In adults it has been shown that serum FGF21 increase after 7 to 10 days of fasting concluding that it contributes to the late stages of adaptive starvation (34). Overall, the physiologic role of FGF21 is to promote the adaptation of metabolism in different conditions and mediate the link between different pathways related to obesity, starvation and stress (34, 35). MS constitutes a constellation of metabolic deregulations, i.e. dyslipidemia and insulin resistance, increased blood pressure and obesity, and FGF21 is a key protective molecule which is up-regulated possibly as a result of overexpression in order to increase its effectiveness in such states.

In pediatric populations, there are limited data on the regulation of FGF21 in MS. Ko, et al.(36) studied children aged 9 years and reported no association between FGF21 and MS defined by the age-modified criteria developed by the National Cholesterol Education Program's Adult Treatment Panel III. Notably, different diagnostic criteria to define MS may be a significant factor in studies on MS in pediatric populations. Reinehr, et al.(37) showed that there was no relationship between FGF21 and obesity, MS defined by the IDF criteria, and nonalcoholic fatty liver disease in children aged 6 to 14. However, the association of FGF21 with features of MS was assessed within the obese population, and thus could not provide information on the distinction between MS and obesity.

Leptin levels have been previously shown to relate to FGF21 levels by not well-known pathways (37). Leptin primarily mediates energy homeostasis and satiety perception, while it
has also been linked to a state of resistance (9, 29, 38). Only a few other studies explored the role of leptin in MS, and found that leptin levels were increased in children with MS in agreement with our study (39, 40).

In the current report, only FGF21 was shown to be associated with the presence of MS independently of BMI, age and gender, suggesting an important diagnostic value of this molecule. Although leptin was also associated with MS in our population, this association did not remain significant after adjustment for BMI, age and gender, probably due to leptin’s regulation primarily by increased fat deposition. Similar to our findings, in a study of 1,138 children using the IDF criteria for MS, leptin and adiponectin correlated with MS but this correlation was found to be mediated by BMI (21).

In contrast to leptin, adiponectin has been described as a molecule with anti-inflammatory and anti-atherosclerotic actions which can protect against vascular damage (41). On this basis, the leptin/adiponectin ratio has been proposed as an atherogenic index (42). In our MS population, adiponectin levels were reduced, albeit non-significantly, and the ratio leptin/adiponectin was significantly increased reflecting the overall deregulation of serum biomarkers to a more atherogenic profile. We also propose the use of the FGF21/adiponectin ratio which was also significantly increased almost threefold in the MS compared to the non-MS group, and remained higher (with borderline significance) in the comparison between MS and non-MS-O children, in contrast to the leptin/adiponectin ratio. These results indicate that the FGF21/adiponectin ratio may be a more sensitive marker than the leptin/adiponectin ratio in the evaluation of MS in children, and also, independent of obesity.

Serum levels of IGFBP1 were decreased in the MS compared to the non-MS group as expected; however, this decrease lost its significance when the MS group was compared to the non-MS-O subgroup, thereby underlining the effect of obesity on the regulation of IGFBP1 (43).
3. Endothelial function and association with biomarkers

Children with MS showed worse endothelial function (i.e. lower normalized FMD) when compared to the nonMS group, and in particular, the nonMS-N subgroup. In contrast, cIMT showed no significant difference between any of the groups, indicating that structural vascular damage might not have occurred yet in this pediatric population.

From the four biomarkers we studied, only FGF21 and leptin were associated with impaired endothelial function. Leptin has been previously shown to be involved in the process of atherosclerosis by regulating the production of different pro-inflammatory cytokines, activating immune cells and increasing oxidative stress (44, 45), thereby indicating that increased leptin levels may promote cardiovascular disease.

In contrast, FGF21 has been shown to have a protective role towards the cardiovascular system by preventing oxidative stress in cardiomyocytes and preventing heart hypertrophy in mice (46, 47). In adults, FGF21 has been associated with subclinical atherosclerosis as shown by a positive correlation to cIMT independently of established cardiovascular risk factors (48). Additionally, FGF21 levels have been found to be augmented in patients with coronary artery disease (49). Therefore, FGF21 may be increased early in cardiovascular disease as a compensatory response. According to our findings in children, the inverse correlation of FGF21 with normalized FMD alongside the independent association of FGF21 with MS underline the dual role of FGF21 as a marker of early metabolic deregulation and endothelial dysfunction.

4. Limitations

The population size in the current study was not large. Furthermore, this study was not designed to include follow-up of the subjects enrolled in order to investigate the predictive
value of biomarkers for pathologic conditions in the future. However, our cross-sectional study showed for the first time that FGF21 is able to identify the presence of MS and early changes in endothelial function in a pediatric population.

In conclusion, metabolic deregulation associated with endothelial dysfunction may be detected early in children and adolescents. FGF21 was shown to be a potent biomarker for identifying the presence of MS in children independently of BMI, age and gender, while it was also correlated with impaired endothelial function. Further research and larger prospective longitudinal studies in children are needed to show the prognostic value of FGF21 in vascular and metabolic health in later life.

**Ethical statement**

All procedures performed in this study were in accordance with the 1964 Declaration of Helsinki and its later amendments. The study was approved by the Bioethics Committee of the University Hospital of Ioannina (2/14-1-2015, 22).

**Conflicts of interest**

No potential conflict of interest relevant to this article was reported.

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<table>
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<th>Variable</th>
<th>MS (n=12)</th>
<th>nonMS (n=66)</th>
<th>MS vs nonMS</th>
<th>MS vs nonMS-N subgroup (n=37)</th>
<th>MS vs nonMS-O subgroup (n=29)</th>
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<td>0.22</td>
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<td>0.16</td>
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<td>Weight (Kg)</td>
<td>69 (64-91)</td>
<td>53 (38-65)</td>
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<td>Height (cm)</td>
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<td>155 (138-166)</td>
<td>158 (141-168)</td>
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<td>(mmol/L)</td>
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<td>HDL</td>
<td>0.90 (0.3-3.7)</td>
<td>1.24 (0.3-3.7)</td>
<td>0.002</td>
<td>1.33 (0.3-3.7)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 1. Laboratory investigations in children with metabolic syndrome (MS) compared to the nonMS group
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Value 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRG (mmol/L)</td>
<td>1.74</td>
<td>0.74</td>
<td>&lt;0.001</td>
<td>0.65</td>
</tr>
<tr>
<td>ApoA (g/L)</td>
<td>1.3</td>
<td>1.4</td>
<td>0.04</td>
<td>1.4</td>
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<tr>
<td>ApoB (g/L)</td>
<td>0.96</td>
<td>0.70</td>
<td>0.002</td>
<td>0.71</td>
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<tr>
<td>ApoB/ApoA</td>
<td>0.75</td>
<td>0.49</td>
<td>&lt;0.001</td>
<td>0.47</td>
</tr>
<tr>
<td>Lp(a) (mmol/L)</td>
<td>0.08</td>
<td>0.29</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>24.0</td>
<td>21.0</td>
<td>0.4</td>
<td>21.5</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>23.5</td>
<td>15.0</td>
<td>0.009</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Continuous variables are presented as median (interquartile range). Comparisons between groups were performed using the Mann-Whitney test. Categorical variables are presented as percentages and comparisons between groups were performed using the chi-square test of Fisher’s exact test as appropriate. P values indicate the comparison against children with MS.

HOMA-IR, homeostasis model assessment-insulin resistance; TChol, total cholesterol; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein; TRG, triglycerides; ApoA, Apolipoprotein A; ApoB, apolipoprotein B; Lp(a), lipoprotein(a); AST, aspartate aminotransferase; ALT, alanine aminotransferase, children with MS (MS), without MS (nonMS), nonMS children with normal BMI (nonMS-N) and nonMS children with increased BMI, i.e. obese/overweight, (nonMS-O).
FIGURE LEGENDS

**Fig. 1** Serum biomarkers (A-D) in the Metabolic Syndrome (MS) compared to the nonMS group. NonMS children were further categorized in children with normal BMI (nonMS-N) and children with increased BMI, i.e. obese/overweight, (nonMS-O). Bars indicate the median values and error bars the 75th percentile of the interquartile range. P values indicate the comparison against children with MS.

**Fig. 2** Vascular indices in the Metabolic Syndrome (MS) compared to the overall nonMS group, to the nonMS children with normal BMI (nonMS-N) and with increased BMI (nonMS-O). A. Brachial flow-mediated dilation (FMD). B. Carotid intima-media thickness (cIMT). Bars indicate the median values and error bars the 75th percentile of the interquartile range. P values indicate the comparison against children with MS.

**Fig. 3** Negative correlations of normalized FMD with FGF21 (A) and leptin (B). Correlations were tested using Spearman’s rho (ρ) correlation coefficient. Best fit curves are shown for visualization purposes.

**Fig. 4** Receiver Operating Characteristic curves for the diagnosis of Metabolic Syndrome (MS). Both FGF21 (A) and leptin (B) showed good discrimination (area under the curve [AUC] >0.75).
Figure 1

A

FGF21 (pg/ml)

MS
nonMS

\[ p = 0.003 \]

\[ p = 0.002 \]

\[ p = 0.016 \]

B

Leptin (ng/ml)

MS
nonMS

\[ p = 0.003 \]

\[ p < 0.001 \]

\[ p = 0.30 \]

C

Adiponectin (pg/ml)

MS
nonMS

\[ p = 0.19 \]

\[ p = 0.11 \]

\[ p = 0.43 \]

D

IGFBP1 (ng/ml)

MS
nonMS

\[ p = 0.028 \]

\[ p = 0.004 \]

\[ p = 0.31 \]

nonMS-N
nonMS-O
nonMS-N
nonMS-O
nonMS-N
nonMS-O
nonMS-N
nonMS-O
Figure 2

A

Normalized FMD

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>nonMS</th>
<th>p=0.06</th>
<th>nonMS-N</th>
<th>p=0.026</th>
<th>nonMS-O</th>
<th>p=0.25</th>
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</thead>
<tbody>
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<td></td>
<td>0.05</td>
<td>0.10</td>
<td></td>
<td>0.07</td>
<td></td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

B

max Carotid IMT (mm)

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>nonMS</th>
<th>p=0.65</th>
<th>nonMS-N</th>
<th>p=0.47</th>
<th>nonMS-O</th>
<th>p=0.98</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.45</td>
<td>0.50</td>
<td></td>
<td>0.52</td>
<td></td>
<td>0.55</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3

(A) Normalized FMD vs. FGF21 (pg/ml)
- rho = -0.24
- p = 0.035

(B) Normalized FMD vs. Leptin (ng/ml)
- rho = -0.34
- p = 0.002