

Research paper

Increased placental growth factor (PIGF) concentrations in children and adolescents with obesity and the metabolic syndrome

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ABSTRACT

OBJECTIVE: Childhood obesity and the Metabolic Syndrome (MetS) are associated with an increased risk for early onset endothelial dysfunction and atherosclerosis. Placental growth factor (PIGF), a member of the vascular endothelial growth factor family, plays an important role in atherosclerosis by stimulating angiogenesis and atherogenic migration of monocytes/macrophages into the arterial wall. The aim of this study was to investigate differences in circulating PIGF concentrations between children with obesity/metabolic syndrome and non-obese children. We have previously shown increased high-sensitivity troponin (hs-TnT) concentrations in children with MetS from the same cohort. **DESIGN:** Fifty-seven obese (49 without and 8 with MetS) and 25 non-obese children (controls) were assessed at the Childhood Obesity Clinic of our Department. Obesity was defined using the IOTF criteria. MetS was defined based on the IDF criteria. PIGF was measured using electrochemiluminescence methodology. **RESULTS:** Mean PIGF concentrations of obese children were significantly higher ($p=0.048$) compared with those of the controls. Analysis of the three groups, the obese (without MetS), the MetS and the control, demonstrated a significant difference in PIGF concentrations ($p=0.035$). Subgroup analysis revealed increased PIGF concentrations in children with the MetS compared to the controls ($p=0.009$). Troponin had a significant positive correlation with PIGF overall ($p=0.003$) and in the obese group ($p=0.046$). **CONCLUSIONS:** Increased serum concentrations of PIGF, a biomarker of angiogenesis, are found in obese children with the MetS compared to non-obese controls, whereas PIGF correlated positively with troponin. Longitudinal studies may reveal the prognostic role of this biomarker in the progression of atherosclerosis in obese children with the MetS.

Key words: Angiogenesis, Cardiovascular risk, Children, Metabolic syndrome, Obesity, PIGF, Troponin

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INTRODUCTION

Childhood obesity and related comorbidities, such as dyslipidemia, arterial hypertension and impaired glucose metabolism, collectively defined as Metabolic Syndrome (MetS), are associated with an increased risk for early onset endothelial dysfunction and atherosclerosis.^{1,2} Angiogenesis, the physiological formation of new blood vessels, is crucial for embryonic and postnatal growth, as well as for a variety of complex biological processes during adulthood, but also constitutes a major pathological mechanism associated with the progression of atherosclerosis.^{3,4} Most atherosclerotic regions are vascularized by a large network of small neo-vessels that play an important role in plaque formation and development. Angiogenesis is induced by hypoxia and inflammation in the atherosclerotic area and is mainly regulated by members of the vascular endothelial growth factor (VEGF) family, their receptors and placental growth factor (PlGF).^{4,6}

Increased serum VEGF concentrations have been found in patients with unstable carotid artery lesions.⁷ It is also well known that the VEGF system accounts for most of the angiogenic activity in the adipose tissue,⁸ whereas several studies have revealed increased serum VEGF concentrations in obese adults and children.⁹⁻¹¹ In addition, a recent study has shown that weight loss was associated with a reduced angiogenic activity in the circulation.¹²

PlGF is highly expressed in placenta throughout gestation, but it is also expressed at a low level in the heart, lung, skeletal muscle, adipose tissue and other organs. There is evidence that PlGF plays an important role in a range of physiological and pathological conditions including preeclampsia, cardiovascular disease (CVD) and tumor progression.^{5,6,13} PlGF, by stimulating angiogenesis and atherogenic migration of monocytes/macrophages into the arterial wall, promoted atherosclerotic intimal thickening in hypercholesterolemic rabbits and macrophage accumulation in early atherosclerotic lesions in apolipoprotein-E deficient mice.¹³ Furthermore, the development of obesity *per se* is associated with significant growth of adipose tissue, i.e. adipogenesis, which is tightly associated with angiogenesis.¹⁴ In murine models of diet-induced obesity, inactivation of PlGF impaired

adipose tissue development, partly resulting from reduced angiogenesis.¹⁵

A very limited number of clinical data have shown increased PlGF concentrations in obese adults with the MetS compared to non-MetS individuals, after adjustment for age, smoking and body mass index (BMI).¹⁶ The same research team examined PlGF concentrations in obese children¹⁰ but did not find significant differences. In our study, we aimed to investigate circulating PlGF concentrations in children with obesity and/or MetS in comparison to non-obese children. We hypothesized higher PlGF concentrations in children with the MetS.

We previously demonstrated increased – although within the normal range – concentrations of high sensitivity troponin (hs-TnT) in the same cohort of children with MetS,¹⁷ compared to the obese group without the MetS and to the non-obese group. Our previous findings imply that subclinical atherosclerosis during childhood may progress more rapidly in obese children with the MetS and that the minimal increase of hs-TnT may be associated with subclinical cardiac damage. Since PlGF is a potential independent predictor of coronary heart disease⁶ in adults, we decided also to examine associations between these two markers, hs-TnT and PlGF, in our cohort.

PATIENTS AND METHODS

We have previously described the study design and methods,¹⁷ which we summarize as follows: The study was approved by the Ethics Committee of “Aghia Sophia” Children’s Hospital and written informed consent was obtained from the participants and their parents. We included children 7-13yrs old, assessed at the Obesity Clinic. Exclusion criteria were: chronic illnesses, chronic use of medication, syndromic obesity, intellectual or psychiatric disorders and chromosomal disorders.

Obesity was defined using the IOTF criteria.¹⁸ BMI z-scores were calculated based on the Greek growth charts.¹⁹ MetS was defined based on the IDF criteria.²⁰ Participants’ pubertal development was determined by physical examination based on the 5 Tanner Stages.²¹ Children at stages (for genital development in boys and breast development in girls) 2-5 were characterized as pubertal.

Three groups were formed: controls (CG), obese without MetS (ObMS-) and obese with MetS (ObMS+). The ObMS- and ObMS+ groups were also pooled to form the combined obesity group (Ob). In addition to obesity, low HDL-C/high triglycerides defined MetS in 7 children, and high triglycerides/high systolic blood pressure in 1 male child.

The blood samples were collected in the overnight fasting state during a scheduled visit at 8.00-9.00 a.m. The blood was centrifuged and the serum obtained was stored at -85°C. Serum glucose, HDL, triglycerides and insulin concentrations were measured with appropriate methodology and HOMA for insulin resistance (HOMA2-IR) and beta-cell function (HOMA2-B) was calculated.²² Serum hs-TnT and PIGF concentrations were measured using an electrochemiluminescence immunoassay (Roche Co., Basel, Switzerland), using the Elecsys/Cobas e411 assays. The intermediate precision Coefficient of Variation was $\leq 10\%$ for hs-TnT and 3.6-4.1% for PIGF.

STATISTICAL ANALYSIS

Continuous variables are presented as mean \pm standard deviation (SD), or median and interquartile range in cases of skewed distributions. Categorical variables are presented as absolute (n) and relative (%) frequencies. Relations between categorical vari-

ables are assessed by Fisher's exact test. Differences in means were tested by the Student t-test, or the Mann-Whitney test for small samples (<30). Individual correlations between continuous variables were assessed by Pearson r, or Spearman rho in small samples (<30).

Univariate analyses were undertaken overall (in the entire population) and stratified according to obesity (CG, Ob), and also according to MetS (CG, ObMS-, ObMS+), to identify possible significant correlates of PIGF. Finally, a linear regression model was used to incorporate and evaluate all parameters significant in the univariate analyses. Size of effect in the final regression model was estimated by Cohen's f^2 . Data were analyzed using Stata 11.0 statistical software (Stata Corp, Texas, USA). Based on pediatric data of PIGF,¹¹ it was estimated that the required sample size, in order to achieve 80% power (with an alpha=0.05) in detecting PIGF differences between controls and obese children, would be 72 individuals per group. The final numbers of participants correspond to a statistical power of 48%.

RESULTS

Eighty-two children were studied: 8 ObMS+, 49 ObMS- and 25 controls. Descriptive statistics are depicted in Table 1. PIGF was not related to age,

Table 1. Anthropometric and Biochemical Data at the Time of the Evaluation

	CG (n=25)	ObMS- (n=49)	ObMS+ (n=8)	Ob (ObMS- & ObMS+, n=57)
Sex, females n (%)	19 (76%)	22 (44.9%)	1 (12.5%)	23 (40.4%)
Age, years ¹	13.2 \pm 1.4	11.1 \pm 1.9	10.4 \pm 2.4	11 \pm 2
Pubertal status, in puberty, n (%)	21 (84%)	24 (49%)	3 (37.5%)	27 (47.4%)
BMI-zscore ¹	0.67 \pm 1.11	3.3 \pm 1.25	3.3 \pm 1.47	3.3 \pm 1.27
PIGF, pg/mL ¹	10.9 \pm 2.8	11.9 \pm 2.8	14.1 \pm 3.4	12.2 \pm 3
HOMA2-IR ¹	2 \pm 0.9	2.6 \pm 1.7	2.4 \pm 1.2	2.6 \pm 1.8
HOMA2-B ¹	174.7 \pm 58.1	199.4 \pm 69.5	177.8 \pm 100.4	195.9 \pm 74.6
Troponin, ng/L ²	3 (3-3) ³	3 (3-3) ⁴	3 (3-6.9) ⁵	3 (3-3) ⁶
Total Cholesterol, mg/dL ¹	165.4 \pm 28.2	180.6 \pm 29.5	180.1 \pm 49.6	180.5 \pm 32.7
LDL, mg/dL ¹	84.1 \pm 30.9	114.5 \pm 27	119.6 \pm 38.2	115.3 \pm 28.6
HDL, mg/dL ¹	55.6 \pm 11.4	50.7 \pm 14.8	36.6 \pm 5.4	48.5 \pm 14.6
Triglycerides, mg/dL ¹	64.9 \pm 24.5	90.2 \pm 41.1	119.4 \pm 52.7	94.6 \pm 43.8

¹mean \pm SD, ²median, (interquartile range), ³10th-90th percentile: 3.0-3.0, ⁴10th-90th percentile: 3-4.3, ⁵10th-90th percentile: 3-21.4, ⁶10th-90th percentile: 3-5.3.

nor to BMI-z scores both overall and in all stratified analyses (all $p > 0.05$). Puberty had no significant effect on PIGF (overall and stratified analyses, $p > 0.05$). In the entire study population, no statistically significant differences were detected according to sex. In CG, males had significantly increased PIGF concentrations compared to females (13.1 ± 3.3 vs. 10.2 ± 2.3 , $p = 0.045$). In the ObMS-, ObMS+ and Ob groups, no such difference was observed.

No correlation between PIGF and HOMA2-IR or HOMA2-B was recorded (overall and stratified analyses, $p > 0.05$). Total cholesterol, LDL and triglycerides were also not related to PIGF (overall and stratified analyses, $p > 0.05$). A weak, negative relation was noted between HDL and PIGF (Pearson's $r = -0.21$, $p = 0.062$). Overall, PIGF was positively related to troponin (Pearson's $r = 0.38$, $p = 0.003$). The stratified analysis revealed that, in controls, PIGF was not related to troponin (Spearman's $\rho = -0.25$, $p = 0.219$). Both in ObMS- and ObMS+ the correlation was positive but not significant ($r = 0.26$ and 0.32 , $p = 0.069$ and 0.428 , respectively). In the Ob group, a clear, positive relation between PLGF and troponin was documented (Spearman's $\rho = 0.26$, $p = 0.046$).

PIGF concentrations differed significantly between CG and Ob (Mann-Whitney test, $p = 0.048$). The analysis of the three groups (CG, ObMS- and ObMS+, Kruskal-Wallis test, $p = 0.035$) demonstrated that this difference occurred due to the significantly higher concentrations of PIGF in the ObMS+ group compared to the CG ($p = 0.009$).

Based on the results of the univariate analysis, we ran a linear regression model including group (3 groups), troponin, HDL and sex as independent covariates. Sex and HDL were not significant factors

and were subsequently removed from the model (Table 2). The outcomes were consistent with the univariate analysis, estimating that children with ObMS+ had on average 2.2 pg/mL higher levels of PIGF compared to the CG. Troponin retained its significant, positive correlation with PIGF. The R^2 of the final model was 0.1622 and Cohen's f^2 was equal to 0.1936, which lies between small (0.02) and medium (0.25) size of effect.

DISCUSSION

Our study revealed that children with the MetS have significantly increased PIGF concentrations, a marker of angiogenesis, in comparison to children without MetS. Obese children, in general, had increased PIGF concentrations compared to the non-obese, but this was mainly due to the extreme effect of the MetS subgroup. We also found higher PIGF concentrations in normal weight males than females, which was not the case in children with obesity and MetS. Lastly, we found that hs-troponin is positively correlated with PLGF in obese children.

A recent study, mentioned previously, examined PIGF concentrations in obese children¹⁰ but did not find significant differences, possibly due to the fact that only childhood obesity and not pediatric MetS was taken into account. However, in the same study, another marker of angiogenesis, VEGF, was associated with BMI z-scores in children, suggesting a potentially concomitant formation of new vessels with the expansion of adipose tissue, starting in childhood.

Published evidence in adults showed increased PIGF concentrations in patients with the MetS,¹⁶ whereas another study demonstrated PIGF expression within human atherosclerotic lesions associated

Table 2. Final regression model showing significant predictors for PIGF

Parameter	b-coefficient (95% ci)	p value	Overall F-test
Troponin	0.29 (0.07, 0.51)	0.010	$p = 0.003$
Group			
ObMS+ vs CG	2.2 (-0.1, 4.5)	0.061	
ObMS- vs CG	0.8 (-0.6, 2.1)	0.250	
ObMS+ vs ObMS-	1.4 (-0.7, 3.6)	0.189	
Constant	10 (8.7, 11.3)	<0.001	

with plaque inflammation and destabilization and clinical manifestations of the disease.²³ However, the role of PIGF in the metabolic syndrome is not totally clear. PIGF binds to the VEGF-1 receptor and forms heterodimers with free VEGF, suggesting a potential role of PIGF in modulating the actions of VEGF.²⁴ PIGF was further proposed, in a study mentioned before,⁶ as an independent predictor of coronary heart disease after adjustment for several confounding factors, such as age, smoking, family history of myocardial infarction, physical activity and BMI, suggesting that PIGF might be involved in the formation of atherosclerotic plaques.

The association between pediatric MetS and PIGF suggests that angiogenesis may begin at an early stage of MetS development and might promote the activation of mechanisms related to the initiation of the atherosclerotic process. This is further supported by the finding that PIGF significantly correlated with hs-TnT, a biomarker related to cardiac damage, only in obese children. Indeed, the Dallas heart study²⁵ found that in a large number of individuals, cardiac (c) TnT was undetectable among healthy subjects and that subjects with minimal cTnT elevation had underlying CVD or a high-risk phenotype for CVD. We have also previously reported in a different cohort of obese children that N-terminal pro-brain natriuretic peptide (NT-proBNP), a direct indicator of functional and structural damage in the cardiovascular system, was increased in obese males with increased blood pressure²⁶ and that this biomarker was associated with adiponectin levels in obese female adolescents.²⁷ The current findings on associations between PIGF and hs-TnT, together with our previous findings on NT-proNP in a different sample of obese children, also strengthens the hypothesis that atherosclerotic risk factors are detectable or minimally elevated at a preclinical stage in children with obesity and obesity-related clinical and metabolic comorbidities.

In our study, males with normal weight had significantly higher PIGF concentrations than normal weight females. This significant gender effect might be mediated by sex hormones, since most of the children in the control group were pubertal. This is consistent with adult studies, where PIGF concentrations are lower in pre-menopausal women than post-menopausal

ones²⁸ in whom, in turn, PIGF concentrations were similar to those of men. Moreover, variations in PIGF concentrations have been described with the phases of the menstrual cycle and regulatory effects of the estrogens on PIGF production have been observed in pregnancy.²⁸

The cross-sectional design of our study cannot ascertain causality in the direction of the associations. Furthermore, the instability of MetS diagnosis during adolescence is another limitation of our data.²⁹ Carotid intima-media thickness measurements, which could contribute significantly to the understanding of our findings, were inadvertently not included in the study.³⁰ Lastly, we solely studied PIGF concentrations as a marker of angiogenesis in children with obesity and the MetS, whereas VEGF, a more established marker of angiogenesis, was not included in the study.

Further studies are required to investigate longitudinally the associations of PIGF with the progression of MetS and atherosclerosis and to clarify whether PIGF could have a clinically significant role in the prediction of cardiovascular risk later in life.

DISCLOSURES

The authors have nothing to declare. There is no conflict of interest.

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