CLINICAL STUDY

Pregnancy-associated plasma protein A in obese children: relationship to markers and risk factors of atherosclerosis and members of the IGF system

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Abstract

Background: Pregnancy-associated plasma protein A (PAPPA) is a large placenta-derived glycoprotein, which serves as a protease of several IGF-binding proteins (IGFBPs). In non-pregnant adults, measurable PAPPA levels were detected and have been implicated in the pathophysiology of atherosclerotic plaques. However, data in children is lacking.

Objective: To study the relationship between PAPPA, markers of atherosclerosis, and members of the IGF system in pediatric obesity.

Patients and design: Eighty-two obese and 52 nonobese children and 1-year longitudinal follow-up study for obese cohort.

Intervention: Outpatient 1-year intervention program based on exercise, behavior, and nutrition therapy.

Main outcome measures: Changes in PAPPA levels, carotid intima media thickness (IMT), weight, blood pressure, lipids, metabolic markers, and members of IGF system.

Results: Baseline PAPPA (PAPPA_{BL}) serum levels did not differ between obese and lean subjects. PAPPA_{BL} correlated significantly with IGF1, IGFBP1, and serum cholesterol. During the 1-year-program mean IMT decreased from 0.66 ± 0.01 to 0.63 ± 0.01 mm ($P < 0.05$) and PAPPA from 1.83 ± 0.12 to 1.58 ± 0.11 μ U/l ($P < 0.00$). In linear regression analysis with IMT after intervention as dependent variable, PAPPA contributed significantly to the observed variance. The longitudinal change of PAPPA correlated significantly with the change of serum triglycerides.

Conclusion: In this cohort of obese children, PAPPA serum levels correlated significantly with other cardiovascular risk factors. The lack of a direct correlation between PAPPA and IMT suggests that the described association of atherosclerotic plaques and increased PAPPA levels might reflect an indirect mechanism of PAPPA with cardiovascular risk factors such as serum lipids rather than a direct effect on the vasculature.

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Introduction

Pregnancy-associated plasma protein A (PAPPA) is a zinc metalloproteinase identified in 1974 as a protein of placental origin in pregnant women (1). Subsequently, PAPPA proved to be a useful marker for Down syndrome during pregnancy (2). Besides the placenta as a major source of PAPPA during pregnancy, several studies reported expression of PAPPA in a number of tissues, such as testis, kidney, colon, (3), and expression during processes of injury repair and remodeling, e.g. in skin healing and vascular smooth muscle cells (4, 5).

Recent studies demonstrated that PAPPA acts as a protease degrading insulin-like growth factor-binding protein 2 (IGFBP2), 4, and 5 (6–9), thereby enhancing local IGF bioactivity. Several animal models underlined

the role of PAPPA as a regulator of growth processes, with PAPPA-deficient animals exhibiting a growth phenotype resembling that of IGF2-deficient mice (10, 11). In contrast, overexpression of PAPPA increases somatic growth and skeletal muscle mass, paralleled by an increase in proteolysis of IGFBP4 and IGF bioavailability (12).

In 2001, Bayes-Genis *et al.* (5) implicated PAPPA in the development of acute coronary syndrome. Based on the function of PAPPA as a protease of IGFBPs, the authors suggested that PAPPA might contribute to progression of atherosclerosis through an increase in the local availability of IGF1. Since then, numerous studies demonstrated a role of PAPPA in the pathophysiology of atherosclerosis (13–15), although this has not been undisputed (16). As a putative explanation for

controversial results, differences in assay composition have been suggested (17). In addition to increased concentrations in cardiovascular disease and increased concentrations of PAPPa have been described in diabetes (18). In adult patients with type 2 diabetes, a previous study found a significant correlation between serum PAPPa concentrations and intima media thickness (IMT) of the common carotid artery, an accepted noninvasive marker of early atherosclerosis (18). Furthermore, a previous study in adults with well-controlled type 2 diabetes found a relationship between glycemic control and serum PAPPa levels (19). We previously demonstrated that an increase in IMT can already be observed in childhood in obese compared with lean children (20).

In this study, we examine whether PAPPa serum concentrations differ between obese and lean children and whether PAPPa serum concentration in pediatric obesity is related to IMT, cardiovascular risk factors, and members of the IGF system. Furthermore, we are interested in changes of PAPPa and potentially related parameters during participation in a 1-year lifestyle intervention program.

Methods

We studied 82 obese children (38 who lost a substantial amount of their overweight and 44 obese children without weight change) over a 1-year period (see Table 1). Fifty-two nonobese healthy children served as controls. In the lean control group, we were able to take only one blood sample when the child's blood was taken for other reasons like during one of the screening visits. Therefore, we were able to determine differences regarding PAPPa and IGFBP4 at baseline, but none of the remaining biochemical parameters obtained in the obesity group and after 1 year.

Written informed consent was obtained from all children and their parents. The study was approved by the local ethics committee of the University of Witten/Herdecke in Germany.

Table 1 Age, weight status (body mass index (BMI) and SDS-BMI), height, sex ratio, and pubertal stage in obese and nonobese children. Data are presented as mean $\pm$ S.E.M. or percentage.

	Obese	Nonobese	P
No.	82	52	
Age (years)	10.7 $\pm$ 0.3	11.1 $\pm$ 0.4	0.362
Weight (kg)	65.3 $\pm$ 2.1	36.4 $\pm$ 1.8	<0.001
BMI (kg/m ²)	28.0 $\pm$ 0.4	17.0 $\pm$ 0.3	<0.001
BMI-SDS	2.5 $\pm$ 0.0	-0.4 $\pm$ 0.1	<0.001
Height (cm)	150.8 $\pm$ 1.7	143.3 $\pm$ 2.8	0.018
Height-SDS	0.85 $\pm$ 0.1	-0.57 $\pm$ 0.2	<0.001
Sex	48.8% male	55.8% male	0.434
Pubertal stage	52.4% prepubertal	46.2 prepubertal	0.617
PAPPa (μ U/l)	1.83 $\pm$ 0.22	1.81 $\pm$ 0.12	0.939
IGFBP4 (μ g/l)	432.5 $\pm$ 11.4	330.9 $\pm$ 12.6	<0.001

The obese children were studied before and after participating in the 1-year lifestyle intervention 'Obel-dicks', which has been described in detail elsewhere (21, 22). Briefly, this outpatient intervention program for obese children is based on physical exercise, nutrition education, and behavior therapy including individual psychological care of the child and his or her family. The nutritional course is based on a fat- and sugar-reduced diet compared to the everyday nutrition of German children.

None of the children in the cohort of the current study suffered from endocrine disorders including type 2 diabetes mellitus, premature adrenarche, or syndromal obesity. All were non-smokers without any regular medication.

Obesity was defined according to the recommendation of the International Task Force of Obesity using population-specific data (23, 24). Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. Because the distribution of body mass index (BMI) is not comparable in children and adults, not even among the various childhood age groups, we used the LMS method to calculate SDS-BMI as a measurement for the degree of overweight. The LMS method was chosen as it summarizes the data in terms of three smooth age-specific curves called lambda (L), mu (M), and sigma (S) based on German population-specific data (24, 25). The M and S curves, respectively, correspond to the median and coefficients of variation (CV) of BMI for German children at each age and gender, whereas the L curve allows for the substantial age-dependent skewness in the distribution of BMI (24, 25). The assumption underlying the LMS method is that after Box-Cox power transformation, the data at each age are normally distributed (25). Using the LMS calculation method described above, substantial weight loss in the course of 1 year was defined as a reduction of SDS-BMI >0.5, because with a reduction of <0.5 SDS-BMI no improvement of insulin resistance and cardiovascular risk factors could be detected in obese children participating in this lifestyle intervention program (21).

The pubertal developmental stage was determined according to Marshall & Tanner (26, 27) and categorized into two groups (prepubertal: boys with pubic hair and gonadal stage I, girls with pubic hair stage and breast stage I; pubertal: boys with pubic hair or gonadal stage $\geq$ II, and pubertal girls with pubic hair stage or breast stage $\geq$ II).

Blood pressure was measured according to the guidelines of the National High Blood Pressure Education Program (NHBPEP) (28). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10 min rest in the supine position using a calibrated sphygmomanometer and averaged. The cuff size of the sphygmomanometer used, based on the length and

circumference of the upper arm, was as large as possible without having the elbow skin crease obstruct the stethoscope (28).

Blood sampling was performed in the fasting state at 0800 h. All serum samples were frozen opaque at -81°C and thawed only once. All measurements were performed in duplicates and averaged.

PAPPA was measured with a specific ELISA (Diagnostic System Laboratories, Inc., Webster, TX, USA; DSL-10-27600). This assay uses an antibody pair that measures dimeric, uncomplexed PAPPA, and PAPPA/pro major basic protein (proMBP) in equimolar concentrations. The minimum detection limit was $0.18\text{ }\mu\text{U/ml}$; intra- and inter-assay CV were $<10\%$. IGF1 was measured with a highly specific chemiluminescence immunoassay (Immulate 1000 IGF1, Diagnostic Products Corporation, Los Angeles, CA, USA) without detectable cross-reactions to proinsulin, insulin, and IGF2. The sensitivity was 20 ng/ml . Intra- and inter-assay CV were $<6\%$.

IGFBP1 levels were assessed by a sandwich ELISA utilizing two specific high-affinity antibodies for this protein (IGFBP1 ELISA Mediagnost, Tuebingen, Germany). Serum was appropriately diluted to measure within the linear range of the assay. The sensitivity of the assay is 0.02 ng/ml ; intra- and inter-assay CV were $<10\%$.

IGFBP3 was measured with highly specific chemiluminescence immunometric assay (Immulate IGFBP3, Diagnostic Products Corporation) without any cross-reactions to IGF2, IGFBP1, and IGFBP2. The sensitivity was $0.02\text{ }\mu\text{g/ml}$. Intra- and inter-assay CV were $<10\%$. IGFBP4 was measured with a specific ELISA (Diagnostic System Laboratories, Inc.; DSL-10-7300). The minimum detection limit was 0.008 ng/ml ; intra- and inter-assay CV were $<10\%$.

Insulin concentrations were measured by microparticle-enhanced immunometric assay (Abbott, Wiesbaden, Germany). Glucose levels were determined by colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargemund, Germany). HDL-cholesterol concentrations were measured by an enzymatic test (HDL-C-Plus Roche Diagnostics) and triglyceride concentrations by a colorimetric assay using a Vitros analyzer (Ortho Clinical Diagnostics). Homeostasis model assessment (HOMA) was calculated by the following formula: resistance (HOMA) = (insulin (mU/l) $\times$ glucose (mmol/l)) / 22.5 (29).

Waist circumference was measured at the narrowest point between the lower rib and the iliac crest during expiration.

We measured IMT by B-mode ultrasound using a 14 MHz linear transducer following a standardized protocol. The measurement was performed at the common carotid artery (CCA) near the bifurcation at the far wall. We measured four values on each side and took the maximum value for statistical purposes because the strongest association between the different measurements of IMT and coronary risk factors in otherwise healthy individuals is achieved by applying

the maximum and not the mean value of IMT (30). The patients were examined in the supine position with the head turned slightly to the side. The same sonographer, who was blinded to the participants' cardiovascular risk factor status, performed all the examinations. In none of the examined patients, we found evidence of plaque.

Statistical analyses were performed using the SPSS Software package, version 17.0. Student's *t*-test for paired and unpaired observations of normally distributed values and for non-normally distributed variables Wilcoxon and Mann–Whitney *U* tests were used when appropriate. Mean values of variables were expressed as mean and S.E.M. Correlations between parameters were calculated by Pearson's correlation coefficient for parameters following a normal distribution and by Spearman's rank coefficient for nonparametric parameters, as tested by the Kolmogorov–Smirnov test. Partial correlations, adjusted for gender, pubertal stage, and age, between changes of PAPPA, IGF1, IGFBP1, 3, 4, serum lipids, fasting glucose and insulin, and anthropometric variables in the course of 1 year were calculated. Parameters that did not follow a normal distribution were log-transformed prior to analysis. Changes were expressed as delta variable calculated by variable at baseline minus variable measured 1 year later. Multiple logistic regression analyses with IMT as a dependent variable including PAPPA, IGF1, IGFBP1, 3, 4, fasting insulin and glucose, triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), blood pressure, age, sex, and pubertal stage were calculated. A *P* value <0.05 was considered as significant; *P* values between 0.05 and 0.1 were considered as indicators of a trend.

Results

Cross-sectional analyses

Obese patients and lean controls did not differ regarding age, gender, and pubertal stage. By definition, BMI and BMI-SDS were significantly higher in the obese group. In addition, obese children were moderately taller with a significantly higher height-SDS (see Table 1).

IGFBP4 was significantly higher in obese children, whereas baseline PAPPA (PAPPA_{BL}) serum levels did not differ between obese and lean subjects (Table 1). In obesity, PAPPA_{BL} correlated significantly with IGF1, IGFBP1, and serum cholesterol, but not with IGFBP3 or 4 serum levels. In addition, IGFBP4 correlated significantly with fasting insulin and HOMA as markers of insulin sensitivity (see Table 2). In lean subjects, PAPPA did not correlate to IGFBP4 or clinical/axiological parameters such as BMI, height, age, or pubertal stage.

At baseline, we found no correlation between PAPPA_{BL} and age, BMI-SDS, waist circumference, IMT, and blood pressure (both SBP and DBP) in obese subjects. We found no differences of PAPPA_{BL} in prepubertal and

Table 2 Correlation of baseline PAPPa, IGFBP4 with other members of the IGF system, plasma lipids, and markers of insulin sensitivity (Spearman's correlation coefficients) in obese children. Numbers in bold indicate statistically significant correlations.

		IGFBP4	IGF1	IGFBP1	IGFBP3	Insulin	HOMA	Cholesterol	Triglycerides	HbA1c	Glucose
PAPPa	<i>r</i>	−0.127	−0.263	0.371	0.082	0.119	−0.132	0.241	0.116	0.010	−0.126
	<i>P</i>	NS	0.026	0.004	NS	NS	NS	0.033	NS	NS	NS
IGFBP4	<i>r</i>		0.172	−0.230	0.366	0.367	0.347	0.022	0.426	0.218	0.034
	<i>P</i>		NS	0.077	0.002	0.001	0.003	NS	0.000	0.074	NS
IGF1	<i>r</i>			−0.554	0.457	0.257	0.264	0.009	0.149	0.000	0.161
	<i>P</i>			0.000	0.000	0.026	0.022	NS	NS	NS	NS
IGFBP1	<i>r</i>				−0.299	−0.574	−0.598	−0.112	−0.281	−0.014	−0.228
	<i>P</i>				0.025	0.000	0.000	NS	0.030	NS	0.080
IGFBP3	<i>r</i>					0.257	0.253	0.305	0.331	−0.005	0.073
	<i>P</i>					0.026	0.029	0.008	0.004	NS	NS
Insulin	<i>r</i>						0.991	0.044	0.470	0.009	0.334
	<i>P</i>						0.000	NS	0.000	NS	0.002
HOMA	<i>r</i>							0.059	0.454	0.010	0.453
	<i>P</i>							NS	0.000	NS	0.000
Cholesterol	<i>r</i>								0.329	0.213	0.028
	<i>P</i>								0.003	0.063	NS
Triglycerides	<i>r</i>									0.005	0.065
	<i>P</i>									NS	NS
Glucose	<i>r</i>										0.027
	<i>P</i>										NS

pubertal subjects (1.7 ± 1.1 $\mu\text{U/l}$ in prepuberty versus 2.0 ± 1.1 $\mu\text{U/l}$ in pubertal subjects; $P=0.19$). At baseline, PAPPa_{BL} did not differ significantly between obese girls and boys (1.7 ± 0.2 vs 2.0 ± 0.2 $\mu\text{U/l}$; $P>0.05$). However, PAPPa after intervention was significantly lower in girls compared with boys (1.3 ± 0.1 in girls versus 1.9 ± 0.2 $\mu\text{U/l}$ in boys; $P=0.002$).

In multiple logistic regression analysis, insulin and serum lipids (both cholesterol and triglycerides), but not PAPPa, contributed significantly to the observed variance in IMT before participation in the intervention program. However, after participating in the program, not only fasting insulin and triglycerides but also PAPPa and fasting glucose contributed significantly to the observed variance in IMT (Table 3). In subgroup

analysis comparing the groups with and without significant weight loss, we found a significant contribution of PAPPa levels to the variance of IMT after intervention only in individuals with weight loss (Table 4). However, subgroup analyses lead to relatively small group sizes; therefore, these results warrant confirmation in larger sized cohorts.

Longitudinal analyses

Thirty-eight of the 82 obese children showed a significant weight loss (defined as a decrease more than 0.5 BMI-SDS; see Table 5). Regarding weight, a decrease from 60.5 ± 2.6 to 58.6 ± 2.5 kg was observed for this subgroup, reflecting a mean weight loss of

Table 3 Multiple backward linear regression analysis with IMT before (A) and after 1 year (B) as the dependant variable and age, sex (0, female; 1, male), pubertal stage (0, prepubertal; 1, pubertal), systolic and diastolic blood pressures (SBP and DBP), IGF1, IGFBP1, 3, 4, PAPPa, fasting insulin and glucose, HbA1c, cholesterol, -HDL, -LDL, and triglycerides as independent variables in 82 obese children.

	Variables					
	β	βSE	Standard β	Partial corr.	<i>P</i>	Corr. model r^2
(A) IMT before 1 year						0.452
Intercept	0.094	0.028				
Insulin	0.009	0.002	0.613	0.650	0.000	
IGFBP3	0.004	0.009	0.234	0.300	0.036	
HbA1c	−0.024	0.017	−0.218	−0.381	0.028	
Triglycerides	−0.001	0.001	−0.313	−0.285	0.007	
(B) IMT after 1 year						0.554
Intercept	0.147	0.037				
Insulin	0.010	0.002	0.520	0.491	0.000	
IGFBP3	0.001	0.000	0.234	0.256	0.075	
HbA1c	−0.003	0.002	−0.235	−0.310	0.030	
Triglycerides	−0.039	0.002	−0.196	−0.315	0.047	

Table 4 Multiple backward linear regression analysis with IMT after participation as the dependant variable and age, sex (0, female; 1, male), pubertal stage (0, prepubertal; 1, pubertal), systolic/diastolic blood pressure (SBP and DBP), IGF1, IGFBP1, 3, 4, PAPPA, fasting insulin and glucose, HbA1c, cholesterol, -HDL, -LDL, and triglycerides as independent variables in obese children with (A) and without (B) significant weight loss.

	Variables					
	β	β SE	Standard β	Partial corr.	P	Corr. model r^2
(A) Obese children with significant weight loss						
IMT						0.423
Intercept	0.157	0.039				
PAPPA	-0.004	0.001	-0.506	-0.669	0.003	
Insulin	0.010	0.004	0.568	0.549	0.022	
Triglycerides	0.000	0.000	0.527	0.604	0.010	
IGFBP1	0.005	0.002	0.572	0.561	0.019	
IGFBP3	0.003	0.002	0.337	0.471	0.056	
LDL	0.000	0.000	0.356	0.512	0.036	
Pubertal stage	-0.006	0.003	-0.341	-0.477	0.053	
Sex	-0.012	0.004	-0.645	-0.650	0.005	
(B) Obese children without significant weight loss						
IMT						0.651
Intercept	0.163	0.114				
PAPPA	0.014	0.010	0.539	0.540	0.017	
Insulin	0.008	0.004	0.520	0.640	0.038	
Fasting glucose	-0.047	0.004	0.600	-0.425	0.070	
Triglycerides	0.001	0.000	4.699	0.456	0.003	
Total cholesterol	-0.005	0.002	-9.417	-0.464	0.045	
HDL	0.004	0.002	3.567	0.456	0.053	
LDL	0.004	0.002	8.588	0.453	0.052	
RRsys	0.000	0.000	-0.418	0.453	0.052	
Sex	0.026	0.024	-0.387	0.479	0.049	

2.9%. In contrast, in the subgroup of 44 children without significant weight loss, weight increased from 69.5 ± 3.0 to 77.0 ± 3.3 kg, reflecting a mean weight gain of 11.3% (see Table 5).

In the longitudinal analyses in the course of 1 year, in obese children with and without weight loss, IMT decreased significantly (delta IMT: 0.03 ± 0.01 mm; $P < 0.05$). In a power analysis on the decrease of IMT, we had a power of 0.999 based on a mean difference of 0.11 ± 0.05 mm in the 42 children with substantial weight loss, a two-side test, and an α error of 0.05. Correspondingly, PAPPA serum levels decreased from 1.83 ± 0.12 to 1.58 ± 0.11 μ U/l, whereas IGFBP4 did not change significantly ($P < 0.005$ for PAPPA; see Fig. 1).

In the 38 children who showed a significant weight loss, markers of insulin sensitivity improved significantly and serum lipids showed a significant decrease (Table 5). Correspondingly, after 1 year (1 y), IMT (baseline 0.66 ± 0.00 vs 1 y 0.55 ± 0.00 mm; $P = 0.000$), SBP (baseline 118.5 ± 2.2 vs 1 y 109.3 ± 2.0 mmHg; $P = 0.004$), DBP (baseline 67.6 ± 2.1 vs 1 y 62.5 ± 1.4 mmHg; $P = 0.016$), and waist circumference (baseline 86.5 ± 1.7 vs 1 y 81.3 ± 1.7 mm; $P = 0.002$) decreased significantly.

In the 44 obese children in whom no significant weight loss could be observed, neither insulin sensitivity nor serum lipids improved (Table 3B). Regarding clinical parameters, IMT increased (IMT_{BL} 0.65 ± 0.00 vs IMT_{after 1 y} 0.69 ± 0.00 mm; $P < 0.001$), whereas RRsys

(systolic blood pressure) (RR_{BL} 117.7 ± 2.3 vs RR_{after 1 y} 115.1 ± 2.2 mmHg; $P > 0.05$), RR dias (RR_{BL} 66.9 ± 1.7 vs RR_{after 1 y} 66.3 ± 1.5 mmHg; $P > 0.05$), or waist circumference (WC_{BL} 92.9 ± 2.1 vs WC_{after 1 y} 92.9 ± 2.2 mm; $P > 0.05$) changed significantly.

Serum PAPPA concentration decreased in both groups during the 1-year program. Mean serum PAPPA concentration after participation in the weight loss program was lower in the group with significant weight loss compared with children without weight loss (1.45 vs 1.69 μ U/l; see Table 5), although this difference showed only a trend of statistical significance ($P = 0.06$).

In the 1-year follow-up period, the changes in PAPPA correlated significantly to changes in triglycerides ($r = 0.243$, $P = 0.017$), but not to changes in cholesterol ($r = -0.03$, $P = 0.491$), changes in fasting glucose ($r = 0.031$; $P = 0.399$), changes in fasting insulin ($r = 0.011$; $P = 0.463$), changes in IGF1 ($r = -0.045$; $P = 0.362$), changes in IGFBP1 ($r = -0.085$; $P = 0.269$), changes in IGFBP3 ($r = 0.132$; $P = 0.147$), and changes in IGFBP4 ($r = 0.123$; $P = 0.155$).

Discussion

This study provides for the first time data on PAPPA and one of its putative substrates, IGFBP4, in obese and lean children and sheds light on the relationship of PAPPA with markers and risk factors of atherosclerosis in obese

Table 5 Change in height and weight status (BMI, SDS-BMI, and SDS-height), PAPPa, members of the IGF system, and plasma lipids in 38 obese children with substantial (decrease in SDS-BMI >0.5) weight loss (group 1) and in 44 obese children without substantial (decrease in SDS-BMI <0.5) weight loss (group 2) over a 1-year period. Data are presented as mean $\pm$ S.E.M.

	Group 1			Group 2		
	Baseline	1-year follow-up	P	Baseline	1-year follow-up	P
BMI	27.2 $\pm$ 3.5	24.2 $\pm$ 3.5	0.000	28.8 $\pm$ 7.1	29.8 $\pm$ 7.5	0.000
BMI-SDS	2.4 $\pm$ 0.06	1.8 $\pm$ 0.07	0.000	2.5 $\pm$ 0.06	2.5 $\pm$ 0.06	0.729
Weight (kg)	60.5 $\pm$ 2.6	58.6 $\pm$ 2.5	0.003	69.5 $\pm$ 3.0	77.0 $\pm$ 3.3	0.000
Height SDS	0.55 $\pm$ 0.1	0.65 $\pm$ 0.1	0.081	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	0.156
Waist circumference (cm)	86.5 $\pm$ 1.7	81.3 $\pm$ 1.7	0.002	92.9 $\pm$ 2.1	92.9 $\pm$ 2.2	0.984
PAPPa (μ U/l)	1.65 $\pm$ 0.2	1.45 $\pm$ 0.2	0.192	1.98 $\pm$ 0.2	1.69 $\pm$ 0.1	0.038
IGFBP4 (μ g/l)	424.9 $\pm$ 19	414.9 $\pm$ 15	0.546	440.0 $\pm$ 13	465.8 $\pm$ 15	0.075
IGFBP1 (ng/ml)	4.5 $\pm$ 0.7	5.3 $\pm$ 1.4	0.414	4.2 $\pm$ 1.1	3.0 $\pm$ 0.6	0.016
IGFBP3 (μ g/ml)	5.3 $\pm$ 0.2	5.5 $\pm$ 0.2	0.340	5.7 $\pm$ 0.2	5.7 $\pm$ 0.2	0.859
IGF1 (ng/ml)	280.9 $\pm$ 32.1	326.5 $\pm$ 25.3	0.039	260.5 $\pm$ 21.4	330.7 $\pm$ 29.7	0.000
Fasting glucose (mg/dl)	83.8 $\pm$ 1.4	88.8 $\pm$ 4.5	0.329	83.8 $\pm$ 1.2	85.9 $\pm$ 1.1	0.125
Insulin (mU/l)	16.0 $\pm$ 1.5	11.6 $\pm$ 1.3	0.004	20.0 $\pm$ 2.1	22.3 $\pm$ 2.4	0.339
HOMA	3.3 $\pm$ 0.3	3.0 $\pm$ 0.8	0.005	4.2 $\pm$ 0.4	4.8 $\pm$ 0.5	0.273
Cholesterol (mg/dl)	169.7 $\pm$ 4.3	158.5 $\pm$ 4.5	0.009	175.9 $\pm$ 4.4	170.7 $\pm$ 3.7	0.074
Triglycerides (mg/dl)	106.2 $\pm$ 9.5	80.8 $\pm$ 6.0	0.001	120.3 $\pm$ 10.4	119.4 $\pm$ 9.0	0.364
Fasting glucose	83.8 $\pm$ 1.4	88.8 $\pm$ 4.5	0.329	83.8 $\pm$ 1.2	85.8 $\pm$ 1.1	0.125
HbA1c	5.41 $\pm$ 0.05	5.39 $\pm$ 0.04	0.571	5.31 $\pm$ 0.07	5.43 $\pm$ 0.04	0.199

children during a 1-year lifestyle intervention program. Carotid IMT is an ultrasonographic marker of atherosclerosis (30, 31), which is associated with coronary heart disease and hypertension in obese adults (32). In a previous study, we demonstrated that arterial abnormalities occur already as early as in childhood, although the mechanisms by which obesity affects IMT are yet unclear. Blood pressure, serum lipids, and glucose have all been found associating with IMT and have thus been discussed as a putative link between obesity and atherosclerosis (20). In this study, we confirmed an important association between serum lipids, glucose metabolism, and early vascular changes. However, our data suggest that in addition to these well-established parameters, early changes in IMT may also be associated with changes in serum PAPPa levels.

PAPPa levels did not differ between obese and lean children. However, despite this lack of association between BMI-SDS and serum PAPPa concentration, PAPPa levels decreased when participating in an intervention program, based on physical exercise, nutrition education, and behavior therapy. In subgroup analysis, PAPPa levels decreased in both groups with and without significant weight loss. Although we found a statistical trend toward lower serum PAPPa concentration after intervention in children with significant weight loss compared with children without weight loss, the mean decrease in PAPPa during the intervention did not reach statistical significance in children with significant weight loss. We assume that the lack of significant PAPPa decrease in children with weight loss results from the already lower baseline serum PAPPa concentration (1.65 vs 1.98 μ U/l in the subgroup without weight loss (WL)).

Currently, no reference data on serum PAPPa levels in different pediatric age groups are available. In our sample, neither in univariate nor in multivariate analyses an association between age and PAPPa was observed. Therefore, we speculate that a weight-independent effect of the lifestyle program might modulate the observed decrease in PAPPa, which occurred in both subgroups (with or without weight loss). However, considering the small age range of our lean and obese patients' samples, we cannot definitely rule out an age-related decline in PAPPa, as so far no other study on serum PAPPa levels in healthy and/or obese children are available.

During the 1-year interval before and after participation, PAPPa, and IMT fell significantly for the whole group, although we cannot clearly attribute this decline to participation in the weight loss program, as we were only able to compare baseline data with a control group. As stated above, in both groups serum PAPPa concentrations after intervention were lower compared with baseline. Lipid levels fell significantly only in the group with weight loss, and only in this subgroup, PAPPa contributed significantly to the observed variance in IMT. This might indicate that in these patients, hyperlipidemia is of dominating importance regarding IMT and thereby masks a putative effect of PAPPa, which becomes apparent only after lifestyle intervention. In subgroup analysis comparing the groups with and without significant weight loss, we found a significant contribution of PAPPa levels to the variance of IMT after intervention only in individuals with weight loss. However, subgroup analyses lead to relatively small group sizes; therefore, these results warrant confirmation in larger sized cohorts.

Figure 1 PAPPA (A) and IGFBP4 serum levels (B) and intima media thickness (C) before and after participation in the weight loss program (mean and s.e.m.).

In addition to a correlation with IGF1 and serum PAPPA levels in our cohort of obese children correlated significantly with cholesterol and IGFBP1. This is in congruence with an observation of Aso *et al.* (18), who found serum cholesterol as an independent predictor of serum PAPPA concentrations in adult patients with type 2 diabetes. This association was additionally observed in asymptomatic hyperlipidemic subjects without clinical signs of atherosclerosis (33). In the animal model

lacking ApoE, the additional knockout of PAPPA did not lead to differences in serum cholesterol, which might indicate that increased cholesterol stimulates PAPPA secretion rather than vice versa (34). However, in this study, in the longitudinal analysis, we found only a significant association between the change in PAPPA and the change in serum triglycerides, but not with the change in cholesterol levels. Thus, at the present point, we suggest that a causal relationship between PAPPA and serum lipids seems likely, but clearly further longitudinal studies including larger cohorts are required to shed more light on this interaction and putative mechanisms.

In contrast to a previous study (19), we did not find a significant correlation between HbA1c and PAPPA concentrations. However, both cohorts were hardly comparable, since in our group of obese, non-diabetic children, all HbA1c levels were within the normal range (mean HbA1c 5.4%), whereas the mentioned study cohort consisted of well-controlled diabetic adults (mean HbA1c 7.1%; controls 5.2%; reference (19)), so that HbA1c levels of our cohort were closer to the control than the study group.

Although in our study IGFBP1 correlated highly significantly to markers of insulin resistance HOMA and fasting insulin, a divergent picture was seen regarding the association of PAPPA with these parameters. In contrast to IGFBP4 and PAPPA did not show any association with HOMA or fasting insulin, but correlated highly significantly with IGFBP1. Although binding only a small fraction of circulating IGF1 and IGFBP1 is considered the only acute regulator of IGF1 bioavailability. Hepatic IGFBP1 expression is inversely related to the portal supply of insulin and reduced fasting serum IGFBP1 levels have been associated with hyperinsulinemia and related disorders in adults (35, 36). Previous studies indicated that a decrease in IGFBP1 levels might precede other indices of insulin resistance in childhood (37–39). However, whether the observed association between PAPPA and IGFBP1 indicates a role of PAPPA in the network influencing insulin sensitivity or is more related to the complex effects of PAPPA on IGFBPs currently remains unclear.

To date, the mechanisms through which PAPPA modulates initiation, maintenance, or progression of the atherosclerotic process remain largely unclear. Previous studies demonstrated that PAPPA acts as a protease and modulates circulating and potentially local IGF1 concentrations by cleaving at least three of six IGF binding proteins, thereby regulating IGF1 bioavailability. Furthermore, local IGF1 bioavailability has been linked to proliferation of vascular smooth muscle cells and modulation of local inflammatory processes, thereby putatively acting pro-atherogenic (40). Exposure of endothelial cells to the pro-inflammatory cytokine tumor necrosis factor α (TNF α) stimulated expression of PAPPA (41). Several animal models supported a pro-atherogenic role of PAPPA. Animals

lacking PAPPa exhibit a longevity phenotype, with a 30% increase in life span (42) and deletion of the *Papp* gene in high-cholesterol-fed, ApoE-deficient animals resulted in a reduction of atheromatous lesions compared with wild-type littermates (36). Targeted disruption of the *Papp* gene was associated with diminished smooth muscle cell response to IGF1 (43). In animals lacking PAPPa and IGF1 serum levels at 104 days of age were lower in both males and females, but this proved not to be of statistical significance (44). In our study, we found a negative correlation between serum levels for PAPPa and IGF1. However, in the current study in obese children, in none of which carotid plaques could be detected, serum IGF1 did not contribute significantly to the observed variance in IMT, neither before nor after intervention. These conflicting results are mirrored in a more recent debate, whether the PAPPa/IGF1 system indeed has a pro-atherogenic or, in the opposite, may play an anti-atherogenic role. Whereas PAPPa levels are increased in patients with unstable coronary or carotid plaques, the evidence of PAPPa as a pro-atherogenic factor in native arteries is low (reviewed in (45)). The same debate takes place for IGF1, with more recent studies demonstrating antioxidant, anti-inflammatory, and potentially atheroprotective effects of IGF1, as opposed to the more traditional view of locally produced IGF1 increasing the neointimal injury response (46). Thus, at present, the observed correlations between PAPPa in the cross-sectional analyses and the changes in the longitudinal part of our study cannot be linked to a clear negative or positive effect on vascular health.

The strength of this study lies in the dual approach of a combination of cross-sectional and longitudinal data. However, our study has a number of potential limitations. First, due to the small sample size of the lean control and ethical restrictions, we were only able to compare selective baseline parameters (PAPPa and IGFBP4), but not post-intervention results of obese children with a lean control group. Although we did not find an association between PAPPa and age, we cannot completely rule out that the observed decline in PAPPa after participation in the lifestyle program is related to the increase in age. Secondly, BMI percentiles were used to classify overweight. Although BMI is a good measure for overweight, one needs to be aware of its limitation as an indirect measure of fat mass. Thirdly, we are not able to differentiate the effect of diet, increased physical exercise, and weight loss on PAPPa or IGFBP4 concentrations due to our study protocol. Fourthly, we determined maximum IMT in this cohort, since in previous studies maximum IMT showed the strongest association to coronary risk factors in otherwise healthy individuals (30). However, we do not know whether our findings related to maximum IMT can be compared with studies using mean IMT determination. Fifthly, the observed associations between PAPPa and IMT, IGF1, cholesterol, and IGFBP1 do not prove a causal

relationship, as we found only a significant correlation of the change in PAPPa with the change in triglycerides, but in none of the above-mentioned parameters. Additional longitudinal studies in healthy and obese subjects covering a larger age range and focusing on the mentioned parameters are required to validate the observations. Finally, as outlined in the Methods section, the assay we used includes an antibody pair that measures dimeric, uncomplexed PAPPa and PAPPa/proMBP in equimolar concentrations. This may explain differences between ours' and previous studies using assays that detect 'pregnancy' PAPPa in form of a covalent complex with pro-MBP.

In summary, in this cohort of obese children, serum PAPPa levels correlated significantly with other cardiovascular risk factors. Cross-sectionally, PAPPa correlated significantly with serum IGF1, IGFBP1, and cholesterol, and longitudinally the change of PAPPa was associated with the observed change of triglycerides. Whether these associations indicate a negative effect of PAPPa on vascular health in these obese children, either through a direct effect or through a more indirect mechanism of PAPPa with cardiovascular risk factors such as serum lipids, remains to be confirmed in subsequent studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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