Relationship Between Plasminogen Activator Inhibitor-1 Antigen, Leptin, and Fat Mass in Obese Children and Adolescents

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Hyperleptinemia may be associated with cardiovascular risk and is linked with parameters of fibrinolytic processes in adults. We studied whether body fatness, leptin, and insulin interact with plasminogen activator inhibitor-1 antigen (PAI-1-Ag) and tissue-type plasminogen activator antigen (tPA-Ag) in obese children and adolescents. Twenty-three boys (mean ± SD: age, 10.7 ± 3.3 years; body mass index [BMI], 28.7 ± 5.4 Kg/m^2) and 19 girls (age, 11.9 ± 2.7 years; BMI, 29.4 ± 4.8 Kg/m^2) were investigated. Body fat mass (FM) in the children was calculated by bioelectrical impedance analysis, and blood samples were obtained for leptin, insulin, C-peptide, PAI-1-Ag, and tPA-Ag. The children were divided into 3 subgroups according to maturation. Maturity was associated with greater adiposity and higher levels of leptin and C-peptide, but insulin and PAI-1-Ag were not different between prepubertal, pubertal, and late/postpubertal children. PAI-1-Ag was associated with leptin and insulin, but not after adjustment for fatness. PAI-1-Ag was independently associated with tPA-Ag ($r = .36$, $P < .02$). Multiple regression analysis showed that tPA-Ag failed to reach the level of significance ($P = .07$), but FM contributed to the variation in PAI-1-Ag (adjusted $R^2 = .29$). The BMI was the main determinant for the variation in leptin (adjusted $R^2 = .386$) and in insulin (adjusted $R^2 = .60$, all $P < .001$). Neither gender, maturation, chronological age, or leptin contributed significantly to the variation in either PAI-1-Ag or tPA-Ag. Our data suggest that adiposity and other variables contribute to higher levels of PAI-1-Ag. Leptin seems not to be independently linked with fibrinolytic parameters, but an unfavorable metabolic and fibrinolytic risk profile might emanate from the obese pubertal stage.

THE PRODUCT of the ob gene, leptin, is secreted by adipocytes and has several physiological mechanisms in rodents and humans.[1-3] Leptin levels are elevated in obese adults[4] and children,[5] indicating that they may be resistant to the effects of leptin.[6] The significance of elevated leptin is unclear; however, recent findings have shown that hyperleptinemia in addition to insulin resistance is strongly related to the components of a metabolic syndrome of cardiovascular risk.[7] Furthermore, a nested case-referent study showed plasma leptin to be associated with an increased risk for first-ever hemorrhagic stroke, independent of other risk markers for cardiovascular disease.[8]

Plasminogen activator inhibitor-1 (PAI-1) is the main physiological inhibitor of plasminogen activation in blood.[9] PAI-1 is elevated in obesity, suggesting that adipose tissue itself is a source of PAI-1.[10,11] Although PAI-1 mRNA is expressed in subcutaneous and omental adipocytes, the visceral body fat compartment seems to be the main source of PAI-1,[12-14] and a greater amount of visceral adipose tissue is associated with an increased cardiovascular risk.[15,16] A variety of hormones and cytokines may be involved in the expression of PAI-1. There is evidence that insulin might elevate PAI-1,[17] and insulin also increases ob gene expression.[18,19] However, it is possible that the actions of insulin on PAI-1[11,20,21] and leptin[22,23] depend on the metabolic state and level of fatness of an individual. Interestingly, insulin resistance, hyperleptinemia, and dysfibrinolysis may coexist, as elevated leptin was shown to be linked with low levels of tissue-type plasminogen activator (t-PA) activity and high levels of PAI-1 activity.[24]

An increase of ponderosity in children is associated with a worsening of cardiovascular risk,[25,26] and changes in the lipoprotein profile might contribute to later health outcomes.[27-29] However, there is a lack of data as to whether interactions of leptin, insulin, and PAI-1 are even present in childhood and juvenile obesity. We therefore investigated whether fatness, leptin, and (or) insulin might contribute independently to the levels of fibrinolytic parameters in obese children and adolescents.

SUBJECTS AND METHODS

Subjects

Twenty-three boys (mean ± SD: age, 10.7 ± 3.3 years; body mass index [BMI], 28.7 ± 5.4 Kg/m^2) and 19 girls (age, 11.9 ± 2.7 years; BMI, 29.4 ± 4.8 Kg/m^2) were investigated. Obesity was defined as a BMI greater than the 90th percentile for age and sex. Maturation of the children was estimated according to the standards of Tanner and Whitehouse[30] using pubic hair and breast development in girls and pubic hair and testicular volume in boys.

Children were judged as healthy by medical examination, and written informed consent was provided by the parents. The study was approved by the local ethics committee. All anthropometric and metabolic characteristics are shown in Table 1.

Laboratory Methods

Blood samples were obtained after an overnight fast and analyzed for leptin, insulin (Linco Research, St. Charles, MO), and C-peptide (CIS, Paris, France) by radioimmunoassay. Blood samples for measurement of fibrinolytic parameters were obtained with plastic tubes containing 0.1 mol/L sodium citrate. The plasma was separated by centrifugation at 3,000 × *g* for 10 minutes at 4°C and stored at −70°C. tPA-antigen (tPA-Ag) and PAI-1-antigen (PAI-1-Ag) were determined by enzyme-linked immunosorbent assay (Kabi, Vienna, Austria).

Measurement of Body Composition

Measurement of the fat-free mass (FFM)[31] was performed by bioelectrical impedance analysis (BIA Akern-RJL 101/S, RJL Systems,

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Table 1. Anthropometric, Metabolic, and Fibrinolytic Parameters of the Obese Children

Parameter	All Children (N = 42)	Boys (n = 23)	Girls (n = 19)	*P*
Age (yr)	11.2 ± 3.1	10.7 ± 3.3	11.9 ± 2.7	.20
BMI (kg/m^2)	29 ± 5.1	28.7 ± 5.4	29.4 ± 4.8	.68
FM (kg)*	32.2 ± 14	30.7 ± 14.05	34 ± 14.2	.47
%FM	45.4 ± 7.9	45.2 ± 7.9	45.7 ± 8.1	.86
WHR†	0.96 ± 0.06	0.98 ± 0.04	0.94 ± 0.06	.08
Serum leptin (ng/mL)	27.45 ± 19.7	26.9 ± 23.3	28.1 ± 14.8	.37‡
Serum insulin (μU/mL)	21.8 ± 17.5	22.05 ± 20.9	21.5 ± 12.8	.56‡
C-peptide (ng/mL)	2.65 ± 1.7	2.3 ± 1.15	3.1 ± 2.1	.31
tPA-Ag (μg/L)§	3.5 ± 1	3.35 ± 0.9	3.7 ± 1.2	.35
PAI-1-Ag (ng/mL)‖	40.05 ± 18.7	39.7 ± 14.9	40.4 ± 22.4	.91

NOTE. Data are presented as the mean ± SD. *P* values are for the gender difference.

*Body composition values were only available for 21 boys and 18 girls.

†Ten boys and 13 girls.

‡After $\log_{10}$ transformation.

§Twenty-one boys and 18 girls.

‖Twenty boys.

Clinton, MI). Fat mass (FM) was calculated as the difference between body weight and FFM. The percent FM (%FM) is expressed as the relative amount of FM for a given body weight. Waist and hip circumferences were measured to the nearest 0.5 cm and the waist to hip ratio (WHR) was calculated in a subsample of children (13 girls and 9 boys).

Statistics

Data that were not normally distributed were $\log_{10}$-transformed (leptin and insulin). The Mann-Whitney *U* test and ANOVA were used to compare variables between groups where appropriate. In the case of a significant difference, post hoc Bonferroni correction was used. The Kruskal-Wallis test was used if variances were not normally distributed. Correlations between variables of interest were calculated using Pearson's correlation coefficient and Spearman's rank correlation. Partial correlation analysis was performed to adjust for the influence of confounding variables. The independence and significance of variables were tested by stepwise multiple regression analysis. A maximum of 4 independent variables were entered into the equation. The significance level for *P* values was set at 5%.

RESULTS

No significant gender differences were found for all available anthropometric and metabolic characteristics (Table 1). Significant maturation-dependent differences were found for the BMI, FM, %FM, log leptin ($P = .008$), and C-peptide ($P = .0025$). Children were therefore divided into 3 subgroups according to maturation : (1) prepubertal stage, 14 boys and 3 girls, (2) pubertal stage, 5 boys and 10 girls, and (3) late/postpubertal stage, 4 boys and 6 girls. The BMI ($P = .0034$), FM ($P = .00017$), %FM ($P = .044$), and log leptin were higher in the pubertal group versus the prepubertal group ($P = .0041$), but log leptin was higher in pubertals versus late/postpubertal children ($P = .04$ by *U* test). C-peptide levels were higher in pubertals than in prepubertals ($P = .0017$), but were not significantly different from the values in the late/postpubertal group. Log insulin was not significantly different between stages of maturation ($df = 2$, $F = 3.41$, $P = .043$, after Bonferroni correction), perhaps due to the differences in sample size (Fig 1A). Likewise, PAI-1-Ag was not different between stages of maturation ($df = 2$, $F = 4.58$, $P = .1$; Fig 1B) and no maturation-dependent differences were found for tPA-Ag.

Univariate Correlations Between Estimates of Adiposity and Metabolic and Fibrinolytic Parameters

The results of univariate correlation analyses for all children are shown in Table 2. Log leptin, log insulin, and C-peptide were correlated with the BMI and indices of fatness in children (all $P < .0001$). The association between log leptin and FM was of greater magnitude in girls ($r = .78$, $P < .0001$) compared with boys ($r = .52$, $P = .01$; Fig 2A). tPA-Ag was not associated with the BMI or FM; however, the association between tPA-Ag and %FM barely failed to reach statistical significance ($r = .27$, $P = .06$). PAI-1-Ag was correlated with the BMI, %FM, and FM ($P = .0003$; Fig 2B). PAI-1-Ag was also correlated with log insulin, C-peptide, and log leptin, but not after adjustment for adiposity. Log leptin was correlated with log insulin, but not after adjustment for adiposity. PAI-1-Ag was independently correlated with tPA-Ag after controlling for the BMI, FM ($r = .36$, $P = .016$), or %FM ($r = .34$, $P = .02$).

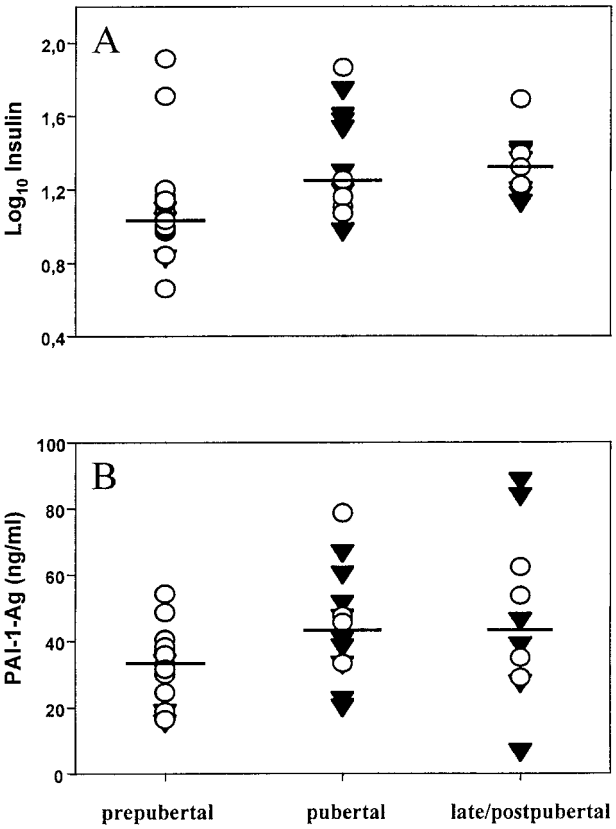

Fig 1. (A) Insulin and (B) PAI-1-Ag in obese children according to stage of maturation. $\log_{10}$ insulin levels were not significantly different between stages of maturation after post hoc Bonferroni correction (ANOVA, *df* = 2, F = 3.41, *P* = .043). Differences between stages of maturation for PAI-1-Ag were not significant (by Kruskal-Wallis test). (○) Boys; (▼) girls. The bar represents the median. Note that only 3 girls were included in the prepubertal group.

Table 2. Univariate Correlation Matrix Between Estimates of Adiposity and Humoral Parameters

	Body Mass	BMI	FM	%FM	WHR	Log_{10} Leptin	Log_{10} Insulin	C-Peptide	tPA-Ag	PAI-1 Ag
Age	.82‖	.51§	.67‖	.20	.01	.39‡	.45‡	.41‡	.13	.35†
Body mass	—	.85‖	—	—	—	—	—	—	—	—
BMI	—	—	—	—	—	—	—	—	—	—
FM	.93‖	.88‖	—	.78‖	—	—	—	—	—	—
%FM	.53§	.68‖	—	—	—	—	—	—	—	—
WHR*	.1	.04	.12	.21	—	—	—	—	—	—
Log_{10} leptin	.61‖	.70‖	.63‖	.54§	−.01	—	—	—	—	—
Log_{10} insulin	.72‖	.78‖	.69‖	.50§	.13	.54§	—	—	—	—
C-peptide	.60‖	.63‖	.63‖	.49§	.16	.47§	.74‖	—	—	—
tPA-Ag	.1	.02	.23	.27	.31	−.01	−.02	.06	—	—
PAI-1-Ag	.47‡	.45‡	.54§	.49‡	.14	.33†	.44‡	.37‡	.41‡	—

*Data for WHR were only available for 10 boys and 13 girls.
†$P < .05$.
‡$P < .01$.
§$P < .001$.
‖$P < .0001$.

When PAI-1-Ag was divided by tPA-Ag on a molar basis to enable a proxy measure of the balance between the fibrinolytic parameters, the resulting ratio was correlated with FM in all children ($r = .47$, $P = .002$; Fig 2C). No significant association was found between the WHR and any of the variables (Table 2).

Correlations in Prepubertal, Pubertal, and Late/Postpubertal Children

In prepubertal children, the BMI was a better correlate of PAI-1-Ag ($r = .45$, $P = .046$) than FM or %FM (both $P > .07$). In pubertal children only, PAI-1-Ag was significantly correlated with FM ($r = .50$, $P = .039$). No significant correlation between PAI-1-Ag and adiposity was found in the late/postpubertal group (all $P > .1$ by Spearman's rank test).

In the prepubertal group, PAI-1-Ag was not correlated with tPA-Ag after controlling for the BMI ($r = .38$, $P = .09$). In the prepubertal group and pubertal group, the ratio of PAI-1-Ag and tPA-Ag was not significantly correlated with the FM, %FM, or BMI (Fig 2C). Only in the late/postpubertal group was this ratio significantly correlated with FM ($r = .71$, $P = .02$) and %FM ($r = .74$, $P = .018$ by Spearman's rank test). Chronological age was included as a surrogate covariate for sexual maturation (data not shown). However, in prepubertal, pubertal, and late/postpubertal children, age had no significant influence on correlations with outcome variables, perhaps due to the increase in fatness with age.

Multiple Regression Analyses

Multiple regression models were performed on the basis of the bivariate correlations (Table 2). The independence of variables was further tested by stepwise regression (Table 3). When the age, gender, maturation, BMI, and log insulin were included in the regression model, only the BMI remained as the main determinant for the variation in log leptin (adjusted $R^2 = .38$, $P < .0001$). The BMI of the children also contributed to the variation in insulin (adjusted $R^2 = .51$, $P < .0001$). FM did not reach the level of significance when age, log leptin, log insulin, and tPA-Ag were included in the model. Only tPA-Ag barely failed to reach significance ($P = .07$). However, FM contributed significantly to the variation in PAI-1-Ag ($R^2 = .288$, $P = .0006$) and PAI-1-Ag contributed to the variation in tPA-Ag ($R^2 = .15$, $P = .0012$) (Table 3).

DISCUSSION

This study has investigated the associations between leptin and fibrinolytic parameters in obese children and adolescents. Our results suggest that any association between PAI-1-Ag and leptin depends mainly on estimates of adiposity, eg, the BMI and FM, in children.

Leptin and PAI-1-Ag were shown to be related to adipose tissue,[4,5,10-14] with subcutaneous adipose tissue being the main source of circulating leptin.[32-37] Body fat distribution has an impact on leptin, and the known gender difference in leptin levels is related to the subcutaneous fat depot and sex steroids.[38] The association between FM and leptin was of greater magnitude in obese girls in our study versus the obese boys, although the slope of the linear regression model was not different between boys and girls (Fig 2A). The WHR as a proxy measure of body fat distribution was not related to leptin, even after adjustment for gender and hip circumference (not shown). However, the lack of an association between the WHR and leptin could be due to the small number of available WHR data.

Leptin is associated with the visceral body fat compartment in boys, but not in girls.[38] Because visceral body fat is also a main determinant for PAI-1,[12-14] one would expect a linear increase in PAI-1 (and leptin) with FM in boys. A linear relationship for leptin and PAI-1-Ag with FM is suggestable for all children except the prepubertal group. Due to the fact that only 3 girls were included in that group, this finding is solely related to obese prepubertal boys. As FM accumulates and maturation proceeds, the data distribution might indicate that at a higher level of FM, PAI-1-Ag is not positively related to FM (Fig 2B). However, it is possible that this simply reflects an insufficient number of obese children studied in the median range of body FM.

For the PAI-1-Ag/tPA-Ag ratio, the best-fitting equation showed an exponential relationship with FM in all children. The increase in FM, due to maturation is associated with an increase in the PAI-1-Ag/tPA-Ag ratio (Fig 2C). Whether the evolution of this ratio reflects primarily the modification of PAI-1-Ag

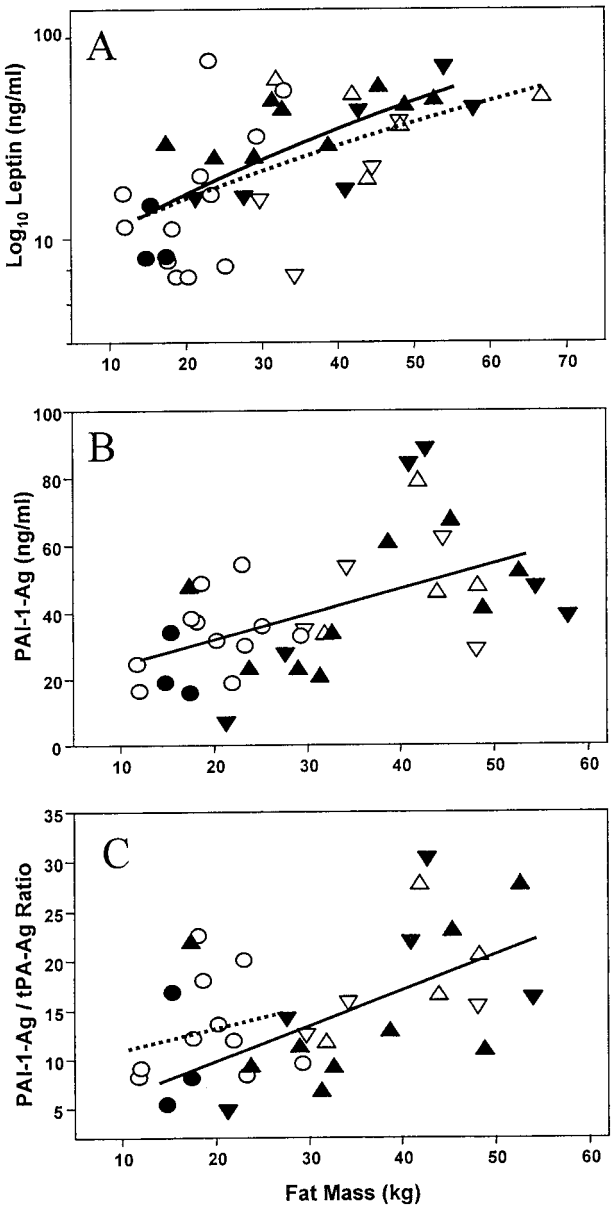

Fig 2. (A) Linear regression analysis between log_{10} leptin (ng/mL) and FM (kg) in all children (y = 0.899 + 0.013x, r = .63, $P < .0001$). Regression line: (···) boys; (—) girls. Note the logarithmic scale. (B) Linear regression analysis between PAI-1-Ag (ng/mL) and FM (kg) in all children (y = 15.67 + 0.78x, r = .54, P = .0003). The regression line is indicated. (C) Linear regression analysis between the molar ratio of PAI-1-Ag/tPA-Ag and FM in obese children for the prepubertal group separately (y = 9.42 + 0.17x, r = .16, P = .3) and the pubertal and late/postpubertal groups combined (y = 3.99 + 0.33x, r = .48, P = .013). Regression line: (···) prepubertal group; (—) pubertal and late/postpubertal groups. When all children were considered together, the best-fitting equation showed exponential relationship ($y = 7.92^{0.017x}$, r = .47, $P < .001$). All data are shown according to the stage of maturation and gender of the children. Prepubertal, (○) boys and (●) girls; pubertal, (△) boys and (▲) girls; late/postpubertal, (▽) boys and (▼) girls.

remains to be determined. However, this ratio was not significantly associated with the FM or BMI in prepubertal and pubertal children, but it was subject to great variability at this early stage.

With ongoing obesity and pubertal development, an unfavorable fibrinolytic risk profile might arise. This is further emphasized by the increase in C-peptide in obese children throughout maturation. However, log-transformed insulin was not significantly different between maturation stages after accounting for the different number of children in each group. Insulin and insulin sensitivity were shown to be involved in the induction of PAI-1[17] and fibrinolytic activity.[20] Basal and stimulated insulin levels increase with advancing puberty,[39] and recent findings in rats have shown a cause-effect relationship between the increased deposition of fat and the reduced ability to store glucose in skeletal muscle after puberty.[40] There is also increased leptin resistance in rats as they grow to maturity.[41] Combined, these findings suggest that insulin resistance, presumably reflected by higher levels of C-peptide, might be accompanied by leptin resistance, thus leading to higher PAI-1-Ag in pubertal and adolescent obesity.

However, after adjustment for adiposity, no independent association remained between metabolic and fibrinolytic parameters. This was further confirmed by multiple regression analysis, which found no main influence of leptin or insulin on PAI-1-Ag and vice versa. FM was the main predictor for the variation in PAI-1-Ag. However, after controlling for other variables in the multiple regression model, it is obvious that multicolinearity might exist and it is doubtful that the role of the FM is independent.

It was not unexpected that the BMI contributed to log leptin, although other known and independent determinants for leptin, eg, gender, age, maturation, or insulin, were also included in the regression model (Table 2). However, the adjusted R^2 values, for both leptin and PAI-1-Ag were not of great magnitude. Other parameters therefore also may have contributed to the variation in PAI-1-Ag and leptin. Although PAI-1-Ag contributed 14% to the variation in tPA-Ag, PAI-1-Ag cannot be viewed independently of adiposity and the measured variables. Both fibrinolytic parameters therefore seem to be under the influence of fatness and associated hormonal and metabolic parameters.

The BMI was also the main determinant for log insulin, and children with higher PAI-1-Ag were older, had a higher BMI and a larger FM, and exhibited higher leptin levels than children with lower PAI-1-Ag (not shown). Although log insulin did not increase significantly with the stage of puberty (Fig 1A), we may consider that maturity, due to its increase in FM, is associated with an altered insulin metabolism.

There are some limitations of the present study that should be mentioned. First of all, the study was cross-sectional and the number of children in each of the 3 subgroups was small. Furthermore, the number of boys and girls in the prepubertal group and pubertal group was not equal. Additionally, we did not control for these associations in normal-weight children. We therefore cannot rule out the possibility that an independent relationship between fibrinolytic parameters and metabolic

Table 3. Multiple Regression Analysis With Leptin, Insulin, PAI-1-Ag, and tPA-Ag as Dependent Variables

	Multiple Regression Model			Stepwise Regression Model		
Dependent Variable	Independent Variables	β	95% CI	Independent Variables	β	95% CI
Log_{10} leptin	Gender	0.09, $P = .26$	±0.15	BMI	0.04	±0.17
	Age	0.02, $P = .33$	±0.04	Intercept = 0.155		
	Maturation	−0.09, $P = .25$	±0.15	Adjusted $R^2 = .38$, $P < .0001$		
	BMI	0.04, $P = .001$	±0.02			
	Log_{10} insulin	−0.02, $P = .91$	±0.4			
Log_{10} insulin	Gender	0.06, $P = .29$	±0.12	BMI	0.04	±0.12
	Age	−0.002, $P = .88$	±0.03	Intercept = 0.08		
	Maturation	0.06, $P = .36$	±0.12	Adjusted $R^2 = .51$, $P < .0001$		
	BMI	0.03, $P = .001$	±0.19			
	Log_{10} leptin	−0.09, $P = .46$	±0.25			
	PAI-1-Ag	9×10^{-5}, $P = .27$	$\pm 1.6 \times 10^{-4}$			
PAI-1-Ag	Age	−4.1, $P = .87$	±50.8	FM	17.3	±9.3
	FM	13.4, $P = .13$	±17.5	Intercept = 269.1		
	Log_{10} leptin	8.0, $P = .97$	±539	Adjusted $R^2 = .288$, $P = .0006$		
	Log_{10} insulin	182.9, $P = .56$	±640			
	tPA-Ag	7.4, $P = .07$	±8			
tPA-Ag	PAI-1-Ag	0.016	±0.012	Intercept = 41		
				Adjusted $R^2 = .15$, $P = .0012$		

characteristics, eg, leptin and insulin, is already present during the dynamic phase of pubertal development and covered by the accumulation of certain adipose tissue depots.

In summary, we found that the adiposity of obese children is associated with PAI-1-Ag. Although an unfavorable fibrinolytic risk profile may already emanate from the pubertal stage, leptin did not contribute independently to the variation in PAI-1-Ag. Whether leptin and other factors in addition to FM may mediate metabolic and fibrinolytic processes in obesity during late adolescence remains to be elucidated.

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