# Infantile Obesity: A Situation of Atherothrombotic Risk?

Miguel Valle, Félix Gascón, Rosario Martos, F. Javier Ruz, Francisco Bermudo, Rafael Ríos, and Ramón Cañete

**Obesity is a major risk factor for cardiovascular disease frequently associated with hypertension, dyslipidemia, and diabetes. In recent years, alterations in the hemostatic system have been added to these dysfunctions. We analyzed some of these alterations in coagulation and fibrinolysis in obese children (6 to 9 years old) of both sexes. We studied 61 obese children (mean body mass index [BMI], 22.35 kg/m$^2$; 95% confidence interval [CI], 21.82 to 22.87) and 70 non-obese children (mean BMI, 16.58 kg/m$^2$; 95% CI, 16.24 to 16.93) as a control group. The obese subjects presented significantly elevated values for insulin ($P < .001$), tissue-plasminogen activator ([t-PA] $P < .001$), plasminogen activator inhibitor-1 ([PAI-1] $P < .001$), and fibrinogen ($P < .001$) with respect to the control group. We found no significant differences in the concentration of glucose and fragment 1 + 2 of prothrombin (F1 + 2). In the obese subjects, insulin, PAI-1, and F1 + 2 were positively correlated with the BMI. On the other hand, t-PA was correlated with insulin and PAI-1 but not with the BMI. Therefore, in the obese children, there was an increment of the risk factors for cardiovascular disease.**



OBESITY is an independent risk factor for the development of cardiovascular disease.[1,2] Recently, it has been reclassified by the American Heart Association as a major modifiable risk factor for coronary heart disease.[3] It is frequently associated with disorders like hypertension, diabetes, carbohydrate intolerance, hyperinsulinemia, and dyslipidemia, presenting a common situation of insulin resistance.[4,5]

In recent years, obesity has been associated with alterations in the hemostatic system characterized by an activation of clotting and inappropriate fibrinolysis.[6,7] In obese subjects, an increase of fibrinogen and plasminogen activator inhibitor-1 (PAI-1), among others, has been described, both factors associated with an increment of cardiovascular events.[8-10] The production of PAI-1 by adipose tissue,[11,12] possibly induced by insulin,[13,14] can contribute significantly to the elevation of plasma PAI-1 observed in the insulin resistance syndrome. This increment determines a decrease of the fibrinolytic activity in plasma,[15,16] which can have an important role in the development of cardiovascular illness in obesity, closing the relationship between obesity, insulin resistance, and cardiovascular disease. Another parameter indicative of the hypercoagulant state is fragment 1 + 2 of prothrombin (F1 + 2), which is of great use as a marker of prethrombotic states. This parameter has been less studied in obese subjects.

In obese children, these dysfunctions have not been sufficiently clarified. Thus, we have analyzed in obese children some aspects of coagulation and fibrinolysis that have been related to obesity and insulin resistance in the mature patient, considering that they constitute in themselves an important risk factor for cardiovascular disease. Also, we attempted to correlate these parameters with carbohydrate metabolism and anthropometric measures.

*From the Clinical Laboratory, Valle de los Pedroches Hospital, Pozoblanco, Córdoba; the Basic Health Zones of Palma del Río and Hinojosa del Duque, Córdoba; and the Pediatric Department, Reina Sofia Hospital, Córdoba, Spain.*

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*Address reprint requests to Miguel Valle, MD, Clinical Laboratory, Valle de los Pedroches Hospital, 14400 Pozoblanco (Córdoba), Spain.*



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## SUBJECTS AND METHODS

### *Subjects*

We performed a cross-sectional study of cases and controls in obese children of both sexes. For an error $\alpha$ of 5% and an error $\beta$ of 0.10 (to detect a minimum increase of 25% in the fasting insulin level), the minimum group size required for the sample was calculated as 60 children. We studied a total of 131 children aged between 6 and 9 years. All subjects were in Tanner stage 1.[17] Sixty-one were obese with a body mass index (BMI) above the 90th percentile, and 70 were non-obese as a group control (BMI <90th percentile). We used growth curves for our population.[18]

The study groups were formed with children from several schools. First, the corresponding pediatricians informed the different schools of the study to be performed. Simultaneously, written consent was requested from all parents of the possible participants. After this, we selected the children among those who accepted participation in the project. The selection was made in the pediatric health care clinic, and they were divided into groups according to the BMI. Children with primary hyperlipidemia, hypertension, diabetes, or carbohydrate intolerance were excluded from the study. Any child in pharmacological treatment was also excluded. All children had a similar life-style without a significant physical training program.[19]

### *Methods*

Blood samples were collected after a 12-hour fast from a vein in the antecubital fossa without venous occlusion. All collections were made between 8 and 9 AM. Whole-blood specimens were collected in different tubes to obtain serum and plasma. After clotting was complete in serum tubes, we centrifuged the samples and removed the serum for assay. Plasma was obtained by mixing 1 part sodium citrate (0.11 mol/L) with 9 parts whole blood and centrifuging to 3,000 × *g* for 15 minutes at 4°C immediately following blood extraction. The plasma was separated in aliquots and frozen immediately at −45°C until analysis. For PAI-1, we used plasma obtained in extraction tubes containing sodium citrate, citric acid, and inhibitors of platelet aggregation (Diatube H; Stago Diagnoses, Asnieres-sur-Seine, France).

Glucose and insulin levels were measured in serum, and the other parameters in plasma. Glucose was determined with a glucose oxidase method in a random-access analyzer (Axon; Bayer Diagnostics, Tarrytown, NY). Insulin was quantified by a microparticle immunoassay in an automatic analyzer (IMx; Abbott Laboratories, Chicago, IL).

We used antigenic immunoassay methods for the quantification of tissue-plasminogen activator ([t-PA] Coliza t-Pa; Chromogenix, Mölndal, Sweden), PAI-1 (Asserachrom PAI-1; Stago Diagnoses), and F1 + 2 (micro Enzygnost F1 + 2; Behring Diagnostics, Marburg, Germany) in a microtiter plate analyzer (Labotech; Cormédica, Barcelona, Spain). Fibrinogen was measured by a quantitative assay using thrombin in an

automatic analyzer (Electra 1600; Ortho Clinical Diagnostics, Madrid, Spain).

Tests were performed in the fewest possible series (depending on the commercial kits), with a coefficient of variation always less than 5% (intraassay) and 9.5% (interassay) for the different techniques.

### *Statistical Analysis*

Statistical assessment was made with Microstat (Ecosoft, Indianapolis, IN) or GraphPAD InStat (GraphPAD Software, San Diego, CA). The abnormal values (outliers) were excluded by Reed's method.[20] Results are expressed as the mean ± SEM with a 95% confidence interval (CI). The distribution of each variable was tested for departure from the gaussian distribution using the $\chi^2$ test for goodness-of-fit to normal distribution and the variance equality controlled by Snedecor's F test. The comparison between mean values for the groups was made by Student's unpaired *t* test. Statistical significance was set at a *P* level less than .05.

The correlation between variables was evaluated by Pearson's correlation coefficient and regression analysis. The multivariate regression analysis was performed using the stepwise method. It is important that the statistical procedure includes a regression check. We must verify that the distribution of residuals is normal and their variance is constant. In this study, the normality of residuals was checked by the $\chi^2$ test, and graphic analysis of residuals was performed to provide information about the constancy of variance. Diagnostic measures were used for the detection of problematic observations. An observation was considered an outlier if its residual was more than 3 standard error of estimate (standard deviation about the regression line) (Sy.x). For each variable, potential confounding factors ($.05 < P < .2$) were evaluated by analysis of raw and adjusted regression coefficients.

## RESULTS

For the control group and obese subjects, there were no significant differences in the age or sex ratio of each group (Table 1).

The mean values (obese *v* control) for insulin (95% CI, 7.19 to 9.21 *v* 5.38 to 6.58 μU/mL), t-PA (95% CI, 3.54 to 4.21 *v* 2.61 to 3.27 ng/mL), PAI-1 (95% CI, 29.67 to 46.35 *v* 15.38 to 25.64 ng/mL), and fibrinogen (95% CI, 2.72 to 3.01 *v* 2.31 to 2.55 g/L) were significantly higher in the obese group. There were no significant differences in the mean concentration of glucose (95% CI, 4.661 to 4.819 *v* 4.669 to 4.814 mmol/L) or F1 + 2 (95% CI, 0.52 to 0.70 *v* 0.50 to 0.67 nmol/L) (Table 2).

We studied the correlation between variables in the obese group with a univariate analysis (Table 3). However, the study with a technical multiple regression showed that for the insulin value ($r = .425$, $P = .005$), only the waist to hip ratio (WHR) was an independent predictive factor (partial $r = .289$, $P = .031$), while the coefficient of regression for the BMI, when corrected for the WHR, was not significant. Using this same statistical technique, both fibrinogen and the BMI were independent predictive factors for F1 + 2 ($r = .436$, $P = .005$). For t-PA ($r = .413$, $P = .038$), PAI-1 lost its statistical significance when corrected for the insulin value, while this was maintained as an independent predictive factor (*r* partial = .332, $P = .044$).

**Table 1. Descriptive Statistics for the Study Groups (mean ± SEM)**

| Variable | Control (n = 70) | Obese (n = 61) | *P* |
|---|---|---|---|
| Sex ratio (male/female) | 29/41 | 22/39 | NS |
| Age (yr) | 7.72 ± 0.10 | 7.69 ± 0.12 | NS |
| Weight (kg) | 27.01 ± 0.57 | 67 ± 0.80 | <.001 |
| Height (cm) | 127.18 ± 0.92 | 129.39 ± 0.84 | NS |
| BMI (kg/m²) | 16.58 ± 0.18 | 22.35 ± 0.27 | .001 |
| WHR | 0.84 ± 0.01 | 0.85 ± 0.01 | NS |
| WTR | 1.46 ± 0.01 | 1.43 ± 0.01 | NS |

Abbreviations: WTR, waist to thigh ratio; NS, not significant.

**Table 2. Mean Values for Glucose, Insulin, and Hemostatic Parameters (mean ± SEM)**

| Parameter | Control (n = 70) | Obese (n = 61) | *P* |
|---|---|---|---|
| Glucose (mmol/L) | 4.741 ± 0.037 | 4.740 ± 0.040 | .984 |
| Insulin (μU/mL) | 5.98 ± 0.31 | 8.20 ± 0.51 | <.001 |
| PAI-1 (ng/mL) | 20.51 ± 1.90 | 38.14 ± 3.01 | <.001 |
| t-PA (ng/mL) | 2.94 ± 0.19 | 3.88 ± 0.13 | <.001 |
| F1+2 (nmol/L) | 0.59 ± 0.04 | 0.61 ± 0.05 | .729 |
| Fibrinogen (g/L) | 2.43 ± 0.06 | 2.86 ± 0.07 | <.001 |

## DISCUSSION

In obese children aged 6 to 9 years, we found hemostatic dysfunctions similar to those described for adult obesity. These dysfunctions are considered risk factors for the development of atherothrombotic disorders.[8-10,15,16] Furthermore, we found a positive correlation for some of these parameters with insulin and anthropometric measures.

Obesity is one of the most frequent diseases in Western civilization. It is associated with an increase of morbidity-mortality and a reduction of life expectancy. Obese mature subjects have a higher frequency of hypertension, hyperinsulinism, diabetes, and dyslipidemia and a higher cardiovascular risk.[5,21,22] This situation of increased atherogenic risk was identified by Reaven[4] as the insulin resistance syndrome, or syndrome X. In recent years, hemostatic alterations have been added to these dysfunctions. These alterations may explain some aspects related to the high cardiovascular risk in obesity.

In obese children, although the higher frequency of hypertension and dyslipidemia has been described, other factors related to obesity in adults (especially related to the fibrinolytic system) have not been sufficiently described.[23] We studied children between 6 and 9 years old because the second increase in the development of adipose tissue appears at this stage of growth. This development seems to have great predictive value for the risk of obesity in adolescence and adulthood.[24]

PAI-1, the most important physiological inhibitor in endogenous fibrinolysis,[15,16] is increased in obese subjects.[6,25] This increase may be responsible for the progressive deterioration of fibrinolytic activity that occurs with the increment of weight.[26,27] We found elevated levels of PAI-1 in obese children and, as described in adults,[6,28-30] these levels were positively correlated

**Table 3. Significant Univariate Correlations Between the Different Variables in the Obese Group**

| Variables | Slope | Intercept | Sy.x | *r* | *P* |
|---|---|---|---|---|---|
| Insulin *v* BMI | 0.576 | −4.982 | 3.444 | .326 | .013 |
| Insulin *v* WHR | 28.01 | −15.73 | 3.414 | .349 | .008 |
| F1+2 *v* fibrinogen | 0.087 | 0.273 | 0.140 | .346 | .011 |
| F1+2 *v* BMI | 0.021 | 0.040 | 0.127 | .328 | .018 |
| t-PA *v* PAI-1 | 0.014 | 3.277 | 1.246 | .322 | .046 |
| t-PA *v* insulin | 0.101 | 2.904 | 1.086 | .378 | .019 |
| PAI-1 *v* BMI | 5.857 | −94.11 | 24.81 | .428 | .005 |

with the BMI. The production of PAI-1 for adipose tissue[11,12] and the elevated insulin levels[13,14] are possibly implicated in the origin of this increase of PAI-1. In our case, we found significantly elevated basal plasma levels of insulin in the obese group, positively correlated with the BMI and WHR. Nevertheless, only this last factor is independently predictive, since in the obese children studied herein, insulin is related more to the distribution of fat (WHR) than to the total fat (BMI). Although a positive correlation between PAI-1 and basal insulin has been described in adults,[6,28,29] we found no correlation between the parameters.

Besides insulin, other factors may be implicated in the plasma elevation of PAI-1 observed in the insulin resistance syndrome. In this way, although an in vitro increment has been found for PAI-1 production stimulated by insulin in endothelioid and hepatic cell culture,[31-33] in vivo, the acute elevation of insulin in plasma had no effect on the concentration of PAI-1 in humans.[34] The mechanism responsible for the plasma elevation of PAI-1 remains unexplained, although throughout this process, the adipose tissue seems to play an important role. In our case, we excluded an overload test with glucose due to the difficulty in obtaining voluntary groups of this age, and because a good correlation exists between basal insulin levels and insulin resistance parameters in children.

Inappropriate fibrinolysis has been correlated with an increment in the risk for coronary artery disease.[35,36] In the obese adult, elevated basal levels of PAI-1 and t-PA have been described.[25,37] However, after venous occlusion, the PAI-1 concentration remains high but the t-PA level (activator factor of the fibrinolysis) decreases in comparison to the non-obese.[25] The elevated basal level of t-PA could be explained by a feedback mechanism in answer to the increase of PAI-1 to maintain a minimal fibrinolytic activity. The formation of circulating t-PA/PAI-1 complexes would be responsible for the elevated basal concentration of t-PA and PAI-1, and it is not contradictory to a reduced fibrinolytic activity.[9,38,39] In the group of obese children studied here, we found values similar to those described in adults, with elevated basal concentrations of t-PA and PAI-1 in comparison to the non-obese. t-PA was correlated with the basal insulin concentration, and PAI-1 with the BMI. Considering the age of the groups, we decided not to perform determinations after venous occlusion.

In most patients with coronary artery disease, there is an increment in thrombin formation.[40,41] F1 + 2, a good marker of thrombin generation in plasma,[42] is considered a marker of thrombotic activity. A positive correlation between the fibrinogen concentration and F1 + 2 has been described.[43] We found no significant differences in the plasma levels of F1 + 2, although in the obese group, these values were slightly higher and were positively correlated, the same as PAI-1, with the BMI. F1 + 2 was also correlated positively with the fibrinogen concentration.

Fibrinogen is considered an independent marker for the development of cardiovascular disease,[8,44,45] and it was significantly higher in obese children. These elevated values are similar to those described in obese adults.

In conclusion, in obese children (Tanner stage 1), there is a risk for atherothrombotic events, with an increment of factors of cardiovascular risk such as elevated levels of insulin, PAI-1, and fibrinogen. Also, as others have described in adults,[37] we find a positive correlation between the BMI and prethrombotic factors like the PAI-1. Although further studies are necessary regarding the knowledge of the hemostatic dysfunctions associated with infantile obesity, the available data support preventive measures at an early age.

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