**Abstract:** The aim of the study was to investigate the Plasminogen activator inhibitor-1 (PAI-1) expression in obese children and to clarify its role with respect to left ventricular (LV) function.

**Patients and methods.** This study included 69 obese children and adolescents ad 40 lean healthy controls. Children were considered obese according to body mass index (BMI) percentile for age and sex curves of growth for our population. Each subject's fat distribution was assessed by measuring waist hip ratio (WHR). Obese children with peripheral fat distribution were excluded. Exclusion criteria included hypertension, endocrine, cardiovascular, renal, insulin dependent or independent diabetes mellitus and smoking habits. Laboratory investigations included measurement of plasma PAI-1 antigen. Determination of total serum cholesterol, low density lipoprotein, high density lipoprotein, triglycerides, blood glucose and fasting serum insulin. Echocardiograph study was obtained by two dimensionally guided M mode. **Results:** BMI and WHR were significantly higher in obese compared to lean children (P<0.001). In addition fasting blood glucose, fasting insulin, HOMA and Triglyceride were significantly higher in obese than in lean children. Left ventricular mass (LVM) and LVM/H were significantly higher in obese compared to controls (P<0.001) while left ventricular systolic (EF%, FS %) and diastolic function (E/A ratio, deceleration time) did not differ between the two groups P>0.05. Plasma PAI-1 were significantly higher in obese compared to controls P=0.03. A significant direct correlation was revealed between PAI-1 in comparison to WHR, fasting insulin and LVM/H. Plasma PAI-1 and WHR were independent predictors of LVM/H. **Conclusions:** Obese children with central fat distribution showed an increase in plasma PAI-1 antigen. Also PAI-1 contributes directly to the complication of obesity including type 2 diabetes and cardiovascular disease.

**Key words:** Childhood obesity-plasminagen activator inhibitor- Cardiovascular disease.

**INTRODUCTION**

Obesity is an independent risk factor for the development of cardiovascular thrombotic disease. (Larsson, 1991). The increased incidence of cardiovascular disease may be associated with elevated levels of coagulation factors (e.g. factor VII, fibrinogen) and plasminogen activator inhibitor-1 (PAI-1) in plasma, which have been observed in obese patients. (Yamamoto et al., 2005), (McGill et al., 1994). Plasminogen activator inhibitor-1 is the primary inhibitor of plasminogen activation in vivo, and increased PAI-1 in plasma compromises the normal fibrin clearance mechanisms promoting thrombosis (Yamamoto and Saito, 1998) which can taken the form of coronary artery disease.( Kohler and Grant, 2000).

In obese humans, increased plasma PAI-1 levels have been correlated with the amount of visceral fat, suggesting that adipose tissue is the primary source of PAI-1 in this condition. (Shimomura et al., 1996), (Morange et al., 1999).

Plasminogen activator inhibitor-1 expression in cultured adipocytes is strongly up regulated by glucocorticoids (Samad et al., 1996), insulin (Samad and Loskutoff, 1996), tumor necrosis factor - α (Konkle et
Overweight subjects without overt heart disease have subclinical changes in left ventricle (LV) structure and function even after adjustment for mean arterial pressure, age, gender and LV mass. (Turkbey et al., 2010). Nevertheless, few data are available on the relationship between obesity, haemostatic activity and left ventricular function. (Licata et al., 1995).

So the aim of this study was to investigate the PAI-1 expression in obese children and to clarify its role with respect to LV function.

Patients and methods:

Subject:

This study included 69 obese children and adolescents (27 male and 42 female) and 40 lean healthy controls (16 males and 24 females) were included in the study. Obese children were recruited from individuals attending the obesity clinic of the National Research Center (NRC). Lean controls were chosen from a group of subjects undergoing clinical evaluation and found to be healthy. Parental consent for all children was obtained according to the form approved by the Ethics committee of the NRC.

Children were considered obese according to body mass index (BMI) percentile for age and sex curves of growth for our population. (Egyptian growth curves, 2011). Cutoff values for obesity were a BMI above the 95th percentile for age and sex. Lean controls were selected on basis of BMI values below the 85th percentile for age and sex. Each subject's fat distribution was assessed by measuring waist hip ratio (WHR) in the standing position. (Licata et al., 1992), (Scaglione et al., 1992). Central fat distribution was defined on the basis of the sex-specific 85th percentile of WHR values. (Egyptian growth curves, 2011). In view of this, the cutoff value for central obesity was considered at least 0.81 for females and 0.92 for males. According to these criteria, obese children with peripheral fat distribution (WHR < 0.81 for female and < 0.92 for male) were excluded.

Systolic (SBP) and diastolic blood pressure (DBP) was measured with a standard clinical sphygmonanometer using a stethoscope placed over the brachial artery pulse. The cuff used was appropriate for the size of the child’s upper right arm. Exclusion criteria included hypertension, endocrine, cardiovascular, renal disease, insulin dependent or independent diabetes mellitus and smoking habits.

Preliminary investigations included determination of total serum cholesterol, Low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, blood glucose, and fasting serum insulin.

Laboratory Methods:

Ten ml of venous blood samples were drawn after 12-14 hours fasting. 5ml blood was drawn into a tube containing 0.1 ml sodium citrate and the remaining 5 ml blood was drawn in a plain tube. Blood was centrifuged at 3000 xg for 5min at room temperature. Citrated plasma and serum were separated and stored at -80°C until analysis. Repeated freezing and thawing cycles were avoided.

Plasminogen activator inhibitor-1 antigen was measured by available commercial enzyme linked immunosorbent assay (ELISA) method (Zymutest PAI, hyphen Biomed, Neuville Sur Oise, France, Lot number: 081030E).

Insulin was measured by Immulite 1000 automated analyzer by chemiluminescence.

Lipid profile (total cholesterol, triglycerides, HDL and LDL) and fasting blood sugar were measured by Olympus Au 400 clinical chemistry analyzer.

Echocardiography study:

Echocardiography study was performed with a vivid 3 expert Norway using 3 and 7 MHZ Transducers. All subjects were studied without sedation while they were lying quietly in the supine position. Chamber dimensions and wall thickness were obtained by two dimensionally guided M mode according to the recommendation of the American society of Echocardiography. (Sahn et al., 1978). Left ventricular mass (LVM) was calculated according to the Devereux method. (Deverux et al., 1986). LVM was also related to body height (LVM/H) using the recommendation that LVM should be indexed to height instead of body surface area for a more accurate evaluation of left ventricular hypertrophy. (Levy et al., 1988). Left ventricular systolic function was determined by measuring the ejection fraction (EF %) and fractional shortening (FS %). (Gutgesell et al., 1997). The LV diastolic functions were evaluated by measuring peak early diastolic filling velocity (E), peak late diastolic filling velocity (A), E/A ratio and deceleration time (DT). (Spirito et al., 1986).

Statistical methods:

Statistical package for social science (SPSS) program version 9 was used for analysis of data. Data were summarized as mean ± SD. Student's t-test for quantitative independent variables was used for analysis of difference between two groups. Pearson's bivariate correlation was used. Linear regression analysis was obtained to calculate correlation coefficients. In all test P<0.05 was considered statistically significant.
Results:

Lean and obese children were comparable with regard to age, systolic and diastolic blood pressure (P>0.05). BMI and WHR were significantly higher in obese compared to lean children (P<0.001). In addition fasting blood glucose, fasting insulin, HOMA index and Triglyceride were significantly higher in obese than in lean children (P<0.001). Table 1.

Table 1: Characteristics of obese and lean children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Obese (n=69)</th>
<th>Lean (n=40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>11.19 ± 3.62</td>
<td>11.45 ± 3.44</td>
<td>0.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.35 ± 5.4</td>
<td>18.83 ± 4.3</td>
<td>0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.88 ± 0.05</td>
<td>0.82 ± 0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>104 ± 7.12</td>
<td>105 ± 8.51</td>
<td>0.9</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>68.63 ± 9.41</td>
<td>69.67 ± 6.01</td>
<td>0.5</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>89.16 ± 12.36</td>
<td>79.87 ± 9.31</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting insulin (mcIu/ml)</td>
<td>6.98 ± 3.77</td>
<td>4.02 ± 2.13</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.37 ± 0.73</td>
<td>0.93 ± 0.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>170.83 ± 34.93</td>
<td>167.13 ± 20.04</td>
<td>0.5</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>107.82 ± 43.39</td>
<td>86.07 ± 18.78</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>41.5 ± 9.08</td>
<td>41.13 ± 6.12</td>
<td>0.8</td>
</tr>
<tr>
<td>LDL- cholesterol (mg/dl)</td>
<td>111.47 ± 29.7</td>
<td>105.87 ± 17.55</td>
<td>0.3</td>
</tr>
</tbody>
</table>

BMI: body mass index    WHR: waist hip ratio
SBP: Systolic blood pressure               DBP: diastolic blood pressure
HDL: high density lipoprotein   LDL: low density lipoprotein
FBG: fasting blood glucose
P-value <0.05 is significant

Measurements of left ventricular structure and function were demonstrated in Table 2. LVEdD, LVEsD, LVM and LVM/H were significantly higher in obese compared to non obese children P<0.001. While left ventricular systolic function (EF%, FS %) and diastolic function (E wave, A wave, E/A ratio and deceleration time) did not differ between the two groups P> 0.05.

Table 2: Measurements of left ventricular structure and function in obese and lean children.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Obese (n=69)</th>
<th>Lean (n=40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEdD (mm)</td>
<td>43.72 ± 8.06</td>
<td>39.56 ± 5.1</td>
<td>0.001</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>28.54 ± 5.7</td>
<td>24.73 ± 3.6</td>
<td>0.001</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>121.35 ± 56.03</td>
<td>77.34 ± 22.04</td>
<td>0.001</td>
</tr>
<tr>
<td>LVM/H (g/m)</td>
<td>77.52 ± 23.6</td>
<td>55.86 ± 13.8</td>
<td>0.001</td>
</tr>
<tr>
<td>EF%</td>
<td>67.99 ± 2.4</td>
<td>66.89 ± 3.9</td>
<td>0.07</td>
</tr>
<tr>
<td>FS%</td>
<td>34.79 ± 4.4</td>
<td>35.96 ± 3.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Mitral E wave (m/s)</td>
<td>0.95 ± 0.16</td>
<td>0.9 ± 0.12</td>
<td>0.1</td>
</tr>
<tr>
<td>Mitral A wave (m/s)</td>
<td>0.59 ± 0.12</td>
<td>0.61 ± 0.16</td>
<td>0.6</td>
</tr>
<tr>
<td>Mitral E/A</td>
<td>1.64 ± 0.32</td>
<td>1.63 ± 0.27</td>
<td>0.8</td>
</tr>
<tr>
<td>Deceleration time (ms)</td>
<td>189.43 ± 43.2</td>
<td>188.58 ± 38.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

LVEdD: left ventricular end diastolic dimension  
LVESD: left ventricular end systolic dimension  
LVM: left ventricular mass  
FS: fractional shortening  
EF: ejection fraction  
P-value <0.05 is significant

Plasma PAI-1 was significantly higher in obese compared to lean children (4.402 ± 3.85 vs. 2.723 ± 0.96 P= 0.03) Fig. 1.

A significant direct correlation was revealed between PAI-1 in comparison to WHR (r=0.319, P=0.01), fasting insulin (r=0.306, p=0.009) and LVM/H (r=0.339, P= 0.001) Fig. 2, 3.

Stepwise multiple regression analysis of LVM/H (dependable variable) versus predictor variables in obese children was demonstrated in Table 3. Plasma PAI-1 and WHR were independent predictors of LVM/H.

Discussion:

Obesity is an important risk factor for cardiovascular disease especially when there is a central fat distribution. (Licata et al., 1994).

This study was done on 69 children with central obesity. Several studies. (Licata et al., 1994), (Mutch et al., 2001) and (Taeye et al., 2005) indicated that obese subjects with central body fat distribution may be characterized by abnormalities in coagulation function and fibrinolytic activity. These included higher levels of factor VII antigen, fibrinogen, plasminogen, PAI-1 activity and basal tPA. In our study there was a significant increase of PAI-1 in obese compared to lean children (P< 0.001) and significant direct correlation between central obesity and PAI-1. Plasma PAI-1 has been a focus of study for 20 years, it is not clear where plasma...
PAI-1 is synthesized, and two sources were originally proposed, endothelium and the liver. Another potential source that has emerged is adipose tissue. (Kluft et al., 1988).

### Table 3: Stepwise multiple regression analysis of left ventricular mass/Height (LVM/H) (dependable variable) versus predictor variables in obese children.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Predictor variables</th>
<th>Regression coefficients (b)</th>
<th>Beta coefficients</th>
<th>Constant</th>
<th>Adjusted R²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVM/H</td>
<td>WHR</td>
<td>356.1</td>
<td>0.322</td>
<td>-47.52</td>
<td>0.211</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>PAI-1</td>
<td>445.1</td>
<td>0.285</td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

WHR: waist hip ratio  
PAI-1: Plasminogen activator inhibitor -1  
Predictor variable that did not enter the equation: serum insulin.  
P-value <0.05 is significant.

**Fig. 1:** Measurement of plasminogen activator inhibitor-1 (PAI-1) in obese and lean children.

**Fig. 2:** Correlation between waist hip ratio (WHR) and plasminogen activator inhibitor-1 in obese children and controls.

The range of plasma PAI-1 is rather variable even among healthy individuals and it shows distinct circadian variations. (Kruithof et al., 1988). Even against this variable background Erickson et al. (1999) found that elevation in plasma PAI-1 has been consistently observed in a range of diseases and in patients with features that predispose them to cardiovascular disease, including increased body mass, central fat distribution, raised blood pressure, increased plasma insulin level and advancing age.

Studies on human subjects by Cigolini et al. (1996) and Ferguson et al (1998) have revealed differences in PAI-1 release from different fat depots. A direct link has been shown between plasma PAI-1 and visceral fat area in obese and non-obese children and adults. Plasma PAI-1 secretion by human adipocytes has been reported...
to be more pronounced in visceral fat than in subcutaneous fat. A phenomenon also reported in animal models, analysis of rat adipose tissue suggested that both visceral and subcutaneous fat could synthesize PAI-1 but that the increased expression associated with obesity is related only to increases in visceral fat PAI-1 expression (Shimomura et al., 1996). An interesting study by kockx and his colleagues (1999) on obese males and females in which visceral fat was measured by magnetic resonance imaging showed that visceral fat was directly correlated with plasma PAI-1 antigen. Alessi et al. (1989) found that visceral and subcutaneous fat were comparable in their production of PAI-1 while Eriksson et al. (1989) found higher PAI-1 mRNA in subcutaneous fat compared with visceral fat and a higher rate of synthesis of PAI-1 antigen.

![Fig. 3: correlation between left ventricular mass/ height (LVM/H) and plasminogen activator inhibitor-1 in obese children and controls.](image)

In the current study there was a significant increase in fasting serum insulin \( P > 0.001 \) and direct correlation between PAI-1 and serum insulin. Insulin has been shown to be correlated with PAI-1 in several studies Kooistra et al. (1989), Anfosso et al. (1993), Nordt et al. (1995) and indeed relationships between PAI-1 and BMI, triglyceride level and blood pressure are regarded as being secondary to this relationship. Efforts to explain this relationship by its up regulation of PAI-1 in cultured cells, including endothelial cells and hepatocytes have shown inconsistent results. This inconsistency may reflect effects of insulin on both uptake of glucose and gene expression by using insulin resistant cells, the up regulation is observed.(Samad et al., 2000) Administration of exogenous insulin to rabbits, mice.(Nordt et al., 1995) and human subjects (Carmassi et al., 1999) resulted in elevated plasma PAI-1 concentration.

D’Agostino et al. (2004) reported that insulin resistance is almost universally present in obese individuals and is widely recognized to promote the development of vascular inflammation and thrombosis. While Festa et al. (2002) found that elevated PAI-1 levels are associated with insulin resistance irrespective of obesity.

In our study despite insulin levels were higher in obese than in lean children no evidence indicated independent effect of fasting insulin on the change in PAI-1 activity. This is agreed with Licata et al. (1995) who reported the same results.

In the present study there was a significant increase in LVM and LVM/ H (i.e. LV hypertrophy) in obese compared to lean children while the LVEF% and FS% was normal and by linear regression analysis the PAI-1 and WHR were independent predictor of LVM/H. (Mean of age of our patients was 11.19 ± 3.6). Study by Licata et al. (1995) on obese subjects younger than 40 years found that LVM and LVEF were significantly affected and this may support a relationship between coagulation activity and silent left ventricular dysfunction. These data may be of interest since general epidemiological studies suggest that high levels of fibrinogen, factor VII and PAI-1 may be causal significance in the development of ischemic heart disease. (Levy et al., 1988) Results obtained by Sobel et al. (2006) indicate that the content of PAI-1 in the heart increases with age. Plasminogen activator inhibitor-1 appears to predispose to fibrosis by inhibiting degradation of extravascular fibrin that serves as a scaffold for deposition of collagen. (Sobel et al., 1989) Cardiac fibrosis has been implicated as a determinant of negative ventricular remodeling after myocardial infarction and congestive heart failure particularly heart failure with preserved systolic function. (Fogo, 2003) , (Zaman et al., 2004).

As PAI-1 appears to be the common thread in the pathology of obesity, diabetes and cardiovascular disease, treatment options ameliorating both metabolic changes associated with insulin resistance syndrome and
decreasing PAI-1 levels might decrease prothrombotic and proinflammatory states. (Festa et al., 2002). A study by Taeye et al. (2005) found that inhibition of the rennin angiotensin system by ACE inhibitors in insulin resistant obese humans reduce PAI-1 levels. Given the important role that PAI-1 appears to play in the cardiovascular consequences of obesity as well as in the development of diabetes and obesity itself, PAI-1 can be identified as an attractive target for direct inhibition.

In conclusion, obese children with central fat distribution showed an increase in plasma PAI-1 antigen. Plasminogen activator inhibitor-1 contributes directly to the complications of obesity including type 2 diabetes and cardiovascular disease including silent left ventricular dysfunction.

The study recommended that future research will expand our understanding of the mechanisms of adipogenesis and elucidate the molecular pathology of obesity. Such studies will almost certainly identify potential therapeutic targets for the treatment of obesity; PAI-1 is one promising target with direct antagonists currently in development.

REFERENCES


