Plasminogen Activator Inhibitor-1, Body Fat and Insulin Action in Aging Women

Shawna McMillin and Alice S. Ryan*
Department of Medicine, University of Maryland School of Medicine, USA

Abstract
Plasminogen activator inhibitor-1 (PAI-1) over-expression is linked to obesity, insulin resistance, and age. We hypothesized that aerobically trained women athletes would have reduced PAI-1 regardless of age compared to sedentary controls and levels would be associated with hyperinsulinemia. Plasma PAI-1 was measured in women athletes who were young (YA, n=19, VO2max=53.7±1.1ml/kg/min) and older (OA, n=18, VO2max=46.6±1.5ml/kg/min) and compared to 19 sedentary controls (YC, n=6, VO2max=35.9±1.2ml/kg/min; OC, n=13, VO2max=22.1±1.7ml/kg/min). PAI-1 levels did not differ between YA and OA but was 23% higher in OC compared to OA (P<0.05). PAI-1 was inversely associated with VO2max, directly to %body fat, and subcutaneous abdominal fat, fasting leptin, insulin, and first-phase and second-phase insulin response during a hyperglycemic clamp. The current results suggest that older athletes have low PAI-1 levels possibly due to high levels of physical fitness, reduced body fat, and increased insulin action and may contribute to low atherothrombosis and improved cardiovascular health.

INTRODUCTION
Human adipose tissue secretes plasma plasminogen activator inhibitor-1 (PAI-1), an inhibitor of both urokinase type plasminogen activator and tissue-type plasminogen activator. PAI-1 production increases with obesity and may be similar [1] or higher [2] in visceral than subcutaneous abdominal adipose tissue. PAI-1 is emerging as an independent risk factor in the development of cardiovascular disease and insulin resistance [3]. Moreover, PAI-1 levels have been associated with cardiovascular morbidity and mortality in both men and women in prospective studies [4]. An impaired fibrolytic activity is considered by some to be one of the components of the metabolic syndrome [3]. Physical activity may influence PAI-1 levels as one study [5] demonstrated lower PAI-1 levels in males participating irregular sporting activities than age-matched inactive individuals, elderly athletes, and post-MI patients.

We have previously shown that highly trained women athletes did not have an increase in body fat typically associated with aging and a sedentary lifestyle [6]. In addition, insulin sensitivity was preserved among women athletes as a function of age [7]. We hypothesized that aerobically trained athletes would have reduced PAI-1 levels regardless of age compared to sedentary controls and that levels would be associated with hyperinsulinemia. The purpose of the present study was to determine PAI-1 levels in a subset of this unique group of highly trained insulin sensitive women athletes [6,7], compare them to healthy insulin resistant controls, and to investigate the relationships between PAI-1 levels and total and central adiposity and glucose metabolism.

MATERIALS AND METHODS
Subjects
Fifty-six women (37 athletes and 19 controls) between the ages of 18-69 years were recruited for participation in the study. The athletes in this investigation are from a cohort that had already been studied in our laboratory before this study. Women were grouped by athletic status and age (young athlete, YA; young control, YC; older athlete, OA; older control, OC). Athletes were swimmers, runners, and triathletes who were training for collegiate, local, and national competitions. The control volunteers were healthy sedentary women who had not participated in a regular exercise program for a minimum of six months prior to the study. All women were weight stable (no weight change of >2 kg for the previous 2 wk). Other athletes who swam averaged 14,000 yards/wk at an intensity of ~1:20 min/100 yards. All runners averaged 27-30 miles/wk at an average 7-8 min/mile pace. Some of these athletes also cycled at ~18 mph for ~65-95 miles/wk. The control volunteers were healthy sedentary women who had not participated in a regular exercise program for a minimum of six months prior to the study. All women were weight stable (no weight change of >2 kg for the previous 2 wk). Subjects were screened by medical history, physical examination, fasting blood profile, 2 hr oral glucose tolerance test, and a graded exercise treadmill test. All subjects...
were non-smokers, free of diabetes [8] and cardiovascular disease (by history and physical exam as well as treadmill stress test), and were not on any medications known to influence glucose metabolism. All methods and procedures were approved by the Institutional Review Board of the University of Maryland. All subjects provided written informed consent.

**Body composition**

Fat mass, lean tissue mass and bone mineral content (BMC) were determined by dual-energy X-ray absorptiometry (DXA) (Model DPX-L or Prodigy LUNAR Radiation Corp., Madison, WI). Fat-free mass (FFM) was calculated as lean tissue plus BMC. Computed tomography (CT) scanning of the abdomen was performed using a GE High Speed Advantage 9800 Scanner to quantify visceral and abdominal subcutaneous fat, as previously described [7,9].

**Maximal oxygen consumption (VO\textsubscript{2max})**

VO\textsubscript{2max} was measured during a progressive treadmill test to subjective exhaustion [6]. To qualify for the study, athletes had to reach a VO\textsubscript{2max} of ≥ 50 and 40 ml · kg·min\textsuperscript{-1} for ages ≤39 and ≥ 50 years, respectively. Swimmers were allowed a 5 ml· kg·min\textsuperscript{-1} difference in these criteria because VO\textsubscript{2max} is underestimated when they are not tested swimming. All controls had to have a VO\textsubscript{2max} < 40 ml · kg·min\textsuperscript{-1}.

**Hyperglycemic/hyperinsulinemic-euglycemic clamps**

All subjects were weight and activity stabilized prior to testing. A newly developed three-step clamp was performed in volunteers as previously published [7]. Six older controls did not undergo the glucose clamp. In athletes, clamps were performed 24-36 hours after the last exercise bout whereas the controls had no prior exercise bout. In brief, the clamp consisted of 3 steps: 1) a hyperglycemic clamp for one hour (+5.4 mmol/l above basal) followed by 2) an hour of glycemic recovery to basal glucose level and immediately followed by 3) a hyperinsulinemic-euglycemic clamp (240 pmol m\textsuperscript{-min}) for two hours and a half hour recovery. Previously published results are presented in this manuscript and include first (0-10 min) and second (10-120 min) phase insulin response and glucose utilization during the last hour of the euglycemic clamp [7].

**Homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR)** was calculated [(fasting insulin (µU/ml) x fasting glucose [mmol/l])/22.5 as described by Matthews [10].

**Analysis of blood samples**

Blood samples were collected in heparinized syringes. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Immunoactive insulin and leptin was determined by RIA (DiaPharma Group, Inc., West Chester, OH). All samples were run in duplicate with CV<10%.

**Statistical analyses**

All data were analyzed using the Statistical Analysis System (SPSS). Standard methods were used to compute means, standard errors of the mean and Pearson correlation coefficients. Comparisons between groups were tested with independent t-tests. Data are expressed as mean ± standard error of the mean (SEM) and P values below 0.05 were regarded as statistically significant.

## RESULTS

### Physical and metabolic characteristics

Subject characteristics are presented in (Table 1). Although age group comparisons between athletes and controls for VO\textsubscript{2max} and body composition, and some of these metabolic outcomes have been previously published, the addition of new OC leads us to report these comparisons here. VO\textsubscript{2max} was 33% higher in the YA than YC and 53% higher in the OA than OC (P<0.01). Body fat was 27% lower in YAs and OA vs. OC (P<0.01). HOMA-IR was not different between YA and YC (1.35 ± 0.09 vs. 1.56 ± 0.22) but was significantly lower in OA compared to OC (1.06 ± 0.08 vs. 2.21 ± 0.24, P<0.001).

Comparison of plasma PAI-1 activity levels did not differ between YA and OA but were higher in YC than YA and OC (P<0.01) (Figure 1). PAI-1 levels were 23% higher in OC compared to OA (P<0.01).

### Table 1: Physical and Metabolic Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Young Athletes (n=19)</th>
<th>Older Athletes (n = 18)</th>
<th>Young Controls (n = 6)</th>
<th>Older Controls (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>24.9±1.7</td>
<td>51.3±2.0</td>
<td>21.8±1.0</td>
<td>56.2±3.0‡</td>
</tr>
<tr>
<td><strong>BMI (kg/m\textsuperscript{2})</strong></td>
<td>20.9±0.4</td>
<td>20.6±0.4</td>
<td>20.8±1.1</td>
<td>26.9±1.2†</td>
</tr>
<tr>
<td><strong>VO\textsubscript{2max} (ml/kg/min)</strong></td>
<td>53.7±1.1</td>
<td>46.6±1.5</td>
<td>36.0±1.2‡</td>
<td>22.1±1.7†</td>
</tr>
<tr>
<td><strong>Percent Body Fat</strong></td>
<td>19.4±1.2</td>
<td>22.6±1.6</td>
<td>28.0±2.9†</td>
<td>41.2±2.1†</td>
</tr>
<tr>
<td><strong>Fat-Free Mass (kg)</strong></td>
<td>45.7±1.0</td>
<td>43.5±1.4</td>
<td>37.5±1.7†</td>
<td>39.2±1.5</td>
</tr>
<tr>
<td><strong>Visceral Adipose Tissue Area (cm\textsuperscript{2})</strong></td>
<td>24.6±1.9</td>
<td>51.8±6.0</td>
<td>39.9±9.1†</td>
<td>105.3±7.2‡</td>
</tr>
<tr>
<td><strong>Subcutaneous Abdominal Adipose Tissue Area (cm\textsuperscript{2})</strong></td>
<td>99.9±9.9</td>
<td>124.8±17.5</td>
<td>159.4±35.2</td>
<td>208.3±26.8†</td>
</tr>
<tr>
<td><strong>Fasting Leptin (mmol/l)</strong></td>
<td>4.4±0.7</td>
<td>3.7±0.5</td>
<td>10.7±2.0</td>
<td>13.0±2.2†</td>
</tr>
<tr>
<td><strong>Fasting Insulin (pmol/l)</strong></td>
<td>40±2</td>
<td>32±2</td>
<td>46±5</td>
<td>50±4‡</td>
</tr>
<tr>
<td><strong>PAI-1 activity (ng/mL)</strong></td>
<td>1.65±0.04</td>
<td>1.60±0.03</td>
<td>1.81±0.08†</td>
<td>2.09±0.17†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Significantly different YA vs. YC and OA vs. OC: †P<0.01, ‡P<0.001.
Figure 1 Plasma PAI-1 activity levels do not differ among young athletes (YA, n=19) and older athletes (OA, n=18); however, older athletes have significantly lower PAI-1 activity compared to older sedentary controls (OC, n=13; young controls: YC, n=6,† P< 0.05).

Figure 2 A) Relationship of maximal oxygen consumption (VO2max) to plasma PAI-1 levels (r = -0.71, P<0.001). B) Relationship of percent body fat to plasma PAI-1 activity levels (r = 0.66, P<0.001).

Relationships of PAI-1 to body fat and insulin action

In the total group, PAI-1 activity levels was inversely associated with VO2max, r = -0.71, P<0.001, (Figure 2A) and directly associated with percent body fat r = 0.66, P<0.001, (Figure 2B), and subcutaneous abdominal fat (r = 0.66, P<0.01), but not with visceral fat. PAI-1 levels were associated with fasting leptin (r = 0.75, P < 0.001), fasting insulin (r= 0.69, P<0.001), and first-phase (0-10 min) (r= 0.61, P<0.01) and second-phase insulin response (10-60 min) (r= 0.55, P<0.05) from the hyperglycemic clamp but not with glucose utilization determined during the hyperinsulinemic-euglycemic clamp. PAI-1 levels were associated with insulin resistance by HOMA-IR (r=0.54, P<0.001).

DISCUSSION

The present study was designed to evaluate the influence of very high levels of physical activity on fibrinolytic function in women across the age span. Our findings are consistent with our hypothesis that highly trained athletes have lower levels of PAI-1 than sedentary women. PAI-1 levels were not different in
older versus younger athletes suggesting that endurance training contributes to low atherothrombosis and may offset the negative effects of aging. Finally, we show that PAI-1 levels are associated with obesity and insulin resistance.

Fitness or VO_{max} is an important determinant of PAI-1 levels. Physically active men [11] and women [12,13] have lower PAI-1 activity than young adults who were inactive. This is consistent with studies in athletes where lower PAI-1 activity were observed in male athletes compared to sedentary men [5]. Our results in older women athletes suggest that high levels of endurance training reduce PAI-1 levels compared to sedentary women and that this training likely attenuates or diminish the effects of aging on the coagulation system.

Elevated levels of PAI-1 predict the development of type 2 diabetes, independent of numerous factors, namely age, sex, BMI, and physical activity [14,15]. Previous studies have shown that plasma PAI-1 is associated with plasma insulin [12-14] and insulin resistance by HOMA in premenopausal obese women [16]. We also found these associations with fasting insulin and HOMA-IR in lean athletes and sedentary women. Our data add to the literature that PAI-1 was associated with insulin sensitivity by first and second phase insulin response during a hyperglycemic clamp. Although we cannot say that these associations would hold at the tissue level, other studies indicate that abdominal PAI mRNA levels are associated with HOMA [17] and glucose utilization [18]. Potential mediators in these associations could include inflammatory markers [19,20] as deletion of TNF receptors in animal models results in a direct reduction in plasma PAI-1 levels and obesity [21]. Further evidence is necessary to elucidate the role of cytokine secretion in PAI-1 levels in physically active and obese women.

Ageing elevates the expression of PAI-1, thus contributing to an increased risk for a thrombotic event [22]. Plasma PAI-1 levels were similar among younger and older women athletes suggesting that increased fitness levels through competitive training and performance benefits the coagulation system and may offset any deleterious effects of age. It remains to be elucidated whether older lean sedentary women can reduce PAI-1 levels to that of older athletes through an exercise program or if increased physical activity reduced the role of local tissue secretion of PAI-1. We also found that PAI-1 levels were associated with total body fat and subcutaneous abdominal fat but not with visceral fat in these women. Results are inconclusive as to whether PAI-1 is related to visceral fat and these relationships may be dependent on gender or level of obesity. Plasma PAI-1 levels were associated with visceral fat in young men [23,24] and young non-obese women [24] but not obese premenopausal women [23]. Because visceral fat stores were so low in the athletes, this depot may not be as important as the subcutaneous fat in levels of PAI-1. Additionally other adipose cell sources such as liver, muscle, and ectopic fat deposits may contribute to plasma PAI-1 levels. Further studies in women athletes as they age would provide longitudinal evidence of maintaining high fitness levels.

CONCLUSION

We demonstrated that PAI-1 levels are associated with obesity and insulin resistance which may be prevented with high levels of physical activity in young and old women. Endurance training may be advantageous throughout a women’s life span to counteract the negative effects of aging on fibrinolytic activity.

ACKNOWLEDGEMENTS

Our appreciation is extended to the participants in the study. We also thank Drs. Dariush Elahi and Barbara Nicklas. This study was supported by funds from: the Baltimore VA Medical Research Service, VA Research Career Scientist Award, VA Merit Award and Baltimore VA GRECC, NIH grants R01-AG19310, R01-AG 030075, P30 AG028747, P30 DK072488.

REFERENCES


