Metabolic Factors, Adipose Tissue, and Plasminogen Activator Inhibitor-1 Levels in Type 2 Diabetes
Findings From the Look AHEAD Study

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Objective—Plasminogen activator inhibitor-1 (PAI-1) production by adipose tissue is increased in obesity, and its circulating levels are high in type 2 diabetes. PAI-1 increases cardiovascular risk by favoring clot stability, interfering with vascular remodeling, or both. We investigated in obese diabetic persons whether an intensive lifestyle intervention for weight loss (ILI) would decrease PAI-1 levels independently of weight loss and whether PAI-1 reduction would be associated with changes in fibrinogen, an acute phase reactant, or fibrin fragment D-dimer (D-dimer), a marker of ambient coagulation balance.

Methods and Results—We examined 1-year changes in PAI-1, D-dimer, and fibrinogen levels; adiposity; fitness; glucose; and lipid control with ILI in 1817 participants from Look AHEAD, a randomized trial investigating the effects of ILI, compared with usual care, on cardiovascular events in overweight or obese diabetic persons. Median PAI-1 levels decreased 29% with ILI and 2.5% with usual care (P<0.0001). Improvements in fitness, glucose control, and high-density lipoprotein cholesterol were associated with decreased PAI-1, independently of weight loss (P=0.03 for fitness, P<0.0001 for others). Fibrinogen and D-dimer remained unchanged.

Conclusion—Reductions in PAI-1 levels with ILI in obese diabetic individuals may reflect an improvement in adipose tissue health that could affect cardiovascular risk without changing fibrinogen or D-dimer levels.

Clinical Trial Registration—URL: http://clinicaltrials.gov/ct2/show/NCT00017953. Unique identifier: NCT00017953.

Key Words: diabetes mellitus ■ fibrinolysis ■ obesity ■ adipokines ■ fitness

Circulating plasminogen activator inhibitor-1 (PAI-1) levels are predictive of incident cardiovascular disease (CVD) in the general population.1 It is reasonable to expect elevation of PAI-1 to contribute to increased CVD risk in persons with type 2 diabetes (T2DM).2 Several mechanisms may explain the association of PAI-1 with CVD. PAI-1 favors intravascular fibrin deposition and promotes clot stability by inhibiting plasmin production from its inactive precursor, plasminogen.3 PAI-1 may also increase cardiovascular risk by inhibiting fibrinolysis in the vessel wall, interfering with vascular remodeling and promoting the development of an unstable plaque phenotype.4 In addition, PAI-1 is considered an acute phase protein5 and could, as an inflammatory mediator, increase CVD risk. Given the association of PAI-1 with CVD, we anticipate that an intervention that reduces PAI-1 could yield a benefit through 1 or several of these pathways.

PAI-1 is synthesized in multiple tissues, and its regulation is complex and incompletely understood. The secretion of PAI-1 by adipose tissue is increased in obese subjects because of an increase in adipose tissue mass6,7 and because of the activation of a proinflammatory phenotype within the adipose tissue microenvironment.8,9 In T2DM, not only increased adipose tissue mass but other metabolic disturbances, including hyperinsulinemia, hyperglycemia, and dyslipidemia, alter adipose tissue function and lead to increased production and circulating levels of PAI-1.10,11 Reductions in PAI-1 levels have been observed in obese nondiabetic individuals with weight loss,12,13 but the effects of weight loss in persons with T2DM and the independent contribution...
of changes in fitness and of improved glucose and lipid control on PAI-1 levels have not been evaluated in the setting of a clinical trial. The overall aim of this study was to investigate whether an intensive lifestyle intervention for weight loss (ILI) would, compared with usual care, decrease PAI-1 levels in obese persons with T2DM and whether an improvement in fitness and in metabolic factors known to affect adipose tissue function could contribute, independently of weight change, to the reduction in PAI-1 levels. Furthermore, to improve our understanding of the implications of PAI-1 reduction with ILI on cardiovascular risk in diabetic individuals and given the substantial epidemiological evidence supporting the association of fibrinogen and d-dimer with CVD (upper versus lower tertile risk of 1.8 for both), we also investigated whether the changes in PAI-1 with ILI were associated with changes in fibrinogen, an established acute phase reactant, changes in fibrin fragment d-dimer (d-dimer), a marker of ambient coagulation balance, or both. We hypothesized that despite the advanced degree of obesity and the metabolic disturbances commonly seen in T2DM, ILI would decrease PAI-1 levels to a greater extent than usual care. We also hypothesized that an improvement in metabolic factors and in fitness with ILI would, independently of adiposity changes, decrease PAI-1 levels. Our third and final hypothesis was that given that PAI-1 is a mild acute phase reactant and a major regulator of fibrinolysis, the reduction in PAI-1 levels with ILI would be associated with decreases in fibrinogen and d-dimer levels.

Research Design and Methods

Study Design

We evaluated 1817 individuals, generally corresponding to the first half of Look AHEAD (Action for Health in Diabetes) participants from 15 of 16 clinic sites, who had PAI-1 and fitness data at baseline and 1 year. Look AHEAD is a randomized clinical trial designed to examine whether a behavioral lifestyle intervention for weight loss will reduce cardiovascular events and overall mortality in overweight/obese subjects with T2DM.

The Look AHEAD study design, subject characteristics, and lifestyle intervention components have been described. Briefly, subjects were randomized to ILI, aiming for a 7% weight loss from baseline, or to a diabetes support and education (DSE) arm, which served as the control. ILI participants attended 3 group sessions and 1 individual encounter per month during the first 6 months of the study, followed by 2 group sessions and 1 individual appointment per month thereafter, supporting behavioral change to increase physical activity to 175 weekly minutes of moderate-intensity exercise, reduce caloric and saturated fat intake, and change macronutrient composition to improve glycemic control. The activity program relied on at-home exercise, which for most participants consisted of brisk walking. The energy intake goal was 1200 to 1500 kcal/day for persons <114 kg and 1500 to 1800 kcal/day for those ≥114 kg. Liquid meal replacement for 2 daily meals was encouraged during the first 6 months to help with portion control. Subjects were asked to keep food and physical activity diaries, counting only bouts of ≥10-minute duration for the activity goal. DSE participants received 3 group health information sessions during the year. All participants continued care with their primary providers. The institutional review boards of the participating centers approved Look AHEAD and this ancillary study.

Laboratory, Anthropometric, and Fitness Determinations

PAI-1, d-dimer, and fibrinogen were measured in the University of Vermont Laboratory for Clinical Biochemistry Research as described. Briefly, PAI-1 was measured in duplicate in platelet-free plasma by ELISA (Asserachrom No. 00249, Stago, Parsippany, NJ). This assay is sensitive to all plasma forms of PAI-1 (average interassay coefficient of variation was 8.9% over 8 different controls). D-Dimer was measured by the STAR automated coagulation analyzer (Stago) using an immunoturbidometric assay (Liatest D-DI) with 2 anti-human monoclonal antibodies specific to d-dimer and 4 controls (average interassay coefficients of variation for mean values of 2.18 and 0.24 μg/mL were 6.3% and 12.3%, respectively, and estimated at 23% for the 25th percentile [0.18 μg/mL]). Fibrinogen was quantified, by the STAR automated coagulation analyzer, using a clot-rate method (Stago; average interassay coefficient of variation was 5.9% over 10 different controls).

Determination of fitness using submaximal effort on a graded exercise stress test in metabolic equivalents and procedures for obtaining anthropometric measures, hemoglobin A1c (HbA1c), glucose, and lipids in Look AHEAD have been described.

Statistical Analysis

Descriptive statistics, including median and interquartile range (IQR), were determined for PAI-1, d-dimer, and fibrinogen levels at baseline and for their 1-year changes from baseline. Differences between the ILI and DSE arms in variable 1-year changes were evaluated with the 2-sample t test or the Wilcoxon rank sum test. Bivariate associations of 1-year changes were evaluated with the Spearman correlation coefficients, adjusting for age and gender with partial correlation analyses, and tested for trend across quartiles of change by treatment arm.

In the multivariable regression analysis, log transformation was applied to PAI-1 to correct for its nonnormal distribution, and the difference between baseline and 1-year log-transformed PAI-1 values was calculated and treated as the outcome variable. Models were fitted to examine the effects of changes in metabolic variables of interest on PAI-1 change. Variables shown not to be significantly different between ILI and DSE in their 1-year changes were excluded. Changes in metabolic variables and in fitness were entered into separate regression models to evaluate their contribution to PAI-1 change, either alone or in combination, after adjusting for baseline PAI-1 level, demographics, clinic site, CVD history, diabetes duration, current smoking, and treatment with statins and thiazolidinediones. A dichotomous indicator for treatment group (ILI versus DSE) was included in all models to examine the significance of the treatment effect. Multicollinearity between related metabolic variables was excluded using Spearman correlation coefficients before inclusion in the regression models (all <0.4). Type I error rate was fixed at 0.05 for all analyses. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Baseline Characteristics

Participants were middle-aged, obese, and sedentary, with mean fitness values below the 20th percentile for their age (Table 1). PAI-1 levels (median [IQR]) were elevated at 45.42 ng/mL (25.26, 75.46 ng/mL; range reference: 4 to 43 ng/mL). Median (IQR) fibrinogen levels were in the high normal range at 376.5 mg/dL (330, 431 mg/dL; reference range: 203 to 404 mg/dL) and d-dimer median (IQR) levels were normal at 0.26 μg/mL (0.17, 0.39 μg/mL; reference range: 0.06 to 0.77 μg/mL). The geometric means for PAI-1 and d-dimer levels were 42.46 ng/mL and 0.27 μg/mL, respectively. Baseline characteristics of 230 participants,
Fibrinogen, median (IQR), mg/dL 376.0 (326.0, 431.0) 379.0 (334.0, 431.0)
PAI-1 median (IQR), ng/mL 14.6 (12.7) 17.2 (14.3)
D-Dimer median (IQR), ng/mL 75.42 (24.33, 175.75) 110.23 (44.61, 240.8)
Fitness, glucose control, and HDL-C with ILI contributed, levels compared with usual care and that improvements in was sufficient to achieve significant reductions in PAI-1 moderate weight loss with ILI sustained over a 1-year period. Our study shows that in obese individuals with T2DM, P/H11021 0.0001 for HbA1c and HDL-C, with ILI levels. Finally, and contrary to our initial hypothesis, ILI did not change fibrinogen or D-dimer levels, pointing to complex physiological relationships between PAI-1 inflammation and coagulation balance.

PAI-1 levels are elevated in diabetes; in this study, they were more than twice those of healthy subjects in the Multi-Ethnic Study of Atherosclerosis (assays also performed at the University of Vermont Laboratory for Clinical Biochemistry Research)20 and higher than those seen in nondiabetic obese12 or prediabetic adults.13 In support of our main hypothesis, ILI effected a greater reduction in PAI-1 levels (29% from baseline) than did usual care (2.5% reduction) in the physiological relationships of PAI-1 inflammation and coagulation balance.

Discussion
Our study shows that in obese individuals with T2DM, moderate weight loss with ILI sustained over a 1-year period was sufficient to achieve significant reductions in PAI-1 levels compared with usual care and that improvements in fitness, glucose control, and HDL-C with ILI contributed, independently of adiposity change, to the lowering of PAI-1 levels. Finally, and contrary to our initial hypothesis, ILI did not change fibrinogen or D-dimer levels, pointing to complex physiological relationships between PAI-1 inflammation and coagulation balance.

Table 2. Variable Changes at 1 Y by Treatment Arm

<table>
<thead>
<tr>
<th>Variable</th>
<th>ILI (n = 957)</th>
<th>DSE (n = 860)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔWeight, mean (SD), kg</td>
<td>–8.9 (7.6)</td>
<td>–8.0 (5.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔBMI, mean (SD), kg/m²</td>
<td>–3.2 (2.6)</td>
<td>–0.3 (1.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔWaist circumference, mean (SD), cm</td>
<td>–7.7 (9.3)</td>
<td>–1.0 (7.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔFasting glucose, mean (SD), mmol/L</td>
<td>–1.23 (2.45)</td>
<td>–0.39 (2.59)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔHba1c, mean (SD), %</td>
<td>–0.7 (1.0)</td>
<td>–0.2 (0.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔLDL-C, mean (SD), mmol/L</td>
<td>–0.11 (0.67)</td>
<td>–0.13 (0.74)</td>
<td>0.66</td>
</tr>
<tr>
<td>ΔHDL-C, mean (SD), mmol/L</td>
<td>0.08 (0.18)</td>
<td>0.04 (0.17)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔTriglycerides, mean (SD), mmol/L</td>
<td>–0.36 (1.29)</td>
<td>–0.15 (1.1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔFitness (submaximal), mean (SD), MET</td>
<td>1.0 (1.4)</td>
<td>0.2 (1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔPAI-1, median (IQR) ng/mL</td>
<td>–13.4 (38.6, 2.7)</td>
<td>–1.1 (19.4, 20.8)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>ΔD-Dimer, median (IQR) µg/mL</td>
<td>0.0 (0.1, 0.1)</td>
<td>0.0 (0.1, 0.1)</td>
<td>0.57†</td>
</tr>
<tr>
<td>ΔFibrinogen, median (IQR), mg/dL</td>
<td>5.0 (33.0, 47.0)</td>
<td>10.0 (25.0, 42.0)</td>
<td>0.21†</td>
</tr>
</tbody>
</table>

Δ indicates change scores using the raw scale; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent. *P values are unadjusted and evaluate treatment differences on variable changes using the unpaired t test except where indicated. †P values are unadjusted and evaluate treatment differences on variable changes using the Wilcoxon rank sum test.

Figure. One-year changes in PAI-1 in the DSE arm vs the ILI arm by quartiles (Q) of variable change. A, For DSE: Q1, <1.1; Q2, –1.1 to <0.15; Q3, –0.15 to <0.64; Q4, ≥0.64. For ILI: Q1, –4.32, Q2, –4.32 to <–2.75; Q3, –2.75 to <1.39; Q4, ≥1.39. B, For DSE: Q1, <0.6; Q2, 0.0 to 0.8; Q3, 0.8 to 1.7; Q4, ≥1.7. C, For DSE: Q1, <–2.0; Q2, 0.0 to <1.0; Q3, 1.0 to <5.0; Q4, ≥5.0. For ILI: Q1, <1.0; Q2, 1.0 to <3.0; Q3, 3.0 to <7.0; Q4, ≥7.0. D, For DSE: Q1, <–0.6; Q2, 0.0 to <0.2; Q3, 0.2 to <0.3; Q4, ≥0.3. For ILI: Q1, <–1.2; Q2, 1.2 to <–0.6; Q3, 0.6 to <0.1; Q4, ≥0.1.

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Table 3. Metabolic Variables as Predictors of PAI-1 Change With 1-Y ILI

<table>
<thead>
<tr>
<th>Model*</th>
<th>B-Coefficient</th>
<th>SE</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model A</td>
<td></td>
<td></td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.47</td>
<td>0.034</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in BMI</td>
<td>0.10</td>
<td>0.007</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model B</td>
<td></td>
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</tr>
<tr>
<td>ILI vs DSE</td>
<td>-0.20</td>
<td>0.038</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>Change in weight</td>
<td>0.03</td>
<td>0.003</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model C</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.19</td>
<td>0.038</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>Change in waist circumference</td>
<td>0.02</td>
<td>0.002</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model D</td>
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<td></td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.32</td>
<td>0.035</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>Change in fasting glucose</td>
<td>0.01</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model E</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.41</td>
<td>0.033</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in triglycerides</td>
<td>0.001</td>
<td>0.0002</td>
<td>0.0002</td>
<td></td>
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<tr>
<td>Model F</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.39</td>
<td>0.034</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in HbA1c</td>
<td>0.17</td>
<td>0.018</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model G</td>
<td></td>
<td></td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.46</td>
<td>0.034</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in triglycerides</td>
<td>0.001</td>
<td>0.0002</td>
<td>0.0002</td>
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<tr>
<td>Model H</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.45</td>
<td>0.034</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in HDL-C</td>
<td>-0.02</td>
<td>0.003</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model I</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.40</td>
<td>0.035</td>
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<tr>
<td>Change in fitness</td>
<td>-0.10</td>
<td>0.014</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model J</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.33</td>
<td>0.035</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in HbA1c</td>
<td>0.15</td>
<td>0.018</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in fitness</td>
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<td>0.014</td>
<td>&lt;0.0001</td>
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<tr>
<td>Model K</td>
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<tr>
<td>ILI vs DSE</td>
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<td>0.038</td>
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<tr>
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<td>0.08</td>
<td>0.006</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in HbA1c</td>
<td>0.13</td>
<td>0.017</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in triglycerides</td>
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<td>0.014</td>
<td>0.026</td>
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<tr>
<td>Model L</td>
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<tr>
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<td>&lt;0.0001</td>
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<td>Change in BMI</td>
<td>0.08</td>
<td>0.007</td>
<td>&lt;0.0001</td>
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<td>Change in HbA1c</td>
<td>0.13</td>
<td>0.018</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in triglycerides</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.45</td>
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<tr>
<td>Model M</td>
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<tr>
<td>ILI vs DSE</td>
<td>-0.15</td>
<td>0.038</td>
<td>&lt;0.0001</td>
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<tr>
<td>Change in BMI</td>
<td>0.08</td>
<td>0.008</td>
<td>&lt;0.0001</td>
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<td>Change in HbA1c</td>
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<tr>
<td>Change in HDL-C</td>
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<td>Change in triglycerides</td>
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<td>Change in fitness</td>
<td>-0.03</td>
<td>0.014</td>
<td>0.04</td>
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</tbody>
</table>

Each model (A to M) was analyzed independently and adjusted for baseline PAI-1 level, demographics, clinic site, history of CVD, diabetes duration, smoking, and thiazolidinedione and statin use, with difference between baseline and 1-y log-transformed PAI-1 values as outcome variable.
Our study has several limitations. First, our PAI-1 assay measured total PAI-1 and was not specific for the active form. However, preliminary experiments in our laboratory found that it correlates highly with 2 frequently used assays: 1 measuring uncomplexed PAI-1 (active and latent free PAI-1; in-house immunoassay; \( R = 0.82 \)) and 1 commercial assay measuring total PAI-1 (Biopool Tintelize immunoassay; \( R = 0.80 \)). Furthermore, PAI-1 antigen and activity are strongly correlated \( (R = 0.77) \). Given the relatively stringent blood collection requirements for an activity assay, coupled with the multicenter nature of Look AHEAD, and the fact that much of the epidemiological data linking PAI-1 to CVD was assembled with assays for either uncomplexed or total PAI-1 (eg, Thogersen et al\(^3\)), we chose the automated total PAI-1 assay. We also evaluated the use of citrate plasma compared with a specialty collection tube (Biopool Stabilyte, Trinity Biotech USA) and found excellent correlation \( (R = 0.99) \). Second, Look AHEAD did not measure insulin levels, and the effects of insulin on PAI-1 change could not be directly assessed. It is possible that the association of hyperglycemia and PAI-1 could be explained in part by the presence of hyperinsulinemia. However, there is ample evidence that hyperglycemia is able to increase PAI-1 secretion independently of insulin change.\(^{26,45}\)

In summary, our findings show that ILI decreases and normalizes PAI-1 levels in stable obese diabetic persons compared with usual care and that the decrease is associated not only with moderate reductions in adiposity but also with improvements in fitness, glucose, and HDL-C levels, factors known to affect adipose tissue function and proinflammatory adipokine production. The absence of effects on fibrinogen, an acute phase reactant, supports the position that the decreases in PAI-1 levels with ILI result mainly from its effects on adipose tissue inflammation rather than being a consequence of systemic changes in inflammatory status. Finally, we show that despite the large reduction in PAI-1 with ILI, there were no changes in D-dimer, a marker of ambient coagulation balance. These results support expanding the role of PAI-1 to that of a marker of adipose tissue health. Future results from Look AHEAD will determine whether decreases in PAI-1 levels with ILI will reduce cardiovascular events.

**Acknowledgments**

Members of the Look AHEAD Research Study Group are listed in the Supplemental Data (Appendix), available online at http://atvb.ahajournals.org. The authors thank Elaine S. Cornell, also a member of the Look AHEAD Obesity, Inflammation and Thrombosis Research Group, for her support with the assays. Parts of this study were presented in abstract form at the 70th Scientific Sessions of the American Diabetes Association in New Orleans, LA, June 2010.

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**Disclosures**

None.
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