Metabolic Factors, Adipose Tissue, and Plasminogen Activator Inhibitor-1 Levels in Type 2 Diabetes

Findings From the Look AHEAD Study

L. Maria Belalcazar, Christie M. Ballantyne, Wei Lang, Steven M. Haffner, Julia Rushing, Dawn C. Schwenke, F. Xavier Pi-Sunyer, Russell P. Tracy, and the Look AHEAD (Action for Health in Diabetes) Research Group

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. (Arterioscler Thromb Vasc Biol. 2011;31:00-00.)

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 reagent, 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 macro-nutrient 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.

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 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.

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 immunoturbidimetric 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).
Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ILI (n=957)</th>
<th>DSE (n=860)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>57.5 (7.1)</td>
<td>57.7 (7.2)</td>
</tr>
<tr>
<td>Gender, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>391 (40.9)</td>
<td>356 (41.4)</td>
</tr>
<tr>
<td>Females</td>
<td>566 (59.1)</td>
<td>504 (58.6)</td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>651 (68.1)</td>
<td>574 (66.7)</td>
</tr>
<tr>
<td>African American</td>
<td>124 (13.0)</td>
<td>115 (13.4)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>91 (9.5)</td>
<td>78 (9.1)</td>
</tr>
<tr>
<td>Native American</td>
<td>58 (6.1)</td>
<td>60 (7.0)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>8 (0.8)</td>
<td>8 (0.9)</td>
</tr>
<tr>
<td>Other/mixed</td>
<td>24 (2.5)</td>
<td>25 (2.9)</td>
</tr>
<tr>
<td>Duration of diabetes,*</td>
<td>6.5 (6.3)</td>
<td>6.6 (6.2)</td>
</tr>
<tr>
<td>History of CVD, † No. (%)</td>
<td>118 (12.3)</td>
<td>99 (11.5)</td>
</tr>
<tr>
<td>Metabolic syndrome, No. (%)</td>
<td>890 (93.0)</td>
<td>797 (92.7)</td>
</tr>
<tr>
<td>Current tobacco use,* No. (%)</td>
<td>34 (3.6)</td>
<td>26 (3.0)</td>
</tr>
<tr>
<td>Statin therapy, No. (%)</td>
<td>399 (41.7)</td>
<td>343 (39.9)</td>
</tr>
<tr>
<td>Thiazolidinedione therapy, No. (%)</td>
<td>243 (25.4)</td>
<td>233 (27.1)</td>
</tr>
<tr>
<td>Insulin therapy, No. (%)</td>
<td>146 (15.3)</td>
<td>122 (14.2)</td>
</tr>
<tr>
<td>Estrogen replacement,* No. (%)</td>
<td>168 (29.7)</td>
<td>146 (29.0)</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>102.0 (20.1)</td>
<td>101.4 (18.7)</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>36.3 (6.3)</td>
<td>36.1 (5.9)</td>
</tr>
<tr>
<td>Waist circumference, mean (SD), cm</td>
<td>114.6 (14.8)</td>
<td>114.4 (14.2)</td>
</tr>
<tr>
<td>BMI</td>
<td>36.3 (6.3)</td>
<td>36.1 (5.9)</td>
</tr>
<tr>
<td>Fasting, mean (SD), mg/dL</td>
<td>8.5 (2.4)</td>
<td>8.7 (2.7)</td>
</tr>
<tr>
<td>HbA1c, mean (SD), %</td>
<td>7.3 (1.2)</td>
<td>7.4 (1.2)</td>
</tr>
<tr>
<td>Total cholesterol, mean (SD), mmol/L</td>
<td>5.0 (1.0)</td>
<td>4.9 (1.0)</td>
</tr>
<tr>
<td>LDL-C, mean (SD), mmol/L</td>
<td>2.9 (0.8)</td>
<td>2.9 (0.8)</td>
</tr>
<tr>
<td>HDL-C, mean (SD), mmol/L</td>
<td>1.1 (0.3)</td>
<td>1.1 (0.3)</td>
</tr>
<tr>
<td>Triglycerides, mean (SD), mmol/L</td>
<td>2.1 (1.5)</td>
<td>2.0 (1.4)</td>
</tr>
<tr>
<td>PAI-1 median (IQR), ng/mL</td>
<td>46.53 (26.20, 75.63)</td>
<td>44.61 (24.33, 75.14)</td>
</tr>
<tr>
<td>D-Dimer median (IQR), µg/mL</td>
<td>0.26 (0.17, 0.39)</td>
<td>0.26 (0.17, 0.39)</td>
</tr>
<tr>
<td>Fibrinogen, median (IQR), mg/dL</td>
<td>376.0 (326.0, 431.0)</td>
<td>379.0 (334.0, 431.0)</td>
</tr>
</tbody>
</table>

*Self-reported.
†Self-reported prior myocardial infarction, stroke, transient ischemic attack, angioplasty/stent, coronary artery bypass graft, carotid endarterectomy, abdominal aortic aneurysm, or heart failure.

Belalcazar et al Modulation of PAI-1 in People With Type 2 Diabetes

Changes in Variables of Interest With ILI

ILI participants had significant improvements in adiposity, fitness, glucose, and lipid control at 1 year compared with those randomized to DSE, as observed for the overall Look AHEAD sample.18 (Table 2). There were no differences in low-density lipoprotein cholesterol between ILI and DSE at 1 year. One-year PAI-1 levels (median [IQR]) dropped 13.4 (−38.6, 2.7) ng/mL from a baseline of 46.53 (26.2, 75.63) ng/mL in the ILI group (29% reduction) and 1.1 (−19.4, 20.8) ng/mL from a baseline median (IQR) of 44.61 (24.33, 75.14) ng/mL in the DSE group (2.5% reduction) (P<0.0001 for difference between groups). No change in d-dimer or decrease in fibrinogen levels was documented in either group.

Changes in PAI-1 levels were not associated with changes in d-dimer or fibrinogen levels (Spearman correlation coefficients, adjusted for gender and age, of −0.03 [P=0.22] and 0.03 [P=0.24], respectively). Greater improvements not only in adiposity but also in fitness, glucose control, high-density lipoprotein cholesterol (HDL-C) levels (Figure), and triglycerides (not shown) with ILI were found to be associated with greater decreases in PAI-1 levels (P for trend, <0.0001 for all). Separate analysis in the DSE arm showed progressive change in PAI-1 across quartiles of change only for body mass index (BMI) and HbA1c (P for trend, <0.001 for both). These findings do not suggest important effect modification on the relationship between treatment arm and PAI-1.

Metabolic Predictors of 1-Year Change in PAI-1

Regression analyses showed that not only adiposity change (measured by BMI, weight, and waist changes) but also each of the changes in glucose (HbA1c and fasting glucose) and lipid (triglycerides and HDL-C) control and in fitness with ILI predicted a decrease in PAI-1 levels (log-transformed for analysis) at 1 year (P<0.001 for all) (Table 3, models A to I). Change in waist accounted for 5% of the variance in PAI-1 change (33% with ILI), after adjusting for baseline PAI-1 level, demographics, medical history, and medication use, and was not a better predictor of PAI-1 change compared with change in BMI or change in weight, which explained 6% to 7% of the variance in PAI-1 change (models B and C, 34% and 35%, respectively, of the variance in PAI-1 change with ILI). Given that change in BMI was a stronger predictor of change in PAI-1 than change in waist was, we chose to include change in BMI in the remainder of our analyses.

Improvement in fitness with ILI, again in the presence of multiple covariates, explained 2% of the variance in PAI-1 change (model I, 30% of the variance in PAI-1 change with ILI) and remained associated with PAI-1 change after adjusting for changes in glucose control (model J, P<0.0001) and in adiposity (model K, P=0.026). Change in triglycerides with ILI was not associated with PAI-1 change when changes in glucose control and BMI were taken into account (model L, P=0.45). Change in glucose control, HDL-C, and fitness remained significantly associated with PAI-1 change in the full model after adjusting for adiposity change (model M, those from the remaining cohort (12% and 15%, respectively).19
Table 2. Variable Changes at 1 Y by Treatment Arm

<table>
<thead>
<tr>
<th></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>–0.8 (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>–13.3 (–36.2, 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.

P<0.0001 for HbA1c and HDL-C, P=0.04 for fitness) and together accounted for 10% of the variance in PAI-1 change (38% of the variance in PAI-1 change with ILI) independently of baseline PAI-1 levels, individual demographic characteristics, history of CVD, diabetes duration, smoking, and thiazolidinedione use.

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.

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) and higher than those seen in nondiabetic obese or prediabetic adults. 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

![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, <–0.4; Q2, –0.4 to 0.0; Q3, 0.0 to 0.8; Q4, >0.8. For ILI: Q1, <–0.0; Q2, 0.0 to 0.8; Q3, 0.8 to 1.7; Q4, >1.7. C, For DSE: Q1, <–2.0; Q2, –2.0 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, –0.6 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.](http://atvb.ahajournals.org/)

![IQR for change in PAI-1 • Mean PAI-1 change](http://atvb.ahajournals.org/)
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>$R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model A</td>
<td>-0.47</td>
<td>0.034</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model B</td>
<td>-0.20</td>
<td>0.038</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model C</td>
<td>0.10</td>
<td>0.007</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model D</td>
<td>-0.19</td>
<td>0.035</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model E</td>
<td>0.03</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model F</td>
<td>-0.32</td>
<td>0.035</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model G</td>
<td>-0.41</td>
<td>0.033</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model H</td>
<td>-0.45</td>
<td>0.034</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model I</td>
<td>-0.02</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model J</td>
<td>-0.40</td>
<td>0.035</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model K</td>
<td>-0.10</td>
<td>0.014</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Model M</td>
<td>-0.16</td>
<td>0.038</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</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 data did not support our hypothesis that reductions in PAI-1 levels with ILI would be associated with changes in fibrinogen and D-dimer. Studies in nondiabetic individuals suggest that moderate weight loss does not alter fibrinogen levels\textsuperscript{12,13} and that major weight loss, on the order of a 40% reduction from baseline,\textsuperscript{35} is necessary to see a decrease in levels. Fibrinogen is an acute phase protein, but unlike PAI-1, it is synthesized only in liver and is not subject to adipose tissue control. We know from previous work in Look AHEAD that ILI decreases C-reactive protein, another marker of systemic inflammation.\textsuperscript{36} Recent data suggest that, like PAI-1, C-reactive protein is synthesized not only by hepatocytes but also by several nonhepatic cells, including adipocytes.\textsuperscript{37,38} The unaltered fibrinogen levels with ILI suggest that the effects of moderate improvements in adiposity, metabolic control and fitness on inflammation in obese people with T2DM may be derived from improvements in adipose tissue function rather than in relation to changes in the acute phase response. Although nonadipose tissue sources of PAI-1, including liver, endothelial cells, and platelets, could have contributed to the change in PAI-1 levels with ILI in our study, studies investigating the origin of circulating PAI-1 suggest that the source of PAI-1 may differ by age and health status and that in obesity, adipose tissue is a major source.\textsuperscript{5,39,40} Our results are also in agreement with a factor analysis in healthy individuals that showed that PAI-1 clustered with a body mass factor and not with an interleukin-6-dependent inflammatory factor that included fibrinogen.\textsuperscript{41}

The absence of associated changes in D-dimer with ILI, despite the important reduction in PAI-1 levels, was unexpected. PAI-1 is a major regulator of fibrinolysis,\textsuperscript{3} and D-dimer is a measure of ambient coagulation balance that includes intraluminal fibrinolysis.\textsuperscript{42} Both coagulation (resulting in fibrin formation) and fibrinolysis (resulting in clot dissolution) have to occur for D-dimer to be formed.\textsuperscript{42} Similar findings have been observed with weight loss in younger, less obese persons without diabetes.\textsuperscript{12} Our results may be explained by the relatively normal D-dimer levels found in our stable ambulatory participants with T2DM, levels that indicate that ongoing fibrin formation and dissolution were not elevated. On the basis of these findings, one could hypothesize that elevated PAI-1 may exert an effect on clotting only in the setting of a relatively large stimulation, such as that occurring in the presence of a ruptured atherosclerotic plaque. An alternative hypothesis would be that if there were a CVD benefit associated with a decline in PAI-1 with ILI, it would not be through regulation of ongoing, so-called ambient coagulant balance and blood-based clot formation but rather through its effect in the vessel wall.\textsuperscript{4} PAI-1 expression in the vessel wall is increased in the presence of diabetes,\textsuperscript{43} and Sobel et al have proposed a deleterious effect of elevated tissue-based PAI-1 on vessel remodeling, leading to an increased risk of plaque rupture.\textsuperscript{4}

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 \(( R = 0.77 )\).\textsuperscript{44} 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\textsuperscript{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.\textsuperscript{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.

Sources of Funding

Look AHEAD is sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases and co-sponsored by the National Heart, Lung and Blood Institute, National Institute of Nursing Research, Office of Research on Women’s Health, National Center on Minority Health and Health Disparities, and Centers for Disease Control and Prevention. Additional sources of funding for Look AHEAD are listed in the Supplemental Data. Work by the Look AHEAD Ancillary Study Group Obesity Inflammation and Thrombosis was supported by the National Heart, Lung and Blood Institute, Grants HL090514 (to C.M.B.) and HL090514-02S1 (to L.M.B.).

Disclosures

None.
References


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

L. Maria Belalcazar, Christie M. Ballantyne, Wei Lang, Steven M. Haffner, Julia Rushing, Dawn C. Schwenke, F. Xavier Pi-Sunyer, Russell P. Tracy and the Look AHEAD (Action for Health in Diabetes) Research Group

Arterioscler Thromb Vasc Biol. published online April 21, 2011;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2011/04/21/ATVBAHA.111.224386

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2011/04/21/ATVBAHA.111.224386.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Look AHEAD Research Group and Support

Clinical Sites
The Johns Hopkins Medical Institutions: Frederick L. Brancati, MD, MHS; Jeff Honas, MS; Lawrence Cheskin, MD; Jeanne M. Clark, MD, MPH; Kerry Stewart, EdD; Richard Rubin, PhD; Jeanne Charleston, RN; Kathy Horak, RD

Pennington Biomedical Research Center: George A. Bray, MD; Kristi Rau; Allison Strate, RN; Brandi Armand, LPN; Frank L. Greenway, MD; Donna H. Ryan, MD; Donald Williamson, PhD; Amy Bachand; Michelle Begnaud; Betsy Berhard; Elizabeth Caderette; Barbara Cerniauskas; David Creel; Diane Crow; Helen Guay; Nancy Kora; Kelly LaFleur; Kim Landry; Missy Lingle; Jennifer Perault; Mandy Shipp; Kathy Horak, RD; The University of Alabama at Birmingham: Cora E. Lewis, MD, MSPH; Sheikilya Thomas MPH; Monika Safford, MD; Vicki DiLillo, PhD; Charlotte Bragg, MS, RD, LD; Amy Dobelstein; Stacey Gilbert, MPH; Stephen Glasser, MD; Sara Hannum, MA; Anne Hubbell, MS; Jennifer Jones, MA; DeLavallade Lee; Ruth Luketic, MA, MBA, MPH; Karen Marshall; L. Christie Oden; Janet Raines, MS; Cathy Roche, RN, BSN; Janet Truman; Nita Webb, MA; Audrey Wrenn, MAEd

Harvard Center: David M. Nathan, MD, Heather Turgeon, RN, BS, CDE; Kristina Schumann, BA; Enrico Cagliero, MD; Linda Delahanty, MS, RD; Kathryn Hayward, MD; Ellen Anderson, MS, RD; Laurie Bissett, MS, RD; Richard Ginsburg, PhD; Valerie Goldman, MS, RD; Virginia Harlan, MSW; Charles McKitrick, RN, BSN, CDE; Alan McNamara, BS; Theresa Michel, DPT, DSc CCS; Alexi Poulos, BA; Barbara Steiner, EdM; Joclyn Tosch, BA

Joslin Diabetes Center: Edward S. Horton, MD; Sharon D. Jackson, MS, RD, CDE; Osama Hamdy, MD, PhD; A. Enrique Caballero, MD; Sarah Bain, BS; Elizabeth Bovaird, BSN, RN; Ann Goebel-Fabbri, PhD; Lori Lambert, MS, RD; Sarah Ledbury, MEd, RD; Maureen Malloy, BS; Kerry Ovalle, MS, RCEP, CDE

Beth Israel Deaconess Medical Center: George Blackburn, MD, PhD; Christos Mantzoros, MD, DSc; Kristinia Day, RD; Ann McNamara, RN

University of Colorado Health Sciences Center: James O. Hill, PhD; Marsha Miller, MS, RD; JoAnn Phillip, MS; Robert Schwartz, MD; Brent Van Dorsten, PhD; Judith Regensteiner, PhD; Salma Benchekroun MS; Ligia Coelho, BS; Paulette Cohrs, RN, BSN; Elizabeth Daeninck, MS, RD; Amy Fields, MPH; Susan Green; April Hamilton, BS, CCRC; Jere Hamilton, BA; Eugene Leshchinskiy; Michael McDermott, MD; Lindsey Munkwitz, BS; Loretta Rome, TRS; Kristin Wallace, MPH; Terra Worley, BA
Baylor College of Medicine  John P. Foreyt, PhD\textsuperscript{1}; Rebecca S. Reeves, DrPH, RD\textsuperscript{2}; Henry Pownall, PhD\textsuperscript{3}; Ashok Balasubramanyam, MBBS\textsuperscript{3}; Peter Jones, MD\textsuperscript{3}; Michele Burrington, RD; Chu-Huang Chen, MD, PhD; Allyson Clark, RD; Molly Gee, MEd, RD; Sharon Griggs; Michelle Hamilton; Veronica Holley; Jayne Joseph, RD; Patricia Pace, RD; Julieta Palencia, RN; Olga Satterwhite, RD; Jennifer Schmidt; Devin Volding, LMSW; Carolyn White

University of California at Los Angeles School of Medicine  Mohammed F. Saad, MD\textsuperscript{1}; Siran Ghazarian Sengardi, MD\textsuperscript{2}; Ken C. Chiu, MD\textsuperscript{3}; Medhat Botrous; Lisa Jones, RN; Lynne Lichtermann, RN, BSN; Shirley Vosburg, RD, MPH; and J. Lee Taylor, MEd, MBA

The University of Tennessee Health Science Center

\textit{University of Tennessee East.} Karen C. Johnson, MD, MPH; Carolyn Gresham, RN; Stephanie Connelly, MD, MPH; Amy Brewer, RD, MS; Mace Coday, PhD; Lisa Jones, RN; Lynne Lichtermann, RN, BSN; Shirley Vosburg, RD, MPH; and J. Lee Taylor, MEd, MBA

\textit{University of Tennessee Downtown.} Abbas E. Kitabchi, PhD, MD; Helen Lambeth, RN, BSN; Debra Clark, LPN; Andrea Crisler, MT; Gracie Cunningham; Donna Green, RN; Debra Force, MS, RD, LDN; Robert Kores, PhD; Renate Rosenthal PhD; Elizabeth Smith, MS, RD, LDN; and Maria Sun, MS, RD, LDN; and Judith Soberman, MD\textsuperscript{3}

University of Minnesota  Robert W. Jeffery, PhD\textsuperscript{1}; Carolyn Thorson, CCRP\textsuperscript{2}; John P. Bantle, MD\textsuperscript{3}; J. Bruce Redmon, MD\textsuperscript{3}; Richard S. Crow, MD\textsuperscript{3}; Scott Crow, MD\textsuperscript{3}; Susan K Raatz, PhD, RD\textsuperscript{3}; Kerrin Brelje, MPH, RD; Carylyne Campbell; Jeanne Carls, MEd; Tara Carmean-Mihm, BA; Emily Finch, MA; Anna Fox, MA; Elizabeth Hoelscher, MPH, RD, CHES; La Donna James; Vicki A. Maddy, BS, RD; Therese Ockenden, RN; Birgitta I. Rice, MS, RPh CHES; \textbf{Error! Contact not defined.}, BS; Ann D. Tucker, BA; Mary Susan Voeller, BA; Cara Walcheck, BS, RD

St. Luke’s Roosevelt Hospital Center  Xavier Pi-Sunyer, MD\textsuperscript{1}; Jennifer Patricio, MS\textsuperscript{2}; Stanley Heshka, PhD\textsuperscript{3}; Carmen Pal, MD\textsuperscript{3}; Lynn Allen, MD; Diane Hirsch, RNC, MS, CDE; Mary Anne Holowaty, MS, CN

University of Pennsylvania  Thomas A. Wadden, PhD\textsuperscript{1}; Barbara J. Maschak-Carey, MSN, CDE\textsuperscript{2}; Stanley Schwartz, MD\textsuperscript{3}; Gary D. Foster, PhD\textsuperscript{3}; Robert I. Berkowitz, MD\textsuperscript{3}; Henry Glick, PhD\textsuperscript{3}; Shiriki K. Kumanyika, PhD, RD, MPH\textsuperscript{3}; Johanna Brock; Helen Chomentowski; Vicki Clark; Canice Crerand, PhD; Renee Davenport; Andrea Diamond, MS, RD; Anthony Fabricatore, PhD; Louise Hesson, MSN; Stephanie Krauthamer-Ewing, MPH; Robert Kuehnel, PhD; Patricia Lipschutz, MSN; Monica Mullen, MS, RD; Leslie Womble, PhD, MS; Nayyar Iqbal, MD

University of Pittsburgh  David E. Kelley, MD\textsuperscript{1}; Jacqueline Wesche-Thobaben,
RN, BSN, CDE²; Lewis Kuller, MD, DrPH³; Andrea Kriska, PhD³; Janet Bonk, RN, MPH; Rebecca Danchenko, BS; Daniel Edmundowicz, MD³; Mary L. Klem, PhD, MLIS³; Monica E. Yamamoto, DrPH, RD, FADA³; Barb Elnyczky, MA; George A. Grove, MS; Pat Harper, MS, RD, LDN; Janet Krulia, RN, BSN, CDE; Juliet Mancino, MS, RD, CDE, LDN; Anne Mathews, MS, RD, LDN; Tracey Y. Murray, BS; Joan R. Ritchea; Jennifer Rush, MPH; Karen Vujevich, RN-BC, MSN, CRNP; Donna Wolf, MS

The Miriam Hospital/Brown Medical School, Rena R. Wing, PhD¹; Renee Bright, MS²; Vincent Pera, MD³; John Jakicic, PhD³; Deborah Tate, PhD³; Amy Gorin, PhD³; Kara Gallagher, PhD³; Amy Bach, PhD; Barbara Bancroft, RN, MS; Anna Bertorelli, MBA, RD; Richard Carey, BS; Tatum Charron, BS; Heather Chenot, MS; Kimberley Chula-Maguire, MS; Pamela Coward, MS, RD; Lisa Cronkite, BS; Julie Currin, MD; Maureen Daly, RN; Caitlin Egan, MS; Erica Ferguson, BS, RD; Linda Foss, MPH; Jennifer Gauvin, BS; Don Kieffer, PhD; Lauren Lessard, BS; Deborah Maier, MS; JP Massaro, BS; Tammy Monk, MS; Rob Nicholson, PhD; Erin Patterson, BS; Suzanne Phelan, PhD; Hollie Raynor, PhD, RD; Douglas Raynor, PhD; Natalie Robinson, MS, RD; Deborah Robles; Jane Tavares, BS

The University of Texas Health Science Center at San Antonio, Steven M. Haffner, MD¹; Maria G. Montez, RN, MSHP, CDE²; Carlos Lorenzo, MD³

University of Washington / VA Puget Sound Health Care System, Steven Kahn MB, ChB¹; Brenda Montgomery, RN, MS, CDE²; Robert Knopp, MD³; Edward Lipkin, MD³; Matthew L. Maciejewski, PhD³; Dace Trence, MD³; Terry Barrett, BS; Joli Bartell, BA; Diane Greenberg, PhD; Anne Murillo, BS; Betty Ann Richmond, MEd; April Thomas, MPH, RD

Southwestern American Indian Center, Phoenix, Arizona and Shiprock, New Mexico, William C. Knowler, MD, DrPH¹; Paula Bolin, RN, MC²; Tina Killean, BS²; Cathy Manus, LPN³; Jonathan Krakoff, MD³; Jeffrey M. Curtis, MD, MPH³; Justin Glass, MD³; Sara Michaels, MD³; Peter H. Bennett, MB, FRCPC³; Tina Morgan³; Shandii Begay, MPH; Bernadita Fallis RN, RHIT, CCS; Jeanette Hermes, MS, RD; Diane F. Hollowbreast; Ruby Johnson; Maria Meacham, BSN, RN, CDE; Julie Nelson, RD; Carol Percy, RN; Patricia Poorthunder; Sandra Sangster; Nancy Scurlock, MSN, ANP-C, CDE; Leigh A. Shovestull, RD, CDE; Janelia Smiley; Katie Toledo, MS, LPC; Christina Tomachee, BA; Darryl Tonemah PhD

University of Southern California, Anne Peters, MD¹; Valerie Ruelas, MSW, LCSW²; Siran Ghazarian Sengardi, MD²; Kathryn Graves, MPH, RD, CDE; Kati Konersman, MA, RD, CDE; Sara Serafin-Dokhan

Coordinating Center
Wake Forest University, Mark A. Espeland, PhD¹; Judy L. Bahnson, BA²; Lynne Wagenknecht, DrPH³; David Reboussin, PhD³; W. Jack Rejeski, PhD³, Alain
Bertoni, MD, MPH; Wei Lang, PhD; Gary Miller, PhD; David Lefkowitz, MD; Patrick S. Reynolds, MD; Paul Ribisl, PhD; Mara Vitolins, DrPH; Michael Booth, MBA; Kathy M. Dotson, BA; Amelia Hodges, BS; Carrie C. Williams, BS; Jerry M. Barnes, MA; Patricia A. Feeney, MS; Jason Griffin, BS; Lea Harvin, BS; William Herman, MD, MPH; Patricia Hogan, MS; Sarah Jaramillo, MS; Mark King, BS; Kathy Lane, BS; Rebecca Neiberg, MS; Andrea Ruggiero, MS; Christian Speas, BS; Michael P. Walkup, MS; Karen Wall, AAS; Michelle Ward; Delia S. West, PhD; Terri Windham

Central Resources Centers
DXA Reading Center, University of California at San Francisco Michael Nevitt, PhD; Susan Ewing, MS; Cynthia Hayashi; Jason Maeda, MPH; Lisa Palermo, MS, MA; Michaela Rahorst; Ann Schwartz, PhD; John Shepherd, PhD

Central Laboratory, Northwest Lipid Research Laboratories Santica M. Marcovina, PhD, ScD; Greg Strylewicz, MS

ECG Reading Center, EPICARE, Wake Forest University School of Medicine Ronald J. Prineas, MD, PhD; Teresa Alexander; Lisa Billings; Charles Campbell, AAS, BS; Sharon Hall; Susan Hensley; Yabing Li, MD; Zhu-Ming Zhang, MD

Diet Assessment Center, University of South Carolina, Arnold School of Public Health, Center for Research in Nutrition and Health Disparities Elizabeth J Mayer-Davis, PhD; Robert Moran, PhD

Hall-Foushee Communications, Inc.
Richard Foushee, PhD; Nancy J. Hall, MA

Federal Sponsors
National Institute of Diabetes and Digestive and Kidney Diseases: Barbara Harrison, MS; Van S. Hubbard, MD PhD; Susan Z. Yanovski, MD

National Heart, Lung, and Blood Institute: Lawton S. Cooper, MD, MPH; Jeffrey Cutler, MD, MPH; Eva Obarzanek, PhD, MPH, RD

Centers for Disease Control and Prevention: Edward W. Gregg, PhD; David F. Williamson, PhD; Ping Zhang, PhD

Funding and Support
This study is supported by the Department of Health and Human Services through the following cooperative agreements from the National Institutes of Health: DK57136, DK57149, DK56990, DK57177, DK57171, DK57151, DK57182, DK57131, DK57002, DK57078, DK57154, DK57178, DK57219, DK57008, DK57135, and DK56992. The following federal agencies have contributed support: National Institute of Diabetes and Digestive and Kidney Diseases; National Heart, Lung, and Blood Institute; National Institute of Nursing
Research; National Center on Minority Health and Health Disparities; Office of Research on Women’s Health; and the Centers for Disease Control and Prevention. This research was supported in part by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases. The Indian Health Service (IHS) provided personnel, medical oversight, and use of facilities. The opinions expressed in this paper are those of the authors and do not necessarily reflect the views of the IHS or other funding sources.

Additional support was received from The Johns Hopkins Medical Institutions Bayview General Clinical Research Center (M01RR02719); the Massachusetts General Hospital Mallinckrodt General Clinical Research Center (M01RR01066); the University of Colorado Health Sciences Center General Clinical Research Center (M01RR00051) and Clinical Nutrition Research Unit (P30 DK48520); the University of Tennessee at Memphis General Clinical Research Center (M01RR0021140); the University of Pittsburgh General Clinical Research Center (M01RR000056 44) and NIH grant (DK 046204); and the University of Washington / VA Puget Sound Health Care System Medical Research Service, Department of Veterans Affairs; Frederic C. Bartter General Clinical Research Center (M01RR01346).

The following organizations have committed to make major contributions to Look AHEAD: Federal Express; Health Management Resources; Johnson & Johnson, LifeScan Inc.; Optifast-Novartis Nutrition; Roche Pharmaceuticals; Ross Product Division of Abbott Laboratories; Slim-Fast Foods Company; and Unilever.

1 Principal Investigator
2 Program Coordinator
3 Co-Investigator
All other Look AHEAD staff members are listed alphabetically by site.

Other
Dr. Dawn Schwenke is also a Research Health Scientist at the Phoenix VA Health Care System