Amelioration of the inhibition of fibrinolysis in elderly, obese subjects by moderate energy intake restriction\textsuperscript{1–3}

Jorge Calles-Escandon, Douglas Ballor, Jean Harvey-Berino, Philip Ades, Russell Tracy, and Burton Sobel

ABSTRACT A possible cause of accelerated atherothrombosis in the syndrome of insulin resistance appears to be an elevated blood concentration of plasminogen activator type-1 (PAI-1). Insulin resistance occurs with aging, attributable partly to increased adiposity. Scarce information exists regarding the effects of weight loss in elderly, obese individuals on PAI-1 concentrations. Consequently, weight loss (9 ± 1 kg) was induced by energy intake restriction in 19 elderly, obese individuals, and its effect on fibrinolytic system peptides was measured. Initially elevated PAI-1 concentrations decreased by 50%, with a simultaneous decrease in the concentration of tissue-type plasminogen activator (t-PA)/PAI-1 complexes but no significant change in t-PA (P < 0.05). The increase in PAI complexes correlated with weight and fat mass losses (r = 0.46, P < 0.05 for both). No correlation was seen between fibrinolytic system variables and baseline concentrations of substrates or insulin, but the change in PAI-1 correlated with the change in plasma triacylglycerols (r = 0.58, P < 0.05). Results indicate that energy restriction sufficient to induce moderate weight loss leads to diminution of elevated plasma PAI-1 and relief of inhibition of the fibrinolytic system in elderly, obese subjects. The extent that these changes are associated with a decrease in the progression of vasculopathy, weight loss in elderly, obese individuals may be a useful means to reduce cardiovascular morbidity and mortality.


KEY WORDS Atherosclerosis, obesity, coronary disease, elderly people

INTRODUCTION The syndrome of insulin resistance (1, 2) (sometimes called syndrome X) is characterized by features, occurring alone or in combination, that include obesity, hypertension, non-insulin-dependent diabetes mellitus, and hypertriacylglycerolemia (3, 4). It is associated with increased atherosclerosis and mortality attributable to coronary artery disease (5). Mechanisms underlying the accelerated atherothrombosis remain somewhat obscure. However, elevated concentrations of plasminogen activator inhibitor type-1 (PAI-1), reported recently by several groups, have been implicated (6–10). Elevated PAI-1 may accelerate atherosclerosis (11–13) by limiting endogenous fibrinolysis, thereby exposing luminal surfaces of arteries to persistent or recurrent microthrombi and hence clot-associated miogens (14) as a result of an imbalance between thrombosis and thrombolysis favoring thrombosis (15). Hyperinsulinemia (16, 17), elevated triacylglycerol concentrations (16, 18), and obesity (19) have each been associated with increased concentrations of PAI-1 in blood. Insulin increases PAI-1 gene expression and synthesis in cells of hepatic origin in culture and appears to induce high PAI-1 concentrations in blood in vivo as judged from studies of animals given infusions of insulin or proinsulin (20, 21). Adipocytes in vitro elaborate PAI-1 in response to diverse cytokines (22), perhaps contributing to the elevated PAI-1 in plasma seen with obesity.

Insulin resistance occurs with aging (23), a phenomenon attributable at least in part to an increase in adiposity (24). Aging is also associated with elevated PAI-1 concentrations in blood and accelerated atherosclerosis, a major cause of overall morbidity and mortality in the elderly (25, 26). Amelioration of obesity decreases insulin resistance (27) and improves cardiovascular risk and function (28). However, little information is available regarding the effects of weight loss in elderly, obese individuals on concentrations of PAI-1 in plasma.

Severe weight loss in young or middle-aged, morbidly obese subjects decreases the concentrations of PAI-1 in plasma (29–35). However, very intense weight-loss programs are not applicable for widespread implementation in elderly subjects. In many studies, dietary restriction has been combined with exercise training (29), confounding attribution of results to weight loss per se because exercise can by itself affect outcome.

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variables, including plasma PAI-1 concentrations (36–38). The present study was designed to determine whether a program of easily attainable, moderate weight loss, induced by voluntary dietary restriction, attenuates the otherwise elevated plasma PAI-1 concentrations seen in elderly, obese subjects.

SUBJECTS AND METHODS

Subjects

Inclusion criteria for participating subjects were as follows: age 60–70 y; body mass index (BMI; in kg/m²) > 32; absence of history, signs, or symptoms of heart disease or clinically overt diabetes; blood pressure < 160/90 mm Hg; absence of smoking; and absence of treatment with any medication known to alter energy metabolism or the coagulation or fibrinolytic systems. Results of screening blood tests (blood cell count, electrolytes, thyrotropin, free thyroxine, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) were normal as was glucose tolerance, defined by the criteria of the National Diabetes Data Group (39). All participating subjects provided written, informed consent on a form approved by the Committee for Human Research in the Medical Sciences of the University of Vermont.

Weight-loss program

Subjects were trained to restrict their daily energy intake by a moderate amount (3700–4600 kJ/d deficit) with a regimen that maintained a prudent balance of macronutrients: 25% of energy from fat, 60% of energy from carbohydrate, and 15% of energy from protein. Subjects adhered to the dietary program for 11 wk, during which time they met weekly in small groups with dietary counselors in sessions that included monitoring of body mass and review of dietary diaries. The participants were instructed in behavior modification (being schooled in strategies for self-monitoring, stimulus control, problem solving, social assertion, goal setting, and relapse prevention) and principles of good nutrition. The weight-loss program was supervised by one of the investigators (JRH) and conducted in the Clinical Research Center (CRC) of The University of Vermont.

Body density was measured by underwater weighing, with correction for residual lung volume (helium dilution), and percentage body fat was estimated by using the Siri equation (40). Skinfold thicknesses and diameters were measured by a single observer. Fat distribution was estimated with the waist-to-hip ratio.

Assays

For all assays, citrated blood was prepared as platelet-free plasma, with centrifugation at 4 °C at 3000 × g for 10 min. PAI-1 was measured in plasma with the use of an enzyme-linked immunosorbent assay (ELISA) originally developed by Declerck et al (41). This method is sensitive to the free forms of PAI-1, both latent and active, but not to the complexed forms. Quality control was established with two control plasma samples (both with approximately normal PAI-1 concentrations), and longitudinal drift was assessed by using a lyophilized plasma control sample. In regular use in our laboratory, this assay has a CV of 9%. Each sample was assayed at a fixed dilution, in duplicate. The standard curve was made from a pooled plasma sample calibrated with purified material pro-
vided by Desiree Collen’s laboratory in Leuven, Belgium.

Phlebotomy was performed between 0800 and 0900 because there is considerable variation in PAI-1 concentrations from morning to evening (up to a twofold decrease over the course of the day).

We measured tissue-type plasminogen activator (t-PA) by the ELISA method of Holvoet et al (42), which uses three different t-PA–specific monoclonal antibodies. This assay is specific for t-PA, measuring both the free and complexed forms. Standardization and quality control were established as for PAI-1. In regular use in our laboratory this assay has a CV of 5.1% as judged from results with a control with a slightly elevated, abnormal value and 10% with a normal control.

We measured t-PA/PAI-1 complexes and plasmin-α2-antiplasmin (PAP) complexes by the methods of Alessi et al (43) and Holvoet et al (44), respectively. Both are two-site monoclonal antibody–based ELISAs. They are specific for the complexes and do not detect the individual components. Standardization and quality control were established as for PAI-1. In regular use in our laboratory the t-PA/PAI-1 assay has a CV of 14% and the PAP assay has a CV of 3.3%.

Plasma insulin was measured with radioimmunooassay at the CRC. Plasma glucose was measured with an automated method based on glucose oxidase (YSI Instruments, Yellow Springs, OH). Lipids (cholesterol, triacylglycerols, and fractionation of cholesterol into LDL and HDL) were measured at the Clinical Chemistry Laboratory of the Medical Center, Hospital of Vermont.

Statistical analysis

Data were analyzed with software from the NUMBER CRUNCH Statistical Systems (NCSS 6.0; Kaysville, UT). Student’s t test (two-tailed) for paired samples was used to compare mean values of variables before and after weight loss. Simple- and multiple-linear-regression techniques were used to delineate descriptors of changes in fibrinolytic system variables and associations among variables. The data had a normal distribution; therefore, log transformation was not necessary. P values < 0.05 were considered to be significant. Data are presented as means ± SEMs unless otherwise noted.

RESULTS

Nineteen (11 females, 8 males) of the original 20 volunteers (12 females, 8 males) completed the 11-wk weight-loss program. Because the analysis did not reveal any sex-related differences, data were pooled. Physical characteristics of subjects before and after weight loss are shown in Table 1. Average weight loss was 9 ± 1 kg (P < 0.005). The weight loss comprised 29% fat-free mass and 71% fat mass. Skinfold thicknesses decreased significantly by 15% (32 ± 4 mm, P < 0.001). In contrast, the waist-to-hip ratio did not change significantly.

Laboratory results before and after weight loss are shown in Table 2. The serum insulin concentration declined by 50% without significant changes in plasma glucose. Triacylglycerol concentrations decreased significantly but total cholesterol (5.8 ± 0.25 compared with 5.7 ± 0.38 mmol/L), LDL cholesterol (3.62 ± 0.18 compared with 3.5 ± 0.24 mmol/L), and HDL cholesterol (0.88 ± 0.09 compared with 0.94 ± 0.012
mmol/L) did not change significantly. Plasma concentrations of PAI-1 before weight loss were elevated to values similar to those of younger, obese individuals with or without diabetes (6). The PAI-1 concentration decreased by 50%, associated with a simultaneous decrease in the concentration of t-PA/PAI-1 complexes (Table 2). There was no significant change in the concentration of t-PA in blood, although the mean value after weight loss was lower than the value before weight loss, probably reflecting the change in the t-PA/PAI-1 complex. Changes in PAI-1 and t-PA are consistent with an overall decrease in inhibition of the fibrinolytic system. Consistent with this interpretation, the concentration of the PAP complex increased significantly, by ~20%, indicative of the augmented fibrinolytic system activity in vivo.

For the subjects as a group, the magnitude of the decline in plasma PAI-1 concentration correlated with that of the decrease in body weight (r = 0.45, P < 0.05) and the loss of fat (r = 0.35, P < 0.05) as shown in Figure 1. The magnitude of the increase in the concentration of plasma PAP complexes correlated with weight loss and fat mass loss (r = 0.53 and r = 0.60, respectively; P < 0.05 for both). No correlation was seen between fibrinolytic-system variables and baseline concentrations of substrates or insulin, but the change in PAI-1 was correlated with the change in plasma triacylglycerols (r = 0.48, P < 0.05). When putative predictors of the change in PAI-1 were entered in a model of multiple-linear-regression analysis, the only significant descriptor of the change in PAI-1 concentrations was the decrease in body weight, with values identical to those from the simple-linear-regression analysis (r = 0.45, P < 0.01).

DISCUSSION

Our results indicate that moderate energy intake restriction sufficient to induce moderate weight loss leads to considerable diminution of initially elevated plasma PAI-1 concentrations and relief of inhibition of the fibrinolytic system in elderly, obese subjects. Because aging and obesity are associated with insulin resistance (1–4), accelerated atherosclerosis (1, 5), and increased cardiovascular morbidity and mortality (25, 26, 45), and because the vasculopathy seen in insulin-resistant states is associated with an imbalance between coagulation and fibrinolysis (6–10) favoring thrombosis, we hypothesized that weight loss could retard the progression of vascular disease by attenuating one of its proximate causes. The relative prothrombotic state known to occur in the syndrome of insulin resistance has been attributed to increased circulating (6) and/or local (22, 46, 47) concentrations of PAI-1, now thought by many to be a hallmark of such states (4, 7–10, 18, 19, 48). A considerable body of information indicates that sustained compensatory hyperinsulinemia (20, 21, 49) and hypertriacylglycerolemia (16, 18) are associated with increased synthesis and elaboration of PAI-1.

Massive weight loss normalizes initially elevated concentrations of PAI-1 in plasma in severely obese young and middle-aged (29–35, 50, 51) subjects. However, massive weight loss entails serious risk, including lethal arrhythmia (52). Thus, massive weight loss is not suitable for treatment of potentially frail, elderly, obese subjects who may harbor occult age-associated cardiovascular abnormalities and therefore are particularly prone to complications. In a recent study of middle-aged obese individuals, more modest weight loss induced by energy intake restriction combined with exercise training normalized fibrinolysis (29). However, it was not clear whether the weight loss, the exercise, or both were responsible. Exercise training alone augments fibrinolytic system activity in patients with

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**TABLE 1**

Physical characteristics before and after weight loss

<table>
<thead>
<tr>
<th></th>
<th>Before (n = 19)</th>
<th>After (n = 19)</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>61.00 ± 1.12</td>
<td>—</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.00 ± 1.96</td>
<td>—</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.40 ± 3.80</td>
<td>87.80 ± 3.50²</td>
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<tr>
<td>Fat mass</td>
<td>41.70 ± 2.08</td>
<td>35.60 ± 2.21²</td>
</tr>
<tr>
<td>Fat-free mass (mm)</td>
<td>54.70 ± 3.07</td>
<td>52.20 ± 2.93³</td>
</tr>
<tr>
<td>Skinfold thickness (mm)</td>
<td>211.0 ± 8.1</td>
<td>171.0 ± 10.3²</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.890 ± 0.023</td>
<td>0.880 ± 0.020</td>
</tr>
</tbody>
</table>

¹ ± SEM.
²,³ Significantly different from before weight loss: ² P < 0.001, ³ P < 0.005.

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**TABLE 2**

Variables in blood before and after weight loss

<table>
<thead>
<tr>
<th></th>
<th>Before (n = 19)</th>
<th>After (n = 19)</th>
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<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Insulin (mmol/L)</td>
<td>57.0 ± 18.4</td>
<td>29.0 ± 8.7²</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>2.72 ± 0.04</td>
<td>1.88 ± 0.03²</td>
</tr>
<tr>
<td>PAI-1 (nmol/L)</td>
<td>56.40 ± 8.70</td>
<td>36.50 ± 7.58³</td>
</tr>
<tr>
<td>t-PA/PAI-1 (nmol/L)</td>
<td>2.16 ± 0.15</td>
<td>1.76 ± 0.12⁴</td>
</tr>
<tr>
<td>t-PA (nmol/L)</td>
<td>8.02 ± 0.95</td>
<td>6.74 ± 0.73</td>
</tr>
<tr>
<td>PAP complex (nmol/L)</td>
<td>3.22 ± 0.15</td>
<td>3.70 ± 0.21²</td>
</tr>
</tbody>
</table>

¹ ± SEM. PAI-1, plasminogen activator inhibitor type-1; t-PA, tissue-type plasminogen activator; PAP, plasmin/antiplasmin.
²,⁴ Significantly different from before weight loss: ² P < 0.05, ⁴ P < 0.01, ³ P < 0.001, ⁴ P < 0.03.
coronary artery disease (53) and in previously sedentary healthy individuals.

Our study was performed to determine whether elevated PAI-1 concentrations in plasma could be normalized by moderate weight loss induced by means widely applicable to elderly subjects, a growing population in which cardiovascular morbidity and mortality is high (26) and one that will comprise > 30% of the total US population by the year 2000. The 11-wk program was designed to induce moderate weight loss by voluntary restriction of energy intake without inducing imbalances in macro- or micronutrient distribution. The behavioral component of the program was developed to be particularly applicable to the age groups studied, and the therapeutic goals were defined in terms other than aesthetic. The moderate, easily achievable 9 ± 1 kg weight loss induced led to striking improvement of fibrinolytic-system variables. Thus, initially elevated PAI-1 concentrations in plasma decreased by 50%, accompanied by a reciprocal increase in concentrations of PAP complexes and a decrease in t-PA/PAI-1 complexes. To the extent that these changes are associated with a decrease in rate of progression of vasculopathy, moderate weight loss in elderly, obese individuals may be a useful adjuvant to other therapies to reduce cardiovascular morbidity and mortality and lower health care costs for elderly individuals by decreasing the incidence of cardiovascular events requiring hospitalization.

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REFERENCES


