Plasminogen Activator Inhibitor–1, Tissue-Type Plasminogen Activator, and Fibrinogen

Effect of Dieting With or Without Exercise in Overweight Postmenopausal Women

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Abstract This study assessed the short- and long-term effects of an energy-restrictive diet with or without exercise on plasminogen activator inhibitor–1 antigen (PAI-1 Ag) and PAI-1 activity, tissue-type plasminogen activator antigen (TPA Ag), and fibrinogen serum levels. Healthy, overweight postmenopausal women (age, 53.8±2.5 years; body mass index, 25 to 42 kg/m^2; n=121) were randomly assigned to one of three groups: control, 4200-kJ/d diet, or 4200-kJ/d diet with combined aerobic and anaerobic exercise. PAI-1 activity and PAI-1 Ag, TPA Ag, and fibrinogen levels were measured at baseline, after a 12-week intervention, and after a further 6-month follow-up. PAI-1 Ag and activity and TPA Ag were positively correlated with serum triglyceride levels, the abdominal-to–total-body fat ratio (as assessed by total-body dual-energy x-ray absorptiometry), fasting blood glucose, and systolic BP and negatively with HDL cholesterol and sex hormone–binding globulin. The diet led to profound decreases and normalization of PAI-1 activity (≈50%), PAI-1 Ag (≈30%), and TPA Ag (≈29%), but exercise conferred no additional effect. Fibrinogen did not change. At follow-up there were no longer any significant changes ($P>.05$). In conclusion, PAI-1 Ag and activity as well as TPA Ag seem to be part of the metabolic syndrome X. The diet made the blood less thrombogenic in the short term with no effect of the added exercise. (*Arterioscler Thromb Vasc Biol.* 1996;16:381-385.)

Key Words • diet • plasminogen activator inhibitor–1 • fibrinogen • exercise • postmenopausal

In the industrialized world, CVD is currently the leading cause of death and disability in women.[1] Several recently established risk factors for CVD include high levels of PAI-1 activity, TPA Ag, and fibrinogen.[2-5] High levels of PAI-1 activity have been suggested as being part of syndrome X and as the link between insulin resistance and CVD.[3,6] In women, an android sex hormone status may also take part in this syndrome.[7-9]

Weight loss by dieting seems to lower PAI-1 activity and TPA Ag, whereas the reported effects on fibrinogen conflict.[10,11] The effect of adding exercise to an energy-restrictive diet has not been reported. We have shown that 12 weeks of an energy-restrictive diet in overweight postmenopausal women resulted in a 10-kg (13%) weight loss and to profound improvements (20% to 30%) in several cardiovascular risk factors without any additional effect from exercise.[12] At a 6-month follow-up the weight loss was about 8 kg and there were still significant improvements in some of the risk factors for CVD.[13] The present study, which presents an extension of those two reports,[12,13] assessed the short- and long-term effects of an energy-restrictive diet with or without exercise on PAI-1, TPA, and fibrinogen in overweight postmenopausal women. Associations of PAI-1, TPA, and fibrinogen with sex hormones and cardiovascular risk factors are also discussed.

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Methods

Subjects and Study Design

Healthy, overweight postmenopausal women (age, 53.8±2.5 years; body mass index, 25 to 42 kg/m^2; n=121) were initially randomized to one of three groups for 12 weeks of intervention: a diet-only group (n=51), a diet-plus-exercise group (n=49), and a control group (n=21).[12] Briefly, the diet consisted of an obligatory basis of the formula diet NUPO (Oluf Mørk Biochemie A/S), which supplied 65 g protein and 1.6 MJ daily (within which international recommendations are met) and an additional 2.6 MJ daily from food freely chosen according to a "counter diet system."[14] The exercise consisted of supervised combined aerobic exercise (≥70% of VO_2 max) and resistance weight training (≥65% of maximum weight-lifting capacity) for 1 hour, increasing to 1½ hours, three times per week.

There were no statistically significant differences between groups in baseline values; 118 women completed the 12-week study, the results of which are available.[12] Briefly, the maximum oxygen uptake (33% versus 12%) and the loss of fat tissue mass (−9.6 versus −7.8 kg) were significantly increased, whereas the lean tissue mass was preserved (0.0 versus −1.2 kg) in the diet-plus-exercise group compared with the diet-only group. At the end of the 12-week intervention, the women were encouraged to continue to exercise and to use the counter diet system to avoid weight gain. After 6 months on their own, the women

Selected Abbreviations and Acronyms
Ag = antigen
BP = blood pressure
CVD = cardiovascular disease
HDL-C = HDL cholesterol
PAI-1 = plasminogen activator inhibitor–1
SHBG = sex hormone–binding globulin
TPA = tissue-type plasminogen activator

(n=110) were reexamined.[13] Baseline correlations and changes in parameters other than PAI-1, TPA, and fibrinogen are available.[9,12,13]

The study was performed in accordance with the Declaration of Helsinki II and with the approval of the ethics committee of Copenhagen County.

Body Composition

Total as well as abdominal (from the first to the fourth lumbar intervertebral disk) body composition was measured by using a total-body dual-energy x-ray absorptiometry scanner (DPX, Lunar Radiation Corp, software version 3.2).[15-17] Total-body bone mineral density, fat tissue mass, and lean tissue mass were measured. The fat tissue mass is not solely adipose tissue, but the sum of the fatty elements of all the soft tissues. Similarly, the lean tissue mass is not an anatomic entity but represents the sum of all chemically fat-free soft tissue elements.

Biochemistry

Blood samples were taken in the morning after an overnight fast of at least 12 hours. If the time of blood sampling between samples for individual subjects differed by more than 1 hour, the samples for PAI-1 and TPA were not analyzed (due to a well-known individual circadian variation). The samples were stored at −80°C until analyzed.

Blood (4.5 mL) was collected in tubes containing 3.13% citrate (0.5 mL) and centrifuged at 2000*g* for 10 minutes at room temperature. PAI-1 activity was then determined spectrophotometrically by using the reagent spectrolyse/pL (No. 101201, Biopool AB) (intra-assay variation, 4.4% to 9.3%; reference range, 5.5±5 IU/mL). The mean of duplicate measurements was used. PAI-1 Ag and TPA Ag levels were measured by using enzyme-linked immunosorbent assays: COALIZA PAI-1 (intra-assay, 4% to 6%; interassay variation, 6% to 8%; reference range, 40.0±29 ng/mL) and COALIZA t-PA, which determines free TPA Ag as well as TPA Ag bound by PAI-1 (intra-assay variation, 7%; interassay variation, 4% to 7%; reference range, 1 to 12 ng/mL) (both from Chromogenix AB). Fibrinogen was measured photometrically by using a Cobas Mira Plus after proteolytic cleavage with batroxobin (Fibrinogen Kinetic, Boehringer Mannheim, catalogue No. 524484; reference range, 2.0 to 4.0 g/L).

HDL-C was isolated by using a phosphotungstic acid–$MgCl_2$ precipitation technique.[18] Serum total cholesterol, triglyceride, and HDL-C levels were determined enzymatically by Chem1 (CHOD-PAP method, Technicon Instruments). VLDL and LDL cholesterol levels were calculated according to the formula of Friedewald et al.[19]

Total serum testosterone and SHBG, which primarily binds testosterone and is believed to be an indicator of androgen status,[20] were measured in unextracted serum by a radioimmunoassay and an immunoradiometric assay, respectively (Coat-a-count, Diagnostic Products Corp). Estradiol-17-β (anti-17-beta-oestradiol-6-BSA-serum; BioMakor) and androstenedione (specific antibody; Wien Laboratories Inc) were measured by using a radioimmunoassay. The mean of duplicate measurements was used for all hormone determinations.

BP was measured once after 10 minutes of supine rest by using a digital BP meter (A&D) read to the nearest 5 or 10 mm Hg.

TABLE 1. Spearman Rank Correlation Coefficients in Overweight Postmenopausal Women

	PAI-1 Ag (n=87)	TPA Ag (n=79)	PAI-1 Activity (n=90)	Fibrinogen (n=118)
Abdominal/total fat tissue mass	.32†	.29†	.25*	NS
Waist-to-hip ratio	.31†	.37‡	.40‡	NS
Systolic blood pressure	.25*	.25*	.28†	NS
Diastolic blood pressure	NS	NS	NS	.28†
Triglycerides	.39‡	.51‡	.50‡	NS
HDL-C	NS	−.27*	−.26*	NS
Fasting blood glucose	.56‡	.53‡	.54‡	NS
SHBG	−.44‡	−.46‡	−.52‡	NS
Weight	NS	NS	NS	NS
Fat tissue mass	NS	NS	NS	.26†
Lean tissue mass	NS	NS	NS	NS
Total testosterone	NS	NS	NS	.26†
Androstendione	NS	NS	NS	NS
Estradiol	NS	NS	NS	NS
Total cholesterol	NS	NS	NS	NS
LDL cholesterol	NS	NS	NS	NS

*$P<.05$, †$P<.01$, ‡$P<.001$.

Calculations and Statistical Analysis

The abdominal-to–total-body fat tissue mass and waist-to-hip circumference ratios were calculated as indicators of fat distribution.

The Statistical Analysis System (SAS Institute Inc) was used for all analyses. Because a test of normality (proc UNIVARIATE) revealed that PAI-1 activity and PAI-1 Ag, TPA Ag, and fibrinogen levels were not normally distributed ($P<.05$), the nonparametric medians and 95% confidence intervals are reported. Differences in changes between groups were compared by using the nonparametric Kruskal-Wallis test (proc NPAR1WAY). The nonparametric Spearman rank correlation coefficients were also computed. Significance was established as $P>.05$.

Results

As shown in Table 1, PAI-1 activity and PAI-1 Ag and TPA Ag levels were positively correlated with abdominal fat distribution, systolic BP, fasting blood glucose, and serum triglycerides and negatively correlated with HDL-C and SHBG. Fibrinogen, on the other hand, correlated positively with diastolic BP, fat tissue mass, and total testosterone concentration.

The baseline levels of PAI-1 activity and PAI-1 Ag (Table 2) were high compared with the reference ranges of the assays, whereas the levels of TPA and fibrinogen were more within the reference ranges.

After the 12 weeks of intervention, PAI-1 Ag (by ≈30%), TPA Ag (by ≈29%), and PAI-1 activity (by ≈46%) were significantly decreased in both the diet-only and diet-plus-exercise groups compared with the control group (Table 2 and the Figure).

During the follow-up period, PAI-1 Ag, TPA Ag, and PAI-1 activity were significantly increased in both intervention groups compared with the control group (Table 2). Therefore, there were no significant changes in PAI-1 Ag, TPA Ag, and PAI-1 activity from baseline to the end of the follow-up period in either the diet-only or diet-plus-exercise group compared with the control group (Table 2 and the Figure).

TABLE 2. Changes in Fibrinolytic Factors and Fibrinogen in Overweight Postmenopausal Women

	Control (n=13-20)	Diet-Only (n=33-50)	Diet+Exercise (n=33-48)	*P*
PAI-1 activity, IU/mL				
Intervention	−0.5 (−9.3; 5.1)	−5.4 (−28.5; 3.8)	−5.9 (−31.7; 0.9)	.004
Follow-up	−0.5 (−12.6; 6.8)	1.7 (−5.7; 15.9)	1.3 (−11.0; 19.5)	.02
Total	−0.9 (−12.3; 5.2)	−2.8 (−22.4; 8.2)	−5.9 (−24.1; 7.9)	.07
PAI-1 Ag, ng/mL				
Intervention	−5 (−83; 210)	−28.5 (−239; 59)	−36.5 (−496; 21)	.01
Follow-up	−56 (−130; 473)	−2.5 (−50; 498)	−1.0 (−83; 128)	.001
Total	−33 (−180; 455)	−34.5 (−248; 362)	−51.0 (−370; 106)	.5
TPA Ag, ng/mL				
Intervention	−0.1 (−6.5; 2.4)	−1.6 (−5.2; 4.3)	−1.6 (−9; 4.3)	.02
Follow-up	−0.8 (−2.4; 1.6)	0.5 (−5.3; 2.8)	0.8 (−6.2; 4.1)	.01
Total	−0.5 (−7.7; 1.4)	−1.1 (−2.9; 1.2)	−1.3 (−7.3; 0.7)	.6
Fibrinogen, g/L				
Intervention	0.0 (−0.4; 0.9)	0.0 (−0.8; 0.9)	−0.1 (−1.8; 0.7)	.2
Follow-up	0.3 (−0.7; 1.1)	0.1 (−0.9; 1.2)	0.0 (−1.0; 1.0)	.4
Total	0.5 (−0.3; 1.1)	0.1 (−0.7; 1.1)	−0.1 (−1.6; 1.0)	.01

Values shown are median (95% confidence interval). Baseline values were PAI-1 activity, 11.1 (0; 37.6); PAI-1 Ag, 91.0 (30; 350); TPA Ag, 5.2 (2.9; 18.8); and fibrinogen, 2.8 (2.2;4.0). Probability values reflect the Kruskal-Wallis test of differences in changes between the three groups. Intervention indicates changes after 12 weeks compared with baseline; Follow-up, changes in follow-up period after 9 months compared with 12 weeks; and Total, changes after 9 months compared with baseline (combined intervention and follow-up period).

There was no change in fibrinogen level after the 12 weeks of intervention. However, fibrinogen was significantly decreased from baseline to the end of the follow-up period in the diet-only and diet-plus-exercise groups compared with the control group due to a nonsignificant increase during the follow-up period in the control group.

There were no significant differences between changes in the diet-only and diet-plus-exercise groups in PAI-1 activity or PAI-1 Ag, TPA Ag, or fibrinogen levels after the intervention or at follow-up ($P>.05$).

Discussion

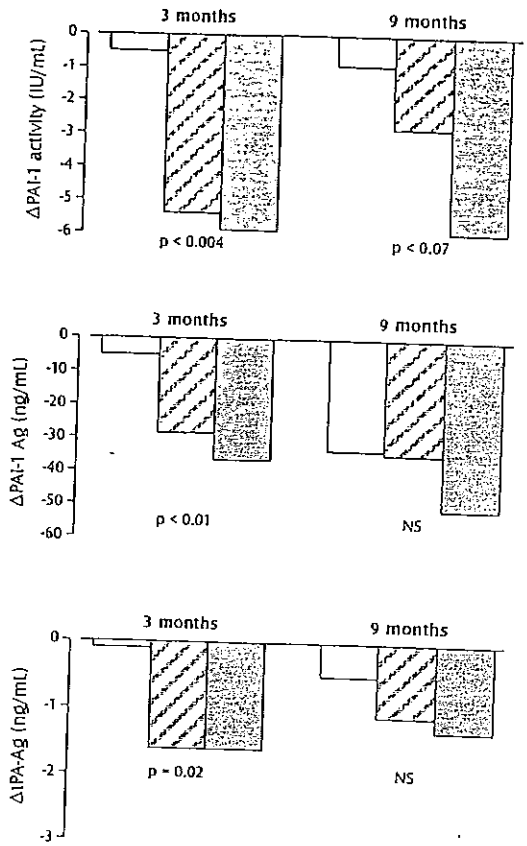
Our results agree with the existence of a metabolic syndrome X within healthy, nondiabetic, normotensive, overweight, postmenopausal women. This syndrome seems to include not only an abdominal fat distribution, low HDL-C levels, hypertriglyceridemia, high BP, and high blood glucose but also a high PAI-1 activity as well as an android sex hormone status (as indicated by the negative association with SHBG). This is in accordance with previous reports.[3,7,21-24] We and others have found that fibrinogen is associated with obesity.[2,11,25,26] On the other hand, in the present overweight postmenopausal women, fibrinogen was not associated with fat distribution, which is in contrast to the findings of others.[25,27]

In our randomized controlled study of healthy overweight postmenopausal women, PAI-1 Ag and PAI-1 activity were increased at baseline. The diet led to a profound decrease in PAI-1 Ag but also to a decrease in TPA Ag. There was an ≈50% decrease in the PAI-1 activity, which dropped into the reference range of the assay. On the other hand, there was no effect on fibrinogen levels, which were within the normal range from baseline. Besides our study, only one randomized controlled study of the effect of weight loss by dieting on PAI-1 Ag, TPA Ag, and fibrinogen levels is available.[11] Six months of dieting with a weight loss of 7 to 9 kg led to a 31% decrease in PAI-1 Ag and a 24% decrease in TPA Ag but no significant changes in fibrinogen. As reviewed by Folsom et al,[11] this is in accordance with uncontrolled studies of PAI-1 activity and PAI-1 Ag and TPA Ag levels. However, the effect of weight loss by dieting on fibrinogen in uncontrolled studies differs between the studies.[10,11] The importance of control groups in intervention studies of fibrinolytic factors and fibrinogen is stressed by the spontaneous variation with season in these parameters.[28,29] In our study PAI-1 Ag and TPA Ag tended to decrease and fibrinogen to increase in the control group during the follow-up period. This may result from a seasonal variation, since the intervention was during the summer and the follow-up was during the winter.

Myocardial infarction and reinfarction seem to be associated with high levels of PAI-1 activity,[5,30] and spontaneous lysis of thrombi seems to be dependent on PAI-1 activity.[31] Recent studies have shown a significant improvement in survival after myocardial infarction when treatment includes dietary intervention.[32,33] A possible mechanism could be a positive effect of the dietary intervention on thrombogenesis through decreased coagulation (lowering of fibrinogen) and increased fibrinolysis (lowering of PAI-1 activity).

Exercise in general has a positive effect on health.[34] The nonacute effect of exercise without energy restriction on PAI-1 and fibrinogen is not clear.[29,35-37] The effect of addition of exercise to an energy-restrictive diet has never been reported. In our study of overweight postmenopausal women there was no additional effect of exercise on PAI-1 activity, PAI-1 Ag, TPA Ag, or fibrinogen. An explanation for the lack of effect of exercise may be the profound effect of the diet itself, which may leave no room for a relatively weaker exercise effect.

At the follow-up 6 months after the intervention, there were no longer any convincing significant changes in PAI-1 activity, PAI-1 Ag, TPA Ag, or fibrinogen. The women in the two intervention groups had gained only



Changes in fibrinolytic factors after 3 months of intervention and an additional 6 months of follow-up (9 months total) in overweight postmenopausal women. Probability values reflect the Kruskal-Wallis test of differences in changes between the three groups. Open bars indicate control group; hatched bars, diet-only group; gray bars, diet-plus-exercise group.

about 2 kg and had thus maintained an ≈8-kg loss in body weight. This could indicate that fibrinolysis is more influenced by the energy-restrictive diet than by weight loss.

In summary, the balance between coagulation and fibrinolysis in overweight postmenopausal women tended to become less thrombogenic in the short term due to the diet with no additional effect of exercise.

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