Intense Nonpharmacological Intervention in Subjects With Multiple Cardiovascular Risk Factors: Decreased Fasting Insulin Levels But Only a Minor Effect on Plasma Plasminogen Activator Inhibitor Activity

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Increased plasma levels of insulin and the fibrinolytic inhibitor, plasminogen activator inhibitor type 1 (PAI-1), are two new cardiovascular risk factors. The extent to which these two risk factors can be affected by nonpharmacological intervention modalities has not been convincingly proven in subjects at high risk for cardiovascular disease. This study assesses the effects on fasting plasma insulin and plasma PAI-1 activity of an intervention program including a low-fat, high-fiber diet and regular daily physical exercise. The intervention was implemented by a 1-month learning and training session in a full-boarding wellness center and included a follow-up evaluation after 12 months. The study was conducted on 108 subjects (31 men and 77 women) referred for multiple risk factor treatment. After 1 month of intense intervention, the physical condition improved significantly, and this effect was maintained during the year. The body mass index (BMI), fasting plasma insulin, and plasma lipids were significantly decreased. In women, PAI-1 activity was significantly reduced. At follow-up study in both sexes, the BMI and fasting insulin were still significantly decreased. In women, PAI-1 activity reverted to the preintervention level. In subjects with multiple risk factors, ie, the insulin resistance syndrome, the plasma insulin level can be reduced by an intense nonpharmacological program. Also, at least in women, plasma PAI-1 activity can be modestly modified. However, the magnitude of the decrease in PAI-1 activity was probably too small to reverse the hypofibrinolytic state characteristic of these subjects.

IN RECENT YEARS, a number of new hemostatic and metabolic factors have been identified as indicators of an increased risk to develop cardiovascular disease.[1,2] A special clustering of risk factors has also been identified, referred to as the insulin resistance syndrome.[3-6] In this syndrome, an increased peripheral insulin resistance is coupled to obesity, especially of the upper-body type, hypertriglyceridemia, high plasma insulin, and hypertension. It appears that impaired fibrinolysis, with low tissue plasminogen activator (tPA) activity and high plasminogen activator inhibitor type 1 (PAI-1) activity, also is part of this syndrome.[7,8]

The association of high plasma levels of insulin and the hemostatic risk factor PAI-1 with an increased cardiovascular risk in prospective studies[9-13] raises the question of whether it is possible to alter these factors by intervention. It has been shown that PAI-1 activity is higher in physically inactive compared with physically active healthy individuals.[14] PAI-1 activity can be acutely reduced in healthy adult men by maximal exercise.[15] Bicycle training acutely decreases PAI-1 activity in post–myocardial infarction patients, but not to the level of age-matched healthy individuals.[14] Six months of intense endurance exercise resulted in a significant reduction in PAI-1 activity in a group of elderly healthy men,[16] as did 3 months of daily running in young sedentary men.[17]

Dietary intervention has also been tried to improve fibrinolysis. A reduction of the fat content in the diet from 40 energy% to 30 energy% proved to be ineffective on the fibrinolytic system.[18,19] A fish oil food supplement containing a high proportion of ω-3-polyunsaturated fatty acids also seemed to affect fibrinolysis only marginally or not at all.[20] An epidemiological study showed that frequent intake of vegetables and fruit was associated with low levels of PAI-1 activity.[21] In a small-scale experimental study, fiber food supplementation resulted in reduced plasma PAI-1 activity.[22] In another experimental low-fat, high-fiber diet study, PAI-1 activity remained unchanged but tPA activity increased significantly.[23] These observations must be interpreted with caution, as changes in body weight are known to have a great impact on fibrinolytic variables.[24-26]

Several studies have shown strong positive associations between the levels of insulin, triglycerides, and PAI-1 activity, respectively.[8,27] An attempt to decrease triglycerides with the lipid-lowering agent gemfibrozil also significantly decreased insulin levels.[28] Using the antidiabetic biguanide metformin to decrease serum insulin also reduced serum triglycerides and PAI-1 activity.[29]

The aim of the present study was to evaluate the effects of an intense 1-month life-style intervention with a fat-reduced, fiber-rich food regimen and increased physical activity on fasting insulin and PAI-1 activity in subjects with multiple cardiovascular risk factors. A second aim was to evaluate the long-term effects of the life-style intervention at a 12-month follow-up study.

SUBJECTS AND METHODS

Subjects and Study Design

One hundred fifty-four patients consecutively referred to Sorsele Wellness Center from September 1990 to May 1991 for modification of cardiovascular risk factors were considered for this uncontrolled intervention study. Forty-five subjects who declined to participate (n = 14) in the study or did not attend their 12-month follow-up appointment (n = 31) were excluded from the study. Individuals not participating in the follow-up study were contacted by phone. One

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participant had a decrease in PAI-1 activity during the first month of intervention of greater than 5.5 standard deviations. Clinical history and laboratory findings suggested an unusually high intake of alcohol in the period before the study, and after considering known experimental findings regarding the association between alcohol and PAI-1 activity,[30] he was excluded from the study. The remaining 108 patients, 31 men aged 31 to 64 years and 77 women aged 17 to 67 years, formed the basis of this study. The participants were referred because of the combination of overweight and other cardiovascular risk factors, ie, hypertension, atherogenic lipid profile, and/or impaired glucose tolerance. At the start of the study, 34% of the participants (n = 104) were using antihypertensive, 3% lipid-lowering, 5% antidiabetic, and 8% heart medication. Approximately 60% of the participants used none of these medications. Eighty-four patients (78% of the participants) had a body mass index (BMI) of 30 kg/m^2 or greater. Six participants (five men) had a history of angina and four (three women) a history of myocardial infarction 1 to 4 years before the study. One of the postmyocardial infarction patients had severe angina. The other three were considered as uncomplicated. Forty-six percent (35 of 77) of the women were considered postmenopausal when using 51 years as the median age of menopause in the population. Three women were using estrogen replacement therapy.

The life-style modification program was implemented during a 1-month stay with full boarding at Sorsele Wellness Center, owned by local health authorities and admitting patients from Västerbotten County on referral from general practitioners and hospital clinics. The training program included approximately 2 to 2.5 hours of daily scheduled aerobic physical activity of low to moderate intensity such as brisk walks, gymnastics, bicycling, and swimming. The intensity of the physical exercise was aimed at a level of 60%, and for short periods up to 80%, of the estimated age-adjusted peak heart rate. The diet during the stay was calculated to contain approximately 20% of energy from fat and a relatively high fiber content, ie, with particular emphasis on vegetables and root vegetables. Table 1 shows the daily mean intake of certain important nutrients served at the wellness center. The mean values were calculated from the menus of 3 randomly selected days. The recommended portion sizes were calculated to give a daily energy intake of 7.6 MJ in men and 6.3 MJ in women. The participants were instructed only to eat the food provided by the wellness center. Consumption of alcohol was not allowed, and smoking cessation was strongly encouraged.

A physical examination was performed on arrival at the wellness center, after 1 month of intervention, and at follow-up study after 12 months. A single blood pressure measurement was performed after 5 minutes' rest with the subjects in the recumbent position. Body weight was measured using an electronic balance. During weighing and measurement of height, the subjects removed their shoes but wore a shirt and trousers. The BMI was calculated as weight in kilograms divided by height in meters squared. The circumference of the smallest part of the waist and the thickest part of the hip in the standing position was measured, and the waist to hip ratio was calculated. Blood samples for lipids, insulin, and PAI-1 activity were obtained, and oxygen consumption was assessed by an ergometer bicycle test. A self-rating questionnaire concerning dietary habits, alcohol intake, and level of physical activity was administered at the beginning of the study and sent home to the participants at 6 and 24 months from baseline. When the participants were sent home after the first month of intense training, they were supplied with a home-training program to maintain the changes in dietary habits and physical activity. The program was based on four principles: to increase the fiber content in the breakfast, to increase the amount of vegetables and root vegetables at lunch and dinner, to keep a record of food intake in excess of regular meals, and to engage in physical exercise for at least 30 minutes two to three times or more per week.

The patients provided informed consent according to the Helsinki Declaration. The study was approved by the Research Ethics Committee of Umeå University.

Table 1. Daily Intake of Certain Nutrients per 6.3 MJ at Sorsele Wellness Center

Nutrient	Value
Carbohydrates	
g	236
Energy%	64
Fat	
g	31
Energy%	18
Protein	
g	65
Energy%	18
Fiber (g)	32
Vitamin E (mg)	5.8
Ascorbic acid (mg)	299
Cholesterol (mg)	21
Saturated fatty acids (g)	10.8
Monounsaturated fatty acids (g)	8.4
Polyunsaturated fatty acids (g)	9.1
P/S ratio	0.9

NOTE. Values are the mean intake from 3 randomly selected days.
Abbreviation: P/S, polyunsaturated to saturated fat.

Estimation of Oxygen Consumption

Maximal oxygen consumption was estimated using the linear relationship between heart rate, workload, and oxygen uptake. The heart rate was measured during submaximal exercise using a bicycle ergometer (model 668; Monark, Varberg, Sweden). Age-adjusted and gender-specific tables of heart frequency in relation to workload were used.[31] The ability of the submaximal test to predict maximal oxygen uptake has been questioned, and differences of up to 30% between actual and predicted maximal oxygen consumption have been reported.[32] However, using the submaximal exercise test to predict maximal oxygen consumption on a group level seems justified.[33]

Blood Sampling

Blood samples were drawn in the morning (7:30 to 10:00 AM) after an overnight fast to reduce confounding by the known diurnal variation of fibrinolytic variables. No physical exercise was permitted at least 8 hours before blood sampling. Venous blood was taken in EDTA tubes to obtain plasma for analyses of insulin and PAI-1 activity. The plasma samples were snap-frozen within 1 hour and stored at −80°C. Serum was used for the lipid variables, which were analyzed on fresh samples.

Laboratory Procedures

The activity of PAI-1 in plasma was determined with an activity assay based on adding an excess of tPA to the sample (40 IU/mL) and quantifying the remaining tPA with the activity assay. The activity is given in arbitrary units per milliliter, where 1 U PAI-1 is defined as the amount of PAI-1 that inhibits 1 IU of the international tPA standard. The reagent kit for assay of tPA and PAI-1 activity (Spectrolyse/Fibrin) was purchased from Biopool (Umeå, Sweden).[34,35] Plasma insulin was determined by radioimmunoassay (Phadeseph Insulin RIA; Pharmacia Diagnostics, Uppsala, Sweden). Serum cholesterol and triglycerides were determined by an enzymatic method using kits (Boehringer, Mannheim, Germany). The serum high-density lipoprotein (HDL) cholesterol level was measured after precipitation of the other lipoproteins with sodium phosphowolframate/magnesium chloride.

Statistical Analysis

The Statistical Analysis System version 6.11 (SAS Institute, Cary, NC) was used. Some of the main variables (PAI-1 activity, oxygen consumption, fasting plasma insulin, and serum triglycerides) had a skewed distribution. Logarithmic transformation of these variables did not alter the results of the study. Distributions are expressed as the mean and standard error of the mean (SE) or 95% confidence interval (CI) of the mean. Hypothesis testing on differences in mean values was made by repeated-measures ANOVA[36] and using contrasts where baseline values acted as controls. A double-sided unpaired Student's *t* test was also used. Differences in proportions were tested by Fisher's exact test. Spearman's correlation coefficients were calculated. Multiple regression analyses were performed using the stepwise strategy.

RESULTS

Clinical and laboratory parameters before and after intervention are shown in Table 2. There were no significant differences in age between the genders. The men were aged 48.7 ± 9.1 years (mean ± SD), and women 47.2 ± 11.9 years. During the month of intense intervention at the wellness center, the body weight, BMI, waist to hip ratio, blood pressure, cholesterol, triglycerides, and HDL cholesterol were significantly reduced in both men and women. There was also a small reduction in fasting plasma glucose. However, at 12 months' follow-up study, serum levels of cholesterol and triglycerides and blood pressure in men had returned to preintervention levels. In contrast, the body weight, BMI, waist to hip ratio, and blood pressure in women were still reduced. In both sexes, there was a significant decrease in HDL cholesterol during the first month of intense treatment. In contrast, at follow-up study after 12 months, HDL cholesterol was increased significantly compared with baseline. During the first month of training, oxygen consumption increased approximately 25% to 30% ($P < .001$), and this increased level of physical fitness was maintained during the whole year of observation. At the start of the study, 15 participants (five men and 10 women) were smokers. During the month of intense intervention, five participants stopped smoking. At follow-up study, two of these had resumed smoking. The nonattendants at follow-up study had approximately the same age distribution as the study group. A phone interview was possible in 20 of these individuals. On average, they reported a weight decrease during 12 months of the same magnitude as the study group, 5.4 kg in men and 6.2 kg in women.

Among the main variables, there was a significant decrease in fasting insulin during the first month of intense treatment, and this was also confirmed at follow-up study. PAI-1 activity was reduced during the first intense part of the treatment, but the decline reached significance only in women ($P < .05$). In the samples obtained at 12 months, PAI-1 activity was reverted to levels not significantly different from the levels at entry.

Table 3 shows there was a significant positive correlation between the body weight, BMI, waist to hip ratio, and fasting insulin, respectively. A low BMI was associated with a low fasting insulin. This was applicable to both men and women and to all three measurement occasions. In addition, there was also a positive correlation, especially in men, between fasting insulin and triglycerides. The same pattern of relationships, although weaker, was seen between PAI-1 activity and the other cardiovascular risk factors.

To detect and quantify the impact of a change in the BMI on fasting insulin and PAI-1 activity, the whole group of participants (men and women) were dichotomized according to the degree of change in the BMI during the first month of intense treatment ($\Delta_{0\text{-}1}$BMI) and between baseline and follow-up study after 12 months ($\Delta_{0\text{-}12}$BMI), respectively. The short-term difference in body weight ($\Delta_{0\text{-}1}$BMI) did not contribute to a significant change between the subgroups in oxygen consumption and fasting insulin level (Table 4). The difference in PAI-1 activity

Table 2. Clinical and Laboratory Characteristics Before Intervention, After a 1-Month Period of Diet and Exercise, and at Follow-up Study at 12 Months (mean ± SE)

Characteristic	Men (n = 31)			Women (n = 77)		
	Before	1 Month	12 Months	Before	1 Month	12 Months
Body weight (kg)	107.2 ± 3.7	101.5 ± 3.5‡	101.3 ± 3.8‡	87.0 ± 1.3	82.7 ± 1.3‡	82.1 ± 1.3‡
BMI (kg/m²)	33.6 ± 1.0	31.8 ± 1.0‡	31.7 ± 1.1‡	32.6 ± 0.5	31.0 ± 0.5‡	30.8 ± 0.5‡
Oxygen consumption (L/min)	2.1 ± 0.06	2.7 ± 0.09‡	2.7 ± 0.12‡	2.1 ± 0.05	2.6 ± 0.05‡	2.6 ± 0.06‡
Physical fitness (mL O_2/kg · min)§	20.4 ± 0.9	27.7 ± 1.3‡	28.1 ± 1.8‡	24.2 ± 0.6	32.1 ± 0.7‡	31.8 ± 0.9‡
Fasting insulin (μU/mL)	15.0 ± 1.3	11.3 ± 1.2‡	12.6 ± 1.2*	12.5 ± 0.7	10.3 ± 0.6‡	10.9 ± 0.6‡
PAI-1 (U/mL)	30.0 ± 3.4	25.1 ± 4.4	28.4 ± 4.3	26.7 ± 2.0	22.6 ± 1.5*	26.3 ± 2.0
Waist to hip ratio	0.98 ± 0.01	0.96 ± 0.01†	0.96 ± 0.01*	0.90 ± 0.008	0.89 ± 0.008*	0.88 ± 0.008‡
Systolic BP (mm Hg)	137 ± 4.2	131 ± 3.2*	139 ± 2.9	135 ± 2.2	127 ± 1.8‡	128 ± 1.9‡
Diastolic BP (mm Hg)	86 ± 2.0	81 ± 2.1†	83 ± 1.9	81 ± 1.3	77 ± 1.2‡	77 ± 1.2†
Cholesterol (mmol/L)	6.8 ± 0.3	5.3 ± 0.3‡	6.6 ± 0.2	6.4 ± 0.17	5.2 ± 0.13‡	6.5 ± 0.16
Triglycerides (mmol/L)	2.5 ± 0.2	1.6 ± 0.1‡	2.2 ± 0.2	1.8 ± 0.07	1.5 ± 0.07‡	1.8 ± 0.10
HDL cholesterol (mmol/L)	0.97 ± 0.05	0.87 ± 0.04*	1.17 ± 0.07‡	1.14 ± 0.03	0.98 ± 0.02‡	1.3 ± 0.03‡
Fasting plasma glucose (mmol/L)	5.1 ± 0.4	4.7 ± 1.4*	5.8 ± 3.0‖	4.7 ± 0.2	4.4 ± 0.1*	4.9 ± 0.2¶

NOTE. Hypothesis testing for statistical significance of differences between 1 month *v* before and 12 months *v* before was performed with repeated-measures of ANOVA and using contrasts.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

§Measured as oxygen uptake per kilogram body weight and corrected for age and sex (Åstrand's nomogram).

‖n = 23.

¶n = 63.

Table 3. Spearman Correlation Coefficients Between Fasting Insulin, PAI-1, and Certain Known Cardiovascular Risk Factors

	Fasting Insulin			PAI-1		
Risk Factor	Before	1 Month	12 Months	Before	1 Month	12 Months
Body weight						
Men	.61‡	.56†	.71‡	.36*	.47†	.38*
Women	.47‡	.50‡	.45‡	.42‡	.47‡	.55‡
BMI						
Men	.72‡	.57‡	.71‡	.37*	.44*	.50†
Women	.42‡	.51‡	.47‡	.53‡	.54‡	.54‡
Oxygen consumption						
Men	−.15	−.02	−.45*	.13	−.17	−.39*
Women	.17	.12	−.002	.21	.08	.12
Waist to hip ratio						
Men	.47†	.54†	.62‡	.36†	.54†	.51†
Women	.47‡	.45‡	.46‡	.42‡	.38‡	.46‡
Systolic BP						
Men	.24	.50†	.17	.10	.46*	.06
Women	.21	.16	.20	.01	−.13	−.02
Diastolic BP						
Men	.26	.45*	.20	.25	.49†	.29
Women	.26*	.05	.07	.16	.01	−.01
Cholesterol						
Men	−.17	.33	.24	.18	.14	.38*
Women	−.02	.04	−.23*	−.18	.06	−.16
Triglycerides						
Men	.47†	.61‡	.62‡	.48†	.56†	.68‡
Women	.25*	.49‡	.26*	.21	.41‡	.31†
HDL cholesterol						
Men	.06	−.29	−.16	.16	−.21	−.40*
Women	−.16	−.26*	−.26*	−.22	−.29*	−.43‡

*$P < .05$.
†$P < .01$.
‡$P < .001$.

between the subgroups was more pronounced, even if it did not reach statistical significance. At follow-up study, the half of the participants with the largest decrease in the BMI, also had the largest decrease in fasting insulin and PAI-1 activity. They also had a higher increase in oxygen consumption.

To evaluate the importance of a change in triglycerides on fasting insulin and PAI-1 activity, the participants were divided in a similar fashion. Table 5 shows that during the first month of intervention, there was no difference between the subgroups in the BMI or oxygen consumption. Fasting insulin was significantly more reduced in the group with a large change in triglycerides. There was also a large, although nonsignificant, decrease in PAI-1 activity. There were no differences in the change in fasting insulin or PAI-1 activity between the high- and low-triglyceride subgroups during the whole year of observation. This was despite the fact that the group with the large change in triglycerides also had a large decrease in the BMI.

Table 6 shows the results of a self-rating questionnaire administered to the participants at 6 and 24 months from baseline. The purpose of the questionnaire was to estimate how well the subjects followed the prescribed diet and level of physical activity. There were no major differences in food habits at baseline between our study group and the general population in northern Sweden with the same age distribution and after adjustment for differences in gender.[37] The frequency of consumption of breakfast with a high fiber content was 44% in our study, and the frequency of consumption of low-fat margarine or no fat on a sandwich was 52%, and of regular use of root vegetables 49% (Table 6). The corresponding figures in the northern Sweden multinational monitoring of trends and determinants in cardiovascular disease (MONICA) survey of 1990 were calculated to be 39%, 57%, and 46%, respectively. There was a significant increase at both 6 and 24 months in the proportion of participants who reported eating a high-fiber breakfast, using fat-free or low-fat margarine on bread, and performing physical exercise at least twice per week. They also consumed more root vegetables ($P = .05$). Generally, there was low alcohol consumption among the participants. According to the questionnaire, more than 90% of the subjects used strong beer, wine, and/or hard liquor less than once per week (data not shown).

Stepwise multiple regression analyses were performed with fasting insulin and PAI-1 activity, respectively, as response variables. To avoid colinearity problems, all multiple regression models were kept restricted and the criterion for the explanatory variables to enter and stay in the models was a significance level of less than .15. Sex was included in all models as a class variable and remained nonsignificant. The first two models (PAI-1 activity) included the change in PAI-1 activity during the first month (12 months) of intervention as the response variable and the corresponding changes during the first month (12 months) in the BMI, oxygen consumption, fasting plasma insulin, and serum triglyceride as the explanatory variables. The coefficient of determination (R^2) was .15 (.13), and the only significant explanatory variable during the first month of intense intervention was the change in fasting insulin ($P < .01$). At follow-up study, the change in fasting insulin ($P < .01$) and change in the BMI ($P < .05$) contributed significantly to the model. Two similar models (fasting insulin) with the change in fasting insulin as a response variable and the change in the BMI,

Table 4. Comparison of the Change in Mean Values and Calculation of the Difference and 95% CI After All Participants Were Divided Into Two Groups Depending on the Change in BMI Between Baseline, 1 Month ($\Delta_{0\text{-}1}$), and 12 Months ($\Delta_{0\text{-}12}$) of Treatment, Respectively

	$\Delta_{0\text{-}1}$BMI				$\Delta_{0\text{-}12}$BMI			
Parameter	High (n = 54)	Low (n = 54)	Difference	95% CI	High (n = 54)	Low (n = 54)	Difference	95% CI
ΔBMI (kg/m²)	−2.1	−1.2	−0.9	−1.1, −0.8	−3.2	−0.4	−2.8	−3.2, −2.4
ΔO_2 uptake (L/min)	0.57	0.52	0.05	−0.05, 0.15	0.64	0.41	0.23	0.06, 0.40
ΔfP insulin (μU/mL)	−3.0	−2.5	−0.5	−2.4, 1.3	−3.0	−0.7	−2.3	−3.9, −0.7
ΔPAI-1 (U/mL)	−6.8	−1.7	−5.1	−10.6, 0.4	−4.7	2.8	−7.5	−13.2, −1.9

Abbreviation: fP, fasting plasma.

Table 5. Comparison of the Change in Mean Values and Calculation of the Difference and 95% CI After All Participants Were Divided Into Two Groups Depending on the Change in Serum Triglycerides Between Baseline, 1 Month ($\Delta_{0\text{-}1}$), and 12 Months ($\Delta_{0\text{-}12}$) of Treatment, Respectively

	$\Delta_{0\text{-}1}$TG				$\Delta_{0\text{-}12}$TG			
Parameter	High (n = 54)	Low (n = 54)	Difference	95% CI	High (n = 53)	Low (n = 54)	Difference	95% CI
ΔTG (mmol/L)	−0.9	0.0	−0.9	−1.1, −0.7	−0.5	0.4	−0.9	−1.1, −0.8
ΔBMI (kg/m²)	−1.7	−1.6	−0.1	−0.3, 0.1	−2.5	−1.2	−1.3	−1.9, −0.6
ΔO_2 uptake (L/min)	0.54	0.55	−0.01	−0.11, 0.09	0.60	0.44	0.16	−0.02, 0.34
ΔfP insulin (μU/mL)	−4.1	−1.3	−2.8	−4.6, −1.1	−2.1	−1.6	−0.5	−2.1, 1.2
ΔPAI-1 (U/mL)	−6.2	−2.4	−3.8	−9.3, 1.8	−0.9	−1.0	0.1	−5.7, 6.0

Abbreviation: TG, triglycerides.

oxygen consumption, and triglycerides as explanatory variables showed an R^2 of .10 (.07), with the change in triglycerides ($P < .01$) as the only significant explanatory variable in the first month of intervention and the change in the BMI ($P < .01$) as the only significant variable at follow-up study.

DISCUSSION

In the present study, there was a significant decrease in PAI-1 activity in women during the first month of controlled and intense intervention. Concomitantly, in both men and women, there was a significant decrease in the BMI, a significant increase in oxygen consumption, and a significant decrease in serum fasting insulin, verifying that the intervention was efficiently implemented. At 12-month follow-up study, the subjects still had a significantly lower BMI and higher oxygen consumption, implying, on average, a continued change in dietary habits and increased physical activity. This was also indicated by the self-rating questionnaires that were sent to the participants at 6 and 24 months from baseline. Furthermore, at follow-up study, there was still a significantly lower fasting insulin, but PAI-1 activity was reverted toward the preintervention level. One special problem accompanying the measurement of PAI-1 activity and, to some extent, fasting insulin is the large intraindividual variability.[38] One explanation for this could be the known diurnal variation in PAI-1 activity. For practical reasons, blood sampling was performed at any time during a 2.5-hour period in the morning. A relative reduction in PAI-1 activity three times the highly significant decrease in the BMI did not reach significance. Nevertheless, the intervention influenced the insulin level to a greater extent than PAI-1 activity, and also for the whole duration of the study.

Table 6. Changes in Food Habits and Physical Activity Measured by a Self-Rating Questionnaire at Baseline, 6 Months, and 24 Months of Follow-up Study

	All Subjects (%)		
Parameter	Baseline (n = 60)	6 mo (n = 60)	24 mo (n = 60)
Breakfast with high fiber content (ie, cereal or porridge)	44	73†	66*
Fat-free or low-fat margarine on sandwich	52	67†	67†
Use of root vegetables ≥3-5 times per week	49	75	81
Physical activity ≥2-3 times per week	63	85†	74†

NOTE. Hypothesis testing for differences in proportions between 6 months and baseline and 24 months and baseline was performed with Fisher's exact test.

*$P < .05$.

†$P < .001$.

Changes in body weight and body fat distribution together with changes in triglycerides seemed to have the largest influence on fasting insulin and PAI-1 activity (Table 3). The half of the participants who had the largest decrease in BMI at follow-up study also had a significant decrease in both fasting insulin and PAI-1 activity compared with the half of the participants who had the smallest weight loss (Table 4). We do not think this difference was due to regression toward the mean. There was no difference in the baseline BMI between the half of the participants with the largest weight decrease and the half with the smallest weight decrease at follow-up study. This result is reminiscent of earlier studies (using other assays for fibrinolytic activity) by Ogston and McAndrew,[39] which suggested that an improved fibrinolytic activity persisted during active weight loss but reverted when body weight reached a plateau or even when the weight decline was decelerated. The reduction in PAI-1 activity in our study obtained after a 1-month intervention period of substantially negative calorie balance seemed to persist during the whole year (11 months) after discharge, provided no reversion to a higher body weight took place during that period.

It must be emphasized that the participants in this study were referred for treatment because of multiple cardiovascular risk factors. PAI-1 activity in the study group was generally pathologically high, considering the clinical criterion for hypofibrinolysis of 15 U/mL used in our laboratory.[21] Most intervention studies,[18,22,23] but not all,[40] that favorably affected tPA activity and/or PAI-1 activity have been performed in healthy subjects. The long-term (months) effects of physical exercise on PAI-1 activity reported in elderly men and in young sedentary men were observed in apparently healthy individuals.[16,17] The low impact on PAI-1 activity levels in this study can consequently be a reflection of the differences in the pattern of PAI-1 change between healthy subjects and subjects with multiple cardiovascular risk factors. It could also be attributed to a too-low intensity in the intervention. However, the magnitude of change in weight and aerobic fitness in this study seems to represent the maximum achievable on a long-term basis in a group of multirisk patients. A possible limitation of our study is that we do not account for constitutional, possibly genetic, differences that may determine how PAI-1 activity reacts to life-style change.[41]

Multiple regression analyses showed that changes in PAI-1

activity could be predicted by changes in fasting insulin and BMI. Furthermore, changes in fasting insulin were predicted by changes in serum triglycerides. This triad of interrelated associations has been reported in several previous studies,[7,27] and was also recently shown by our group to be present in a cross-sectional population-based study.[8] Similar results were also seen in a recent observational study where insulin sensitivity was estimated by the minimal model method.[42] Strong support for this reasoning comes from two pharmacologic intervention studies. Administration of the antidiabetic biguanide metformin, which is considered to decrease insulin resistance and thereby fasting insulin, also decreased PAI-1 activity.[29] Administration of the lipid-lowering agent gemfibrozil for the purpose of decreasing serum triglycerides also decreased fasting insulin.[28] Taken together, all of these studies are in line with recent experimental data supporting the idea of insulin or its precursors as regulators of plasma PAI-1 activity, either directly by enhancing the production of PAI-1 in endothelial or liver cells[43,44] or indirectly by increasing the synthesis of the triglyceride-rich very–low-density lipoprotein (VLDL) from the liver.[45] Increased VLDL levels, at least in vitro, stimulate endothelial cells to increase the production and/or release of PAI-1.[46]

The interplay between the control of PAI-1 levels by factors amenable to life-style changes and genetic control is a challenging subject of future studies. Such studies should attempt to reduce the large intraindividual variability of PAI-1 activity by using repeated measurements on every intervention level.

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