Effect of Weight Change and Metformin on Fibrinolysis and the von Willebrand Factor in Obese Nondiabetic Subjects

The BIGPRO1 Study

M. ALINE CHARLES, MD
PIERRE MORANGE, MD
EVELINE ESCHWÈGE, MD
PHILIPPE ANDRÉ, MD
PHILIPPE VAGUE, MD, PHD
IRÈNE JUHAN-VAGUE, MD, PHD
ON BEHALF OF THE BIGPRO STUDY GROUP

OBJECTIVE — Insulin resistance is associated with hypofibrinolysis. Metformin has been shown to improve insulin sensitivity and fibrinolysis. Its action on fibrinolysis and the von Willebrand factor was evaluated in the Biguanides and the Prevention of the Risk of Obesity (BIGPRO)1 trial in nondiabetic men (n = 151) and women (n = 306) aged between 34 and 65 years with a central fat distribution and a mean BMI of 32.5 kg/m^2.

RESEARCH DESIGN AND METHODS — The subjects were randomly allocated to a 1-year treatment with metformin (850 mg b.i.d.) or placebo, in addition to diet and exercise recommendations.

RESULTS — Plasminogen activator inhibitor 1 (PAI-1) activity and antigen decreased significantly but similarly by 30 and 40%, respectively, in both the placebo and the metformin groups. This decrease occurred mainly in subjects who lost weight. Metformin did not have any significant additional effect on PAI-1. In contrast to the results for PAI-1, there was a significantly greater decrease in tissue-type plasminogen activator (tPA) antigen in the metformin than in the placebo group (mean ± SD: −1.1 ± 3.1 vs. 0.2 ± 3.2 ng/ml, $P < 0.02$). The von Willebrand factor (vWF) also decreased significantly more in the metformin group (−0.17 ± 0.42 vs. −0.05 ± 0.38 U/l, $P < 0.02$).

CONCLUSIONS — Weight loss was the main factor associated with the decrease in PAI-1, in accordance with the recent demonstration of production of PAI-1 by adipocytes. Metformin had a significant effect on two factors, tPA antigen and vWF, mainly secreted by the endothelial cells, which suggests an effect of the drug on the production or the metabolism of these two hemostatic proteins.

Diabetes Care 21:1967–1972, 1998

The insulin resistance syndrome is a disorder that associates central obesity, high blood pressure, hyperinsulinemia, glucose intolerance, hypertriglyceridemia and hypo-HDL-cholesterolemia (1,2). A decreased fibrinolytic capacity has recently been shown in this syndrome (3). This decreased capacity may be at least partly responsible for the cardiovascular risk associated with the syndrome. Indeed, hypofibrinolysis—as reflected by an increased clot lysis time or by high plasma levels of plasminogen activator inhibitor 1 (PAI-1) or of tissue-type plasminogen activator (tPA) antigen (which evaluates mainly the inactive inhibitors/tPA complexes)—has been found to be a cardiovascular risk factor in several epidemiological studies (4–7). The Biguanides and the Prevention of the Risk of Obesity (BIGPRO)1 trial (8) was performed to assess whether metformin, an antidiabetic drug that has been shown to increase insulin sensitivity (9), would have an effect on the insulin resistance syndrome parameters in nondiabetic subjects with central obesity. Because a beneficial effect of metformin on fibrinolysis has been reported in several studies (10–15), an evaluation of its effect on fibrinolysis and on other hemostatic parameters was included in this trial.

RESEARCH DESIGN AND METHODS

A total of 457 nondiabetic men (33%) and women, free of cardiovascular disease, were recruited from hospital outpatient clinics and included in the BIGPRO1 trial. The detailed methodology of this randomized double-blind controlled trial and its primary results have already been published (8). The inclusion criteria were a high waist-to-hip ratio (≥0.95 for men, ≥0.80 for women) and an age between 35 and 65 years. The subjects were randomly allocated, with stratification by center (n = 32, see APPENDIX) and by sex, to a 1-year oral treatment with metformin (850 mg twice a day) or placebo, in addition to usual diet and exercise recommendations. A clinical examination was performed every 3 months, and venous blood samples were collected at entry, 6 months, and 1 year. A total of 324 subjects completed the trial (72% in the metformin and 70% in the placebo group, NS). Baseline characteristics of these subjects were similar to those of the subjects who did not finish the trial, except that the completers were more often treated

From INSERM (National Institute of Health and Medical Research) Unit 21 (M.A.C., E.E., P.A.), Villejuif; the Laboratory of Hematology (P.M., I.J.-V.) CRI INSERM, Marseille; and the Department of Diabetology (P.V.), University Hospital La Timone, Marseille, France.

Address correspondence and reprint requests to Dr. M. Aline Charles, INSERM U 21, 16 avenue Paul Vaillant Couturier, F-94807 Villejuif, France. E-mail: charles@vjf.inserm.fr.

Received for publication 8 May 1998 and accepted in revised form 31 July 1998.

E.E. has received funds from Lipha.

Abbreviations: BIGPRO, Biguanides and the Prevention of the Risk of Obesity; PAI-1-act, plasminogen activator inhibitor 1 activity; PAI-1-ag, plasminogen activator inhibitor 1 antigen; tPA-ag, tissue-type plasminogen activator inhibitor; vWF, von Willebrand factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Correlation coefficients between hemostatic parameters and parameters of the insulin resistance syndrome at inclusion*

	PAI-1-act	PAI-1-ag	tPA-ag	vWF
Age	−0.08	−0.03	0.09*	0.26‡
BMI	0.29‡	0.33‡	0.21‡	0.17‡
Waist-to-hip ratio	0.13†	0.18‡	0.22‡	−0.01
Systolic blood pressure	0.11*	0.17‡	0.17‡	0.11*
Diastolic blood pressure	0.06	0.14†	0.15‡	0.03
Fasting glucose	0.23‡	0.26‡	0.18‡	0.05
Fasting insulin	0.42‡	0.45‡	0.34‡	0.11*
Fasting triglyceride	0.29‡	0.34‡	0.36‡	0.004
HDL cholesterol	−0.19‡	−0.20‡	−0.21‡	0.001

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

for hypertension and had a slightly lower mean HDL cholesterol concentration (8).

Laboratory investigations

Aliquots of blood samples drawn during the trial were immediately centrifuged and frozen. They were then sent to the central laboratories, in Lille (INSERM U325) for glucose, creatinine, and lipid measurements, and in Marseille (Endocrinology and Hematology Departments of La Timone Hospital) for insulin, blood coagulation, and fibrinolysis measurements. Blood was drawn into fluoride Vacutainer tubes for glucose and into EDTA Vacutainer tubes for lipids and insulin. Glucose, cholesterol, and triglyceride were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany) adapted to an automatic analyzer (Hitachi 717). Plasma insulin was determined by radioimmunoassay using a commercially available kit (CIS; Bio Industrie, Gif-sur-Yvette, France). For determination of hemostatic parameters, blood was drawn without stasis and collected on trisodium citrate (0.011 mol/l final concentration) in the presence of platelet inhibitors (Diatube; Diagnostica Stago, Asnières, France) and immediately cooled on ice. PAI-1 activity (PAI-1-act) was determined using a commercially available kit (Biopool, Umea, Sweden) according to Ericksson et al. (16). PAI-1 antigen (PAI-1-ag), tPA antigen (tPA-ag), and von Willebrand factor (vWF) were evaluated with an enzyme-linked immunosorbent assay with kits from Diagnostica Stago, according to Declerck et al. (17) for PAI-1-ag and to Holvoet et al. (18) for tPA-ag.

Statistical analysis

Correlations between continuous variables were expressed with Pearson coefficients after logarithmic transformation for the four hemostatic variables and for BMI, insulin, and triglyceride to normalize their distributions. Partial correlations were computed to take into account the effect of potential confounders. Spearman coefficients were used for correlations with age.

An analysis of variance with repeated-measures design (PROC MIXED procedure of SAS) was performed to test for the effect of treatment, time, and the interaction between time and treatment. In case of interaction, the changes in the metformin and placebo groups were compared at different times with *t* tests. All analyses were done with the SAS package (SAS Institute, Cary, NC).

RESULTS

At the inclusion visit, the 457 subjects had a median (range) age of 49 (34–65) years and a BMI of 32.5 (22.0–60.5) kg/m^2. Correlations of tPA-ag with PAI-1-act and PAI-1-ag were 0.46 and 0.58, respectively ($P < 0.001$). The tPA-ag only was also related to the vWF ($r = 0.24$, $P < 0.001$). The three fibrinolytic parameters were highly positively related to fasting insulin and triglyceride concentrations and more weakly to the other clinical and biological variables of the insulin resistance syndrome (Table 1). In contrast, vWF showed only low correlations with BMI, systolic blood pressure, and fasting insulin but was more positively associated with age.

Effect of treatment with metformin

The baseline characteristics of the subjects who completed the trial are shown in Table 2 by treatment group. During the 1-year duration of the trial, weight and fasting insulin concentration decreased in both groups, but more so in the metformin group (mean 1-year ± SD change in weight: metformin, −2.0 ± 6.2; placebo, −0.8 ± 5.5 kg, $P < 0.06$; and in fasting insulin: metformin, −29 ± 50; placebo, −20 ± 35 pmol/l, $P < 0.06$). There was also a favorable effect of metformin on fasting glucose concentrations (mean 1-year change ± SD: metformin, 0.2 ± 1.0; placebo, 0.4 ± 1.2 mmol/l, $P < 0.05$) (8). The baseline, 6-month, and 1-year concentrations of hemostatic parameters are shown in Table 3. There was no significant difference between the concentrations at baseline for any of the parameters. PAI-1-act and PAI-1-ag concentrations significantly decreased during the trial (significant time effect in the analysis of variance, $P < 0.001$) but decreased similarly in both the placebo and metformin groups. In contrast, tPA-ag and vWF decreased more in the metformin group, and there was a significant difference

Table 2—*Comparison between treatment groups at entry visit for subjects who completed the trial*

	Metformin	Placebo
n	164	160
Age (years)	49.7 ± 6.3	49.2 ± 6.9
Men (%)	35	32
Smokers (%)	21	15
BMI (kg/m^2)	33.3 (24.6–45.1)	33.0 (24.0–45.4)
Waist-to-hip ratio	0.94 ± 0.09	0.94 ± 0.08
Systolic blood pressure (mmHg)	134 ± 16	133 ± 17
Diastolic blood pressure (mmHg)	81 ± 10	82 ± 11
Antihypertensive treatment (%)	34	32
Fasting blood glucose (mmol/l)	5.3 ± 0.8	5.2 ± 0.6
Fasting insulin (pmol/l)	96 (42–246)	96 (48–198)
Total cholesterol (mmol/l)	5.7 ± 1.0	5.4 ± 1.1
Triglyceride (mmol/l)	1.6 (0.7–3.4)	1.6 (0.7–3.5)

Quantitative variables are expressed as arithmetic means ± SD or as geometric means (95% tolerance limits).

in 12-month changes between the two groups (tPA ag: −1.1 ± 3.1 vs. −0.2 ± 3.2 ng/ml, $P < 0.02$; vWF: −0.17 ± 0.42 vs. −0.05 ± 0.38 U/l, $P < 0.03$). The greater decrease in the metformin group was already apparent at 6 months for tPA-ag but was more evident after 1 year of treatment (interaction between time and treatment, $P < 0.02$) (Table 3). For vWF, the effect of metformin was seen only after 1 year of treatment (interaction between time and treatment, $P < 0.02$) (Table 3).

Effect of changes in weight, insulin, and triglyceride concentrations on hemostatic parameters

Among the subjects with a complete 1-year follow-up, 150 subjects gained weight (mean ± SD weight gain: 3.1 ± 3.0 kg) and 174 lost weight (weight loss: 5.3 ± 4.8 kg). The subjects who lost weight showed, on average, a greater decrease of all hemostatic parameters, and this was seen in both the placebo and the metformin groups (no significant interaction between treatment and weight change) (Fig. 1).

The weight change during the trial was positively associated with change in fasting insulin ($r = 0.30$, $P < 0.001$) and triglyceride ($r = 0.22$, $P < 0.001$) concentrations. However, after adjustment for the changes in fasting insulin and triglyceride, the correlations between changes in weight and in PAI-1-act or tPA-ag remained significant (Table 4).

CONCLUSIONS

At inclusion in the BIGPRO1 trial, there were significant associations between the three fibrinolysis parameters and nearly all the variables of the insulin resistance syndrome that were measured (Table 1). The strongest correlations were seen for fasting insulin concentrations with PAI-1-act and PAI-1-ag. These results are in concordance with the literature, where correlations with fasting insulin concentrations of the same order have been reported in population-based studies (19) and in subjects with hypertension, hypertriglyceridemia, angina, obesity, or diabetes (20–22). The mechanism behind this association is not precisely known. In vitro, insulin, proinsulin, and VLDL have been shown to stimulate PAI-1 production by the hepatocytes or the endothelial cells (23–26). However, in vivo, acute administration of insulin or triglyceride does not increase PAI-1 concentration (22,27,28).

Although the liver and the endothelial cells are considered the main sources of

Table 3—*Comparison of the mean values of the hemostatic parameters in the metformin and placebo groups at inclusion, 6 months, and 12 months by analysis of variance in the subjects who attended the three visits*

	Metformin	Placebo	P_{time}	$P_{treatment}$	$P_{interaction}$
n	147	139	—	—	—
PAI-act (IU/ml)					
Inclusion	12 (3–57)	12 (3–57)	—	—	—
6 months	8 (1–55)	8 (1–52)	—	—	—
12 months	6 (1–70)	7 (1–71)	0.001	0.9	—
PAI-ag (ng/ml)					
Inclusion	40 (7–220)	38 (6–240)	—	—	—
6 months	29 (4–196)	30 (5–196)	—	—	—
12 months	27 (5–162)	28 (5–162)	0.001	0.9	—
tPA-ag (ng/ml)					
Inclusion	9.3 (4.6–18.7)	9.0 (4.3–18.9)	—	—	—
6 months	8.3 (3.5–19.3)	8.5 (4.0–18.3)	—	—	—
12 months	8.1 (3.6–18.3)	8.8 (4.3–18.2)	—	—	0.02
vWF (U/l)					
Inclusion	1.3 (0.6–2.6)	1.2 (0.6–2.7)	—	—	—
6 months	1.2 (0.6–2.5)	1.2 (0.5–2.5)	—	—	—
12 months	1.1 (0.5–2.3)	1.2 (0.5–2.5)	—	—	0.02

Data are geometric means (95% CI). *P* values are given for the interaction between time and treatment or for the effects of time and treatment in the absence of interaction.

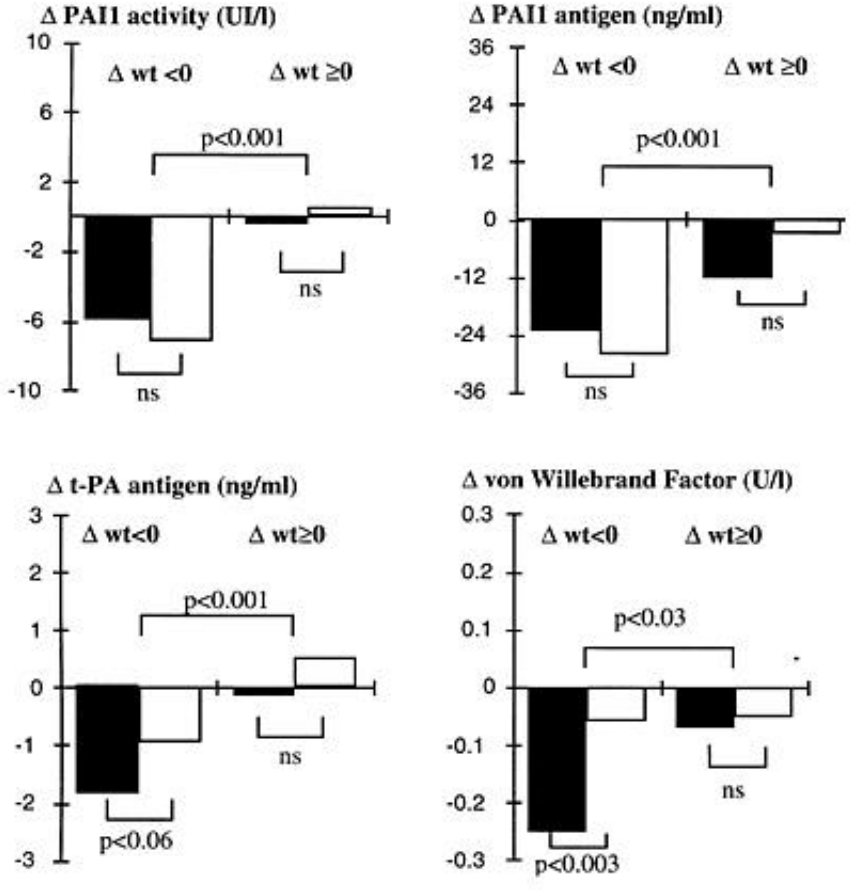

Figure 1—*Changes over 1 year in hemostatic parameters in subjects who lost or gained weight according to treatment group. Mean weight loss and gain were not significantly different between the metformin group (■) and the placebo group (□): −5.8 vs. −4.7 and 3.2 vs. 3.1 kg, respectively.*

Table 4—*Correlations between 1-year changes in fibrinolytic parameters and 1-year changes in weight, before and after adjustment for concomitant changes in fasting insulin and triglyceride concentrations*

	Δ PAI-act	Δ PAI-ag	Δ tPA-ag
Before adjustment			
Δ weight	0.24‡	0.12*	0.30‡
After adjustment			
Δ weight (adj)	0.18†	0.06	0.22†

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

plasma PAI-1, it has recently been shown that adipose tissue, especially the visceral adipose tissue, produce PAI-1 and may be involved in the PAI-1 increase in obesity (29–33). PAI-1-act and PAI-1-ag decreased by 30 and 40% during the 1-year course of the BIGPRO study in both the placebo and the metformin groups. It occurred mainly in the subjects who lost weight during the follow-up, as shown in Fig. 1. Weight loss was accompanied by decreases in fasting insulin and triglyceride concentrations. However, the multivariate analysis suggests that the changes in plasma insulin and triglyceride did not fully account for the relationship between changes in PAI-1-act and in weight. Changes in diet and physical activity that were recommended in the two groups may have contributed by themselves or by promoting weight loss to the decrease in PAI-1 (34).

A 1-year treatment with metformin had no significant effect on PAI-1-act or PAI-1-ag, as these two parameters decreased similarly in both the placebo and the metformin groups (Table 3). Although there was a small but significantly greater decrease in weight and fasting insulin concentration in the metformin group, it did not translate into a greater decrease in PAI-1 level. Several studies in the literature (10–15) have concluded that metformin treatment may improve fibrinolysis. However, three of these studies (10–12) were uncontrolled studies for which there is no way to untangle a real effect of the drug from an effect of, for example, weight loss. Two other randomized studies reported a greater decrease of PAI-1-ag (13) or PAI-1-act (14) in the metformin than in the placebo group but did not directly compare the changes in PAI-1 between the groups to test whether the difference could be attributed to metformin. Thus, there is only one controlled randomized trial in type 2 diabetic subjects that showed a decrease in PAI-1-act significantly different from that observed in the placebo group (15). In that study, there was no significant difference between the metformin and placebo groups for weight and fasting insulin concentration changes, but there were significant differences in changes of glucose and triglyceride concentrations. If metformin exerts an effect on PAI-1 through its action on weight or on glucose, insulin, or triglyceride concentrations, the difference in the basal concentrations and in the magnitude of the changes in these variables between this study and the present study may explain the different results.

In contrast to an absence of effect of metformin on PAI-1, there was a specific effect of the drug on tPA-ag in our study after 6 months and 1 year of treatment. A randomized triple cross-over study comparing the effect of metformin, metoprolol, and placebo in 18 nonobese, nondiabetic middle-aged men reported a similar significant effect of metformin on tPA-ag, whereas PAI-1 level was not significantly affected by metformin (35). There was also a significantly greater decrease of vWF concentrations in the metformin group, which was seen later, however, (only at the 1-year evaluation) than the effect on tPA-ag. It is possible that metformin influences the metabolism of these two hemostatic proteins. However, both tPA-ag and vWF are mainly secreted by the endothelial cells and are considered markers of endothelial damage (7,36). There are animal data suggesting a vascular effect of the drug: reduction of the hyperpermeability induced by alloxan in normoglycemic or hyperglycemic hamsters (37); and protection against the loss of vasomotion induced by diabetes in bats (38). There are also uncontrolled in vivo studies in nondiabetic humans describing a favorable effect of metformin in vascular pathology, such as cyclic edema (39) and peripheral arterial disease (40). Therefore, the effect that we observed with metformin on two markers of endothelial damage may correspond to an indirect action of metformin on the endothelial cells as the consequence of a more general vascular effect of the drug.

In conclusion, our results confirm that hypofibrinolysis, as measured by a high PAI-1 activity, is associated with the insulin resistance syndrome. Obesity, hyperinsulinemia, and hypertriglyceridemia are all potentially involved in the increase of the PAI-1 level. Whatever the exact mechanism(s), our results emphasize the beneficial effect of even modest weight loss: on average, weight loss was 0.9 and 2.3% of the initial weight in the placebo and metformin group, respectively. In subjects with central obesity who, on average, lost weight, no significant additional effect of metformin treatment on fibrinolysis was found. However, metformin showed a specific and significant effect on tPA-ag and vWF concentrations. Treatment by metformin may alter the metabolism of these proteins or may have beneficial consequences for the endothelium, possibly through a more general vascular effect as suggested by animal studies.

Acknowledgments— This work was supported by grants from the National Institutes of Health and Medical Research (INSERM) and the National Health Insurance for Wage Earners (CNAMTS), as well as by funds from the Lipha Pharmaceutical Company.

We thank A. D. Blann, PhD, FIBMS, for his useful advice.

APPENDIX

BIGPRO Study Group

Michel Safar, MD, Chairman (INSERM U337, Paris); Eveline Eschwège, MD, M. Aline Charles MD, MPH, Annick Fontbonne, MD, PhD, Patricia Lavoine, (INSERM U21, Villejuif); Jean-Marie Bard, PhD, Jean-Charles Fruchart, MD (INSERM U325, Lille); Gilles Bouvenot, MD, Charles Caulin, MD, Pierre Grandmottet, MD, Patrice Queneau, MD (National Pedagogic Association for the Teaching of Therapeutics, APNET); Bernard Bégaud, MD (School of Medicine, University of Bordeaux); Irène Juhan-Vague, MD, PhD, Philippe Vague, MD, PhD (University of Marseille); Philippe André, MD, Françoise Isnard, MD (LIPHA); Jean-Marie Cohen, MD (OPEN/ROME).

BIGPRO clinical centers

Amiens: Regional University Hospital, Department of Internal Medicine (Head: J.P. Ducroix. Investigator: N. Vanwymeersch);

Angers: Regional University Hospital, Department of Diabetology (Head: M. Marre. Investigator: M. Hallab); Besançon: J. Minjoz Regional University Hospital, Department of Endocrinology-Diabetology (J. Massol. Investigators: M. Grandmottet, V. Llorca); Bondy: Jean Verdier AP-HP University Hospital, Department of Endocrinology-Diabetology (Head: P. Attali. Investigators: P. Valensi, M. Attalah); Bordeaux: Saint-André Regional University Hospital, Department of Internal Medicine (Head: J. Paccalin. Investigator: C. Fromenteau); Brest: Morvan Regional University Hospital, Department of Internal Medicine (Head: D. Mottier. Investigator: L. Bressollette); Corbeil: Gilles-de-Corbeil Hospital, Department of Metabolic Diseases (Head: G. Charpentier. Investigators: C. Diaz, F. Goebel); Dijon: Bocage Regional University Hospital, Department of Endocrinology-Diabetology (Head: J.M. Brun. Investigator: J.M. Petit); Grau-du-Roi: Medical and Surgical Center, Department of Diabetology and Nutrition (Head: J.L. Richard. Investigator: S. Jureidini); Grenoble: Regional University Hospital, Department of Endocrinology (Head: S. Halimi. Investigator: C. Talantikit); Lille: Regional University Hospital, Department of Endocrinology-Diabetology (Head: P. Fossati and Dr. Borys' private practice. Investigator: J.M. Borys); Lyon: Lyon Sud Regional University Hospital, Department of Internal Medicine (Head: D. Vital-Durand. Investigator: R. Nové-Josserand); Lyon: Lyon Sud Regional University Hospital, Department of Endocrinology-Diabetology (Head: J. Orgiazzi). Investigator: N. Chatenay-Laniel; Lyon: Edouard Herriot Regional University Hospital, Department of Nephrology (Head: M. Laville. Investigator: P. Morel); Lyon: Antiquaille Regional University Hospital, Department of Endocrinology (Head: F. Berthezène. Investigator: M.P. Larmaraud); Marseille: La Timone Regional University Hospital, Department of Endocrinology-Diabetology (Head: P. Vague. Investigator: C. Mattei): Nancy: Regional University Hospital, Department of Internal Medicine (Head: J. Schmitt. Investigator: D. Wahl); Nantes: Hôtel-Dieu Regional University Hospital, Department of Endocrinology-Diabetology (Head: B. Charbonnel. Investigator: P. Blanchard); Nîmes: Carémeau Hospital, Department of Internal Medicine (Head: J. Jourdan. Investigator: P. Guillot); Nîmes: Carémeau Hospital, Department of Medicine T (Head: M. Rodier. Investigator: C. Gouzes); Paris: Broussais AP-HP University Hospital, Department of Internal Medicine (Head: M. Safar. Investigators: B. Darné, F. Ferhaoui); Paris: Laënnec AP-HP University Hospital, Department of Internal Medicine (Head: F.C. Hugues. Investigator: C. Le Jeunne); Paris: Lariboisière AP-HP University Hospital, Emergency Department (Head: C. Caulin. Investigator: O. Chassany); Paris: Saint-Antoine AP-HP University Hospital, Department of Endocrinology (Head: P. Aubert. Investigator: L. Ponsoye); Poitiers: Regional University Hospital, Department of Endocrinology-Diabetology (Head: R. Maréchaud and Dr. Breux's private practice. Investigator: M. Breux); Reims: Robert Debré Regional University Hospital, Department of Endocrinology (Head: M. Leutenegger. Investigator: H. Grulet); Rennes: Hôtel-Dieu Regional University Hospital, Department of Cardiology A (Head: J.C. Daubert. Investigators: F. Paillard, B. Bompais); Roubaix: Roubaix Hospital, Department of Metabolic Diseases (Head: J.L. Grenier. Investigators: P. Gross, F. Gentholz); Saint-Etienne: Bellevue Regional University Hospital, Department of Endocrinology (Head: B. Estour. Investigator: H. Villard); Saint-Laurent-du-Var: Arnaud Tzanck Institute, Department of Endocrinology-Diabetology (Head: N. Balarac. Investigator: N. Balarac); Toulouse: Purpan Regional University Hospital, Department of Internal Medicine (Head: B. Chamontin. Investigator: T. Brillac); Tours: Bretonneau Regional University Hospital, Department of Internal Medicine (Head: J.L. Guilmot. Investigator: E. Diot).

References

1. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1988
2. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–194, 1991
3. Juhan-Vague I, Thompson SG, Jespersen J, on behalf of the ECAT Study Group: Involvement of the hemostatic system in the insulin resistance syndrome: a study of 1500 patients with angina pectoris. *Arterioscler Thromb* 13:1865–1873, 1993
4. Meade TW, Ruddock V, Stirling Y, Chakrabarti T, Miller GJ: Fibrinolytic activity, clotting factors and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet* 342:1076–1079, 1993
5. Thompson SG, Kienast J, Pyke SDM, Haverkate F, Van de Loo JCW: Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med* 332:635–641, 1995
6. Juhan-Vague I, Pyke SDM, Alessi MC, Jespersen J, Haverkate F, Thompson SG, on behalf of the ECAT Study Group: Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *Circulation* 94:2057–2063, 1996
7. Jansson JH, Nilsson TK, Olofsson BO: Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary artery disease: a 7 year follow-up. *Circulation* 88:2030–2034, 1993
8. Fontbonne A, Charles MA, Juhan-Vague I, Bard JM, André P, Isnard F, Cohen JM, Grandmottet P, Vague P, Safar ME, Eschwège E, the BIGPRO Study Group: The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution. *Diabetes Care* 19:920–926, 1996
9. Bailey CJ, Turner RC: Metformin. *N Engl J Med* 334:574–579, 1996
10. Chakrabarti R, Hocking ED, Fearnley GR: Fibrinolytic effect of metformin in coronary-artery disease. *Lancet* ii:256–259, 1965
11. Fearnley GR, Chakrabarti R, Evans JF: Effect of ethyloestrenol combined with phenformin or with metformin on platelet stickiness and serum-cholesterol in patients with occlusive vascular disease. *Lancet* ii:1004–1007, 1968
12. Menon IS: Metformin and stanozolol in blood fibrinolytic activity. *Br Med J* 1:289–290, 1971
13. Grant PJ: The effects of metformin on the fibrinolytic system in diabetic and non-diabetic subjects. *Diab Metab* 17:168–173, 1991
14. Vague P, Juhan-Vague I, Alessi MC, Badier C, Valadier J: Metformin decreases the high plasminogen activator inhibition capacity, plasma insulin and triglyceride levels in non-diabetic obese subjects. *Thromb Haemost* 57:326–328, 1987
15. Nagi DK, Yudkin JS: Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects. *Diabetes Care* 16:621–629, 1993
16. Ericksson E, Ranby M, Gyzander E, Risberg B: Determination of plasminogen activator inhibitor in plasma using t-PA and a chromogenic single-point poly-D-lysine stimulated assay. *Thromb Res* 50:91–101, 1988
17. Declerck PJ, Alessi MC, Verstreken M, Kruithof EKO, Juhan-Vague I, Collen D: Measurement of plasminogen activator inhibitor 1 in biological fluids with a murine monoclonal antibody based enzyme linked immunosorbent assay. *Blood* 71:220–225, 1988
18. Holvoet P, Cleemput H, Collen D: Assay of

human tissue type plasminogen activator (t-PA) with an enzyme linked immunosorbent assay (ELISA) based on three murine monoclonal antibodies to t-PA. *Thromb Haemost* 54:684–687, 1985
19. Lindahl B, Asplund K, Eliasson M, Evrin PE: Insulin resistance syndrome and fibrinolytic activity: the Northern Sweden MONICA Study. *Int J Epidemiol* 25:291–299, 1996
20. Toft I, Bonaa KH, Ingebretsen OC, Nordoy A, Birkeland KI, Jenssen T: Gender differences in the relationships between plasma plasminogen activator inhibitor-1 activity and factors linked to the insulin resistance syndrome in essential hypertension. *Arterioscler Thromb Vasc Biol* 17:553–559, 1997
21. Asplund-Carlson A, Hamsten A, Wiman B, Carlson LA: Relationship between plasma plasminogen activator inhibitor-1 activity and VLDL triglyceride concentration, insulin levels and insulin sensitivity: studies in randomly selected normo and hypertriglyceridaemic men. *Diabetologia* 36:817–825, 1993
22. Juhan-Vague I, Alessi MC, Vague P: Increased plasma plasminogen activator inhibitor-1 levels: a possible link between insulin resistance and atherothrombosis. *Diabetologia* 34:457–462, 1991
23. Alessi MC, Juhan-Vague I, Kooistra T, Declerk PJ, Collen D: Insulin stimulates the synthesis of plasminogen activator inhibitor 1 by the human hepatocellular cell line HepG2. *Thromb Haemost* 60:491–494, 1988
24. Sironi L, Mussoni L, Prati L, Baldassarre D, Camera M, Banfi C, Tremoli E: Plasminogen activator inhibitor type-1 synthesis and mRNA expression in hepG2 cells are regulated by VLDL. *Arterioscler Thromb Vasc Biol* 16:89–96, 1996
25. Schneider DJ, Nordt TK, Sobel BE: Stimulation by proinsulin of expression of plasminogen activator inhibitor type-1 in endothelial cells. *Diabetes* 41:890–895, 1992
26. Stiko-Rahm A, Wiman B, Hamsten A, Nilsson J: Secretion of plasminogen activator inhibitor-1 from cultured human umbilical vein endothelial cells induced by very low density lipoprotein. *Arteriosclerosis* 10:1067–1073, 1990
27. Nagi DK, Tracy R, Pratley R: Relationship of hepatic and peripheral insulin resistance with plasminogen activator inhibitor-1 in Pima Indians. *Metabolism* 45:1243–1247, 1996
28. Grant PJ, Kruithof EKO, Felly CP, Felber JP, Bachmann F: Short-term infusions of insulin, triacylglycerol and glucose do not cause acute increases in plasminogen activator inhibitor-1 concentrations in man. *Clin Sci* 79:516–516, 1990
29. Lundgren CH, Brown SL, Nordt TK, Sobel BE, Fujii S: Elaboration of type-1 plasminogen activator inhibitor from adipocytes: a potential pathogenetic link between obesity and cardiovascular disease. *Circulation* 93:106–110, 1996
30. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I: Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes* 46:860–867, 1997
31. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y: Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nature Med* 2:800–803, 1996
32. Samad F, Yamamoto K, Lookutoff DJ: Distribution and regulation of plasminogen activator inhibitor 1 in murine adipose tissue in vivo. *J Clin Invest* 97:37–46, 1996
33. Cigolini M, Targher G, Bergamo Andreis IA, Tonoli M, Agostino G, De Sandre G: Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. *Arterioscler Thromb Vasc Biol* 16:368–374, 1996
34. Schulman S, Lindmaker P, Johnsson H: Influence of changes in lifestyle on fibrinolytic parameters and recurrence rate in patients with venous thromboembolism. *Blood Coagul Fibrinolysis* 6:311–316, 1995
35. Landin K, Tengborn L, Smith U: Effects of metformin and metopolol CR on hormones and fibrinolytic variables during a hyperinsulinemic, euglycemic clamp in man. *Thromb Haemost* 71:783–787, 1994
36. Blann AD: Von Willebrand factor and the endothelium in vascular disease. *Br J Biomed Sci* 50:125–134, 1993
37. Colantuoni A, Bertuglia S, Donato L: Effects of metformin on microvascular permeability in diabetic syrian hamsters. *Diabete Metab* 14:549–553, 1988
38. Bouskela E: Effects of metformin on the wing microcirculation of normal and diabetic bats. *Diabete Metab* 14:560–565, 1988
39. Valensi P, Behar A, Andre P, Wiernsperger N, Attali JR: The effects of metformin on the capillary permeability to albumin in women patients with cyclic edema. *Angiology* 46:401–408, 1995
40. Sirtori CR: Clinical experience with metformin in patients with peripheral vascular disease. *Diabete Metab* 14:601–607, 1988