Effects of Troglitazone on Blood Concentrations of Plasminogen Activator Inhibitor 1 in Patients With Type 2 Diabetes and in Lean and Obese Normal Subjects

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Low plasma fibrinolytic activity in association with increased plasma plasminogen activator inhibitor 1 (PAI-1) levels has been linked to an increased risk of atherosclerosis in obesity and type 2 diabetes. We tested the hypothesis that troglitazone, which improves insulin sensitivity and lowers plasma insulin levels in insulin-resistant obese subjects and patients with type 2 diabetes, would also lower circulating PAI-1 antigen concentrations and activity. We assessed insulin sensitivity (5-h, 80 mU · m^{-2} · min^{-1} hyperinsulinemic-euglycemic clamp) and measured plasma PAI-1 antigen and activities and tissue plasminogen activator (tPA) in 14 patients with type 2 diabetes and 20 normal control subjects (10 lean, 10 obese) before and after 3 months of treatment with troglitazone (600 mg/day). At baseline, plasma PAI-1 antigen levels after an overnight fast were significantly higher in the obese (33.5 ± 4.7 μg/l) and type 2 diabetic subjects (54.9 ± 6.3 μg/l) than in the lean control subjects (16.3 ± 3.2 μg/l; $P < 0.01$ and $P < 0.001$, respectively). Troglitazone decreased plasma PAI-1 antigen concentrations in the diabetic patients (36.8 ± 5.0 μg/l; $P < 0.001$ vs. baseline), but the reduction in the obese subjects did not reach statistical significance (baseline, 33.5 ± 4.7; after troglitazone, 25.6 ± 5.2 μg/l). Changes in plasma PAI-1 activity paralleled those of PAI-1 antigen. The extent of the reduction in plasma PAI-1 antigen concentrations in the diabetic patients after troglitazone correlated with the reductions in fasting plasma insulin ($r = 0.60$, $P < 0.05$), nonesterified fatty acid ($r = 0.63$, $P < 0.02$), and glucose concentrations ($r = 0.64$, $P < 0.02$) but not with the improvement in glucose disposal rates during the glucose clamps. Three nonresponders to troglitazone with respect to effects on insulin sensitivity and fasting glucose and insulin levels also had no reduction in circulating PAI-1. In conclusion, troglitazone enhances fibrinolytic system activity in insulin-resistant type 2 diabetic patients. This effect appears to be intimately linked to its potential to lower plasma insulin levels and improve glycemic control through its peripheral tissue insulin-sensitizing effects. *Diabetes* 49:633–639, 2000

Accelerated atherosclerosis is an important cause of morbidity and mortality in insulin-resistant obese subjects and patients with type 2 diabetes (1,2). Low fibrinolytic activity in blood is a well-recognized major risk factor not only for thrombosis, but also for atherosclerosis (3,4). Deficient fibrinolysis is due primarily to increased concentrations in blood of plasminogen activator inhibitor 1 (PAI-1), which rapidly binds to and inactivates tissue plasminogen activator (tPA) (5). PAI-1 concentrations and activity are increased in obese subjects (6–8) and patients with type 2 diabetes (8–13). The increases may contribute to an excess risk of cardiovascular disease (4,12–14).

PAI-1 levels correlate with a number of variables that cosegregate in subjects with the insulin resistance syndrome (15) including BMI, waist-to-hip ratio, and fasting plasma insulin, triglyceride, and apolipoprotein B levels (4,6–10). The relative contributions of the liver (16), vascular endothelial cells (17,18), and adipocytes (19,20) to circulating PAI-1 levels in health and disease states remain unclear. A key role for adipose tissue in the elevated PAI-1 levels of obesity and type 2 diabetes is suggested by the finding of markedly increased PAI-1 mRNA levels in subcutaneous adipose tissue that correlate inversely with whole-body insulin sensitivity and exhibit a diurnal variation similar to that of PAI-1 in blood (20).

Results of several studies suggest that hyperinsulinemia is the primary determinant of elevated PAI-1 in subjects with obesity and type 2 diabetes (4,6,9). Support for the hypothesis that hyperinsulinemia secondary to insulin resistance explained the elevated PAI-1 levels came from studies showing that insulin increased PAI-1 synthesis by hepatocytes (16) and vascular endothelial cells (17,18). However, no increase in plasma PAI-1 is found following infusion of insulin under euglycemic conditions in normal subjects (21–24). Moreover, intensive treatment of type 2 diabetic patients with insulin lowered PAI-1 in blood (10), implying that factors other than insulin must be important as well. The finding that acute hyperinsulinemia does increase plasma PAI-1 levels in normal subjects when plasma glucose, nonesterified fatty acids (NEFAs), and triglyceride levels are increased concomitantly (24) underscores the potential importance of interaction

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AU, arbitrary units; CV, coefficient of variation; HGO, hepatic glucose output; NEFA, nonesterified fatty acid; PAI-1, plasminogen activator inhibitor 1; R_a, rate of glucose appearance; R_d, rate of glucose disappearance; tPA, tissue plasminogen activator.

between insulin and these other variables in influencing PAI-1 concentrations in blood.

Troglitazone, a thiazolidinedione, lowers blood glucose in type 2 diabetic patients primarily by enhancing peripheral tissue sensitivity to insulin (25–29). The insulin-sensitizing action of troglitazone, seen also in insulin-resistant obese nondiabetic subjects (29), is accompanied by a reduction in fasting and postprandial insulin levels. Thus, if chronic hyperinsulinemia is an important determinant of elevated PAI-1 with obesity and type 2 diabetes, treatment with troglitazone should lower PAI-1 in blood. Such an effect would have important clinical implications, particularly because treatment of type 2 diabetic patients with sulfonylureas increases PAI-1 (30). Thus, troglitazone might be a particularly useful adjunct to therapy in type 2 diabetes for reasons independent of its glucose-lowering potential.

Our aim was to examine the effects of treatment with troglitazone on PAI-1 and tPA antigen concentrations and activity in blood in patients with type 2 diabetes and lean and obese normal subjects. We examined the relationships between changes in these variables and effects of troglitazone on insulin sensitivity, glycemic control, and blood lipids. In view of the suggestion that adipocytes may be an important source of PAI-1 in obesity and type 2 diabetes (19,20) and the profound effects of troglitazone on adipocyte gene expression and metabolism (25,31,32), including an enhancement of adipocyte sensitivity to insulin, we also examined the influence of troglitazone on the response of PAI-1 in blood to a 5-h infusion of insulin under euglycemic clamp conditions.

RESEARCH DESIGN AND METHODS

Fourteen patients with type 2 diabetes and 20 normal control subjects (10 lean, BMI <27kg/m^2, and 10 obese, BMI >27 kg/m^2) participated in a study of the effects of 3 months of treatment with troglitazone on PAI-I antigen levels and activity in blood. Normal glucose tolerance was verified in all the lean and obese nondiabetic subjects based on results with a 75-g oral glucose tolerance test. None of the control subjects were taking any medication known to alter glucose tolerance. The clinical characteristics of the subjects are shown in Table 1. None of the patients had clinical evidence of long-term complications of diabetes. All subjects had normal renal and liver function tests. Six of the diabetic patients were being treated with sulfonylureas, four with metformin, two with combination therapy with a sulfonylurea and either insulin or metformin, and two with diet alone. The diabetic patients were asked to continue their usual diet but to discontinue the use of oral hypoglycemic agents for at least 2 weeks before study. Each subject underwent an 80 mU · m^{-2} · min^{-1} hyperinsulinemic-euglycemic clamp with measurement of plasma lipids, insulin, PAI-1, and tPA levels and activity at baseline and again after 3 months of treatment with 600 mg/day troglitazone. In 12 of the normal subjects (6 lean, 6 obese) and 9 of the type 2 diabetic patients, blood for PAI-1 and tPA antigen and activities was also obtained at the end of the hyperinsulinemic-euglycemic clamps before and after troglitazone to delineate the influence of troglitazone on the response of these variables to a 5-h insulin stimulus.

The study was approved by the Human Subjects Internal Review Board of the University of California, San Diego; written informed consent was obtained from each subject.

Hyperinsulinemic-euglycemic clamps. Studies were performed after a 10- to 12-h overnight fast. At 0300, an 18-gauge cannula was inserted into an antecubital vein and a constant infusion of [3-^{3}H]glucose (0.25 μCi/min) (New England Nuclear, Boston, MA) was started. For blood sampling, a venous cannula was inserted retrograde into a distal forearm vein, the hand being maintained in a hand warmer at 70°C. After each blood sample was taken, this cannula was flushed with 0.15 mol/l NaCl in water. After four basal blood samples were taken between 0800 and 0830 for estimation of plasma glucose concentration and specific activity and serum insulin concentration, an intravenous infusion of insulin (Humulin S; Eli Lilly, Indianapolis, IN) diluted in 0.15 mol/l saline containing 1% wt/vol human albumin was begun at 80 mU · m^{-2} · min^{-1} from a Harvard syringe pump. Potassium and phosphate were given intravenously to compensate for the intracellular movement of these ions and to maintain normal blood levels. Plasma glucose was measured at 5-min intervals by the glucose oxidase method (YSI 2700 analyzer; Yellow Springs Instruments, Yellow Springs, OH) and the blood glucose level was clamped at 5 mmol/l for 5 h by adjustment of the rate of infusion of a solution of 20% (wt/vol) glucose in water (33). The 20% glucose solution was labeled with [3-^{3}H]glucose to maintain plasma glucose specific activities during the clamp close to basal levels (33). Blood samples for glucose concentration and specific activity were taken every 20 min and for insulin concentrations every 30 min until 270 min, and then every 10 min until 300 min.

Analytical procedures. Plasma and infusate [^{3}H]glucose specific activity were determined as previously described (34). Insulin was measured by a double-antibody technique (35). The intra- and interassay coefficients of variation were 6.8 and 7.9%, respectively. Plasma NEFAs were determined with the use of an acyl-CoA oxidase–based colorimetric kit (NEFA-C; Wako, Richmond, VA) with intra- and interassay coefficients of variation (CVs) of 2.4 and 3.3%.

tPA and PAI-1 were measured in duplicate in EDTA plasma by enzyme-linked immunosorbent assays with recombinant tPA (Activase; Genentech, South San Francisco, CA) used as a standard. Although citrate is generally used as anticoagulant, EDTA plasma may be used for the assay of PAI-1 and tPA (36); in our laboratory, results with citrate and EDTA plasma, separated under the conditions used in this study, are virtually identical. Standard curves were obtained with acidified pooled plasma spiked with tPA to provide a total range of detectable PAI-1 activity of 40 arbitrary units (AU)/ml. Samples were assayed under identical conditions after dilution 40-fold with assay buffer (Tris HCl, pH 8.3) containing 0.01% vol/vol Tween 80. Standards and samples were pipetted into wells of an enzyme-linked immunosorbent assay plate (Nunc, Roskilde, Denmark) before addition of the chromogenic substrate S-2251 (DiaPharma, West Chester, OH) (final concentration 22 mg/l), plasminogen 104 U/l final (Enzyme Research Laboratories, South Bend, IN), cyanogen bromide fibrinogen fragments (30 mg/l final), and tPA 7 U/l final. Incubations were conducted at 37°C for 2.5 h, and plates were read in a spectrophotometer at 405 nm. The method detects active and latent forms of free PAI-1 and PAI-1 complexed to plasminogen activators. The tPA method detects both free and tPA complexed to PAI-1. The CV of both procedures used was 9%.

Calculation of glucose kinetics. In the basal state, rates of glucose appearance (R_a) and disappearance (R_d) were calculated by dividing the [3-^{3}H]glucose infusion rate by the plasma glucose specific activity (the mean of values in the four basal plasma samples). During the clamp, total R_a and R_d were calculated from the [3-^{3}H]glucose data using the non–steady-state equations of Steele (37). A distribution volume of 0.19 l/kg and a pool fraction of 0.5 were used in the calculation (38). In the diabetic patients, basal R_d was corrected for urinary glucose excretion. Hepatic glucose output was calculated by subtracting the exogenous glucose infusion rate from the total R_a.

Statistical analysis. Results are expressed as means ± SE unless otherwise indicated. Base 10 logarithmic transformation of plasma PAI-1 activity and triglyceride and fasting insulin levels was used to normalize their distributions. The significance of differences within groups was tested by Student's paired t test and between groups by analysis of variance followed by Tukey's multiple comparison test. Correlations were sought by Pearson's least-squares method. A P value of <0.05 was considered statistically significant.

TABLE 1
Clinical characteristics of the subjects

	Normal subjects		Diabetic patients
	Lean	Obese	
n	10	10	14
Sex (M/F)	8/2	8/2	11/3
Age (years)	48 ± 2	43 ± 3	51 ± 3
Weight (kg)	74 ± 3	103 ± 4	105 ± 5
BMI (kg/m^2)	24.4 ± 0.6	34.2 ± 1.1	33.3 ± 1.39
Fasting plasma glucose (mmol/l)	4.9 ± 0.1	5.1 ± 0.1	11.2 ± 0.8
HbA_{1c} (%)	5.2 ± 0.1	5.1 ± 0.1	8.59 ± 0.44
Fructosamine (μmol/l)*	172 ± 7	180 ± 11	258 ± 13

Data are n or means ± SE. *Normal laboratory range, 160–260 μmol/l.

RESULTS

Body weight and glycemic control. Body weight did not change significantly with 3 months of troglitazone therapy in any group. Fasting plasma glucose was 20% lower after 3 months of troglitazone therapy in the diabetic patients

TABLE 2
Fasting plasma insulin and lipid levels before and after 3 months of treatment with troglitazone (600 mg/day)

	Lean (n = 10)		Obese (n = 10)		Diabetic patients (n = 14)	
	Before	After	Before	After	Before	After
Insulin (mU/l)	3.3 ×/÷ 1.16	3.1 ×/÷ 1.27	11.1 ×/÷ 1.11*	7.1 ×/÷ 1.16†	16.9 ×/÷ 1.22*	11.8 ×/÷ 1.28‡
Triglyceride (mg/dl)	93 ×/÷ 1.35	87 ×/÷ 1.53	142 ×/÷ 1.38	116 ×/÷ 1.47‡	201 ×/÷ 1.44§	152 ×/÷ 1.30‡
NEFA (mmol/l)	0.31 ± 0.03	0.41 ± 0.04	0.38 ± 0.04	0.35 ± 0.05	0.59 ± 0.05*	0.43 ± 0.04†
Cholesterol (mg/dl)						
Total	186 ± 10	191 ± 10	169 ± 8	183 ± 11	190 ± 12	178 ± 9
HDL	51.2 ± 4.8	54.2 ± 5.8	35.5 ± 2.0§	37.9 ± 2.6	36.3 ± 1.7*	44.8 ± 3.8‖
LDL	133 ± 14	135 ± 15	130 ± 13	138 ± 13	125 ± 12	113 ± 11

Data are means ± SE or means ×/÷ for log-transformed data. *P < 0.001 and §P < 0.005 compared with before-troglitazone values in the lean control subjects. †P < 0.02, ‡P < 0.05, and ‖P < 0.005 compared with values before troglitazone therapy within groups.

(11.2 ± 0.8 vs. 9.1 ± 0.7 mmol/l; P < 0.02) but unchanged in the lean (4.9 ± 0.1 vs. 4.8 ± 0.1) and obese (5.1 ± 0.1 vs. 4.9 ± 0.1 mmol/l; NS) normal subjects. The small decline in serum fructosamine levels in the diabetic patients with troglitazone therapy did not reach statistical significance (baseline 258 ± 13, after troglitazone 239 ± 14 μmol/l; 0.05 < P < 0.1). Plasma fructosamine levels did not change with troglitazone therapy in either normal control group.

Plasma insulin and lipid levels. Plasma insulin levels after an overnight fast were significantly higher in the obese and type 2 diabetic patients than in the lean control subjects (both P < 0.001) but were not significantly different between the diabetic and obese subjects (Table 2). Fasting insulin levels were lower after troglitazone in the diabetic (P < 0.05) and obese (P < 0.02) subjects but were unchanged in the lean normal subjects (Table 2).

Plasma triglyceride levels were higher in the type 2 diabetic patients than in the lean control subjects (P < 0.005) (Table 2), as were plasma NEFAs (P < 0.001). Total cholesterol did not differ between the groups, but HDL cholesterol was lower in both the diabetic and obese subjects than in the lean control subjects (Table 2). Troglitazone therapy in the diabetic patients was associated with a decrease in plasma triglyceride and NEFA levels (P < 0.05 and P < 0.02 vs. baseline, respectively) and an increase in HDL cholesterol (P < 0.005) (Table 2). A small reduction in triglyceride levels was also noted in the obese subjects (P < 0.05). No significant change in any of the other lipid variables occurred in the obese or lean normal subjects given troglitazone (Table 2). The extent of suppression of plasma NEFA levels in the diabetic patients correlated with the improvement in glycemic control as reflected by the reductions in fasting plasma glucose (r = 0.55, P < 0.05) and plasma fructosamine (r = 0.60, P < 0.05) levels.

Insulin sensitivity. Mean plasma insulin levels during the last 40 min of the glucose clamps were similar before and after troglitazone and did not differ between groups (baseline: lean 130 ± 10, obese 128 ± 7, diabetic 139 ± 7 mU/l; after troglitazone: lean 122 ± 8, obese 125 ± 7, diabetic, 120 ± 5 mU/l). R_d during the last 40 min of the first euglycemic clamps was lower in the type 2 diabetic patients (3.76 ± 0.40 mg · kg^{-1} · min^{-1}) than in the lean (11.14 ± 0.47 mg · kg^{-1} · min^{-1}) or obese (7.15 ± 0.66 mg · kg^{-1} · min^{-1}) subjects (P < 0.001 for both). R_d during the last 40 min of the clamp was significantly increased after 3 months troglitazone therapy compared with baseline in both the type 2 diabetic patients (5.25 ± 0.65 mg · kg^{-1} · min^{-1}; P < 0.005) and obese subjects (9.11 ± 0.79 mg · kg^{-1} · min^{-1}; P < 0.005) but not in the lean subjects (baseline 11.14 ± 0.47, after troglitazone 10.61 ± 0.46mg · kg^{-1} · min^{-1}). Hepatic glucose output (HGO) was suppressed completely during the last 40 min of the clamp in the lean control subjects (0.01 ± 0.13) and obese subjects (0.15 ± 0.19 mg · kg^{-1} · min^{-1}); it was a little higher in the diabetic patients (0.42 ± 0.11 mg · kg^{-1} · min^{-1}; P < 0.05 vs. lean control subjects). Troglitazone had no significant effect on HGO during the glucose clamps in any of the groups.

Plasma PAI-1 and tPA antigen levels and activity. Plasma PAI-1 antigen levels after an overnight fast (Fig. 1) were higher in the obese (33.5 ± 4.7 μg/l) and type 2 diabetic (54.9 ± 6.3 μg/l) subjects than in the lean control subjects (16.3 ± 3.2 μg/l; P < 0.01 and P < 0.001, respectively) and higher in the diabetic than the obese subjects (P < 0.05). Plasma PAI-1 activity showed a similar pattern: the diabetic patients had markedly increased plasma PAI-1 activity in comparison with the lean normal subjects, whereas plasma PAI-1 activity in the obese subjects was intermediate (Fig. 1). When the lean and obese groups were combined, plasma PAI-1 antigen levels correlated with BMI (r = 0.69, P < 0.001), the common logarithm of fasting insulin levels (r = 0.68, P < 0.001), and fasting plasma NEFA levels (r = 0.44, P < 0.05) (Fig. 2) but not significantly with the logarithm of fasting plasma triglyceride levels (r = 0.34, NS) or insulin sensitivity (R_d at the end of the glucose clamps) (r = –0.41, NS). In the diabetic patients, PAI-1 antigen correlated with BMI (r = 0.72, P < 0.005) but not significantly with fasting plasma insulin levels (r = 0.43, NS), plasma lipids, or insulin sensitivity (r = –0.28, NS) (Fig. 2) and was unrelated to baseline measures of glycemic control (fasting plasma glucose, r = 0.28; HbA_{1c}, r = 0.10; fructosamine, r = 0.23, all NS).

Plasma tPA antigen concentrations were higher in obese (10.6 ± 1.0 μg/l) and type 2 diabetic (12.7 ± 0.4 μg/l) subjects than in lean control subjects (5.8 ± 0.8 μg/l) (P < 0.001 for both) but not significantly different between the obese and diabetic groups (Fig. 1). Troglitazone therapy in the diabetic patients led to a significant decrease in both plasma PAI-1 antigen (54.9 ± 6.3 to 36.8 ± 5.0 μg/l; P < 0.001) and PAI-1 activity (43.5 ×/÷ 1.20 to 26.0 ×/÷ 1.18; P < 0.005). In two of the diabetic patients, basal PAI-1 antigen concentrations showed either no change or increased by 18 μg/l following troglitazone therapy. These two patients may be regarded as nonresponders to troglitazone in

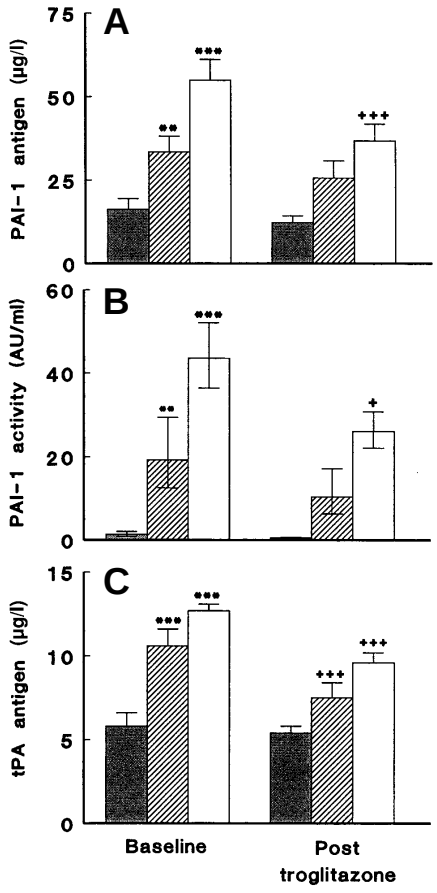

FIG. 1. Fasting plasma PAI-1 antigen (A), PAI-1 activity (B), and tPA antigen levels (C) (mean ± SE) before and after 3 months of treatment with troglitazone 600 mg/day in 10 lean normal subjects (■), 10 obese subjects (▨), and 14 type 2 diabetic patients (□). The PAI-1 activity data were logarithmically transformed (base 10) before calculation of the mean and SE, hence the asymmetry about the mean. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with fasting levels in lean normal subjects; $+P < 0.05$, $+++P < 0.001$ compared with levels within the same group before troglitazone therapy.

that their serum fructosamine levels increased by 37 and 33 µmol/l, respectively, their insulin sensitivity decreased by ~10%, and their fasting plasma insulin levels increased by 4 and 11 mU/l, respectively, compared with values at baseline. In the obese subjects, the reduction in PAI-1 antigen and activity with troglitazone did not reach statistical significance (PAI-1 antigen: 33.5 ± 4.7 to 25.6 ± 5.2 µg/l, PAI-1 activity: 19.2 ×/÷ 1.53 to 10.4 ×/÷ 1.66 AU/ml). This was due primarily to relatively large increments in both of these variables in one subject in this group. No change in either PAI-1 antigen concentration or PAI-1 activity was seen with troglitazone therapy in the lean normal subjects (Fig. 1). Fasting plasma tPA antigen concentration decreased with troglitazone therapy in both the diabetic and obese subjects (Fig. 1, both $P < 0.001$). However, from a stoichiometric point of view, the changes in PAI-1 were much more marked than those in tPA.

The decrease in plasma PAI-1 antigen concentrations and activity in the diabetic patients after troglitazone correlated with the reduction in fasting plasma insulin levels (PAI-1 antigen, $r = 0.60$, $P < 0.05$; PAI-1 activity, $r = 0.67$, $P < 0.01$). These relationships also held when the diabetic and obese groups were combined (PAI-1 antigen, $r = 0.58$, $P < 0.005$; PAI-1 activity, $r = 0.60$, $P < 0.002$). The extent of the fall in basal PAI-1 antigen levels with troglitazone therapy in the diabetic patients also correlated with the extent of reduction of fasting plasma glucose ($r = 0.64$, $P < 0.02$) and fructosamine ($r = 0.61$, $P < 0.02$) levels and with the fall in plasma NEFA levels ($r = 0.63$, $P < 0.02$), but not with the reduction in plasma triglyceride concentration or improvement in insulin sensitivity. Although after troglitazone therapy plasma PAI-1 antigen levels were not significantly correlated with the logarithm of the fasting plasma triglyceride levels, in either the obese ($r = 0.62$, $0.05 < P < 0.10$) or diabetic ($r = 0.23$, NS) subjects, plasma tPA levels were in both the obese ($r = 0.75$, $P < 0.02$) and diabetic ($r = 0.59$, $P < 0.05$) subjects.

Effect of acute hyperinsulinemia with and without troglitazone on plasma PAI-1 and tPA levels. In the diabetic patients studied before troglitazone therapy, PAI antigen concentrations were lower at the end of the 5-h hyperinsulinemic-euglycemic clamps (38.9 ± 6.1 µg/l) than in the basal state (53.6 ± 5.4 µg/l; $P < 0.05$). There was a corresponding reduction in plasma PAI-1 activity (42.4 ×/÷ 1.22 to 9.6 ×/÷ 1.47 AU/ml; $P < 0.001$), but the small reduction in plasma tPA antigen levels was not statistically significant (12.06 ± 0.50 to 11.13 ± 0.55 µg/l; NS). Plasma PAI-1 antigen and activity also fell during the glucose clamps in the 12 normal subjects (6 lean and 6 obese) in whom these measurements were made at the end of the glucose clamps (PAI-1 antigen, 26.0 ± 3.4 to 17.1 ± 2.9, $P < 0.05$; PAI-1 activity, 8.7 ×/÷ 1.55 to 1.5 ×/÷ 1.48 AU/ml; $P < 0.001$) (Fig. 3). Plasma tPA antigen, however, was not significantly different in the normal subjects at the end of glucose clamps (8.3 ± 1.2 µg/l) compared with basal levels (8.9 ± 1.2 µg/l). Whereas troglitazone lowered the basal PAI-1 antigen and activity levels in the type 2 diabetic and obese normal subjects, it did not influence the response to the glucose clamps in either the diabetic or normal subjects. Thus, plasma PAI-1 antigen concentrations and activities at the end of the glucose clamps were very similar before and after treatment with troglitazone (Fig. 3).

DISCUSSION

Low plasma fibrinolytic activity in association with increased plasma PAI-1 levels has been linked to an increased risk of atherosclerosis in obese subjects and patients with type 2 diabetes (4,5,14). In keeping with results in previous studies, plasma PAI-1 antigen concentrations and activity were markedly increased in the diabetic patients we studied and to a lesser extent in the obese nondiabetic subjects (Fig. 1). Consistent with results from others (4,6–10), we found that PAI-1 levels in the nondiabetic subjects correlated with a number of variables that cosegregate in subjects with the insulin resistance syndrome, including BMI, fasting plasma insulin, and NEFA levels (Fig. 2). A positive correlation between plasma PAI-1 levels and BMI was found also in the diabetic patients (Fig. 2).

The suggestion that chronic hyperinsulinemia in association with insulin resistance is the primary determinant of elevated plasma PAI-1 levels in obesity and type 2 diabetes (4,6,9) prompted us to examine the influence of treatment with troglitazone in diabetic and nondiabetic subjects on plasma PAI-1 antigen concentrations and activity. As expected, troglitazone improved peripheral tissue insulin sensitivity and lowered fasting plasma insulin levels in both the type 2 diabetic patients and insulin-resistant obese non-

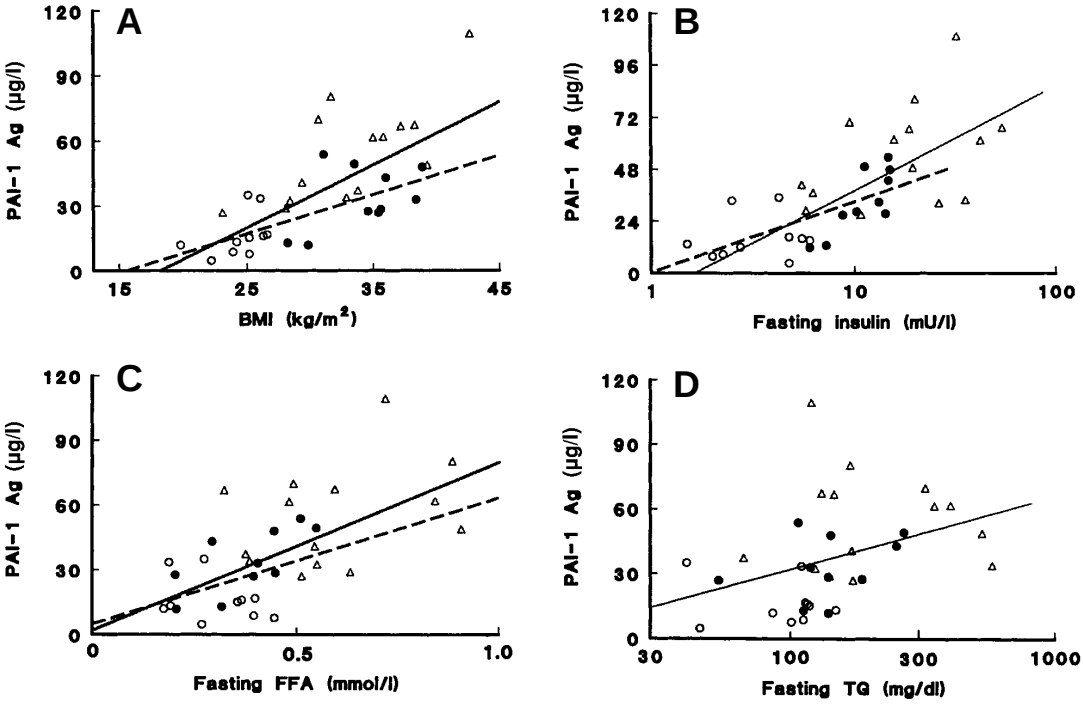

FIG. 2. Relationships between fasting plasma PAI-1 antigen levels and BMI (*A*), fasting plasma insulin levels (log scale) (*B*), fasting plasma NEFA levels (*C*) and fasting plasma triglyceride levels (log scale) (*D*) in 10 lean normal subjects (○), 10 obese subjects (●), and 14 type 2 diabetic patients (△). In the 20 nondiabetic subjects (- - -), PAI-1 antigen was directly correlated with BMI ($r = 0.69$, $P < 0.001$), fasting insulin ($r = 0.68$, $P < 0.001$), and fasting NEFAs ($r = 0.44$, $P < 0.05$). In the diabetic patients, PAI-1 antigen correlated with BMI ($r = 0.72$, $P < 0.005$). For all three groups combined (—), PAI-1 antigen correlated with BMI ($r = 0.71$, $P < 0.001$), fasting plasma NEFAs ($r = 0.63$, $P < 0.001$), and the common logarithms of fasting plasma insulin ($r = 0.71$, $P < 0.001$) and triglyceride levels ($r = 0.38$, $P < 0.05$).

diabetic subjects (Table 2). In the diabetic patients, a 20% reduction in fasting plasma glucose levels and a decrease in fasting plasma NEFA and triglyceride levels (Table 2) were seen. Plasma PAI-1 antigen and activity in the diabetic patients after an overnight fast decreased by 33 and 40%, respectively, after 3 months of treatment with troglitazone compared with baseline values (Fig. 1). Plasma PAI-1 antigen and activity tended to fall as well in the obese nondiabetic subjects. The response in this group did not, however, reach statistical significance ($0.1 < P < 0.05$ for PAI-1 antigen).

The increased plasma PAI-1 antigen concentrations in the diabetic and obese subjects were accompanied by increased plasma tPA antigen. The latter decreased after troglitazone in both the obese subjects and type 2 diabetic patients (Fig. 1). tPA antigen circulates in blood largely bound to PAI-1. As PAI-1 levels increase, a higher proportion of tPA is complexed with PAI-1. The enzymatically inactive PAI-1/tPA complexes are cleared more slowly than active, free tPA so that total plasma tPA levels increase along with those of PAI-1, even though tPA activity falls (39). Thus, tPA antigen concentrations are paradoxically inversely related to fibrinolytic system activity (39). When plasma PAI-1 levels decrease, the clearance of tPA antigen is increased. This may explain the fall in tPA antigen concentrations we observed after troglitazone. In type 2 diabetic patients treated intensively with continuous subcutaneous insulin, a similar reduction in tPA antigen levels accompanied the reduction in PAI-1 levels (10).

Glycemic control is generally not considered to be an important determinant of plasma PAI-1 concentrations (4,10,30,40). The lack of any relationship between plasma PAI-1 antigen and activity levels and parameters of glycemic control in the diabetic patients at baseline would support

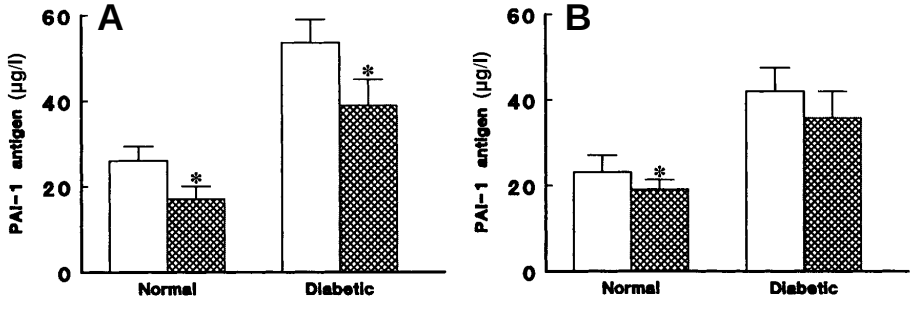

FIG. 3. Plasma PAI-1 antigen levels (means ± SE) after an overnight fast (□) and at the end of a 5-h hyperinsulinemic-euglycemic clamp (▨) in 12 normal subjects (6 lean and 6 obese) and 9 type 2 diabetic patients before (*A*) and after (*B*) 3 months of treatment with troglitazone (600 mg/day). *$P < 0.05$ compared with overnight fasted PAI-1 antigen values.

this view. However, we did find that the decrease in PAI-1 antigen levels with troglitazone (Fig. 1) correlated with both the extent of the fall in fasting plasma glucose concentration ($r = 0.64$, $P < 0.02$) and the reduction in plasma fructosamine levels ($r = 0.61$, $P < 0.02$). The extent to which plasma PAI-1 antigen levels and activity were lowered after troglitazone in the diabetic patients studied is comparable to that seen with intensive insulin therapy (10), even though the improvement in glycemic control in our diabetic patients was rather modest. This finding suggests that factors other than glycemic control play a role. Improved glycemic control in the diabetic patients was accompanied by lower plasma NEFA levels and an improved lipid profile, raising the possibility that the reduction in lipid levels was a more proximate determinant of the decline in plasma PAI-1 antigen levels and activity. Indeed, the improvements in glycemic control and plasma NEFA levels were directly related, and the decline in plasma NEFA levels was found also to correlate with the reduction in plasma PAI-1 antigen levels ($r = 0.63$, $P < 0.02$). Although troglitazone led to a significant reduction in fasting plasma triglyceride and increased HDL cholesterol levels in our subjects, none of these changes correlated with the reduction in PAI-1 antigen and activity levels.

Consistent with a role for chronic hyperinsulinemia as a determinant of the elevated plasma PAI-1, we found that the decline in plasma PAI-1 antigen and activity with troglitazone was correlated with the extent of reduction in fasting insulin levels in the diabetic patients (PAI-1 antigen, $r = 0.60$, $P < 0.05$; PAI-1 activity, $r = 0.67$, $P < 0.01$). These relationships also held in the diabetic and obese groups combined ($r = 0.58$, $P < 0.005$, and $r = 0.60$, $P < 0.002$, respectively). Our obese subjects had much lower fasting insulin levels than the obese women with the polycystic ovary syndrome studied by Ehrmann et al. (41), which probably explains why troglitazone, which lowered fasting insulin levels by 66% in these women, induced a more marked reduction in plasma PAI-1 antigen levels and activity than in our obese subjects. Our findings in the diabetic patients differ from those of Sironi et al. (42), who found no effect of 8 weeks troglitazone therapy on plasma PAI-1 levels in patients with type 2 diabetes. This difference is most likely due to their use of only 200 mg/day of troglitazone, which had no significant effect on fasting plasma insulin levels (42). The shorter duration of therapy may also have been a factor, since the maximal insulin-sparing effect of troglitazone may not be attained until 3–4 months of treatment (43).

The relative contributions made by the liver, adipose tissue, and vascular endothelium to circulating PAI-1 levels is unclear. Several studies have suggested that adipose tissue may be an important source and play a role in the elevated PAI-1 levels found in obesity and type 2 diabetes (19,20). Increased PAI-1 mRNA expression was found in subcutaneous adipose tissue from obese and type 2 diabetic patients (20).

Earlier studies suggested that visceral fat was particularly important (19,44), with PAI-1 production rates being higher in visceral than subcutaneous adipocytes (19). However, because subcutaneous adipocytes make a greater contribution to total fat mass, it is likely that both visceral and subcutaneous fat depots are important sources of circulating PAI-1. In addition to increasing skeletal muscle insulin sensitivity, troglitazone has profound effects on adipose tissue gene expression and metabolism (25,31,32). It was therefore of interest to examine the influence of troglitazone on the PAI-1 response to acute hyperinsulinemia. Consistent with results in previous studies (21,23,24), PAI-1 levels tended to fall during the hyperinsulinemic-euglycemic clamps in the normal subjects and more strikingly in the diabetic patients with elevated basal PAI-1 levels. Plasma PAI-1 levels undergo a diurnal variation, with highest levels in the early morning and lower levels in the afternoon (22,24,45,46). Irrespective of whether the fall in PAI-1 levels during the glucose clamp was a direct effect of insulin or in part due to this diurnal rhythm, troglitazone did not influence the PAI-1 response to the clamps. Thus, although basal PAI-1 levels were lower after 3-month treatment with troglitazone, levels at the end of the glucose clamps were unaffected (Fig. 2).

It has been suggested that some of the beneficial effects of troglitazone on cardiovascular risk factors may be independent of its insulin-sensitizing effects (25). Our findings suggest that the favorable effects on fibrinolytic system capacity are linked intimately to increased insulin sensitivity for several reasons. First, the reductions in plasma PAI-1 levels were related directly to reductions in plasma insulin, NEFA, and glucose levels. Second, the two diabetic patients in whom glycemic control deteriorated, as evidenced by large increases in serum fructosamine levels along with a small reduction in insulin sensitivity and increase in fasting insulin levels, exhibited either no change or an increase in plasma PAI-1. This suggests that nonresponders to troglitazone with respect to insulin sensitivity and glycemic control are likely to be nonresponders also with respect to improvement in fibrinolytic system capacity.

In conclusion, the results of the present study demonstrate that treatment with troglitazone enhances otherwise markedly inhibited fibrinolytic system impairment in insulin-resistant obese subjects and patients with type 2 diabetes by enhancing insulin sensitivity.

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