Effect of Combination Glipizide GITS/Metformin on Fibrinolytic and Metabolic Parameters in Poorly Controlled Type 2 Diabetic Subjects

WILLIAM T. CEFALU, MD[1]
DAVID J. SCHNEIDER, MD[1]
HAROLD E. CARLSON, MD[2]
PHYLLIS MIGDAL, MD[2]
LEONIL GAN LIM, MD[2]
MERIAM P. IZON, MD[2]
ANOOP KAPOOR, MD[3]
AUDREY BELL-FARROW, MBA, MHA[4]
JAMES G. TERRY, MS[4]
BURTON E. SOBEL, MD[1]

OBJECTIVE — Epidemiological studies have implicated increased plasminogen-activated inhibitor 1 (PAI-1) as a marker or predictor of accelerated coronary atherosclerotic disease in type 2 diabetes. We sought to determine whether metabolic control, independent of its oral mode of implementation, affects PAI-1 in patients with marked hyperglycemia.

RESEARCH DESIGN AND METHODS — A total of 91 subjects were screened, subjected to a 4-week drug washout, and randomized to daily treatment with glipizide GITS (maximum 20 mg, $n = 46$) or metformin (maximum 2,550 mg, $n = 45$) as monotherapy. After monotherapy, combination therapy was initiated by adding the second agent to the regimen. Plasma glucose (fasting and postprandial), HbA_{1c}, fructosamine, and PAI-1 were assayed before and after randomization and sequentially thereafter in all subjects; hepatic glucose output (HGO) and abdominal fat distribution were each measured in a subset of subjects.

RESULTS — Glycemic control was markedly impaired at baseline (mean HbA_{1c} 10.4 ± 0.2% glipizide GITS; 10.0 ± 0.2% metformin) but improved comparably with each agent as monotherapy and in combination ($P < 0.0001$ vs. baseline), as assessed with meal tolerance studies, fructosamine values, and HGO. Body weight and abdominal fat distribution did not change significantly in either group. PAI-1 concentrations were extraordinarily high (5- to 10-fold more than normal) at baseline (202 ± 12 ng/ml glipizide GITS; 201 ± 13 ng/ml metformin) but declined comparably, and significantly, after treatment with either agent as monotherapy and decreased further with combination therapy.

CONCLUSIONS — When hyperglycemia is profound, increases in PAI-1 are also profound. Control of hyperglycemia with either glipizide GITS, an insulin secretagogue, or metformin as monotherapy comparably ameliorates elevated PAI-1.

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From the [1]Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont; the [2]Department of Medicine, State University of New York at Stony Brook, Stony Brook, New York; [3]Medical Service, Northport VA Medical Center, Northport, New York; and [4]Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Address correspondence and reprint requests to William T. Cefalu, Associate Professor of Medicine, University of Vermont College of Medicine, UHC Campus, Arnold 3433, One South Prospect Street, Burlington, VT 05401. E-mail: william.cefalu@uvm.edu.

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Abbreviations: ACS, acyl-CoA synthase; AUC, area under the curve; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; FBG, fasting blood glucose; FFA, free fatty acid; FPG, fasting plasma glucose; HGO, hepatic glucose output; LOCF, last observed clinical finding; MRI, magnetic resonance imaging; NEFA, nonesterified (free) fatty acids; PAI-1, plasminogen-activated inhibitor 1; SUNY, State University of New York at Stony Brook; t-PA, tissue-type plasminogen activator; UKPDS, U.K. Prospective Diabetes Study; WFU, Wake Forest University.

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

In type 2 diabetes, complications from cardiovascular disease (CVD) remain the major cause of morbidity and mortality. Type 2 diabetes confers at least a twofold increase in CVD risk, negates the cardioprotection afforded nondiabetic women, and is considered a "risk equivelant" for CVD (1–5). The multifactorial determinants of CVD include blood lipids, smoking, sex, and hypertension. However, these and other traditional major risk factors, in combination, do not fully explain the increased CVD risk in patients with type 2 diabetes. Accordingly, other determinants may be important (6–8). Epidemiological evidence has implicated one such factor, increased concentration of plasminogen-activated inhibitor 1 (PAI-1), as a marker or predictor of accelerated coronary atherosclerotic disease (9–12).

Increased PAI-1 accompanies insulin resistance (8). In association with hyperglycemia, hyperlipidemia, and increased insulin, PAI-1 concentrations increase in blood in vivo in normal human subjects and in experimental animals (13,14). VLDL, hypertriglyceridemia, and elevated free fatty acids (FFAs) can augment synthesis of PAI-1 in vitro through interactions with the PAI-1 promoter (15,16). Alternatively, the hyperglycemia associated with type 2 diabetes could influence the synthesis and secretion of PAI-1 directly, as judged from results of in vitro studies (17). Whether effective clinical treatment of hyperglycemia alters PAI-1 levels provides the rationale for the current study.

In the U.K. Prospective Diabetes Study (UKPDS) (18), a 1% difference in mean HbA_{1c} was associated with a 14% difference in risk for myocardial infarction; however, although glycemic control was comparable with the various agents as monotherapy in the UKPDS, monotherapy rarely provided adequate control over the long term (19), and combination

therapy with oral antidiabetic agents then becomes necessary (19–21).

Therapies with insulin and sulfonylureas, such as those evaluated in the UKPDS, although initially beneficial on glycemic control, resulted in weight gain and increased insulin levels (22). Obesity (visceral fat in particular) is associated with increased PAI-1 levels, and it has not been established whether the weight gain that accompanies improved glycemic control negates the otherwise favorable effects of glycemic control on CVD. Extended release sulfonylurea formulations, compared with older sulfonylureas, are associated with less weight gain and lower fasting insulin levels (23,24). Their potential benefit with respect to impaired fibrinolysis has not yet been assessed.

In view of the apparent equivalence of both extended release sulfonylureas and metformin in improving glycemic control despite the presumably differential effects on hyperinsulinemia, the present study was designed to determine whether metabolic control, per se, independent of its mode of implementation, would affect concentrations of PAI-1 in blood similarly in subjects with type 2 diabetes and marked hyperglycemia. Further, the efficacy of the clinical intervention on glycemic control, hepatic glucose production, and body fat distribution was assessed.

RESEARCH DESIGN AND METHODS

Subjects

Subjects aged 35–70 years with type 2 diabetes diagnosed at least 6 months before onset of the study were assessed. All subjects had been previously maintained on a stable dose of oral sulfonylureas for a minimum of 2 months and were required to have an HbA_{1c} >7% at the time of enrollment. All procedures were approved and conducted in strict compliance with each institution's human research guidelines.

Design of the study

The study was an open label, randomized, parallel, titration study. It utilized a 4-week washout period (period 1), a 6-week period of treatment with glipizide GITS (Glucotrol XL) or metformin (Glucophage) as monotherapy (period 2), and a 12-week period of glipizide GITS and metformin in combination (period 3).

Period 1. After informed consent was signed and entry criteria had been met, subjects were instructed about a weight maintenance diet and home blood glucose monitoring, and oral agents were then discontinued. At the end of the 4-week drug washout, fasting glucose, HbA_{1c}, fructosamine, PAI-1 levels, and meal tolerance testing was determined. Body fat distribution was assessed with magnetic resonance imaging (MRI) scans, and hepatic glucose output (HGO) studies were performed. A fasting glucose >11.1 mmol/l was required for continued inclusion of subjects in the study.

Period 2. Subjects were randomized to one of two experimental groups consisting of either glipizide GITS or metformin as monotherapy. Subjects receiving glipizide GITS were initially treated with 5 mg every morning, and increased in 5-mg increments (to a maximum of 20 mg q a.m.) at 1-week intervals or until the fasting blood glucose (FBG), assayed in samples obtained by fingerstick, was ≤7.8 mmol/l. Subjects randomized to metformin initially received 850 mg daily, increased in 850-mg increments (to a maximum of 850 mg t.i.d.) at 2-week intervals until the FBG was ≤7.8 mmol/l.

If the subject had a fasting plasma glucose (FPG) ≤7.8 mmol/l, the same dose of glipizide GITS or metformin was continued throughout period 2. Glycemic control and PAI-1 measures, in addition to abdominal MRI scans and HGO assay results, were obtained at the end of period 2. FPG ≥7.8 mmol/l was required for continuation of subjects into period 3.

Period 3. Subjects who had been inadequately controlled (fasting glucose >7.8 mmol/l) on the final dosage of metformin at the end of period 2 were continued on the same dosage of metformin and given, in addition, 5 mg of glipizide GITS every morning. The daily dose of glipizide GITS was titrated upward in 5-mg increments at 1-week intervals (to a maximum of 20 mg) on the basis of FBG values.

Subjects who had been inadequately controlled (fasting glucose >7.8 mmol/l) on the final dose of glipizide GITS at the end of period 2 were maintained on that dose and given, in addition, 850 mg of metformin daily. The dose of metformin was titrated upward in 850 mg dose increments at 2-week intervals based on FBG values, to a maximum of 2,550 mg daily (850 mg t.i.d. with meals). At the end of period 3, assays of study variables were repeated.

Study variables

Body fat content in the abdominal area (total abdominal, intra-abdominal, and subcutaneous) was assessed in all subjects ($n = 44$) at one site (WFU) with the use of MRI at the level of the umbilicus as previously described (25). HbA_{1c} was assessed by ion-exchange high-performance liquid chromatography (Bio-Rad, Hercules, CA), fructosamine by nitrotetrazolium blue assay (Roche, Indianapolis, IN), and glucose by hexokinase photometric assay (Roche). All tests were performed in a central laboratory (MedLab, San Antonio, TX). Glucose tolerance was assessed by performing a mixed meal (Sustacal, Mead Johnson, Evansville, IN) challenge with determination of blood glucose and insulin levels at 0, 0.5, 1, 1.5, 2, and 3 h after challenge.

PAI-1 concentration was determined by enzyme-linked immunosorbent assay (ELISA) (TintElize; BioPool, Umea, Sweden) in fasting, morning plasma samples obtained from blood anticoagulated with trisodium citrate (0.129 mol/l, pH 6.0, 1:10 vol/vol) after centrifugation (30,000$g \times$ min). Samples were stored at −70°C until assay. Nonesterified (free) fatty acid (NEFA) concentrations in serum were determined with Wako NEFA *C* Kits and an enzymatic colorimetric method that entails acylation of CoA by the fatty acids in the presence of added acyl-CoA synthase (ACS).

HGO was assessed in all subjects ($n = 17$) at one of the study sites (SUNY). Each HGO measurement was initiated at 0800 h after a 10-h overnight fast. An upper-extremity intravenous line was inserted for infusion of test substances. A second cannula was placed retrograde in the opposite hand for collection of venous blood, arterialized by warming the arm in a heated box (65°C). Deuterated (6,6-$^2H_2$) glucose was given intravenously as a priming dose (2.72 mg/kg) followed by a constant infusion of 0.034 mg · kg^{-1} · min^{-1} for 210 min. Serum glucose and the mole fraction percent excess deuterated glucose were measured every 5 min during the final 30 min of the infusion, during which the blood glucose concentration was stable. Endogenous glucose production was calculated with the use of standard isotope dilution methods (26).

Statistical analysis. The two treatment groups were compared using the least-squares means of the change from the monotherapy baseline to end of mono-

Table 1—*Metabolic and body fat parameters*

Parameters	Glipizide GITS			Metformin		
	Baseline	Mono Rx	Combo Rx	Baseline	Mono Rx	Combo Rx
Glycemic						
HbA_{1c} (%)	10.4 ± 1.5	10.1 ± 1.5*	8.2 ± 1.4†	10.0 ± 1.1	9.9 ± 1.2	7.0 ± 0.7†
Fructosamine (μmol/l)	432 ± 80	374 ± 83†	298 ± 81†	398 ± 54	355 ± 74†	234 ± 38†
Fasting glucose (mmol/l)	16.7 ± 0.4	12.3 ± 0.5†	9.6 ± 0.4†	16.3 ± 0.4	12.6 ± 0.5†	8.5 ± 0.3†
Meal tolerance						
Glucose AUC ($mmol \cdot min \cdot l^{-1}$)	4,555 ± 691	3,418 ± 723†	2,766 ± 715†	4,392 ± 692	3,252 ± 727†	2,223 ± 425†
Insulin AUC ($pmol \cdot min \cdot l^{-1}$)	33,636 ± 20,214	45,672 ± 24,810†	47,676 ± 25,524†	35,514 ± 20,412	45,882 ± 27,396†	64,098 ± 32,328†
Body fat						
Body weight (kg)	94 ± 18	94 ± 17	97 ± 19	95 ± 18	94 ± 16	96 ± 19
IAF mass (cm^2)	132 ± 54	128 ± 50	142 ± 55	175 ± 61	175 ± 78	184 ± 63
Total abdominal fat mass (cm^2)	476 ± 195	459 ± 190	504 ± 193	462 ± 108	452 ± 92	463 ± 113
HGO ($mg \cdot kg^{-1} \cdot min^{-1}$)	3.1	2.3‡	2.1‡	2.9	2.4‡	1.9‡

Data are means ± SD. *$P < 0.02$, †$P < 0.0001$, ‡$P < 0.05$ vs. baseline. IAF, intra-abdominal fat.

therapy using ANCOVA. The ANCOVA model contained factors for treatment, center, and treatment-by-center interaction along with the baseline value as a covariate. Within each treatment group, the change from the monotherapy baseline to the final visit of the monotherapy period (last observed clinical finding [LOCF]) was calculated for each subject. A paired *t* test was performed to assess whether there was a significant within-treatment change after treatment. Similarly, the changes in the measured parameters from the combination therapy baseline to the final visit of the combination therapy period (LOCF) were estimated for each subject. A paired *t* test was performed to assess whether there was a significant within-treatment change after combination therapy. For the meal challenge tests, the total area under the curve (AUC) for postprandial plasma glucose and insulin was estimated for each subject at each visit using a linear trapezoidal method. The change from monotherapy baseline AUC to end of monotherapy was computed and compared between the two treatment groups using the two-sample *t* test. In addition, paired *t* tests were used to determine whether there were significant changes in these three AUCs within each treatment arm.

RESULTS

Demographics. A total of 131 subjects were screened and entered the washout; 91 subjects completed study entry requirements and qualified for the monotherapy phase by having FPG values >11.1 mmol/l 4 weeks after medications had been withdrawn; and 45 subjects were randomized to treatment with metformin (30 men/15 women) and 46 (25 men/15 women) to glipizide GITS as monotherapy. The duration of diabetes did not differ between treatment groups (glipizide GITS 7.7 ± 4.5 years vs. metformin 6.3 ± 4.3 years), nor was there a significant difference in age of subjects (glipizide GITS 53.5 ± 9.1 years vs. metformin 55.8 ± 7.2 years). BMI was not different between groups (glipizide GITS 32.5 ± 6.2 kg/m^2 vs. metformin 32.1 ± 5.6 kg/m^2). There were no other differences in subject characteristics at baseline (Table 1). Figure 1 demonstrates the protocol design, study periods, number of subjects advancing to each phase, and reasons for discontinuation.

Metabolic control. After 6 weeks of monotherapy, FPG had fallen significantly in those treated with either metformin or glipizide GITS. Both declines were highly significant compared with baseline values but did not differ significantly between groups (Table 1). There was a further, and significant, decrease in FPG at the end of the combination treatment (Table 1).

HbA_{1c} fell slightly in both groups over the 6 weeks of monotherapy. The 0.3% decrease in the glipizide GITS–treated group was statistically significant ($P < 0.02$), but the 0.1% decrease in the metformin-treated group was not. However, at the end of combination treatment, HbA_{1c} was significantly decreased in both groups ($P = 0.0001$) (Table 1).

Serum fructosamine fell significantly and comparably in both monotherapy groups ($P < 0.0001$) and subsequently in both combination therapy groups ($P < 0.0001$) (Table 1). Glucose tolerance assessed with determination of AUC during the meal study improved significantly and comparably with both agents after 6 weeks of monotherapy ($P < 0.0001$) and decreased further with combination therapy (Table 1). Insulin AUC observed during the tolerance test increased slightly with both agents as monotherapy; when glipizide GITS was added to metformin, a further increase was noted.

Body composition and body weight. Body weight and BMI did not change significantly with either glipizide GITS or metformin as monotherapy or in combination with both agents (Table 1). In addition, total abdominal and intra-abdominal fat, as assessed with MRI scanning, did not change with either agent or with the combination of both agents (Table 1).

HGO. Endogenous glucose production fell with monotherapy with either agent (Table 1), but the decrease was significant only in the glipizide GITS subjects ($P = 0.003$). The percentage decrease in HGO was somewhat greater with glipizide GITS monotherapy (23%) compared with metformin (13%), but the difference was not

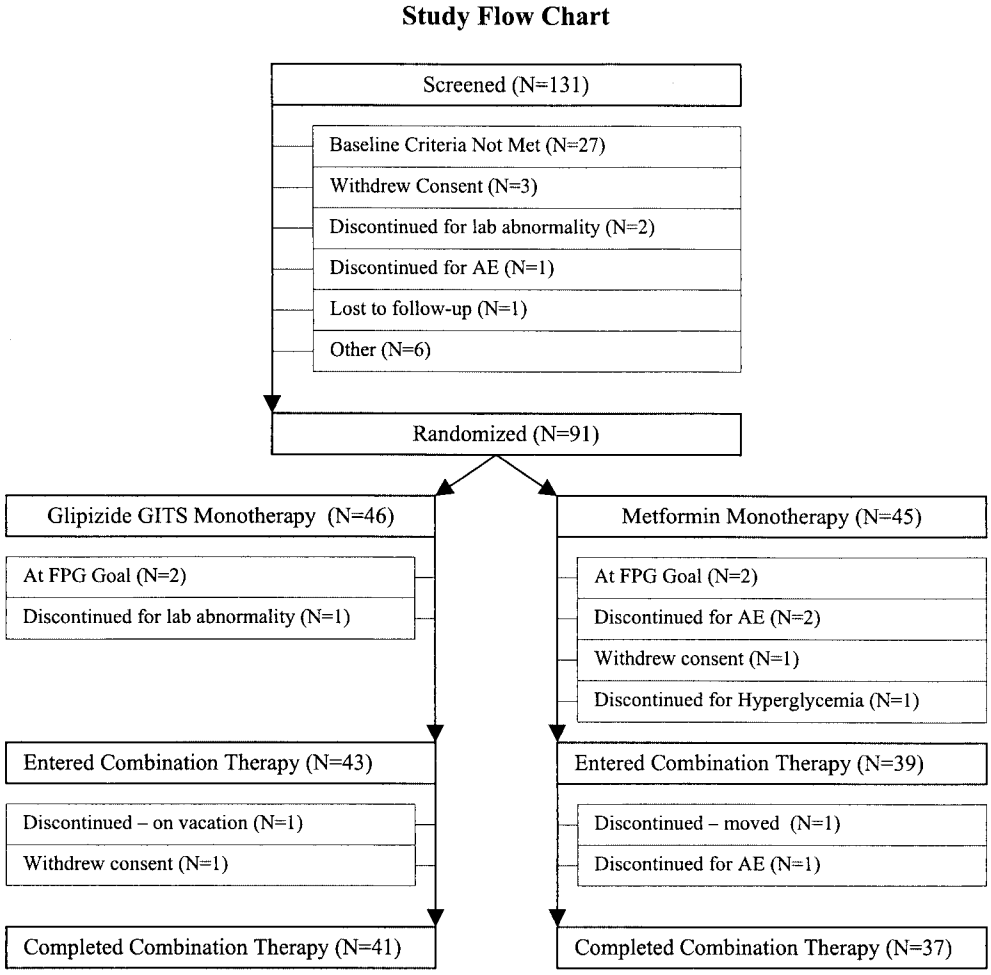

Figure 1—*Study flow chart.*

statistically significant. Combination therapy resulted in a further 16% reduction in HGO from the end of the monotherapy treatment phase to the end of the combination therapy phase ($P = 0.006$ for all 17 subjects) (Table 1).

NEFA and PAI-1. As shown in Fig. 2*A*, both agents significantly reduced NEFA concentrations in blood. When the agents were used in combination, NEFA was reduced further and significantly compared with baseline values. Decreased PAI-1

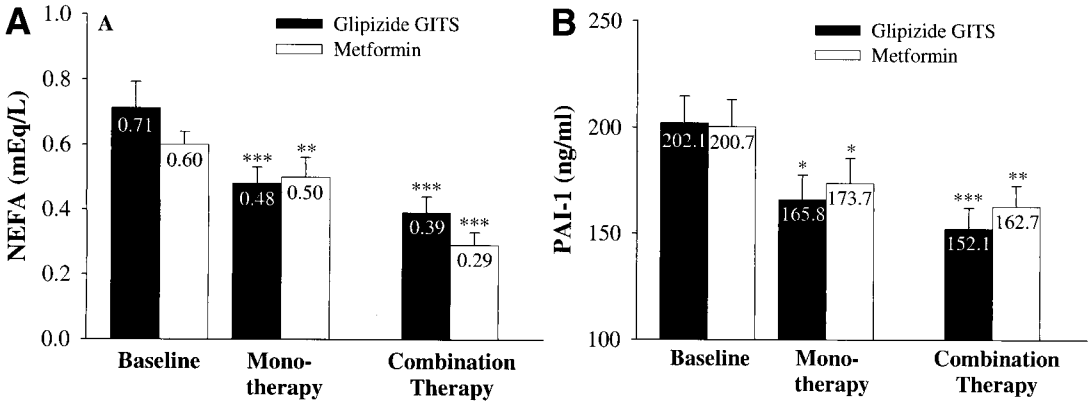

Figure 2—*Demonstrates NEFAs and PAI-1 at baseline and at the end of monotherapy and combination therapy. A robust effect was seen with monotherapy, with a further reduction with combination therapy for both NEFA* (A) *and PAI-1* (B). *P < 0.05, **P < 0.005 and ***P < 0.001 *versus baseline. Data are means ± SE.*

was seen with improved metabolic control regardless of the agent used in the monotherapy phase (Fig. 2*B*); the concentration of PAI-1 in blood was decreased further and significantly when both agents were used in combination (Fig. 2*B*).

CONCLUSIONS — The goal of this study was to determine the relation between metabolic control and PAI-1 levels in patients with type 2 diabetes. Our results indicate that monotherapy with either an extended-release sulfonylurea preparation (glipizide GITS) or metformin improved metabolic control, as assessed by FPG, meal tolerance, glycated protein levels, and serum FFA. GHb did not change markedly with monotherapy, which may have been because of the short treatment period, or perhaps the washout period was too short to allow equilibrium for HbA_{1c}. However, serum fructosamine, a shorter-term objective marker for glycemic control, mirrored the changes observed for the FPG and meal tolerance studies. Additive effects on all parameters of glycemic control were seen when the two agents were used in combination (Table 1).

Both drugs, alone and in combination, had a neutral effect on body weight and body fat distribution. Weight gain is commonly observed with regimens that improve glycemia; in the UKPDS, sulfonylureas or exogenous insulin induced greater weight gain than that seen with the use of metformin or diet alone (22). However, we have shown that monotherapy with glipizide in the extended-release formulation provides a weight neutral result compared with placebo, a finding confirmed with the present results (24). Although the mechanism by which the newer sulfonylureas result in a more neutral effect on weight is not known, a more modest increase in ambient insulin secretion between meals, in addition to less hypoglycemia, may partially account for the difference (23,24). The lack of weight gain with glycemic control may be particularly relevant with respect to the fibrinolytic system when consideration is given the role that adipocytes and visceral fat play in the elaboration of PAI-1 (27–30). It is important to note, however, that subjects were instructed on weight maintenance diets for this study as weight loss may have had favorable and comparable effects on PAI-1 levels.

Both agents decreased HGO when given as monotherapy but achieved statistical significance with the glipizide GITS. Combination therapy resulted in a further and significant decrease in HGO. The potential mechanism to explain this observation could be a relatively greater impact of reduced glucose toxicity compared with the direct effect of a drug, but the precise mechanism is not known. Although it appeared that glipizide GITS was more effective than metformin in reducing HGO in the monotherapy phase of the study, this may be secondary to the more rapid titration that occurred with glipizde GITS therapy. The role that decreased HGO played in altering PAI-1, outside of decreasing glycemia, is not known and was not addressed in this study. The decreased HGO was reflected in the reduced FBG. Further, the insulin response to a mixed meal was noted to actually improve after glycemic control with either agent. This increase in insulin response may also have been secondary to a reduction in glucose toxicity, as this increase was also observed with metformin.

PAI-1 concentrations were markedly elevated (~200 ng/ml) in our poorly controlled subjects compared with values reported in diabetic subjects who were less obese and less hyperglycemic (~30–50 ng/ml) (31). Antigen was measured rather than activity because of the greater stability of its concentration compared with activity in frozen, shipped, and stored samples. Directional changes in the two are generally concordant. In general, increases in PAI-1 antigen are associated with PAI-1 activity and with stoichiometrically much more modest increases in tissue-type plasminogen activator (t-PA) because clearance of t-PA/PAI-1 complex is less rapid than that of free t-PA (32). Elevated PAI-1 antigen is tantamount to increased PAI-1 activity (33). Regardless of how high PAI-1 values are, t-PA rarely exceeds 12 ng/ml in people with diabetes. Because of these considerations and costs, t-PA was not measured. PAI-1 concentrations, however, fell with either metformin or glipizide GITS monotherapy, indicating that the derangement in poorly controlled patients can be ameliorated by improving glycemic control, regardless of the mechanism (insulin sparing or insulin providing) through which it is achieved; further decreases in PAI-1 concentrations occurred during the combination therapy period. Thus, improved metabolic control exerted favorable effects on PAI-1 concentrations, thereby favorably modifying a known CVD risk factor in type 2 diabetes. Although increased insulin alone does not increase PAI-1 in normal human subjects (34), endogenous hyperinsulinemia and hyperlipidemia induced by infusion of glucose plus intralipid do (14). Others have shown that glibenclamide either increases (35) or fails to change elevated PAI-1 (36) in contrast to insulin or troglitazone, which attenuate the elevations.

Diminished release of PAI-1 from adipocytes, visceral fat, and other sites may reflect reductions in plasma FFAs, triglycerides, and glucose (14,37). Such favorable changes can be expected in response to therapy that improves metabolic control and diminishes glucose toxicity. In view of the implicated and potential contributions of elevated PAI-1 to atherogenesis, formation of vulnerable atherosclerotic plaques prone to rupture, and precipitation of acute coronary syndromes, our present observations underscore the value of metabolic control, per se, in retarding progression of macro- as well as microvascular disease.

In summary, we demonstrate that improved metabolic control in type 2 diabetes can significantly decrease elevated concentrations of PAI-1. The decrease in PAI-1 was induced by drugs with dissimilar effects on insulin secretion (i.e., glipizide GITS and metformin), emphasizing the important contribution that metabolic control has on this process. Thus, the favorable effects on PAI-1 concentrations induced by the improved metabolic control from use of a sulfonylurea/metformin combination diminished a known CVD risk in type 2 diabetes.

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