To determine whether the hypercoagulable state of patients with complications of diabetes can be reversed toward normal, a group of insulin-dependent individuals with proteinuria was treated with intensive insulin protocols. A statistically significant ($P<.001$) improvement in control of diabetes was achieved (mean±SEM glycosylated hemoglobin, 9.51%±0.35% at baseline to 8.36%±0.39% at 12 months; and mean±SEM advanced glycosylated end products, 14.8±2.8 U/mL at baseline to 8.4±1.5 U/mL at 12 months). There were statistically significant decreases in 2 procoagulant factors: mean±SEM baseline elevated plasma factor VII, 128.69%±5.63% at baseline to 106.24%±3.43% at 12 months ($P=.002$); and mean±SEM plasma fibrinogen, 12.3±0.7 µmol/L (417.3±24.7 mg/dL) at baseline to 10.2±0.7 µmol/L (348.8±22.6 mg/dL) at 12 months ($P=.04$).

Throughout the study, lipid fractions did not change significantly. Because plasma factor VII and fibrinogen concentrations were elevated while cholesterol and triglyceride concentrations were not, more attention should be paid to procoagulants as markers for thromboembolic complications in diabetic patients undergoing intensive insulin therapy.

Patients with diabetes mellitus and impaired renal function have a high mortality rate due to an increased incidence of cardiovascular events with a high prevalence of symptomatic and asymptomatic coronary arterial disease.\(^1\) Because thrombosis plays an important role in acute coronary syndromes, and elevated levels of fibrinogen and other hemostatic factors have been found in patients with microvascular complications of diabetes, we determined the relation between intensive insulin therapy and hemostatic factors in these high-risk patients.

In diabetic patients, the development of nephrotic syndrome with azotemia is associated with hyperlipidemia and an increased incidence of thromboembolic cardiovascular events.\(^2,4\) Cholesterol, triglycerides, fibrinogen, and factor VII are synthesized in the liver and recognized as cardiovascular risk factors; their levels are increased in patients with type 1 diabetes mellitus.\(^5,6\) There have been few prospective observations relating the interaction between intensive insulin treatment and hemostatic factors in patients with type 1 diabetes mellitus who also have renal dysfunction.

**RESULTS**

For the 17 patients (aged 42.0±1.8 years; range, 33-63 years) described in the Table, the glycosylated hemoglobin level, AGEs, and hemostatic factors were outside of the normal range at baseline. Body weight, plasma viscosity, and serum lipids did not change significantly during the study. The levels of glycosylated hemoglobin and AGEs decreased significantly. Plasma factor VII and plasma fibrinogen levels also decreased significantly. The mean plasma fibrinolytic activity was 1.4 times normal at baseline, decreasing to 0.7 times normal at 12 months, while the level of PAI-1 did not change significantly ($P=.09$) from
PATIENTS AND METHODS

The criteria for enrollment included the following: the onset of insulin dependence before the age of 35 years; albuminuria, with a urinary albumin level of more than 0.1 g/dl; or proteinuria, with a urinary protein level of more than 0.3 g/dl, on 2 separate 24-hour urine collections; and a creatinine clearance of greater than 0.50 ml/s (30 ml/min). Patients were followed up for at least 12 months as part of a multicenter study that had as its purpose the evaluation of the effect on renal function of a regimen of 4 injections of subcutaneous insulin per day vs a similar regimen to which a weekly intravenous infusion of insulin had been added.7 Enrollment required a workup of several weeks to assess the adequacy of control of diabetes and blood pressure. All patients were maintained on a diet of 0.8 g of protein per kilogram of body weight diet to maintain ideal body weight. At each interval, levels of whole blood glycosylated hemoglobin, serum cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, and 24-hour urine for total protein and creatinine were obtained. Under an additional protocol, each patient at the Joslin Diabetes Center, Boston, Mass, underwent standardized phlebotomy (at 8 AM, before any infusions) at baseline and at 6 and 12 months to determine the plasma fibrinogen level, the fibrinolytic activity, the plasminogen activator inhibitor (PAI-1) antigen, and plasma viscosity. Fibrinogen levels were determined by measuring clotting times according to Clauss.8 Plasma factor VII antigen was determined by enzyme-linked immunosorbent assay using a commercially available kit (Asserchrom VII; AG Diagnostica SIAGO, Parsippany, NJ). Antigen levels of PAI-1 were determined by enzyme-linked immunosorbent assay using kits purchased from Biopool International, Ventura, Calif. Fibrinolytic activity was measured in euglobulins (fibrin plate method) as described by Brakman.9 Glycosylated hemoglobin was measured colorimetrically following separation by high-pressure liquid chromatography (Nichols Laboratories, San Juan Capistrano, Calif). The levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by the enzymatic colorimetric method with an autoanalyzer (Boehringer-Mannheim/Hitachi Rueil Diagnostics, Chicago, Ill). Very low-density lipoprotein cholesterol was calculated as triglyceride divided by 5. Low-density lipoprotein cholesterol was calculated by the Friedwald formula: low-density lipoprotein cholesterol = total cholesterol – very low-density lipoprotein cholesterol – high-density lipoprotein cholesterol. Levels of advanced glycosylated end products (AGEs) were measured in plasma by enzyme-linked immunosorbent assay using polyclonal antibodies to AGE-modified proteins (Picower Institute for Medical Research, Manhasset, NY).10

All 23 patients recruited at the Joslin Diabetes Center were enrolled in this study. Of these, 4 did not complete 12 months of study for medical reasons: gangrenous cholecystitis, peripheral vascular ischemia, injury from a motor vehicle crash, and inadequate venous access; 2 did not complete 12 months of study because of a personal decision. Thus, 17 patients completed the study. For clarity of presentation, repeated-measures testing is only included in statistical analysis and in the Table if results were available at all data points for each test. Results from statistical analysis of all data collected (including data from patients in whom an insufficient quantity of blood was available at one session) were virtually identical with results presented in the Table. Patients receiving weekly infusions of insulin were not different from those not receiving insulin infusions for glycosylated hemoglobin level, AGEs, or results of procoagulant studies; therefore, we combined the groups for analysis. Two patients received cholesterol synthesis blockers throughout the study.

Frequency data were tested for significance using the χ² test for independence following the implementation of the Yates correction for continuity. In cases in which the expected frequencies were less than 5, the Fisher exact test was used. Interrelations among the variables were tested for significance using Pearson product moment correlations. Temporal data were analyzed using a 2-way repeated-measures analysis of variance followed by the Newman-Keuls test to determine the significance among time-period means. Repeated-measures testing was performed only when all data points were present. All data are expressed as frequencies or means, with SEMs as a measure of dispersion. An α level of .05 was considered statistically significant. All analyses were done using SAS statistical software (SAS Institute Inc, Cary, NC).

In our patients, the baseline levels of plasma factor VII, fibrinogen, fibrinolytic activity, and whole blood viscosity were elevated; the level of PAI-1 was decreased. During the study, the levels of glycosylated hemoglobin and AGEs significantly decreased with intensive efforts to maintain normal blood glucose. Mean levels of plasma fibrinogen and factor VII decreased significantly. Because fibrinolytic potential was increased, the elevation of plasma fibrinogen is best explained by acceleration of synthesis in the liver. Fibrinogen synthesis is inhibited by the administration of insulin. In 3 reports,11-13 hyperfibrinogenemia associated with insulinopenia was corrected by insulin repletion. Normal subjects challenged by hyperinsulinemia demonstrate a reversible increase in fibrinogen synthesis.11 In patients with type 1 diabe-
Decrease in insulin-dependent patients with type 2 diabetes mellitus, acute withdrawal of insulin with prompt replacement caused fibrinogen synthesis to increase, then decrease. In insulin-dependent patients with type 2 diabetes mellitus, intensive control of hyperglycemia over several weeks was associated with a reversible, accelerated turnover of fibrinogen. Plasma factor VII levels have been shown to increase in normal subjects following a meal or with a hyperglycemic-hyperinsulinemic clamp. Despite improved glycemic control with insulin therapy, lean patients with type 2 diabetes mellitus demonstrated no significant decrease in elevated factor VII activity over 6 months. Insulin-dependent patients with diabetes who have an elevated blood glucose, serum triglycerides, or urinary albumin level also demonstrate increased plasma concentrations of factor VII. We hypothesize that control of glycemia over 6 to 12 months decreased pathological activation of hepatic synthesis of plasma factor VII and fibrinogen.

At baseline in our study, fibrinolytic activity was elevated and PAI-1 was decreased. However, at 12 months, fibrinolytic activity had been significantly reduced without a proportional increase in PAI-1. Excessive fibrinolysis has also been demonstrated in a group of patients with type 1 diabetes mellitus (with similar microvascular complications) to be enhanced, with no proportional relation to tissue plasminogen activation. This reduction in fibrinolytic activity without participation of PAI-1 implies the presence of an alternative pathway. Polymorphonuclear leukocyte elastase has been suggested as an alternative pathway of fibrin degradation in patients with a biologically activated state, such as uncontrolled diabetes. It is possible that catabolism or inflammation may signal activation of immediate-phase reactants, such as elastase, from white blood cells increasing fibrinolytic activity. We hypothesize that control of glycemia over 6 to 12 months decreased pathological activation of an alternative pathway that was responsible for accelerated fibrinolysis.

Our results demonstrate that improvement of glycemic control in diabetic patients with microangiopathy caused fibrinogen synthesis to increase, then decrease.
pathic complications alters procoagulant factors favoring arterial disease. Results of the multicenter study have demonstrated that control of blood glucose by four injections of rapid-acting insulin per day was associated with a loss of creatinine clearance of 0.13 mL/s (7.7 mL/min) per year. Patients randomized to the same insulin schedule plus pulsatile intravenous insulin therapy 1 day per week experienced a loss of creatinine clearance of 0.04 mL/s (2.2 mL/min) per year (P<0.04). We consider that a program of intensive insulin therapy that can normalize levels of glycosylated hemoglobin and AGEs in diabetic patients with microvascular complications will eventually be shown to protect patients from cardiovascular injury by attenuating the inflammatory cascade that stimulates excess fibrinogen production. While further studies will be needed to assess the clinical impact of these findings on thromboembolic event rates, our findings support the recommendation that tight glycemic control “will substantially reduce the macrovascular complications of diabetes.”

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