Relationship Between Autonomic Function and Plasma Fibrinogen, Viscosity, and Elements of Fibrinolytic Activity in Diabetic Nephropathy

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Twenty-three insulin-dependent diabetics with proteinuria (3.3 g/day: range 0.3 to 8.9) and azotemia (creatinine clearance: 58 mL/min, range 30 to 112) were tested for 24-h mean arterial blood pressure; instantaneous heart rate variations to a computerized protocol involving timed ventilation, assumption of upright posture, and Valsalva maneuver; plasma fibrinogen, viscosity, fibrinolytic activity, and plasminogen activator inhibitor. These were to test the hypothesis that autonomic dysfunction is associated with altered concentrations of plasma fibrinogen, fibrinolytic activity, viscosity, and plasminogen activator inhibitor. We have previously shown the absence of a correlation between level of blood pressure, clinical and standard laboratory testing, and the results of the autonomic function testing protocol used in this study. In this group of patients, plasma fibrinogen concentration was correlated (positively) with mean arterial pressure and (negatively) with heart rate variation in response to the Valsalva maneuver. The greater the mean arterial pressure or the worse the Valsalva results, the higher the plasma fibrinogen concentration. In addition, patients with one or no abnormal autonomic function tests had a mean fibrinogen of less than 400 mg/dL compared to the group of patients with two or more abnormal tests who had a mean fibrinogen of 500 mg/dL. In patients with demonstrated parasympathetic abnormalities, postural heart rate variation testing also discerned a differential in plasma fibrinogen. Lower concentration of plasminogen activator inhibitor throughout the day, and greater fibrinolytic activity in the morning were also noted to be present in patients with abnormal heart rate response to the Valsalva maneuver. We conclude that there are relationships between high blood pressure, autonomic function, and hemostatic factors favoring thrombogenesis that may be related by common mechanisms and treatments in the diabetic with kidney disease. © 1997 American Journal of Hypertension, Ltd. Am J Hypertens 1997;10:454–461

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A n increased rate of myocardial infarctions in the morning hours has been explained by increased sympathetic nervous system function upon arising, leading to more rapid heart rate, higher systemic blood pressure, and more rapid platelet aggregation. Decreased parasympathetic stimulation has been associated with a greater number of sudden deaths because of ventricular arrhythmias.\textsuperscript{1-4} In type I diabetes mellitus parasympathetic dysfunction often precedes clinical neuropathic symptoms.\textsuperscript{5,6} It follows that abnormal circadian variation of blood pressure control proceeds directly from altered autonomic function in diabetics. We have recently begun to document aspects of diabetic peripheral and autonomic neuropathy to correlate these with standard risk factors for cardiovascular complications. At baseline there appears to be no statistically significant relationship between standard risk factors for macrovascular disease and the severity of diabetic autonomic dysfunction, even when age, duration of diabetes, and level of renal dysfunction are taken into account.\textsuperscript{7} To date there have been no reports evaluating relationships between dysfunctions observed in the autonomic nervous system of diabetics and a full panel of fibrinolytic/procoagulant blood tests now available on a research basis.

The interface between atherosclerotic plaque disruption, concentrations of lipids, generation or lysis of fibrin, and the activity of the sympathoadrenal system during early morning hours has been the focus of much clinical research. The majority of studies have focused on large groups of patients at low risk for cardiovascular events, or on postmortem data. The study of a group of patients at high risk for such events may provide useful information regarding trigger mechanisms. The variable timing of atherosclerotic events (myocardial infarction, sudden death, and stroke) has been the subject of theories regarding the mechanisms that trigger them.\textsuperscript{8,9} There appears to be a circadian distribution of ischemic events, along with some of the markers of risk suggesting relationships between autonomic nervous system function and variations in blood pressure, heart rate, and blood markers for coagulation/fibrinolysis. In vitro and in vivo testing has suggested that the morning peak in myocardial events may be blunted by $\beta$-adrenergic blockade.\textsuperscript{10-12} The blunting of heart rate increase in the morning and of pressor response upon awakening have usually but not always been sufficient to explain the beneficial effect of $\beta$-adrenergic blockade.\textsuperscript{13,14} $\beta$-Blockers decrease the risk of plaque rupture because flexibility and tensile strength of the fibrous cap are increased when heart rate is diminished.\textsuperscript{15} Once microangiopathy is present (renal), a large percentage of diabetics will have coronary arterial disease that is asymptomatic.\textsuperscript{16-20}

**HYPOTHESIS**

Accelerated atherosclerosis with an increased incidence of cardiovascular events is because of increased concentrations of procoagulants, of cholesterol related lipids, and perhaps of other blood constituents (advanced glycosylated end-products, homocysteine) that may increase blood viscosity or change blood rheology. Conditions in which atherosclerosis and cardiovascular event rate have been shown to be increased are often associated with autonomic dysfunction (sudden death after myocardial infarction). We postulate that there is an interrelationship between altered autonomic control of cardiovascular tone (parasympathetic defect/sympathetic dominance) and the concentration of some blood constituents responsible for increased cardiovascular events in diabetics with nephrotic syndrome.

**MATERIALS AND METHODS**

We studied 23 insulin-dependent juvenile onset diabetics (16 men, 7 women) between the ages of 21 and 65 years (mean age 39.5 years) who had: 1. onset of insulin dependence before the age of 35; 2. albuminuria > 0.1 g/day or proteinuria > 0.3 gm/day on two separate 24-h urine collections; 3. creatinine clearance of more than 30 mL/min; 4. ability to be followed for a period of 18 months at the Joslin Clinic; 5. willingness to accept randomization; and 6. absence of pregnancy or an acute or chronic illness that would render testing uninterpretable (drug dependence, active hepatitis, recent myocardial infarction, or stroke, etc.). These diabetics were prospectively and consecutively enrolled as part of a multicenter study involving seven institutions in the United States. Using an 18-month follow-up period, comparisons of a regimen including 3 to 4 injections of insulin per day were made with a similar regimen in which a weekly infusion of intravenous insulin was added.\textsuperscript{21} Enrollment in this study required a workup of several weeks to collect the required urine samples and confirm the adequacy of control of diabetes mellitus and blood pressure. To have interpretable data, we attempted to avoid use of any drug that was, at the time, known to exert major effects on the autonomic nervous system that would interfere with either the patient’s sensation of hypoglycemic reactions or with the testing of cardiac autonomic function. The patients at the Joslin Clinic underwent cardiac autonomic function tests that included computerized measurements of heart rate variation (NDX, q Med Inc., Lawrence Harbor, NJ) during a period of timed ventilation, standardized Valsalva maneuver (expiration against a 40 mm Hg mercury column), recumbent to a standing position change, and 24-h ambulatory blood pressure measurements (90207 SpaceLabs, Redmond, WA). In
addition to the autonomic studies, each patient underwent a standardized phlebotomy at 8 AM and 4 PM at baseline and at 3-month intervals for plasma fibrinogen, plasma viscosity, plasminogen activator inhibitor antigen, factor 7, von Willebrand factor, fibrinolytic activity, and aggregation response of platelets to ADP, epinephrine, and collagenase.

For this study, to avoid questions of mixing normals into our abnormal group, we have strictly defined heart rate variations (HRV) as abnormal only if timed ventilation caused < 10% variation (heart rate ratio < 1.09), Valsalva response resulted in < 20% variation (heart rate ratio < 1.19), or if postural change < 5% (heart rate ratio < 1.04) was observed. These numbers are clearly abnormal for all ages included in this study. The day/night blood pressure ratio was considered to be abnormal if there was < 5% variation. For purposes of this study we defined day blood pressures as those occurring in the 16 h from 8 AM to midnight, and night blood pressures as those recorded in the 8 h from midnight to 8 AM. To further classify autonomic function results we defined several different classifications of autonomic dysfunction: ie, by a) individual test abnormalities; b) number of tests abnormal (0 to 1 v 3 to 4); c) selective sympathetic testing in patients whose parasympathetic tests were abnormal (n = 17) by the criteria so stated; and d) creating groups of parasympathetic test dysfunction (timed ventilation HRV or Valsalva HRV abnormalities) versus sympathetic dysfunction (postural HRV or day/night mean arterial pressure variation abnormalities). Hemoglobin A1C (high pressure liquid chromatography, Nichols Institute, Van Nuys, CA) was considered high if > 9.0% for purposes of this study.

Frequency data were tested for significance using $\chi^2$ for independence following the implementation of Yates correction for continuity. In cases where expected frequencies < 5, Fisher’s exact test was used. For quantitative variables, unpaired t tests were used to test for significance between the means of various classification variables (gender, normal-abnormal stratification of cardiac autonomic function). Because, in some cases, stratification groups differed significantly in mean age, the same variables were compared using analyses of covariance with age as the covariant. Interrelationships among the variables were tested for significance using Pearson’s Product Moment correlations. All data are expressed as frequencies or means with standard deviations as a measure of dispersion. An $\alpha = 0.05$ was considered statistically significant. All analyses were carried out using SAS software (SAS Institute, Cary, NC).22

RESULTS

Demographics This group of 23 patients entered the study with a 24-h mean arterial pressure (MAP) of 99.7 mm Hg (daytime MAP 100.4 mm Hg, nighttime MAP 98.1 mm Hg). Glycohemoglobin A1C mean was 9.5% (range 6.4 to 11.8), hematocrit mean 40% (range 31 to 53), serum albumin mean 3.7 g/dL (range 3.0 to 4.5), serum cholesterol mean 232 mg/dL (range 122 to 402), HDL cholesterol 51 mg/dL (range 32 to 82), triglyceride 151 mg/dL (range 44 to 489), serum uric acid 5.8 mg/dL (range 4.6 to 8.4), serum creatinine 1.8 mg/dL (range 1.0 to 3.6), creatinine clearance 58 mL/min (range 30 to 112), and urine albumin 3.3 g/24 h (range 0.3 to 8.9).

There were no significant correlations between any of the above and any of the four autonomic function tests described individually or by groupings.

Coagulation Tests Mean plasma fibrinogen was 424 mg/dL (range 238 to 711), mean plasma viscosity 1.24 centipoise (range 1.10 to 1.43), fibrinolytic activity was 166.3 mm² (range 60 to 264), and plasminogen activator inhibitor was 7.41 ng/mL (range 2.4 to 30.9). A significant positive correlation between plasma fibrinogen and mean arterial pressure for 24 h was noted (Figure 1). Plasma viscosity had a significant positive correlation with both glycohemoglobin and plasma fibrinogen, although there appeared to be no statistically significant relationship between glycohemoglobin and serum fibrinogen. In Figure 2 there is a significant negative correlation between plasma fibrinogen concentration and heart rate variation during the Valsalva maneuver (ie, the more abnormal the Valsalva result, the higher the plasma fibrinogen). In both figures plasma fibrinogen levels (220 to 780 mg/dL) were distributed above 300

![Figure 1](https://academic.oup.com/ajh/article-abstract/10/4/454/156796/24-10-97-11-49-42-eajha-EL-AJH-APRIL-1997-VOL-10-NO-4-PART-1)

**FIGURE 1.** Relationship of 24-h mean arterial blood pressure to plasma fibrinogen. 24-h mean arterial pressure = 81.5 mm Hg + (0.0434 × fibrinogen); Pearson coefficient = 0.47 (P < .03).
test results (3 to 4 tests abnormal) have a statistically significantly higher plasma fibrinogen level in both the AM and PM than their counterparts with normal (0 to 1 abnormal result) autonomic test results (AM 511 mg/dL, PM 489, 373).

In patients with parasympathetic dysfunction, sympathetic testing included both assumption of upright posture HRV and day/night MAP variation. In Figure 5 plasma fibrinogen concentrations were similar throughout the day in both the abnormal and normal postural HRV groups. Plasma fibrinogen concentrations were higher for the abnormal postural HRV group (P < .05 for the PM only) compared to the normal postural HRV group. Day/night MAP variation again did not correlate with plasma fibrinogen level.

For the purposes of comparison, creating groups of pure parasympathetic versus pure sympathetic dysfunction led to groups that were too small to make meaningful comparisons.

**DISCUSSION**

Treated hypertensive diabetics in this study had a high prevalence of abnormal autonomic function tests. There appeared to be a relationship between asymptomatic autonomic dysfunction and the concentrations of intravascular blood constituents thought to be related to thromboembolic events. We observed a correlation between abnormal heart rate variation test results, and plasma fibrinogen concentration. It is unlikely that this is a result merely of hemococoncentration or vasoconstrictor tone induced by the unopposed sympathetic system. Individuals with nephrotic syndrome from any cause may be expected to have an increased concentration of plasma fibrinogen, but, to our knowledge, there have been no studies correlating the degree of hyperfibrinogenemia with autonomic nervous control. The mechanism for hyperlipidemia and hyperfibrinogenemia in nephrotic syndrome may hinge on hepatic synthesis of multiple proteins (albumin, fibrinogen, and lipoprotein) in response to hypoalbuminemia with hypoviscosity. We have no information testing the possibility that the autonomic nervous system contributes directly to the mechanism responsible for hyperlipidemia/hyperfibrinogenemia in the nephrotic syndrome. We hypothesized that diabetics with both severe autonomic dysfunction and significantly elevated plasma fibrinogen concentrations were at the highest risk for thromboembolic events when compared with individuals demonstrating only one of these abnormalities.

Study patients who were capable of maintaining a Valsalva maneuver with relatively normal results had lower (morning and afternoon) plasma fibrinogen levels, and higher (morning and afternoon) plasminogen activator inhibitor levels than did their counter-
FIGURE 3. Relationship of Valsalva maneuver heart rate variation results (normal versus abnormal) to plasma fibrinogen concentration, fibrinolytic activity, and plasminogen activator inhibitor concentrations during morning and afternoon. Panel A: Significant difference in plasma fibrinogen concentration between normal and abnormal Valsalva result groups in both morning and afternoon hours ($P < .05$). Panel B: Significance difference in plasma fibrinolytic activity between normal and abnormal Valsalva result groups noted in the morning hours only ($P < .05$). Panel C: Significant difference in plasma plasminogen activator inhibitor concentration between normal and abnormal Valsalva result groups in both morning and afternoon hours ($P < .05$).

Parts whose Valsalva results were markedly abnormal. Patients whose Valsalva HRV results were markedly abnormal had significantly increased plasma fibrinogen and increased fibrinolytic activity with decreased concentration of plasminogen activator inhibitor. In this patient cohort there was a relationship between an intact heart rate response to Valsalva maneuver and normal control of intrinsic prothrombotic and thrombolytic activity, as well as a relationship between loss of heart rate variation to the Valsalva maneuver and abnormal prothrombotic and thrombolytic activity. It is possible that patients who do not vary their heart rates with the increase of intrathoracic pressure engendered by the Valsalva maneuver are demonstrating a highly stimulated sympathetic system. These patients may be at relatively higher risks...
resulting from hyperfibrinogenemia. In the hypertensive diabetic, loss of parasympathetic innervation leads to vasoconstriction and platelet aggregation. The hyperlipidemia associated with proteinuria contributes to plaque formation. Plaque rupture is most likely potentiated by unopposed sympathetic nerve stimulation. Beta blocker therapy may be justified despite the risks of hypoglycemia to prevent plaque rupture. A new antagonist of endothelin-A receptor will be of immediate use when available.29

We have observed relationships between injury to the autonomic nervous system and changes in the common pathway of blood coagulation indicating a clustering of risk factors in the azotemic diabetic. Specifically, higher plasma fibrinogen levels were distributed among the group of patients whose Valsalva tests were more abnormal. Improved glucose control has been shown to improve elevated fibrinogen levels over several weeks of intensive insulin therapy.30 Increased plasma fibrinogen contributes to more vigorous platelet aggregation. Increased plasma fibrinogen results in both morning and afternoon hours (P < .05).

for cardiovascular events than those patients whose sympathetic tone is less active, and may benefit from efforts to reduce sympathetic stimulation. Follow-up studies will be necessary to elucidate the cardiovascular thromboembolic event rates in these subgroups.

Mechanisms for diabetic neuropathy are thought to involve ischemia resulting from low flow in the vasa vasora because of a) increased plasma viscosity; b) increased blood clotting; c) impaired red blood cell and platelet flexibility leading to slow capillary flow; d) hypoxia in tissues that require high flow in capillaries because of an impaired release of oxygen from glycosylated hemoglobin; e) capillary injury at points of binding of advanced glycosylated end-products to their receptors leading to platelet adhesion; and f) increased release of endothelin and von Willebrand factor from endothelium in diabetics with small vessel complications.25,26

The severity of vessel wall injury and the prothrombotic milieu within both the vessel and the circulating plasma determine the rapidity and severity of thrombosis.27 Once the vessel has been injured the potent vasoconstrictor endothelin is released. The presence of thrombin further accelerates the release of endothelin, which induces chemotaxis, promotes replication of fibroblasts, accelerates contraction of collagen fibroblasts, and acts as a stimulator of microvascular platelet thrombi.28 Thus endothelin reinforces both the unopposed sympathetic vasoconstrictor risk factor in diabetics, and the atherosclerotic thrombotic process

FIGURE 4. Relationship of parasympathetic and sympathetic dysfunction (HRV, day/night MAP) to plasma fibrinogen concentration. Significant difference for normal versus abnormal test results in both morning and afternoon hours (P < .05).

FIGURE 5. Relationship between HRV on assumption of upright posture and plasma fibrinogen in patients with evidence for parasympathetic dysfunction by abnormal HRV during timed ventilation. Significant difference between normal and abnormal result group during afternoon hours only (P < .05).
heart attack with a test of global fibrinolytic activity. Surprisingly the plasminogen activator inhibitor levels were not elevated in our type I diabetic patients with high plasma fibrinogens, To test the predictive accuracy of plasma fibrinogen versus plasminogen activator inhibitor, future prospective studies must control for normal versus abnormal autonomic function. We suggest that correlations between clinical events and either the coagulation system or the autonomic system but not all three are potentially flawed.

CONCLUSIONS

In hypertensive diabetic nephropathy after the onset of proteinuria, we observed a relationship between abnormalities in autonomic function and hemostatic factors favoring thrombogenesis. Because it appears that there may be a relationship between relative sympathetic dominance, vascular tone and the homeostasis of the coagulation cascade, we believe that autonomic function testing should be extended to a larger population of diabetics. The appearance of a sympathetic dominant profile may be an indication for the use of beta blockers in these hypertensive insulin dependent diabetics despite the risk of hypoglycemic reactions. The use of calcium channel blockers that do not increase the baseline tachycardia in these patients may be justified on clinical grounds. We are not aware of any prospective studies which relate the presence of coronary arterial disease to both prothrombotic and autonomic modulation of sympathovagal activity in diabetes: a possible explanation for altered temporal onset of cardiovascular disease. Circulation 1992;86:1443–1452.

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