Plasma Prekallikrein

A Risk Marker for Hypertension and Nephropathy in Type 1 Diabetes

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The relevance and significance of the plasma kallikrein/kinin system as a risk factor for the development of vascular complications in diabetic patients was explored in a cross-sectional study. We measured the circulating levels of plasma prekallikrein (PK) activity, factor XII, and high−molecular weight kininogen in the plasma of 636 type 1 diabetic patients from the Diabetes Control and Complications Trial/Epidemiology and Diabetes Intervention and Complications Study cohort. The findings demonstrated that type 1 diabetic patients with blood pressure ≥140/90 mmHg have increased PK levels compared with type 1 diabetic patients with blood pressure <140/90 (1.53 ± 0.07 vs. 1.27 ± 0.02 units/ml; $P < 0.0001$). Regression analysis also determined that plasma PK levels positively and significantly correlated with diastolic (DBP) and systolic blood pressures (SBP) as continuous variables ($r = 0.17$ and 0.18, respectively; $P < 0.0001$). In multivariate regression analysis, the semipartial r^2 value for PK was 2.93% for SBP and 2.92% for DBP ($P < 0.0001$). A positive correlation between plasma PK levels and the urinary albumin excretion rate (AER) was also observed ($r = 0.16$, $P < 0.0001$). In categorical analysis, patients with macroalbuminuria had a significantly higher level of plasma PK than normoalbuminuric patients (1.45 ± 0.08 vs. 1.27 ± 0.02 units/ml; $P < 0.01$), whereas microalbuminuric patients had an intermediate PK value (1.38 ± 0.05 units/ml; P = NS). Among patients in the microalbuminuric subgroup, we observed a positive and independent correlation between PK and AER in univariate and multivariate regression analysis ($r = 0.27$, $P < 0.03$; $n = 63$). We concluded that in type 1 diabetes, *1*) PK levels are elevated in association with increased blood pressure; *2*) PK levels are independently correlated with AER and are categorically elevated in patients with macroalbuminuria; and *3*) although the positive correlation between PK and AER within the subgroups of patients with microalbuminuria suggest that PK could be a marker for progressive nephropathy, longitudinal studies will be necessary to address this issue. *Diabetes* 52: 1215–1221, 2003

Hypertension is a common comorbid disease of diabetes, and is a major risk factor for the development of macrovascular and microvascular complications of diabetes (1–3). Furthermore, predisposition to hypertension is thought to be an important determinant in a subset of type 1 diabetic patients who develop diabetic nephropathy and, subsequently, cardiovascular disease (4). Although advanced diabetic nephropathy secondarily worsens hypertension, the rising blood pressure may also be an early contributor to vascular damage.

Diabetic nephropathy is the leading cause of end-stage renal failure, and is clinically manifested by albuminuria, hypertension, and a progressive decline in the glomerular filtration rate (5–7). It is estimated that 30–40% of patients with type 1 diabetes develop progressive nephropathy (7). Although the association of chronic hyperglycemia and diabetic nephropathy is well established, the risk factors and cellular signaling mechanisms that link hyperglycemia and glomerular injury are not fully defined (8). Because the prevalence of diabetic nephropathy is on the rise, the need to define the risk factors and pathophysiological mechanisms that are operative in this disorder has intensified.

Accumulating evidence supports a relation between activity of the kallikrein/kinin system (KKS) and the development of hypertension and renal impairment. Two forms of kallikrein exist: one in tissue and another in plasma. Tissue kallikrein, which is mainly expressed in glandular tissue, kidney, vasculature, and brain, preferentially acts on low−molecular weight kininogen substrate to release lysyl-bradykinin. Plasma kallikrein preferentially acts on high−molecular weight kininogen (HK) substrate to release bradykinin (BK) (9). BK, the principal effector of the plasma KKS, is generated both systemically and locally within the vessel wall, where it acts in a paracrine or autocrine fashion to influence vascular tone

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ACEI, ACE inhibitor therapy; AER, albumin excretion rate; APTT; BK, bradykinin; BP, blood pressure; CI, confidence interval; DBP, diastolic BP; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology and Diabetes Intervention and Complications; GFR, glomerular filtration rate; HK, high−molecular weight kininogen; KKS, kallikrein/kinin system; MUSC, Medical University of South Carolina; OR, odds ratio; PK, prekallikrein; RPF, renal plasma flow; RVR, renal vascular resistance; SBP, systolic BP.

and ultrastucture (10–15). Recent studies have suggested that the KKS is the physiological counterbalance to the renin-angiotensin system (16). The same enzyme, prolylcarboxypeptidase, that degrades angiotensin II is the endothelial cell prekallikrein (PK) activator (17).

We have previously shown that type 1 diabetic patients at risk for developing nephropathy (i.e., those with elevated glomerular hemodynamics) show increased renal tissue kallikrein and kinin production (18). In addition, diabetic rats with moderate hyperglycemia show increased renal and urinary excretion of active kallikrein and kinin, in conjunction with reduced renal vascular resistance (RVR) and an increased glomerular filtration rate (GFR) and increased renal plasma flow (RPF) (19,20). Acute treatment of hyperfiltering diabetic rats with aprotinin, a kallikrein inhibitor, or with a B2-kinin receptor antagonist, increases the RVR and reduces the GFR and RPF (19,20). This indicates that tissue kallikrein and kinins help to mediate renal hyperfiltration in diabetes.

The relevance and significance of the plasma KKS to the development of vascular disease in diabetic patients has not been explored. Changes in the plasma levels of these components are predictive of the degree of activation of this system and/or clearance of this system's components. In the present study, elevation of the plasma PK activity is shown to positively associate with the development of hypertension and macroalbuminuria in patients with type 1 diabetes, enrolled in the Diabetes Control and Complications Trial/Epidemiology and Diabetes Intervention and Complications Study (DCCT/EDIC).

RESEARCH DESIGN AND METHODS

Study population. The study population was the North American DCCT/EDIC cohort, which comprised 1,325 type 1 diabetic patients from an original 1,441 DCCT subjects. The original DCCT cohort consisted of men and women ages 13–40 years with diabetes of 1–15 years' duration at study entry (21) who were enrolled between 1983 and 1989. Half of the patient population was randomly assigned to conventional diabetes treatment and the other half was assigned to intensive diabetes treatment. In 1993, the DCCT study was stopped after an average follow-up time of 6.5 years, when intensive treatment was clearly shown to reduce the risks of retinopathy, nephropathy, and neuropathy (21). The patients were then invited to enroll in the EDIC, a multicenter, longitudinal observational study of the development of macrovascular complications and further progression of microvascular complications (22). At EDIC baseline in 1994, the average age of the DCCT/EDIC cohort was 35 years (range 19–50 years), with 54% of the cohort being male and the mean duration of diabetes being 12 ± 5 years.

In 1996, a collaborative project (Markers and Mechanisms for Macrovascular Disease in Type 1 Diabetes) was undertaken by the Medical University of South Carolina (MUSC) and EDIC. Its goal was to identify new markers, risk factors, and mechanisms for vascular disease in type 1 diabetic patients using plasma samples shipped directly from participating EDIC clinics to MUSC. In all, 25 of the 28 EDIC clinics participated, and between 1997 and 1999, fasting plasma samples were collected from subjects for KKS component measurements. The Institutional Review Boards of MUSC and all participating DCCT/EDIC clinics approved the study, and written informed consent was obtained from each patient participant.

EDIC procedures. On the approximate anniversary of enrolling in the DCCT, each EDIC subject had a standardized annual history and physical examination, including a detailed evaluation of overall health, diabetes management, occurrence of diabetic complications, development of new disease, and medications used. Annual evaluations also include HbA_{1c}, resting electrocardiograms, and arm blood pressure (BP) measurements. BP and HbA_{1c} measurements were done at the same time as blood sampling. BP was measured in the right arm using a mercury column sphygmomanometer while the patient was in the sitting position. Renal function was assessed every second year and included measurements of the urinary albumin excretion rate (AER) in a standardized 4-h collection (21). The DCCT has defined microalbuminuria as an AER of 40–300 mg/24 h; albuminuria, as an AER ≥300 mg/24 h; and normal albumin excretion, as an AER ≤40 mg/24 h. In all, ~98% of all AER measurements in the EDIC subjects were carried out within 1 year of blood sampling.

Assay of kallikrein-kinin system components. Plasma was collected from each participant into a 10-ml tube containing 3.2 g/dl sodium citrate. The specimens were centrifuged at room temperature for 15 min at 1,800*g*. The plasma supernatant was removed with a polypropylene pipette and aliquoted into 0.5-ml plasma samples in polypropylene and stored at −20°C at study site for less than 1 month, then shipped to MUSC and frozen at −80°C until assay. Only plasma samples that were thawed once were used in the analysis. Factor XII:coagulant and HK:coagulant were measured using deficient plasma in a single-stage APTT-based assay using APTT Reagent (Organon Technika, Durham, NC), as previously reported (23). The HK procoagulant assay was performed with total kininogen-deficient plasma (a generous gift of the late Mayme Williams, Philadelphia, PA). Plasma PK was activated with ~0.4 nmol/l Hageman factor fragment (betaFXIIa), and the formed plasma kallikrein was detected by hydrolysis of the chromogenic substrate H-D-Pro-Phe-Arg-paranitroanilide (S2302) (DiaPharma, Franklin, OH) according to the published procedure, and was expressed as units per milliliter (24). The intra-assay coefficient of variance was 0.85% (24). The plasma PK level in normal subjects determined by using this assay was 1.02 ± 0.18 units/ml (n = 22) (24). Therefore, each unit of PK was defined as the amount of activity associated with 1 ml of pooled normal plasma. Pooled normal human plasma and factor XII−deficient plasma were purchased from George King (Overland Park, KS).

KKS components were measured sequentially in 641 subjects out of the total cohort as they appeared at study sites for scheduled visits. The clinical characteristics of the subjects in which KKS components were measured were compared to those of the remaining EDIC subjects. No differences in age, sex, HbA_{1c}, BP, AER, DCCT treatment group, duration of diabetes, or BMI were observed between the two groups.

Statistical analysis. The association among plasma PK, BP (systolic and diastolic), and AER was examined using a multiple linear regression model. BP and AER were treated as dependent variables, whereas plasma PK was used as the main independent variable. Linear regression diagnostics suggested performing a logarithmic transformation of AER to accommodate the nonnormal distribution of AER. Other covariates (age, HbA_{1c}, duration of diabetes, sex, and DCCT treatment group) were accounted for in all multiple regression models. The square of the multiple partial correlation coefficient was calculated to estimate the increase in the variance of the dependent variable obtained by introducing that variable into a model already including all the others.

Multiple logistic regression models were estimated to assess the association between plasma PK and hypertension status (systolic/diastolic BP ≥140/90 or taking hypertension medication) or between plasma PK and micro-/macroalbuminuria status (AER ≥40 vs. AER ≤40 mg/24 h). The odds ratio (OR) of hypertension and albuminuria per unit increase in PK levels was calculated along with the 95% confidence interval (CI). Goodness-of-fit was evaluated using the Hosmer-Lemeshow χ^2 statistic. Multiple logistic regression models were adjusted for age, HbA_{1c}, duration of diabetes, sex, DCCT treatment group, anti-hypertensive medication, and documented hypertension. All statistical analyses were performed using SAS, and the values are expressed as means ± SE.

RESULTS

KKS components in type 1 diabetes. The circulating levels of plasma PK, factor XII, and HK measured in 636 subjects are shown in Fig. 1. There were no significant differences in the plasma levels of PK, factor XII, or HK between male and female subjects. PK levels were symmetrically distributed among the patients and ranged from 0.2 to 3.0 units/ml, with a mean value of 1.29 ± 0.01 units/ml. Plasma factor XII levels ranged from 0.01 to 9.2 units/ml, with a mean value of 1.06 ± 0.03 units/ml. Plasma HK levels ranged from 0.1 to 5.0 units/ml, with a mean value of 1.34 ± 0.03 units/ml. In cross-sectional analyses, plasma PK levels positively correlated with HK (r = 0.21, $P < 0.0001$) and XII (r = 0.08, $P < 0.04$).

Table 1 shows the relation of plasma PK, HK, and factor XII with biochemical parameters in the patient cohort. Univariate analysis showed BMI (r = 0.1, $P < 0.01$), HbA_{1c} (r = 0.16, $P < 0.0001$), and mean BP (r = 0.19, $P < 0.0001$) to be significantly and positively correlated with PK,

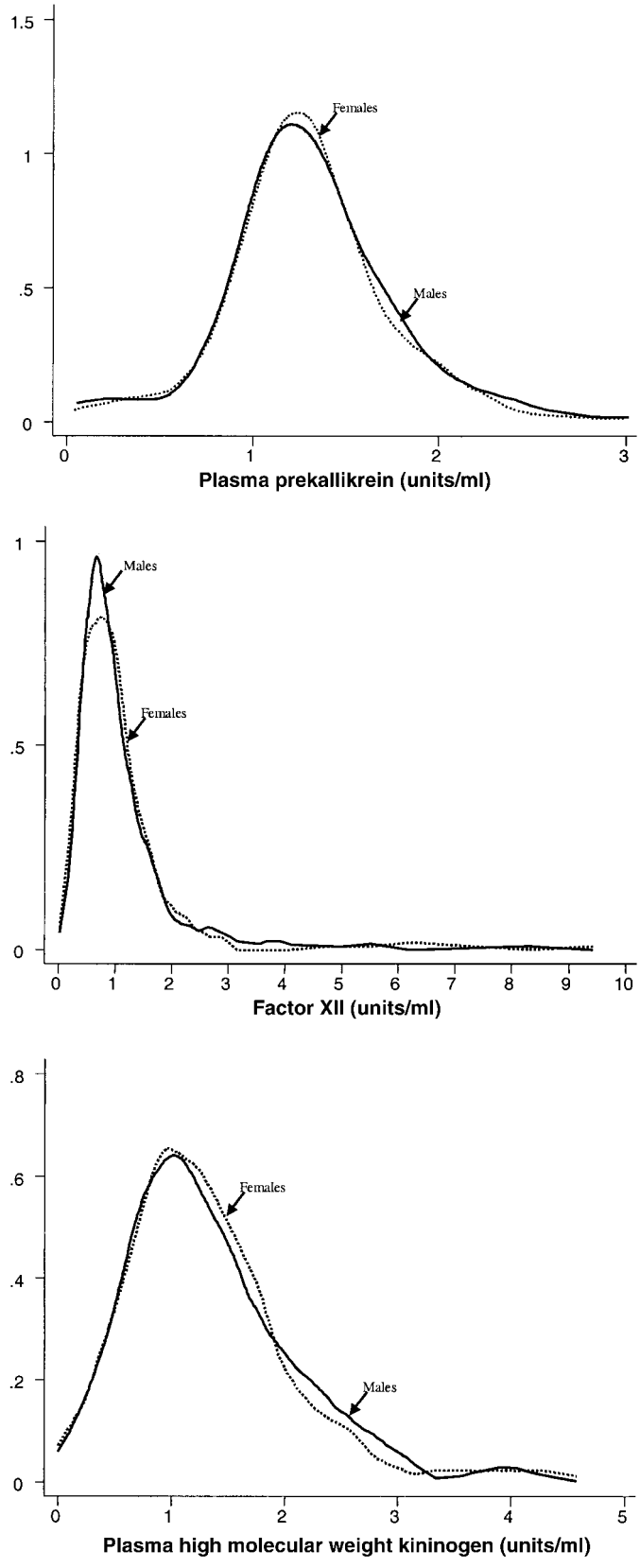

FIG. 1. Frequency of density distribution of plasma PK, factor XII, and high–molecular weight kininogen in male and female subjects in the DCCT/EDIC cohort of type 1 diabetic patients (n = 636 patients).

whereas body weight (0.08, $P < 0.05$), waist-to-hip ratio ($r = 0.08$, $P < 0.04$), and HbA_{1c} ($r = 0.09$, $P < 0.02$) were significantly and positively correlated with HK.

Relation between plasma levels of KKS components and blood pressure. The results shown in Fig. 2 demonstrate that plasma PK levels positively correlated with diastolic blood pressure (DBP; $r = 0.17$, $P < 0.0001$) and systolic blood pressure (SBP; $r = 0.18$, $P < 0.0001$; n = 636). The relation between SBP and DBP and plasma factor XII or HK values was not statistically significant (data not shown). A multivariate linear regression model for BP was also constructed (Table 2). PK levels, age, and male sex were significant independent correlates of both SBP and DBP. The semipartial r^2 value for PK was 2.93% for SBP and 2.92% for DBP ($P < 0.0001$).

We next analyzed the data categorically with respect to current BP status. Figure 3 demonstrates that type 1 diabetic patients with blood pressure values ≥140/90 mmHg had increased plasma PK levels compared with type 1 diabetic patients with a BP <140/90. Mean plasma PK levels in patients with BP ≥140/90 mmHg were increased compared with the level in patients with BP <140/90 mmHg (1.53 ± 0.07 vs. 1.27 ± 0.02 units/ml; n = 52 and 492, respectively; $P < 0.0001$). Because some of the hypertensive patients were on ACE inhibitor therapy (ACEI), we examined whether ACEI influenced the level of plasma PK in this patient cohort. The plasma PK levels in patients treated with ACEI were similar to that in patients not treated with ACEI (1.35 ± 0.04 vs. 1.28 ± 0.02 units/ml; n = 92 and 549, respectively; $P < 0.145$). Among the 549 patients used for the analysis with no ACEI treatment, ~91% of patients were normotensive and ~9% were hypertensive and being treated with BP-lowering medications other than ACEI.

The cumulative distribution of plasma PK levels versus hypertension status (all patients diagnosed with hypertension compared with those who remained normotensive) is shown in Fig. 4. The patients who developed hypertension had a significantly higher level of plasma PK compared with patients who did not develop hypertension (1.39 ± 0.04 vs. 1.26 ± 0.02; $P < 0.002$).

A multiple logistic regression model determined the strength of the association of plasma PK with hypertension. After controlling for age, duration of diabetes, sex, DCCT intensive group, hypertension medications, and HbA_{1c}, there was a 72% increase in the risk of being hypertensive for every one unit increase in PK level (OR = 1.72, 95% CI 1.09–2.74; $P < 0.021$).

Relation between plasma prekallikrein and albumin excretion rate. Because 30–40% of type 1 diabetic patients develop diabetic nephropathy, we determined if PK levels correlated with the degree of AER. On cross-sectional analyses, there was a significant positive correlation between PK levels and log AER ($r = 0.16$, $P < 0.0001$) (Fig. 5). Our findings also indicated that diabetic patients with AER ≥300 mg/24 h had a significantly higher level of plasma PK compared with diabetic patients with AER ≤40 mg/24 h (1.45 ± 0.08 vs. 1.27 ± 0.02; n = 34 and 535, respectively; $P < 0.01$). Diabetic patients with AER 40–300 mg/24 h had an intermediate plasma PK level of 1.38 ± 0.05 mg/24 h (n = 63) (Fig. 6); however, the mean value was not significantly different from that in the

TABLE 1
Simple regression analyses for plasma prekallikrein, kininogen, and factor XII

	Correlation coefficients	*P*
Plasma prekallikrein (units/ml)		
Body weight (kg)	0.05	0.2433
BMI (kg/m^2)	0.10	0.0127
Waist-to-hip ratio	0.07	0.0939
Current HbA_{1c}	0.16	0.0001
Duration of diabetes (years)	0.04	0.3345
DCCT intensive Group	0.00	0.9650
Age (years)	0.01	0.7361
Mean blood pressure (mmHg)	0.19	0.0001
High–molecular weight kininogen (units/ml)		
Weight (kg)	0.08	0.0477
BMI (kg/m^2)	0.06	0.1451
Waist-to-hip ratio	0.08	0.0415
Current HbA_{1c}	0.09	0.0183
Duration of diabetes (years)	0.03	0.3961
DCCT intensive group	0.05	0.2185
Age (years)	0.00	0.9263
Mean blood pressure (mmHg)	0.05	0.2582
Factor XII (units/ml)		
Body weight (kg)	0.01	0.7536
BMI (kg/m^2)	–0.03	0.4048
Waist-to-hip ratio	0.04	0.3480
Current HbA_{1c}	0.05	0.2176
Duration of diabetes (years)	0.00	0.9996
DCCT intensive group	0.05	0.1733
Age (years)	0.04	0.3257
Mean blood pressure (mmHg)	0.01	0.7944

normo- and macroalbuminuric subgroups. To further explore whether PK was associated with microalbuminuria, we performed a univariate regression analysis between PK and AER in patients with AER 40–300 mg/24 h and observed a positive and significant correlation (slope = 4.07 + 0.29; $r = 0.27$, $P < 0.03$; $n = 63$). No correlation was observed between plasma PK and AER in normoalbuminuric patients with AER ≤40 mg/24 h (slope = 2.00 + 0.14; $r = 0.08$, $P < 0.06$; $n = 535$). In a multivariate linear regression, plasma PK was independently correlated with log AER (Table 3). The semipartial r^2 values for PK, age, SBP, DCCT intensive group, and HbA_{1c} were 0.69, 0.82, 7.09, 2.33, and 5.35%, respectively.

The cumulative distribution of plasma PK by AER is shown in Fig. 7. The data demonstrated that a greater number of patients with AER ≥300 mg/24 h had elevated PK values above any given PK threshold compared with patients with AER ≤40 mg/24 h.

DISCUSSION

This study represents the first attempt to define the role of the plasma KKS in type 1 diabetes. Our findings provide the first evidence that augmented PK levels positively and independently associate with hypertension and albuminuria, an association that could contribute to the development of vascular disease in type 1 diabetic patients.

The plasma KKS is comprised of a group of plasma proteins that mediate their effects on the vasculature through the release of BK (9,15,16). Recently, it has been shown that endothelial cells express a unique PK activator that converts PK to kallikrein and that factor XII is activated secondary to PK (16,17,25). In our study, we observed a positive correlation between plasma PK and HK as well as PK and factor XII. The increase in PK levels in diabetes may result from either a decrease in PK activation or an increase of its synthesis. Such a process could arise in diabetes, where vascular permeability and reduced vasodilatory responses have been recognized to occur in endothelium. Dysfunction of vascular endothelium may, then, provide a link between albuminuria and atherosclerotic cardiovascular disease (26–28).

An alternative explanation for the elevated PK levels seen in these patients is increased synthesis. In this regard, our findings demonstrated that HbA_{1c} positively and significantly correlated with plasma PK and HK levels. These epidemiological findings establish an association between metabolic control and KKS components, but do not define the cellular mechanisms or factors that modulate plasma PK synthesis. Because PK is produced mostly in the liver and plasma levels of this protein fall dramatically in liver disease, we have initiated studies to directly assess the

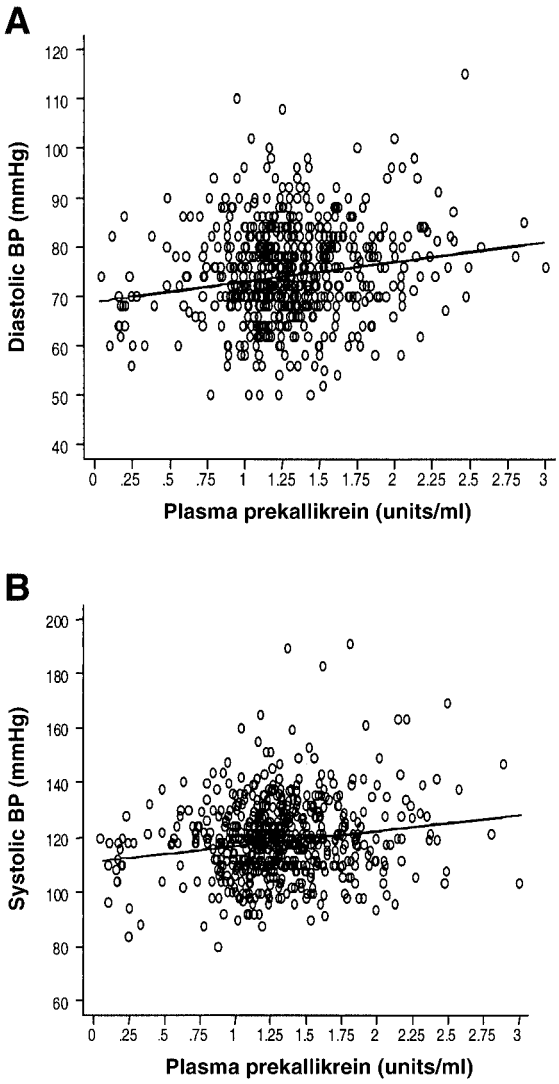

FIG. 2. Continuous variable correlation plots between PK and DBP (*A*) and SBP (*B*). A significant positive correlation was observed between plasma PK and DBP (*y*-axis intercept = 112.27, slope = 5.62, $r = 0.17$, $P < 0.0001$) and PK and SBP (*y*-axis intercept = 69.86, slope = 4.00, $r = 0.18$, $P < 0.0001$, $n = 636$).

TABLE 2
Multiple linear regression models for blood pressure

	Slope	SE	*P*	Partial r^2 (%)
Systolic blood pressure				
Intercept	98.6	4.70	0.000	—
Plasma prekallikrein (units/ml)	5.53	1.28	0.000	2.93
Age (years)	0.93	0.39	0.018	0.89
Duration of diabetes (years)	0.10	0.05	0.06	0.57
Sex	7.99	1.08	0.000	8.12
DCCT intensive group	–1.76	1.07	0.101	0.43
HbA_{1c}	0.19	0.40	0.637	0.04
Diastolic blood pressure				
Intercept	60.27	3.20	0.000	—
Plasma prekallikrein (units/ml)	3.76	0.87	0.000	2.92
Age (years)	0.44	0.27	0.103	0.43
Duration of diabetes (years)	–0.10	0.04	0.816	0.01
Sex	4.94	0.74	0.000	6.80
DCCT intensive group	–0.56	0.73	0.446	0.09
HbA_{1c}	0.50	0.28	0.073	0.52

role of glucose on plasma PK synthesis in liver cells (29). Previous studies have also reported increased PK levels in patients with diabetes, and in one study (30) an association between plasma PK levels and blood glucose was observed (30,31). Another possible explanation for increased levels of plasma PK in albuminuric patients is that the circulating kallikrein inhibitor, C1 inhibitor, could be reduced via urinary excretion. The smaller molecular mass protein C1 inhibitor (107 kDa) is more likely to be eliminated during glomerular disease than PK, which circulates in a complex with HK with a combined molecular mass of 220 kDa. However, this situation is unlikely because factor XII (80 kDa), which is known to become deficient in nephrotic patients, has a plasma level that positively correlates with plasma PK (32). If reduced C1 inhibitor levels from urinary loss account for an apparent increase in plasma PK, then factor XII levels should also be reduced. Such was not the situation in the diabetic patients examined. Our assay measures activated and nonactivated PK activity and represents total PK mass rather than circulating PK activity. This is independent of C1's

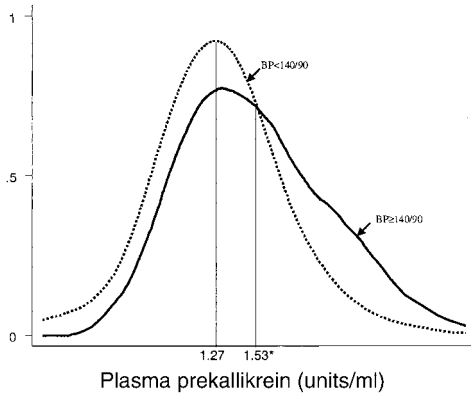

FIG. 3. Distribution curves showing the plasma PK values in diabetic patients with BP ≥140/90 mmHg and <140/90 mmHg. The curve demonstrates that type 1 diabetic patients with BP ≥140/90 mmHg have increased plasma PK levels compared with diabetic patients with a BP <140/90 mmHg ($P < 0.0001$; $n = 632$). The data were controlled for age, duration of diabetes, sex, treatment group (intensive versus conventional), and hypertension medications.

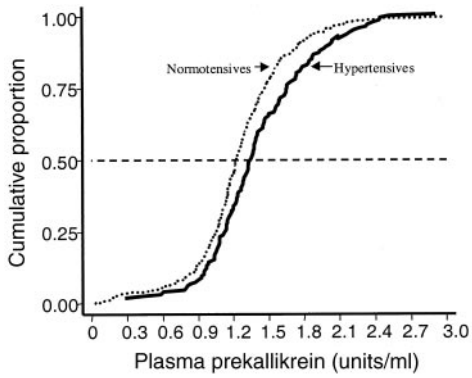

FIG. 4. Plasma PK versus cumulative distribution of hypertension status in the DCCT/EDIC cohort. The plasma PK level in patients with hypertension was significantly higher than patients with normal blood pressure ($P < 0.0023$; $n = 632$).

ability to alter PK-specific activity. Thus indirect evidence suggests that the increased PK levels are not caused by artifactual loss of an inhibitor to kallikrein or increased specific activity of the formed enzyme. At this point, however, the information present does not completely exclude increased synthesis in PK in these diabetic patients.

The mechanisms by which diabetes and hypertension interact to accelerate renal damage are as yet undefined. Both conditions are associated with renal endothelial denudation, mesangial cell proliferation, basement membrane changes, and impaired endothelium-dependent relaxation of blood vessels (33,34). Few interventions have been shown to slow the progression of renal disease in diabetic patients. These include intensive glycemic control, blood pressure regulation, and treatment with ACE inhibitors (2,3,8). Despite these interventions, diabetic patients still progress with time to develop end-stage renal disease. The factors responsible for these maladaptive signals leading to end-stage renal failure are still undefined. Our findings indicate that plasma PK levels are positively and independently associated with blood pressure elevation and AER in type 1 diabetic patients. Whether the increase in plasma PK levels we observed in diabetic patients is the cause of injury or the result of injury is yet to be determined.

The renoprotective effects conferred by ACEI could be

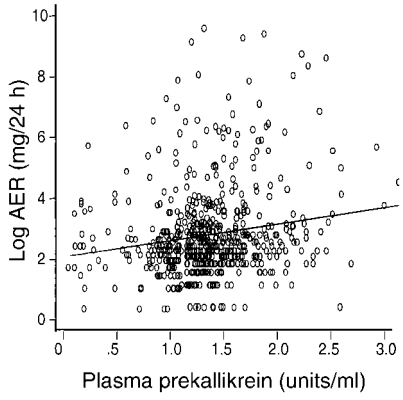

FIG. 5. Continuous variable correlation plots between plasma PK and log AER. A significant correlation was observed between PK and log AER (y-axis intercept = 2.00, slope = 0.54, $r = 0.16$, $P < 0.0001$, $n = 636$).

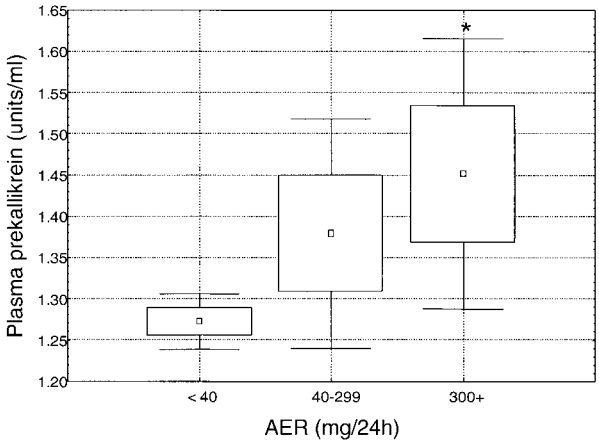

FIG. 6. Box and Whisker plot showing the relation between mean level of plasma PK and AER status. The plot demonstrates that diabetic patients with AER ≥300 mg/24 h have a significantly higher level of plasma PK compared with diabetic patients with AER ≤40 mg/24 h (1.45 ± 0.08 vs. 1.27 ± 0.02; n = 34 and 535, respectively; P < 0.01).

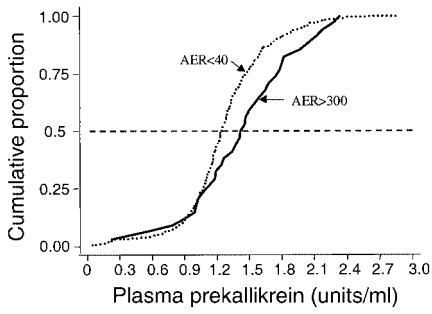

FIG. 7. Plasma PK versus cumulative distribution of nephropathy. The data demonstrate that all the patients with AER ≥300 mg/24 h had a significantly higher level of plasma PK compared with patients with AER ≤40 mg/24 h.

mediated via reduced conversion of angiotensin I to angiotensin II or via decreased degradation of BK, as ACE is the principal enzyme mediating its catabolism. Recent interventional studies have shown that angiotensin receptor blockers also confer renal protection, suggesting that angiotensin II actions are involved in nephropathy pathogenesis (35,36). Even so, a number of studies have suggested that elevated kinins are responsible for at least some of the protective effects of ACEI (37–39).

We have previously published data indicating that BK and related products of the plasma KKS may play an injurious role as well as a protective role. Indeed, under normal physiological conditions, BK is a potent stimulator of nitric oxide release from renal and endothelial cells, which result in vasodilatation because of effects on underlying vascular smooth muscle cells (40,41). However, when the endothelium is denuded or dysfunctional, as is observed in disease states such as hypertension or diabetes, BK can act directly on mesangial and vascular smooth muscle cells (VSMCs) to induce contraction and to activate multiple signaling pathways, in a manner similar to those activated by vasoconstrictors, ultimately resulting in changes in cell proliferation and increased matrix deposition (42–46). Thus BK can protect from vascular disease associated with diabetes when there is an intact endothelium, but could aggravate vascular disease when endothelium is damaged.

In summary, these investigations demonstrated that plasma PK levels are a marker for vascular disease in patients with type 1 diabetes. The mechanism for the elevation of the plasma PK levels is not known, but could arise from either decreased activation or increased synthesis. Additional studies are underway to determine whether increased PK precedes the rise in blood pressure and/or albuminuria in type 1 diabetes.

TABLE 3
Multiple linear regression models for log AER

	Slope	SE	P	Partial r^2 (%)
Intercept	–1.97	0.59	0.001	—
Plasma prekallikrein (units/ml)	0.26	0.12	0.039	0.69
Age (years)	–0.09	0.04	0.025	0.82
Duration of diabetes (years)	0.01	0.01	0.065	0.56
Sex	0.05	0.11	0.676	0.03
DCCT intensive group	–0.40	0.10	0.000	2.33
SBP (mmHg)	0.03	0.00	0.000	7.09
HbA_{1c}	0.23	0.04	0.000	5.36

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