OBJECTIVE — Whereas increased urinary excretion of type IV collagen, which is believed to reflect renal overproduction of this extracellular matrix protein in early diabetic nephropathy, has been confirmed in several studies, examination of serum concentrations of this analyte has yielded conflicting results. We sought to clarify the relationship between early renal dysfunction in diabetes and circulating type IV collagen concentrations.

RESEARCH DESIGN AND METHODS — We measured serum (human) collagen IV concentrations by immunoassay in 109 patients with type 1 or type 2 diabetes and various amounts of albuminuria extending from the normo- to the macroalbuminuric range, and we examined its relationship to albumin excretion and to serum creatinine levels.

RESULTS — Serum collagen IV concentrations (mean ± SEM) were not significantly different in normalalbuminuric (219 ± 10 ng/ml), microalbuminuric (209 ± 6 ng/ml), or macroalbuminuric (206 ± 7 ng/ml) diabetic subjects or in nondiabetic normal volunteers (206 ± 10 ng/ml). Collagen IV concentrations showed no significant correlation ($P > 0.25$) with albumin excretion ($r = -0.001$), HbA$_1c$ ($r = 0.030$), or serum creatinine ($r = -0.161$) and were unrelated to urinary excretion of collagen IV in the subset of subjects in whom these data were available.

CONCLUSIONS — The results of this cross-sectional analysis discount the utility of measurement of the serum concentration of collagen IV as an indicator of early renal dysfunction in diabetes. Increased urine excretion of collagen IV without a significant change in the serum concentration is consistent with a renal origin of this analyte in early diabetic nephropathy.

Diabetes Care 24:1324–1327, 2001

The well-described structural abnormalities in early diabetic nephropathy are observed in both type 1 and type 2 diabetes (1–4) and underlie the eventual encroachment of the glomerular filtration surface area that leads to renal failure (5,6). The association of diabetic glomerulopathy with increased renal production of type IV collagen, a prominent constituent of the thickened basement membrane and expanded mesangium (7–10), has prompted measurement of the concentration of this extracellular matrix protein in biologic fluids in the hope that such measurements might serve as a useful indicator of early diabetic renal disease (11–16). Indeed, the excretion of collagen IV has been found to be increased in type 1 (13,17) and type 2 (13–16) diabetes, without (15–17) or with (11,14,17,18) microalbuminuria or overt proteinuria (11,13,17). However, examination of serum levels of collagen IV in diabetic subjects has yielded discordant results; there are reports of a decrease in type 1 and type 2 diabetes (13), no change in type 2 diabetes without or with microalbuminuria (14), and an increase in patients with type 2 diabetes and proteinuria or renal insufficiency (11). The reasons for this discordance are unknown but may relate to various immunoassay constructs and/or various populations.

To clarify relationships between early diabetic renal dysfunction and circulating type IV collagen concentrations, we measured this analyte by immunoassay in 109 patients with type 1 or type 2 diabetes who had normal or increased albumin excretion extending to the proteinuric range. We excluded patients with a serum creatinine level $\geq 1.7$ mg/dl (≥150 μmol/l) in order to avoid a potential confounding influence of overt renal failure on serum concentrations of this protein and to allow comparison with our previous study (18). We report that serum collagen IV in patients with type 1 or type 2 diabetes and microalbuminuria or proteinuria does not significantly differ from that in normalalbuminuric diabetic subjects or in nondiabetic normal volunteers.

RESEARCH DESIGN AND METHODS — The study group consisted of 109 subjects with a diabetes duration of 5–35 years and a mean age of 58.8 ± 1.2 years. A total of 26 patients had type 1 diabetes (age 48.5 ± 0.6 years), and 83 patients had type 2 diabetes (age 61.0 ± 1.0 years) according to the American Diabetes Association (ADA) criteria. The patients were all under active management and were receiving insulin and/or oral hypoglycemic agents, and they executed an informed consent to participate in the study, which was approved by the Institutional Review Board. Measurement of HbA$_1c$ (analysis by high-pressure liquid chromatography) and serum creatinine concentrations were performed in a commercial laboratory. Blood for serum collagen IV assay and urine for measurement of albumin and creatinine were obtained twice at a 1-month interval in each patient, and the values were averaged. Because the purpose of this study was to assess relationships between early compromise in renal function and serum collagen IV, serum creatinine between 0.5 and 1.7 mg/dl was required for inclusion. This range corre-
Clinical characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Type 1 diabetic subjects</th>
<th>Type 2 diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>109</td>
<td>26</td>
<td>83</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.8 ± 1.2 (45–74)*</td>
<td>48.5 ± 0.4 (45–51)†</td>
<td>61.0 ± 1.0 (52–74)‡</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>98/11</td>
<td>26/0</td>
<td>72/11</td>
</tr>
<tr>
<td>HbA1c</td>
<td>8.16 ± 0.15 (5.8–12.1)</td>
<td>7.45 ± 0.41 (5.8–11.9)</td>
<td>8.27 ± 0.16 (5.8–12.1)</td>
</tr>
<tr>
<td>Urine A.C. (µg/mg)</td>
<td>192.9 ± 26.1</td>
<td>293.2 ± 70.8</td>
<td>161.9 ± 25.4</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.09 ± 0.07 (0.50–1.70)</td>
<td>1.07 ± 0.04 (0.50–1.30)</td>
<td>1.09 ± 0.09 (0.70–1.70)</td>
</tr>
</tbody>
</table>

Data are means ± SEM (range). *Median age 53 years; †median age 48 years; ‡median age 61 years.

**RESULTS** — Table 1 summarizes the clinical characteristics of the study population. As a group, the patients had suboptimum control of their diabetes with a mean HbA1c of 8.2% (range 5.8–12.1%). The mean HbA1c was significantly higher in patients with type 2 than in patients with type 1 diabetes (8.27 ± 0.16 and 7.45 ± 0.41%, respectively). Serum creatinine concentrations were ≤1.7 mg/dl (≤150 µmol/l) in all patients. A total of 65 patients exhibited microalbuminuria (range 28–278 µg/mg creatinine, mean ± SEM 109 ± 9), 22 patients had overt proteinuria (351–1,747 µg/mg, 603 ± 78), and 22 patients had normal albumin excretion (3.9–23.5 µg/mg, 13.7 ± 1.4). The high prevalence of increased albumin excretion in this study population reflects the study design, which, to assess relationships between early nephropathy and collagen IV levels, recruited patients who were likely to have albuminuria, the established predictive marker of diabetic renal dysfunction. None of the study subjects had evidence of urinary tract infection or other obvious diseases that might be responsible for proteinuria.

Serum collagen IV concentrations ranged from 73–303 ng/ml in diabetic patients and from 167–256 ng/ml in 10 nondiabetic normal volunteers. Previous studies have reported values ranging from 50–200 ng/ml in patients with type 1 or type 2 diabetes and in nondiabetic control subjects (11,13,16). The mean ± SEM collagen IV concentration was 219 ± 10 ng/ml in normoalbuminuric diabetic subjects, 209 ± 6 ng/ml in microalbuminuric diabetic patients, 206 ± 7 ng/ml in diabetic patients with proteinuria, and 206 ± 10 ng/ml in 10 nondiabetic normal volunteers with albumin excretion ≤10 µg/mg. Serum collagen IV concentrations showed no significant correlation with albumin excretion in the total study group (r = −0.001) (Fig. 1A) or in the microalbuminuric (r = −0.013) or proteinuric (r = 0.277) subgroups. Collagen IV also did not correlate significantly with glycemic control, assessed by HbA1c (r = 0.030), or with serum creatinine concentrations (r = 0.161) (Fig. 1B). Measurements of urinary collagen IV excretion, performed as previously described (18), were available for 55 of the 109 patients, and no correlation between urinary and serum collagen IV levels was found (r = 0.102). Additionally, in accordance with our previous study, subjects were divided into two groups, RSC ≤100 (serum creatinine ≥1.0 mg/dl) and RSC >100 (serum creatinine <1.0 mg/dl), a value at which decline in filtration function with time can begin to be detected (18,21,26,27). The mean serum collagen IV in patients with an RSC value ≤100 (215 ± 6 ng/ml, n = 62) was not significantly different from that in patients with an RSC value >100 (204 ± 7 ng/ml, n = 47).

**CONCLUSIONS** — We have reported that urinary excretion of type IV collagen antibodies to human albumin in standard or sample competed in the soluble phase with human albumin in all albumin standard or sample reactions. The assay is sensitive to 0.1 µg/ml and shows linearity with the log of concentration between 10 and 1,000 µg/ml. Intra- and interassay coefficients of variation were 3 and 4–6%, respectively. Creatinine was measured in the same sample by a colorimetric method (Sigma, St. Louis, MO) in order to calculate the urine albumin-to-creatinine ratio (A:C). This ratio is regarded as one of the best accepted means for assessing urinary albumin excretion, and it is recommended by the ADA and by Warram et al. (20–24), who used it to establish sex-specific ranges delineating normalalbuminuria, microalbuminuria and overt proteinuria, and levels of microalbuminuria associated with declining glomerular filtration function (21). The definition of microalbuminuria by Warram et al. (21,24) provides a lower limit of 17 µg/mg for men and a lower limit of 25 µg/mg for women.

**Collagen IV immunoassay**

The immunoassay for quantitation of type IV collagen used type IV collagen purified from human placenta (Collaborative Biomedical Products, Bedford, MA) and rabbit anti-human type IV collagen antibody (Biodesign, Kennebunk, ME) according to a previously described methodology (19) that has been reported in detail (18,25). The assay is sensitive to 5 ng/ml and shows a linear inverse relationship with the log of concentration between 5 and 2,000 ng/ml. Intra- and interassay coefficients of variation were 7%, and the average intraindividual variation between the two specimens was 8.5%. The anticollagen IV antibody showed no reactivity with other human serum proteins. Glucose ≤50 mmol/l did not interfere in the assay.

Statistical analysis was performed using unpaired t tests to compare means. Correlation between analytes were determined by linear regression analysis.
collagen is increased in diabetic patients and that it correlates significantly with filtration function, as assessed by the RSC, in patients with albuminuria and a serum creatinine ≤1.7 mg/dl (18). This correlation pertained regardless of whether albumin excretion was in the low (≤100 µg/mg creatinine) or high (>100 µg/mg) range and suggested that urinary collagen IV excretion is a more useful indicator than albumin excretion to detect diabetic renal disease entering a phase of compromised filtration function (18). This interpretation was supported by the observation that urine albumin excretion showed insignificant correlation with the RSC and by results of longitudinal studies showing that not all patients with microalbuminuria progress to clinical nephropathy with an overt reduction in filtration function and that some patients progress without antecedent microalbuminuria (28–30). These considerations prompted this study, which attempted to determine if analogous relationships pertained with respect to serum collagen IV concentrations and serum creatinine and/or urine albumin in patients with early diabetic renal dysfunction. The results show that there is no increase in circulating collagen IV in any category of diabetic renal involvement (i.e., normo-, micro-, or macroalbuminuria), that the serum concentration of type IV collagen shows no correlation with either serum creatinine or urine albumin excretion, and that the serum collagen IV concentration is unchanged in patients at an increased risk for compromise in filtration function, as assessed by an RSC ≤100. Serum collagen IV also shows no correlation with glycemic control, as assessed by HbA1c levels, or with urinary collagen IV excretion in patients for whom data were available. The observation that changes in serum collagen IV do not accompany an increased urine collagen IV excretion in diabetic patients with microalbuminuria is consistent with a renal origin of this analyte in early diabetic nephropathy. In summary, this cross-sectional analysis discounts the utility of serum collagen IV concentrations as an indicator of early renal dysfunction in diabetes.

Acknowledgments—Supported in part by National Institutes of Health Grants DK 054608 and DK 454143.

Figure 1—Study population values and correlation analyses between (A) serum type IV collagen (ng/ml) versus urine albumin (A:C [µg/mg], r = −0.001) and (B) serum type IV collagen versus creatinine (mg/dl) (r = 0.161). ○, Type 1 diabetes; ●, Type 2 diabetes.
The authors acknowledge Yulin Jin for providing technical assistance.

References


