Connective Tissue Growth Factor Is Increased in Plasma of Type 1 Diabetic Patients With Nephropathy

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OBJECTIVE — Connective tissue growth factor (CTGF) is strongly upregulated in fibrotic disorders and has been hypothesized to play a role in the development and progression of diabetes complications. The aim of the present study was to investigate the possible association of plasma CTGF levels in type 1 diabetic patients with markers relevant to development of diabetes complications.

RESEARCH DESIGN AND METHODS — Plasma CTGF levels (full-length and NH_2-terminal fragments) were determined in 62 well-characterized patients with type 1 diabetes and in 21 healthy control subjects. Correlations of these plasma CTGF levels with markers of glycemic control, platelet activation, endothelial activation, nephropathy, and retinopathy were investigated.

RESULTS — Elevated plasma NH_2-terminal fragment of CTGF (CTGF-N) levels were detected in a subpopulation of type 1 diabetic patients and were associated with diabetic nephropathy. Stepwise regression analysis revealed contribution of albuminuria, creatinine clearance, and duration of diabetes as predictors of plasma CTGF-N level. Elevation of plasma CTGF-N levels in patients with retinopathy was probably due to renal comorbidity.

CONCLUSIONS — Plasma CTGF-N levels are elevated in type 1 diabetic patients with nephropathy and appear to be correlated with proteinuria and creatinine clearance. Further studies will be needed to determine the relevance of plasma CTGF as a clinical marker and/or pathogenic factor in diabetic nephropathy.

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Diabetic patients frequently develop severe chronic complications like cardiovascular disease, nephropathy, neuropathy, and retinopathy. Characteristics of these complications are macro- and microvascular damage, extracellular matrix (ECM) accumulation, and eventually chronic fibrosis. It has been shown (1–4) that growth factors play an important role in the development of these diabetes complications. One of the important growth factors involved in ECM accumulation and fibrotic processes is transforming growth factor (TGF)-β (1). In both type 1 and type 2 diabetes, plasma levels of TGF-β were correlated with the presence of diabetes complications (5,6).

Connective tissue growth factor (CTGF) is another important growth factor implicated in the development of diabetes complications. CTGF is a 38-kDa, cystein-rich secreted protein that was originally cloned from human umbilical vein endothelial cells (7). Strong CTGF expression has been reported (8,9) in atherosclerotic aorta and in renal mesangial cells cultured in high-glucose medium. CTGF was also shown (10–12) to be strongly expressed in glomeruli of diabetic patients and animals with nephropathy. Similarly, the magnitude of urinary CTGF excretion was related to the severity of diabetic nephropathy (DN) in a cross-sectional study (13) of patients with type 1 diabetes. Both high glucose concentrations and advanced glycation end products (AGEs) are able to induce ECM production via CTGF (9,14). CTGF itself can act as a downstream mediator of TGF-β in ECM synthesis, but TGF-β–independent regulation of CTGF has also been reported (9,15). Therefore, the analysis of CTGF plasma levels of diabetic patients might provide important additional information about the involvement of this growth factor in the development of diabetes complications. Different fragments of the CTGF protein have been detected in vitro and in vivo, and at least some of these have biological activity. The CTGF molecule, which consists of four modules, is mostly cleaved between modules II and III, yielding fragments of 16–20 kDa, but smaller fragments have also been identified (16,17). The relative contribution to fibrotic processes of full-length compared with fragmented CTGF remains to be established.

The aim of the present study was to investigate the possible association of plasma CTGF levels in 62 well-

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Abbreviations: AGE, advanced glycation end product; BMP, bone morphogenetic protein; CTGF, connective tissue growth factor; CTGF-N, NH_2-terminal fragment of CTGF; DN, diabetic nephropathy; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; TGF, transforming growth factor; vWF, von Willebrand factor.

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characterized type 1 diabetic patients with DN and diabetic retinopathy as well as with general patient characteristics and other markers relevant to the development of diabetes complications, i.e., glycemic control, endothelial activation, and platelet activation (2,18–20).

RESEARCH DESIGN AND METHODS

For the present study, we analyzed plasma samples obtained from 62 well-characterized patients with type 1 diabetes and 21 healthy control subjects. All gave their informed consent before participation in the study. The study protocol has been approved by the local ethics committee/institutional board and was conducted according to the principles of the Declaration of Helsinki. After an overnight fast and abstaining from vigorous physical activity during the previous 24 h, patients and control subjects presented at the outpatient clinic between 8:00 and 10:00 A.M., bringing their 24-h urine. Demographic and relevant medical history data were recorded, including age, sex, duration of diabetes, insulin dose, comorbidity, medication, and smoking habits. Blood pressure was measured with a sphygmomanometer in the sitting position; the median of three successive measurements was noted. Height and weight were measured to determine BMI. Retinopathy was scored by a single experienced ophthalmologist by fundoscopic examination and examination of the clinical charts.

Blood was collected by puncture of an antebrachial vein using sodium heparin, citrate, or PECT as anticoagulants, dependent on the assay. PECT medium (400 μl/4.5 ml polypropylene tube), used to prevent ex vivo platelet activation, is composed of equal volumes of solutions A, B, and C, in which A = 282 nmol/l prostaglandin E_1 and 1.9 mmol/l Na_2CO_3, B = 30 mmol/l theophylline in PBS, and C = 270 mmol/l EDTA. To further avoid artificial platelet and leukocyte activation, blood samples were immediately cooled on ice and platelets were depleted by centrifuging the samples for 60 min at 4,000*g* and 4°C within 1 h after collection. All plasma samples were stored at −70°C until analysis.

Measurement of markers for glycemic control and nephropathy

Concentrations of HbA_{1c} were determined in citrate plasma by means of high-performance liquid chromatography. Plasma creatinine concentrations were determined by automated spectrophotometrical assay using Creatinine-PAP (peroxidase-antiperoxidase). In 24-h urine samples, creatinine was measured using the Jaffé method, albumin was determined with an immunonephelometric assay, and renal creatinine clearance was calculated from these data.

Measurement of markers for platelet and endothelial activation

Von Willebrand factor (vWF) antigen was determined by sandwich enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human vWF as "capture" antibody and horseradish peroxidase–conjugated rabbit anti-human vWF as "detecting" antibody (Dakopatts, Glostrup, Denmark). β-Thromboglobulin, a marker for in vivo platelet activation, and platelet factor 4, a marker for ex vivo platelet activation, were determined in platelet-depleted PECT plasma by means of sandwich ELISAs according to the manufacturer's instructions (Asserachrom; Diagnostica Stago) (21).

ELISAs for plasma CTGF (NH_2-terminal and full length) and TGF-β

Plasma content of full-length and N-terminal fragments (CTGF-N) of CTGF were determined by means of two separate sandwich ELISAs, each using two distinct monoclonal antibodies against human CTGF (FibroGen, South San Francisco, CA). Microtiter plates were coated overnight at 4°C with capture antibody and blocked with BSA. Citrate plasma was diluted 5- or 10-fold in assay buffer, and a 50-μl diluted sample was added to each well together with 50 μl biotinylated CTGF detection antibody. After incubation for 2 h at 37°C followed by incubation with streptavidin-conjugated alkaline phosphatase for 1 h at room temperature, plates were washed and 100 μl of substrate solution containing *p*-nitrophenyl phosphate was added to each well. After 20 min of color development, absorbance was read at 405 nm. Purified recombinant human CTGF (FibroGen) was used for calibration. Both monoclonal antibodies used in the CTGF-N sandwich ELISA specifically bind distinct epitopes on the N-terminal half of the CTGF protein. This assay detects both CTGF-N as well as the full-length CTGF protein. In the full-length CTGF ELISA, the capture antibody binds the COOH-terminal part of the CTGF protein, whereas the detecting antibody (the same as in the CTGF-N ELISA) binds the NH_2-terminal part of the CTGF protein. To avoid confusion due to differences in the molecular weight of full-length and different fragments of CTGF, all levels were expressed as picomoles per liter. The detection limit of these assays was 4 pmol/l for CTGF-N and 8 pmol/l for full-length CTGF, and intra- and interassay variations were 6 and 20%, respectively.

TGF-β1 was determined in PECT plasma by means of sandwich ELISA according to the manufacturer's protocol (R&D Systems, Minneapolis, MN).

Statistical analysis

Data are expressed as mean ± SD. Mann-Whitney analysis and Kruskal-Wallis analysis followed by Dunn's method were performed to determine differences in plasma CTGF-N levels between groups. Forward stepwise regression analysis was used to compare CTGF-N levels with general patient characteristics, glycemic control, endothelial activation, platelet activation, albuminuria, and creatinine clearance. Because data were not normally distributed, Spearman's correlations were calculated between CTGF-N levels and HbA_{1c}, albuminuria, and TGF-β1 levels. In all cases, $P < 0.05$ was considered significant.

RESULTS

Plasma CTGF-N level is elevated in DN

Levels of full-length CTGF were below the sensitivity limit of our sandwich ELISA in all control as well as diabetic plasma samples tested, although full-length recombinant CTGF was readily detectable if spiked in these plasma samples. This means that these plasma samples contained <80 pmol/l of full-length CTGF (not shown). In contrast, CTGF-N was readily detectable in the majority of these same plasma samples. No significant difference was found in CTGF-N levels between control subjects and the total group of diabetic patients, but the variation was much larger among samples from diabetic patients (138 ± 136 pmol/l in diabetic patients vs. 103 ± 51 pmol/l in control subjects) (Fig. 1*A*). Patients were divided into subgroups according to the

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level of albuminuria, i.e., normoalbuminuria (<30 mg/day), microalbuminuria (30–300 mg/day), and overt proteinuria (i.e., DN, >300 mg/day). This revealed significant differences in plasma CTGF-N levels between DN patients and control subjects and between DN and normoalbuminuric patients (Fig. 1*B*). Furthermore, a very wide variation in plasma CTGF-N levels was noted within the subgroup of microalbuminuric patients (203 ± 203 pmol/l).

Plasma CTGF-N level correlates with HbA_{1c}

General patient characteristics and markers of glycemic control, endothelial activation, platelet activation, and nephropathy of healthy subjects and normoalbuminuric, microalbuminuric, and DN diabetic patients are summarized in Table 1.

HbA_{1c} levels in the diabetic patients correlated, albeit only weakly, with plasma CTGF-N ($R = 0.355$, $P = 0.005$) (Fig. 1*C*). This is in line with induction of CTGF expression by high ambient glucose and AGEs observed previously in vitro (9,14). No significant correlation between plasma CTGF-N and HbA_{1c} was found within any of the subgroups. This might be due to the relatively small numbers of microalbuminuric and DN patients.

Plasma CTGF-N correlates with albuminuria and creatinine clearance

Forward stepwise regression analysis showed that albuminuria, creatinine clearance, and duration of diabetes are independent predictors of plasma CTGF-N level. Plasma CTGF-N most strongly correlated with albuminuria ($R = 0.572$, $P < 0.001$) (Fig. 1*D*), whereas albuminuria and creatinine clearance together yielded a cumulative R of 0.667, with $P < 0.001$. Correlation of CTGF-N with albuminuria, creatinine clearance, and duration of the diabetes together resulted in a cumulative R of 0.759, with a $P = 0.001$. Addition of further parameters did not significantly contribute to this correlation. Within the group of DN patients, the plasma CTGF-N levels showed a tendency to correlate with albuminuria, but this was not statistically significant ($n = 10$, $R = 0.553$, $P = 0.097$). When, instead of plasma CTGF-N level, albuminuria or creatinine clearance was taken as a dependent variable in the forward stepwise regression analysis of this dataset, plasma CTGF-N level was identified as the strongest independent predictor ($R = 0.572$ and 0.514, respectively). It thus appears that plasma CTGF-N levels are correlated with markers for nephropathy.

Plasma TGF-β1 levels

Since TGF-β1, unlike CTGF (F.A.V.N., P.R., unpublished observations) is abundant in platelets and released during platelet activation, reliable estimates of "in vivo" plasma TGF-β1 levels can be obtained only in the absence of significant ex vivo platelet activation (i.e., platelet factor 4 <10 units/l). Despite careful plasma collection and processing, only 21 diabetic and 5 control samples met this criterion. In these 26 samples, TGF-β1 levels did correlate with CTGF-N levels ($R = 0.671$, $P < 0.001$), but no significant difference in plasma TGF-β1 levels was observed between healthy subjects and diabetic patients. Within the group of 21 diabetic patients, TGF-β1 correlated with HbA_{1c} ($R = 0.477$, $P = 0.029$). This is stronger than the correlation between plasma CTGF-N and HbA_{1c}.

In subgroups defined by degree of albuminuria, no significant difference was found between TGF-β1 levels, although there seemed to be a trend to higher levels in patients with DN as compared with diabetic patients without DN ($P = 0.114$) (data not shown). Due to small numbers, it was not possible to include TGF-β1 as a parameter in forward stepwise regression analysis.

Plasma CTGF-N levels in patients with retinopathy

Diabetic retinopathy is almost invariably present in patients with DN. Plasma CTGF-N levels appeared to be significantly elevated in patients with retinopathy compared with those without retinopathy ($P < 0.001$) (Fig. 1*E*). However, of the 17 patients with diabetic retinopathy who had >180 pmol/l of CTGF-N in their plasma (upper limit of normal control subjects), only 4 were normoalbuminuric, whereas 4 of the remaining 13 were microalbuminuric and 9 proteinuric (i.e., had DN). Plasma CTGF-N levels of normoalbuminuric patients with retinopathy ($n = 23$) were not different from those of normoalbuminuric patients without retinopathy ($n = 17$).

CONCLUSIONS — Changes in growth factor balance are important in the development of chronic complications of diabetes. In this study, we observed that plasma CTGF-N levels are significantly elevated in patients with DN and correlated with markers for DN and glycemic control. Full-length CTGF levels were below the detection limit of our ELISA in all diabetic and normal plasma samples tested. This absence of detectable full-length CTGF in plasma might be related to technical limitations (sensitivity of the full-length ELISA) or to its clearance via COOH-terminal interaction with matrix components and (scavenging) receptors and to proteolysis, e.g., by matrix metalloproteases, plasmin, and elastase, which have all been reported to cleave CTGF (16).

It has been reported (10–13,22) that CTGF mRNA and protein levels are significantly increased in kidney tissue and urine of patients as well as experimental animals with DN. Adler et al. (23) found equally elevated CTGF mRNA levels in glomeruli of microalbuminuric ($n = 5$) and DN ($n = 6$) diabetic patients. We now add to this notion that CTGF-N levels are elevated in plasma of almost all type 1 diabetic patients with DN and also in about one-third of microalbuminuric patients, but only in a small minority of normoalbuminuric patients. Plasma CTGF-N levels of patients with DN as a group differed significantly from those of normoalbuminuric patients and healthy control subjects. Due to very wide scatter, levels in microalbuminuric patients were not significantly different from those in healthy control subjects or normoalbuminuric patients and also not significantly different from levels in patients with DN. Since there was a difference in age between healthy control subjects and microalbuminuric and DN diabetic patients, we also measured plasma CTGF-N in an additional group of 20 healthy subjects whose mean age (42 ± 8 years) was comparable with that of the studied microalbuminuric and DN diabetic patients. In this additional control group, we found a mean plasma CTGF-N level of 120 ± 72 pmol/l, which is in the same range as the plasma CTGF-N levels in the other healthy control group and the normoalbuminuric diabetic patient group. It

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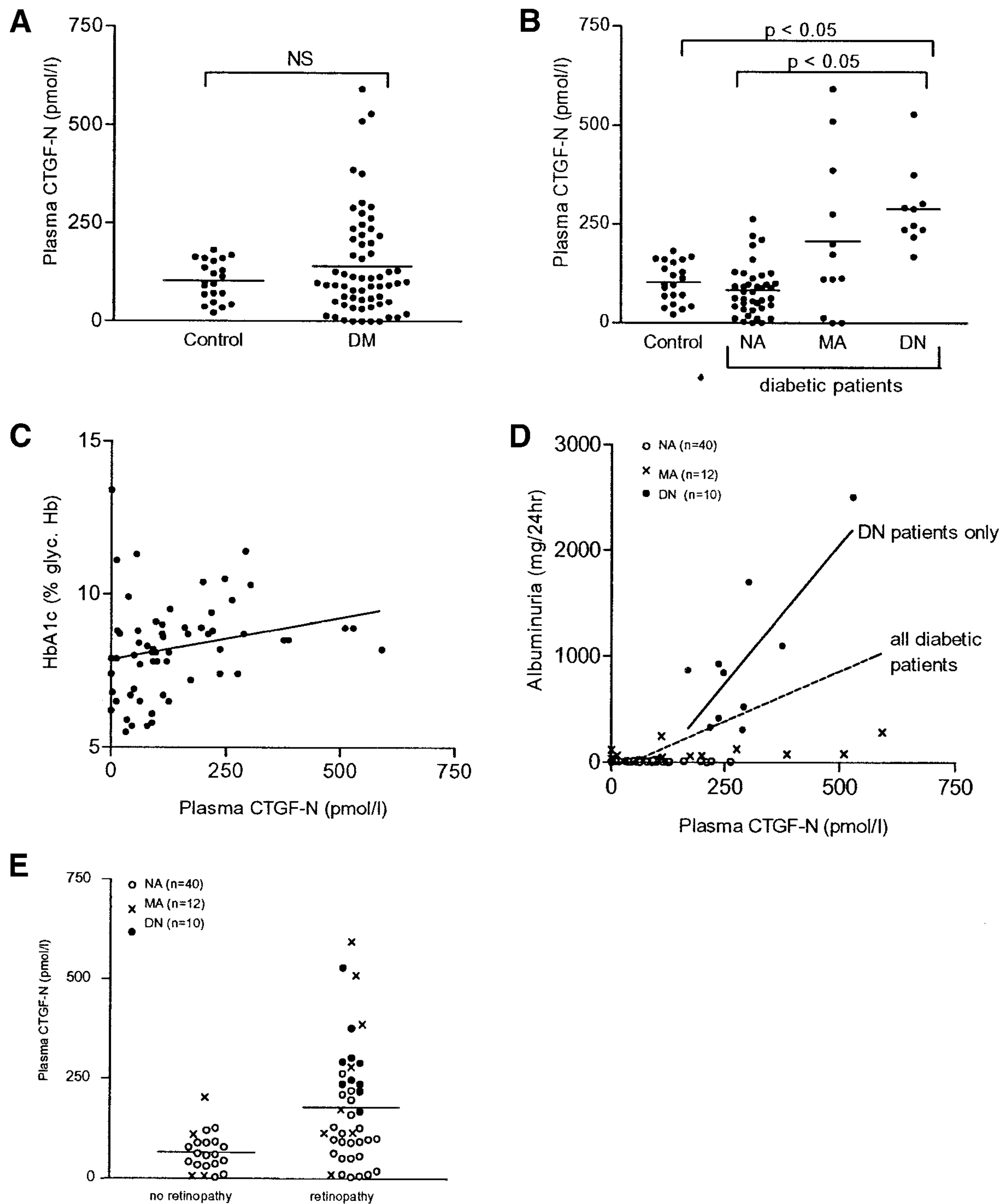

Figure 1—*Plasma CTGF-N levels and correlations in control subjects and different subgroups of type 1 diabetic patients. A: Plasma CTGF-N levels in healthy control subjects (103 ± 51 pmol/l [mean ± SD]) and all diabetic patients (138 ± 136 pmol/l) (P = 0.765). B: Distribution of plasma CTGF-N fragment levels in the different patient subgroups according to albuminuria: healthy control subjects, 103 ± 51 pmol/l; normoalbuminuric (NA), 80 ± 66; microalbuminuric (MA), 203 ± 203; and DN, 290 ± 101 (mean ± SD). A significant difference (P < 0.05) was observed between control subjects and DN patients and between normoalbuminuric and DN patients. C: Correlation between plasma CTGF-N levels and HbA_{1c} in all diabetic patients. Spearman's correlation:* R = *0.355, P = 0.005. D: Correlations between plasma CTGF-N and albuminuria in the total group of type 1 diabetic patients and in the subgroup of DN patients only. Diabetic patients (*n = *62):* R = *0.572, P < 0.001. DN patients only (Spearman's correlation,* n = *10):* R = *0.553,* P = *0.097. E: Distribution of plasma CTGF-N levels in diabetic patients with and without retinopathy: no retinopathy, 61 ± 57 pmol/l, and retinopathy, 178 ± 148 (mean ± SD) (P < 0.001). Elevated plasma CTGF-N levels are almost exclusively found in microalbuminuric and DN patients. ○, normoalbuminuric; ×, microalbuminuric; ●, DN.*

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Table 1 —*General and clinical parameters of healthy control subjects and type 1 diabetic patients*

	Control subjects	Type 1 diabetic patients		
		NA	MA	DN
General patient characteristics				
n (men)	21 (11)	40 (19)	12 (6)	10 (6)
Age (years)	30 ± 6	37 ± 10	45 ± 9	43 ± 10
Duration of diabetes (years)	—	18.3 ± 12.0	23.0 ± 8.8	26.2 ± 6.2
BMI (kg/m^2)	23.4 ± 3.3	23.2 ± 2.7	22.0 ± 2.0	22.6 ± 2.1
Systolic blood pressure (mmHg)	129 ± 15	132 ± 18	141 ± 18	152 ± 22
Glycemic control				
HbA_{1c} (%)	5.2 ± 0.4	8.0 ± 1.7	8.3 ± 1.1	9.2 ± 1.2
Platelet activation				
β-Thromboglobulin (units/l)	28.5 ± 8.8	27.8 ± 6.4	33.4 ± 6.5	34.6 ± 9.9
Endothelial activation				
vWF (%)	78 ± 18	103 ± 31	111 ± 23	129 ± 43
Nephropathy				
Albuminuria (mg/24 h)	9.2 ± 7.4	8.9 ± 5.1	102 ± 82	953 ± 690
Creatinine clearance (ml/min)	137 ± 22	131 ± 37	120 ± 46	87 ± 34

Data are means ± SD. MA, microalbuminuric; NA, normoalbuminuric.

was not possible to include these additional 20 control subjects in the study because other parameters were not determined in these individuals.

The apparent discrepancy with the similarly elevated kidney mRNA levels in all five microalbuminuric patients and six patients with DN reported by Adler et al. (23) might indicate that plasma CTGF level is not solely determined by renal CTGF expression. Moreover, microalbuminuric patients are heterogeneous with respect to progression. A recent meta-analysis by Caramori et al. (24) revealed a 30–45% risk of progression of microalbuminuria to proteinuria over 10 years compared with 30% remission to a normoalbuminuric state and stabilization of microalbuminuria in the remaining patients. In this respect, it is of interest that our microalbuminuric group showed a remarkable interindividual variation in plasma CTGF-N levels, with significantly elevated levels in 4 of 12 microalbuminuric patients. Obviously, it will be important to obtain follow-up data of these and other microalbuminuric diabetic patients to investigate whether high plasma CTGF-N levels might predict progression of microalbuminuria to DN.

The major finding in forward stepwise regression analysis was that plasma CTGF-N levels were associated with albuminuria, creatinine clearance, and duration of diabetes. The strongest correlation was found with albuminuria, one of the main characteristics of DN, and a negative but almost equally strong correlation was found with creatinine clearance. Identical results were obtained when the MDRD (modification of diet in renal disease) formula and Cockcroft-Gault calculations were used as estimates of glomerular filtration rate instead of creatinine clearance. CTGF (36–38 kDa), and even more its fragments (∼10–20 kDa), can be expected to pass the glomerular filter into the primary urine, although dimerization and other protein interactions might prevent filtration in normal glomeruli. In DN, proteinuria is accompanied by a progressive decline of glomerular filtration. Therefore, elevated plasma CTGF-N levels in patients with DN might (at least in part) reflect loss of renal clearance. In conjunction with albuminuria and creatinine clearance, duration of diabetes was identified as a third factor that significantly contributed to plasma CTGF-N levels. No colinearity between albuminuria, creatinine clearance, and duration of diabetes was observed, which indicates that in this analysis these three parameters can be considered as independent factors. Nevertheless, a certain degree of interdependence might be assumed because DN tends to develop about 10–15 years after the onset of type 1 diabetes, starting with microalbuminuria, followed by proteinuria and later progressive loss of renal function.

Although CTGF has originally been cloned from endothelial cells and atherosclerotic aorta (7,8), markers for endothelial or platelet activation (vWF and β-thromboglobulin) did not contribute to prediction of plasma CTGF-N levels. Plasma CTGF-N levels were significantly elevated in the group of diabetic patients with retinopathy compared with patients without retinopathy, but patients with retinopathy who had no signs of renal complications did not have elevated plasma CTGF-N levels. Therefore, the elevated plasma CTGF-N levels in the group of retinopathy patients are likely due to associated nephropathy rather than to the retinopathy itself. However, this does not necessarily mean that CTGF is more important in nephropathy than in retinopathy. In retinal endothelial cells and in pericytes, CTGF is induced by vascular endothelial growth factor (25). We have observed altered CTGF distribution in retinas of diabetic patients with increased positivity of pericytes, which might relate to capillary basement membrane thickening characteristic of diabetic retinopathy (E. Kuiper, R.O.S., R.G., unpublished results). Based on the size and perfusion of the organs, however, local production of CTGF in the kidney can be expected to contribute more to plasma levels than local production in the retina.

The observed correlation of plasma CTGF levels with markers for nephropathy raises questions as to the relevance of known determinants of the development of chronic diabetes complications to regulation of CTGF expression. It is known that CTGF and its major inducer, TGF-β, can be both induced by elevated levels of glucose and AGEs (9,14) and that strict glycemic control is important in the prevention of development and progression of diabetes complications (2). Therefore, a correlation might be expected between CTGF, TGF-β1, and HbA_{1c} levels in diabetic plasma. In our study, we indeed found a correlation between plasma CTGF-N and TGF-β1 ($r = 0.671$, $P \leq 0.001$) and weaker correlations between CTGF and HbA_{1c} ($r = 0.355$, $P = 0.005$) and between TGF-β1 and HbA_{1c} ($r = 0.477$, $P = 0.029$). The latter is in agreement with previous observations (5) in a much larger population of diabetic subjects. Forward stepwise regression analysis, however, revealed no significant addition of HbA_{1c} to the prediction of plasma CTGF-N levels from albuminuria, creatinine clearance, and duration of dia-

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betes. This suggests that, as is known for the development of diabetes complications, CTGF expression might be determined largely by factors other than glycemic control alone.

Given its biological activity in terms of fibrosis and matrix accumulation, it is possible that CTGF is not only a marker, but also a pathogenic factor in the development of DN and other complications. CTGF is involved in the induction of the expression of ECM components by high-glucose concentrations in renal mesangial cells and fibroblasts in vitro (12,26). Under diabetic conditions, TGF-β and IGF-1 are upregulated, while bone morphogenetic protein (BMP) is downregulated (3,27). Both inhibition of TGF-β and supplementation of BMP-7 protect against (progression of) experimental DN (28,29). CTGF can bind TGF-β, BMPs, and IGF-1 and is also able to influence their activity. It enhances IGF-1–induced secretion of collagens I and III, and it increases the profibrotic activity of TGF-β while inhibiting BMP activity (26,30,31). Thus, CTGF, induced by hyperglycemia, AGEs, or other pathways, might have an important impact on tissue remodeling and fibrosis by its contribution to the diabetes-related growth factor imbalance. Given the impressive elevation of urinary CTGF excretion in DN, it is possible that the nephropathy-associated increase of plasma CTGF reflects, at least in part, increased renal production in addition to the expected effect of reduced renal clearance (13). In this way, plasma CTGF might be involved in nephropathy-associated aggravation of systemic (and particularly cardiovascular) complications of diabetes.

In summary, we have observed that in patients with type 1 diabetes, elevated plasma CTGF-N levels were associated with DN and correlated with level of albuminuria, impairment of creatinine clearance, and poor glycemic control. Longitudinal studies will be needed to further investigate the possible clinical value of CTGF detection as a marker for incipient or progressive diabetes complications and, in particular, DN. To assess the possible importance of increased CTGF levels for development and progression of these disorders, animal studies will be required in which CTGF expression and activity is manipulated against a background of diabetes.

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References

1. Border WA, Noble NA: Interactions of transforming growth factor-β and angiotensin II in renal fibrosis. *Hypertension* 31: 181–188, 1998
2. Sheetz MJ, King GL: Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 288:2579–2588, 2002
3. Flyvbjerg A: Putative pathophysiological role of growth factors and cytokines in experimental diabetic kidney disease. *Diabetologia* 43:1205–1223, 2000
4. Gupta S, Clarkson MR, Duggan J, Brady HR: Connective tissue growth factor: potential role in glomerulosclerosis and tubulointerstitial fibrosis. *Kidney Int* 58: 1389–1399, 2003
5. Chaturvedi N, Schalkwijk CG, Abrahamian H, Fuller JH, Stehouwer CD: Circulating and urinary transforming growth factor β1, Amadori albumin, and complications of type 1 diabetes: the EURODIAB prospective complications study. *Diabetes Care* 25:2320–2327, 2002
6. Pfeiffer A, Middelberg-Bisping K, Drewes C, Schatz H: Elevated plasma levels of transforming growth factor-β1 in NIDDM. *Diabetes Care* 19:1113–1117, 1996
7. Bradham DM, Igarashi A, Potter RL, Grotendorst GR: Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. *J Cell Biol* 114: 1285–1294, 1991
8. Oemar BS, Werner A, Garnier JM, Do DD, Godoy N, Nauck M, Marz W, Rupp J, Pech M, Luscher TF: Human connective tissue growth factor is expressed in advanced atherosclerotic lesions. *Circulation* 95:831–839, 1997
9. Murphy M, Godson C, Cannon S, Kato S, Mackenzie HS, Martin F, Brady HR: Suppression subtractive hybridization identifies high glucose levels as a stimulus for expression of connective tissue growth factor and other genes in human mesangial cells. *J Biol Chem* 274:5830–5834, 1999
10. Ito Y, Aten J, Bende RJ, Oemar BS, Rabelink TJ, Weening JJ, Goldschmeding R: Expression of connective tissue growth factor in human renal fibrosis. *Kidney Int* 53:853–861, 1998
11. Riser BL, Denichilo M, Cortes P, Baker C, Grondin JM, Yee J, Narins RG: Regulation of connective tissue growth factor activity in cultured rat mesangial cells and its expression in experimental diabetic glomerulosclerosis. *J Am Soc Nephrol* 11:25–38, 2000
12. Wahab NA, Yevdokimova N, Weston BS, Roberts T, Li XJ, Brinkman H, Mason RM: Role of connective tissue growth factor in the pathogenesis of diabetic nephropathy. *Biochem J* 359:77–87, 2001
13. Gilbert RE, Akdeniz A, Weitz S, Usinger WR, Molineaux C, Jones SE, Langham RG, Jerums G: Urinary connective tissue growth factor excretion in patients with type 1 diabetes and nephropathy. *Diabetes Care* 26:2632–2636, 2003
14. Twigg SM, Chen MM, Joly AH, Chakrapani SD, Tsubaki J, Kim HS, Oh Y, Rosenfeld RG: Advanced glycosylation end products up-regulate connective tissue growth factor (insulin-like growth factor-binding protein-related protein 2) in human fibroblasts: a potential mechanism for expansion of extracellular matrix in diabetes mellitus. *Endocrinology* 142:1760–1769, 2001
15. Blom IE, van Dijk AJ, Wieten L, Duran K, Ito Y, Kleij L, deNichilo M, Rabelink TJ, Weening JJ, Aten J, Goldschmeding R: In vitro evidence for differential involvement of CTGF, TGFbeta, and PDGF-BB in mesangial response to injury. *Nephrol Dial Transplant* 16:1139–1148, 2001
16. Hashimoto G, Inoki I, Fujii Y, Aoki T, Ikeda E, Okada Y: Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor 165. *J Biol Chem* 277:36288–36295, 2002
17. Ball DK, Surveyor GA, Diehl JR, Steffen CL, Uzumcu M, Mirando MA, Brigstock DR: Characterization of 16- to 20-kilodalton (kDa) connective tissue growth factors (CTGFs) and demonstration of proteolytic activity for 38-kDa CTGF in pig uterine luminal flushings. *Biol Reprod* 59:828–835, 1998
18. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH: Diabetic nephropathy (Position Statement). *Diabetes Care* 26 (Suppl. 1):S94–S98, 2003
19. Stehouwer CD, Fischer HR, van Kuijk AW, Polak BC, Donker AJ: Endothelial dysfunction precedes development of microalbuminuria in IDDM. *Diabetes* 44: 561–564, 1995
20. Tschoepe D, Ostermann H, Huebinger A, Ziegler D, Wiefels K, Gries FA: Elevated platelet activation in type I diabetics with chronic complications under long-term near-normoglycemic control. *Haemostasis* 20:93–98, 1990
21. Kaplan KL, Owen J: Plasma levels of platelet secretory proteins. *Crit Rev Oncol He-*

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matol 5:235–255, 1986

22. Riser BL, Cortes P, Denichilo M, Deshmukh PV, Chahal PS, Mohammed AK, Yee J, Kahkonen D: Urinary CCN2 (CTGF) as a possible predictor of diabetic nephropathy: preliminary report. *Kidney Int* 64:451–458, 2003
23. Adler SG, Kang SW, Feld S, Cha DR, Barba L, Striker L, Striker G, Riser BL, LaPage J, Nast CC: Glomerular mRNAs in human type 1 diabetes: biochemical evidence for microalbuminuria as a manifestation of diabetic nephropathy. *Kidney Int* 60:2330–2336, 2001
24. Caramori ML, Fioretto P, Mauer M: The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? *Diabetes* 49:1399–1408, 2000
25. Suzuma K, Naruse K, Suzuma I, Takahara N, Ueki K, Aiello LP, King GL: Vascular endothelial growth factor induces expression of connective tissue growth factor via KDR, Flt1, and phosphatidylinositol 3-kinase-akt-dependent pathways in retinal vascular cells. *J Biol Chem* 275:40725–40731, 2000
26. Lam S, Verhagen NAM, van der Geest RN, van Nieuwenhoven FA, Blom IE, Aten J, Goldschmeding R, Daha MR, van Kooten C: Connective tissue growth factor and IGF-I are produced by human renal fibroblasts, and cooperate in the induction of collagen production by high glucose. *Diabetes* 52:2975–2983, 2003
27. Wang SN, Lapage J, Hirschberg R: Loss of tubular bone morphogenetic protein-7 in diabetic nephropathy. *J Am Soc Nephrol* 12:2392–2399, 2001
28. Chen S, Carmen Iglesias-de la Cruz M, Jim B, Hong SW, Isono M, Ziyadeh FN: Reversibility of established diabetic glomerulopathy by anti-TGF-beta antibodies in db/db mice. *Biochem Biophys Res Commun* 300:16–22, 2003
29. Lund RJ, Davies MR, Hruska KA: Bone morphogenetic protein-7: an anti-fibrotic morphogenetic protein with therapeutic importance in renal disease. *Curr Opin Nephrol Hypertens* 11:31–36, 2002
30. Wang S, Denichilo M, Brubaker C, Hirschberg R: Connective tissue growth factor in tubulointerstitial injury of diabetic nephropathy. *Kidney Int* 60:96–105, 2001
31. Abreu JG, Ketpura NI, Reversade B, De Robertis EM: Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. *Nat Cell Biol* 4: 599–604, 2002

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