Matrix Metalloproteinase-2 Dysregulation in Type 1 Diabetes

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OBJECTIVE — Dysregulation of matrix metalloproteinase (MMP)-2 may contribute pathologically to the development of diabetes complications, including diabetic retinopathy and coronary and peripheral arterial disease. Our objective was to explore whether systemic MMP-2 dysregulation could be demonstrated in type 1 diabetes and to determine how MMP-2 concentration relates to disease status.

RESEARCH DESIGN AND METHODS — In this cross-sectional study, MMP-2 concentrations and MMP-2 activity were measured in plasma and timed urine samples from 93 type 1 diabetic and 50 healthy control subjects, aged 14–40 years. Relationships between MMP-2 concentrations in these biological fluids and subject characteristics (sex, age, and duration of type 1 diabetes), indexes of glycemic control (A1C, fasting plasma glucose, and continuous glucose monitoring system average daily glucose), and measurements of renal function (urinary albumin excretion and glomerular filtration rate) were examined.

RESULTS — Urine and plasma MMP-2 concentrations and plasma MMP-2 activity were all significantly elevated in type 1 diabetic subjects compared with those in control subjects. Urine MMP-2 concentrations, in particular, were correlated with several clinical parameters that infer increased risk for diabetic comorbidity and specifically for diabetic nephropathy, including higher A1C, longer duration of disease, evidence of renal hyperfiltration, and the presence of microalbuminuria.

CONCLUSIONS — Urine and plasma MMP-2 concentrations are dysregulated in type 1 diabetes; urinary excretion of MMP-2, in particular, might provide a unique biomarker of diabetes-induced intrarenal pathologic processes.

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Matrix metalloproteinases (MMPs) constitute a group of enzymes that hydrolyze protein components of the extracellular matrix (1). The subgroup of MMPs known as gelatinases, specifically gelatinase A (MMP-2) and gelatinase B (MMP-9) digest collagen, denatured collagens (i.e., gelatins), laminin, elastin, and fibronectin, among other substrates (2), and have been implicated in the pathological processes that contribute to fibrotic diseases, tumor progression, and inflammation(1,3,4).

Dysregulation of gelatinase activity has also been implicated in the pathophysiology of diabetes complications. Specifically, gelatinase concentrations are increased in the systemic circulation (MMP-9 [5]) and in the vitreous (MMP-2 [6] and MMP-9 [7]) of type 1 diabetic patients with diabetic retinopathy. Elevated retinal levels of MMP-2 and MMP-9 have also been demonstrated in an animal model of diabetic retinopathy (8). Increased circulating concentrations of MMP-2 have been observed in pediatric patients with type 1 diabetes who developed microangiopathy over a 5-year interval (9). Systemic concentrations of MMP-2 and MMP-9, in addition to gelatinase activity levels, are also increased in patients with type 2 diabetes and peripheral arterial disease (10).

Data suggesting a link between MMP-2 dysregulation and diabetic nephropathy also exist but appear contradictory. Rodent models of diabetes reveal decreased expression and/or proteolytic activity of MMP-2 in renal tissues (11–13). High glucose culture conditions also decrease MMP-2 secretion by mesangial cells in vitro (14). Human studies, however, yield other results. Expression of membrane-type 5 matrix metalloproteinase, a protease that functions to convert pro–MMP-2 to its active form, is upregulated in human diabetic kidney tissue samples, localized to renal tubules (15). In addition, MMP-2 protein and MMP-2 enzyme activity are elevated in the protein extracts from these kidney tissue samples (15).

In our study, we investigated whether MMP-2 dysregulation could be implicated in the pathogenesis of diabetes complications, with focused attention on diabetic nephropathy, by measuring concentrations of MMP-2 in the plasma and urine of a large cohort of patients with type 1 diabetes and by examining correlations between observed differences in MMP-2 concentrations and various indexes of renal function and glycemic control.

RESEARCH DESIGN AND METHODS

— Subjects with type 1 diabetes and age-matched healthy control subjects, aged 14–40 years, were recruited from clinics at the University of Arkansas for Medical Sciences (UAMS) or Arkansas Children's Hospital and surrounding communities. Approval was obtained from the Institutional Review Board of UAMS. Exclusion criteria included *1*) concurrent use of medications that alter MMP activity (i.e., tetracycline or glucocorticoids), *2*) type 2

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Abbreviations: CGMS, continuous glucose monitoring system; CrCl, creatinine clearance; FPG, fasting plasma glucose; GFR, glomerular filtration rate; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; UAE, urinary albumin excretion; UAMS, University of Arkansas for Medical Sciences.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

diabetes, *3*) a history of other chronic systemic inflammatory or autoimmune disease or malignancy, *4*) pregnancy, and *5*) concurrent ketonuria. Subjects were also excluded if the baseline evaluation revealed any site of active infection. Control subject data were excluded from final analyses if a subject was incidentally found to have albuminuria.

Two study visits were conducted 3–5 days apart. Visit 1 included *1*) a medical history and physical examination, *2*) ascertainment of demographic data (age, sex, race, and duration of type 1 diabetes), *3*) fasting venipuncture laboratory measurements of plasma glucose (FPG), A1C, C-peptide, and serum creatinine, and *4*) insertion of a continuous glucose monitor sensor (Minimed CGMS, MMT-7102; Medtronic, Northridge, CA). At visit 2, 24-h urine samples collected between visits 1 and 2 were returned, 3- to 5-day CGMS data were downloaded, and FPG, A1C, C-peptide, and serum creatinine measurements were repeated. Urine collections were used for measurement of a timed microalbumin excretion rate. Estimated creatinine clearance (CrCl) and glomerular filtration rate (GFR) (using the Cockcroft-Gault and Modification of Diet in Renal Disease study equations, respectively [16]) were also calculated.

Clinical assays

FPG and C-peptide (using an immunochemiluminesence assay; normal range, 0.4–3.3 ng/ml for subjects aged 10–16 years and 0.9–4.0 ng/ml for subjects aged >16 years) were measured by the UAMS General Clinical Research Center Core Laboratory. A1C, serum creatinine, complete urinalysis (visit 1), and urinary albumin and creatinine concentrations (24-h urine collection, visit 2) were measured by LabCorp (Dallas, TX).

MMP-2 and tissue inhibitors of metalloproteinase 1 and 2 measurements

MMP-2 concentrations were measured in plasma (5 μl, diluted 1:10) and in timed urine collections (50 μl, undiluted) using the Fluorokine MultiAnalyte Profiling assay from R&D Systems (Minneapolis, MN). Specimens were analyzed in duplicate on a Luminex 100 Bioanalyzer (Luminex, Austin, TX) as previously described (minimal detection limit, 25.4 pg/ml) (17,18).

Plasma MMP-2 activity was measured using the Matrix Metalloproteinase-2 Biotrak Activity Assay System (GE Healthcare, Piscataway, NJ). This assay has a range of 0.75–12 ng/ml and sensitivity of 0.19 ng/ml. This assay has not been validated for use in urine; although the assay of plasma samples yielded informative results, results from all but six urine samples were <0.19 ng/ml.

Tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 concentrations in plasma were measured using the Quantitative Human TIMP-1 and TIMP-2 immunoassays from R&D Systems. Minimal detectible concentrations were 0.08 ng/ml for TIMP-1 and 0.01 ng/ml for TIMP-2.

Statistical analysis

A sample size target of 50 subjects/group would provide ~80% power to detect a between-group difference for MMP-2 of 0.5 SD. This power is further increased by a larger type 1 diabetic cohort. Results for plasma MMP-2, FPG, A1C, C-peptide, and serum creatinine obtained from visits 1 and 2 were averaged. Exploratory data analyses (summary statistics, scatter plots, and box plots) were used to examine the distribution of and relationship between variables. Because variables were not normally distributed, nonparametric statistical analyses (Mann-Whitney tests) were used. Data are presented as median values with a minimum to maximum range and as means ± SEM or ± SD, as indicated. Statistical significance was defined as $P < 0.05$. We also used classification tree analysis to find "cut point" levels for MMP-2 concentrations, which best indicated those with type 1 diabetes (19,20). For relative risk analyses, odds ratios and 95% CIs are reported.

RESULTS

Baseline characteristics

Fifty control subjects and 93 subjects with type 1 diabetes were evaluated. Control and type 1 diabetic groups were comparable with respect to sex (52 vs. 47% female, respectively); racial distribution (84 vs. 91% Caucasian), and baseline BMI (25.2 ± 4.8 vs. 24.8 ± 4.4 kg/m^2). The diabetic subgroup was slightly younger (control 24.1 ± 6.8 years vs. type 1 diabetic 19.3 ± 6.3 years; $P < 0.001$). Therefore, additional analyses, as detailed below (see RELATIONSHIP OF MMP-2 TO AGE) were conducted to examine any potential effect of age on MMP-2 results.

Expected differences between the control and type 1 diabetic subgroups were confirmed by baseline measurements of FPG (81.2 ± 6.5 vs. 155.3 ± 65.1 mg/dl, respectively; $P < 0.001$), A1C (4.97 ± 0.3 vs. 8.49 ± 1.85%; $P < 0.001$), C-peptide (0.84 ± 0.49 vs. 0.14 ± 0.14 ng/ml; $P < 0.001$), and 3- to 5-day average glucose by the CGMS (88.0 ± 10.2 vs. 170.5 ± 47.7 mg/dl; $P < 0.001$). Of the type 1 diabetic subjects, 59% were being treated with insulin injections, and 41% were using insulin pumps.

A history of hypertension ($n = 4$), retinopathy ($n = 5$), nephropathy ($n = 3$), or neuropathy ($n = 0$) was ascertained by self-report. During the baseline physical examination and as a result of the 24-h urine collection, an additional three subjects demonstrated evidence of sensory neuropathy, and nine subjects were identified as having microalbuminuria. (Dilated funduscopic examination was not a component of the protocol.) Among the type 1 diabetic subjects, the median urinary albumin excretion (UAE) was 12.2 mg/g creatinine and microalbuminuria (30–299 mg/g creatinine) was present in 12 subjects (microalbuminuria range 30.4–280.3 mg/g creatinine). No subjects in this study displayed macroalbuminuria (≥300 mg/g creatinine).

MMP-2, TIMP-1, and TIMP-2 concentrations in biological fluids

MMP-2 concentrations were significantly elevated in the urine and plasma of type 1 diabetic subjects compared with control subjects. The increase in urine MMP-2 was apparent whether analyzed as *1*) an undiluted urine concentration (control 48.4 ± 10.2 [mean ± SEM] vs. type 1 diabetic 184.9 ± 31.3; $P < 0.001$), *2*) a urine MMP-2–to–urine creatinine ratio (Table 1), or *3*) total MMP-2 excretion per day for the 135 of 143 subjects who provided a complete 24-h urine sample (Table 1). When all study subjects were analyzed together, a weak correlation between plasma MMP-2 and urine MMP-2–to–creatinine ratios was seen (Table 2). Similar to MMP-2 protein concentrations, MMP-2 activity was also significantly increased in the plasma of type 1 diabetic subjects (control 193.3 ± 163.0 vs. type 1 diabetic 292.7 ± 190.2 ng/ml; $P < 0.005$). Moreover, MMP-2 activity in plasma was correlated both with plasma MMP-2 concentrations ($R = 0.453$, $P < 0.001$) and urine MMP-2–to–creatinine ratios ($R = 0.331$, $P < 0.001$). No sex differences were seen for urine or plasma MMP-2 values.

The activity of MMPs is tightly regulated by a family of TIMPs (TIMPs 1–4);

MMP-2 preferentially binds TIMP-2, whereas MMP-9 preferentially binds TIMP-1. To determine whether the increase in plasma MMP-2 concentrations was offset by a simultaneous upregulation of inhibitor, plasma concentrations of TIMP-1 and TIMP-2 were measured. Neither TIMP-1 nor TIMP-2 differed between the type 1 diabetic and control groups (TIMP-1 88.7 ± 30.2 [mean ± SEM] vs. 93.2 ± 29.0 ng/ml, respectively; TIMP-2 66.2 ± 25.2 vs. 66.5 ± 19.5 ng/ml, respectively).

Relationship of MMP-2 to age

When age was examined as a continuous variable, values for the urine MMP-2–to–creatinine ratio and total MMP-2/day, but not plasma MMP-2, were very weakly inversely correlated with age (MMP-2–to–creatinine ratio $R = -0.181$, $P < 0.05$; total MMP-2/day $R = -0.202$, $P < 0.05$). However, when MMP-2 values were compared in subjects who were aged ≤18 years as a group ($n = 64$) with those who were aged 19–40 years ($n = 79$), plasma MMP-2 concentrations were significantly higher among the younger cohort, whereas the urine MMP-2–to–creatinine ratio and total MMP-2/day displayed a trend toward higher values in younger subjects but did not reach statistical significance. To exclude the possibility that the younger mean age of the type 1 diabetic subgroup alone accounted for the increase in MMP-2 values seen in type 1 diabetes, comparisons were made between the subset of type 1 diabetic subjects and control subjects who were aged 19–40 years (adult subgroup: control, $n = 42$; age [mean ± SEM] 25.8 ± 0.9 years; type 1 diabetic, $n = 37$; age 24.8 ± 1.1 years; between-group age comparison, $P = 0.3$). A similar comparison was made between the subset of type 1 diabetic subjects and control subjects who were aged ≤18 years (adolescent subgroup: control, $n = 8$, age 15.5 ± 0.3 years; type 1 diabetic, $n = 56$, age 15.6 ± 0.2 years). Consistent with differences reported for the entire study population, for subjects aged 19–40 years, urine concentrations of MMP-2, the urine MMP-2–to–creatinine ratio, and total MMP-2/day were significantly higher among subjects with type 1 diabetes (Table 1). For subjects aged 14–18 years, a trend toward higher values in the type 1 diabetic subjects was also evident, although these differences did not attain statistical significance. Plasma MMP-2 concentrations were significantly higher in type 1 diabetic subjects compared with control subjects for the entire study population and for the adolescent subgroup (Table 1).

Relationship of MMP-2 to duration of disease

Among subjects with type 1 diabetes, values for the urine MMP-2–to–creatinine ratio and total MMP-2/day were elevated in subjects with a duration of disease of >3 years ($n = 70$), compared with those with a ≤3 year history of type 1 diabetes ($n = 23$). Specifically, for subjects with duration of diabetes of >3 vs. ≤3 years, median MMP-2–to–creatinine ratio concentrations were 113.4 vs. 29.8 pg/g, respectively ($P < 0.05$), and median total MMP-2/day values were 151,766 vs. 45,466 pg/day ($P < 0.05$). This difference was seen despite the fact that the mean age of type 1 diabetic subjects with a longer duration of disease was slightly older (20.2 ± 0.8 years) than the mean age of type 1 diabetic subjects with a ≤3 year history of type 1 diabetes (16.7 ± 0.6 years). In contrast, plasma MMP-2 concentrations were not different between those subjects with duration of type 1 diabetes of >3 vs. ≤3 years.

Relationship of MMP-2 to glycemic control

Values for the urine MMP-2–to–creatinine ratio and total MMP-2/day were correlated positively with A1C and CGMS average daily glucose and less strongly correlated with FPG (Table 2). Moreover, urine MMP-2–to–creatinine ratio and total MMP-2/day were significantly higher in type 1 diabetic subjects whose A1C was ≥8.25% vs. <8.25% ($P < 0.001$ for both urine values) and in subjects whose CGMS average daily glucose was ≥140 vs. < 140 mg/dl ($P < 0.001$ for both urine values). Urine MMP-2 relationships also differed as a function of sex; specifically, correlations with FPG, A1C, and CGMS average daily glucose were stronger in female subjects (Table 2). In contrast, no correlations between plasma MMP-2 concentrations and A1C, FPG, or CGMS average daily glucose were demonstrated. However, plasma MMP-2

Table 1—*MMP-2 concentrations*

	Urine MMP-2 concentrations						Plasma MMP-2 concentrations (pg/ml)		
	MMP-2–to–creatinine ratio (pg/g creatinine)			Total MMP-2/day (pg/day)					
	All	19–40 years	≤18 years	All	19–40 years	≤18 years	All	19–40 years	≤18 years
Control	54.1 ± 13.1	52.5 ± 9.3	53.2 ± 10.6	75,126 ± 10,437	70,077 ± 8573	101,004 ± 48,048	191,241 ± 14,470	196,215 ± 15,677	165,126 ± 38,578
Median (range)	25.6 (8.9–618.6)	31.4 (9.4–323.94)	47.1 (8.8–109.0)	53,340 (11,430–430,840)	53,340 (11,430–271,902)	60,265 (13,970–430,840)	158,792 (66,132–552,713)	167,138 (66,132–552,713)	116,688 (82,457–358,751)
n	49	41	8	49	41	8	50	42	8
Type 1 diabetes	242.2 ± 36.9	171.6 ± 28.0	260.0 ± 51.6	312,302 ± 45,828	284,088 ± 71,779	330,750 ± 59,936	273,986 ± 19,947	214,388 ± 18,681	313,363 ± 29,724
Median (range)	72.6 (13.24–1644.1)	147.5 (10.7–657.9)	76.6 (9.9–1746.9)	116, 805 (7,620–2,119,595)	97,104 (26,670–1,742,205)	122,883 (7,620–2,119,594)	229,650 (69,612–1,329,410)	195,910 (72,920–559,238)	267,426 (69,612–1,329,410)
n	93	37	56	86	34	52	93	37	56
P value	<0.001	<0.0001	NS	<0.001	<0.0001	NS	<0.005	NS	<0.05
Total *n*	142	78	64	135	75	60	143	79	64

Data are means ± SEM unless otherwise indicated.

Table 2—*Plasma and urine MMP-2 concentrations: correlation with glycemic control and renal function*

	Plasma MMP-2 (pg/ml)	Urine MMP-2 (pg/ml)	Urine total MMP-2 (pg/ml)	FPG (mg/dl)	A1C (%)	CGMS (mg/dl)	GFR (ml/min per 1.73 m^2)	CrCl (ml/min)	UAE (mg/g)
Urine MMP-2–to–creatinine ratio									
All (*n*)	142	142	135	141	142	138	142	142	137
R	0.190	0.781	0.677	0.264	0.540	0.421	0.453	0.315	0.555
P value	0.024	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
Type 1 diabetes (*n*)		93	86		93	89	93	93	88
R		0.767	0.645		0.500	0.296	0.404	0.301	0.532
P value	NS	<0.001	<0.001	NS	<0.001	0.005	<0.001	0.003	<0.001
Control (*n*)		49	49	49		49			49
R		0.654	0.368	0.301		0.289			0.383
P value	NS	<0.001	0.009	0.036	NS	0.044	NS	NS	0.007
Female (*n*)		69	68	69	69	69	69	69	68
R		0.722	0.729	0.450	0.630	0.537	0.562	0.467	0.674
P value	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Male (*n*)	73	73	67		73	69	73		69
R	0.354	0.883	0.792		0.423	0.272	0.349		0.326
P value	0.002	<0.001	<0.001	NS	<0.001	0.024	0.002	NS	0.006

NS, not significant at $P < 0.05$.

activity was weakly correlated with A1C ($R = 0.249$, $P = 0.004$) and CGMS average daily glucose ($R = 0.318$, $P < 0.001$).

Relationship of MMP-2 to renal function

For subjects with type 1 diabetes, values for the urine MMP-2–to–creatinine ratio and total MMP-2/day were correlated positively with UAE, GFR, and CrCl (Table 2). Again, these relationships were strongest among women. Similar correlations with UAE, GFR, or CrCl were not demonstrated for plasma MMP-2 concentrations, although weak correlations were demonstrated between MMP-2 activity in plasma and UAE ($R = 0.278$, $P = 0.001$) or GFR ($R = 0.278$, $P < 0.001$).

To examine the relationship between urinary concentrations of MMP-2 and predictors of diabetic nephropathy, values for the urine MMP-2–to–creatinine ratio and total MMP-2/day were compared in diabetic subjects with UAE of <30 mg/g creatinine ($n = 76$) and in those with microalbuminuria (≥30 mg/g creatinine; $n = 12$). A statistically significant increase was seen in the urine MMP-2–to–creatinine values (UAE <30 mg/g creatinine [mean ± SEM]: 169.9 ± 23.7 pg/g; UAE ≥30 mg/g creatinine: 622.4 ± 172.3 pg/g; $P = 0.02$) and in total MMP-2/day (UAE <30 mg/g creatinine: 252,892 ± 41,532 pg/ml; UAE ≥30 mg/g creatinine: 678,663 ± 177,584; $P = 0.003$). Urine MMP-2–to–creatinine ratios were also compared by subset for both control and type 1 diabetic subjects with UAE of *1*) <10 mg/g creatinine, *2*) 10–30 mg/g creatinine, or *3*) > 30 mg/g creatinine (type 1 diabetic subjects only, as dictated by study exclusion criteria for control subjects). MMP-2–to–creatinine ratios were as follows: *1*) UAE <10 mg/g creatinine: control 31.7 ± 7.7 vs. type 1 diabetic 108.5 ± 37.6 pg/g; $P = 0.01$; *2*) UAE 10–30 mg/g creatinine: control 81.6 ± 26.7 vs. type 1 diabetic 205.2 ± 31.1 pg/g; $P = 0.01$; and *3*) UAE >30 mg/g creatinine: type 1 diabetic 718.4 ± 171.8 pg/g; $P < 0.001$ for comparison with both control subsets. A progressive increase in the urine MMP-2–to–creatinine ratio concentrations was seen among type 1 diabetic subjects, with the increase in MMP-2 concentration noted even when the UAE was not yet clinically abnormal.

Urine MMP-2–to–creatinine ratios were further examined among the subset of type 1 diabetic subjects who demonstrated evidence of hyperfiltration. Urine MMP-2–to–creatinine ratios were higher ($P < 0.05$) in those subjects with type 1 diabetes and GFR >130 ml/min per 1.73 m^2 ($n = 25$; 405.4 ± 100.0 pg/g) compared with those with GFR ≤130 ml/min per 1.73 m^2 ($n = 68$; 158.4 ± 22.5 pg/g) or CrCl >130 ml/min (300.2 ± 63.8) compared with those with CrCl ≤130 ml/min (165.3 ± 29.7). Among those type 1 diabetic subjects with GFR >130 ml/min per 1.73 m^2, an increase in urine MMP-2-to-creatinine ratios was seen for those with microalbuminuria (UAE <30 mg/g creatinine: 229.7 ± 61.1pg/g; UAE ≥30 mg/g creatinine: 853.5 ± 218.3 pg/g; $P < 0.05$).

Multiple regression analysis

Tree analysis demonstrated that if the urine MMP-2–to–creatinine ratio was >65 pg/g or if the urine total MMP-2/day value was >66,720 pg/ml, markers for elevated risk, such as hyperglycemia, hyperfiltration, and microalbuminuria, could be established (Table 3).

CONCLUSIONS — We have demonstrated a marked increase in urinary excretion of MMP-2 in type 1 diabetic subjects compared with healthy control subjects. Moreover, urine MMP-2 concentrations were correlated with several known risk factors for diabetic comorbidity in general and diabetic nephropathy in particular, including elevated A1C, longer duration of type 1 diabetes, evidence of renal hyperfiltration, the presence of microalbuminuria, and female sex (21). We have also demonstrated a significant increase in plasma concentrations of MMP-2 and MMP-2 activity in type 1 diabetes. No concurrent increase in TIMP-1 or TIMP-2 concentrations was detected, confirming a specific increase in circulating gelatinase activity in type 1 diabetes.

A weak inverse correlation between urine MMP-2 concentrations and age was also noted, consistent with our previous report demonstrating that urinary MMP-2

Table 3—*Relative risk calculations*

Parameter*	A1C >7.5% or not	A1C >8.25% or not	CGMS average blood glucose >140 mg/dl or not	Duration of diabetes >3 years or not	GFR >130 ml/min per 1.73 m^2 or not	UAE >30 mg/g or not
A	4.62 (2.27–9.42)	4.97 (2.24–11.83)	6.43 (3.06–13.52)	2.49 (0.95–6.51)	4.55 (1.69–12.24)	NA
B	4.25 (2.02–8.95)	4.78 (1.99–11.46)	3.71 (1.79–7.69)	4.24 (1.51–11.94)	3.34 (1.15–9.67)	10.48 (1.31–83.84)
C	7.05 (3.28–15.14)	6.88 (3.09–15.31)	6.96 (3.22–15.08)	2.28 (0.85–6.12)	4.66 (1.84–11.80)	21.74 (2.71–174.09)

Data are odds ratios (95% CI). *A, urine MMP-2–to–creatinine ratio >65 pg/g; B, urine total MMP-2 >66,720 pg/day; C, urine MMP-2–to–creatinine ratio >65 pg/g and total MMP-2 >66,720 pg/day. NA, not analyzed. (No subjects with type 1 diabetes and UAE >30 mg/g had urine MMP-2–to–creatinine ratio values <65 pg/g.)

activity in healthy pubertal subjects is increased over values observed among adults (22). These findings highlight the necessity of using age-matched study cohorts for analysis of gelatinase dysregulation in type 1 diabetes.

Hyperglycemia-induced upregulation of MMP-2 has been demonstrated in the arterial vasculature in vivo (23) and in various vascular components in vitro, including endothelial cells (24), macrophages (24), and vascular smooth muscle cells (25). Therefore, the increase in plasma MMP-2 concentrations and MMP-2 activity in type 1 diabetes could be indicative of increased vascular synthesis of MMP-2 or could reflect the systemic transport of MMP-2, which is being overproduced in other tissues. Several possibilities exist to account for the increased urinary concentrations of MMP-2 including *1*) hyperfiltration of circulating MMP-2 due to increased glomerular basement membrane permeability, *2*) diabetes-induced changes in renal tubular handling of the MMP-2 filtered load, or *3*) increased production or secretion of MMP-2 by renal tissues in response to hyperglycemia.

It is notable that urinary concentrations of MMP-2 were correlated with higher A1C values, with higher average glucose values, and with duration of diabetes of >3 years. In addition, urine MMP-2 concentrations were highest in those subjects demonstrating renal hyperfiltration and/or microalbuminuria. Because these parameters are risk factors associated with the development and/or progression of diabetic nephropathy, in particular (26), one could speculate that urinary secretion of MMP-2 might reflect intrarenal MMP-2 dysregulation, contributing to the pathophysiology of nephropathy. This hypothesis could only be confirmed by longitudinal, histological data demonstrating tissue-specific dysregulation of MMP-2, which precedes clinical nephropathy. However, intrarenal MMP-2 expression or MMP-2 activity is increased in other examples of renal pathological conditions (27,28). In addition, MMP-2 and MMP-9 are produced by cultured glomerular podocytes, and podocyte MMP production in culture can be modified by numerous growth factors, by cytokines, and by high ambient glucose levels (29–31). Moreover, renal expression of MMP-2 is both absolutely necessary and sufficient for inducing the transformation of renal tubular epithelium to the myofibroblastic phenotype, a critical step heralding the development of renal interstitial fibrosis in conditions such as diabetic nephropathy (32,33). In keeping with this possibility is the fact that although the between-group comparisons of MMP-2 filtered load between control and type 1 diabetic subjects demonstrated an average ~2-fold increase in type 1 diabetes, urinary MMP-2 excretion in the type 1 diabetic group was >4-fold higher. Consequently, an increase in renal production of MMP-2 may contribute, in part, to the increase in urinary MMP-2 concentrations.

Certain limitations of our study must be acknowledged. This study does not establish a tissue source for the increase in plasma and urine MMP-2 concentrations nor does it establish a causal link between MMP dysregulation and the onset of renal lesions. In addition, any relationship of these results to other diabetes complications cannot be established, as the incidence of hypertension, retinopathy, and neuropathy were only ascertained by history and not by clinical documentation.

In summary, plasma and urine MMP-2 concentrations and plasma MMP-2 activity are elevated in type 1 diabetes. Because higher MMP-2 concentrations were associated with clinical parameters that are known to confer increased risk for diabetic comorbidity, including diabetic nephropathy, we raise the possibility that upregulation of MMP-2 activity might play a role in the pathogenesis of diabetes complications.

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