Insulin-Like Growth Factor Binding Proteins (IGFBPs) in Serum and Urine and IGFBP-2 Protease Activity in Patients With Insulin-Dependent Diabetes Mellitus

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Diabetes mellitus and glucose dysregulation have significant effects on the circulating level of insulin-like growth factor-I (IGF-I) and IGF binding proteins (IGFBPs). In the present study, serum and urine IGFBP (IGFBP-1, -2, and -3) and serum IGF-I and -II levels were measured by radioimmunoassay (RIA) in 27 patients with type 1 diabetes aged 9 to 48 years compared with 9 healthy subjects aged 10 to 28 years. The patients were divided into 3 groups according to the amount of albumin excreted in 24 hours. The macroalbuminuria group (>500 mg/24 h) had elevated serum IGFBP-1 and -2 and decreased IGF-I levels ($P < .01$ *v* normal controls). Serum IGFBP-3 and IGF-II were not different among the patient groups and controls ($P > .05$). The mean urinary IGFBP-1 was decreased in all 3 patient groups compared with the controls ($P < .05$). Urinary IGFBP-2 and IGFBP-3 were increased in patients with macroalbuminuria. Immunoblot analysis showed increased low–molecular-weight fragments of urinary IGFBP-2 in the poorly controlled diabetics, and direct evidence for increased urinary IGFBP-2 proteolytic activity could be demonstrated in both the microalbuminuric and macroalbuminuric groups. Low–molecular-weight fragments of urinary IGFBP-3 were also increased in both the microalbuminuric and macroalbuminuric groups. In conclusion, alterations of IGFBPs in urine and serum are related to metabolic control in diabetic patients, and there is an increase of urinary IGFBP-2 protease activity in poorly controlled diabetics. The changes in serum IGFBP concentrations (eg, increases in IGFBP-1 and IGFBP-2) may lead to alterations in the availability of IGF-I to peripheral tissues.

NEPHROPATHY is one of the major complications associated with diabetic angiopathy and is one of the leading causes of death among diabetic patients. Early indicators include an enlargement of the kidney, increase in the glomerular filtration rate (GFR), increase in renal plasma flow, and microalbuminuria, which may be related to a number of factors, including growth hormone (GH) and the insulin-like growth factors (IGF-I and -II).[1-3] IGF-I has been shown to bind to renal tissue in vitro, to increase glomerular mesangial cell proliferation, and to stimulate extracellular matrix accumulation. These changes are preceded by an increase in the renal content of IGF-I. It has been shown that human glomerular mesangial cells synthesize and secrete IGF-I and IGF binding proteins (IGFBPs),[4] and both may play a role in the pathogenesis of diabetic nephropathy. The human IGFBP family consists of at least 6 proteins whose structure has been determined. The IGFBPs are thought to play a significant role in the regulation of IGF-I and IGF-II activity. IGFBPs are present in human and rat serum,[5] amniotic fluid,[6] lymph,[7] preovulatory follicular fluid,[8] seminal plasma,[9] milk,[10] and normal urine.[11] Recently, it has been shown that IGFBP-1 and -5 mRNAs are expressed in renal tissue.[12,13] High levels of IGF binding activity in the glomerulus suggest that at least some of these IGFBP mRNAs are translated into proteins that are available to bind to IGFs. These findings suggest that a focal increase in IGFBPs may account for the accumulation of IGF-I, which then leads to altered kidney function. Diabetes-associated alterations in metabolism appear to affect the abundance of both IGF-I and IGFBPs. Recently, it was demonstrated that IGF-I and -II and IGFBP-3 levels are low and IGFBP-1 levels are high in serum from poorly controlled diabetics, and these components of the IGF system return to control levels with insulin therapy.[14,15]

Urinary IGFBP profiles have been shown to reflect changes in serum IGFBPs, and therefore have the potential to serve as diagnostic markers in disease states. Although the urinary excretion of IGFBPs has not been well characterized, concentrated urine from healthy subjects has a characteristic IGFBP pattern.[11] In acute and chronic renal failure, there is increased urinary IGFBP-1 and increased IGFBP-3 protease activity.[16] There is no correlation between urinary IGFBP-1 and serum creatinine.[16] Urinary IGFBP profiles and IGFBP protease activity have not yet been defined in diabetic patients.

One of the earliest indicators of diabetic renal disease is an increase in the urinary excretion of albumin.[17] Other proteins of low molecular weight, such as β2-microglobulin, may be elevated in the urine of diabetic patients with overt nephropathy and have been proposed as a reliable measure for the early detection of renal functional impairment.[18] However, the clinical value of IGFBP measurements in diabetic nephropathy has not been clarified. Therefore, IGFBPs represent another group of low–molecular-weight proteins whose secretion and clearance is altered in diabetic patients with renal disease, and a determination of the types of alteration has the potential to add to the understanding of the pathogenesis of diabetic nephropathy.

SUBJECTS AND METHODS

Patients

A total of 36 patients aged 9 to 48 years with insulin-dependent diabetes mellitus (IDDM) of 3 to 35 years' duration were recruited from the Diabetes Care Center at Texas Medical Center. Informed consent was obtained from all of the subjects. Patients were selected randomly. They were treated with insulin at a dosage of 0.5 to 1.5 U/kg/d. Upon enrollment, each subject collected two 24-hour urine samples at

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1-month intervals for measurement of albumin. When the two values were compared, there was no substantial difference in the albumin content. Therefore, an aliquot of the second urine sample was stored at −20°C for measurement of IGFBPs and protease activity. Urine glucose and ketone levels were measured in all urine samples on the same day. Blood samples were taken when the second urine sample was provided. All blood samples were collected after an overnight fast. Serum measurements of hemoglobin A_{1c} (HbA_{1c}), IGF-I, IGF-II, IGFBPs, blood urea nitrogen, creatinine, albumin, and globulin were obtained from the patients. A complete physical examination, blood pressure measurement, fundus examination, and pubertal staging were performed for each patient. The patient's usual insulin and diet regimens were not altered. IDDM patients with urinary tract infection, failure to thrive, or poorly controlled hypertension or those who were taking angiotensin-converting enzyme inhibitors, steroids, nephrotoxic agents, and diuretics were not included in the study. Three patients who were taking low doses of β-blockers were included.

IDDM patients were grouped as follows according to the level of albuminuria in 24 hours: (1) normoalbuminuria (albumin < 30 μg/24 h, n = 12), (2) microalbuminuria (albumin 30 to 300 μg/24 h, n = 9), and (3) macroalbuminuria (albumin > 300 μg/24 h, n = 6). Nine healthy individuals aged 10 to 28 years were selected as a control group.

Urine samples were prepared for analysis by electrophoresis as previously described by Hasegawa et al.[11] Briefly, 10 mL of each sample was dialyzed against 1 L deionized distilled water for 12 hours at 4°C. The procedure was repeated twice. A 1-mL aliquot of each sample was placed into 1.7-mL siliconized microfuge tubes and lyophilized to dryness. The samples were then reconstituted with 30 μL Laemmli buffer and analyzed on 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). After clotting at 4°C, 1.0 μL serum from the patients and normal controls was also analyzed by SDS-PAGE.

Radioimmunoassay

Serum IGF-I and IGF-II levels were measured by radioimmunoassay (RIA) as described previously.[19] The human IGFBP-1 concentration in the sera and urine of 36 diabetic patients and 9 controls was measured by RIA.[20] Urine samples were not concentrated prior to assay. Between 5 and 13 μL serum and 100 μL urine were used. The interassay and intraassay coefficient of variation (CV) was 8% and 10% for sera and 7% and 9% for urine, respectively. Human IGFBP-2 in the sera and urine of 36 diabetic patients and 9 controls was measured by RIA.[21] Serum (0.7 to 1.0 μL) or 20 μL urine was added. The interassay and intraassay CV was 6.8% and 10.5% for sera and 9.4% and 7.5% for urine, respectively. Human IGFBP-3 concentrations in the sera and urine of 36 diabetic patients and 9 controls were also measured by RIA.[22] The sera were diluted 1:10 in the assay buffer, and then 2.5 to 5.0 μL of this dilution was analyzed. Fifteen to 100 μL urine was assayed directly. The interassay and intraassay CV was 4% and 6% for sera and 5.4% and 8.6% for urine, respectively.

Western Ligand and Immunoblot Analysis

Western blots of serum and urine proteins were performed as described previously.[7,11] Ligand blotting of the Western blots was performed using ^{125}I-IGF-I prepared and iodinated as previously described.[19] Serum (1 to 2 μL) or 10 μL urine concentrate was electrophoresed on 12.5% SDS-PAGE under nonreducing conditions as described by Hasegawa et al.[11] The proteins were then transferred to nitrocellulose paper (0.45-μm pore size) using a semidry electroporetic transfer unit with Towbin buffer (15 mmol/L tris, 120 mmol/L glycine, and 20% methanol). For immunoblotting, Western blots were washed with Tris-buffered saline (TBS) containing 0.03% Triton X-100 and blocked with TBS containing 3% bovine serum albumin (BSA) for 30 minutes followed by incubation with the specific primary antiserum using a 1:1,000 dilution in TBS plus 1% BSA. The blots were rinsed extensively with TBS containing 0.03% Triton X-100. They were then incubated for 3 hours with a 1:5,000 dilution of a goat anti-rabbit immunoglobulin G–alkaline phosphatase conjugate in TBS plus 1% BSA. The filters were washed with TBS and blocked with TBS with 1% BSA; 1.0×10^6 cpm ^{125}I-IGF-I was added to 4.0 mL buffer and the filters were incubated overnight. The nitrocellulose was washed, dried, and placed in the cassette with film. The autoradiographic signal was visualized after 4 to 6 days' exposure time.

Proteolytic Activity

To test for the presence of IGFBP-2 protease activity, 2 to 4 μL (adjusted for total urine volume) unconcentrated urine or 2 μL serum from the samples were incubated alone or in combination with purified IGFBP-2 (50 ng) in 200 μL 0.05-mol/L Tris buffer containing 5 mmol/L calcium chloride, pH 7.4, for 24 hours at 37°C. After incubation, 45 μL was removed and mixed with 5 μL 10× Laemmli sample buffer. The samples were heated and the proteins were separated by 12.5% SDS-PAGE. Western ligand blots and immunoblots were then prepared as previously described.[23]

Statistical Analysis

The data are presented as the mean ± SD. Differences among the groups were tested with ANOVA. Correlations among IGFBP concentrations, age, IDDM duration, HbA_{1c}, and albuminuria were assessed by linear regression analysis.

RESULTS

Clinical characteristics of the study population are presented in Table 1. The patients with macroalbuminuria were older and had a longer duration of diabetes. HbA_{1c} was significantly elevated compared with the control level in all 3 groups, but did not differ among the diabetic groups. The insulin dosage did not differ between patient groups. The GFR was significantly reduced in the macroalbuminuric group.

Table 1. Clinical Characteristics of the IDDM Patient and Controls

Characteristic	Controls	Patients		
		Normoalbuminuric	Microalbuminuric	Macroalbuminuric
Age (yr)	15.5 ± 1.8 (10-20)	15.6 ± 1.2 (9-25)	19.2 ± 2.8 (12-35)	32.6 ± 5.9 (15-48)*†
Duration of diabetes (yr)	—	6.42 ± 1.2 (2-15)	10.4 ± 2.7 (5-28)	18.5 ± 5.1 (3-35)†
Insulin dosage (IU/kg/d)	—	1.3 ± 0.4 (0.9-1.6)	1.1 ± 0.01 (0.9-1.5)	1.3 ± 0.3 (1.1-1.8)
HbA_{1c} (%)	6 ± 0.1 (5.5-6.6)	11.08 ± 0.8 (5.9-15.6)*	13.4 ± 0.8 (9.7-16.9)*	14.2 ± 0.9 (11.6-16.6)*
GFR (mL/min/1.73 m^2)	125.4 ± 10.6 (100-167)	141.3 ± 21.2 (98-171)	122.5 ± 22.9 (79-163)	72.5 ± 12.5 (31-97)†
Urinary albumin excretion (mg/24 h)	11.6 ± 2.7 (3-27)	11.5 ± 2.2 (3-24)	61.3 ± 9.3 (32-112)*	425.6 ± 63.1 (320-712)*†

NOTE. Data are presented as the mean ± SD (range).

*$P < .05$ *v* control.

†$P < .05$ *v* normal and microalbuminuric groups.

The macroalbuminuric group showed a significant reduction of serum IGF-I compared with the control or normoalbuminuric subjects ($P < .05$; Table 2 and Fig 1A). Among the patient groups, there was no significant difference in IGF-II levels ($P > .05$; Fig 1B).

Serum IGFBP-1 levels were significantly elevated in the microalbuminuric and macroalbuminuric patients compared with the control group ($P < .05$ and $P < .01$, respectively; Table 2 and Fig 1C). For serum IGFBP-2, values tended to be higher in the normoalbuminuric and microalbuminuric groups versus the control nondiabetic group, but the difference was significant only in the macroalbuminuric group ($P < .01$; Fig 1D). Serum IGFBP-3 levels did not show significant differences between the patient groups and controls ($P > .05$; Fig 1E).

Urinary IGFBP-1 levels were progressively lower in the normoalbuminuric group versus the macroalbuminuric group, and values were significantly lower in the macroalbuminuric group versus the normoalbuminuric diabetic group ($P < .01$; Fig 1F). In the normoalbuminuric diabetic group, urinary IGFBP-2 was significantly lower than the value in the macroalbuminuric group ($P < .05$; Fig 1G). Urinary IGFBP-3 levels were significantly higher in the macroalbuminuric group versus the normoalbuminuric and control groups ($P < .05$; Fig 1H).

Possible relationships between serum IGFBP-1, -2, and -3 and the age, IDDM duration, HbA_{1c}, and GFR were evaluated for each patient group and the controls (Table 3). Urinary IGFBP-1 showed a negative correlation with the age and duration of diabetes for the microalbuminuric group but not the other groups. There was a significant positive correlation between the GFR and urinary IGFBP-1 excretion in both the normoalbuminuric and microalbuminuric groups. Serum IGFBP-2 was positively correlated with glycemic control (HbA_{1c}), age, and duration of diabetes for the macroalbuminuric and microalbuminuric groups. There was a strong inverse correlation between IGF-I and serum IGFBP-1 in the macroalbuminuric and control groups and between serum IGFBP-2 and IGF-I in the microalbuminuric and macroalbuminuric groups, respectively (Table 3). IGF-II levels in the normal and macroalbuminuric groups correlated with IGF-I. When the degree of albuminuria was compared by the urinary level of IGFBP-1, -2, and -3 in patients with microalbuminuria or macroalbuminuria, there was no significant correlation in either group.

Immunoblot analysis of urine from microalbuminuric and macroalbuminuric patients showed intact IGFBP-2 and 3 IGFBP-2 fragments with a M_r estimate of approximately 26, 22, and 19 kd (Fig 2A).The control subjects and 2 normoalbuminuric diabetics showed mostly intact IGFBP-2 and a minimal amount of fragments. Western ligand blot analysis of urine samples from microalbuminuric and macroalbuminuric patients showed that only the intact IGFBP-2 band (31 kd) bound IGF-I (Fig 2B). No clearly detectable IGFBP-2 fragment bands were present in serum from the normoalbuminuric and microalbuminuric groups (Fig 3). In contrast, IGFBP-2 fragment bands were easily detectable in serum samples from all 4 patients that were analyzed in the macroalbuminuric group.

To determine if the urine contained IGFBP-2 protease activity, urine samples from microalbuminuric and macroalbuminuric groups were analyzed (Fig 4). Analysis of the urine from all 3 groups showed that IGFBP-2 proteolytic activity was increased compared with the urine from control subjects. The size of the fragments generated by this protease in vitro was similar to those present in the urine of these patients. The protease activity was inhibited by phenylmethylsulfonyl fluoride (PMSF) but not by EDTA (Fig 5A and B). These results are consistent with the presence of a cation-independent serine protease. Immunoblot analysis showed that a major IGFBP-3 fragment with a M_r estimate of approximately 22 kd was present in urine samples from microalbuminuric and macroalbuminuric patients (Fig 6). In contrast, almost no IGFBP-3 (intact or fragment) was detectable in the normoalbuminuric group.

DISCUSSION

IGF-I, IGF-II, and IGFBP levels are reportedly altered in patients with IDDM. A variety of studies have shown that patients with poorly controlled diabetes have low serum IGF-I levels,[14,15,24,25] although other studies have reported normal[26] or elevated[27] levels. Serum IGF-I levels have been correlated with serum HbA_{1c} in some diabetic groups[28] but not others.[29,30] These differences between studies could be due to the different degrees of glycemic control, varying effects of pubertal stage, age, duration of diabetes, and variation in IGFBP concentrations. In our study, macroalbuminuric patients had low IGF-I when compared with microalbuminuric and normoalbuminuric patients, and there was a negative correlation between serum

Table 2. Serum and Urine IGFBP-1, -2, and -3 and Serum IGF-I and IGF-II in Patient and Control Groups

Parameter	Controls (n = 9)	Patients		
		Normoalbuminuric (n = 12)	Microalbuminuric (n = 9)	Macroalbuminuric (n = 6)
IGF-I (ng/mL)	508 ± 197 (264-789)	397 ± 192 (36.3-822)	345 ± 179 (89-602)	298 ± 203 (92-503)*
IGF-II (ng/mL)	856 ± 112 (702-1,042)	922 ± 283 (518-1,427)	878 ± 139 (730-1,189)	1,021 ± 329 (570-1,313)
S-IGFBP-1 (ng/mL)	27.3 ± 6.5 (25-41)	64.1 ± 38.2 (23-205)	82 ± 65 (24-398)†	185 ± 131 (23-428)*
S-IGFBP-2 (ng/mL)	145 ± 57 (88-269)	179 ± 84 (60-419)	199 ± 95 (124-374)	387 ± 185 (103-666)*‡
S-IGFBP-3 (ng/mL)	3,828 ± 529 (3,092-4,668)	3,549 ± 1,146 (1,424-4,704)	3,326 ± 646 (2,560-4,172)	2,853 ± 1,091 (1,220-3,992)
U-IGFBP-1 (μg/g Cr)	5.3 ± 3.3 (0.78-19.8)	5.8 ± 3.1 (1.86-13.9)	2.5 ± 1.4 (0.67-6.27)	1.87 ± 1.2 (0.87-3.58)‡
U-IGFBP-2 (μg/g Cr)	7.1 ± 6.7 (1.65-23.4)	5.6 ± 4.9 (0.57-33.5)	4.8 ± 4.1 (0.63-14.3)	12.5 ± 11.1 (0.37-29.2)‡
U-IGFBP-3 (μg/g Cr)	35.1 ± 24.6 (4.4-57)	36.7 ± 24.9 (5.7-170)	28.4 ± 20.7 (5.4-65)	46.5 ± 29.5 (21-72)†‡

NOTE. Data are presented as the mean ± SD (range).

Abbreviations: Cr, creatinine; S, serum; U, urine.

*$P < .01$ *v* control group.

†$P < .05$ *v* control group.

‡$P < .05$ *v* normoalbuminuric group.

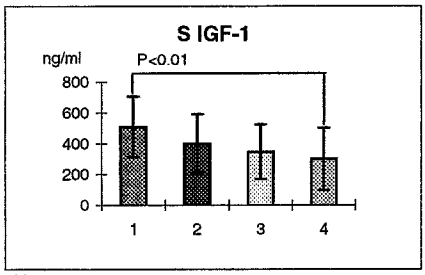


Figure 1a

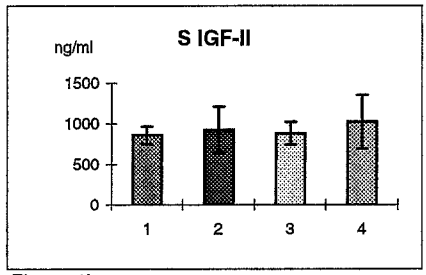
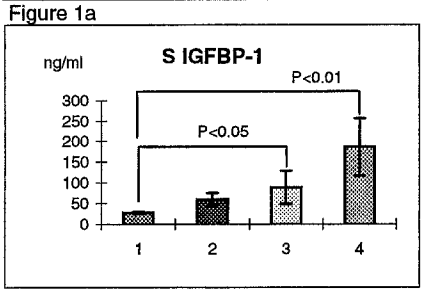


Figure 1b



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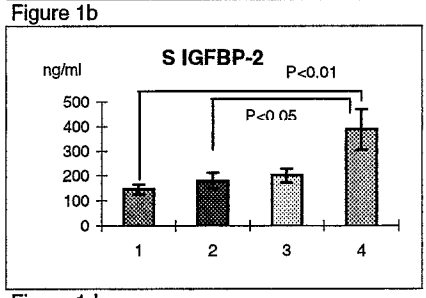

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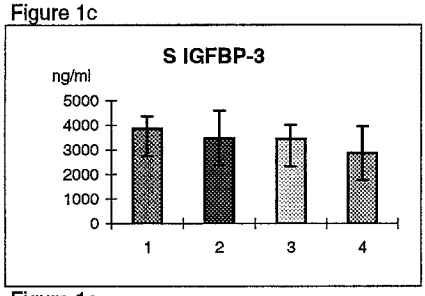

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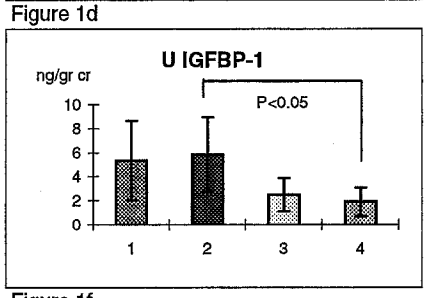

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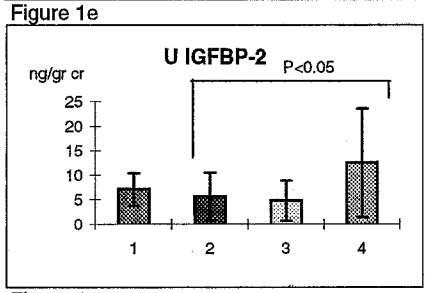

Figure 1g

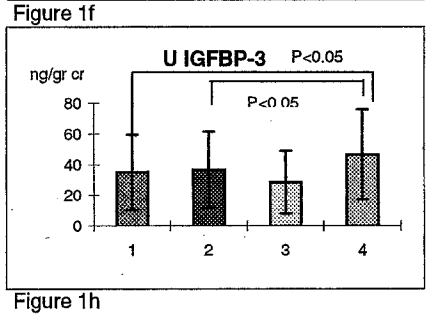

Figure 1h

Fig 1. Serum (S) IGF and IGFBP and urinary (U) IGFBP levels in control and diabetic subjects. Numbers on the x-axis denote the patient group: 1, control; 2, normoalbuminuric; 3, microalbuminuric; and 4, macroalbuminuric. Results are expressed as the mean ± SD.

Table 3. Correlations (*r*) Between Urine and Serum IGFs, IGFBP-1, -2, and -3, and Clinical Parameters as Evaluated by Regression Analysis

Variable	IGF-I	IGF-II	S-IGFBP-1	S-IGFBP-2	S-IGFBP-3	U-IGFBP-1	U-IGFBP-2	U-IGFBP-3
Age		$r = .81$ M†		$r = .63$ m† $r = .93$ M*		$r = -.84$ m*		
Duration		$r = -.86$ M*		$r = .80$ m* $r = .81$ M†		$r = -.75$ m†		
HbA_1C	$r = -.68$ M			$r = .86$ M* $r = .69$ m†			$r = .74$ m† $r = .74$ N	
GFR						$r = .75$ m† $r = .78$ N†		$r = .69$ m†
IGF-I			$r = -.74$ C† $r = -.90$ M*	$r = -.59$ N†	$r = .92$ M*		$r = -.84$ M*	
IGF-II	$r = .61$ N† $r = .90$ M*				$r = .96$ M*			$r = .73$ m†

NOTE. Only significant correlations are depicted.

Abbreviations: C, control; N, normoalbuminuric; m, microalbuminuric; M, macroalbuminuric.

*$P < .01$.

†$P < .05$.

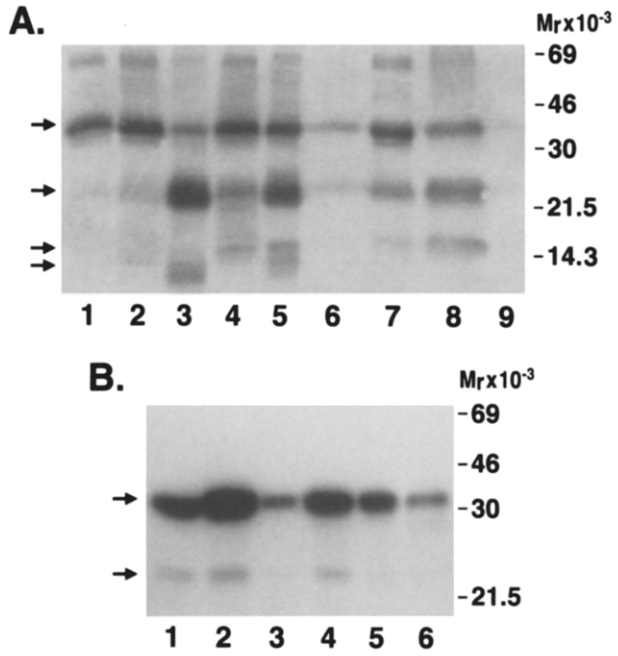

Fig 2. Immunoblot of IGFBP-2 in urine from diabetic patients and normal controls. Lane 1, positive control, 50 ng IGFBP-2; lane 2, normal control; lanes 3-5, macroalbuminuric group; lanes 7-8, microalbuminuric group; lanes 6 and 9, normoalbuminuric group. Top arrow represents intact IGFBP-2 at 34 kd. The 3 additional arrows represent IGFBP-2 fragments at 25, 22, and 19 kd. (B) Western ligand blot of urine from the 3 patient groups. Lanes 1-2, macroalbuminuric group; lanes 3-5, microalbuminuric group; lane 6, normoalbuminuric group. Arrows denote intact IGFBP-2 and the 22-kd fragment.

IGF-I and HbA_{1c}. In contrast, we did not find a significant correlation between serum HbA_{1c} or the amount of albuminuria and serum IGF-I in the other 2 patient groups. Based on previous studies, the low serum IGF-I levels detected in this group are probably due to a decreased synthesis of IGF-I resulting from poorly controlled diabetes, an increased urinary loss of serum IGFBP complexes, or both; however, an effect of age in this group cannot be totally excluded as a variable contributing to the lower levels. Janssen et al[29] reported that the age-related decline in IGF-I was not different in normal controls

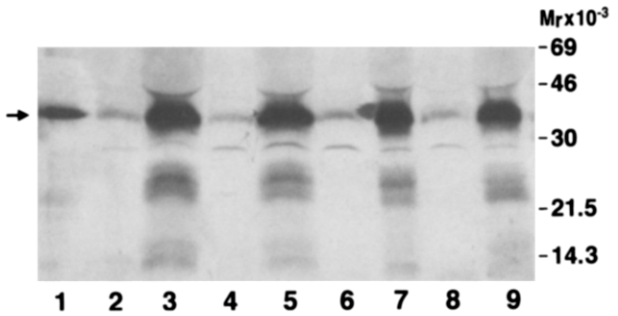

Fig 3. IGFBP-2 immunoblot of 2 μL serum from the patients. Lane 1, IGFBP-2 50 ng; lanes 2 and 8, normoalbuminuric group; lanes 3, 5, 7, and 9, macroalbuminuric group; lanes 4 and 6, microalbuminuric group. Arrow denotes the position of the intact protein. Four fragment bands were detectable in the serum from macroalbuminuric patients.

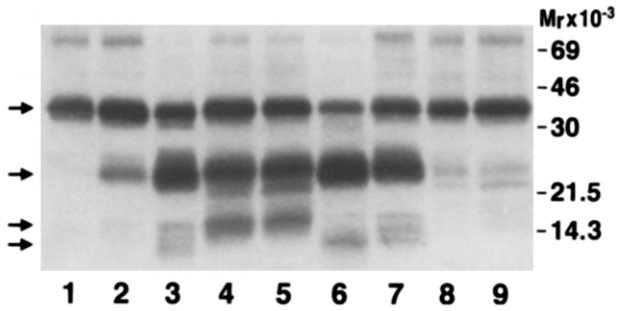

Fig 4. Western immunoblot showing degradation of IGFBP-2 by a urinary protease from patients and controls. IGFBP-2 (50 ng) was mixed with buffer alone (lane 1) or 2 μL urine from the patients (normoalbuminuric group, lane 2; microalbuminuric group, lanes 3-5; macroalbuminuric group, lanes 6 and 7) and normal controls (lanes 8 and 9). After a 14-hour incubation, the proteins were separated and then immunoblotted for IGFBP-2. Intact IGFBP-2 is detected at 31 kd (top arrow). Fragments with a *M*, estimate of 23, 17, and 14 kd were detected. Fragment bands were of much greater intensity in the microalbuminuric and macroalbuminuric patients.

and diabetics. Therefore, it seems likely that the age difference in this study is less important compared with other variables such as the duration and severity of diabetes and a reduced GFR.

In contrast to IGF-I, less information has been reported regarding IGF-II in diabetic patients. Normal IGF-II values have been reported in diabetic adults and adolescents.[31] In two studies in human diabetic subjects, as well as streptozotocin-induced diabetic rats, serum IGF-II was elevated.[32,33] In this study, serum IGF-II levels were not depressed or increased in any of the patient groups regardless of metabolic control. This suggests that urinary loss of serum IGFBP complexes is not the sole reason for low serum IGF-I in the macroalbuminuric group, since this also should have resulted in a low IGF-II.

IDDM is associated with increased serum IGFBP-1. IGFBP-1 levels are inversely related to serum insulin levels in healthy and diabetic subjects, and there is some dependence on glucose control.[15,25,34,35] Insulin suppresses circulating IGFBP-1 levels via inhibition of hepatic IGFBP-1 synthesis. In our subjects with microalbuminuria and macroalbuminuria, IGFBP-1 levels were significantly higher than the control levels. This is unlikely due to age alone, since IGFBP-1 values change minimally between ages 15 and 32, and there was a significant increase in IGFBP-1 in the microalbuminuric group, with a mean age of 19 years. In some studies, there has been a significant relationship between elevated IGFBP-1 levels and metabolic control in poorly controlled diabetics, eg, HbA_{1c} more than 9%.[14,25,29,36] Although we did not find any correlation between IGFBP-1 and HbA_{1c}, there was a strong inverse correlation between IGF-I and IGFBP-1 levels in the macroalbuminuric group. Our findings of a correlation between IGF-I and IGFBP-1 in this group are in accordance with the findings of Strasser-Vogel et al,[37] but others have reported no correlation.[38] The increase of IGFBP-1 is probably due to increased hepatic synthesis as a result of a decrease in insulin, although a decrease of IGF-I also could be a contributing factor.[39]

Experimental data in rats suggest that insulin may inhibit IGFBP-2 synthesis.[40] In the present study, serum IGFBP-2 was

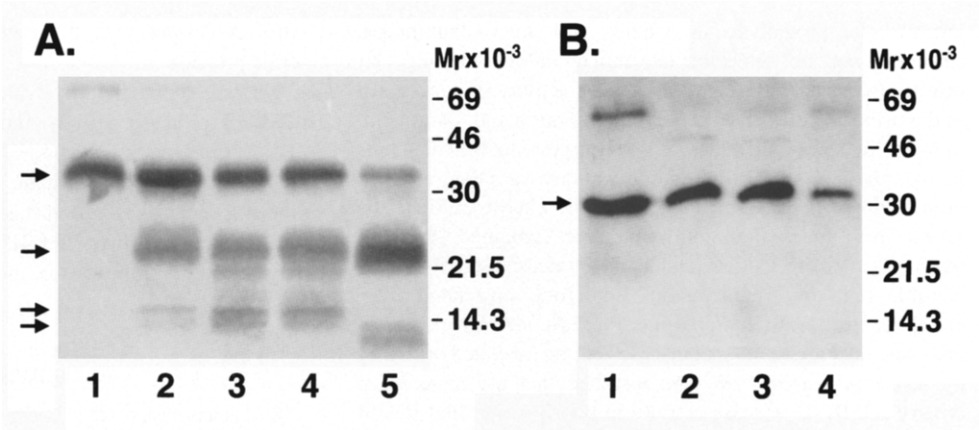

Fig 5. Effect of protease inhibitors on urinary IGFBP-2 protease activity. IGFBP-2 proteolysis was performed in the presence of (A) EDTA or (B) PMSF (10 mmol/L). The products of the reaction were detected by immunoblotting. IGFBP-2 protease activity was not inhibited by EDTA, but was inhibited by PMSF. (A) Lane 1, IGFBP-2 standard 50 ng; lanes 2 and 3, microalbuminuric group; lanes 4 and 5, macroalbuminuric group. (B) Lanes 1 and 2, microalbuminuric group; lanes 3 and 4, macroalbuminuric group.

increased in the macroalbuminuric group. It was positively correlated with the duration of diabetes and the HbA_{1c} level in microalbuminuric and macroalbuminuric patients. These findings suggest that this increase is due to poor glycemic control or insufficient insulin treatment.

In poorly controlled IDDM, IGFBP-3 levels tend to be low despite high levels of circulating GH.[41] Although we could not find any statistical differences in the plasma IGFBP-3 concentration, patients with macroalbuminuria had a tendency for reduced serum IGFBP-3 levels compared with the other patient groups. We found a strong positive correlation between IGFBP-3 and IGF-I and -II in the macroalbuminuric group, but this correlation did not exist in the other groups. Plasma IGFBP-3 and IGF-I levels often change coordinately in catabolic states such as IDDM or dietary protein restriction, and in some cases, IGFBP-3 protease activity is increased.[41] These appear to be the major factors that account for the reduction in IGFBP-3.

IGFBP-3 protease activity has been observed in sera from patients with poorly controlled diabetes, but urinary IGFBP-3 protease activity in diabetic patients has not been reported.[40] Recently, increased urinary IGFBP-3 protease activity has been reported in children with chronic renal failure and GH deficiency.[15,42] Hasegawa et al[11] found that the urine IGFBP profile in normal subjects showed mainly IGFBP-2, IGFBP-3, and a variable presence of other smaller proteins such as IGFBP-1

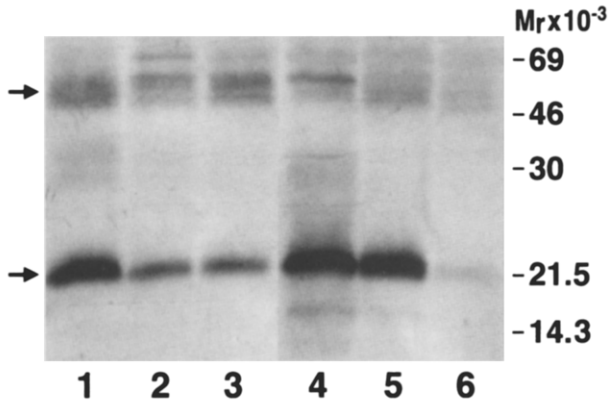

Fig 6. Western immunoblot of IGFBP-3 in urine samples from the microalbuminuric group (lanes 1-3), macroalbuminuric group (lanes 4 and 5), and normoalbuminuric group (lane 6). Most IGFBP-3 is present as a 23-kd fragment as shown by the arrow.

and IGFBP-4. Gargosky et al[42] demonstrated that IGFBP-3, not IGFBP-2, appears to be the dominant form of IGFBP in the urine of healthy subjects. Our study demonstrates that urinary IGFBP-3 was significantly increased in the macroalbuminuric group compared with the other diabetic groups. There was a correlation between individual patient values for urinary IGFBP-3 and albumin. The presence of proteinuria in this group undoubtedly contributed to the increased value. We also found that the 23-kd IGFBP-3 fragment band was more intense than the intact IGFBP-3 in urine samples from the microalbuminuric and macroalbuminuric patients. This suggests that there is an increase in the proteolysis of IGFBP-3 in the urine of albuminuric patients, and that the increase in the immunoreactive low–molecular-weight form of IGFBP-3 is due to either an increase of IGFBP-3 protease activity in plasma or an increase in the excretion of intact IGFBP-3 with subsequent proteolysis in urine.

We did not find any correlation between the urinary IGFBP-1 level and the age or duration of diabetes. Urinary IGFBP-1 was significantly decreased in the macroalbuminuric group, suggesting that a decreased GFR or impaired renal synthesis of IGFBP-1 causes this change. Recently, it has been shown that IGFBP-1 mRNA is abundant in the medullary area.[43] Abrass et al[44] demonstrated that cells cultured from rabbit collecting ducts secrete IGFBP-1. In addition, it was shown that IGFBP-1 expression is inversely related to plasma glucose in the diabetic rat kidney, and the highest binding activity was demonstrated in the kidney of moderately hyperglycemic rats.[45] In our study, normoalbuminuric patients and controls had high levels of urinary IGFBP-1 in the face of decreased serum IGFBP-1 levels, whereas in the microalbuminuric and macroalbuminuric groups, the converse was true. Therefore, since plasma IGFBP-1 is increased, it appears that a decrease in the renal production of IGFBP-1 may be responsible for decreased urinary IGFBP-1 in the poorly controlled groups.

Urinary IGFBP-2 has been much less well investigated than the other IGFBPs in diabetes. Low–molecular-weight proteins are normally ultrafiltered in the glomerulus at higher rates. In our study, the level of intact urinary IGFBP-2 was highest in the macroalbuminuric group, whereas serum IGFBP-2 was increased in microalbuminuric and macroalbuminuric patients. Low–molecular-weight fragments of IGFBP-2 were detected in the urine of macroalbuminuric and microalbuminuric patients. It is doubtful that these fragments originated as a result of

intravascular proteolysis in patients with microalbuminuria, since they were not detectable in serum by immunoblotting. Our results also show that IGFBP-2 protease activity was increased in the urine of patients with microalbuminuria and macroalbuminuria. Taken together, these results suggest that the fragments in the urine of microalbuminuric patients are the result of intrarenal or urinary IGFBP-2 proteolytic cleavage. Since the serum from macroalbuminuric patients contained IGFBP-2 fragments and the urine contained protease activity, it is possible that the fragments in this group originated from proteolysis in serum and/or urine. Protease activity in normal urine and most urine in renal disease is often mediated primarily by serine proteases.[16] We demonstrated that the increase of urinary IGFBP-2 protease activity in poorly controlled IDDM patients is completely inhibited by PMSF but not by EDTA in vitro, consistent with the presence of a cation-independent serine protease.

We have shown the first evidence for the presence of urinary IGFBP-2 protease activity and confirm the presence of IGFBP-3 fragments in patients with IDDM, and our data indicate that this increase in enzymatic activity may be related to poor glycemic control. If confirmed, these data suggest that the measurement of urinary IGFBP-2 or IGFBP-3 protease activity may be useful as an index of metabolic control in patients with IDDM.

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