Beta₂-microglobulinaemia: a sensitive index of diminishing renal function in diabetics

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Abstract

A sensitive single measure of diminishing renal function is of importance in attempts to modify the progression of diabetic nephropathy. In 12 insulin-dependent diabetics with proteinuria plasma concentrations of beta₂-microglobulin were found to correlate more closely than plasma creatinine concentrations or creatinine clearance with glomerular function as measured by clearance of ⁵¹Cr-EDTA. The plasma beta₂-microglobulin concentration was raised in all patients with diminished glomerular filtration rate (below 80 ml/min/1.73 m²). By contrast, in two of these patients plasma creatinine concentration was normal. Plasma beta₂-microglobulin concentrations were stable throughout the day and not affected by food intake, unlike plasma creatinine concentrations, which rose in the afternoon and evening and after a meat meal. Plasma beta₂-microglobulin concentrations were the same in venous and capillary blood, the capillary blood being readily self-collected. Concentrations of beta₂-microglobulin were stable for up to 24 hours when whole blood was stored at 4°C; adding aprotinin inhibited loss of beta₂-microglobulin for up to seven days.

The results of this study suggest, therefore, that measuring beta₂-microglobulin concentrations is a simple and accurate method of detecting minor degrees of renal impairment and monitoring the effects of treatment.

Introduction

Renal failure is a major cause of disability and death in insulin-dependent diabetics.¹ New approaches to treatment seek to prevent or delay the onset of diabetic nephropathy and to slow, arrest, or even reverse the course of established diabetic kidney disease. The glomerular filtration rate (GFR) measured at intervals, particularly after the onset of proteinuria, is often used to indicate the rate of decline in renal function. Precise techniques for accurately determining GFR are complex, often require the administration of radioactive substances, and use expensive equipment for measurement. Plasma creatinine concentration and endogenous creatinine clearance remain the most widely used estimates of GFR in clinical practice, but accuracy, performance, and interpretation are not without problems.² Creatinine is secreted by the renal tubule, its plasma concentration is dependent on muscle mass, and its production is diminished in patients with renal failure and affected by protein intake.³

Beta₂-Microglobulin is a low-molecular-weight protein (11 800 daltons) with a Stokes radius of 16 Å that constitutes part of the histocompatibility antigens on cell membranes and is freed into body fluids after dissociation from them during their normal turnover.⁴⁻⁶ It is released at constant rate in normal subjects^{7, 8}

and readily filters through the glomerular capillary wall, over 99.9% being reabsorbed and catabolised in the proximal tubules⁹⁻¹¹ with virtually no return of the filtered protein to the circulation. Plasma concentration of beta₂-microglobulin is therefore theoretically a highly suitable index of GFR and indeed has been used to assess renal function in patients with non-diabetic renal disease.^{12, 13}

We studied a group of insulin-dependent diabetics with proteinuria due to diabetic nephropathy, relating plasma beta₂-microglobulin concentrations to GFR measured by clearance of ⁵¹Cr-EDTA and comparing them with estimations of plasma creatinine concentrations, inverse creatinine concentrations (1/creatinine concentrations × 10³), and creatinine clearance. We also investigated the effect of time of day, food intake, and the feasibility of self-collection and storage of capillary blood samples for beta₂-microglobulin measurements.

Patients and methods

Table I shows the clinical features of the 12 insulin-dependent diabetics with proteinuria recruited without conscious selection from the Guy's Hospital diabetic clinic. A group of 12 normal subjects individually matched for age (range 28-62 years, mean 45.2) and sex (nine men, three women) served as controls. All 24 subjects gave fully informed consent to participation in the study, which was approved by the hospital ethical committee. All subjects were within 10% of their ideal body weight,¹⁴ had normal liver function tests, and were free of lymphoproliferative and autoimmune disease. Urine culture was sterile in all. Systolic and diastolic pressures were below 130 mm Hg and 90 mm Hg respectively in the controls, who had no history or clinical or biochemical evidence of renal disease.

The GFR as assessed in the diabetics by the technique of single-shot injection of ⁵¹Cr-EDTA^{15, 16} was used as the reference value in this study. A blood sample for assay of beta₂-microglobulin and creatinine concentrations was taken in the fasting state from each subject and the results compared with the GFR as assessed by clearance of ⁵¹Cr-EDTA. In the diabetics samples were also taken two-hourly for 24 hours. During this 24-hour period all urine was collected. On separate occasions a carbohydrate meal (bread and jam without butter; 50 g carbohydrate) and a protein meal (250 g boiled beef) were eaten by six controls and a fat meal (2.25 ml of double cream and 0.75 g carbohydrate/kg body weight) by three others.

Simultaneous venous and capillary blood samples were collected (the capillary samples by the subjects themselves) before and three hours after the start of each meal. The specially constructed, plastic capillary collecting tubes (Sarstedt Ltd, West Germany) were sealed and centrifuged and 20 µl of plasma separated. In four subjects four capillary samples were taken at the same time; one sample was assayed the same day and the others left for one, three, or seven days, either at room temperature or at 4°C, before separation and assay. In two subjects 1 ml of venous blood was added to tubes containing 100 µl of aprotinin, a proteinase inhibitor (10 000 kallikrein inactivating units/ml), and stored unspun at 4°C for one, three, or seven days.

Plasma beta₂-microglobulin concentrations were measured by radioimmunoassay (Phadebas, beta₂-micro test, Pharmacia Diagnostics AB, Uppsala, Sweden); creatinine concentrations were determined in plasma and urine by an autoanalyser technique (Technicon method no SE4-0011 FH4). The coefficients of variation for creatinine and beta₂-microglobulin were 2.6% and 6.2% respectively within assays and 3.0% and 9.9% between assays. Clinical proteinuria was screened for with the semi-quantitative strips Albustix, and albuminuria was measured by an accurate radioimmunoassay method.¹⁸

The statistical relation between clearance of ⁵¹Cr-EDTA and the other variables measured in the diabetics was evaluated by linear regression analysis. Although over the range of values with which we

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were concerned the relations between β_2 -microglobulin and creatinine concentrations, on the one hand, and GFR, on the other, did not deviate from linearity, over wider ranges of values the relation is hyperbolic for β_2 -microglobulin concentrations and asymptotic for creatinine concentrations.¹³ Log-conversion linearises the relations over the complete range of values,¹² and therefore linear regressions of β_2 -microglobulin and creatinine concentrations on GFR were also calculated by using double logarithmic scales. Paired *t* tests were used for statistical analysis. Data are presented as means $\pm$ SEM.

Results

All diabetics had been selected because they had albuminuria (0.3 to over 10 g/l); one patient (case 9) had a nephrotic syndrome. Urinary albumin excretion rates ranged from 105 to 3530 μ g/min (mean $\pm$ SEM 1293.8 $\pm$ 322.2 μ g/min). Table I shows estimates of GFR for each patient as assessed by clearance of ^{51}Cr -EDTA.

A strong negative correlation ($r = -0.96$; $p < 0.001$) was found between plasma β_2 -microglobulin concentrations and GFR in the 12 diabetics

FIG 2—Relation between plasma creatinine concentrations and clearance of ^{51}Cr -EDTA (GFR) in 12 diabetics with proteinuria ($r = -0.89$; $p < 0.001$).

Conversion: SI to traditional units—Plasma creatinine: 1 μ mol/l $\approx$ 11.3 μ g/100 ml.

TABLE I—Clinical features of patients

Case No	Sex	Age (years)	Duration of diabetes (years)	Duration of proteinuria (years)	Other diabetic complications*	Blood pressure† (mm Hg)	Clearance of ^{51}Cr -EDTA (ml/min/1.73 m ²)
1	F	34	20	7	BR, AN, and PN	120/80	32
2	M	53	26	2	PR, PN, and AN	150/90	87
3	M	29	16	3	PR (blind) and AN	126/84	81
4	M	57	14	1	BR, PN, and AN	152/94	70
5	M	57	15	3	PR, PN, and AN	142/90	55
6	M	59	15	2	BR, PN, and AN	160/98	64
7	F	34	27	7	PR (blind), PN, and AN	114/72	23
8	M	55	14	1.5	BR and PN	142/94	48
9	F	48	22	2	PR, PN, and AN	128/80	45
10	M	33	19	4	BR and AN	124/76	68
11	M	30	17	9	PR	130/80	80
12	M	46	31	4.5	PR, PN, and AN	160/100	36.5

GFR = Glomerular filtration rate.

* BR = Background retinopathy. AN = Autonomic neuropathy. PN = Peripheral neuropathy. PR = Proliferative retinopathy.

† Mean of six estimates taken over 24 hours (sitting).

FIG 1—Relation between plasma β_2 -microglobulin concentrations and clearance of ^{51}Cr -EDTA (GFR) in 12 diabetics with proteinuria (●) ($r = -0.96$; $p < 0.001$). ▲ Plasma β_2 -microglobulin concentrations in 12 controls.

(fig 1). In all diabetics in whom the GFR was below 80 ml/min/1.73 m² plasma β_2 -microglobulin concentrations exceeded the highest concentration in the controls. The range of plasma β_2 -microglobulin concentrations in the 12 controls was 0.9–2.3 mg/l (mean $\pm$ SEM 1.5 $\pm$ 0.09 mg/l). Figure 2 shows that plasma creatinine concentrations also correlated highly with GFR in the 12 diabetics ($r = -0.89$, $p < 0.001$); but in two patients with GFRs of 64 and 70 ml/min/1.73 m² the plasma creatinine concentration (90 μ mol/l and 100 μ mol/l respectively (1.02 and 1.13 mg/100 ml)) was within the normal range, which in our laboratory is 30–110 μ mol/l (0.34–1.24 mg/100 ml). Concentrations in the 12 controls ranged between 71 and 110 μ mol/l (0.8 and 1.24 mg/100 ml) (mean $\pm$ SEM 89.9 $\pm$ 3.23 μ mol/l; 1.02 $\pm$ 0.04

mg/100 ml). The correlations between log plasma β_2 -microglobulin concentration and log plasma creatinine concentration on the one hand and log GFR on the other were $r = -0.94$ and $r = -0.88$ respectively. In the 12 diabetics the correlation coefficient between plasma β_2 -microglobulin and creatinine concentrations was 0.80 ($p < 0.01$). Inverse creatinine concentration showed a positive correlation with clearance of ^{51}Cr -EDTA ($r = 0.85$; $p < 0.001$), but creatinine clearance correlated less strongly with this ($r = 0.67$; $p < 0.05$).

FIG 3—Diurnal variation of plasma creatinine and β_2 -microglobulin concentrations in 12 diabetics with proteinuria. Arrows show times of meals. Significance of difference from fasting values: * $p < 0.02$; ** $p < 0.01$; *** $p < 0.005$.

Conversion: SI to traditional units—Plasma creatinine: 1 μ mol/l $\approx$ 1.3 μ g/100 ml.

The diabetics showed no diurnal variation in plasma β_2 -microglobulin concentration, whereas their plasma creatinine concentrations rose significantly above fasting values in the afternoon and evening (fig 3), returning to the lower values by next morning. Tables II and III give individual diurnal profiles for plasma β_2 -microglobulin and creatinine concentrations. In the controls plasma β_2 -microglobulin concentrations were not affected by the carbohydrate meal (1.3 ± 0.13 mg/l v fasting value 1.2 ± 0.06 mg/l), protein meal (1.2 ± 0.05 mg/l v fasting value 1.2 ± 0.06 mg/l), or fat meal (1.3 ± 0.17 mg/l v fasting value 1.3 ± 0.14 mg/l), but creatinine concentrations increased

Discussion

In this study of diabetics with proteinuria the plasma β_2 -microglobulin concentration indicated accurately and sensitively the degree of loss of renal function as measured independently by clearance of ^{51}Cr -EDTA. Concentrations of β_2 -microglobulin correlated more closely with GFR than did plasma creatinine concentrations. β_2 -Microglobulin concentrations were always above the normal range when the GFR was below

TABLE II—Individual diurnal profiles of plasma β_2 -microglobulin concentrations (mg/l) in 12 diabetics with proteinuria

Case No	Time											
	0800	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600
1	5.4	5.4	5.3	5.4	5.3	5.4	5.3	5.6	5.5	5.4	5.3	5.6
2	2.0	2.0	1.9	2.1	2.0	2.0	2.0	2.1	2.1	2.0	2.1	2.1
3	2.3	2.3	2.2	2.4	2.4	2.3	2.4	2.3	2.4	2.2	2.4	2.3
4	2.8	2.8	2.6	2.8	2.8	2.6	2.5	2.7	2.8	2.7	2.6	2.8
5	4.0	4.0	4.1	4.1	4.2	4.3	4.2	4.4	4.2	4.3	4.0	4.0
6	3.7	3.8	3.7	3.7	3.8	3.8	3.7	3.7	3.8	3.7	3.7	3.8
7	7.2	7.4	7.5	7.0	7.5	7.4	7.2	7.0	7.0	7.0	7.1	7.1
8	4.7	4.8	5.0	4.7	4.7	4.5	4.8	4.8	5.1	4.7	4.7	4.7
9	5.2	5.2	5.2	5.2	5.3	5.2	5.3	5.4	5.2	5.3	5.3	5.2
10	3.3	3.3	3.2	3.2	3.1	3.2	3.2	3.3	3.3	3.2	3.2	3.2
11	2.3	2.3	2.3	2.2	2.3	2.3	2.4	2.4	2.3	2.3	2.3	2.3
12	4.4	4.4	4.4	4.4	4.5	4.4	4.5	4.5	4.6	4.4	4.4	4.3
Mean $\pm$ SEM	3.95 $\pm$ 0.44	3.97 $\pm$ 0.46	3.95 $\pm$ 0.47	3.93 $\pm$ 0.43	3.99 $\pm$ 0.46	3.95 $\pm$ 0.45	3.96 $\pm$ 0.45	4.02 $\pm$ 0.44	4.02 $\pm$ 0.44	3.93 $\pm$ 0.44	3.93 $\pm$ 0.44	3.95 $\pm$ 0.44

TABLE III—Individual diurnal profiles of plasma creatinine concentrations ($\mu\text{mol/l}$) in 12 diabetics with proteinuria

Case No	Time											
	0800	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600
1	209	231	223	231	231	231	234	238	234	237	240	231
2	106	106	101	106	106	108	111	111	106	101	101	104
3	105	115	110	118	142	121	114	127	118	115	113	110
4	100	113	123	123	125	127	113	125	118	126	103	113
5	135	161	173	182	157	164	178	155	160	153	142	132
6	90	98	97	107	97	105	105	100	100	99	98	92
7	210	213	200	240	200	271	251	215	248	240	240	210
8	143	163	143	158	175	145	165	179	156	158	158	148
9	156	166	156	180	186	184	177	147	141	141	152	152
10	150	161	157	156	161	152	143	147	147	133	138	150
11	98	90	94	100	98	103	99	100	98	92	80	90
12	214	144	182	200	206	192	234	225	229	144	180	
Mean $\pm$ SEM	143 $\pm$ 13.4	146.7 $\pm$ 12.9	146.6 $\pm$ 12.3	158.4 $\pm$ 14.1	157 $\pm$ 12.8	158.6 $\pm$ 15.3	160.3 $\pm$ 15.9	155.7 $\pm$ 14.0	154.6 $\pm$ 15.6	144.9 $\pm$ 14.0	144.8 $\pm$ 16.7	139.2 $\pm$ 13.9
Significance				p < 0.01	p < 0.02	p < 0.02	p < 0.005	p < 0.01	p < 0.02			

Conversion: SI to traditional units—Creatinine: 1 $\mu\text{mol/l} \approx 11.3 \mu\text{g/100 ml}$.

TABLE IV—Plasma creatinine concentrations ($\mu\text{mol/l}$) fasting and three hours after a protein meal (250 g boiled beef) in six controls

Case No	Creatinine concentration	
	Fasting	After protein meal
13	104	145
14	79	128
15	105	139
16	100	148
17	105	148
18	105	170
Mean $\pm$ SEM	99.7 $\pm$ 4.2	146.3 $\pm$ 5.6*

* Significance of difference from mean fasting value: p < 0.001.
Conversion: SI to traditional units—Creatinine: 1 $\mu\text{mol/l} \approx 11.3 \mu\text{g/100 ml}$.

significantly after the protein meal (table IV). No significant difference in plasma β_2 -microglobulin concentrations between venous blood (1.16 ± 0.07 mg/l) and capillary blood (1.15 ± 0.07 mg/l) was found in either fasting or postprandial samples. β_2 -microglobulin concentrations fell slowly in whole blood left in capillary tubes at room temperature. At 4°C concentrations were stable for up to 24 hours but declined slowly afterwards (table V). There were no losses in storage when aprotinin was added to the whole blood of two subjects.

80 ml/min/1.73 m², but plasma creatinine concentrations were sometimes within the normal range in patients with a GFR as low as 64 ml/min/1.73 m². The existence of this so-called blind area for plasma creatinine concentrations has also been recognised in other renal disease.¹³ The GFR may fall by half without a detectable change in plasma creatinine concentration, and normal creatinine concentrations may conceal gross renal dysfunction in the young and the very old.² The poor correlation between creatinine clearance and clearance of ^{51}Cr -EDTA highlights the formidable problems associated with accurate urine collection,² even under supervision in hospital. Plasma β_2 -microglobulin concentration is stable and independent of time of day and food intake in both diabetics and normal subjects.¹⁹ By contrast, plasma creatinine concentration shows a clear diurnal variation in diabetics with proteinuria, and similar changes have been observed in normal subjects.²⁰ These variations are likely to be related to food intake, and we have shown, like others,³ that plasma creatinine concentration is affected by protein intake, a finding that undermines the supposed principal advantage of creatinine measurements.

The radioimmunoassay for β_2 -microglobulin requires only 10–20 μl of plasma, which is easily obtained from finger-prick blood taken into a capillary tube by the patient himself. Loss of plasma β_2 -microglobulin is probably due to proteolytic enzymes, which may be inhibited by adding to whole blood small quanti-

TABLE V—Effect of storage of whole blood at room temperature and 4°C on plasma β_2 -microglobulin concentration in four controls, and effect of adding aprotinin (10 000 kallikrein-inactivating units/ml) in two subjects

Sample No	Room temperature				4°C				4°C + aprotinin (10 000 KIU/ml)			
	Day 0	Day 1	Day 3	Day 7	Day 0	Day 1	Day 3	Day 7	Day 0	Day 1	Day 3	Day 7
1	1.23	1.04	1.15	0.75	1.23	1.17	1.12	1.03				
2	1.20	1.17	0.87	0.89	1.20	1.20	0.99	0.94				
3	1.39	1.14	1.07	0.78	1.39	1.37	1.31	1.18	1.30	1.24	1.23	1.30
4	1.10	0.96	0.88	0.78	1.10	1.06	0.94	0.96	1.21	1.25	1.24	1.24
Mean $\pm$ SEM	1.23 $\pm$ 0.06	1.08 $\pm$ 0.04	0.99 $\pm$ 0.07	0.80 $\pm$ 0.03	1.23 $\pm$ 0.06	1.20 $\pm$ 0.06	1.09 $\pm$ 0.08	1.02 $\pm$ 0.05				
Significance		p < 0.05	p < 0.05	p < 0.01		NS	p < 0.02	p < 0.005				

ties of aprotinin, which might be used to coat the capillary collection tubes.

An ideal endogenous substance for estimating GFR should be easily measurable, have a low molecular weight, pass freely through the glomerular capillary wall, have a constant rate of production, and be excreted or catabolised only by the kidney. β_2 -Microglobulin possesses most of these qualities. Its concentration differs slightly between the sexes, increases with age,²¹ and is raised in patients with liver²² and malignant disease^{23–26} and in immune disorders.^{27–28} These factors must be taken into account when drawing inferences about renal function. With these caveats raised plasma β_2 -microglobulin concentrations, in diabetic nephropathy as in other renal disease,^{12–13} reflect sensitively even minor decreases in filtration rate and provide an accurate assessment of moderate to severe renal dysfunction. Measured serially within individual patients β_2 -microglobulin seems likely to be of even greater value, though this remains to be established.

Increasing evidence supports the view that restoring normal blood glucose concentrations in animals with experimentally induced diabetes induces regression in their glomerulopathy,²⁹ and some evidence exists that optimal glycaemic control may reduce the incidence of nephropathy³⁰ and improve early functional abnormalities in man.³¹ A simple and accurate tool to detect minor degrees of renal impairment and monitor the effects of treatment is thus of great scientific and practical value. It should provide a useful adjunct to the existing, more commonly used methods. The cost of the reagent for estimating β_2 -microglobulin concentration is higher (70p per sample) than that for estimating serum creatinine concentration, but this disadvantage is offset by the better information given by the method, particularly in respect of increasing therapeutic optimism for earlier intervention in diabetic renal disease.

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