Urinary excretion of advanced glycation endproducts in patients with type 2 diabetes and various stages of proteinuria

N Turk¹, A Mornar², V Mrzljak², Z Turk³

SUMMARY
Objective: The objective of the study was to detect AGE-immunoreactive proteins in urine, and to evaluate AGE excretion at various stages of diabetic nephropathy in type 2 diabetes assessed by the level of proteinuria.

Methods: AGEs were measured in 24-h urine collection of patients with normoalbuminuria (N) (n = 22), microalbuminuria (Mi) (n = 31), macroalbuminuria (Ma) (n = 28), and overt proteinuria with elevated serum creatinine level (PC) (n = 26). A competitive ELISA with polyclonal anti-AGE antibodies was used to monitor AGE excretion.

Results: Multiple comparison of urine AGE content among various stages of proteinuria showed significant differences (summary p < 0.000). Fifty percent of samples from the group of normoalbuminuria, and only 15% of samples from the group of microalbuminuria patients were AGE negative. However, there was no significant difference in AGE excretion between the patients with persistent proteinuria and elevated serum creatinine, and those with macroalbuminuria (PC vs Ma, p = 0.265). None of the samples from these two groups of patients with highest AGE content in 24-h urine was negative for AGE-immunoreactivity. In addition, the ratio between 24-h urinary AGEs and urinary albumin excretion was calculated to determine whether total 24-h urinary AGE content is an index of the toxic form of albumin released in the course of diabetic nephropathy. The ratio values were log-transformed and bivariate comparison showed significant differences between the N vs Mi (p = 0.006) and Mi vs Ma (p = 0.000) groups. However, there was no significant difference (p = 0.407) between values in the Ma and PC groups of patients. Multiple stepwise regression analysis indicated a relationship of urinary AGE-immunoreactivity with creatinine clearance values (r = 0.52, p < 0.001).

Conclusion: The study demonstrated the presence of AGE-immunoreactivity in the urine of diabetic patients with various stages of proteinuria. Study results pointed to creatinine clearance as the principal predictor of AGE excretion. Therefore, the measurement of urinary AGE appears to offer limited extra information in patients with impaired renal function.

Key-words: Diabetic proteinuria - Advanced glycation endproduct - Renal function.

Diabetes Metab 2004,30,187-92

RÉSUMÉ

Excrétion urinaire de produits avancés de glycation chez des diabétiques de type 2 avec des niveaux variés de protéinurie


Patients et méthodes : Les AGEs ont été mesurés dans les urines de 24 h chez des patients avec normoalbuminurie (N) (n = 22), microalbuminurie (Mi) (n = 31), macroalbuminurie (Ma) (n = 28), et protéinurie avec élévation de la créatininémie (PC) (n = 25). Un dosage ELISA par compétition utilisant des anticorps anti-AGE polyclonaux a été utilisé pour montrer l’excrétion d’AGE.

Résultats : Les comparaisons multiples du contenu urinaire en AGE selon différents niveaux de protéinurie ont montré des différences significatives (résumé p < 0.000). Une absence d’AGE était notée dans 50 % des échantillons dans le groupe normoalbuminurique, et seulement 15 % de ceux du groupe macroalbuminurique. Cependant, il n’y avait pas de différence significative dans l’excrétion d’AGE entre les patients avec protéinurie persistence et créatininémie élevée, et ceux du groupe macroalbuminurique (PC vs Ma, p = 0,265). Aucun échantillon de ces deux groupes de patients avec le contenu en AGE le plus élevé dans les urines de 24 h n’était négatif pour l’immunoréactivité AGE. En outre, le ratio entre les AGEs urinaires de 24 h et l’excrétion urinaire d’albumine a été calculé pour déterminer si le contenu total en AGE des urines de 24 h est un index de la forme toxique d’albumine libérée au cours de la néphropathie diabétique. Les valeurs du ratio ont été log-transformées et une comparaison bivariée a montré des différences significatives entre les groupes N vs Mi (p = 0.006) et Mi vs Ma (p = 0.000). Cependant, il n’y avait pas de différence significative (p = 0.407) entre les valeurs des groupes Ma et PC. L’analyse en régression multiple a pas à pas a montré une relation de l’immunoréactivité AGE urinaire avec la clairance de la créatinine (r = 0,52, p < 0,001).

Conclusion : Cette étude démontre la présence d’une immunoréactivité AGE dans les urines de patients diabétiques avec différents niveaux de protéinurie. Les résultats montrent que la clairance de la créatinine est le principal prédicteur de l’excrétion d’AGE. Ainsi, la mesure des AGE urinaires apparaît offrir une information supplémentaire limitée chez les patients ayant une fonction rénale altérée.

Mots-clés : Protéinurie diabétique - Produits avancés de glycation - Fonction rénale.

1 Merkur University Hospital
2 School of Pharmacy & Biochemistry
3 Vuk Vrhovac University Clinic for Diabetes, Zagreb, Croatia.

Address correspondence and reprint requests to:
Z Turk. Vuk Vrhovac University Clinic for Diabetes, Dugi dol 4A, HR-10000 Zagreb, Croatia.
zturk @ idb.hr
Received: May 13rd, 2003; revised: January 14th, 2004

Chronic hyperglycemia is the primary etiologic factor in the pathogenesis of diabetic microvascular disease (nephropathy, retinopathy, and neuropathy) and accelerated atherosclerosis. Investigations of the mechanisms connecting hyperglycemia and long-term complications of diabetes emphasize the role of nonenzymatic glycation [1]. Glucose is known to promote the linkage of proteins to form advanced glycation endproducts (AGEs). In the glycation process, sugar is chemically bound to the protein free amino group through a sequence of chemical reactions described by the chemist Maillard. Glycated residues called Amadori products are formed first, and are thereafter transformed to stable covalent adducts collectively called AGEs, by a series of dehydration, cleavage and rearrangements [2]. Until recently, a high glucose concentration alone was considered to play a primary role in Maillard reactions to form AGE macromolecules in vivo. Recent data have shown that, although glucose is the main precursor of AGE compounds, many other intermediary metabolites, i.e. toxic aldehydes, also are actively involved in the formation of AGEs. Such intermediaries are generated during the course of glycolysis (methylglyoxal) or along the polyl pathway, or may even be produced by carbohydrate auto-oxidation (glyoxal) [3]. A considerable number of studies have demonstrated AGE accumulation in a variety of tissues, thus contributing to the development of long-term complications [1, 4].

Diabetic nephropathy is characterized by abnormal deposits of matrix material in the glomerular mesangium, leading to glomerulosclerosis. The accumulation of collagen-related proteins in the glomerular extravascular matrix causes progressive capillary occlusion. Immunohistochemical studies have shown that AGEs accumulate in both glomerular and tubular cells in experimental and human diabetic nephropathy [5-10]. AGEs also interact with specific receptors expressed on the surface of micro- and macrovascular cells, including renal mesangial cells. The finding suggests that the ligand-receptor interaction might be important in the genesis of diabetic nephropathy [4, 11].

Development of overt proteinuria and declining renal function are the hallmarks of diabetic nephropathy. However, before the onset of overt proteinuria, there are various functional changes including renal hyperfiltration and increasing permeability to macromolecules such as albumin. The kidney appears to be at least one of the key organs eliminating AGEs. Therefore, AGE kinetics might be very different between persons with normal renal function and patients suffering from renal failure. To date, there have been only a few studies monitoring urinary AGE excretion in human diabetes, and the results are controversial [9, 12-16]. In the present study, we evaluated the relationship between urinary AGE excretion in patients with various stages of proteinuria in correlation with other relevant renal parameters.

**Patients and methods**

**Patients**

Serum and urine samples were obtained on routine glycemia control from 106 (m/f = 55/51) type 2 diabetic patients (mean age 61.6 yrs; range, 33 to 88, median 66; and diabetes duration from 1 to 39 yrs, median 12 yrs). The patients were divided into 4 groups: normal renal function with normoalbuminuria ([N]≤ 30 mg/24 h) (n = 22), normal renal function with microalbuminuria ([Mi] 30-300 mg/24 h) (n = 31), normal renal function with macroalbuminuria ([Ma] ≥ 300 mg/24 h) (n = 28), and persistent proteinuria with elevated serum creatinine ([PC] creatinine > 130 µmol/l) (n = 25). Normal renal function was defined as serum creatinine < 130 µmol/l with creatinine clearance > 1.10 ml/s for male, and serum creatinine < 110 µmol/l with creatinine clearance > 0.80 ml/s for female subjects.

Diabetic retinopathy, diagnosed ophthalmoscopically by an ophthalmologist and graded according to the European protocol, was found in 56 of 106 patients. The diagnosis of diabetic neuropathy was made by neurologic examination according to international classification (subjective discomforts, clinical signs of disease, EMG) in 60.4% of study patients. A history of macrovascular disease (myocardial infarction, coronary artery disease or peripheral arterial disease) was present in 31% of study patients. Hypertension was confirmed in 91 of 106 patients who were treated with antihypertensive medication. Clinical characteristics of the study subjects are shown in Table I. Diabetic patients were treated with diet (n = 11), hypoglycemic drugs (n = 33) or insulin (n = 36), whereas 26 patients were on combined insulin/hypoglycemic agent therapy. The experimental procedure was designed and carried out in accordance with ethical standards for human subjects, as formulated in the Declaration of Helsinki.

Twenty healthy normoglycemic volunteers recruited from laboratory staff with normal creatinine clearance and serum creatinine served as controls.

**ELISA measurement of AGE-proteins**

Multiple rabbit immunization (New Zealand strain) with AGE rabbit albumin was used to prepare AGE specific antiserum, as previously described [17]. For the performance of a competitive ELISA with polyclonal antibodies, one half of immunoplate was coated by adding 0.1 ml AGE-human serum albumin, 0.5 µg/well in 0.05 mol/l carbonate buffer, pH 9.6, while the other half of the plate was coated with native human albumin and served for the detection of non-specific binding. Human serum diluted 1: 5 with PBS-Tween-20 or urine samples diluted from 1: 2 to 1: 20 were added to the wells, followed by 0.05 ml of anti-AGE antiserum (dilution 1: 2000). The plate was incubated at room temperature for 3 h and overnight at 4 °C. After rinsing, the plate incubation continued with 0.1 ml of secondary anti-
clear supernatant was diluted with malonic buffer and ana-
B is absorbance after the addition of competitor, and B0 is
standard in calibration curve and expressed relative to AGE-albumin
protein concentration of 100 mgEq/l to 3.12 mgEq/l. Competi-
sional double dilutions of AGE-HSA, corresponding to pro-
tion was stopped with 0.1 ml of 1 mol/l NaOH and optical
density was measured on an ELISA reader at 405/630 nm.

Biochemical variables
Routine chemical analyses were performed on an Olym-
pus UA 600 analyzer. Serum and urine creatinine concentra-
tions were determined by an enzyme assay. Urinary albumin
excretion rate was measured using an immunoturbidimetric
assay. Urinary total protein quantification was performed by Biuret method, applied to a concentrate obtained by mem-
brane filtration (Minicon-B-15, Amicon, USA). This
method has the advantage of equal sensitivity for each indi-
vidual protein in the sample.
To measure creatinine clearance, serum and 24-h urine
samples were collected. Creatinine clearance was calculated
from serum and urine creatinine concentrations and urine
volume.

Determination of hemoglobin A1c. Samples were prepared
according to Jeppsson et al. [18]: heparinized blood (600 µl)
was incubated with saline for 4 h at 37 °C, and centrifuged.
The supernatant was discarded and the erythrocyte pellet
was hemolyzed by the addition of H2O and CCl4. The sepa-
ration of cell ghosts was accomplished by centrifugation, and
clear supernatant was diluted with malonic buffer and ana-
lyzed. HbA1c was separated from other hemoglobins by cat-
ion exchange chromatography (fast protein liquid chroma-
tography system, Mono S HR 5/5 column at pH 5.7) and
expressed as percentage of the total amount of hemoglobin.

Statistical analysis
Data are expressed as mean ± SD unless stated otherwise.
The distribution of particular variable was tested for nor-
mality. Between-group comparisons were tested by Mann-
Whitney U test. Kruskal-Wallis ANOVA test was used for
multiple group comparison. Relationships between two
variables were assessed by Spearman’s rank correlation coef-
cient. Regression analysis was used as a simultaneous test
for the relationship among multiple variables. The level of
significance was set at p < 0.05.

Results
To evaluate the relationship between urinary AGE-
modified proteins in various stages of proteinuria, we mea-
sured AGE-immunoreactivity in 24-h urine of diabetic pa-
tients with normoalbuminuria (N), microalbuminuria (Mi),
macroalbuminuria (Ma), and overt proteinuria with el-
levated serum creatinine level (PC) using an ELISA method.
The four groups of patients were matched according to gly-
cemia control (p = 0.074) and age (p = 0.732), and their met-
bolic data are presented in Table II.
There was no evident difference in AGE serum level
(p = 0.26) among diabetic groups with normoalbuminuria
(N; 10.4 ± 3.9 µgEq/ml), microalbuminuria (Mi; 12.1 ±
4.2 µgEq/ml) and macroalbuminuria (Ma; 11.2 ± 4.6 µgEq/ ml). However, in the group with persistent proteinuria and
elevated serum creatinine, AGE-level was slightly increased
(PC: 14.3 ± 6.8 µgEq/ml, p < 0.05). In addition, we mea-
sured total 24-h urinary AGE content, which revealed, by
multivariate comparison (summary p < 0.000), significant
differences among diabetic patients with various stages of
diabetic proteinuria, as shown in Figure 1. AGE content in
urine was examined as both a categorical variable (yes/no)

Table I
Clinical characteristics of type 2 diabetic patients with various stages of proteinuria.

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria (&lt; 30 mg/d)</th>
<th>Microalbuminuria (30-300 mg/d)</th>
<th>Macroalbuminuria (&gt; 300 mg/d)</th>
<th>Persistent proteinuria (creatinine &gt; 130 µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>31</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Age (yrs, range)</td>
<td>59 (38-88)</td>
<td>62 (33-85)</td>
<td>63 (35-82)</td>
<td>63 (39-84)</td>
</tr>
<tr>
<td>Disease duration (yrs, range)</td>
<td>12 (2-24)</td>
<td>13.5 (1-26)</td>
<td>13.7 (3-39)</td>
<td>17 (2-34)</td>
</tr>
<tr>
<td>Retinopathy (n, %)</td>
<td>10 (45)</td>
<td>16 (52)</td>
<td>15 (54)</td>
<td>15 (60)</td>
</tr>
<tr>
<td>Neuropathy (n, %)</td>
<td>11 (50)</td>
<td>16 (52)</td>
<td>17 (61)</td>
<td>18 (72)</td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>13 (59)</td>
<td>25 (80)</td>
<td>28 (100)</td>
<td>25 (100)</td>
</tr>
<tr>
<td>Macrovascular disease * (n, %)</td>
<td>4 (18)</td>
<td>10 (32)</td>
<td>8 (29)</td>
<td>11 (44)</td>
</tr>
</tbody>
</table>

*History of myocardial infarction, coronary artery disease or peripheral arterial disease.
and a continuous variable. AGE-immunoreactivity was tested in 20 healthy volunteers and 85% of samples were negative. In normoalbuminuric diabetic patients urinary AGEs ranged from 0-8.12 mgEq/d, median 1.82 mgEq/d, and AGE-immunoreactivity was not detected in 50% of samples. Median value of urinary AGEs in the group of patients with microalbuminuria was higher 6.96 mgEq/d (range 0-23.3) and only 15% of samples were negative. Furthermore, there was a difference in total 24-h urinary AGEs between the group of patients with micro- and macroalbuminuria (Ma; median 14.2 mgEq/d, range 4-47.1). In the group of patients with renal impairment (persistent proteinuria with elevated serum creatinine) median value of urinary AGEs was 16.7 mgEq/d (range 4.2-67.6). None of the samples from the two groups with highest AGE content in 24-h urine was AGE negative. In addition, we calculated the ratio between 24-h urinary AGEs and urinary albumin excretion to determine whether total 24-h urinary AGE content is an index of toxic form of albumin released in the course of diabetic nephropathy. The ratio values were log-transformed to give a distribution appropriate for sample comparison. Bivariate comparison yielded a significant difference between N vs Mi (p = 0.006) and Mi vs Ma (p = 0.000) groups. However, there was no statistically significant difference (p = 0.407) between the values in Ma and PC group of patients. We observed no correlation between circulating AGEs and total 24-h urine AGEs. Multiple stepwise regression analysis of the relationship between 24-h urinary AGEs and metabolic and functional parameters pointed to creatinine clearance as the main predictor of urinary AGE excretion (Tab III). There was also a positive and significant correlation with disease duration and total proteinuria.

Additionally, diabetic patients were divided into two subgroups according to the creatinine clearance level. Normal renal function was defined as creatinine clearance >1.10 ml/s for male and >0.80 ml/s for female subjects. Renal impairment was present in 56 patients (creatinine clearance 0.65 ± 0.27 ml/s, serum creatinine 172 ± 122 µmol/l), whereas 50 patients had normal renal function (creatinine clearance 1.36 ± 0.51 ml/s, serum creatinine 104 ± 22 µmol/l). Increased urinary albumin excretion was observed in both subgroups [755 (472-1037 C.I.95%) vs 599 (378-739 C.I.95%), p = 0.69]. Regarding AGE in 24-h urine volume between the patients with impaired (11.5 ± 13.6) and normal renal function (14.9 ± 12.9), one-way ANOVA showed a difference in AGE content [F(1, 30) = 6.39, p = 0.0167]. In the subgroup with impaired renal function, when urinary AGE was con-

### Table II

Metabolic data of type 2 diabetic patients with various stages of proteinuria.

<table>
<thead>
<tr>
<th>Metabolic Parameter</th>
<th>Normoalbuminuria (&lt;30 mg/d)</th>
<th>Microalbuminuria (30-300 mg/d)</th>
<th>Macroalbuminuria (&gt;300 mg/d)</th>
<th>Persistent proteinuria (creatinine &gt;130 µmol/l)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>8.61 ± 1.75</td>
<td>8.60 ± 1.74</td>
<td>9.03 ± 1.21</td>
<td>7.88 ± 1.59</td>
<td>0.074</td>
</tr>
<tr>
<td>UAE (mg/d)</td>
<td>22.5 ± 7.7</td>
<td>161.8 ± 91</td>
<td>913.3 ± 605</td>
<td>1458 ± 922</td>
<td>0.000</td>
</tr>
<tr>
<td>Total proteinuria (g/d)</td>
<td>0.039 ± 0.015</td>
<td>0.197 ± 0.153</td>
<td>1.83 ± 1.78</td>
<td>2.70 ± 2.03</td>
<td>0.000</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>M: 104 ± 14</td>
<td>M: 108 ± 18</td>
<td>M: 115 ± 115</td>
<td>M: 239 ± 158</td>
<td>0.000</td>
</tr>
<tr>
<td>Creatinine clearance (ml/s)</td>
<td>M: 1.02 ± 0.3</td>
<td>M: 1.30 ± 0.86</td>
<td>M: 1.16 ± 0.4</td>
<td>M: 0.78 ± 0.4</td>
<td>0.051</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.85 ± 0.9</td>
<td>5.36 ± 1.83</td>
<td>6.41 ± 1.23</td>
<td>6.64 ± 2.0</td>
<td>0.014</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.58 ± 1.39</td>
<td>2.05 ± 1.53</td>
<td>3.16 ± 2.79</td>
<td>2.59 ± 1.67</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.44 ± 0.98</td>
<td>3.33 ± 1.5</td>
<td>4.09 ± 1.17</td>
<td>4.47 ± 1.83</td>
<td>0.007</td>
</tr>
</tbody>
</table>

UAE, urinary albumin excretion. All values are mean ± SD. p level was assessed by Kruskal-Wallis ANOVA test.

**Figure 1**

Scatterplot of total advanced glycation endproduct (AGE) content in 24-h urine collection of type 2 diabetic patients with normoalbuminuria (N) (n = 22), microalbuminuria (Mi) (n = 31), macroalbuminuria (Ma) (n = 28) and persistent proteinuria with elevated serum creatinine (PC) (n = 25). Bivariate comparison yielded significant between-group differences for N vs Mi, p = 0.0006; and Mi vs Ma, p = 0.009. However, there was no significant difference between the Ma and PC (p = 0.265) groups of patients.
with overt proteinuria (PC) the increase in AGE in the group with microalbuminuria. However, in patients the level of AGEs significantly exceeded the level measured samples were AGE negative. In the group with macroalbuminuria excretion was significantly increased and only 15% of these in the group of patients with microalbuminuria, AGE immunoreactivity was not detected in 50% of these samples. In the subgroup with normal creatinine clearance values (beta = 0.42, p = 0.019). This relationship was absent in the subgroup with normal creatinine clearance values.

Discussion

The objective of the present study was to detect AGE-immunoreactivity to confirm the presence of advanced glycation-derived proteins in urine, and to evaluate AGE excretion in various stages of proteinuria. Findings of particular AGEs [9, 14, 16, 20, 21] or unspecified AGEs [12, 13, 19, 22] have been reported in the literature. However, because of the great heterogeneity of the structures collectively known as AGEs, it has not yet been precisely determined which AGE or AGES are natively dominant. Therefore, our study was designed to measure total AGE content in 24-h urine by a competitive immunoassay, i.e. solid-phase bound AGE-human serum albumin and polyclonal anti-AGE antibodies.

As for immunologically reactive AGE proteins in urine, significant differences were recorded among patients with various stages of diabetic proteinuria (Fig 1). In normoalbuminuric individuals, AGE excretion was significantly lower than in other study groups, the more so, AGE-immunoreactivity was not detected in 50% of these samples. In the group of patients with microalbuminuria, AGE excretion was significantly increased and only 15% of these samples were AGE negative. In the group with macroalbuminuria, none of the urine samples was AGE negative, and the level of AGEs significantly exceeded the level measured in the group with microalbuminuria. However, in patients with overt proteinuria (PC) the increase in AGE-immunoreactive proteins did not reach statistical significance. Accordingly, the finding that AGE-immunoreactivity did not follow overt proteinuria appears to suggest that urinary AGES associated with albumin or minor protein fragments were detected. Such a line of reasoning is consistent with previous reports that 80% of serum AGES in diabetics with nephropathy were detected in low molecular weight fractions [23, 24]. In our study, there was a significant difference in urinary AGE excretion between the patients with impaired renal function (creatinine clearance 0.65 ± 0.27 ml/s) and subjects with normal renal function (1.36 ± 0.51 ml/s). Multiple stepwise regression analysis emphasized a significant correlation between urinary AGE-immunoreactivity and creatinine clearance values. Therefore, we are inclined to believe that urinary AGE excretion is influenced mainly by renal function. This also suggests that the higher serum AGE content in the PC group was due to their less efficient excretion. Our results support the study of Wagner et al. [14] demonstrating a decreased urinary excretion of Nε-(carboxymethyl) lysine, a particular AGE compound, in diabetic patients with impaired renal function. However, a recently published study of Morcos et al. [9] describes a significantly higher urinary CML level in type 2 diabetic patients with overt nephropathy, probably because renal function was not severely impaired in their study group, i.e. the patients were not analyzed according to their creatinine clearance values.

Furthermore, a significant correlation between serum AGE level and serum creatinine was also recorded in our study patients, which is consistent with literature data [12, 14]. Our results are consistent with those of Yanagisawa et al. [12], who measured AGE-specific fluorescence in serum and urine. They found a higher level of urinary fluorescence for AGE-peptide in patients with persistent proteinuria with elevated serum creatinine, and in those requiring hemodialysis. Prolonged retaining of glycated products in the circulation due to their enhanced production or insufficient excretion may be an additional pathologic factor in the biology of the micro- and macrovascular disease in diabetes. Concerning renal microvascular pathology, the advanced glycation process may play a role for several potential mechanisms: (i) AGES are able to stimulate the production of extracellular matrix and inhibit its degradation; (ii) AGE proteins also interact with specific receptors, and mediate the production of cytokines and growth factors, thereby regulating the growth and proliferation of the various renal cell types; (iii) AGE significantly interact with the local intrarenal renin-angiotensin system; and (iv) AGE can promote the generation of reactive oxygen radicals [25-27].

In conclusion, the present study demonstrated AGE-immunoreactivity in the urine of patients with various stages of proteinuria. Study results pointed to creatinine clearance as the main predictor of AGE excretion, thus the measure-

Table III

Multiple stepwise regression analysis of the relationship between total 24-h urinary AGE content and metabolic and renal function parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient of multiple correlation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/s)</td>
<td>0.52</td>
<td>0.001*</td>
</tr>
<tr>
<td>Total proteinuria (g/d)</td>
<td>0.36</td>
<td>0.007*</td>
</tr>
<tr>
<td>Disease duration (yrs)</td>
<td>0.36</td>
<td>0.002*</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.22</td>
<td>0.055 (NS)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>0.13</td>
<td>0.28 (NS)</td>
</tr>
<tr>
<td>UAE (mg/d)</td>
<td>0.08</td>
<td>0.97 (NS)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.09</td>
<td>0.97 (NS)</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>0.015</td>
<td>0.91 (NS)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>0.09</td>
<td>0.79 (NS)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.015</td>
<td>0.89 (NS)</td>
</tr>
</tbody>
</table>

Advanced glycation and proteinuria

Diabetes Metab 2004,30,187-92 • © 2004 Masson, all rights reserved 191
ment of urinary AGEs would provide only limited extra information in patients with renal function impairment.

References