URINARY EXCRETION OF FLUORESCENT ADVANCED GLYCATION END PRODUCTS (AGES) IN THE ELDERLY

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Advanced glycation and lipoxidation end-products (ALEs), generically named as glycoxidation end-products (AGEs), contribute to the pathogenesis of several age-related conditions (1, 2, 3), apart from their participation in microvascular complications of diabetes mellitus (4). Circulating levels reflect endogenous synthesis and renal excretion in diabetics, especially those with renal failure (5). Recent studies highlight the contribution of dietary intake and intestinal absorption (6), in diabetics as well as in non-diabetic humans (18).

AGEs are proinflammatory molecules, able to generate reactive oxygen species (ROS), deplete from antioxidant molecules (7) and induce production of cytokines (TNFα, IL-1β and IL-6), vascular adhesion molecule-1 (VCAM-1), and C reactive protein (CRP) among others (8). These mechanisms seem to account for the enhanced cardiovascular risk associated to age, diabetes and end stage renal disease 9). Vascular damage requires binding to specific receptors, activation of NFκ and the cytokine cascade, leading to a proinflammatory and procoagulatory state (10). Oxidative reactions are implicated both in the synthesis of AGEs and their pathogenic effects (11).

Since AGEs are heterogeneous moieties, originated from the nonenzymatic reaction of reducing sugars with proteins, lipids, and nucleic acids, there are multiple structurally identified AGEs. Among them, N-carboxymethyllysine (CML), pentosidine, pyrraline, imidazolone, and other glycated proteins or peptides of dissimilar molecular weights. They can be measured through competitive ELISA, chromatography, and fluorescence. In this study we measured urinary concentrations of AGEs, through a simple and inexpensive fluorescence method, the Flow Injection Assay (FIA) (5).

Our aim was to compare urinary excretion of AGEs in diabetic and non-diabetic elderly, looking for its association with dietary intake, nutritional status and hsCRP as a general inflammation and global risk indicator.

Subjects and Methods

After signing a written informed consent, 86 elderly subjects (ages 70-89 y, 9 men), including 19 stable type 2 diabetics, were assessed as follows: a medical story, physical examination and nutritional assessment was carried out, including anthropometric variables (weight, height, waist and hip circumferences) and body composition using a bone double beam densitometer (Lunar Corporation, Madison Wisconsin, USA). A fasting blood sample was obtained to measure serum glucose, creatinine, lipoproteins and A1c haemoglobin (HbA1c, through standard automated systems, using Roche kits), insulin (DPC kit), and C reactive protein (hsCRP) (EIA3954, DRG, International NJ, USA). Creatinine serum levels averaged 0.8 ± 0.2, and exceeded 1.5 g/dL in only one subject; creatinine clearance was calculated through the MDRD equation (12). Diagnosis of diabetes was based on clinical history of long-term treatment with diet, oral antidiabetic agents or insulin.

A second morning urine sample was also obtained to measure fluorescent small-sized urinary AGE-peptides by FIA, in a HPLC system as described (5). This methodology does not identify any particular adduct. Briefly, the samples were treated with trichloroacetic acid (TCA), then centrifuged, injected at a flow rate of 0.5 ml/min to the flow system, driven by a Merck-Hitachi L-6200 pump to the fluorescence detector. 50mg/L AGE-BSA (EMD Biosciences, La Jolla, CA) after hydrolysis with proteinase K as a standard (intrassay error = 2,26 %).

A dietary recall, with emphasis on estimation of AGEs, (specifically N-carboxy-methyl and ethyl-lysine [CML and CEL respectively]), according to Goldberg et al (6), was obtained in those volunteers with urinary AGEs in the upper and lower quartiles.

Results

Table 1 depicts variables in diabetics and non-diabetic elderly. Among the latter, urinary AGEs correlated weakly with age (Spearman R= 0.25, p= 0.04). No association was found with serum glucose, HbA1c, lipoproteins, insulin, creatinine, creatinine clearance, nor body mass index (BMI), both including or excluding diabetics. As shown in figure 1, bone mineral content, femoral and spine mineral density were negatively associated with AGE excretion, both when including (Spearman R = -0.29, -0.31 and -0.24 $p < 0.05$) or excluding diabetics (Spearman R = -0.31, -0.38, and -0.33 $p < 0.05$). Fat free mass assessed by DEXA was also negatively correlated

with urinary AGEs (Spearman R = - 0.21 p = 0.05 in the whole sample, - 0.34, p = 0.005 if diabetics are excluded).

Table 1
Urinary AGEs and clinical laboratory variables in diabetic and non-diabetic elderly subjects

	Diabetic (n = 19)	Non-Diabetic (n = 67)	p =
Urinary AGEs *	5.1 ± 2.6	4.0 ± 1.8	0.08
Age (years)	75 ± 4	77 ± 4	0.02
Creatinine (mg/dL)	0.75 ± 0.2	0.82 ± 0.2	0.18
Creatinine Clearance (ml/min)	92 ± 31	79 ± 17	0.02
Glucose (mg/dL)	141 ± 47	93 ± 13	< 0.001
Hemoglobin A1c (%)	7.9 ± 1.4	6.2 ± 0.6	< 0.001
BMI (k/m2)	29.8 ± 4.1	27.5 ± 6.8	0.16
Fat mass (k)	27.6 ± 7.3	25.4 ± 8.4	0.29
Lean body mass (k)	38.6 ± 6.2	36.9 ± 5.4	0.26
Bone mineral content (k)	1.95 ± 4.4	1.95 ± 3.5	0.89
Total cholesterol (mg/dL)	187 ± 42	205 ± 47	0.13
HDL Cholesterol (mg/dL)	51 ± 16	57 ± 14	0.13
Tryglicerides (mg/dL)	198 ± 157	147 ± 80	0.058
Insulin (uU/mL)	14.5 ± 21	9.4 ± 7	0.10
C reactive protein	2.9 ± 2.1	2.6 ± 1.8	0.65

Data expressed as Means ± Standard Deviations; * uAGEs expressed as fluorescence intensity/g urinary creatinine x 10^{10}; Statistical significance estimated by Student´s T test or Mann-Whitney U test

Dietary recalls for estimation of AGE ingestion were performed in 13 subjects with urinary AGE concentration within the lowest quartile (2.3 ± 0.3 U/g creatinine), and in 9 elderly within the highest quartile of urinary AGE excretion (7.0 ± 1.9 U/g creatinine). According to these recalls, AGE ingestion was distributed normally and did not differ between groups (table 2). However, dietary protein was significantly higher in subjects with elevated urinary AGE excretion. Bone mineral content, femoral and spine mineral density were not associated with protein ingestion.

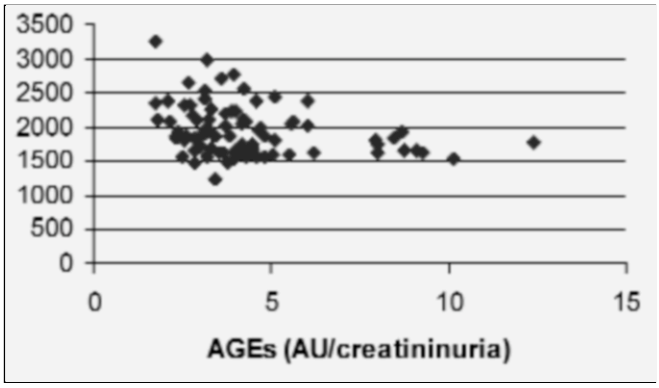
Table 2
Dietary intake amongs study groups

	High urinary AGEs (n = 9)	Low urinary AGEs (n = 13)	p =
AGE (kU/d)	4985 ± 2645	4742 ±1643	0.79
Energy (kcal/d)	1442 ± 175	1361 ± 307	0.48
Protein (g/d)	62 ± 17	46 ± 10	0.009
Fat (g/d)	37 ± 12	31 ± 8	0.74
Carbohydrate (g/d)	212 ± 35	220 ± 65	0.2
Fiber (g/d)	5.1 ± 1	4.9 ± 2	0.77

AGE = Advanced Glycoxidation End-products; Data expressed as Means ± Standard Deviations; Statistical significance estimated by Student´s T test or Mann-Whitney U test

Serum CRP did not correlate with urinary AGE excretion nor dietary intake, but showed a relation with BMI, waist and hip circumferences and fat mass assessed by DEXA, both including (Spearman R = 0.35, 0.31, 0.32, and 0.43 respectively, p< 0.05) and excluding diabetics (Spearman R = 0.44, 0.43, 0.44 and 0.40 respectively, p < 0.05).

Figure 1
Correlation plot between urinary AGEs and Bone Mineral Content assessed by DEXA in 86 subjects



Discussion

Elevated serum AGEs were described in diabetics (13), and thought to be generated endogenously, given the "favourable" environment of hyperglycaemia, impaired glomerular filtration and hemodialysis (14). These Maillard reaction products can also be originated in foodstuff during the cooking process (15), and it has been shown that dietary sources are relevant (16). Roughly 10 % of dietary precursors of AGEs are absorbed, and one third excreted by the kidneys (17). Reducing cooking time and temperature, can modulate serum concentrations of these substances, and most importantly, modify oxidative stress and the cytokine cascade (18). However, AGE levels and their relevance in normoglycemic individuals are now under investigation (17), so we aimed to measure AGEs mainly in non-diabetic elderly; we included few diabetic patients with relatively preserved renal function, as positive controls. This sample size was not adequate to obtain differences in AGE excretion between groups.

Since fluorescence is a well known property of most AGEs, detection through fluorescence spectroscopy has been used, although it cross-reacts with serum proteins like albumin. In our experience, urinary detection of fluorescent peptides appears less critically dependent on renal function than serum assessment in serum samples. In the present study, AGEs did not correlate with creatinine levels (ranging from 0.4-1.7 mg/dL) nor the calculated clearance (30- 167 ml/min), although methods for estimating renal function have limitations (12). In other series (unpublished data), urinary AGEs correlated with renal function mostly when it was severely impaired. A recent report showed that low molecular weight AGEs measured through this system predict mortality among hemodialysis patients (19).

Several findings of this study are clinically relevant. First we

found that, although slightly less than in diabetics, noticeable quantities of urinary AGEs are excreted in healthy non-diabetic elders, and can be detected through an inexpensive laboratory method. Third AGEs urinary excretion was, at least partly, determined by dietary patterns (particularly protein intake), unlike diabetics, whose AGE levels are also related to metabolic control.

As estimation of AGE dietary content computes CML and CEL, the lack of correlation between AGE intake and urinary fluorescent AGEs was not unexpected. However AGEs were associated with protein intake, in agreement with proposed mechanisms of AGE formation in foods, derived from chemical reaction of fats and protein (6). In addition, we studied Chilean elderly subjects of low socioeconomic income, consuming low-protein diets (around 40 g/d) (20), with scarce animal protein and milk ingestion and a large proportion of vegetal proteins, mostly as bread and other cereals, and negligible intake of processed food. This places them in the low AGE diet according to Goldberg´s classification (the cutoff is 16,000 kU AGE/d). However in a subgroup of elders consuming over 50 g/d protein, AGE ingestion and excretion were slightly higher, compared with low protein consumers (6093 ± 2898 versus 4331 ± 1710 kU AGE/day, $p = 0.09$ and 5.3 ± 0.84 versus 2.98 ± 0.34 U/g creatinine respectively, $p = 0.03$). Calcium intake was ensured by oral supplementation (800 mg/day).

The low AGE ingestion and excretion found in this study could also explain the lack of correlation between AGEs and hsCRP, considered a good quality global risk indicator. The latter was more connected to fat accumulation in this sample, as has been described (21).

Several age-related conditions have been attributed to accumulation of AGEs in tissues such as arteries, lens, cartilage, bone, nervous system, etc. In this sample, AGEs excretion was not related with indicators of cardiovascular risk, but to bone mass loss assessed by DEXA. The latter has been attributed to accumulation of the products mostly in cortical bones (22), and secondary alteration of bone turnover and remodelling (23, 24).

In conclusion, the contribution of dietary sources to urinary excretion of AGE and cardiovascular risk, assessed by CRP, was not evident in this sample of Chilean non diabetic elderly, probably due to the extremely low dietary intake of these substances, except in a subgroup with higher animal protein intake. Nevertheless urinary AGEs, and not dietary protein, were negatively associated with bone density, confirming its relation to age related bone loss.

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