



Orally absorbed reactive glycation products (glycotoxins): An environmental risk factor in diabetic nephropathy

(food nonenzymatic glycation products/renal/vascular/diabetes disease/glycotoxins/Maillard products)

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ABSTRACT Endogenous advanced glycation endproducts (AGEs) include chemically crosslinking species (glycotoxins) that contribute to the vascular and renal complications of diabetes mellitus (DM). Renal excretion of the catabolic products of endogenous AGEs is impaired in patients with diabetic or nondiabetic kidney disease (KD). The aim of this study was to examine the oral absorption and renal clearance kinetics of food AGEs in DM with KD and whether circulating diet-derived AGEs contain active glycotoxins. Thirty-eight diabetics (DM) with or without KD and five healthy subjects (NL) received a single meal of egg white (56 g protein), cooked with (AGE-diet) or without fructose (100 g) (CL-diet). Serum and urine samples, collected for 48 hr, were monitored for AGE immunoreactivity by ELISA and for AGE-specific crosslinking reactivity, based on complex formation with ¹²⁵I-labeled fibronectin. The AGE-diet, but not the CL-diet, produced distinct elevations in serum AGE levels in direct proportion to amount ingested ($r = 0.8$, $P < 0.05$): the area under the curve for serum ($\approx 10\%$ of ingested AGE) correlated directly with severity of KD; renal excretion of dietary AGE, although normally incomplete (only $\approx 30\%$ of amount absorbed), in DM it correlated inversely with degree of albuminuria, and directly with creatinine clearance ($r = 0.8$, $P < 0.05$), reduced to $< 5\%$ in DM with renal failure. Post-AGE-meal serum exhibited increased AGE-crosslinking activity (two times above baseline serum AGE, three times above negative control), which was inhibited by aminoguanidine. In conclusion, (i) the renal excretion of orally absorbed AGEs is markedly suppressed in diabetic nephropathy patients, (ii) daily influx of dietary AGEs includes glycotoxins that may constitute an added chronic risk for renal-vascular injury in DM, and (iii) dietary restriction of AGE food intake may greatly reduce the burden of AGEs in diabetic patients and possibly improve prognosis.

A growing body of evidence suggests that many of the effects of hyperglycemia on diabetic vascular and renal tissues are mediated by late products of glucose–protein or glucose–lipid interactions, called advanced glycation endproducts (AGEs) (1, 2). These interactions lead to the formation of unstable, reactive intermediates that readily form intra- and intermolecular covalent crosslinks (3) or glycoxidation products (4). Both the half-life and the internal microenvironment of a protein or lipid dictate the number and stages of AGE modifications present, ranging from reactive intermediates to late (nonreactive) AGEs (1–4). Endogenous AGEs are known to contribute to vascular and renal dysfunction (1, 2). Their turnover is proximally regulated in part by specific cellular

receptors, which participate in uptake and degradation of AGE-modified proteins (5, 6), and distally by the kidney. Urinary AGE clearance correlates directly with creatinine clearance (Ccr) (7, 8); thus, persons with diabetes mellitus (DM) and renal disease display elevated serum AGE levels and reduced urinary AGE excretion (7, 8). Because uncleared reactive AGEs can readily form new crosslinks with plasma or tissue components—e.g., low density lipoprotein (LDL) (9) or collagen (8)—AGE burden and associated damage worsens with diabetic kidney disease (KD). Indeed, the infusion of pre-formed AGEs into healthy animals initiates selective gene dysfunction, including those of key cytokines, growth factors, and extracellular matrix proteins associated with diabetes-like renal and vascular pathology (1, 2, 10).

Advanced glycation products or Maillard reaction products also form in foods during heating (11–14). Oral bioavailability studies of such products in defined glucose/casein or glucose/glycine diets, estimated at $\approx 10\%$ (14) led to its dismissal as biologically insignificant. Known only for reducing nutritional value of proteins (11, 12, 14), the impact of life-long AGE influx has not been considered as a potential source of toxicity. Major obstacles to studying the biology of this process include the unstable and heterogeneous nature of the chemical moieties involved and the lack of sensitive methodology (11); recently, the latter has been facilitated with new AGE-specific immunological assays (15, 16).

Using such methodology, we have evaluated the bioavailability, kinetics, and renal elimination of food-derived chemically, and immunologically reactive AGE substances in both healthy and diabetic patients, with and without impaired renal function. The evidence points to a markedly impaired renal elimination system for food-derived AGEs in diabetics with renal disease, raising concerns about an added risk in this population.

METHODS

Preparation of the AGE and Control Meals. Standardized meals were prepared from fresh chicken egg white (55.5 g protein) alone (CL-diet) or mixed with D-fructose (100 g) (AGE-diet) and heated at 90°C for 1–3 hr. Before ingestion, the prepared egg whites were homogenized, aliquots saved for protein (17) and AGE determinations (15, 16). Each meal was freshly prepared.

Clinical Studies and Patient Population. Forty-three subjects [38 male and female DM in-patients (15 type I, 23 type II)], aged 16–84 years, with or without diabetic complications,

Abbreviations: AGE, advanced glycation endproducts; AUC, area under the curve; Ccr, creatinine clearance; DM, diabetes mellitus; KD, diabetic kidney disease; macro-AU, macroalbuminuria; micro-AU, microalbuminuria; LDL, low density lipoprotein.

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as well as 5 nondiabetic healthy subjects (NL), aged 24–53 years, participated in this study after obtaining informed consent. The clinical and laboratory data on these subjects are provided in Table 1. All patients were ambulatory, free of acute illness, and had been admitted to the metabolic unit for improvement of glycemic control. None of the patients presented evidence of diabetic gastroparesis. Nondiabetic controls were healthy volunteers. Patients were grouped according to severity of KD, as follows: no KD (albuminuria, <20 $\mu\text{g}/\text{min}$), microalbuminuria (micro-AU; 20–200 $\mu\text{g}/\text{min}$), macroalbuminuria (macro-AU; >200 $\mu\text{g}/\text{min}$), and severe renal insufficiency or failure ($\text{Ccr} < 50 \text{ ml}/\text{min}$).

Study Protocol. The 3-day study period comprised a 24-hr pre-meal test period, followed by meal ingestion between 8:00–8:30 a.m. in place of breakfast, and a 48-hr post-meal evaluation period. Diabetic subjects were maintained on their diabetic diets, as provided by the metabolic ward, divided into 15–20% protein, 30–35% fat, and 45–50% carbohydrates, while breakfast carbohydrates were supplemented with apple juice. In addition to the test meal, 0.5–1 liter of tea or mineral water was consumed by all subjects. In several instances, the same patient (DM/micro-AU, $n = 3$) was crossed-over from the AGE-diet to the CL-diet, separated by 1 week.

Serum samples (2 ml) were collected 24 hr before as well as before the test meal (time 0). Blood was then collected at 0.5-hr intervals for the first 4 hr, then at 1-hr intervals for the next 5 hr, or as indicated. All fractions were stored at -20°C .

Urine samples were collected over the 24-hr pretest period and at 2- to 4-hr intervals for 48 hr posttest meal, as indicated. Ccr rate (ml/min) was based on the formula $(U_{\text{cr}}/P_{\text{cr}}) \times (U_{\text{v}}/1,440)$.

Assessment of AGE Content. The concentration of AGEs in all specimens was measured by a competitive AGE-specific ELISA (15, 16) with minor modifications. Before assay, serum samples were diluted 1:5 in PBS and digested with 0.1% (wt/wt) proteinase K (Sigma) at 37°C overnight. Proteinase K was inactivated at 70°C for 1 hr. AGE data were expressed as AGE units per ml of serum. Urinary AGEs and urinary protein levels were expressed as AGE units excreted per hour and mg protein excreted per hour, respectively.

Aliquots of eight randomly selected AGE- and CL-diet preparations as well as several representative types of food and beverages consumed by the participants of this study as part of their regular diets were also tested for AGE content by ELISA (15, 16). Results (Tables 2–4) are expressed as mean \pm SD units AGE/mg solid food or units AGE/ml liquid. In addition to the polyclonal rabbit anti-AGE-RNase routinely used in this assay (15), an AGE-specific mAb (kindly provided by Alteon, Ramsey, NJ) was also used, with identical results (data not shown) (16).

Determination of Serum AGE Chemical Reactivity. The crosslinking reactivity of food-derived AGEs in serum fractions was assayed as follows. To remove non-AGE serum components, baseline (premeal) or AGE-rich (postmeal) serum samples (1 ml/patient) were diluted 1:5 with PBS and

passed over a Sepharose column onto which chicken lysozyme (Sigma), an AGE binding substrate (18), was immobilized. The lysozyme-bound components were eluted with 0.1% NaOH and immediately neutralized with 6 M HCl. Aliquots of the AGE-enriched serum eluates (5 mg/ml), or human IgG (Sigma) (5 mg/ml), used as negative control, were incubated with ^{125}I -labeled fibronectin fragments (Sigma) (6 ng/ml, specific activity, $15 \times 10^5 \text{ cpm}/\text{ng}$) in the presence or absence of aminoguanidine at 37°C for up to 72 hr in the presence of proteinase inhibitors (1 mM phenylmethylsulfonyl fluoride/1 mM EDTA/0.02% NaN_3). After gradient gel electrophoresis (4–20% SDS) and autoradiography, high molecular weight complex formation ($>200 \text{ kDa}$) in each mixture was quantitated by densitometric and phosphorimage analysis. The presence of AGEs in these complexes (AGE specificity) was confirmed by Western blot analysis using anti-AGE antibodies (19, 20). Data were expressed as percent increase in phosphorimage (PI units/mg) above the control (IgG) value.

Calculations/Statistics. After the oral meal tests, changes in AGE levels in serum and urine AGE kinetics were calculated based on the trapezoidal formula (21). In brief, serum and urine AGE values were plotted against time and the area under the curve (AUC) representative of a 48-hr period ($\text{AUC}_{1-48\text{hr}}$) was integrated according to the standard formula: $S = (t_2 - t_1)/2 \times (C_2 + C_1)$. Results were expressed in units AGE \times hr/ml after subtracting the baseline (endogenous) levels. Comparisons of the mean $\text{AUC}_{1-48\text{hr}}$ among different groups were based on one-way ANOVA (with post hoc comparison by Student's Newman-Kuels test) and between AGE-diet and CL-diet-induced changes were based on one-way ANOVA, with *a priori* contrasts to compare CL-diet to the remaining groups. Significant correlations were those with a P value < 0.05 , based on regression analysis.

RESULTS

A greater than 200-fold increase in AGE immunoreactivity was found after cooking egg whites with fructose (AGE-diet, 1617 ± 348 units AGE/mg), as compared with the CL-diet (7.0 ± 1.6) (Fig. 1). Similarly, analysis of selected dietary samples after cooking indicated massive increases ($\times 4$ - to 100-fold) in AGE concentration, influenced by the nutrient content (protein or fat vs. carbohydrate), as well as by the amount of heat applied (Table 2). In the absence of protein or heat, levels of AGE did not correlate with high sugar content, as in orange juice and Sprite (carbonated beverage), nor did the absence of sugar predict low AGE content, as in preparations containing preformed AGE-like caramel additives (e.g., Classic Coca-Cola or Diet Coke) (Table 4). Based on the combined amount of AGEs present in the food types analyzed (Tables 2–4), the AGE-rich meal used in these studies contained about three times more than the corresponding amount of AGEs present in a single meal of a regular diet. Each patient ingested between 15 and 55 g of egg-white protein ($\approx 11.1 \text{ g}$ per 100 g egg white). The total amount of AGE-modified protein

Table 1. Clinical and laboratory characteristics of study subjects

Oral test	DM	DM/KD	<i>n</i>	Age, years	Duration, years	UAER, $\mu\text{g}/\text{min}$	Scr, mg/dl	Ccr, ml/min	HbA _{1c} , %
CL-diet	DM	None	3	49 \pm 18	21 \pm 25	7.7 \pm 4.7	0.88 \pm 0.14	139 \pm 68	8.9 \pm 2.0
		Micro-AU	4	48 \pm 17	10 \pm 9	75 \pm 73.8	0.88 \pm 0.15	100 \pm 24	9.0 \pm 1.1
		Macro-AU	1	67	17	539	1.09	84	13.4
AGE-diet	Non-DM	None	5	41 \pm 15	0	5 \pm 2	0.82 \pm 0.10	134 \pm 17	5.2 \pm 0.6
		DM	7	41 \pm 19	12 \pm 17	6 \pm 4	0.78 \pm 0.14	104 \pm 7	8.9 \pm 1.9
	DM	Micro-AU	9	65 \pm 16	16 \pm 9	74 \pm 37	0.93 \pm 0.21	87 \pm 35	9.5 \pm 1.9
		Macro-AU	10	59 \pm 11	20 \pm 11	904 \pm 987	1.20 \pm 0.36	85 \pm 20	10.7 \pm 2.5
		RF	4	55 \pm 12	16 \pm 12	1,432 \pm 738	2.68 \pm 1.40	25 \pm 11	8.9 \pm 1.7

AGE-diet, egg white and fructose; CL-diet, control diet (egg white); UAER, urinary albumin excretion rate; Scr, serum creatinine; HbA_{1c}, hemoglobin A_{1c}; RF, renal failure.

Table 2. AGE content in common foods

Food	Nutrient content, g/100 g of food			Cooking conditions		AGE content			
	Protein	Carbohydrate	Fat	Temperature, °C	Time, min	Units/g protein before cooking	Units/g protein postcooking	Fold increase post/before cooking	Units/100 g of food postcooking
Cereal (granola)	10	43	29	175	25	4,730	19,340	×4	193,400
Pastry (donut)	7	46	14	160	5	2,590	60,820	×24	425,740
Cake (Berlin)	6.4	45	18	200	50	2,220	131,000	×59	838,400
Duck skin (roasted)	35	67	4	220	110	2,350	236,180	×101	6,259,000

ingested by each patient varied (AGE-diet: $27\text{--}76 \times 10^6$ units/patient; CL-diet: $\approx 0.345 \times 10^6$ units/patient); however, there was a significant correlation between amount ingested per person and the resulting elevation in serum AGE kinetics, calculated as AUC (Fig. 1B, $r = 0.8$, $P < 0.05$).

As suggested above, the AGE-diet, but not the CL-diet, produced distinct elevations in serum as well as in urine AGE levels above the pretest baseline (Fig. 2), while blood glucose levels remained unchanged. On average, serum AGE levels rose 2 hr postingestion and peaked at 4–6 hr, after which they returned to baseline with kinetics that varied with the presence and severity of KD. As shown in Fig. 2A, compared with AGE elevations in nondiabetic subjects that lasted 18–20 hr, AGE levels in diabetics with macro-AU returned to baseline in 36–48 hr, whereas, in diabetics with severe renal disease, increased AGE levels persisted beyond 48 hr postingestion.

Consistent with the serum AGE effects, urinary AGE levels in normal individuals peaked within 4 hr and returned to baseline no later than 24 hr postmeal, whereas, in diabetic patients, even those with micro-AU, urinary AGE excretion did not rise until 8 hr postmeal (Fig. 2B). In addition, in diabetic patients, there was a distinct prolongation of the excretory peak, not returning to baseline until 40–48 hr postingestion (Fig. 2B). Of note, during the study, no abnormal rises in protein or albumin were found in the urines of patients with normal renal function, nor were there any changes from baseline in the other groups (data not shown), indicating that the increased serum AGE levels were largely contributed to by small molecular weight AGE substances. When the same patients who first received the AGE-diet were crossed over to the CL-diet 1 week later, their responses were identical to those patients, each of whom received a single test (data not shown).

To determine the impact of kidney dysfunction on the kinetics and clearance of orally absorbed AGEs, the average serum or urine AGE AUC over 48 hr were compared among the different patient groups, each reflecting different degrees of kidney impairment resulting from diabetes (Table 1). The AUCs of serum AGEs derived from the AGE meal correlated directly with albuminuria, a measure of declining kidney function (Fig. 3A). In parallel, the total 48-hr urinary excretion of orally absorbed AGEs by each patient group correlated inversely with the severity of kidney disease (Fig. 3B). Comparison of the diet-induced, 48-hr excretion of AGEs with Cr using regression analysis revealed a significant correlation ($r = 0.8$, $P < 0.05$) (Fig. 3C). Generally, based on the formula: serum AUC \times plasma volume, the total amount of orally absorbed AGEs found in blood was equal to $\approx 10\%$ of that estimated to be present in the ingested meal. Of that, only 30%

Table 3. AGE content of common condiments

Condiment	Content, g/15 ml serving*			AGE, units/15 ml serving
	Protein	Carbohydrate	Fat	
Maple syrup	0	53	0	795
Brown rice vinegar	1	1	0	2,100
Soy sauce	1	3	0	8,700

*15 ml = 1 teaspoon.

was excreted in the urine of persons with normal renal function over the subsequent 48 hr, and it decreased proportionately to as low as $<5\%$ in those with severe renal disease (Fig. 3B).

The *in vitro* exposure of AGE-enriched serum fractions collected during the peak test period to native fibronectin, a protein abundant in plasma and matrix, resulted in a nearly 2-fold increase in large molecular weight aggregate formation above the pre-test serum, and a 3-fold increase above control IgG (Fig. 4). In both cases, aggregate formation was blocked in the presence of the AGE crosslink inhibitor, aminoguanidine (Fig. 4).

DISCUSSION

The pathogenicity of endogenous, glucose-derived AGEs in human tissues has been the subject of intense investigation over the last 20 years (1–4). As reported in the recent literature, the pluripotent effects of AGEs range from multiple gene activation to well established proatherosclerotic and glomerulosclerotic effects involving cytokine and growth factor modulation, lipid oxidation and albuminuria (1–4, 22–26). Of particular significance has been the realization that the process of degradation of tissue-bound AGEs exposes a new pool of previously internal, highly reactive AGE intermediates in circulation, herein termed glycotoxins. Some of these serum AGE derivatives have been found capable of reacting with new proteins, (e.g., LDL, α_2 -microglobulin, collagen) propagating oxidative modifications or forming new AGE crosslinks *in vitro* and *in vivo* (8, 9, 22). Studies have already established that individuals with impaired renal function are unable to efficiently excrete glycotoxins in the urine, resulting in abnormally high AGE concentrations in the blood and tissues (7, 8). When infused in normal animals, exogenously formed glycotoxins produced distinct diabetic-like vascular and renal lesions (24–26).

These studies, against the background of existing evidence for heat-promoted AGEs in human diets (11), led us to question the impact of the daily influx of food-derived AGE analogs, especially in the context of existing diabetic nephropathy.

The precise chemical characterization of heat-derived sugar-addition products due to their complex and unstable nature, has been limited to only a few derivatives among many hundreds of glucose-protein adducts (11–14) (Fig. 5). Using antibodies that recognize lipid-AGEs as well as protein-AGEs (9, 19), we have confirmed previous reports that conventional

Table 4. AGE content of common beverages

Beverage	Content, g/cup*			AGE, units/cup
	Protein	Carbohydrate	Fat	
Sprite (soda)	0	26	0	475
Orange juice	<1	23	0	600
Tea	0	0	0	2,025
Coffee	0	0	0	2,200
Classic Coca-Cola (soda)	0	27	0	8,500
Diet Coke (soda)	0	0	0	9,500

*250 ml = 1 cup.

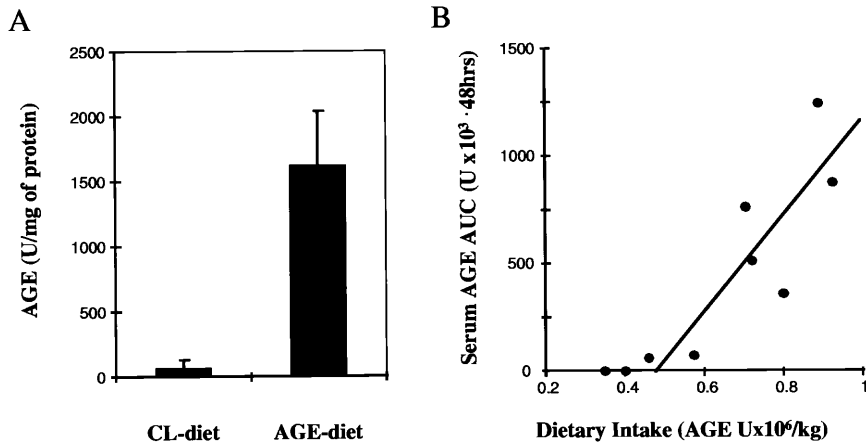


FIG. 1. (A) Levels of immunoreactive AGEs in test diets, consisting of egg white and fructose (AGE-diet) or without fructose (CL-diet). Data are expressed as AGE units/mg of protein, and represent the mean \pm SD of eight randomly selected AGE-diet preparations, ingested by eight diabetic patients with normal kidney function. (B) Serum levels of AGE correlate with amount of ingested AGE-diet. Serum values are expressed as area under the curve (AUC AGE units/48 hr). Dietary intake is expressed as the total AGE units ingested/body weight (kg). Data are analyzed by regression analysis ($r = 0.8, P < 0.05$).

diets contain significant amounts of AGE-modified substances which arise during heating. Of note, among the selected foods tested, lipid-rich poultry skin contained far greater amounts of AGEs than those foods high in protein. This may be attributed to the radicals generated most efficiently from lipids during the heating of cutaneous fat, catalyzing the glucose-induced glucoxidative modification of free amine-containing lipids (9, 19). Although the observation is only preliminary, given the prominence of this type of food in the human diet, the deleterious effects of high-fat foods may be in part due to the high content in glycotoxins, above and beyond those due to oxidized fatty acid derivatives (23).

Rather than use a mixed diet to explore absorption, kinetics, and excretion of dietary AGEs, we selected one consisting largely of a single protein, ovalbumin, and the sugar fructose, both common ingredients of daily diets and similar to those already tested in animal studies (11, 14). The total amount of AGE meal was proportional to the AGE content estimated to be consumed daily.

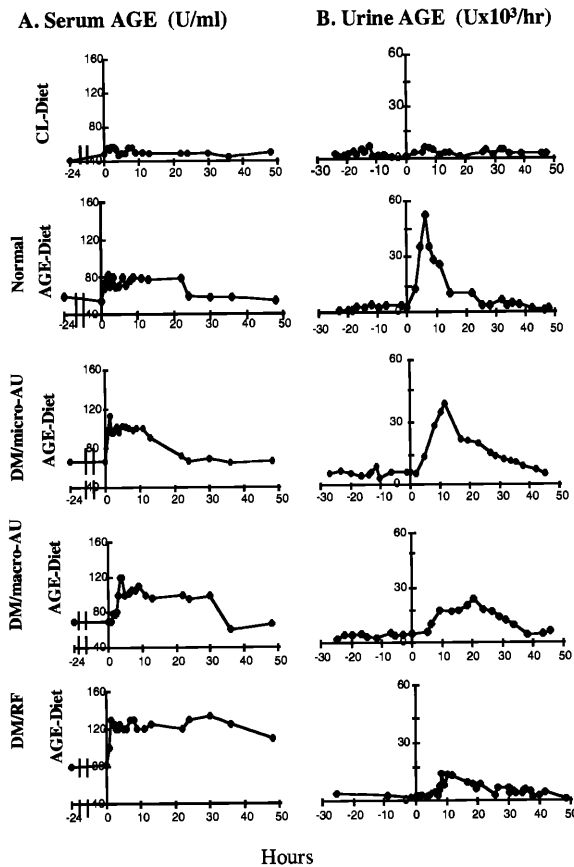


FIG. 2. Diet-induced AGE kinetics in human serum (A) and in urine (B) following a single meal of AGE- or CL-diet. Each curve is representative of the profiles obtained from groups of either normal subjects or diabetic patients with renal disease [DM/micro-AU, DM/macro-AU or DM/renal failure (RF)] and are based on ELISA. Serum values are expressed as AGE units/ml and urinary data as AGE units $\times 10^3$ /hr.

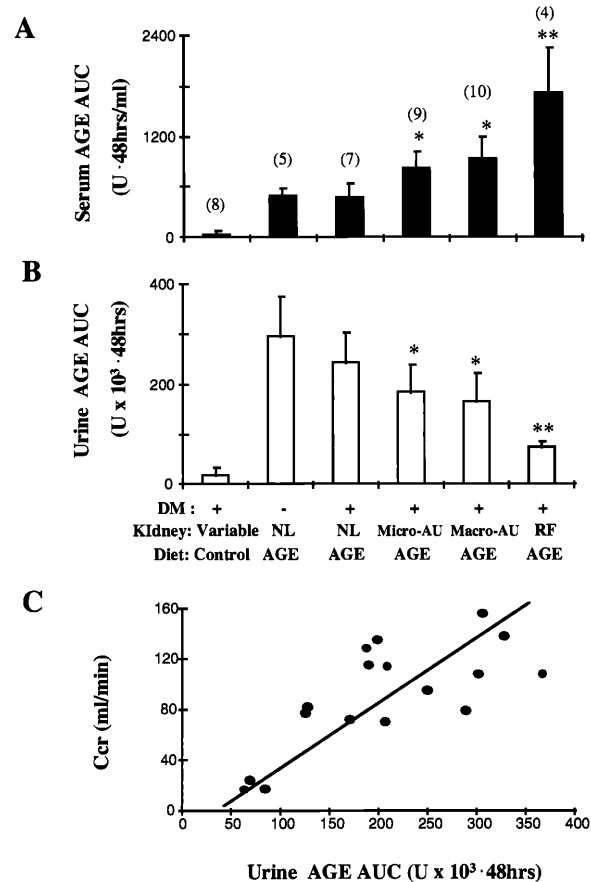


FIG. 3. Comparison of integrated diet-induced increases in serum (A) and urine (B) AGE levels between groups of diabetic patients (DM) and normal subjects (NL), as well as among groups of diabetic patients with different degrees of renal disease (none, micro-AU, macro-AU, or renal failure; RF) after a single meal of AGE- or CL-diet. Data are expressed as the mean AUC in serum (AGE units/ml \times 48 hr), or in urine (AGE units $\times 10^3$ per 48 hr). Significant P values are indicated by asterisk (*, $P < 0.05$; **, $P < 0.01$). Numbers in parenthesis indicate number per group. (C) Correlation by regression analysis of urinary Ccr (ml/min) to the renal excretion of diet-derived AGE. Urine concentration of AGEs is expressed as total area under the curve of AGEs over 48 hr (AUC AGE units $\times 10^3$ per 48 hr) ($r = 0.73, P < 0.05, n = 17$).

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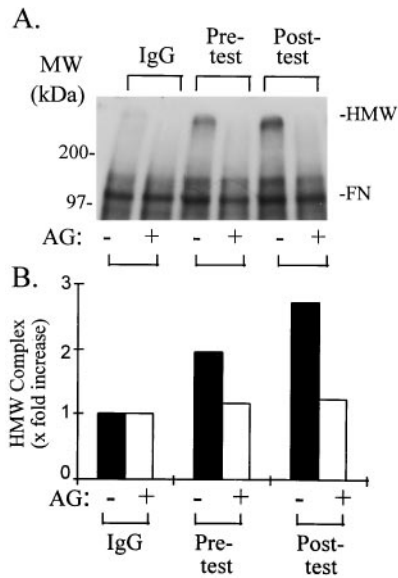


FIG. 4. Chemical reactivity of diet-derived AGEs in human serum. After passing over a lysozyme-linked Sepharose column, serum fractions obtained prior to (pre-test) or after AGE-diet (post-test) (5 mg/ml) were incubated with ^{125}I -labeled fibronectin fragments (FN) (6 ng/ml) for up to 72 hr at 37°C in the presence or absence of aminoguanidine (AG). Human IgG (5 mg/ml) was used as control. High molecular weight complex (HMW) formation in the mixtures was determined by autoradiography (A), and by phosphorimage (PI) analysis (B). Data are expressed as fold increase in PI units/mg protein above the control value (IgG) and represent the mean of three identical experiments, each done in duplicate.

The increases in AGE concentration in serum and urine of normal individuals after ingesting the AGE-rich protein meal confirmed that AGE moieties present in foods survive the digestive process and are transported, as small molecular weight particles into the bloodstream, along with short peptides and amino acids present in the digest, in a manner directly proportional to the amount ingested. Although not more than 10% of ingested AGEs was accounted for in the intravascular space, an additional portion is likely to be distributed to the extravascular space along with endogenously formed AGEs.

Consistent with previous reports, however, (mt)70% of the ingested advanced glycation products escape absorption, probably due to the documented resistance of AGE crosslinks to enzymatic or acid hydrolysis in the digestive tract (11, 13).

Only one-third of the absorbed AGEs appearing in the serum was detected over the ensuing 48 hr in the urine, the fate of the other two-thirds remaining undetermined (11, 13). Although a portion of that could conceivably be excreted slowly, at a rate below baseline over the ensuing days or weeks, a valid explanation is that it is incorporated covalently onto tissues and cells. The enhanced capacity of diet-derived serum AGEs to form complexes with a native protein (e.g., fibronectin) shown here supports this notion and suggests that the daily ingested glycotoxins are retained in various tissues over time. The effective inhibition of the chemical reactivity of diet-derived serum glycotoxins by aminoguanidine further supports their potentially toxic nature. This is also supported by earlier studies of rats fed AGE-modified diets for up to 12 months that showed kidney (and liver) enlargement and cumulative pigment deposition in these organs, or by the chronic infusion of animals with AGEs resulting in accelerated, diabetes-like vascular, and renal lesions (24–26). In addition to targeting matrix components (8), glycotoxins can attach onto the LDL particle (9), resulting in slower clearance and higher plasma LDL concentration, a well known atherogenic condition (9, 19). It can be anticipated that, in humans as well, the steady influx of food-derived AGEs serves as a permanent source of glycotoxins, a part of which is added daily onto the total body burden. Thus, the amount of food-derived AGEs ingested daily over many decades can be regarded as clinically significant, given the striking rise in serum AGE (after a single test) to levels even higher than the level of diabetic renal failure.

Time-related changes in the diet-induced AGE kinetics in diabetics clearly reflected the underlying renal status, which is consistent with the previously observed positive correlation between endogenous AGE and Ccr (7, 8), regardless of the presence or absence of diabetes. This is further validated in the present report by multiple regression analysis, which indicated that, although kidney dysfunction imparted a significant effect on the excretion rate of dietary AGE, the presence of diabetes did not. AGE clearance rates have been found to be slower than that of creatinine, prompting other workers to observe that significant AGE reabsorption occurs at the proximal

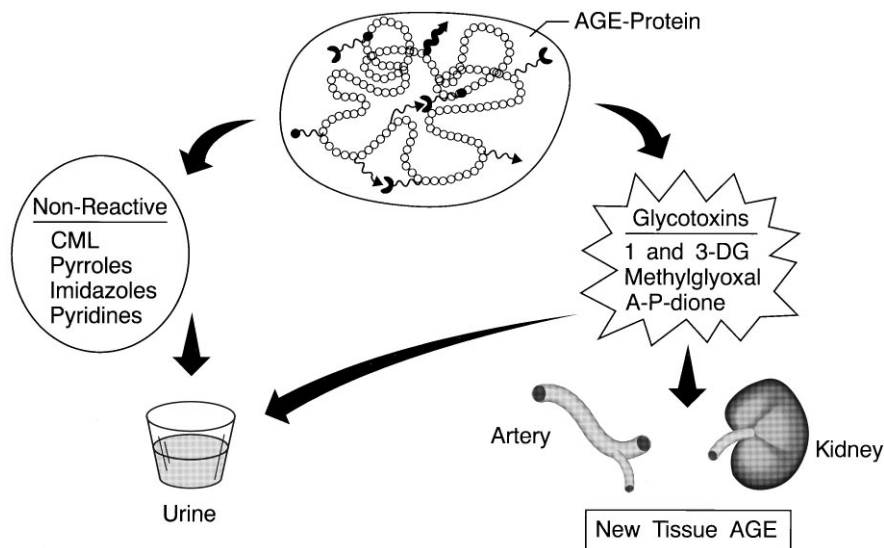


FIG. 5. Schematic representation of the fate of diet-derived AGEs. Cooked foods contain sugar-derived protein or lipid glycation intermediates that may include either nonreactive products such as *N*- ϵ -carboxymethyl-lysine (CML), pyrroles, imidazoles, pyridines (left insert), or reactive intermediates (glycotoxins), such as 1-,3-deoxyglucozone (3-DG), methylglyoxal, protein-linked A-P-dione (right insert). The former are presumably readily excreted in the urine, while the latter may reattach onto serum or tissue components to form new AGEs with the eventual pathological consequences.

tubule (27). In diabetics with KD, the lower amplitude of the urinary AGE peak, together with its significant prolongation, could reflect either underlying loss of filtration surface or of tubular reabsorption although both processes may decline simultaneously. Regardless of each individual's precise renal reserve, it can be speculated that AGE molecules percolating daily through the glomerulus, along with their attendant chemical toxicity and propensity to influence the local haemodynamic environment, could provide a very effective synergism for eventual injury. Other local effects of AGE accumulation on the glomerular structures have been shown to include cell activation and growth-promoting responses (26), linking them to progressive renal sclerosis (25). Eventually, dietary glycotoxins, allowing for high local concentration and time, could bind irreversibly to matrix cell membranes of the systemic vasculature. Although the chemical structures of biologically significant orally absorbed AGEs remains to be defined, their collective adverse effects can no longer be ignored, much like the derivatives of lipid oxidation, which, though structurally elusive, are recognized as proatherogenic (23).

In conclusion, diet-derived AGEs that are absorbed into the bloodstream may represent a major source of chemically and biologically active toxins. These glycotoxins are only partially eliminated in the urine and may exert significant reactivity in the body. Diabetic renal impairment severely impedes the timely elimination of dietary AGEs, potentially compounding the injury inflicted by these agents. On the basis of the data presented, appropriate measures to limit AGE intake, such as eliminating those foods or modes of cooking associated with the highest AGE content, may greatly reduce the already heavy burden of these toxins in the diabetic patient.

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