

## Research and Professional Briefs

## Advanced Glycoxidation End Products in Commonly Consumed Foods

TERESIA GOLDBERG, MS, RD; WEIJING CAI, MD; MELPOMENI PEPPA, MD; VERONIQUE DARDAINE, MD;  
BANTWAL SURESH BALIGA, MD; JAIME URIBARRI, MD; HELEN VLASSARA, MD

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Editor's note: Tables 2-6 that accompany this article are available on-line at [www.adajournal.org](http://www.adajournal.org).

## ABSTRACT

**Objective** Advanced glycoxidation end products (AGEs), the derivatives of glucose-protein or glucose-lipid interactions, are implicated in the complications of diabetes and aging. The objective of this article was to determine the AGE content of commonly consumed foods and to evaluate the effects of various methods of food preparation on AGE production.

**Design** Two-hundred fifty foods were tested for their content in a common AGE marker  $\epsilon$ -N-carboxymethyllysine (CML), using an enzyme-linked immunosorbent assay based on an anti-CML monoclonal antibody. Lipid and protein AGEs were represented in units of AGEs per gram of food.

**Results** Foods of the fat group showed the highest amount of AGE content with a mean of  $100 \pm 19$  kU/g. High values were also observed for the meat and meat-substitute group,  $43 \pm 7$  kU/g. The carbohydrate group contained the lowest values of AGEs,  $3.4 \pm 1.8$  kU/g. The amount of AGEs present in all food categories was related to cooking temperature, length of cooking time, and presence of moisture. Broiling ( $225^\circ\text{C}$ ) and frying ( $177^\circ\text{C}$ ) resulted in the highest levels of AGEs, followed by roasting ( $177^\circ\text{C}$ ) and boiling ( $100^\circ\text{C}$ ).

**Conclusions** The results indicate that diet can be a significant environmental source of AGEs, which may constitute a chronic risk factor for cardiovascular and kidney damage. *J Am Diet Assoc.* 2004;104:1287-1291.

*T. Goldberg is a research dietitian, W. Cai is a research associate, M. Peppia is a postdoctoral fellow, V. Dardaine is a postdoctoral fellow, H. Vlassara is professor and director, Division of Experimental Diabetes and Aging, Department of Geriatrics; B. S. Baliga is an assistant professor, Division of Endocrinology, Department of Medicine; J. Uribarri is an associate professor, Division of Nephrology, Department of Medicine, all at Mount Sinai School of Medicine, New York, NY.*

*Address correspondence to: Helen Vlassara, MD, Mount Sinai School of Medicine, Box 1640, New York, NY 10029. E-mail: [helen.vlassara@mssm.edu](mailto:helen.vlassara@mssm.edu)*

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Advanced glycoxidation end products (AGEs) constitute a group of heterogeneous moieties produced endogenously from the nonenzymatic glycation of proteins, lipids, and nucleic acids (1,2). In addition, AGEs can also form from lipid peroxidation, receiving the name advanced lipoxidation end products (ALEs) (3,4). The number of structurally identified AGEs is growing, and  $\epsilon$ -N-carboxymethyllysine (CML) is one of the better characterized end products frequently used as an AGE/ALE marker in laboratory studies (5).

The pathologic effects of AGEs/ALEs are related to their ability to modify the chemical and biological properties of native molecules by cross-link formation and their ability to bind to several cellular receptors (6,7) promoting cellular oxidative stress and cell activation, importantly of the immune system (8). AGEs have been associated with numerous diabetic (9-15) and renal complications (16,17), as well as with Alzheimer's disease (18).

An unrecognized source of AGEs and ALEs is the modern diet, due to heat treatment of foodstuffs (19-22). Recently, human studies confirmed that about 10% of diet-derived AGEs are absorbed and correlate with circulating and tissue AGE levels (23). Dietary AGE restriction resulted in significant reduction of circulating AGE levels and disease progression in animal models of atherosclerosis (24) and diabetes (25,26), as well as in diabetic patients with normal renal function (27) and in nondiabetic patients with renal failure (28). These findings suggest that dietary AGEs may constitute a chronic environmental risk factor for tissue injury. Prospective interventional studies modulating the dietary AGE content will be necessary to prove the clinical benefits of low AGE diets; however, availability of data on dietary AGE content is currently lacking. We therefore evaluated the AGE content in representative, commonly consumed foods and discuss our findings.

## METHODS

From a menu survey of hospital cafeteria items and local eating establishments, a total of 250 foods were determined to represent foods and culinary techniques typical of a multiethnic urban population. Test items were obtained from Mount Sinai Hospital's central kitchen or were prepared in the Clinical Research Center. Samples of convenience and fast foods were purchased from local establishments. Foods were prepared for standard cooking times with commonly used cooking methods: boiled in water ( $100^\circ\text{C}$ ), broiled ( $225^\circ\text{C}$ ), deep fried ( $180^\circ\text{C}$ ), oven fried ( $230^\circ\text{C}$ ), and roasted ( $177^\circ\text{C}$ ).

Food-derived AGEs and AGE precursors represent a very large number of compounds, and it is presently impossible to account for all of them. Previous studies, however, have shown that they include the well-characterized  $\epsilon$ N-carboxy-methyl and  $\epsilon$ N-carboxy-ethyl-lysine (CML, CEL) derivatives that we have chosen to measure in this study (2-5,22).

Protein and lipid-linked AGE determination was based on a competitive enzyme-linked immunosorbent assay, using a well-characterized anti-CML monoclonal antibody (4G9) (29-31) and expressed as AGE units per milligram of protein or lipid. This AGE value was then multiplied by protein and lipid per gram of food (ESHA Food Processor database version 7.1, 1998, Salem, OR, and manufacturer information for convenience items). The combined protein and lipid-associated AGE content of each sample was expressed as mean  $\pm$  standard error of the mean units per gram of food or units per milliliter of liquid. For data presentation, AGE content was expressed in kilounits (kU) per gram or per milliliter or for a standardized serving size.

## RESULTS

The AGE content for each food group, classified as per American Diabetes Association exchange lists, is shown in Tables 1 through 6. (Tables 2-6 are available on the on-line version of the *Journal*.)

The fat group contained the highest mean AGE food values. Among the items of this group, spreads, including butter and processed cream cheese, margarine, and mayonnaise, showed the highest amounts, followed by oils and nuts (Tables 1 and 2). Thus 5-g servings of butter and oil contained 1,300 and 450 kU AGE, respectively.

High AGE values were also observed for the meat and meat-substitute groups ( $43 \pm 7$  kU/g). Within this group, highest levels were determined for cheeses, followed by beef and poultry, tofu, fish, and whole eggs (Tables 1 and 3). In all categories, exposure to higher temperature achieved a greater AGE content for equal weight of the sample. The trend for AGE values achieved was oven-frying > deep frying and broiling > roasting > boiling. Thus, 90-g servings of chicken breast prepared with these methods yielded 9,000, 6,700, 5,250, 4,300, and 1,000 kU AGE, respectively.

The carbohydrate group contained relatively low amounts of AGE ( $3.4 \pm 1.8$  kU/g). Within this category, the highest AGE content was reported in processed items, followed by grains, legumes, and starchy vegetables and breads (Tables 1 and 4). The lowest AGE values were detected in the milk group, followed by vegetables and fruits (Tables 1 and 4), although infant formula contained ~100-fold more AGE than natural milk.

Microwaving was shown to increase AGE content similar to boiling cooking methods (data not shown).

## DISCUSSION

Our data support the premise that nutrient composition, temperature, method, and duration of heat application affect AGE generation in foods during cooking (19,21).

**Table 1.** Advanced glycoxidation end products (AGE) content of selected foods prepared by standard cooking methods

Food item	AGE <sup>a</sup> (kU/g or /mL of food)
<b>Fats</b>	
Almonds, roasted	66.5 kU/g
Oil, olive	120 kU/mL
Butter	265 kU/g
Mayonnaise	94 kU/g
<b>Proteins</b>	
Chicken breast, broiled $\times 15$ min	58 kU/g
Chicken breast, fried $\times 15$ min	61 kU/g
Beef, boiled $\times 1$ h	22 kU/g
Beef, broiled $\times 15$ min	60 kU/g
Tuna, roasted $\times 40$ min	6 kU/g
Tuna, broiled $\times 10$ min	51 kU/g
Cheese, American	87 kU/g
Cheese, Brie	56 kU/g
Egg, fried	27 kU/g
Egg yolk, boiled	12 kU/g
Tofu, raw	8 kU/g
Tofu, broiled	41 kU/g
<b>Carbohydrates</b>	
Bread, whole-wheat center	0.54 kU/g
Pancake, homemade	10 kU/g
Milk, cow, whole	0.05 kU/mL
Milk, human, whole	0.05 kU/mL
Enfamil (infant formula)	4.86 kU/mL
Apple	0.13 kU/g
Banana	0.01 kU/g
Carrots	0.1 kU/g
Green beans	0.18 kU/g

<sup>a</sup>AGE denotes  $\epsilon$ N-carboxymethyl-lysine (CML)-like immunoreactivity, assessed by enzyme-linked immunosorbent assay based on monoclonal antibody (4G9) (30,31).

Consistent with earlier studies (27,28), there is a clear relationship between AGE content and nutrient composition. Thus, foods high in lipid and protein content show the highest AGE levels. This may result from high levels of free radicals released in the course of various lipoxidation reactions, which catalyze the formation of AGEs and ALEs on amine-containing lipids during cooking of fats and meats. Glycoxidation and lipoxidation are promoted by heat, absence of moisture, and presence of metals, important factors in the production of edible fats (32,33). Thus, CML-like AGEs also form in oils, although protein content is negligible.

Foods that are composed mostly of carbohydrates, eg, starches, fruits, vegetables, and milk, contain the lowest AGE concentrations. However, within this group, commercially prepared breakfast foods and snacks show significant AGE content, eg, 30-g servings of toasted frozen waffles and biscotti contained 1,000 kU AGE and Rice Krispies (Kellogg Co, Battle Creek, MI) contained 600 kU/serving. Items of similar nutrient composition, such as toasted bread, contain only 30 kU AGE/serving. Indeed, several food processing techniques promote glycoxidation. Processing of some ready-to-eat cereals, which

includes heating at temperatures over 230°C, may explain the high AGE content of these products. Also, many cereals and snack-type foods undergo an extrusion process under high pressure to produce pellets of various shapes and densities. This treatment causes major chemical changes, thermal degradation, dehydration, depolarization, and recombination of fragments all of which can promote glycoxidation (34). The AGE difference between pretzels (500 kU/serving) and popcorn (40 kU/serving) might be explained by their different preparation methods.

Temperature and methods of cooking seem to be more critical to AGE formation than cooking time. This is evidenced in the higher AGE values of samples broiled or grilled at 230°C for a short time when compared with samples boiled in liquid media for longer periods. Thus, a serving of chicken breast boiled for 1 hour yielded 1,000 kU AGE, while the same item broiled for 15 minutes yielded 5,250 kU AGE.

The data reported in Tables 1 through 6 enabled us to estimate dietary AGE intake using food records and to develop diets with variable AGE content, which were then applied in designing dietary intervention studies in humans (27,28). In a preliminary survey of the usual daily AGE intake, we analyzed 3-day food records from healthy individuals (n=34). Mean daily AGE intake was 16,000±5,000 kU AGE. These data were used to define a high- or low-AGE diet, depending on whether the estimated daily AGE intake is significantly greater or less than 16,000 kU AGE. A similar investigation in 40 type 2 diabetic patients showed a daily AGE intake of 18,000±7,000 kU AGE, with major proportions of AGE contributed by broiled, fried, grilled, and roasted meat and meat alternatives. Diabetic patients tended to consume more AGE because of the consumption of larger portions of meals rich in meats. Alternative cooking methods, such as boiling and stewing, allow daily AGE ingestion to be reduced by up to 50% keeping the same primary nutrients.

The new information presented herein can be easily integrated into meal patterns that are consistent with those currently recommended against cardiovascular disease and cancer in the general population. Firstly, reduced intake of AGEs can be achieved by reducing high-AGE sources such as full-fat cheeses, meats, and highly processed foods, and increasing the consumption of fish, grains, low-fat milk products, fruits, and vegetables. These guidelines are features of The Dietary Approaches to Stop Hypertension (35) and similar to directives of the American Heart Association (36). Secondly, data on meat and meat substitute preparation clearly showed marked differences in the AGE content of food items subjected to low vs high temperature treatment. Consumers can be directed to the time-honored low-AGE-producing culinary techniques of boiling, poaching, and stewing to prepare palatable menu items. The American Cancer Society also recommends avoidance of exposure of meats to “excessive” heat (37,38) to limit production of potentially carcinogenic compounds generally forming at greater temperatures (>250°C) or when applied for longer periods (>1 hour) (39). Third, the importance of selecting unprocessed nutrients when possible cannot be overemphasized. For instance, AGE content in infant formula (Enfamil) is found to be 100-fold higher than in human or bovine milk

(40). Thus, since AGEs are known immune cell modulators (8), the introduction of infant diets, rich in AGE antigens, may account for the rise in childhood autoimmune diseases such as Type 1 Diabetes (T1D), as suggested in animal studies for this disease (41).

A limitation of the present data is reliance on CML, a single AGE marker, while many other AGEs/ALEs are generated in food (20,21), albeit of unknown significance. In practical terms, however, CML is a commonly measured AGE/ALE compound, used routinely as an indicator of the AGE/ALE burden in numerous animal and human studies (22-31,41).

The results presented herein are preliminary, and systematic food analyses are needed to reveal the chemical nature of pathogenic AGE/ALE substances. This report provides the rationale and the initiation point for a database to be used in clinical studies aiming to evaluate this newly identified dietary factor as a risk for diabetes and other chronic disorders. These findings also support reevaluation of contemporary meal patterns in the context of major health epidemics of today.

## CONCLUSIONS

This article reports on the high content of AGEs in commonly consumed foods, and notes that this is primarily the result of the dry-heat treatment of protein- and lipid-rich foods. This initial body of data can be used as a basis for the design of clinical studies to investigate the effects of manipulating dietary AGE intake to determine whether simple adjustments in the methods of food preparation can have a significant positive impact on health outcomes. Results of these studies will support reevaluation of contemporary dietary habits and enable development of meal pattern recommendations to enhance well-being and limit disease progression. A potential benefit of this knowledge regarding AGE sources is that it will enable individuals to reduce a previously unrecognized dietary risk factor that contributes to the pathologic sequelae seen in normal aging, diabetes, and kidney disease.

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