Diet-Derived Advanced Glycation End Products Are Major Contributors to the Body's AGE Pool and Induce Inflammation in Healthy Subjects

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ABSTRACT: Advanced glycation end products (AGEs) are a heterogeneous group of compounds that form continuously in the body. Their rate of endogenous formation is markedly increased in diabetes mellitus, a condition in which AGEs play a major pathological role. It is also known, however, that AGEs form during the cooking of foods, primarily as the result of the application of heat. This review focuses on the generation of AGEs during the cooking of food, the gastrointestinal absorption of these compounds, and their biological effects *in vitro* and *in vivo*. We also present preliminary evidence of a direct association between dietary AGE intake and markers of systemic inflammation such as C-reactive protein in a large group of healthy subjects. Together with previous evidence from diabetics and renal failure patients, these data suggest that dietary AGEs may play an important role in the causation of chronic diseases associated with underlying inflammation.

KEYWORDS: Maillard reaction; CRP; flow-mediated vasodilatation; endothelial function; nutrition

INTRODUCTION

Advanced glycation end products (AGEs) are a heterogeneous group of compounds with significant prooxidant and proinflammatory actions.[1,2] They form in the body under physiologic conditions produced by the reaction of reducing sugars and reactive aldehydes with the free amino group of proteins, lipids, and nucleic acids.[3] Their rate of endogenous formation is markedly enhanced in diabetes mellitus.[2] They also form externally during the heat processing of food.[4,5] The potential biological role of these exogenous AGEs has generally been ignored because of the assumption that they undergo negligible gastrointestinal (GI) absorption. Recently, however, it has become apparent that dietary AGEs contribute significantly to the

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body's AGE pool,[6–9] and may play a significant pathogenic role in a variety of disease states.[7–15] We will review formation of AGEs during the cooking of food, their GI absorption, and their biological effects. Finally, we will briefly discuss new information on dietary AGEs derived from studies on a large cross-section of healthy subjects.

FORMATION OF AGEs IN FOOD

Heat treatment of foods containing sugars and/or lipids and proteins may result in the formation of AGEs. This nonenzymatic reaction is also called the Maillard reaction in honor of the French chemist who examined the reaction between glycine and glucose in 1912.[4]

Several factors are known to affect AGE generation in foods, including nutrient composition, temperature, humidity, pH, and duration of cooking.[4,5] A large database with the contents of N^{ε}-carboxymethyl-lysine (CML), a commonly measured AGE, in more than 200 food items has recently become available.[5] The average AGE

TABLE 1. AGE content of selected food items[a]

Food item	AGE content
Fats	
Almonds, roasted	66.5 kU/g
Oil, olive	120 kU/g
Butter	265 kU/g
Mayonnaise	94 kU/g
Proteins	
Chicken, broiled (15 min)	58 kU/g
Chicken, fried (15 min)	61 kU/g
Beef, boiled (60 min)	22 kU/g
Beef, broiled (15 min)	60 kU/g
Tuna, roasted (40 min)	6 kU/g
Tuna, broiled (10 min)	51 kU/g
Cheese, American	87 kU/g
Cheese, brie	56 kU/g
Tofu, raw	8 kU/g
Tofu, broiled	41 kU/g
Carbohydrates	
Bread, whole wheat	0.5 kU/g
Pancakes, homemade	10 kU/g
Apples	0.13 kU/g
Bananas	0.01 kU/g
Carrots	0.1 kU/g
Green beans	0.2 kU/g

[a]Data from Goldberg *et al*.[5]
NOTE: AGE denotes CML-like immunoreactivity, assessed by ELISA (4G9mAb)

content for each food group classified as per the American Diabetes Association exchange lists is shown in TABLE 1, adapted from reference 5. In general, foods high in lipid and protein content exhibit the highest AGE levels; for example, the fat and meat groups contain 30- and 12-fold higher AGE content than the carbohydrate group, respectively.[5] Temperature and method of cooking appear to be more critical to AGE formation than time of cooking. This is evidenced by the higher AGE values of samples broiled or grilled at temperatures of 230°C for shorter cooking times when compared to samples boiled in liquid media at 100°C for longer periods.[5] Clearly, meat and meat-derived products, processed by high, dry heat such as in broiling, grilling, frying, and roasting are major sources of AGEs. Since these foods are commonly consumed in the USA, most people are constantly on a high-AGE diet. In fact, when we actually estimated the daily AGE consumption, based on analyses of three-day food records from 90 healthy subjects, the average value was 16,000 ± 5000 AGE kilo units/day (mean ± SD).

INTESTINAL ABSORPTION OF AGEs

The intestinal absorption of AGEs has long been demonstrated yet ignored because of its small magnitude.[16] Recent studies on human subjects, however, have shown a significant increase in plasma AGE levels within two hours following the oral administration of a single AGE-rich meal.[6] To address this issue further, normal Sprague-Dawley rats were fed single- and double-labeled AGE-modified protein diet preparations; within a few hours serum peaks of these radiolabeled AGEs were easily demonstrated.[17]

Several animal studies have shown a correspondence between dietary AGE content and serum and tissue AGE levels.[10–15] Moreover, studies in diabetics and renal failure patients have shown a significant association between dietary and circulating AGE levels.[7–9]

All the above data demonstrate that food-derived AGEs are directly absorbed into the circulation and contribute to the body's AGE pool. The exact mechanism for this absorption, however, remains unclear at present.

BIOLOGICAL EFFECTS OF FOOD-DERIVED AGEs

Food-derived AGEs induce protein cross-linking and intracellular oxidant stress similar to their endogenous counterparts when tested *in vitro* using human-derived endothelial cells.[18] These prooxidant and proinflammatory properties are also found in the circulating AGE fractions derived from these exogenous AGEs. For instance, low-density lipoprotein (LDL) samples obtained from diabetic subjects exposed to a high-AGE diet for several weeks led to a marked increase in MAPK phosphorylation, NF-κB activity, and VCAM-1 secretion when added to cultured human endothelial cells.[19] These results were in contrast to the response to LDL extracted from diabetic subjects under similar glycemic control but exposed to a low-AGE diet.[19]

Experiments performed in different animal models have established a significant role for dietary AGEs in inducing type 1 diabetes mellitus in nonobese diabetic (NOD)

mice,[10] insulin resistance in *db/db (+/+)* mice,[11] atherosclerosis in apoE-deficient mice,[12,13] diabetic nephropathy,[14] or wound healing in NOD and *db/db (+/+)* mice.[15] In a group of diabetic subjects, dietary AGE restriction was associated with significant reduction of two markers of inflammation, plasma C-reactive protein (CRP) and peripheral mononuclear cell TNF-α, as well as of VCAM-1, a marker of endothelial dysfunction.[7] These observations were later extended to chronic renal failure patients on maintenance peritoneal dialysis, in whom dietary AGE restriction was associated with a parallel reduction of serum AGEs and CRP.[8] The parallel changes of serum AGEs and CRP following dietary AGE modifications are highly suggestive of a role for dietary AGEs in inducing inflammation.

More recently, a group of diabetic patients underwent testing of flow-mediated vasodilatation (FMD) in response to the single oral administration of an AGE-rich beverage (without any glucose). Ninety minutes later, serum AGEs increased, while FMD response was markedly impaired.[20] These findings demonstrate an acute harmful effect of dietary AGEs on endothelial function.

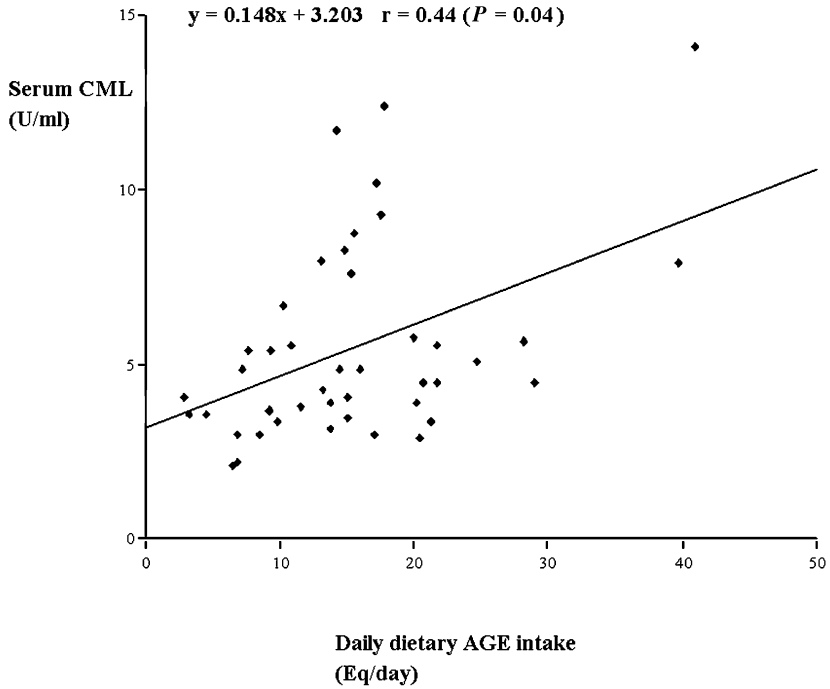

FIGURE 1. Association between dietary AGEs and serum AGE levels in healthy subjects. Fasting blood for measurement of AGEs and three-day food records for assessment of daily AGE intake were obtained in a cross-section of healthy subjects. Serum AGEs were measured by ELISA using a monoclonal antibody against CML, and dietary AGEs were estimated from a database with the dietary AGE content of a large number of food items.

STUDIES IN HEALTHY SUBJECTS

We recently performed a cross-section analysis of 90 healthy subjects. Demographic data, three-day food records, and fasting blood were obtained during the subjects' usual activity and diet. Subjects were given detailed instructions on how to record three-day food records, including cooking methods. A database with the AGE content of a large number of food items was used to estimate the AGE content of food.[5] AGEs were measured by ELISA using a monoclonal antibody against CML. We found significant correlation between dietary AGE content and serum AGEs (FIG. 1), and CRP.

A subgroup of five healthy subjects was exposed to short-term dietary AGEs restriction (daily AGE content reduced fourfold). This reduction of dietary AGEs intake was associated with an average decrease of serum AGE levels by 30–40%. These data match previous findings in diabetics and renal failure patients,[7–9] and imply a major quantitative contribution of dietary AGEs to the body's AGE pool. FIGURE 2 depicts our current understanding of the factors regulating circulating AGE levels.

In summary, the information reviewed above demonstrates that dietary AGEs, present in abundance in food commonly consumed in a "Western-style" diet, contribute significantly to the body's AGE pool. Moreover, these diet-derived AGEs have a significant association with indicators of inflammation in healthy subjects. Together with previous evidence in diabetics and renal failure patients, these data suggest that dietary AGEs may play an important role in the causation of chronic diseases associated with underlying inflammation. The findings also highlight the importance of consuming diets with low AGE content as a preventive measure.

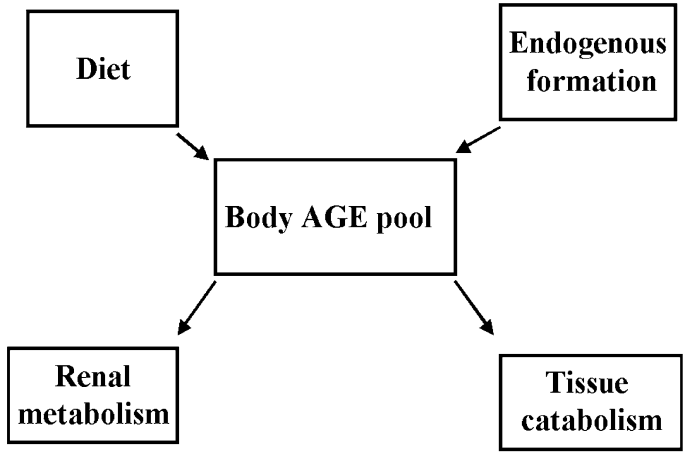

FIGURE 2. Factors determining circulating AGE levels: postulated factors regulating circulating AGEs levels in the body of human subjects. This diagram emphasizes the contribution of dietary AGEs to the body's AGE pool.

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