

Dietary Intake of Advanced Glycation End Products Did Not Affect Endothelial Function and Inflammation in Healthy Adults in a Randomized Controlled Trial^{1–3}

Richard D. Semba,^{4*} Sarah K. Gebauer,⁵ David J. Baer,⁵ Kai Sun,⁴ Randi Turner,⁴ Harry A. Silber,⁴ Sameera Talegawkar,⁶ Luigi Ferrucci,⁷ and Janet A. Novotny⁵

⁴Departments of Ophthalmology and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; ⁵USDA Beltsville Human Nutrition Research Center, Beltsville, MD; ⁶Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; and ⁷National Institute on Aging, Baltimore, MD

Abstract

When food is heated to high temperatures, the characteristic “browning” generates advanced glycation end products (AGEs). AGEs are associated with an increased risk of cardiovascular disease, diabetes, and other adverse outcomes. Whether dietary AGEs are absorbed and are harmful to human health remains highly controversial. The objective of this study was to compare the effects of a diet high or low in AGEs on endothelial function, circulating AGEs, inflammatory mediators, and circulating receptors for AGEs in healthy adults. A randomized, parallel-arm, controlled dietary intervention was conducted for 6 wk with 24 healthy adults, aged 50–69 y, that compared isocaloric, food-equivalent diets that were prepared at either high or mild temperatures. Peripheral arterial tonometry, serum and urine carboxymethyl-lysine (CML), inflammatory mediators (interleukin-6, C-reactive protein, vascular adhesion molecule-1, and tumor necrosis factor- α receptors I and II), soluble receptor for AGEs, and endogenous secretory receptor for AGEs were measured at baseline and after 6 wk of dietary intervention. In the low-AGE diet group, the following changed from baseline to 6 wk (mean \pm SE): serum CML from 763 ± 24 to 679 ± 29 ng/mL ($P = 0.03$) and urine CML from 1.37 ± 1.47 to 0.77 ± 2.01 μ g/mL creatinine ($P = 0.02$). There were no significant changes in serum and urinary CML concentrations from baseline to follow-up in the high-AGE diet group. A high- or low-AGE diet had no significant impact on peripheral arterial tonometry or any inflammatory mediators after 6 wk of dietary intervention. In healthy middle-aged to older adults, consumption of a diet high or low in AGEs for 6 wk had no impact on endothelial function and inflammatory mediators, 2 precursors of cardiovascular disease. This trial was registered at clinicaltrials.gov as NCT01402973. *J. Nutr.* 144: 1037–1042, 2014.

Introduction

Advanced glycation end products (AGEs)⁸ are possible dietary risk factors for adverse health outcomes (1). Major sources of systemic AGEs are endogenous AGEs generated in the body and

exogenous AGEs in foods. When food is heated to high temperatures, the characteristic “browning” generates Maillard reaction products, also known as AGEs. The Western diet is rich in AGEs that are formed when food is processed at high temperatures (2).

AGEs in food are absorbed in the gut, enter the circulation, are deposited in tissues, metabolized, and/or excreted in the urine. AGEs play a role in atherosclerosis by cross-linking collagen in vessel walls, oxidizing of LDL, and increasing inflammation through the receptor for AGE (RAGE) (3). RAGE is also found in the circulation in 2 major forms: 1) RAGE cleaved from the surface of cells; and 2) endogenous secretory RAGE (esRAGE) secreted by cells (4). Older adults with elevated serum carboxymethyl-lysine (CML), a well-characterized circulating AGE, have an increased risk of arterial stiffness (5,6), greater carotid diameter (7), and death (8–10).

Whether AGEs in foods represent a risk to human health remains highly controversial (11). A barrier to understanding

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³ Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

⁸ Abbreviations used: AGE, advanced glycation end product; BHNRC, Beltsville Human Nutrition Research Center; CML, carboxymethyl-lysine; CRP, C-reactive protein; esRAGE, endogenous secretory receptor for advanced glycation end product; RAGE, receptor for advanced glycation end product; RHI, reactive hyperemia index; sRAGE, secretory receptor for advanced glycation end product; TNF- α RI, TNF- α receptor I; TNF- α RII, TNF- α receptor II; VCAM-1, vascular cell adhesion molecule-1.

* To whom correspondence should be addressed. E-mail: rdsemba@jhmi.edu.

whether dietary AGEs are a health risk has been the lack of rigorously conducted, controlled clinical trials of dietary AGEs in healthy adults (12). Human trials involved single meals or oral challenges high in AGEs in patients with diabetes or renal failure (13–15). High dietary intake of AGEs was associated with elevations in inflammatory mediators in diabetics (16). A trial conducted in healthy college students compared diets that were high vs. low in AGEs (17). However, the diets were dissimilar in nutrient content and not isocaloric. Other dietary and lifestyle interventions may influence circulating AGEs (18,19).

Although AGEs have been implicated in many diseases, including atherosclerosis and cardiovascular disease, the impact of restricting dietary AGEs has not been well characterized in healthy adults. We hypothesized that dietary intake of AGEs would affect serum and urinary CML and have an impact on endothelial dysfunction and inflammation. To address these hypotheses, we conducted a controlled dietary intervention that compared the chronic effects of diets that were high vs. low in AGEs in healthy adults.

Participants and Methods

Study design and participants. The study was a randomized, parallel-arm, controlled 6-wk dietary intervention involving 24 community-dwelling adults, aged 50–69 y, at the USDA Beltsville Human Nutrition Research Center (BHNRC) in Beltsville, Maryland, in October and November 2011 (Fig. 1). The study was publicized by advertisements. Potential participants attended an information session at the BHNRC in which the study protocol and informed consent document were reviewed by the project coordinator (S.K.G). A Mini-Mental State Examination (20) was given to all potential participants. Participants who gave written, informed consent returned on a subsequent morning for screening tests that included blood pressure and BMI measurements, fasting venous blood samples, and a spot urine sample collection.

Individuals were eligible if they fit these criteria: 1) aged ≥ 50 and < 70 y; 2) non-smokers; 3) no history of diabetes, thyroid disease, cardiovascular disease, chronic obstructive pulmonary disease, inflammatory bowel disease, liver disease, chronic kidney disease, or Raynaud's disease; 4) not taking regular vitamin supplements or, if taking supplements, willing to discontinue taking supplements during the duration of the study; 5) not taking vitamin B-6 (pyridoxamine) (≥ 50 mg), a known AGE

TABLE 1 Nutrient composition of the study diet¹

	Unit/d
Weight, g	1229
Energy, kcal	1800
Protein, g	77
Carbohydrate, g	244
Fiber, g	13.4
Sugar, g	126
Total fat, g	59
Saturated fat	23.4
trans fat, g	0.9
Cholesterol, mg	485
Vitamin A, IU	7812
Thiamin, mg	1.02
Riboflavin, mg	1.56
Niacin, mg	16.9
Vitamin B-6, mg	1.27
Vitamin B-12, μ g	3.52
Biotin, μ g	26.6
Vitamin C, mg	46.6
Vitamin D, IU	127
Calcium, mg	933
Iron, mg	12.0
Sodium, mg	2211

¹ Diet composition was analyzed using The Food Processor (ESHA Research). The nutrient composition reported represents an example of an 1800 kcal diet. Participants were assigned to a calorie amount to meet their energy requirements.

inhibitor (21); 6) not taking aspirin or nonsteroidal anti-inflammatory medications or, if so, willing to discontinue taking them during the duration of the study; 7) BMI of ≥ 18.5 to < 30 mg/kg²; 8) no major food allergies; 9) no history of eating disorders or other dietary patterns different from the typical American diet; 10) no loss of $> 10\%$ of body weight within the past 12 mo or plan to initiate a weight loss program during the next 2 mo; 11) no medical, psychiatric, or behavioral factors that might interfere with study participation; 12) willing to consume the experimental diets; 13) Mini-Mental State Examination score of < 23 ; 14) TGs ≤ 300 mg/dL, hemoglobin > 11 g/dL, creatinine < 1.5 mg/dL, fasting glucose ≤ 125 mg/dL, and no proteinuria; and 15) gave written, informed consent.

Experimental diets. Participants were matched by sex and BMI (± 3 kg/m²) and randomly assigned to consume either diet using a computer random-number generator. The project coordinator assigned participants to interventions using a color-coded scheme to maintain blinding. Investigators and staff were unaware of the treatment through the intervention and during all analyses. Diets were prepared in the research kitchen of the BHNRC under the observation of a dietitian, research associate, or investigator. The kitchen staff was aware of the treatment assignment. Participants received breakfast and dinner in the dining room of the BHNRC, Monday through Friday. Lunch and weekend meals were provided as carryout for offsite consumption.

Diets consisted of 3 meals per day and 1 evening snack. Using a reference database for AGE content in foods (22), the high-AGE diet was ~ 4 times higher in AGEs than the low-AGE diet. Diets were either high or low in AGEs depending on how the same foods were prepared: 1) beef and chicken were either roasted in the oven at 204.4°C until brown or covered with foil (to prevent browning) and heated to 121.1°C; 2) shrimp was roasted in the oven or steamed; 3) bread products were toasted or untoasted; 4) ice cream was made with high temperature-treated evaporated milk or with regular milk; 5) nuts were toasted or served raw; and 6) croutons for salads were toasted brown or dried to prevent browning.

High- and low-AGE diets were isocaloric and nutrient equivalent (Table 1). Meals were designed using The Food Processor software

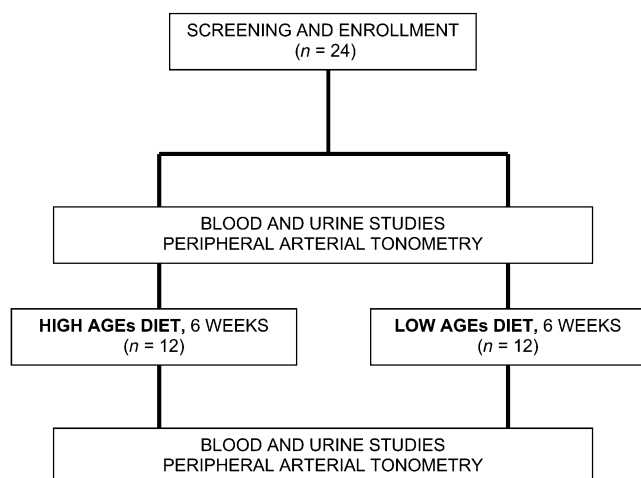


FIGURE 1 Flow diagram of the clinical trial. AGE, advanced glycation end product.

(ESHA Research). Diets comprised traditional American foods incorporated into a 7-d menu cycle. Participants were instructed to consume all and only foods provided by the BHNRC. Meals were fed in amounts that maintained body weight at enrollment. Participants were weighed daily, Monday through Friday, and if a consistent pattern of weight gain or loss were observed, adjustments were made to individual caloric intake [in 837 kJ (200 kcal) increments], if necessary, to keep participants at a steady weight. Participants were encouraged to maintain their normal exercise routine. Daily questionnaires were completed by participants to assess general health, use of over-the-counter medications, and diet and exercise over the past 24 h. The follow-up rate of the participants was 100%.

The Johns Hopkins University School of Medicine and MedStar Health Research Institute institutional review boards approved the protocol. This trial was registered at clinicaltrials.gov as NCT01402973.

Data collection. Demographic and health data were collected using standardized questionnaires at screening. All staff involved with phlebotomy, sample collection, measurement of endothelial function, and administration of questionnaires were unaware of the treatment. Endothelial function and serum and urinary biomarkers were measured on the first day of the 6-wk experimental diet period and the day after completion of the period. Participants were seen in the early morning after a 12-h fast. Endothelial function was measured using peripheral arterial tonometry (EndoPAT; Itamar Medical) (23). The same 2 technicians performed the test during the entire study, and technicians were assigned to do pre- and post-measurements on the same study participants following the protocol of the manufacturer (EndoPAT). Fasting venous blood samples were drawn twice during the study for analyte measurements in duplicate. All laboratory studies were run in a blinded manner. Total cholesterol, HDL cholesterol, TGs, and glucose were measured at enrollment and 6 wk. ELISA was used for measurement of serum and urine CML (Microcoat) (24,25), IL-6, secretory RAGE (sRAGE), vascular cell adhesion molecule-1 (VCAM-1), TNF- α receptor I (TNF- α RI), TNF- α receptor II (TNF- α RII) (all Quantikine; R&D Systems), high-sensitivity C-reactive protein (CRP) (Alpco), and esRAGE (B-Bridge). The interassay and intra-assay CVs for all analytes measured by ELISA were <5%. Plasma TGs and total cholesterol were measured by an enzymatic method (ABA-200 ATC Biochromatic Analyzer; Abbott Laboratories). HDL cholesterol was determined by a dextran sulfate-magnesium precipitation procedure (26). LDL cholesterol was estimated using the Friedewald formula (27). Serum glucose was measured by the glucose oxidase method (Beckman Coulter). Urine creatinine was measured using a colorimetric assay (Creatinine assay kit; Abcam).

Statistical analysis. Primary outcome measures were endothelial function, as indicated by reactive hyperemia index (RHI), and serum and urinary CML concentrations. Secondary outcome measures were CRP, IL-6, VCAM-1, TNF- α RI, TNF- α RII, lipids, fasting plasma glucose, sRAGE, and esRAGE. The sample size of 12 participants in a group gave >80% power to detect the following between the groups: 1) a priori 30% difference in the RHI, given mean RHI of 2.25, $\sigma = 0.58$; 2) 8% difference in urinary CML, given mean log-transformed urinary CML of 7.00, $\sigma = 0.45$; and 3) 3% difference in serum CML, given mean log-transformed serum CML of 6.62, $\sigma = 0.11$, and for all power calculations, $\alpha = 0.05$, and a 2-sided test.

Continuous variables were reported as means \pm SDs or least-square means \pm SEs. Spearman correlations were used to examine relations between variables at baseline. Both repeated-measures ANOVA and multiple linear regression that controls for within-participant repeated measures were used to examine the effects due to age, sex, diet, time, and their interaction. All analyses were conducted using SAS 9.1 (SAS Institute) with a type I error of 0.05.

Results

Characteristics of the study participants at baseline are shown in Table 2. There were no significant differences between the participants in 2 diet groups by age, sex, BMI, and systolic or diastolic blood pressure. Spearman correlations for selected

TABLE 2 Characteristics of the study participants at baseline by dietary group¹

Characteristic	Low-AGE diet (n = 12)	High-AGE diet (n = 12)
Age, y	57.9 \pm 6.0	60.6 \pm 4.3
Sex, %		
Male	41.7	41.7
Female	58.3	58.3
BMI, kg/m ²	26.4 \pm 4.0	26.1 \pm 3.4
Systolic blood pressure, mm Hg	130 \pm 18	126 \pm 13
Diastolic blood pressure, mm Hg	79 \pm 11	75 \pm 10

¹ Presented as means \pm SDs for continuous variables and percentages for categorical variables. AGE, advanced glycation end product.

variables at baseline are shown in Supplemental Table 1. Serum CML was positively correlated with age and negatively correlated with BMI. BMI was positively correlated with IL-6 and CRP. Serum VCAM-1 was positively correlated with TNF- α RI. There was a positive correlation between sRAGE and esRAGE. There were no significant correlations between the remaining variables.

Endothelial function, serum and urinary CML, markers of inflammation, lipids, fasting plasma glucose, and receptors for AGEs at enrollment and 6 wk are shown in Table 3. There were no significant differences in RHI, serum CML, urinary CML, IL-6, CRP, VCAM-1, TNF- α RI, TNF- α RII, total cholesterol, HDL cholesterol, LDL cholesterol, TGs, fasting plasma glucose, sRAGE, or esRAGE between the low- vs. high-AGE diet groups at baseline or the 6 wk follow-up visit. There was an 11% decrease in serum CML ($P = 0.03$) and 44% decrease in urinary CML ($P = 0.02$) from baseline to follow-up within the group that was fed a low-AGE diet. There were no significant changes in serum or urinary CML from baseline to follow-up in the group that was fed a high-AGE diet. There were no significant changes in any of the other measures from baseline to follow-up within each dietary group.

Discussion

The present study showed that, contrary to our original hypothesis, endothelial function after a 12-h fast was not affected by a diet that was high or low in AGEs in healthy adults. To our knowledge, this is the only trial that examined the chronic effects of meals that are high or low in AGEs on endothelial function in healthy adults. Healthy adults were the participants of this study, because modulation of dietary intake of AGEs has been discussed as a possible strategy in reducing risk of diabetes and cardiovascular disease (1). Endothelial function was measured using peripheral arterial tonometry, a tool that was validated (23) and applied for assessment of microvascular function in the Framingham Heart Study (28). Previous studies examined the effect of single meals on flow-mediated dilation in the postprandial period (14,15) and are not directly comparable with the present study. Participants who consumed low-AGE meals had a significant decrease in both serum and urinary CML concentrations by the 6-wk follow-up visit, which verifies that the relative restriction of AGE-rich foods had an effect in lowering circulating and excreted CML. The participants who consumed meals that were high in AGEs did not have a significant increase in serum and urinary CML concentrations by the 6-wk follow-up visit. The lack of an apparent increase may be due to the fact that the usual American

TABLE 3 Endothelial function, serum and urinary CML concentrations, markers of inflammation, and receptors for AGEs at baseline and after 6 wk of dietary intervention in 24 healthy adults, aged 50–69 y¹

Characteristic	Low-AGE diet (n = 12)	P within group, baseline to 6-wk low-AGE diet	High-AGE diet (n = 12)	P within group, baseline to 6-wk high-AGE diet	P between diet groups
Reactive hyperemia index		0.34		0.77	
Baseline	2.29 ± 0.17		2.22 ± 0.17		0.84
6 wk	2.03 ± 0.17		2.31 ± 0.17		0.31
Serum CML, ng/mL		0.03		0.28	
Baseline	763 ± 24		751 ± 24		0.79
6 wk	678 ± 29		711 ± 29		0.42
Urinary CML, μg/mg creatinine		0.02		0.56	
Baseline	1.37 ± 1.47		1.03 ± 1.47		0.21
6 wk	0.77 ± 2.01		1.21 ± 2.01		0.12
Serum IL-6, pg/mL		0.94		0.81	
Baseline	1.48 ± 0.53		2.25 ± 0.53		0.25
6 wk	1.53 ± 0.39		2.09 ± 0.39		0.39
Serum CRP, mg/L		0.60		0.93	
Baseline	2.11 ± 0.42		1.57 ± 0.42		0.39
6 wk	2.62 ± 0.65		1.38 ± 0.65		0.16
Serum VCAM-1, μg/mL		0.30		0.87	
Baseline	1.26 ± 0.23		1.39 ± 0.23		0.95
6 wk	1.01 ± 0.26		1.34 ± 0.26		0.22
Serum TNF-α RI, ng/mL		0.65		0.61	
Baseline	2.03 ± 0.09		1.96 ± 0.09		0.76
6 wk	1.93 ± 0.19		2.07 ± 0.19		0.52
Serum TNF-α RII, ng/mL		0.56		0.52	
Baseline	3.44 ± 0.29		2.94 ± 0.29		0.31
6 wk	3.17 ± 0.30		3.28 ± 0.30		0.85
Plasma total cholesterol, mg/dL		0.08		0.15	
Baseline	191 ± 13		196 ± 13		0.66
6 wk	221 ± 13		220 ± 13		0.96
Plasma HDL cholesterol, mg/dL		0.40		0.98	
Baseline	57 ± 4		67 ± 4		0.09
6 wk	61 ± 5		66 ± 5		0.37
Plasma LDL cholesterol, mg/dL		0.06		0.13	
Baseline	111 ± 11		110 ± 11		0.99
6 wk	141 ± 12		132 ± 12		0.66
Plasma TGs, mg/dL		0.41		0.59	
Baseline	114 ± 19		96 ± 19		0.53
6 wk	91 ± 15		110 ± 15		0.48
Fasting plasma glucose, mg/dL		0.64		0.64	
Baseline	97 ± 3		93 ± 3		0.61
6 wk	93 ± 3		96 ± 3		0.69
Serum sRAGE, ng/mL		0.77		0.74	
Baseline	1.42 ± 0.25		1.78 ± 0.25		0.21
6 wk	1.30 ± 0.19		1.91 ± 0.20		0.07
Serum esRAGE, ng/mL		0.80		0.78	
Baseline	0.52 ± 0.07		0.45 ± 0.07		0.48
6 wk	0.54 ± 0.07		0.48 ± 0.07		0.49

¹ Values expressed as least-square means ± SEs. P values were calculated using multivariate linear regression controlling for within-participant repeated measures that examined effects due to age, sex, diet, time, and interaction of diet × time. AGE, advanced glycation end product; CML, carboxymethyl-lysine; CRP, C-reactive protein; esRAGE, endogenous secretory receptor for AGE; sRAGE, secretory receptor for AGE; TNF-α RI, TNF-α receptor I; TNF-α RII, TNF-α receptor II; VCAM-1, vascular cell adhesion molecule-1.

diet contains AGEs in amounts similar to what was fed in the high-AGE diet. The present study corroborates the inverse relation between BMI and serum CML that was described previously (29). Serum CML may be affected by body fat possibly because CML is preferentially deposited in adipose tissue or because adipocytes affect the metabolism of AGEs (29).

The study is limited in that the AGE content of the experimental diets was estimated using a database for foods that was based on an antibody to CML rather than the gold-

standard HPLC analysis (22). Another limitation is that the AGE composition of the individual meals was not measured using HPLC.

A recent review of trials involving AGE-restricted dietary interventions showed that most trials had limited generalizability and involved dietary interventions of <6 wk duration (12). In addition, the majority of trials were undertaken by the same research group, which may have introduced “similar methodologic constraints into the majority of the studies conducted on

this topic" (12). Many of the trials had poor methodologic quality because of multiple shortcomings, such as not keeping researchers unaware of treatment, inadequate randomization, no provision of sample size and power calculations for main outcomes, no mention of dropouts or withdrawals from the trials, and lack of an intention-to-treat analysis (12).

The present study showed that a diet high or low in AGEs did not influence circulating inflammatory mediators after 6-wk duration. We were unable to corroborate a previous study in which large changes in serum CRP and VCAM-1 concentrations were found in a small sample of adults with diabetes on a high- or low-AGE diet for 6 wk (16). In the present study, we did not measure plasma TNF- α directly, because TNF- α is relatively labile to storage, handling, and freeze thaw (30). Instead, we measured TNF- α RI and TNF- α RII, which can reflect activity of TNF- α (31). A high- or low-AGE diet had no apparent effect on TNF- α RI and TNF- α RII concentrations.

Circulating RAGE and esRAGE were hypothesized to act as decoy receptors by binding with circulating AGEs and thus prevent direct binding of AGEs with cell-surface RAGE (1). Whether modulation of dietary intake of AGEs would influence circulating RAGE and esRAGE is unknown. The present study suggests that a diet high or low in AGEs did not affect circulating RAGE and esRAGE.

The strengths of the present study include the standardized preparation of meals, the control of food intake during the intervention, the nutritional and dietary equivalence of the 2 types of meals that differed only in the temperature used to process the food, and the 100% follow-up rate. Rigorous randomization was undertaken, both groups were comparable at baseline, strict inclusion and exclusion criteria of participants was followed, and study and laboratory staff involved in data collection were unaware of the dietary allocation. To our knowledge, this is the only rigorous, highly controlled dietary intervention in healthy adults that has measured the effects of chronic consumption of high- or low-AGE diet on markers of cardiovascular disease risk. The findings from this study suggest that consumption of a diet high or low in AGEs does not affect endothelial function and inflammation, 2 precursors of coronary artery atherosclerotic disease. It is also possible that any physiologic processes related to dietary AGEs are slow, small, and not detectable by 6 wk but may have a cumulative health impact over many years. Another possibility is that the effects of high dietary AGEs on endothelial function are only seen in the postprandial rather than fasting state. The rate of clearance of dietary AGEs is not well understood. Studies in rats and humans show that urinary AGE concentrations are significantly correlated with dietary AGE intake (32,33). Nonetheless, the lack of measurable effect of dietary AGEs in this intervention demonstrates that, if dietary AGEs were to affect inflammation or endothelial function, the impact of AGEs over the longer term is likely at most small. The results of this study cannot necessarily be generalized to other study populations.

In conclusion, to our knowledge, this paper describes the most rigorously conducted study to date on the impact of dietary AGEs on biomarkers of health. The results support minimal if any effect of dietary AGEs on endothelial function and inflammation, 2 important indicators of health and underlining processes of cardiovascular disease that were implicated previously as targets of negative actions of AGEs.

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research; D.J.B., J.A.N., L.F., R.D.S., and H.A.S. provided essential materials; K.S. analyzed the data; R.D.S., S.K.G., D.J.B., K.S., R.T., H.A.S., S.T., L.F., and J.A.N. wrote the paper; R.D.S., L.F., and J.A.N. had primary responsibility for the final content. All authors read and approved the final manuscript.

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