Dietary Advanced Glycation End Products and Their Role in Health and Disease[1,2]

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ABSTRACT

Over the past 2 decades there has been increasing evidence supporting an important contribution from food-derived advanced glycation end products (AGEs) to the body pool of AGEs and therefore increased oxidative stress and inflammation, processes that play a major role in the causation of chronic diseases. A 3-d symposium (1st Latin American Symposium of AGEs) to discuss this subject took place in Guanajuato, Mexico, on 1–3 October 2014 with the participation of researchers from several countries. This review is a summary of the different presentations and subjects discussed, and it is divided into 4 sections. The first section deals with current general knowledge about AGEs. The second section dwells on mechanisms of action of AGEs, with special emphasis on the receptor for advanced glycation end products and the potential role of AGEs in neurodegenerative diseases. The third section discusses different approaches to decrease the AGE burden. The last section discusses current methodologic problems with measurement of AGEs in different samples. The subject under discussion is complex and extensive and cannot be completely covered in a short review. Therefore, some areas of interest have been left out because of space. However, we hope this review illustrates currently known facts about dietary AGEs as well as pointing out areas that require further research. *Adv Nutr* 2015;6:461–73.

Keywords: nutrition, oxidative stress, inflammation, insulin resistance, RAGE, nutraceutical

Introduction

In October 2014 a meeting on the potential role of dietary advanced glycation end products (AGEs)[14] took place in Guanajuato, Mexico. Increasingly, it has become evident that food-derived AGEs make a substantial contribution to the systemic burden of AGEs and therefore predispose individuals to oxidative stress (OS) and inflammation, which play a major role in the causation of chronic diseases. A group of international experts in different areas of AGE research from the United States, Spain, Mexico, Brazil, Argentina, and Chile presented their work and discussed extensively what is currently known and what areas of research need to be emphasized to increase our understanding of the role of dietary AGEs in health and disease. Herein, we present a summary of the main presentations and discussions that took place during this 3 d symposium. This review is divided into the following 4 sections: *1*) general background on AGEs; *2*) molecular pharmacology of AGEs, in which we discuss how AGEs cause pathologic effects in the body, with particular attention to the receptor for advanced glycation end products

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[14] Abbreviations used: ACE, angiotensin-converting enzyme; AGE, advanced glycation end product; AQC, 6-aminoquinonyl-*N*-hydroxysuccinimidyl-carbamate; CKD, chronic kidney disease; esRAGE, endogenous secretory RAGE; OPA, ortho-phthalaldehyde; OS, oxidative stress; RAGE, receptor for advanced glycation end products; sRAGE, soluble receptor for advanced glycation end products.

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(RAGEs) as well as the potential role of AGEs in neurodegenerative diseases; *3*) tools to prevent AGE accumulation in the body, discussing the role of the diet, including results of clinical trials and physical exercise in reducing AGEs and the potential use of plant-derived products as antiglycative drugs, with special emphasis on agroindustrial by-products; and *4*) AGE analysis, dealing with measurement of AGEs, with special attention to current methodology issues in measuring these compounds in different body fluids and in foods.

General Background on AGEs

AGEs are a large and heterogeneous group of compounds that originate from the spontaneous reaction between reducing sugars and free amino groups in amino acids. This is the classic "Maillard reaction," but we know now that AGEs also can be generated by a variety of other reactions, including the oxidation of sugars, lipids, and amino acids, to create reactive aldehydes that covalently bind to proteins (**Figure 1**). Two commonly measured and well-described AGEs are carboxymethyllysine and methylglyoxal derivatives.

AGEs are important in clinical science because they are associated with OS and inflammation, processes that eventually cause most chronic diseases, including cardiovascular disease, diabetes, chronic kidney disease (CKD), and neurodegenerative diseases. Of note, AGEs cause OS but OS also leads to AGE formation (1).

AGEs were first recognized as endogenous compounds that formed in excess in diabetes due to hyperglycemia (2). It is now clear that they can also be generated in conditions of increased OS, even in the absence of hyperglycemia. Moreover, increasing evidence points to exogenous AGEs (derived mostly from food and tobacco) as important contributors to the body's AGE pool, where they become indistinguishable from endogenous AGEs, both in structure and function (3).

The main factors determining the rate of AGE formation in food include nutrient composition (protein > fat > carbohydrate), temperature and duration of heat application, humidity, pH, and the presence of trace metals (4, 5). Therefore, different cooking methods can substantially affect the AGE content of food without necessarily changing the nutrient composition. For example, using the same amount of chicken, but treating it with different cooking methods for the same length of time, will produce very different AGE content. In general, animal-derived foods cooked at a high temperature for a prolonged period of time and under dry conditions will have the highest AGE content (4, 5).

Molecular Pharmacology of AGEs

AGEs induce pathology by 2 main mechanisms (1). First, AGEs may crosslink proteins, directly altering their structure and therefore their properties and function. Second, AGEs activate intracellular signals through several receptor- and non-receptor-mediated mechanisms, leading to an increased production of reactive oxygen species and inflammatory cytokines. One of the best-studied AGE receptors is RAGE (6, 7).

AGEs are broken down in the body by enzymatic degradation and receptors and then eliminated by the kidneys. Major degradative enzymes include the glyoxalase I and II system. Advanced glycation end product receptor 1 (AGER1) is an AGE receptor that binds, internalizes, and degrades AGEs (8).

RAGE. The biology of RAGE is summarized in **Figure 2**.

Besides recognizing AGEs, RAGE also binds a large number of different ligands, including proinflammatory cytokine-like mediators of the S100/calgranulin family and high-mobility group protein B1, a nuclear protein that is released upon cell necrosis and, when extracellular, can exert

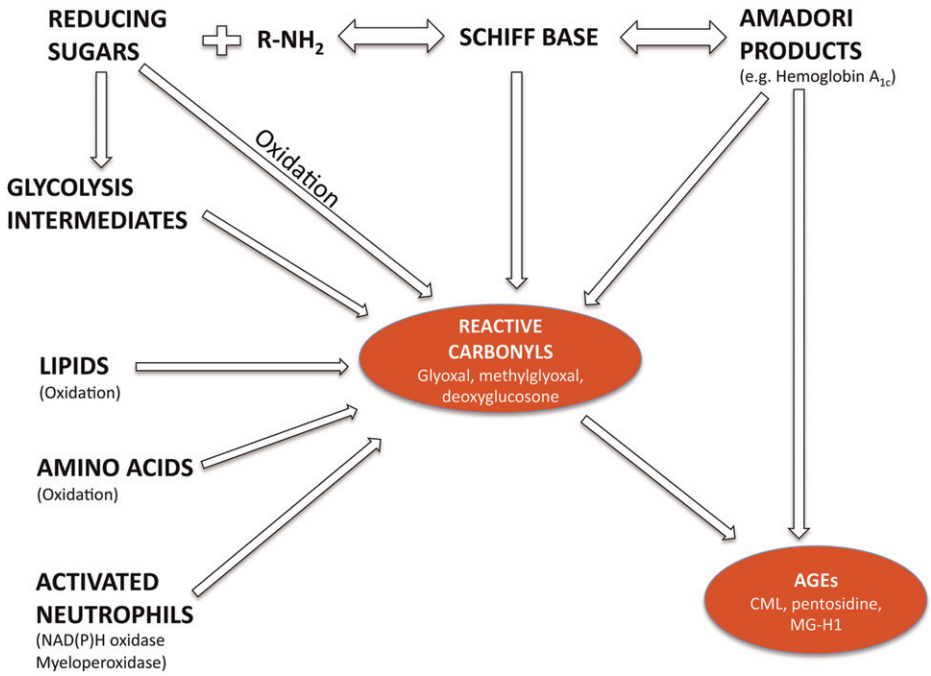

FIGURE 1 Different pathways of AGE formation. This figure schematically depicts the traditional Maillard reaction leading to AGE formation through the initial reaction between reducing sugars and the free amino group of a protein going through the stages of Schiff base and Amadori product formation. The figure also illustrates the many other different pathways that may lead to the formation of AGEs, even in the absence of glucose. AGE, advanced glycation end product; CML, carboxymethyllysine; MG-H1, methylglyoxal-derived hydroimidazolone 1.

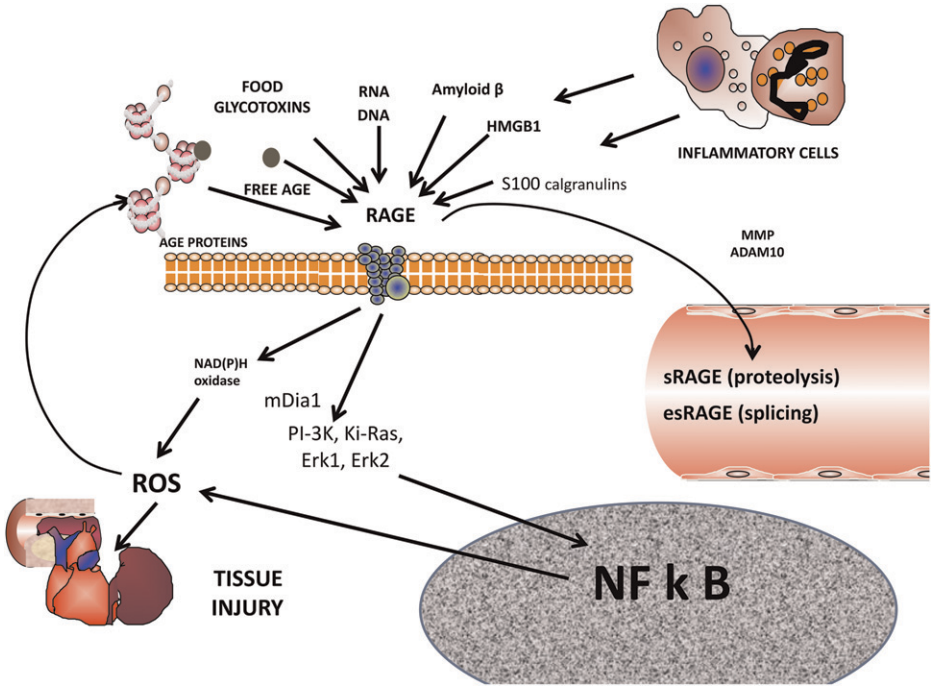

FIGURE 2 RAGE, its ligands, and its main signaling pathways enhance oxidative stress. RAGE is a pattern-recognition protein belonging to the immunoglobulin superfamily of receptors. RAGE recognizes a diversity of ligands encompassing endogenous and food-derived AGEs, DNA, RNA, amyloid fibrils, HMGB1, the leukocyte integrin Mac-1, S100 calgranulins, and modified LDL. Upon activation, RAGE activates NADP oxidase and signaling with activation of NF-κB inducing oxidative stress and inflammation. RAGE increases ROS via NAD(P)H oxidase. RAGE also signals via PI-3K, Ki-Ras, and Erk1 and Erk2. These pathways stimulate the translocation of NF-κB from the cytoplasm to the nucleus in a coordinated manner. Activation produces inflammation and tissue injury sustained by a RAGE-dependent expression of proinflammatory mediators such as MCP-1 and VCAM-1. sRAGEs that bear the ligand-binding domains are present in the circulation and may act as decoy molecules or be surrogate biomarkers. ADAM10, a disintegrin and metalloprotease domain–containing protein 10; AGE, advanced glycation end product; Erk1, extracellular signal-regulated kinase 1; Erk2, extracellular signal-regulated kinase 2; HMGB1, high-mobility group box 1; Ki-Ras, Kirsten rat sarcoma viral oncogene homolog; MCP-1, monocyte chemoattractant protein 1; mDial1, mammalian diaphanous 1; MMP, matrix metalloproteases; PI-3K, phosphatidylinositol-3 kinase; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; sRAGE, soluble receptor for advanced glycation end products; VCAM-1, vascular cell adhesion molecule 1.

proinflammatory activities (6, 7), among many others. Therefore, RAGE, despite its name, is now considered to be a multiligand receptor, playing a key role in inflammatory processes. Although RAGE is expressed at basal levels in healthy tissues, its expression is markedly augmented by a classic amplification loop through the sustained activation of NF-κB and triggered by the increased concentrations of ligands found in pathologic states (9).

RAGE is classified as a pattern-recognition receptor because of its ability to engage classes of molecules rather than individual ligands, in addition to the fact that it shares not only ligands but also signaling pathways with some members of the toll-like receptor family. Pattern-recognition receptors are involved not only in identifying nonself-microbial products such as pathogen-associated molecular patterns, but also "danger" signals (danger-associated molecular patterns—also known as alarmins—from the host that foretell of changes in homeostasis). Of note, many RAGE ligands are now classified as pathogen-associated molecular patterns or danger-associated molecular patterns. A growing body of evidence suggests that RAGE has a substantial role in innate immunity (10).

The availability of a RAGE knockout mouse model has contributed substantially in highlighting the role of RAGE in innate immunity. Through the use of different in vivo models of infection, it has been demonstrated that the inflammatory response, as well as the infiltration of immune cells, is markedly reduced in this mouse model. Additionally, the pathogen dissemination in several organs in a model of peritonitis in RAGE knockout mice was significantly increased (11, 12). This clearly indicates that RAGE knockout produces a dysfunctional innate immune response. It has been demonstrated that RAGE is able to recognize a counter-receptor on the surface of *Helicobacter pylori* favoring not only the adhesion of this pathogen to gastric epithelial cells, but also the inflammatory response triggered by the infection (13). RAGE-targeted knockout significantly reduced adherence and the inflammatory response. These findings are particularly interesting because several lines of evidence, derived from both epidemiologic studies and basic research, have shown that organ-specific carcinogenesis is linked to the development of a chronic local inflammatory milieu (14) as reported for *H. pylori*–induced gastric inflammation and the occurrence of gastric cancer.

Rudolph Virchow first launched the idea about a putative connection between inflammation and cancer in the 19th century. Inflammatory responses play decisive roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis. Immune cells that infiltrate tumors engage in an extensive and dynamic crosstalk with cancer cells in the tumor microenvironment. In this context, RAGE has emerged as a

relevant element that can continuously fuel an inflammatory milieu within the tumor microenvironment. Of note, RAGE ligands are expressed and secreted by cancer cells, as well as by many immune cells within the tumor microenvironment, and interact in an autocrine manner with all these cell types, promoting proliferation, invasion, angiogenesis, and metastasis (15). Additionally, tumors rely primarily on anaerobic metabolism and show a higher rate of glucose uptake and glycolysis, thus leading to higher local formation of AGEs.

Soluble RAGE. In the circulation, there are also soluble forms of the receptor, known as soluble RAGE (sRAGE). The C-terminal truncated form of RAGE mRNA lacks the sequences encoding the transmembrane and cytoplasmic domains (16). The extracellular domain of RAGE produced thereby, as shown in Figure 2, is released from the cells to the circulation (17). The latter has been called endogenous secretory RAGE (esRAGE), and it may play a role in cardiovascular disease (18–20). This esRAGE cancels the effects of AGEs in cultured cells. esRAGE overexpression in mice reverses diabetic vascular dysfunction, suggesting that esRAGE can act as a decoy. A feedback mechanism has been proposed that suggests that esRAGE prevents RAGE signaling and therefore the harmful effects of its activation (18, 21, 22). Other isoforms of sRAGE that may act as decoy receptors are proteolytic fragments of RAGE expressed on the cell surface. Therefore, various types of human sRAGE in plasma could exert protective effects against RAGE-mediated toxicity (22). However, endogenous sRAGE function is far more complex than just a putative decoy against RAGE signaling. For example, in a model of arthritis induced by high-mobility group protein B1, sRAGE acts as a proinflammatory and chemotactic molecule (23).

Because RAGE and esRAGE may be involved in feedback regulation of the toxic effects of RAGE-mediated signaling, recent clinical studies have focused on the potential importance of sRAGE and esRAGE in the circulation in a variety of pathophysiologic conditions. ELISA tests are available to assess concentrations of these circulating forms of RAGE, and multiple studies have been conducted in this area. For example, sRAGE concentrations have been shown to increase after a stroke depending on the clinical evolution (24), and they could potentially become promising prognostic markers. The finding of elevated concentrations of low molecular weight AGEs along with higher sRAGE concentrations in neonates compared with adults supports the concept of sRAGE as a decoy molecule (A Gugliucci, unpublished results, 2014). An inverse correlation has been shown to exist between circulating concentrations of sRAGE and oxidized LDL (malondialdehyde-modified LDL), raising the hypothesis that part of the antiatherosclerotic effects of sRAGE may be related to oxidized LDL quenching (25). Total sRAGE concentrations were shown to be substantially lower in nondiabetic patients with angiographically documented coronary artery disease than in healthy controls of the same age (21). A review of the literature, however, shows that the current evidence regarding the association between sRAGE and vascular disease in diabetes is contradictory with at least one study showing that sRAGE concentrations are positively associated with coronary artery disease in patients with type 1 diabetes (21). Similarly, findings from studies of sRAGE concentrations in cardiovascular disease are also contradictory with reports showing either positive or negative associations (26, 27). If sRAGE indeed plays a defensive role, diets with high concentrations of AGEs might elicit a response in terms of sRAGE concentrations in plasma; however, this has not been reported in the literature. A pilot study conducted to explore postprandial changes in sRAGE after a standard meal showed no significant change, although fasting sRAGE concentrations correlated with the AUC for glucose during an oral glucose tolerance test ($r = 0.69$, $P = 0.029$) (A Gugliucci, unpublished results, 2014). This would suggest adaptation of sRAGE to glycemic loads, but the latter results have to be interpreted with caution, because this was a pilot study with only 10 subjects.

Modulation of sRAGE/RAGE by current therapies. Several currently available pharmacologic agents may regulate esRAGE or sRAGE. Inhibitors of angiotensin-converting enzyme (ACE) increase renal expression of sRAGE in rats, and this is associated with decreases in the expression of RAGE (28). ACE inhibition in diabetic rats and in humans with type 1 diabetes significantly increases sRAGE concentrations (28). Thus, an attractive scenario is that the protective effect of ACE inhibition against progression of renal dysfunction is mediated via regulation of RAGE compared with sRAGE. Other potential agents that may affect circulating sRAGE are thiazolidinediones and statins. Thiazolidinediones might be promising drugs for increasing circulating concentrations of esRAGE and sRAGE (29).

Much remains to be investigated with respect to the role of sRAGE in cardiovascular and metabolic disease; this role may be either active or just a valuable surrogate marker. Moreover, the protective effect may not solely rely on its neutralization of toxic AGEs. As shown in Figure 1, other endogenous ligands of RAGE, such as calgranulin S100A12, can also be involved in the function of sRAGE. The field is wide open for further research on the role of sRAGE as a marker of disease, as well as on its pathophysiologic role.

AGE involvement in neurodegenerative diseases. As reviewed (30) and prompted by recent data (31), AGEs accumulate in an age-related fashion in the brain and other organs of the central nervous system of individuals in many different neurodegenerative diseases, including Alzheimer, Parkinson, and other less common diseases, such as Pick disease, the prionopathies, and amyotrophic lateral sclerosis (32). AGEs accumulate in the target tissue in areas with marked pathology (e.g., the hippocampal regions in Alzheimer, substantia nigra in Parkinson, and ventral spinal cord in amyotrophic lateral sclerosis). Whenever possible, these AGEs should be evidenced by both immunologic and chemical methods (e.g., MS evidence) and further complemented by

proteomic approaches. For instance, in argyrophilic grain dementia, carboxymethyllysine accumulation does not show a significant increase when total proteins are evaluated (33). However, when targets of this modification were identified by 2-dimensional electrophoresis coupled with Western blot and peptide finger printing, several key enzymes, such as isoforms of fructose bisphosphate aldolase, or structural proteins, such as phosphatidyl-ethanolamine binding protein, were clearly more modified in the pathologic situation than in controls. Thus, besides global AGE amounts, information on specific AGE proteome hotspots is also relevant.

Interestingly, in most neurodegenerative processes evaluated, changes in AGE concentrations are found not only in the target locations, but also in anatomically distant regions. For example, in Pick dementia or in Parkinson disease, pathologically spared regions (such as the occipital cortex in the former or parietal cortex in the latter) also show increased glycoxidative damage (34). Thus, in spite of morphologic neuronal conservation, AGE formation takes place in several neuronal regions of the affected individuals in comparison with healthy age- and gender-matched individuals. This suggests that biochemical abnormalities leading to AGE build-up are present along many different tissues and locations in the central nervous system. A naive interpretation would be that cells with a lower capacity of response to AGE generation (i.e., lower protein turnover—either proteasomal or autophagic—or lower reductase or glyoxalase activities for clearing AGE precursors) would be those with a higher propensity for AGE accumulation and therefore dysfunction.

Obtaining qualitative information on which products of the Maillard reaction are formed may also help to understand the source of the AGEs being formed. Measuring carboxymethyllysine, as a glyoxal derivative, or carboxyethyllysine, as a methylglyoxal derivative, is important in order to evaluate the relative contribution of these AGE precursors to the progression of neurodegeneration. Importantly, methylglyoxal and its derivatives, such as carboxyethyllysine, are considered to be by-products of glycolysis (35). Therefore, lower levels of glycolysis flux, such as that present in Alzheimer and other diseases, could lead to lower values of methylglyoxal and, potentially, lower steady-state concentrations of methylglyoxal-derived AGEs.

A number of studies have focused on the potential implications of methylglyoxal and glyoxal, even of exogenous (dietary) origin, in neurodegeneration. Kuhla et al. (36) have observed no major differences in these AGE precursors in cerebrospinal fluid when comparing patients with Alzheimer disease with patients without dementia. However, experimental evidence in murine models demonstrates that glyoxalase I is overexpressed in the temporal cortex pyramidal neurons of P301L transgenic mice (37). This suggests that either endogenous or exogenous AGE precursors reach neurons and glial cells, potentially exerting their deleterious effect. Interestingly, recent data show that methylglyoxal, which is bioavailable from diet, can lead to an increase in the permeability of human brain endothelial cells by its effect on catenin and claudin-5 subcellular distribution. Therefore, dietary AGEs or their precursors could lead to a loss of selective permeability of the blood-brain barrier. Indeed, recent data support this latter hypothesis (31). The evaluation of animal models exposed to a high methylglyoxal diet, as well as the longitudinal assessment of cognition in elderly individuals, strongly suggests that glycotoxins could contribute to loss of cognition. In these experiments, the authors showed that methyglyoxal is inversely related to sirtuin 1 mRNA levels, suggesting that dietary AGEs could inflict their effects through decreased sirtuin 1 expression, which has been shown to increase production of β-amyloid and plaques via a disintegrin and metalloprotease domain–containing protein 10 in a mouse model of Alzheimer disease (38).

Clearly, more work is needed to discriminate the role of dietary AGEs or AGE precursors in neurodegeneration, but the present data support the hypothesis of their implication in neuronal dysfunction. The fact that metabolic factors (e.g., insulin resistance) are now recognized as relevant in neurodegeneration contributes to strengthening this relation (39). Last, but not least, dietary AGE concentrations can be monitored, so further studies can be performed, either retrospectively or prospectively, to potentially control the effect of a modifiable factor (e.g., dietary habits) in neurodegeneration.

Tools to Prevent AGE Accumulation in the Body

The role of the diet. Two large databases with the content of carboxymethyllysine, a commonly measured AGE, in >500 food items are now available to estimate daily dietary AGE intake, as well as to design diets with variable AGE content (4, 5). As mentioned before, foods rich in both protein and fat, mostly of animal origin, and cooked at high and dry heat, such as in broiling, grilling, frying, and roasting, tend to be the richest dietary sources of AGEs, whereas low-fat, carbohydrate-rich foods tend to be relatively low in AGEs. AGEs in food are generated not only from the Maillard reaction, but also by interactions between oxidized lipids and protein, reactions that may give rise to AGEs such as carboxymethyllysine.

Knowledge about the factors determining the amount of AGEs in foods allows us to use culinary techniques that reduce food AGE content without necessarily changing the type and quantity of foods consumed (**Table 1**). The essential concept when introducing a low AGE diet is that it is the manner of cooking, not the actual nutrient composition of the food, that determines its AGE content. For example, stewing or steam-cooking meat, which maintains food moisture during cooking, will generate much less AGE than broiling or frying. Marinating will also have an AGE-reducing effect by lowering the local pH of food (pH effect of lemon or vinegar).

The results from published data describing the effects of introducing a low-AGE diet, as described above, for variable periods of time to different populations in different countries (40–48) are summarized in **Table 2**. Healthy subjects young and old, including patients with diabetes but not

TABLE 1 Examples of high- and low-AGE–containing foods[1]

High-AGE		Low-AGE	
Food item	**AGEs**	**Food Item**	**AGEs**
Beef (fried)	9522	Beef (stewed)	2443
Chicken (roasted)	5975	Chicken (boiled)	2232
Beef, frankfurter (broiled)	10143	Beef, frankfurter (boiled)	6736
Lamb (broiled)	2188	Lamb (boiled)	1096
Salmon (broiled)	3012	Salmon (poached)	2063
Potato (white, French fried)	694	Potato (white, boiled)	17

[1] AGE content is expressed in arbitrary AGE kilounits per 90 g serving for meats and 100 g serving for potatoes. AGE values are taken from the tables in reference 5. AGE, advanced glycation end product.

CKD, and patients with CKD but not diabetes, respond to this intervention with a substantial drop in circulating AGE concentrations. With the exception of one study (46), this reduction in serum AGE concentrations is accompanied by a simultaneous reduction in markers of inflammation, oxidative stress, and endothelial dysfunction. Moreover, in patients with insulin resistance, the low-AGE diet actually improves insulin sensitivity as assessed by HOMA (43, 47).

More recently, a randomized trial comparing sevelamer with calcium carbonate in a group of diabetic patients with CKD showed that treatment with sevelamer, which binds AGEs in vitro, reproduced all the previous findings observed on the low dietary AGE intervention, namely, reduced circulating concentrations of markers of AGEs, OS, and inflammation (49).

Local investigators from Mexico and a group from Chile are currently involved in the design and/or performance of new clinical trials studying the effect of AGE-restricted diets and pharmacologic interventions to limit their intestinal absorption. This raises the important issue that food sources of AGEs would be different in different countries. Currently, the most widely used database with food carboxymethyllysine content measured by ELISA was created in New York City. Through the use of this database, the average dietary AGE intake in a cohort of healthy adults from the New York City area was found to be 14,700 ± 680 AGE kilounits/d (5). Based on this information, a tentative definition of a high- or low-AGE diet will depend on whether the estimated daily AGE intake is significantly greater or less than 15,000 AGE kilounits. The need to generate new data within the content of carboxymethyllysine and other AGEs in other populations with different local foods cannot be overemphasized.

Improving the estimation of dietary AGEs from food questionnaires will facilitate future clinical trials. Measuring dietary variables always has been an approximate endeavor, because bias exists in both study subject and study observer. Measuring dietary AGEs adds difficulty to the task, because dietary AGEs are highly dependent on cooking methods, which are not usually defined in traditional food records. There are several instruments for measuring dietary intake. The 7 d food record with weighing provides a reasonably accurate method of actual intake, but it is very time consuming for participants (50). The FFQ is a good alternative to a food record because it decreases the participant's time burden, and it is very useful when measuring a specific dietary component. The list of foods used in this case should include foods with higher amounts of the nutrient of interest or with moderate amounts, but consumed in larger amounts by the population of interest (51). Even though designing and validating a FFQ specific to AGEs could be time consuming, having a reliable and valid FFQ will increase the accuracy of associations between dietary AGEs and diabetes or other related health issues, decrease participant burden during studies, and decrease analysis time of food records. Thus, a FFQ could help to assess dietary AGEs in a time- and cost-saving manner.

Luevano-Contreras et al. (52) designed a FFQ specific to dietary AGEs and evaluated its reliability and validity. For the design, data from the usual intake of 30 subjects was used. The final instrument included 90 food items, and it measured intake for the past year. To assess reliability and

TABLE 2 Clinical trials with an AGE-restricted diet[1]

Author, year (reference)	Study population	Trial design	Results
Vlassara et al., 2002 (40)	Diabetes (United States)	Crossover	Decrease sAGEs and markers of OS and inflammation
Uribarri et al., 2003 (41)	ESRD, no diabetes (United States)	2 parallel groups (high- and low-AGE)	Decrease sAGEs and markers of inflammation
Vlassara et al., 2009 (42)	Healthy and CKD, no diabetes (United States)	2 parallel groups (high- and low-AGE)	Decrease sAGEs and markers of OS and inflammation
Uribarri et al., 2010 (43)	Diabetes (United States)	2 parallel groups (high- and low-AGE)	Decrease sAGEs, OS, inflammation, and HOMA
Birlouez-Aragon, et al., 2010 (44)	Healthy (France)	2 parallel groups (high- and low-AGE)	Decrease sAGEs and HOMA
Luevano-Contreras et al., 2013 (45)	Type 2 diabetes (Mexico)	2 parallel groups (high- and low-AGE)	Decrease sAGEs and weight
Semba et al., 2014 (46)	Healthy (United States)	2 parallel groups (high- and low-AGE)	Decrease sAGEs but no change in endothelial function and inflammation
Mark et al., 2014 (47)	Overweight women (Denmark)	2 parallel groups (high- and low-AGE)	Decrease urinary AGEs and HOMA
Macias-Cervantes et al., 2015 (48)	Overweight or obese men (Mexico)	3 parallel groups (diet + exercise)	Decrease sAGEs and weight

[1] AGE, advanced glycation end product; CKD, chronic kidney disease; ESRD, end-stage renal disease; OS, oxidative stress; sAGE, soluble advanced glycation end product;

validity, 20 participants with type 2 diabetes filled out the FFQ and 7 d food records 2 times. The FFQ showed good reliability: during validation 75% and 80% of subjects, respectively, were correctly classified into tertiles of AGE consumption, demonstrating moderate to good agreement. Therefore, this FFQ is comparable to 7 d food records for measuring dietary AGEs. Because this FFQ was validated in a group of subjects with type 2 diabetes and a mean age of 56.6 y, it will need further validation before it can be used in other populations.

Role of physical exercise on AGEs. Physical exercise has been shown to induce beneficial metabolic changes in and of itself (53). Many researchers have shown that exercise is more helpful than pharmaceuticals in the secondary prevention of cerebral vascular disease and is as efficient as drugs in preventing the development of diabetes, not to mention that exercise is very inexpensive compared with medications (54, 55). It has been solidly demonstrated that aerobic exercise improves insulin resistance in children, adolescents, and adults (56, 57). Long interventions >6 mo show major health benefits, including improved glucose control and decreased serum lipids, with favorable anthropometric changes. Exercise has also been shown to diminish concentrations of circulating AGEs. Performing a tai chi program 2 times/wk for 12 mo led to decreased serum AGE concentrations in healthy overweight adult patients (58).

A recent 3 mo intervention study with moderate aerobic exercise and/or dietary AGE restriction in a group of overweight or obese Mexican men suggested that each of the applied treatments might have different effects on metabolic disease risk factors (48). Although exercise alone was associated with a decrease in weight, BMI, and waist circumference, the low-AGE diet had the same effects but also decreased concentrations of circulating AGEs. When the low AGE diet was combined with exercise 3–5 d/wk for 20–60 continuous minutes at an intensity of 55–90% of maximum heart rate (following American College of Sports Medicine guidelines), all the benefits of the low-AGE diet were reproduced, and a healthier lipid profile was observed (lower TGs and higher HDL cholesterol compared with other treatments). Exercise alone did not reduce serum AGEs in this study, in contrast with the other studies mentioned above, reflecting perhaps a different intensity of the exercise protocol and different populations (48).

Exercise also appears to reduce sRAGE concentrations. A 6 mo interventional program designed to increase physical activity in elderly subjects resulted in significant reductions in BMI and sRAGE (59).

Toward a nutraceutical approach: Plants and derived products as AGE-regulating agents. The inhibition of AGE formation by some synthetic compounds such as aminoguanidine is well documented. However, this compound has been associated with several adverse effects in vivo. The inhibition of AGE formation might follow several mechanisms involving, e.g., aldose reductase, antioxidant activity, reactive dicarbonyl trapping, sugar autoxidation inhibition, and amino group binding. Many phytochemicals have been tested for their ability to prevent AGE formation. Polyphenols (phenolic acids, flavonoids, and stilbenes) derived from both medicinal and food plants constitute the major group of compounds that have demonstrated the ability to prevent AGE formation. Therefore, they represent a promising alternative for the development of natural medicines with antiglycation activity at a lower cost with fewer side effects than synthetic compounds. As a consequence, the search for natural products that can inhibit AGE formation has increased worldwide recently.

Vegetable and fruit agronomic by-product residues are a rich source of phytochemicals such as polyphenols and dietary fiber (60–62).

Mate is a popular beverage in several South American countries. It consists of a brew of the dried, minced leaves (green or roasted, depending on the region) of *Ilex paraguariensis*. Because mate is rich in polyphenols (60–62), its potential antiglycation effect has also been explored. The results demonstrated a significant, dose-dependent effect from water extracts of *I. paraguariensis* on AGE adduct formation by methylglyoxal in several in vitro protein models, whereas green tea displayed no significant effect. The inhibition of AGE formation is mainly due to chlorogenic acid and was comparable to that obtained with the use of millimolar concentrations of the standard antiglycation agent aminoguanidine. The effect is mainly due to an inhibition of the second phase of the glycation reactions, namely the free-radical–mediated conversion of Amadori products to AGE (60–62).

Only one study, to our knowledge, is available regarding the in vivo effect of mate on the formation of AGEs (63). However, the authors did not find significant differences in concentrations of serum AGEs between the baseline period and 20, 40, and 60 d of mate tea ingestion by diabetic and prediabetic subjects (63).

Agroindustrial by-products. The use of phytocompounds as food ingredients capable of inhibiting the formation of dietary AGEs during food processing is of great interest. In 2010, the Spanish Institute of Food Science Research food bioscience team, headed by María Dolores del Castillo, in collaboration with other scientists from the Spanish National Research Council (GP Blanch, A Cifuentes, E Herrero, M Ibañez, AJ Martinez-Rodriguez, FJ Morales, and C Molina-Rosell), Granada University (MD Mesa), and University of the Republic, Uruguay (A Medrano), started the search for innovative eco-sustainable sources of phytodrugs, including AGE inhibitors, to be applied in nutrition and health, funded by the Spanish Government (Projects AGL2010–17779 and A2/036996/11).

Food production chain sustainability, which refers to waste reduction and the exploration of innovative ways to increase resource efficiency, is of increasing importance. Because the agronomic by-product residues of vegetables and fruits are a rich source of phytochemicals, such as polyphenols and dietary fiber, composting results in a loss of

valuable nutritional compounds. If used in suitable food products and alternative medicine, they could be made available to consumers to improve health. The application of the biorefinery concept may allow this goal to be achieved.

Biorefining refers to fractionating biomass into various separated products that possibly undergo further biological, chemical, biochemical, physical, and/or thermochemical processing and separation. By coproducing relatively high-value chemicals (e.g., fine chemicals, pharmaceuticals, and polymers) the production costs of secondary energy carriers (e.g., transport fuels, heat, and power) potentially could become competitive on the market, especially when biorefining is integrated into the existing chemical, material, and power industries. A biorefinery could, for example, produce one or several low-volume, high-value chemical products, such as food ingredients and phytodrugs to prevent diabetes and its complications caused by the formation of AGEs, and simultaneously a low-value, high-volume liquid transportation fuel, such as biodiesel or bioethanol. The concept of biorefinery is still in the early stages of development in most parts of the world, but it has a promising future (64). The concept has been applied successfully to the sustainable design and synthesis of manufacturing processes for making algal bioproducts (64–66). However, it also can be applied to other products of plant origins.

Safe, effective, and high-quality phytodrugs are being used for treating several diseases. The biopharmaceutical industry has experienced substantial growth in recent years at the international level in response to the demands of today's consumers, with a substantial economic impact. The sustainable production of phytodrugs requires strong political will, human resources, leadership, and effective partnerships between traditional health practitioners, researchers, and the pharmaceutical industry, which can be achieved through the application of the biorefinery concept. A new domain of medicines from the drug molecules extracted from plants, called phytopharmaceuticals or phytodrugs, is expected to hit the market in the near future.

Cost-effective waste materials such as agronomic by-products derived from coffee processing and blueberries are considered food wastes because retailers believe they will be difficult to sell and are disposable, but they have added value as AGE inhibitors and could be used in the production of eco-sustainable phytopharmaceuticals to prevent complications from diabetes and other diseases associated with advanced glycation of proteins and aging. They could also be used as food ingredients to prevent the formation of dietary AGEs during food processing.

Spanish National Research Council scientists have developed a procedure for the extraction of bioactive compounds from coffee silverskin (WO2013004873A1), the only by-product of coffee roasting. The procedure consists of the extraction of coffee silverskin without prior milling with the use of subcritical water at a moderate temperature (50°C or higher) and high pressure (1500 pounds per square inch), although unpressurized water at 100°C can be used as well. Under these conditions, extracts with high antioxidant properties are obtained in 10–20 min (67). The extracts can be considered a natural source of inhibitors of in vitro formation of AGEs and carbonyl stress. The inhibitory effect of these extracts may be associated with their carbonyl-trapping capacities (67).

Recently it has been shown that extracts of blueberry (*Vaccinium corymbosum*) obtained under aqueous conditions at 50°C for 60 min have potential as inhibitors of fluorescent AGE formation. The anti-AGE character of the extract has been associated with the presence of phytochemicals such as cyanidin and chlorogenic acid (A Medrano, unpublished results, 2014).

How to Measure AGE Content

A quantitative approach to the analysis of AGEs and their precursors: Some examples of analytic procedures in different fluid samples. Measuring AGEs in different specimens requires reliable procedures, which is a challenging task in analytic chemistry. Analytic methods provide precise and exact quantitative results for individual compounds or for groups of compounds that share similar optical properties. Such a quantitative approach is extremely important when concentrations of specific compounds are to be compared between experimental groups in dose-response studies, and also in the evaluation of dietary intake of exogenous AGEs. Early fluorometric and spectrophotometric assays took advantage of natural fluorescence, a characteristic of cross-linked structures (pentosidine, pyrraline, glyoxal lysine dimer, methylglyoxal lysine dimer, and others). Some of these procedures are still in use in the evaluation of cumulative tissue damage by AGEs, because they are simple and do not need any sophisticated instrumentation or trained analysts. Of note, considerable effort has been focused on improving the selectivity of these assays. As an example, a procedure based on the sample introduction to the flow system containing 2 detection systems coupled on line is noted (68). For serum analysis, sample pretreatment consisted of protein precipitation (0.15 mol/L trichloroacetic acid) and extraction of lipids (chloroform). Afterward, 20 μL aliquots were injected into the water flow (0.5 mL/min) that carried sample segments to the spectrophotometric and fluorometric detectors, which were connected in a series. The fluorescence signal of AGEs present in the low molecular fraction of the serum (excitation and emission wavelengths 247 nm and 440 nm, respectively) was normalized for peptide content (absorbance at 280 nm). The peptide-derived AGE calibrator was obtained, characterized, and used throughout. The procedure was validated by comparison with a competitive ELISA as applied to samples from diabetic patients and healthy individuals. This method enabled the processing of up to 60 samples/h and, on further development, it was adopted for the analysis of urine, saliva, and skin samples (69).

Because individual compounds of interest in advanced glycation lack distinctive optical properties, more thorough sample treatment and the use of a separation technique have to be introduced for their determination. For example, this

is the case for pentosidine, a well-characterized fluorescent AGE. For the determination of pentosidine in urine, ion-pair reversed-phase LC has been applied. The sample treatment consisted of matrix precipitation with heptafluorobutyric acid (0.1M), dilution of the supernatant, and its introduction to the chromatographic system (25 μL). The mobile phase contained heptafluorobutyric acid as a contra-ion for cationic pentosidine, and acetonitrile and methanol were used as organic modifiers; total chromatographic run was accomplished in 15 min. With fluorometric detection set at 325 nm and 385 nm for excitation and emission, respectively, the procedure was suitable for the determination of pentosidine at pM concentrations. The analytic sensitivity achieved with this method allowed the observation of statistically meaningful differences between pentosidine concentrations in healthy persons compared with diabetic patients with CKD before and after telmisartan treatment (K Wrobel, K Wrobel, C Koenhauser, unpublished data, 2012).

Another AGE of interest is carboxymethyllysine, which is nonfluorescent and can be determined by several techniques (see discussion in section below). If LC with optical spectrometry detection is used, carboxymethyllysine needs to be converted into fluorescent species. Several derivatizing agents have been proposed for this purpose (70), among them, ortho-phthalaldehyde (OPA) (71) and 6-aminoquinolyl-*N*-hydroxysuccinimidyl-carbamate (AQC) (72). It is relevant that OPA reacts with primary amine groups, whereas AQC has reactivity with both primary and secondary amine groups. In food analysis, carboxymethyllysine is usually determined in protein hydrolysate, in which all amino acids and peptides are susceptible for derivatization, so chromatographic resolution becomes an issue. To make separation conditions less rigorous, the feasibility of 2-stage derivatization before carboxymethyllysine determination by reversed-phase HPLC with fluorometric detection was demonstrated. Specifically, OPA was used to saturate primary amine groups. Afterwards, the addition of AQC yielded selective derivatization of secondary amine groups. Because detection conditions for OPA and AQC derivatives were different (λ_{ex} = 330 nm and λ_{em} = 450 nm for OPA, and λ_{ex} = 245 nm and λ_{em} = 395 nm for AQC, respectively), by using specific excitation/emission wavelengths for the detection of AQC derivatives, only the elution of secondary amines was recorded. In doing so, the carboxymethyllysine signal was separated from those of other compounds and the procedure has been successfully applied to screen carboxymethyllysine in food samples (73).

There is increasing demand for quantitative assessment of reactive α-dicarbonyl species as bioindicators of glycation processes and oxidative or carbonyl stress; however, such analysis is truly challenging. The main difficulties include the high reactivity and ubiquitous character of these compounds, their low concentrations, the possible polymerization or formation of adducts with sample components, and de novo generation during sample handling. The chemical complexity of the samples (biological fluids, clinical samples, and food) and the risk of sample contamination from reagents, air, and water should also be noted (74–76). It is clear from the above considerations that the conditions applied at procedural stages before and during derivatization should be as mild as possible, avoiding drastic changes in chemical conditions and prolonged heating. The overview and comparison of analytic procedures, the great majority of them based on suitable precolumn derivatization followed by chromatographic or electrophoretic separation, can be found in the comprehensive reviews (75, 76). Quite surprisingly, very different conditions have been reported; depending on the derivatization reaction, pH ranged from very acidic (pH < 1) to alkaline (pH = 10), the samples were kept during reaction at temperatures from ambient to 85°C, and derivatization times between 10 min and 24 h were reported. In a recent study, 4-methoxy-*o*-phenylenediamine was proposed as a derivatizing reagent, which allowed for relatively mild reaction conditions (neutral pH, 40°C, 4 h in dark), thus minimizing the risk of changes in analyte concentrations before and during the course of derivatization (77). In this novel procedure, diethylglyoxal was used as the internal standard, urine was treated with acetonitrile for matrix precipitation, and, after derivatization, respective quinoxalines were extracted with the use of this same solvent by acidification and salt-induced phase separation. Chromatographic separation of quinoxalines was accomplished on a reversed-phase column with a total chromatographic run of 12 min, which, together with the sample treatment protocol, made the whole procedure relatively fast and simple. It should be stressed, however, that special care was needed to avoid sample contamination and oxidative degradation. The low, sub–parts per billion detection limits evaluated for glyoxal, methylglyoxal, and diacetyl enabled their quantification at physiologic levels in urine from healthy adults, and the procedure was suitable for the detection of concentration differences between samples and subjects in different clinical and exposure conditions (77). This same procedure was adopted for the analysis of honey and high-fructose agave syrup (78). The health impact of exogenous α-ketoaldehydes ingested with food is now under discussion (79). It has been proposed that binding to digestive enzymes in the gastrointestinal tract might reduce their bioavailability; however, this same process would also contribute to the increased formation of AGEs (80, 81). Within this context, an interesting finding (78) was that agave syrups contained higher concentrations of methylglyoxal than did commercial Mexican honey and high-fructose corn syrup. Furthermore, it was also demonstrated that methylglyoxal is at least in part responsible for the nonperoxide bacteriostatic properties of agave-based syrups (78).

In conclusion, it should be noted that analytic procedures based on spectrophotometric or fluorometric detection are reliable tools for quantitative assessment of AGEs and their precursors in a variety of samples. These procedures are relatively simple, fast, and cost-effective; however, authentic standards are needed for assignment of analytic signals, compound confirmation, and calibration. Because the species

involved in advanced glycation processes form a large and heterogeneous group of compounds, of which relatively few are well characterized and commercially available, chromatographic separation with optical spectrometry detection is not always the best choice. In recent decades, application of MS has gained strength in the field of structural characterization and quantification of known, unexpected, or new species. These techniques, especially in combination with powerful chromatographic or electrophoretic separations, have been used to assess glycation product profiles, specific glycation sites, and their molecular structures in different biomolecules both in vitro and in vivo (82–84).

Measurement of carboxymethyllysine in foods: Pros and cons of different methods. Lowering the intake of AGE-rich foods based on information from 2 large databases with the content of carboxymethyllysine measured by ELISA has been reported to decrease the biomarkers of OS, inflammation, and other abnormal states (Table 1).

ELISA methods to measure carboxymethyllysine have several advantages, including relatively high specificity for carboxymethyllysine, speed, and no need for sophisticated laboratory equipment or previous isolation of carboxymethyllysine from the complex food matrix. Drawbacks of the method include the cost of the ELISA kit and the lack of sufficient antibody specificity, depending on the commercial kit used. Results obtained with immunochemical tests are usually expressed as units of carboxymethyllysine/mL (the signal generated by 3.45 μg/mL carboxymethyllysine-modified BSA (carboxymethyllysine 1 nmol/mL) was defined as 1 U carboxymethyllysine/mL), which hampers its application in evaluating carboxymethyllysine content in the edible food portion and comparison among different studies (84).

Although carboxymethyllysine content as measured with the ELISA test has been reported for different food matrices, the method validation (expressed as accuracy, precision, and limit of quantification, among other variables) has not been well described in most of the reports (85, 86).

Because of the above problems with the ELISA test, since the late 1980s, chromatographic methods (GC and LC) have been employed to determine carboxymethyllysine content in foods (85, 87–90). Lately, the development of HPLC and ultra-pressure liquid chromatography equipment, combined with mass spectra detectors (tandem MS), allowed several laboratories to investigate the carboxymethyllysine content in different food matrices and compare their results with those obtained by the immunochemical method (84, 86).

Chromatographic methods require carboxymethyllysine to be extracted from the food matrix. Therefore, several analytic steps must be observed, including sample preparation, reduction (to avoid artifact formation), hydrolysis, protein isolation, and the use of an internal standard. Because food is such a variable and chemically complex matrix and considering that food components such as different types of proteins or lipids can generate AGEs by different pathways, some of the analytic procedural steps may still be considered to be under development and need to be fully standardized. The reduction step, hydrolysis (both acid and enzymatic), and/or protein isolation (ultrafiltration, trichloroacetic precipitation, and Folch extraction) have been tested and discussed by several authors. Whether the matrix composition may interfere with carboxymethyllysine ionization is also an important point to be observed during analysis by MS. Reports making use of chromatographic methods to evaluate carboxymethyllysine in foods usually present the validation variables for the targeted food matrix (84, 86, 91).

As is clear from the above, chromatographic methods require multiple steps for sample preparation, expensive equipment, and reagents, as well as specialized human resources to perform the analysis. Their main advantage is that they provide accurate data on carboxymethyllysine contents and the results are usually reported as analyte concentration directly in the sample.

One intriguing aspect is that results obtained by application of chromatographic methods differ from those obtained with the ELISA by an order of magnitude (84, 90, 91). The main reason for this discrepancy is the poor selectivity of the ELISA; specifically, food components such as fat globules or advanced lipoxidation products may bind to the ELISA antibody, interfering in the final results. It should be stressed that, at present, no fully validated method is available for carboxymethyllysine determination in food, although there have been many procedures internally evaluated and recommended for the analysis of specific food matrices. The important elements of the validation procedure involve the analysis of appropriate certified reference materials and participation in interlaboratory assays, both of these essentially nonexistent in the actual practice of AGE analysis in food. However, this should not invalidate evidence supporting the contribution of dietary AGEs in chronic disease, because, irrespective of the methodology employed for AGE assessment, foods cooked or processed at high temperatures persistently contain elevated concentrations of AGEs.

However, chromatographic methods and ELISAs offer specific advantages and also present certain disadvantages; hence, choosing the most suitable method for specific application depends on several factors, including available laboratory facilities, trained personnel, financial resources, and the aim of the study (chemical food composition, carboxymethyllysine concentration, number of samples, etc.). It is fundamental, however, that the chosen methodology provide reliable data, especially if these data are to be used to implement strategies addressing health conditions, such as diabetes and renal impairment. One of the resolutions of this symposium was to attempt to define the best methods for future estimation of carboxymethyllysine in those foods of highest consumption in different countries.

Conclusions

The evidence discussed in this symposium and presented in this review strongly suggests that the modern diet is an

important contributor to the circulating AGE pool in health and in the disease state. It is further postulated that sustained exposure to these exogenous pro-oxidant substances gradually erodes native defenses, setting the stage for abnormally high OS and inflammation, the precursors of disease. Of importance, however, is that this scenario seems to be reversible by following a rather simple dietary intervention, the low-AGE diet, which has been shown to be a feasible, effective, and safe intervention in different populations and countries. Although a role for dietary AGEs in disease seems to be supported by the literature, there is as yet no agreement among researchers working with dietary AGEs on important aspects of this area of research. At least 4 different areas demand further research: *1*) further definition of the mechanisms involved in absorption, metabolism, and accumulation of dietary AGEs; *2*) an analytic quantification of AGEs in foods; *3*) a search for novel inhibitors of AGE formation in vitro (in food) and in vivo; and *4*) long-term clinical studies with hard endpoints. The 1st Latin American Symposium of AGEs is just the first step toward joining efforts in adding data derived from regional studies, which will contribute to addressing some of these unresolved issues.

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