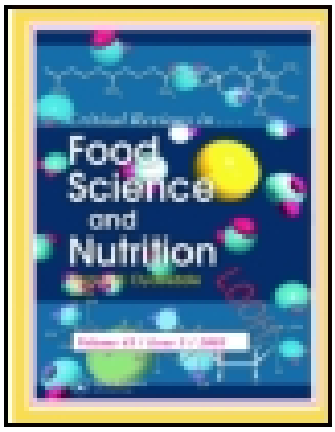

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Advanced Glycation End Products, Inflammation, and Chronic Metabolic Diseases:Links in a Chain?

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Advanced Glycation End Products, Inflammation, and Chronic Metabolic Diseases: Links in a Chain?

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Running title: Advanced Glycation End Products and Inflammation

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Abstract

Advanced glycation end products (AGEs) are a diverse group of compounds produced when reducing sugars react with proteins or other compounds to form glycosylated molecules. AGEs may form endogenously, and glycation of molecules may negatively affect their function. AGEs may also be consumed in food form with dietary AGEs reported to be particularly high in foods treated with high heat: baked, broiled, grilled, and fried foods. Whether dietary AGEs are absorbed in significant quantities and whether they are harmful if absorbed is a question under current debate. The American Diabetes Association makes no recommendation regarding avoidance of these foods, but many researchers are concerned that they may be pro-inflammatory and way worsen cardiac function, kidney function, diabetes and its complications and may even contribute to obesity.

Introduction:

Advanced glycation end products (AGEs) are a diverse group of compounds formed when glucose or other reducing sugars react with amine groups linked to proteins, nucleotide bases or fatty acids, forming glycosylated products. Some of these products include carboyxmethyllysine (CML), hydroimidazolone, pentosidine, glucosepane, and others (Semba, Nicklett 2010).

The Maillard browning reaction in foods, a necessary precursor to the formation of AGEs, was first described by Louis Camille Maillard in 1912, and for many, the Maillard reaction is most familiar as something belonging to the realm of food science, affecting the color, flavor, and acceptability of cooked food. However, increasingly this reaction and the reactions which may follow it are being investigated for their role in negative physiological effects in the body.

The first indirect support for health implications of non-enzymatically glycated proteins came from observations of increased formation of hemoglobin A_{1c} in parallel with poor glucose control in persons with diabetes (Trivelli 1971). Hemoglobin A_{1c} is an Amadori product, a ketoamine formed as the ultimate product of a reaction between glucose and the free amine group on the valine residue of the hemoglobin beta chain (Saudek 2006). It is not an AGE, but it is the standard for determining adequacy of blood glucose control in diabetes today (Saudek 2006). It is used as an indicator of the 3-4 month average of blood glucose values, with goals for patients with diabetes set at a hemoglobinA_{1c} of less than 7% or 6.5% (Saudek 2006). While hemoglobin A_{1c} is not an AGE, this research became an important springboard for future AGE research. The implications of AGEs as possible pro-inflammatory compounds gained further

steam and legitimacy when the receptor for AGEs (RAGE) was cloned in 1992 (Schmidt 1992; Semba, Nicklett 2010). Other receptors for AGE have since been identified.

What are AGEs?

The Maillard browning reaction, which yields a yellowish-brown colored product, precedes the formation of AGEs. In this process, a reducing sugar and amine group react, non-enzymatically forming a Schiff base, which may then form more stable Amadori products (Wu 2011). The first part of this reaction is reversible, so AGEs may *not* result from the initial browning reaction; however, the Amadori products, once formed, lead inexorably to the formation of AGEs. AGEs are produced when the Amadori products are further modified by dehydration, oxidation, rearrangement or other reactions, leading to the formation of cross linked or non-cross linked derivatives of sugar and other molecules (Barlovic 2011; Wu 2011). See Figure 1 (Sell 2012) for a detailed look at how AGEs are formed.

Although AGEs include pyrraline, pentosidine, carboxymethyllysine, glucosepane and some 16 plus other structures (Ames 2007), hemoglobin A_1c, though an Amadori product, is not an AGE. AGEs are found both in food and within the body. Among cross-linked AGEs are crossline, 2-(2-furoyl)-4(5)-(2-furanyl)-1H imidazole (FFI), glyoxal-lysine dimer (GOLD), methyl-glyoxal-lysine dimer (MOLD) and others (Wu 2011). Non cross-linked AGEs include CML, carboxyethyllysine (CEL), pyrraline, and argpyrimidine (Wu 2011).

The substances formed when reducing sugars interact with substances containing a free amino acid group may be called pre-AGEs. In addition, sugars in the body or in food may be oxidized to form dicarbonyls such as glyoxal (GO) and methylglyoxal (MG). GO and MG are

not themselves AGEs and may have other fates, but are thought to have strong AGE potential and the ability to form cross-linked AGEs via reaction with arginine residues. The product of this reaction is the AGE hydroimidazolone. In addition, GO and MG may impact formation of reactive oxygen species. (Ames 2007; Wu 2012)

Measuring AGEs

Although several procedures are available for measuring AGEs, neither a universally accepted technique nor normative values have been established. Immunochemical and instrumental methods are most often employed to measure AGEs. Immunochemical methods (enzyme linked immunosorbent assay or ELISA) have the advantage of requiring less expensive equipment in order to begin quantification, but studies employing these methods have been criticized for being non-specific due to the nature of the antibodies employed and also for reporting AGE in "Units" per average portion (Goldberg 2004; Uribarri 2010; Ames 2008). Instrumental methods used to quantify AGEs in food have included liquid chromatography combined with fluorescence detection or mass spectrometry (HPLC, UPLC) or in some cases gas spectrophotometry (GC) (Ames 2008; Birlouez-Aragon 2004; Hull 2012; Assar 2009; Delgado-Andrade 2006, 2007; Drusch 1998; Yaacoub 2008). Instrumental methods are usually accurate, repeatable and express AGEs in milligrams (mg) per kilogram (kg) of protein, mg/100 grams (g) of food or mg/average portion size.

The problem in comparing published AGEs data are multiple. These include method used for quantification (instrumental versus ELISA) and antibody-dependent differences in ELISA assays that may measure different AGEs products. In addition, despite the identification of at least 20 AGE structures, CML has been most often reported in the literature as a measure of

AGEs (Semba, Nicklett 2010). The use of CML as a proxy for measuring AGEs is based on early studies indicating that CML levels correlate directly with levels of other protein or lipid AGEs (Cai 2008; Brownlee 2001; Requena 1997). However, much remains to be understood about the physiologic significance of the large AGEs family of structures and which should be measured as a marker of AGEs load.

AGEs have multiple receptors including RAGE, AGERs 1, 3, and CD35 receptors, which will be discussed further in the section on "Metabolism and Pathophysiology". In addition, given the discovery that RAGE has multiple ligands and that during an inflammatory response, the binding of the ligand to RAGE leads to increased expression of RAGE (Fritz 2011), it is reasonable to ask whether each ligand has an equal affinity for RAGE and whether CML is the most important ligand in promoting inflammation. The ligands for RAGE identified so far include AGEs, S100 proteins (a protein family consisting of 25 members, which have metal ion binding properties), amyloid beta and amyloid fibrils, high mobility group box 1 protein (HMGB1) and beta2-integrin macrophage-1 antigen (Fritz, 2011). RAGE is classified as a "pattern recognition receptor" because of its ability to allow binding by a diverse group of ligands (Ramasamy 2010). Questions remain regarding whether all ligands for RAGE have been identified, which are most important, and how they may interact with or compete for the RAGE receptor.

Some progress is being made in this regard. A recent study attempted to use UPLC with mass spectrometry (UPLC-MS) to analyze the glycating activity of AGE-precursors. This study identified five AGE structures which did not match any known product, but it also confirmed

that CML and CEL were abundant AGEs formed from well-known AGE precursors such as MG GO and glucosone (Mittelmaier 2011).

Another problem in the AGE literature is that measured AGEs in foods vary based on the quantification method used. Roast chicken breast in which AGEs were quantified using ELISA is reported to have 5,245 kU of AGE per 90 g serving (Goldberg 2004; Uribarri 2010). Using UPLC-MS, a 90 g serving is reported to have 0.4 mg/ serving (Hull 2012). This compares to 825 kU of AGE per 90 g serving of canned salmon (ELISA) and 6.2 mg/90 g (UPLC) (Hull 2012; Goldberg 2004). Thus, according to ELISA measurements, broiled chicken is high in CML whereas canned salmon is low (Uribarri 2010). Conversely, according to UPLC measurements, canned salmon contains high levels whereas broiled chicken contains low levels (Hull 2012).

To further illustrate the point, Kellogg's Corn Flakes have either 70 kU of AGE per 30 gram serving (ELISA) or 1.6 mg per 30 gram serving size (UPLC). Again, this equates to a *very* low measurement for corn flakes compared to most of the foods in the ELISA-based food AGE database and a high measurement of AGE compared to most foods in the UPLC-based food AGE database, exceeding that of braised steak, battered cod and pork sausage but not of whole milk yogurt (Goldberg 2004; Uribarri 2010; Hull 2012). This picture is confusing and needs to be rectified. A recent study comparing the use of UPLC, ELISA and another method to determine the CML content of known samples of glycated bovine serum albumin found good correlation between the various methods; however the r^2 value was just 0.75 for UPLC and ELISA. (Srey 2010) Foods were not tested in this study, thus more work needs to be done to evaluate whether these methods are equally valid in testing CML content of a variety of foods.

Measurement of serum and plasma AGEs in human subjects is less difficult and less controversial. CML is the favorite AGE for measurement, and a few kits are available. However, reporting of data is not in standard units.

Serum AGEs have been reported in the range of 8.5 ± 0.9 Units/ml in men less than age 45, 9.9 ± 1.5 in men older than 60, 7.9 ± 0.7 in women less than age 45 and 10.7 ± 1.1 in women older than 60 in a study of 172 healthy individuals (Uribarri, Negrean 2007). In a group of 60 healthy women and women with polycystic ovarian syndrome (PCOS), healthy women had AGE levels of 5.85 ± 0.89 Units (U)/ml, and women with PCOS had higher levels: 8.7 ± 1.65 U/ml (Diamanti-Kandarakis 2009). Yamagishi (2006), using a different CML antibody for ELISA, has reported serum CML concentrations of 4.1 ± 0.7 U/ml in men and 4.1 ± 0.9 U/ml among 184 healthy participants.

In the Italian In-CHIANTI study CML was reported in nanograms (ng)/ml, and participants with the slowest walking speeds had reported plasma CML levels of 375 ng/ml compared to 343 ng/ml for those with the fastest speeds (Semba, Bandanelli 2010). Using the same assay, a separate study reported CML levels of 344 ng/ml for healthy participants and 390 ng/ml for participants with chronic kidney disease (Semba 2009). Reporting values in standard units might help establish norms for AGEs and make it more useful as a biomarker for inflammation.

Endogenous AGEs:

AGEs have both endogenous and dietary origins and are thought to contribute to oxidative stress within the body. Endogenous AGE formation occurs slowly but may be up-regulated in conditions of metabolic stress such as sepsis and insulin resistance and in disease

states such as diabetes and chronic kidney disease. Hyperglycemia may also lead to up-regulation of pathways other than glycolysis including the polyol pathway, which increases AGE production. Activation of protein kinase C could also stimulate the hexosamine pathway, increasing endogenous AGE. Furthermore, an increase in reactive dicarbonyls and reduced detoxification by the glyoxalase system is thought to lead to a state of carbonyl stress, which may increase endogenous AGEs. (Barlovic 2011)

Exogenous AGEs:

Because AGEs are glycated molecules, one might expect that foods rich in carbohydrates would be important dietary sources. In fact exogenous AGE consumption typically increases in high fat diets and when high temperature cooking methods such as deep-frying, broiling, roasting and grilling are used. This is particularly true for high protein products (Semba, Nicklett 2010). However, CML tables produced by Hull, which employ UPLC, also indicate the presence of increased AGEs in high carbohydrate foods (Hull 2012).

Whether dietary AGEs contribute significantly to deleterious health effects is a topic of active investigation. They were once considered insignificant due to early research showing that small amounts of dietary AGE were absorbed in rats, primarily in the large intestine, with only about 1.5% being found in the liver after absorption (Sgarbieri 1973). A 1997 study in humans found only about 10% of dietary AGEs were absorbed. However, in this study, in persons with normal kidney function, approximately 30% of ingested AGEs were found to be excreted in the urine; whereas, for those with impaired kidney function, as estimated by glomerular filtration rate, as little as five percent of ingested AGE was excreted in the urine. A high AGE diet was

also found to result in significant elevations in serum AGE post feeding despite low absorption (Koschinsky 1997).

In an *in vitro* experimental model assessing diet derived AGEs' effects on glutathione (GSH) and GSH peroxidase (GPx) (indicators of oxidative stress), on human umbilical vein endothelial cells (HUVECs), it was reported that AGEs depleted GSH and increased GPx activity (Cai 2002). The authors concluded that prior to absorption, food-derived AGEs contain carbonyl species, which may induce oxidative damage.

The larger studies which have attempted to create CML food databases are ELISA based: Goldberg 2004 and the follow-up, Uribarri 2010. A limitation of the Goldberg study was that the methods section did not describe whether multiple samples/trials were performed in order to determine AGE. AGEs were reported to be lowest in carbohydrate foods with the lowest levels within this group being found in milk, followed by vegetables and fruits. Broiled beef, and chicken, oils heated to high levels and roasted nuts were among the foods highest in CML. These data have been criticized in part because of the finding that oils were found to be high in CML. Because oils should contain no lysine, some question how they could also be rich in CML. However in a later publication, the researchers hypothesized that extraction and purification procedures accounted for this finding (Uribarri 2010). Elsewhere the same group of researchers analyzed food records from healthy participants and found mean daily AGE intake to be about 14,780 +/- 680 kU AGE (Uribarri 2007; Uribarri 2010) compared to an earlier report of average intakes of 16,000 ± 5000 kU AGE (Goldberg 2004).

A study of a variety of common foods published in 2009 found levels of CML to vary from as low as 0.3 mg/kg of raw milk and 0.35 mg/kg of skim milk to as high as 46.1 mg/kg of

whole meal bread crust (compared to just 4.45 mg/kg of bread crumb) (Assar 2009). Commercial breakfast cereals and ice creams also appear to be sources of AGE (Delgado-Andrade 2006; Drusch 1999). An evaluation of a variety of processing methods for nuts and seeds found that CML production was increased by roasting methods. Thus consumption of cooked foods compared to raw foods increases AGE ingestion (Yaacoub 2008).

Intermediate products or AGE-forming metabolites include methylglyoxl (MG) and glyoxal (GO). In a study evaluating the MG and GO content of soft drinks the trend was for products containing high fructose corn syrup to be relatively high in MG whereas diet drinks were not (Tan 2008). However, another analysis of AGEs in soft drinks employing ELISA found that AGE levels in soft drinks did not correlate with sugar content as long as protein and heat were not present. In fact drinks with caramel additives such as Coke Classic or Diet Coke were found to contain 8500 and 9500 units/cup compared to 475 units/cup in Sprite, 600 in orange juice and around 2000 in coffee and tea (Koschinsky 1997).

Metabolism and Pathophysiology of AGEs

Enzymatic Disposal and Mechanistic Action:

The body has a number of ways for degrading AGEs including the degradative enzymes glyoxalase I and II, which degrade and detoxify the pre-AGEs MG and GO along with aldose reductase and carbonyl reductase, which prevent formation of AGEs. Circulating proteins such as lysozyme, defensins and lactoferrin may also bind AGEs in circulation, preventing them from binding elsewhere within the cells (Vlassara 2008). However, degradation may not always be rapid enough to keep up with production as in cases of hyperglycemia or severe oxidative stress.

In addition, in kidney disease the body appears to decrease its capacity to degrade and excrete AGE to sufficient levels (Koschinsky 1997).

There are also two types of cell surface AGE receptors, one which binds AGE and initiates cell activation and another which binds, internalizes and degrades AGE. The RAGE receptor for AGE is thought to initiate oxidative stress whereas the AGERs 1, 3, and CD35 receptors help to mediate degradation of AGE (Vlassara 2008). Damage caused by AGEs is thought to result from activation of nuclear factor kappa beta (NF-κβ) which leads to up-regulation of genes for cytokines, growth factors and cell adhesion molecules (Kalousova 2005).

Brownlee (2001) described ways in which AGEs may damage target cells leading to diabetic complications. Three primary means of damage may be: modification of cellular proteins by glycation leading to loss of function, extracellular proteins or other molecules modified by AGE might interact differently with their receptors, and receptor-mediated AGE oxidative processes may also cause cellular damage.

In fact most mechanistic explanations of how AGE may cause oxidative damage suggest that AGEs exert influence via receptors. RAGE receptors may be expressed on the surface of a variety of cells including neurons, smooth muscle cell monocytes, endothelial cells, fibroblasts and more (Kalousova 2005). The RAGE receptor may then activate signal transduction pathways including extracellular signal-related kinase (ERK), mitogen activated protein kinase (p38 ^{MAPK}), c-jun N terminal kinases (JNK) and NF-κβ. Thus, transcription of genes may be stimulated which will up-regulate production of growth factors such as tumor necrosis factor (TNF-α), inter-leukin 1 (IL-1) and insulin like growth factor-1 (IGF-1) and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules

(VCAM-1). In addition, it is suggested that DNA may undergo glycation. Thus, AGE modification of DNA could affect regulatory and epigenetic processes at the DNA level (Ramasamy 2005). In addition, RAGE may be differentially spliced forming endogenous secretory RAGE (esRAGE). RAGE may also be cleaved from the cellular surface. The pool of both differentially-spliced and cleaved RAGE is known as soluble RAGE (sRAGE) which may act as a decoy receptor aiding in secretion of AGE (Barlovic 2011).

Pharmacokinetics:

As discussed earlier under exogenous AGEs, one major study has attempted to answer questions regarding the pharmacokinetics of AGEs. In it, 43 subjects, (38 male and 5 female) including 38 with diabetes and 5 healthy participants were given high AGE test meals, which was followed by 48 hours of post meal evaluation. Urine samples were collected 48 hours after the test meal. Changes in serum and urine AGE kinetics were calculated and plotted as the area under the curve (AUC) (Koschinsky 1997). Participants consumed differing quantities of AGE, but there was a correlation between the amount consumed and the area under the curve (elevation in serum AGE). Only 10% of the AGEs ingested were accounted for in serum in this study. Only one-third of the AGEs detected in serum were detected in the urine. Thus, what happened to the other two-thirds was not explained by this study. (Koschinsky 1997)

Other studies related to pharmacokinetics of CML have primarily been done in animals (Somoza 2006; Tessier 2012) with a few exceptions. Birlouez-Aragon et. al. reported higher urinary excretion of CML in participants fed a high AGE diet; however labeled products were not fed, so the relationship was correlational only (Birlouez Aragon 2010).

Pathophysiology:

Healthy Individuals:

There is a paucity of human studies involving healthy adults and AGE. Uribarri and co-investigators correlated calculated dietary AGE content and serum AGE in 90 healthy subjects and found increases in CRP with both dietary AGE and serum AGE (Uribarri 2005). In five participants taken from this group of 90, short term dietary AGE restriction decreased serum AGE levels by 30-40 percent. Based on this single study it is possible to say only that dietary AGE may significantly impact AGE levels even in healthy individuals in spite of low absorption.

Birlouez-Aragon also conducted an AGE feeding study involving healthy individuals with average BMI of 21.8 kg/m^2 (range 18-26.9) and found that after four weeks of feeding a steamed diet (low in CML) versus a conventional diet (comparatively higher in CML), participants on the steamed diet had 5% lower plasma total cholesterol, 10% lower HDL, and 9% lower triglycerides (Birlouez Aragon 2010). However, it should be pointed out that the participants consuming the steamed diet (who were fed *ab lib*) consumed significantly less energy, significantly less total fat, and more vitamin C than the participants consuming the standard diet, thus this data is somewhat confounded.

Obesity

AGEs may play a more prominent role in the health of obese individuals. The literature suggests that AGEs are involved in inflammatory processes, which are often increased in obesity (Ramasamy 2005; Brownlee 2001). The extent to which elevated circulating AGEs are the cause or symptom of disease and how AGEs, RAGE and sRAGE may be influenced by factors such as body fatness and lifestyle is not clear.

In several studies in older adults, fatness and sRAGE appear to be linked. Koyama et. al. reported an inverse correlation of components of the metabolic syndrome with sRAGE. Components demonstrating this inverse relationship included body mass index, TG, HbA1c and insulin resistance, which were all inversely linked with sRAGE in both diabetic and non-diabetic participants (Koyama 2005). Another Japanese group also found sRAGE to be inversely correlated with BMI, waist circumference, AGE intake and alcohol intake (Yamagishi 2006). Norata et. al. also reported that sRAGE was inversely correlated with BMI, waist/hip circumference and fasting glucose with a positive correlation to apolipoprotein A-1 (Norata 2009). Thus, the link between sRAGE and fatness in older adults seems to be established based on several studies done in separate populations.

In studies relating AGEs to BMI, the correlation is usually direct. Semba et. al. used DEXA to establish body fatness and found that total fat mass, truncal fat mass and appendicular fat mass were each correlated with serum CML when controlled for age, sex, BMI, blood pressure, TG, HDL and renal function. This suggests that body fat affects CML and possibly other AGEs perhaps because AGEs may be stored in fatty tissue (Semba 2011). Diamanti-Kandarakis et. al. also found a correlation between AGEs and BMI (Diamanti-Kandarakis 2009), as did a study by Yamagishi et. al. (Yamagishi 2006). However, in a study of obese children and adolescents, levels of AGEs were actually lower in the obese children compared to controls (Sebekova 2009). Given more limited data in this area, the link between AGEs and fatness is suggestive but not confirmed.

Finally Yamagishi et. al. identified a correlation between sRAGE and AGE. However, they found a positive correlation between the two. This seems counter-intuitive given research

suggesting that AGEs increase with increasing BMI whereas the link is opposite for sRAGE. This group of researchers hypothesized that sRAGE may reflect tissue RAGE expression and that sRAGE may increase along with AGE in order to mount a counter-defense (Yamagishi 2006).

In the only study of weight loss and AGEs in obese subjects, Gugliucci et. al. treated 37 Japanese participants (30 females, 7 males) with a low calorie diet (1200 kcals/5020 KJ daily) for 2 months. Participants reduced their BMI, waist circumference and TG but also reduced serum AGEs by 7.2%. Whether the reduction in AGEs was due to decreased fat mass, a reduction in glycation due to caloric restriction, or a decreased intake of dietary AGEs was not assessed. (Gugliucci 2009)

Diabetes and Diabetes-Related Cardiovascular Disease

The link between serum AGE and inflammatory stress in diabetes is comparatively strong. In a study by Tan (2004), serum AGEs were significantly increased in persons with diabetes, and CRP was highly correlated with AGEs. Regression analysis demonstrated that BMI, interleukin-6 (IL-6) and CRP were determinants of AGEs (Tan 2004). An earlier study also by Tan found significant increases in serum AGE in persons with diabetes and found AGEs to be correlated with endothelium-dependent vasodilation (Tan 2002). In addition, dietary AGEs may result in prolonged increases in serum AGEs in persons with diabetes. Koschinsky et. al. evaluated the effect of a single meal containing egg white cooked with or without fructose (with=AGE; without=control) in 38 participants with type 2 diabetes. This study first showed that not only was serum AGE increased with dietary consumption but also that AGE levels

remained elevated in persons with both diabetes and severe renal disease for more than 48 hours after ingestion (Koschinsky 1997).

Studies involving diabetic complications related to AGEs have shown that AGEs are increased in vessels of the retina and in renal glomeruli in persons with diabetes (Brownlee 2001). Animal based studies have also demonstrated fewer diabetic complications, especially microvascular complications, in mice fed reduced AGE diets. Follow-up multicenter trials of an AGE inhibitor (aminoguanidine) demonstrated reduced diabetic retinopathy (Brownlee 2001). While this drug did not progress to clinical trials, other potential AGE drugs include AGE breakers such as ALT 711 (Friedja 2012).

Studies relating to macrovascular complications of diabetes also indicate a role for AGEs. Patients with diabetes randomized to a low AGE diet were found to have less glycated serum LDL and oxidized serum LDL compared to those fed the standard diet (Cai 2002). In the Diabetes Control and Complications Trial, oxidized and glycated LDL were found to correlate well with duration of diabetes, BMI, and lipid levels (Lopes-Virella 2011). Serum AGEs were found to correlate with triglyceride levels in a study of diabetes-related cardiovascular disease, and those with the highest AGEs had the most adverse lipid profiles (Chang 2011).

While mechanisms for damage in diabetes have usually been related to signaling and inflammatory processes as previously described, one study involving both cells and mice with diabetes found that AGEs were acutely toxic to pancreatic beta cells, causing loss of manganese superoxide dismutase (SOD) and increased glucose uptake. In addition, rats fed high AGE diets had progressive insulin secretory defects and beta cell death (Coughlan 2011).

Studies which have attempted to manipulate the diet of persons with diabetes to include fewer AGEs have demonstrated positive results. Negrean et. al. fed persons with type 2 diabetes isocaloric diets with identical ingredients but which were prepared using different cooking methods in order to obtain high and low AGE test meals followed by measurement of endothelial function using flow-mediated dilation (FMD) and Laser-Doppler flowometry. FMD was found to decrease significantly more after the high AGE meal (a decrease which was 1.5 times greater than the low AGE meal change). AGE did not affect glucose, triacylglycerol levels or insulin. No significant changes in inflammatory markers were found (Negrean 2007, Stirban 2008).

A separate report of a similar study by the same group of researchers demonstrated increased serum AGE and increased glycated LDL in 11 diabetic subjects fed a high AGE diet in a two-week crossover study. Thirteen participants in a longer 6 week low or high AGE intervention also were noted to have increased glycated LDL while on the high AGE diet (Vlassara 2002).

In feeding stable diabetic participants and healthy participants an oral AGE challenge beverage with no carbohydrate or lipid, Uribarri et. al, found that maximal arterial dilation after ischemia decreased significantly with no changes in glucose or VCAM-1. This decrease in FMD is thought to be indicative of impaired endothelial function resulting from the AGE bolus (Uribarri, Negrean 2007).

Each study seems to indicate a possible positive impact of a low-AGE diet on diabetes-related cardiovascular diseases. However, even though many studies in the field of diabetes have been done with regard to AGEs, the evidence is regarded as insufficient to warrant changes in dietary advice to persons with diabetes (*Diabetes Care* 2012).

Kidney Disease

AGEs are thought by some to damage nephrons by altering the structure and function of proteins and by injuring cells (Uribarri 2006) due to changes in cellular adhesion molecules and inhibition of processes leading to the production of type IV collagen and laminin fibers (Uribarri 2006). Serum AGEs correlate with inflammatory markers and are inversely associated with creatinine clearance (Vlassara 2009). Proof that AGEs damage renal function is currently lacking, but suggestive data is available.

The impact of dietary AGEs on kidney function has been evaluated in overweight and obese adult males randomized to two weeks on a low and high AGE diet (Harcourt 2011). In this study fat was held constant at 30% with AGE being the primary variable, and AGE was calculated based on the Goldberg values (2004). The researchers found that albumin to creatinine ratios were improved following the low AGE diet. In addition plasma AGE increased following high AGE consumption. Some drawbacks to this study design include likely differences in type of fat in the study due to the dietary approach used.

In a large study of older community-dwelling adults, CML was associated with chronic kidney disease and glomerular filtration rate (GFR), the primary indicator of CKD. This remained true at 3 and 6 year follow-up. While this study shows CML to possibly be a good indicator of renal function, it does not prove that dietary CML negatively impacts GFR (Semba 2009). Similarly in the Baltimore Longitudinal Study of Aging in which 750 adults were followed for 52 years, serum CML was associated with CKD (p=0.003) and decreasing GFR (Semba, Fink 2010).

Studies in animals have demonstrated that aminoguanidine (an AGE inhibitor) decreases kidney disease in aging rats (Vlassara 2009; Li 1996). In addition a life-long restriction of dietary AGEs in mice decreased kidney lesions in aging mice (Vlassara 2009; Cai 2008).

However, Schwedler et. al. found that elevated serum AGEs were not related to mortality in patients on hemodialysis (HD). CML and AGE were definitely lower in healthy controls compared to those on HD, but mortality was not associated with CML (Schwedler 2002).

A fairly scathing critique of the above literature in kidney disease and AGEs research was recently written by Piroddi et. al. This group of researchers measured CML in serum using chemiluminescent detection and immuno-blots and pentosidine using reverse-phase HPLC analysis (Piroddi 2011). CML and pentosidine levels were increased in CKD and HD patients, but intake of CML was lower in participants with CKD or HD. Piroddi et. al. reported a negative correlation between CML intake and plasma CML and a positive correlation of CML and protein intake. They concluded that CML in the plasma does not correlate with dietary intake and should not be restricted in healthy individuals or those on HD. They also claimed that the CML epitope measured by the assay used by Vlassara, Semba and others at Mt. Sinai is present in low abundance in human serum and in food (Piroddi 2011).

Cardiovascular Disease

An animal model developed by Ueno and Koyama has sought to determine whether RAGE is linked to progression of atherosclerosis (Ueno 2010). Variations of RAGE and apolipoprotein E (ApeE) knockout mice were produced and randomly assigned to atherogenic diet and standard chow diet from 6 weeks to 20 weeks of age. The $ApoE^{-/-}RAGE^{-/-}$ mice fed with

either diet had decreased plaques even though no differences in cholesterol or glucose were observed. The $ApoE^{-/-}RAGE^{-/-}$ mice also gained significantly less body weight despite no difference in food intake. Thus, it appears that mice deficient in RAGE have improved body fatness and less atherogenesis, an intriguing finding should it also be found to be true in humans. (Ueno 2010)

Another group of researchers identified a decrease in leptin after high AGE feeding in 20 in-patients with type 2 diabetes. They also found decreases in adiponectin and increases in VCAM-1 and e-selectin (Stirban 2008). The same group did a separate study involving 13 persons with type 2 diabetes and found that a high AGE test meal significantly impaired macrovascular FMD (Stirban 2006). The findings in the animal study together with the studies previously discussed with regard to cardiovascular disease related to diabetes (Cai 2002, Lopes-Virella 2011, Chang 2011, Vlassara 2002, Negrean 2007, Stirban 2008), indicate a possible association between foods high in AGE and impacts on oxidized LDL, glycated LDL, and endothelial dysfunction, all indicators of atherogenesis.

Population based studies have also indicated a possible role for AGEs in cardiovascular disease and mortality. Kilhovd et. al. determined in a Finnish population of persons with and without diabetes that increased serum AGE predicted total and cardiovascular disease-related mortality in women but not men (Kilhovd 1999, 2005, 2007). Similarly, Nin et. al. found plasma AGE (CML and pentosidine) to be linked to fatal and non-fatal cardiovascular disease in 339 patients with type 1 diabetes (Nin 2011). Kiuchi et. al also identified that AGE levels increased with severity of cardiovascular disease in a Japanese population with diabetes (Kiuchi 2001).

Conclusions:

Much has yet to be done in AGEs research. We need to better understand the pharmacokinetics of AGEs. With so little being excreted in the urine or discovered deposited in tissues, what is the fate of the remainder of ingested AGEs? Are there important differences in the influence of AGEs in the body based on disease state and age? Do different AGEs interact with receptors differently with different results? More fundamentally, which AGE is the most important to measure, and can we decide as a scientific community on an ideal way of quantifying AGE in the body and in foods?

Another incomplete area of research includes the interaction between dietary macronutrient composition and serum AGE and sRAGE. Much has been made of the importance of low fat diet in lowering risk of cardiovascular disease. However, cooking methods recommended for lowering fat intake such as broiling, grilling and roasting tend to increase concentrations of AGEs in foods. Current research suggests that AGEs increase oxidative and carbonyl stress, decrease cardiovascular endothelial dysfunction, and contribute to cellular damage related to diabetes and chronic kidney disease. In addition increased body fat and body weight are associated with higher levels of AGE and decreased levels of an important receptor for AGE (sRAGE). Since low-fat diet is often recommended for weight control in obese and overweight persons, it is important to determine to what extent a low fat diet using these commonly advised cooking methods may contribute to AGE load and whether such a low-fat diet may negatively affect inflammatory cytokines thought to be influenced by AGE.

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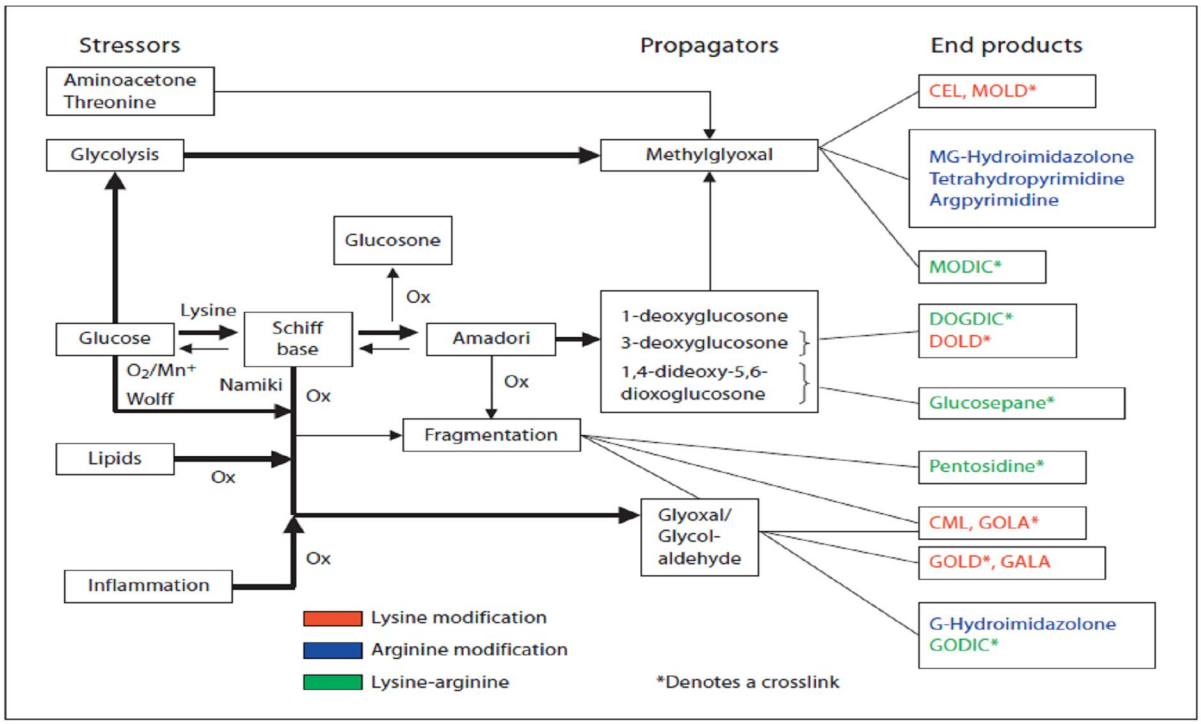

Fig. 1. Conceptual scheme of the Maillard reaction summarizing the major known glycation pathways involved in arterial stiffness. CEL = Carboxyethyl-lysine; CML = carboxymethyl-lysine; DOGDIC = 3-deoxyglucosone-derived imidazolium cross-link; DOLD = 3-deoxyglucosone-lysine dimer; G = glyoxal; GALA = glycolic acid-lysine-amide; GODIC = glyoxal-derived imidazolium cross-link; GOLA = glyoxal-lysine-amide; GOLD = glyoxal-lysine dimer; MG = methylglyoxal; MOLD = methylglyoxal-lysine dimer; MODIC = methylglyoxal-derived imidazolium cross-link.

Figure 1.* Formation of AGEs.

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