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AGEs as a Preventable Cause of Diabetes and its Complications

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1. Introduction

The incidence of diabetes and its complications continues to increase at epidemic proportions[1]. While the etiology of this disease is multifactorial, an increasing amount of evidence points to environmental and lifestyle factors, rather than genetic abnormalities[2,3]**.** T2D is often accompanied by obesity and is characterized by inflammation and insulin resistance, both of which are attributed to increased oxidant stress (OS).

Previous clinical trials[4,5] reinforced the importance of control of hyperglycemia in the pathogenesis of diabetic complications, but more recent studies[6-8] re-introduced the question of non-glucose related risk factors in these pathologies. One of the pathways by which hyperglycemia causes cell injury is via the formation of advanced glycation end products (AGEs). In fact, glucose-derived AGEs still account for many of the complications of diabetes, largely by increasing oxidative stress (OS)[9-11].

However, compelling data indicates that elevated AGEs may cause beta cell injury[12-15] and peripheral insulin resistance prior to hyperglycemia[16,17]. This evidence led to a potentially important view, that an excess of oxidants, including AGEs in Western diets could play an unforeseen role in the initiation of diabetes[18].

High AGEs – Do they precede High Glucose?

Reducing sugars non-enzymatically react with free amino groups of proteins, amine-containing lipids[19,20] and nucleic acids[9]. The entire process is accelerated in diabetes and in conditions of chronic OS, since unopposed ROS are a potent trigger for glycoxidants.

While numerous AGE compounds exist in nature, it is still unclear which are the most pathogenic AGEs. Pentosidine, carboxymethyllysine (CML) and methylglyoxal (MG)-derivatives are among the better-characterized compounds and these are often used as AGE markers[18,21,22].

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AGEs Impair Insulin Secretion and Insulin Action on Insulin Sensitive Tissues

A large body of literature points to the multi-organ injury caused by AGEs, implicating them in most diabetic complications[2,9-11]. Other studies however have began to suggest that AGEs

are also involved in islet β-cell damage[24,25]. Restriction of orally absorbed AGEs significantly protected pancreatic islet morphology and/or function in four models of diabetes: mice susceptible to autoimmune T1D (NOD mice) or T2D (d*b*/*db*+/+ mice)[13,14], as well as fat-induced or age-related diabetes[15,17]. Further evidence affirmed these findings and assigned AGE-mediated β-cell toxicity to the inhibition of Cytochrome-c oxidase and ATP production, causing impaired insulin secretion[12,24,25].

New findings supported AGEs as causes of reduced peripheral insulin responsiveness and glucose utilization. Studies in adipocytes or mice, exposed to specific AGEs, showed that glycoxidants can lead to a decrease in insulin receptor and IRS-1 phosphorylation levels, impairing glucose-uptake, via JNK activation[26]. These findings have began to shift prior dogmas and to introduce the view that prolonged exposure to oxidant overload can deplete native defense and foster defective insulin action, a view that has begun to receive support in the clinical setting[18,21,26].

A New Causative Factor for Diabetes

AGEs are readily introduced into the circulation with nutrients[27] or tobacco smoking[28] long before, or in the absence of diabetes. Certain methods of food processing, particularly heat and dehydration, accelerate the generation of new reactive dicarbonyl derivatives of complex glyco- and lipoxidation reactions[29,30]. Approximately 10% of AGEs in a single AGE-rich meal are absorbed into the circulation, and 2/3rds of this are still in the body 72 hours later[31], an interval sufficient to promote tissue injury. AGEs, such as MG-derivatives are potent inducers of inflammation[30,32,33]. The daily intake of AGEs with regular meals by healthy adults is estimated to exceed the safe range by at least 2-3 fold[34] related the preferred methods of food preparation involving heat**.** This may also account for the recent finding that high serum AGE levels in mothers correlate with those of their infants, predicting higher insulin levels and lower adiponectin[35], changes which may pre-condition infants to early diabetes.

Two large databases containing the AGE content of common foods have been made available[34, 36]. These illustrate how dry heat-processing methods (such as broiling, searing, and frying) significantly increase the protein- and lipid-AGE content of foods, compared to methods that utilize lower temperatures and moisture (i.e. stewing, steaming, boiling). These databases promoted the development of practical tools for assessing dietary AGE intake, as well as for adjusting diets to a lower AGE content, but optimal caloric and nutrient intake. Given to subjects with or without diabetes or with chronic kidney disease, a low AGE dietary regimen proved highly effective in lowering circulating AGEs, inflammation and insulin resistance[21,26]. If confirmed in larger trials, this intervention may help prevent T2D, or improve efficacy of diabetes therapies[21,37].

Another environmental source of AGEs is tobacco. The curing of tobacco involves AGE formation as tobacco leaves are dried in the presence of reducing sugars[28]. Serum AGE or AGE-apolipoprotein-B levels are significantly higher in cigarette smokers than in nonsmokers, and diabetic smokers have higher AGE levels in their arteries and ocular lenses than non-smokers[38].

Human AGE Consumption

Several studies have demonstrated that the diet is a significant source of AGEs for humans and that orally absorbed AGEs directly affect serum AGE levels in patients. Lower levels of serum AGEs were observed in diabetic (with T1D and T2D) patients after 6 weeks on a low-AGE ADA-recommended diet[37]. Prior to these studies, elevated AGE levels in T2D patients had been attributed to endogenous glycemia and OS[9]. Contrary to earlier views, serum AGE levels were found to be independent of age and glycemia, but correlated with established markers of OS and inflammation[18,21]. In addition, there was a significant association between serum AGEs and HOMA, an indicator of insulin resistance, in healthy subjects. These data suggested that the common diet is a significant source of oxidants in non-diabetic or pre-diabetic persons. This may be of particular concern today since many "healthy" subjects with normal BMI tend to consume diets with excessive glycoxidants and may thus be at risk for T2D.

AGEs and their Receptors

AGE catabolism is dependent on both tissue degradation and renal elimination. At the tissue level, there are two main types of cellular AGE receptors characterized. One promotes and perpetuates cell activation and causes tissue injury via in-

creased OS. A multi-ligand receptor, RAGE, represents this category[39]. The opposite type of receptor, AGER1, binds, degrades AGEs and protects tissues from oxidant injury based on studies on cells and mice overexpressing this gene[21,40-44]. Since both regulate multiple cellular pathways, the balance between these two receptors may be critical in the maintenance of normal homeostasis or progression to diabetes. Recent evidence suggests that AGER1 also acts to protect SIRT1, a major deacetylase and regulator of inflammation as well as insulin actions. SIRT1, like AGER1, is suppressed in chronic diabetes, but restored after lowering external oxidant burden by AGE restriction[21].

AGE-peptides normally filter across the glomerular membrane and undergo variable degree of reabsorption or catabolism by the renal proximal tubule, and the rest is excreted in the urine[45]. A precise analysis of the contribution of each of these processes is lacking in humans. The important role of the kidneys in the metabolism and excretion of AGEs is demonstrated by an inverse correlation between serum AGE levels and renal function (glomerular filtration rate) and by the abnormal handling of an oral AGE load by animals and humans with severe renal disease[27,46,47].

Human AGE Receptors

In healthy subjects, ambient levels of AGEs and ROS drive the expression of both AGER1 and RAGE receptors. A short-term increase in AGEs is associated with an increase in RAGE, as well as in AGER1, which maintains the oxidative balance within cells. Similarly, consumption of a low-AGE diet by healthy subjects effectively lowers both AGER1 and RAGE levels[21]. Importantly, AGER1 levels correlate inversely with the intracellular levels of AGEs, and directly with urine AGEs in non-diabetic persons, consistent with its active role in AGE turnover and elimination by the normal kidney. Similar relationships have not been reported for RAGE or sRAGE, although RACE correlates with risk for CVD in T1D patients[21].

The situation is quite different under conditions of chronically elevated AGEs, such as in T2D or chronic kidney disease. Despite full anti-diabetic therapy, RAGE levels remain high, while AGER1 levels are uniformly suppressed[2,17,21,26,33], both indicative of persistent high OS. Interestingly, following 4 months on a low AGE diet, AGER1 levels were restored in diabetic patients, while RAGE levels were significantly suppressed[21]. This pattern suggests that depletion or loss of function of AGER1 gene contributes to diabetic tissue injury, possibly by intracellular AGE accumulation, ROS generation, suppression of NAD+-dependent SIRT1 and further increased ROS. Hyper-activation of RAGE, as well as of other pro-inflammatory gene products, is likely a consequence of the initial failure of defensive mechanisms, such as of AGER1 and SIRT1 to fend off oxidant overload, feeding into a cycle of OS and innate defense depletion.

Modulating AGE intake in humans

There is considerable support for the role for AGEs in human diabetes, as well as for non-diabetic CVD or renal disease[48-52].

Moreover, the concept that nutrient-derived oxidant AGEs could pose risk for acquiring diabetes —not only for diabetic vascular disease— has introduced a paradigm shift, according to which hyperglycemia could be the downstream *effect*, not the *cause* of high AGEs and thus, glucose control is only one of the variables that must be controlled. Without altering blood glucose or HgbA1c levels, a 6-week AGE-restricted diet given to a group of type I and type II diabetic patients led to the significant reduction of indicators of inflammation and endothelial dysfunction, including hsCRP, TNFa and VCAM-1, along with AGE levels[36,37]. More recently, serum AGEs were shown to correlate with fasting insulin and HOMA-IR, as well as in leptin, along with a marked rise in adiponectin in T2D subjects[26] after a longer treatment with AGE-restricted diet (4 months). These changes were consistent with an improvement in insulin sensitivity in these diabetic subjects. There were also a significant increase in AGER1 as well as of SIRT1 levels and decreased mononuclear TNF-α, all consistent with suppressed inflammation in these subjects[21,26].

Fighting AGEs, Not Only Hyperglycemia

Hyperglycemia is a major source of native AGEs. Therefore, intensive treatment of hyperglycemia helps maintain control of AGEs. For instance, in a large cohort of type 1 diabetic patients from the DCCT skin collagen glycation, glycoxidation, and crosslinking were decreased in those undergoing long-term intensive treatment more than in patients on conventional treatment[55,57]. Although the focus was on hyperglycemia[4,58], recently conducted large

studies (ACCORD, ADVANCE, NADT)[6,8,58] failed to produce the anticipated conclusions. In view of the evidence discussed above, having not adjusted for exogenous oxidants or AGEs may have influenced the results. Well-controlled cellular and animal studies suggest that AGEs can incite beta cell injury[12] and that the occurrence and severity of T1D and T2D diabetes and its complications may depend on exogenous oxidant (AGE) overload, beyond hyperglycemia[59].

Preventing New AGE Formation

A number of agents act by inhibiting post-Amadori advanced glycation reactions or by trapping carbonyl intermediates (glyoxal, methylglyoxal, 3-deoxyglucosone) and include aminoguanidine[60-62], benfotiamine[63-65] and pyridoxamine[66,67]. Other agents, such as ORB-9195 are thought to inhibit both glycation and lipoxidation reactions[68,69]. A number of these are expected to become available in the near future.

Several studies have proposed various antioxidants as anti-AGE agents, including vitamin E[70], N-acetylcysteine[71], taurine[72], alpha lipoic acid[73], penicillamine[74], nicanartine[75] and others. More studies are needed to establish the effectiveness of antioxidants, even if the results from trials on a wide range of oral antioxidants have been disappointing, as in the past these were not informed by the current evidence and, thus, were not designed to address the large exogenous overload of oxidants.

Conclusions

There is little controversy surrounding the fact that surplus AGEs contribute to OS and inflammation in diabetes and its complications. Hyperglycemia, a driving force for AGEs, can be substantially greater, if based on pre-existing overt OS. The common diet is a carrier of AGEs, and a key contributor to this pre-existing OS, which eventually impairs innate defenses and drives IR. This is of particular concern in subjects with pre-diabetes. Thus, AGE overload is an area of major clinical relevance as it may be a cause of diabetes. Interventions that reduce both exogenous (food-derived) as well as endogenous (hyperglycemia-derived) AGEs can provide definitive and lasting beneficial effects for diabetes and its complications.

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