Review Article
Advance Glycated End Products in Type 2 Diabetes Mellitus and Role of Dietary Supplement in their Management

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Article Info

Abstract
Advance glycated end products are a heterogeneous group of molecules formed from the non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids. Advance glycation is an irreversible process, its physiological roles are to identify senescent proteins and hence there is an accumulation of advanced glycation end products. Diabetes is currently recognized as an oxidative stress disorder and hyperglycemia is a condition has an important role in the pathogenesis of diabetic complications. Diabetes has an overload of reducing sugars which accelerate advance glycated end products formation in body tissues. Interventions that can reduce advance glycated end products accumulation are also helpful in preventing the development of diabetic complications. Antioxidants can protect the formation of advance glycated end products by blocking the free amino groups on proteins and also trap the carbonyl group. However, the efficiency of these dietary supplements against advance glycated end products in vivo is unknown. The antioxidants are now used as add on to pharmacotherapy in diabetic patients to reduce hyperglycemia and prevent advance glycated end products formation.

1. INTRODUCTION
Diabetes mellitus is a metabolic disorder characterized by increased glucose level i.e. persistent hyperglycemia. It is a chronic disease associated with diabetic complications such as microvascular and macrovascular complications1. Persistent hyperglycemia leads to abnormal changes i.e. formation of advanced glycated end products, oxidative stress and increase of polyol pathway flux. AGEs are implicated in the pathogenesis of diabetic complications2. AGEs accumulation is not just a measure of hyperglycemia, but represents metabolic burden of both hyperglycemia and hyperlipidemia3. Although there have been important advances in the control of diabetes by means of glycemic drugs, insulin, the insulin pump, the diet and interventions that can reduce advance glycated end products accumulation are also helpful in preventing the development of diabetic complications4.

2. ADVANCE GLYCATED END PRODUCTS
Advance glycated end products are heterogeneous end products of non-enzymatic glycation, sequential glycation and oxidation of sugars with free amino groups on proteins, peptides. This sequence of events is known as the Maillard reaction. These are implicated in the pathogenesis of microvascular and macrovascular complications of diabetes. AGEs are generally divided into two types on the basis of chemical structure; one type is fluorescent properties and another one is cross linking structures AGEs5.

2.1 Advance Glycated End Products Formation
In early-stage glycation, the amino group of the body proteins (specifically lysine residues in protein) produces a labile covalent bond with aldehyde group of the physiological sugars to form a Schiff base. This Schiff base, in turn, undergoes molecular rearrangement to form one ketoamine known as Amadori product6. Covalent cross linking of these Amadori products once again with the sugar group in other glycated protein lead to development of irreversibly modified molecules, termed as advanced glycation end products (AGEs) (Figure 1).

Figure 1: Pathways for the formation of advanced glycated end products

Other pathway which may lead to AGEs formation is through auto-oxidation of glucose by reactive oxygen species, and through carbonyl compounds. In particular methylglyoxal, a reactive carbonyl is the most reactive AGE precursor7.

The level of these early advance glycated end products change in response to blood glucose and are reflected in the analysis of HbA1c and glycated albumin to monitor average blood glucose control. However, the irreversibly formed AGEs level does not return to normal when glucose level is normal and accumulates over the lifetime of the proteins8.

In addition to endogenous formation, AGEs are also derived from exogenous sources i.e. diet and tobacco smoke. When sugars are cooked with proteins or fats exogenous glycation and advance glycated endproducts (AGEs) are formed. Temperatures over 120°C (~248°F) greatly accelerate the reactions, however lower temperatures with longer cooking times also promote their
formation. A significant proportion of ingested AGEs are absorbed from foods exposed to high temperatures. AGE levels are seen higher in smokers and individuals on high-AGE diets.

3. INHIBITION OF ADVANCE GLYCATION
Attempts have been made to pharmacologically influence the process of synthesis of AGEs in order to prevent or slow down the formation of AGEs. Different types of AGEs inhibitors have been classified (Table 1).

Table 1: Types of AGEs inhibitors

<table>
<thead>
<tr>
<th>S. No.</th>
<th>TYPE A</th>
<th>TYPE B</th>
<th>TYPE C</th>
<th>TYPE D</th>
<th>TYPE E</th>
<th>TYPE F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspirin</td>
<td>Guanidine</td>
<td>EDTA</td>
<td>Guanidine</td>
<td>Guanidine</td>
<td>ALT-711</td>
</tr>
<tr>
<td>2.</td>
<td>Pyridoxal-5 phosphate</td>
<td>Aminoguanidine</td>
<td>Penicillamine</td>
<td>Aminoguanidine</td>
<td>Aminoguanidine</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Pyridoxamine</td>
<td>Metformin</td>
<td>Carnosine</td>
<td>Carnosine</td>
<td>Metformin</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>-</td>
<td>Thiamine</td>
<td>Vitamin C</td>
<td>L-Arginine</td>
<td>Carnosine</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>-</td>
<td>Vitamin E</td>
<td>Metformin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
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Type A- Prevent sugar attachment
Type B- Block formation of cross-links
Type C- Chelating agents and antioxidants
Type D- Trap reactive carboxyls
Type E- Prevent formation of AGEs from amadori products
Type F- Cross-linkage and AGE breakers that can break cross-links after they form

3.1 Dietary Antioxidants
A dietary antioxidant can be defined as "a substance in food that significantly decrease the adverse effects of reactive species, such as reactive oxygen species (ROS) and nitrogen species on normal physiological function in humans". Studies show that antioxidant compounds such as vitamin C, vitamin E prevents or reverse nerve conduction velocity deficits in experimental model. They have also shown to reduce in vitro and in vivo protein glycation. Vitamin C works in conjunction with vitamin E to maintain its potency. Carnosine, a natural dipeptide, is a major brain and muscle antioxidant which can compete with proteins for binding with sugars with effects on AGEs. Essential trace elements, selenium works with vitamin E to protect cell membrane and tissues. In vitro studies suggest that AGEs-inhibitory activities of antioxidants have been achieved by preventing antioxidative pathways of AGEs formation.

3.2 Synthetic and Natural Advance Glycation End Products-Inhibitors
A pharmacologic approach for the prevention of AGEs formation is the use of carbonyl traps. Promising substances are pyridoxamine, aminoguanidine and the well known metformin. Pyridoxamine, also known as vitamin B6, scavenges α-dialedehydes as well as lipid peroxidation intermediates. The first compound which has been extensively studied in vitro and in vivo to be a powerful inhibitor of AGEs formation is aminoguanidine, which prevents diabetic complications. Aminoguanidine inhibit the formation of CML or CEL, traps reactive carbonyls and a potent inhibitor of nitric oxide synthase. The use of aminoguanidine hindered reduction in glomerular filtration rate reduced 24-h urinaryproteinuria and prevented the deterioration of retinopathy. Ligation of advanced glycation end products (AGEs) with their receptor (RAGE) plays an important role in the development of various secondary complications of diabetes, including atherosclerosis. Monocyte activation, adhesion, and migration are key events in the pathogenesis of atherosclerosis. Ligation of RAGE on monocytes stimulates the production of pro-inflammatory cytokines and chemokines, which can contribute to the development of atherosclerosis. Metformin also inhibit AGEs with the mechanism of trapping methylglyoxal and dicarbonyls. Benfotiamine, a lipid soluble compound, was found to be a potent inhibitor of glycation. This drug was recently shown to block the three major pathways of hyperglycemic damage and was successful in preventing diabetic retinopathy in rats. Benfotiamine prevented both microvascular and macrovascular endothelial dysfunction and oxidative stress induced AGEs rich meal and also the use of licopid acid with benfotiamine normalizes the level of AGEs. The natural compounds curcumin, a constituent of turmeric, also have an anti-AGEs and anti-inflammatoryatory property. Resveratrol prevents the impairment of AGE on macrophage lipid homeostasis partially by suppressing RAGE via PPARα activation, which might provide new insight into the protective role of resveratrol against diabetic atherosclerosis. Resveratrol has been shown to inhibit AGE-induced proliferation and collagen synthesis activity in vascular smooth muscle. Other natural compounds are garlic extract and green tea also prevents formation of AGEs.

4. ADVANCE GLYCATED END PRODUCTS (AGEs) AND DISEASES
The presence of AGEs is correlated with several important diseases. The rate of AGEs formation is accelerated in diabetics as it is often accompanied with hyperglycemia and oxidative stress. This glycation reaction contributes to morbidity of diabetes, end-stage kidney and heart diseases, and it is also involved in the pathophysiology of Alzheimer’s disease, arthritis and aging. AGEs causes intermolecular collagen cross-linking which leads to vascular stiffness, phenomena that are considered to explain partly the increase in diastolic dysfunction and systolic hypertension seen in diabetic. AGEs accumulate in most sites of diabetes complications i.e. the kidney, retina, and atherosclerotic plaques.

4.1 Advance Glycated End Products and Diabetes
It has long been recognized that increased HbA1c, (a precursor of AGEs) levels are associated with a higher incidence of vascular complications and reduced life expectancy in diabetes patients. In addition, the intervention studies to reduce HbA1c lead to lower microvascular and macrovascular lesions and a reduced death rate over several years. Serum levels of AGEs in patients with type 2 diabetes appear higher than those without diabetes. Preliminary studies in diabetic patients have shown that aminoguanidine therapy for 28 days reduces hemoglobin derived AGEs thus providing evidence that aminoguanidine can reduce AGEs in vivo. However, the same study showed that aminoguanidine inhibited only AGEs but had no effect on levels of Amadori products.

4.2 Diabetic Nephropathy
Diabetic nephropathy is the major cause of end-stage renal disease. Pathophysologically, it is characterized by abnormal
Capsaicin alleviate peripheral neuropathy symptoms, works on pain by binding to the VR 1 receptors and striping nerves of substance P, a pain-signaling neurotransmitter. In a clinical study topical capsaicin was applied to the feet of patients with symptomatic diabetic neuropathy which results in improvement of symptoms, including pain perception threshold. Researchers concluded that capsaicin cream was effective, without causing adverse effects on nerve fiber function. Six-week trial of 10 diabetic patients to evaluate the effect of 0.25% of oral vitamin B6 on diabetic peripheral neuropathy revealed symptomatic improvement in all patients 34. Type 2 diabetic patients showed improvement of some clinical features of neuropathy after 3-week treatment of lipid acid. Alpha-lipoic acid treatment improves nerveblood flow and distal nerve conduction and increases endonuclear glucose uptake and energy metabolism in animals 35.

4.3 Diabetic Neuropathy

Diabetic neuropathy is complex in its etiology and manifestations and is produced by metabolic abnormalities (poloy pathway, AGES, oxidative stress), functional abnormalities (reduced nerve conduction) and structural abnormalities such as glycation of axonal cytoskeletal protein i.e. tubulin, actin and neurofilament results in slow axonal transport and degeneration. AGES, also affects growth factors such as fibitin and nerve growth factors contributes to loss of function 43. Hyperglycemia induced AGES on peripheral nerve myelin contribute to segmental demyelination by increasing itsusceptibility to phagocytosis by macrophages. AGES by increasing macrophage recognition and uptake, stimulates macrophage – derived growth factor. This results in proliferation of smooth muscle and arteriogenesis and glycation of extracellular matrix protein laminin leads to impaired regenerative activity in diabetic neuropathy 44.

In diabetic neuropathy AGES and interaction of AGES with its receptor induce oxidative stress, result in upregulation of nuclear factor (NF)-kappaB and various NF-kappaB-mediated proinflammatory genes, and exaggerate neurological dysfunction, including altered pain sensation.

AGE – RAGE interaction producing reactive oxygen species, ROS accumulating AGES and oxidative stress, and AGE quenching oxidative stress. The quenching action of AGE binding on NO is relevant to nerve ischemia. The reduction of NO is one of the most important mechanisms of ischaemic nerve injury 45.

Benfotiamine has beneficial effects on nerve conduction velocity in the mononeuropathy 46 in diabetic patients, and a short-term clinical study showed alleviation of painful neuropathy 47, but long-term human data are still lacking. Capsaicin alleviate peripheral neuropathy symptoms, works on pain by binding to the VR 1 receptors and striping nerves of substance P, a pain-signaling neurotransmitter. In a clinical study topical capsaicin was applied to the feet of patients with symptomatic diabetic neuropathy which results in improvement of symptoms, including pain perception threshold. Researchers concluded that capsaicin cream was effective, without causing adverse effects on nerve fiber function. Six-week trial of 10 diabetic patients to evaluate the effect of 0.25% of oral vitamin B6 on diabetic peripheral neuropathy revealed symptomatic improvement in all patients 34. Type 2 diabetic patients showed improvement of some clinical features of neuropathy after 3-week treatment of lipid acid. Alpha-lipoic acid treatment improves nerveblood flow and distal nerve conduction and increases endonuclear glucose uptake and energy metabolism in animals 35.
amputations.51 Diabetes also affects the heart muscle and causes both systolic and diastolic heart failure. The etiology of this excess cardiovascular morbidity and mortality is not completely clear. Evidence suggests that although hyperglycemia contributes to myocardial damage, it is clearly not the only factor, because both pre-diabetes and the presence of the metabolic syndrome, even in normoglycemic patients, increase the risk of most types of cardiovascular diseases. Heart failure is characterized by a structural or functional cardiac disorder that results in an inability of the heart to fill with or pump out blood combined with dyspnoea or fatigue. AGEs may contribute to the development of heart failure through two pathways, firstly, AGEs affect the physiological properties of proteins in the extracellular matrix and secondly, advanced glycated end products cause multiple vascular and myocardial changes via the interaction with AGE receptors. AGEs can cause diastolic and systolic dysfunction through these pathways. Later, these abnormalities may result in the development and progression of heart failure.

AGEs and diastolic dysfunction: Cross-linking of extracellular matrix proteins is essentially a pathological phenomenon. It results in strengthening tissues ensuring tissue integrity without compromising flexibility. AGEs, however, can bind covalently to other AGEs and form additional cross-links between matrix proteins like collagen, laminin and elastin. Excessive cross-linking caused by AGE accumulation underlines the flexibility of matrix proteins and will induce diastolic dysfunction in the heart. Another pathway by which AGEs could contribute to the development of diastolic dysfunction is via the activation of AGE receptors. Receptor for AGE (RAGE) over-expression was found to reduce the systolic and diastolic intra-cellular calcium concentration.65

AGEs and systolic dysfunction: AGE accumulation may be involved in the development of systolic dysfunction by accelerating the progression of coronary artery disease. AGE-RAGE interaction results in atherosclerosis, thrombosis, and vasoconstriction. By negatively influencing LDL-metabolism, AGEs could further increase the risk of developing atherosclerosis and subsequent myocardial infarction.66,67

AGEs and vascular dysfunction: Endothelial dysfunction is a very important predictor of adverse cardiac events, hospitalization of patients with heart failure, and death. Together with vascular compliance, endothelial dysfunction relates closely with the functional capacity of chronic heart failure patients.74 AGEs impair vascular function by influencing both endothelial function and vascular compliance. AGEs may induce endothelial dysfunction by reducing the availability of the vasodilator nitric oxide (NO).75,76 Furthermore, AGEs can enhance the production of endothelin-1, a potent vasoconstrictor.97 Vascular compliance is influenced by AGE cross-linking in a similar fashion as in myocardial tissue. In human tissues, no AGEs correlate with arterial compliance.82,83

Hyperglycemia contributes to cardiovascular risk and patients with diabetes have increased risk of cardiovascular morbidity and mortality. AGEs accumulate at a much higher rate in diabetics and contribute in the development and progression of cardiovascular disease in diabetes. Serum AGE levels are elevated in diabetic patients with coronary heart disease compared to patients without coronary heart disease.74 AGE levels are further related to other features of cardiovascular disease, such as carotid stenosis and peripheral arterial occlusive disease.66,67

In a study of type 2 diabetic mice with cardiac dysfunction benfotiamine protects against diet-induced cardiac dysfunction through pleiotropic mechanisms, culminating in the activation of pro-survival signaling pathways. Thus, benfotiamine merits attention for application in clinical studies.85 In randomized clinical trials (RCTs), patients with coronary heart disease were administrated omega-3 fatty acid supplements and the result shows significant reduction in CV events. Omega-3 supplements can also slow down the progression of atherosclerosis in these patients.86 Research concerning nutritional regimens has shown that persons who consume large amounts of fruit and vegetables have lower incidences of cardiovascular diseases although the precise mechanisms for this protective effect are elusive. Possible explanations include (a) increased consumption of dietary fiber (b) reduced consumption of dietary cholesterol and other lipids, and (c) increased intake of the antioxidant vitamins (A, C, and E).87

5. CONCLUSION
AGEs act as pro-oxidants and pro-inflammatory agents that mediate the development of diabetes-related complications. Hyperglycemia with advanced glycation end products (AGEs) levels may further accelerate the progression of these complications. The effective dietary supplements are used for their antiglycation activity and also are beneficial to prevent AGEs associated chronic diseases. This review examined the use of supplements for their putative AGE-lowering properties, and effects on complications in diabetic patients. In conclusion, dietary supplements are intended to be used as a therapeutic option in specifically type 2 diabetes patients and also act as add-ons to the pharmacotherapy for a healthy lifestyle; therefore supplements can be used to promote overall good health and prevent disease.

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