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Gluco-Oxidation of Proteins in Etiology of Diabetic Retinopathy

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1. Introduction

Diabetes mellitus a chronic slow progressing catastrophe and is a major medical problem throughout the world. Diabetes causes an array of long-term systemic complications that have considerable impact on patient as well as society, as the disease typically affects individuals in their most productive years (Federman et al., 1994; Bhavsar et al., 2010). In addition, this increase appears to be greater among certain ethnic groups and in developing countries. Diabetic retinopathy is one of the main causes of diabetic complications. It causes visual impairment and finally blindness, a result of long-term accumulated damage to the small blood vessels in the retina. The proportion of blindness due to diabetic retinopathy ranges from close to 0% in most of Africa, to 3–7% in much of South-East Asia and the Western Pacific, to 15–17% in the wealthier regions of the Americas, Europe and the Western Pacific (Resnikoff et al. 2004; Zhang et al., 2010). According to the WHO fact sheet Aug. 2011, 346 million people worldwide have diabetes (World Health Organization [WHO], 2011). About 50% of persons with diabetes are unaware that they have the condition, although about 2 million deaths every year are attributable to complications of diabetes. After 15 years, about 2% of persons with diabetes become blind, and about 10% develop severe visual loss (WHO 2011). After 20 years, more than 75% of patients will have some form of diabetic retinopathy (Barcelo et al., 2003).

Post onset diabetes with increasing age, there is a higher risk of developing diabetic retinopathy and its complications, including diabetic macular oedema or proliferative diabetic retinopathy increases. The exact mechanism by which diabetes causes retinopathy remains unclear, but several theories have been postulated to explain the typical course and

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history of the disease (Crawford et al. 2009). Chronic hyperglycemia exerts protein gluco-oxidation, a process involving the non-enzymatic modification of tissue proteins by physiologic sugars in vivo, appears to play a central role in the pathogenesis of diabetic complications. One mechanism linking uncontrolled hyperglycaemia with tissue damage such as that in diabetic retinopathy is the formation and accumulation of advanced glycation end-products (AGE) (Hammes et al, 1996). *Ex vivo* and in vivo studies have indicated that AGE induce irreversible cross-links in long-living extracellular matrix (Brownlee et al., 1988; Fu et al., 1992; Sell et al., 1992; Sell et al., 1993) and, upon binding to specific cellular proteins, change the local concentrations of cytokines, growth factors and other bioactive molecules (Schleicher & Nerlich 1996; Vlassara et al, 1985). Accumulation of AGEs depends on both sugar concentration and the rate of protein turnover. Thus, some proteins that reach critical levels of AGE modification in sites where diabetic complications occur may turnover too quickly for normal levels of blood glucose to cause functional alterations, while proteins with a longer half-life would continue to be modified over a longer period of time (Brownlee 1995).

The relation between diabetes mellitus and oxidative stress is well known. With the onset of diabetes, persistent and chronic hyperglycemias causes increased production of free radicals through auto-oxidation of glucose, via nonenzymatic protein glycation and enhanced flux of glucose through the polyol pathway (Giugliano et al., 1996). The generation of reactive oxygen species and protein glycation are strictly interconnected (Palm et al, 2003). Levels of serum AGE are increased in diabetes mellitus before they have developed microvascular complications (Berg et al., 1997). These increased serum levels of AGE can predict changes in microvascular morphology in patients with diabetic retinopathy. Proteins containing AGE are highly immunogenic (Reddy et al., 1995) and anti-AGE antibodies were found in the sera of patients with diabetes (Shibayama et al, 1999; Baydanoff et al., 1996). Our research team has hypothesized that increase in the titre of anti-AGE antibodies has a direct role in the pathogenesis of diabetes microvascular complications especially diabetic retinopathy. Detection and characterization of antibodies against gluco-oxidative modified proteins could help in understanding the exact aetiology of gluco-oxidation of protein and diabetic retinopathy. Anti-gluco-oxidative modified proteins antibodies may potentially help in the prediction and /or prognosis of diabetes retinopathy. However the exact pathophysiology is yet to be ascertained.

2. Glycation

Reducing sugars such as glucose (or other reducing sugars as fructose, pentoses, galactose, mannose, ascorbate, xylulose) reacts nonenzymatically with free ε-amino groups in protein, lipids and nucleic acids through a series of reactions forming Schiff's bases and Amadori products to produce AGEs; and this process, also known as the Milliard reactions. In theory, every protein can be modified by glycation. Indeed, many protein-AGE adducts have been identified, *e.g.* glycated fibrinogen, collagen, albumin, herpes simplex glycoprotein B, hemoglobin, β2-microglobulin, and low density lipoprotein (Raj et al., 2000; Cribbs et al., 2000). Albumin is the most abundant protein in human serum, about 35-50 g/liter, and it is prone to glycation (Carter & Ho 1994). Non-enzymatic glycation of albumin occurs at multiple sites; glucose can attach to Lys199, Lys281, Lys439, and Lys525 as well as some other lysine and arginine residues and also at the N-terminal residues of polypeptides (Iberg & Fluckiger 1986). In fact, only a small number of factors are known to result in the variation

of serum albumin. The alteration in the structure of albumin due to uncontrolled hyperglycemia causes vascular complications (Bourdon et al., 1999).

Glycation is a classical covalent reaction in which, by means of N-glycoside bonding, the sugar-protein complex is formed through a series of chemical reactions described for the first time by a chemist Louis Camille Maillard in 1912 (Sing et al., 2001). Maillard reactions are complex, multilayered, and can be analyzed in three steps. (i) The sugar-protein complex is formed first (Amadori rearrangement), an early product of non-enzymatic glycation leading to intermediary products which are precursors of all later compounds. (ii) Formation of numerous intermediary products, some of which are very reactive and continue the glycation reaction. (iii) Final phase consists of polymerization reaction of the complex products formed in the second step, whereby heterogeneous structures named advanced glycation end products (AGE) are formed (Fig. 1).

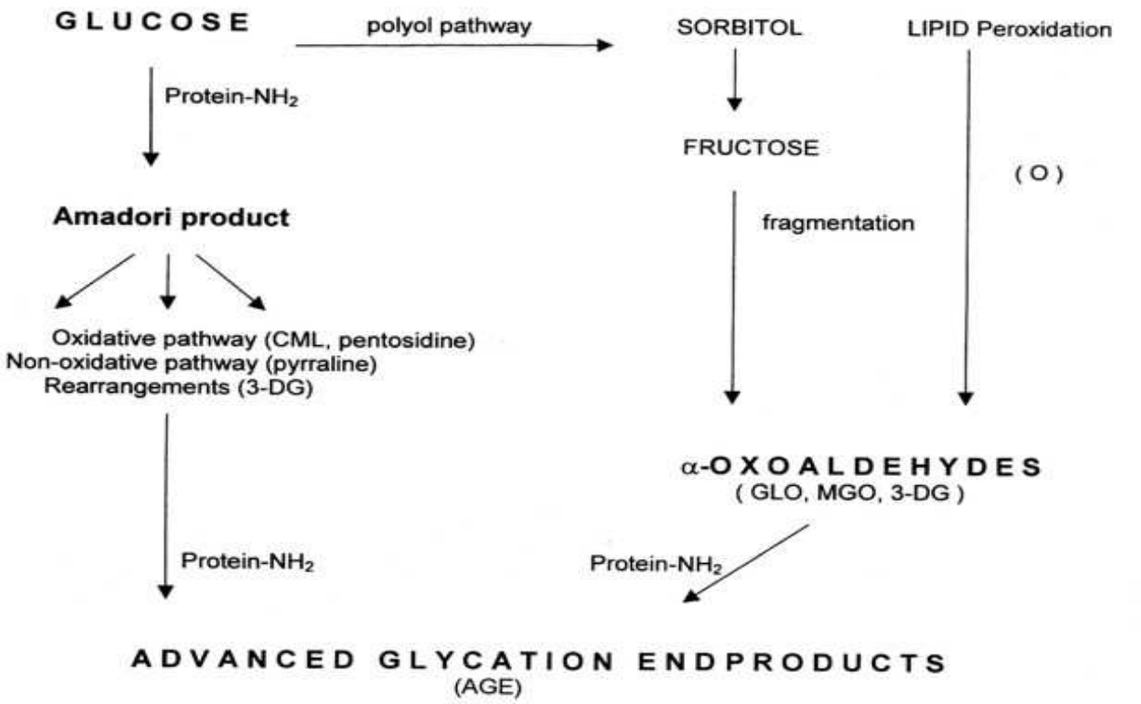


Fig. 1. Schematic presentation of potential pathway leading to AGE formation. The abbreviations given above are represented as, GLO=glyoxal; MG=methylglyoxal; 3-DG=3-deoxyglucosone; CML=carboxymethyl-lysine (Turk, 2001).

AGE constitute a heterogenous group of molecules (Peppa & Vlassara 2005) and its formation takes place continuously within the body during ageing, however it is extremely accelerated in diabetes (Vlassara & Palace 2002; Fu et al., 1996; Thorpe & Baynes 1996; Peppa et al., 2004). Some of the major AGEs are carboxymethyl lysine (CML) and pentosidine and also include many reactive intermediates or AGE-precursors such as 1- or 3-deoxyglucosone, methylglyoxal (MG) and their derivatives. AGE can cause tissue damage by two main pathways: they either form cross-links that disrupts the structure and function of short and long-lived proteins and lipids or they bind with specific and nonspecific cell

surface receptors inducing deleterious consequences, leading to altered intracellular events that induce oxidative stress and inflammation (Vlassara & Palace 2002; Peppa et al., 2002; Peppa et al., 2004; Vlassara 2001). AGE induced pathogenesis of diabetic retinopathy occurs via alteration of small vessel wall integrity and structure, by inducing cytokines, growth factors and increased oxidative stress (Sheetz & King 2002; Vlassara & Palace 2002; Peppa et al., 2002; Peppa et al., 2004; Vlassara 2001; Stitt et al., 1997; Stitt 2001; Yamagishi et al., 2002). *Ex vivo*, retinal endothelial cells exposed to AGE overproduce vascular endothelial growth factor (VEGF) through oxidative stress induction, protein kinase-C pathway activation and abnormal endothelial nitric oxide synthase (eNOS) expression (Mamputu & Renier 2002; Chakravarthy et al., 1998). Retinal organ cultures show an increased glyoxal induced CML formation in association with increased apoptosis and cell death, restored by anti-AGE agents and antioxidants (Mamputu & Renier 2002). Increased AGE accumulation was found in the retinal pericytes of diabetic rats after 8 months of diabetes (Stitt et al., 1997). In addition, exogenous AGE-albumin administration in non-diabetic animals accumulated around and within the pericytes, colocalized with AGE receptors inducing retinal vessel wall thickening and loss of retinal pericytes (Xu et al., 2003; Clements et al., 1998). In humans, it has been found that with the increasing severity of retinopathy there is a proportional increase in AGE accumulation around retinal blood vessels (koya et al., 2002). Glycation of vitreous collagen was also observed in human donor eyeballs (Sulochana et al, 2003). In addition, studies using anti-AGE agents further support the role of AGE in diabetic retinopathy (Yamagishi et al., 2002; Chappey etal., 1997; Reber et al., 2003). AGE have also been linked to the changes associated with diabetic keratopathy through their effect in reducing corneal epithelial cell adhesion (Matsumoto et al., 1997). Furthermore, glycation of the vitreal collagen fibrils leading to dissociation from hyaluronan and resultant destabilization of the gel structure has been associated with vitreous liquefaction and posterior vitreous detachment in diabetes (Stevens 1998; Sebag et al., 1992; Stitt et al., 1998).

3. Pathophysiological mechanism of AGEs formation in diabetic retinopathy

The knowledge of gluco-oxidation of proteins and AGEs has considerably expanded over the years, and a large body of evidence has documented their implication in diabetes-related complications (Singh et al., 2001; Turk et al., 2001; Brownlee 2001; Monnier et al., 2005; Huebschmann et al., 2006). In the process of glycation, AGE peptides that are released as degradation products, which partly occur through proteolysis of the matrix component are commonly named as glycotoxins. Glycated proteins are toxic for neuronal cells, retinal capillary cells, leukocytes, pericytes, and endothelial cells (Takeuchi et al., 2000; Yamagishi et al., 2002; Lyons et al., 2000). Toxicity of glycated polypeptides may be due to the AGE modification or due to the aggregation state of the polypeptides. Glycotoxins are very reactive on entering blood circulation. Non elimination of these proteins through the kidneys leads to recirculating AGE peptides which can generate new AGE products that react with other plasma or tissue components. At this stage, glycation becomes an autonomic process, which significantly accelerates the progress of the complication (Turk 2001).

Immunoglobins are glycated differently according to their class. The glycation of immunoglobulin-M is twofold greater than that of immunoglobulin G, and can be related to

the difference in amino acid composition. Albumin can be glycated at multiple sites. In diabetic patients, excessive glycation of fibrinogen and fibrin has been reported (Chappey et al., 1997). Hemoglobin is glycated at two sites: on the valine residue of the N-terminal β-chains at the ε-amino group of the α and β-chains, and at the N-termini of the α-chains (McDonald et al., 1978). Other intracellular and membrane proteins of red blood cells (RBC) are also glycated, for example Spectrin, a major RBC membrane protein, band 3 transmembrane protein, and band 4-1 (Retnaikes et al., 1987; Bryszewska & Szosland 1988). Hence glycation results in RBC deformability and an increased adherence to endothelium. Membrane proteins of platelets can also be glycated. Increased binding of fibrinogen and platelet aggregation observed in diabetic patients can be related to the glycation of adenosine diphosfate receptors. Lipids can also contribute to the modifications of platelet functions in diabetes (Chappey et al., 1997). Hyperglycemia can also induce protein aggregation which is associated with diabetes and its complications. Gluco-oxidation of proteins induces refolding of globular proteins, accompanied by the formation of cross β-structure (Bouma et al., 2003).

Gluco-oxidation of proteins forms complex and irreversible molecules, which accumulate in the retinal vasculature of patients with diabetes and streptozotocin-induced diabetic rats (Hammes et al., 1999; Stitt et al., 1997) and have been implicated in the development of diabetic retinopathy (Boehm et al., 2004 Genuth et al., 2005). Chronic exposure of the endothelium to AGEs has been shown to increase retinal vascular permeability in vivo (Stitt et al., 2000) and *ex vivo* (Leto et al., 2001). AGEs, however, have also been shown to increase capillary permeability acutely (Sampietro et al., 1987). Activation of AGE recepter (RAGE) and production of oxygen free radicals have been shown to mediate cellular responses to AGEs; however, the signalling pathways involved in the early permeability response are unknown (Kislinger et al., 1999; Bonnardel-Phu et al., 1999).

It has been suggested that, in diabetes, oxidative stress plays a key role in the pathogenesis of vascular complications, both microvascular and macrovascular, and an early marker of such damage is the development of an endothelial dysfunction (Giugliano et al., 1996; Cai & Harrison 2000). Evidence implicates hyperglycemia-derived oxygen free radicals as mediators of diabetic complications. Recently recognized relationship between α-oxoaldehydes and biologically important macromolecules highlights the intermediate step of advanced glycation cascade (Beisswenger et al., 2003a; Beisswenger et al., 2003b; Thornalley 2005). Diabetic individuals may exhibit elevated levels of iron and free copper ions (Cutler 1978; Mateo et al., 1978), which in the presence of glycated proteins *ex vitro* have been shown to generate free radicals (Hunt 1994). The accumulation of glycated material in tissues that contain free copper ion contribute to the generation of free radical mediated damage. These highly reactive species are capable of causing oxidative degradation of protein *ex vivo* (Hunt 1994). The formation of α-dicarbonyl compounds is known to be an essential step for the cross-linking of proteins and subsequent free radical generation (Rahbar & Figarola 2003). Methylglyoxal is increased 5-6 fold; in adult onset, non-insulin dependent diabetes mellitus as compared to healthy individuals. In the presence of oxidative stress, glycation of proteins by methylglyoxal is enhanced. This may underlie the link of glycation and oxidative stress with diabetic complications, and may also contribute to pathological processes of ageing.

Structural and functional modification of host-protein is a common feature of all AGEs irrespective of their generating precursors. Through their effects on the functional properties

of extracellular matrix, intracellular signal transduction and protein function, AGEs may contribute to the pathogenesis of diabetic retinopathy (Poukupec et al., 2003). A mechanism by which AGE-modified proteins may exert their effect is binding to RAGE identified on a variety of cells including endothelial and smooth muscle cells, and by internalization and degradation *via* monocyte/macrophage AGE-receptors. Using radiolabeled AGE proteins it has been shown that several cells, such as human and mouse monocyte, macro-phage and lymphocyte, bind these types of glycated compounds in a relatively selective way (Gilcrease & Hoover 1990; Imani et al., 1993). Gluco-oxidative modified proteins bind to these cells in a saturable manner with a dissociation constant in the range of 50–200 mmol/l^{-1}. The putative receptors for AGE have been isolated from cell membranes and purified, and were reported to have different molecular weights: 30–50 KD for renal tissue, 36–83 KD for a macrophage cell line, 60–90 KD for liver cells (Yang et al., 1991; Skolnik et al., 1991). A carbohydrate-binding protein of 35 KD named Galectin 3 is present on lymphocytes, macrophages, endothelial, mesangial, smooth muscle cells, and fibroblasts, and binds AGE with a higher affinity than other carbohydrates (Vlassara et al., 1995).

Increased hyperglycemia caused protein gluco-oxidation and/or glucose auto-oxidation enhanced formation of AGEs, stimulate the expression of RAGE and hence NADPH oxidase activation. Activation of NADPH oxidase increased the production of free reactive oxygen radicals can up-regulate vascular endothelial growth factor (VEGF) in retinal cells via nuclear transcription factors (eg. NF-kappaB) potentially promoting retinal neovascularisation and increasing permeability to proteins across the retinal barrier. Increased RAGE expression has been found on endothelial cells, vascular smooth muscle cells and cardiac myocytes of diabetic patients (Schmidt et al., 1999). It has been reported that ligation of AGE with RAGE causes activation of intracellular signaling, gene expression, and production of proinflammatory cytokines and free radicals, thus playing an important role in the development and progression of diabetic micro- and macroangiopathy (Kim et al., 2005).

4. Oxidative stress and diabetic retinopathy

Diabetic retinopathy pathogenesis is multifactorial, and the precise mechanisms are unclear. Several mechanisms have been proposed, including enhanced free radical production ROS (Brownlee et al., 1998; Koya & King 1998). Oxidative stress is increased in the retina in diabetes, and it is considered to play an important role in the development of retinopathy (Manikanth et al., 2010; Armstrong et al., 1998). It has been already proved that oxidative stress and hyperglycemia are central to chronic pathogenesis of diabetic retinopathy (Turk 2010). Increased levels of free radicals have a direct effect on *in vivo* protein. Oxidative stress induced modification of proteins is initiated mainly by reactions with hydroxyl radical; however, the course of the oxidation process is determined by the availability of oxygen and superoxide radical or its protonated form ($HO_2^{\cdot}$). Collectively, these ROS can lead to oxidation of amino acid residue side chains, cross-linking of soluble and/or membrane-bound proteins, oxidation of the protein backbone resulting in protein damage and yielding larger aggregates fragmentation. In the meantime, it has been shown that other forms of ROS may yield similar products and that transition metal ions can substitute for hydroxyl and superoxide radicals in some of the reactions (Berlett & Stadtman 1997). Even peptide bonds are subject to oxidative modification by ROS (Adams et al., 1999; Dhalla et al., 2000; Schoonover 2001).

Animal studies have demonstrated that oxidative stress contributes not only to the development of diabetic retinopathy but also to the resistance of retinopathy to reverse after good glycemic control is reinstituted—the metabolic memory phenomenon (Berg et al., 1997). Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose auto-oxidation is one of the major sources of ROS that is generated by oxidative pathways of glycation. Glucose exists in equilibrium with its enediol, which can undergo auto-oxidation to form an enediol radical. This radical reduces molecular oxygen to generate the superoxide radical and becomes oxidized itself to a dicarbonyl ketoaldehyde that reacts with protein amino groups forming a ketoamine, **Fig. 2** (Wolff and Dean 1987a). Ketoamine are similar to, although more reactive, than Amadori products and participate in AGE formation (Ahmed et al., 2005). The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals (Wolff and Dean 1987; Jiang et al., 1990).

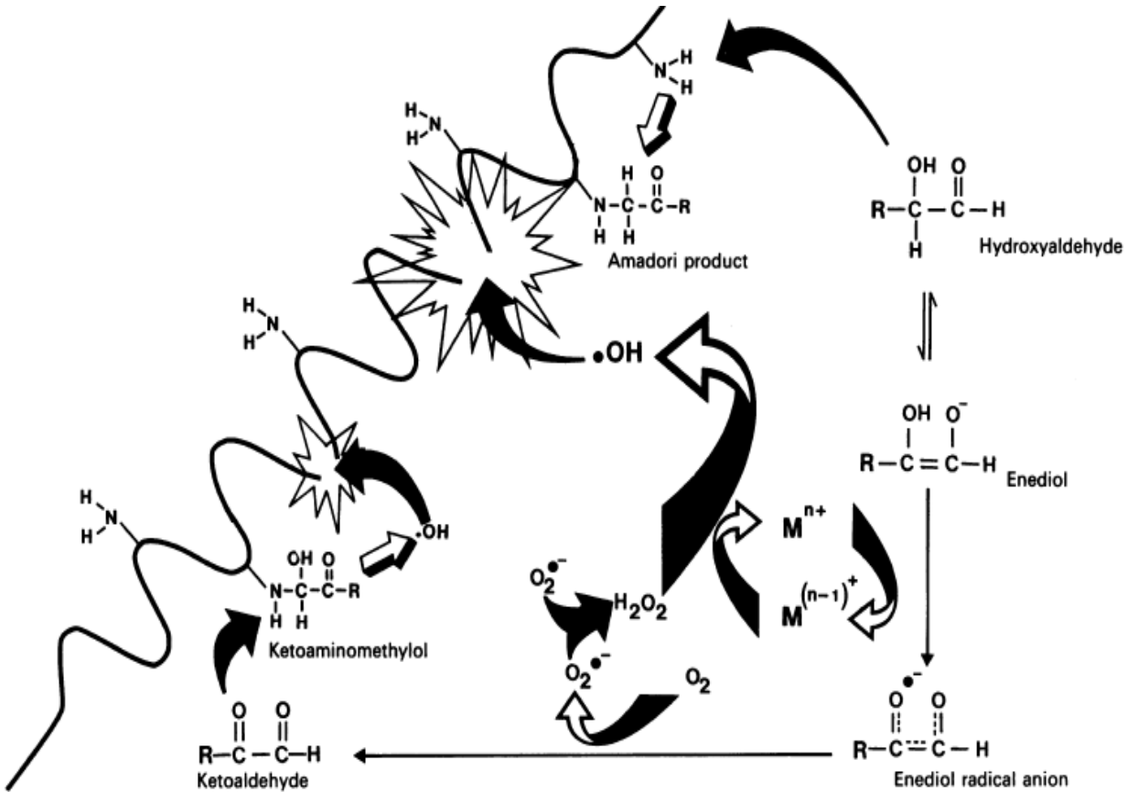


Fig. 2. Role of glucose auto-oxidation in the formation of reactive oxygen species induced protein damage (Wolff and Dean 1987a).

In hyperglycemic conditions, most of the carbonyl compounds generated by glycation need oxidative steps in their formation. The protein dicarbonyl compounds can participate in AGE formation and are referred to as glyco-oxidative products (Liggins & Furth 1997). Studies in diabetic rats showed elevated levels of superoxide in retinal cells with high glucose levels (Du et al., 2003; Cui et al., 2006), as well as increased levels of hydrogen peroxide (Ellis et al., 2000). Normally retinal blood vessels have tight junctions that protect

them from leaking. Prolonged hyperglycemia damages the tight junctions by oxidative stress and the vessels become leaky allowing fluid or blood to seep into the retina, thus resulting in the swelling of the retina (Harhaj & Antonetti 2004). Recently the etiology behind the production of superoxide in endothelial cells in diabetic complications has been elucidated (Brownlee 2001). There are four pathways suggested to be involved in the pathogenesis of diabetic complications due to increased production of free radical (increased polyol pathway flux, increased advanced glycosylation end product formation, activation of protein kinase C, and increased hexosamine pathway flux (Nishikawa et al., 2000; Du et al., 2002). In diabetes, the activities of antioxidant defense enzymes responsible for scavenging free radicals and maintaining redox homeostasis such as SOD, glutathione reductase, glutathione peroxidase, and catalase are diminished in the retina (Kowluru et al., 2001; Haskins et al., 2003). The intracellular antioxidant GSH is probably the most important antioxidant in the cell and acts as an ROS scavenger and modulates intracellular redox state (Meister 1988). The levels of this intracellular antioxidant are decreased in the retina in diabetes (Kern et al., 1994), and enzymes responsible for its metabolism are compromised (Lou 2003). Some nonenzymic antioxidants such as vitamin C, vitamin E, and β-carotene are also depressed during hyperglycemia induced oxidative stress (Ford et al., 2003).

5. Autoantibodies in diabetes complications

The lack of an immune response to self when responses to environmental antigens are retained is due to immunological tolerance. The role of tolerance, or lack of tolerance, is important to the understanding of autoimmune diseases and transplantation immunobiology (Mackay 2000). A loss of natural tolerance (to self) underlies all autoimmune diseases. Many more individuals develop autoimmune phenomena than autoimmune disease. Immune-mediated (Type I) diabetes results from an organ-specific autoimmune-mediated loss of insulin-secreting β cells. This chronic destruction process involves both cellular and hormonal components detectable in the peripheral blood, months or even years, before the onset of clinical diabetes (Kukreja & Maclaren 1999). In order to elicit an immune response, a molecule must be recognized as non-self by the biological system.

Proteins containing AGE are highly immunogenic and anti-AGE antibodies have been found in the sera of patients with diabetes (Reddy et al., 1995; Shibayama et al, 1999; Baydanoff et al., 1996). Several AGE structures have been identified including pyrraline, pentosidine (Sell & Monnier 1989), (carboxymethyl)lysine (Ahmed et al., 1986), and crosslines (Nakamura et al., 1992). Immunological studies using antibodies specific for these compounds have confirmed their presence in vivo (Ienaga et al., 1995). However, it is still not known whether one of these compounds contributes, as a major AGE structure, to the pathogenesis of these diseases, or whether other structures may involve in this process. Immunological approaches have been attempted to determine the major AGE structures expressed in vivo. Using AGE-BSA as an antigen, researchers prepared a monoclonal anti-AGE antibody (6D12) in mice as well as a polyclonal anti-AGE antibody in rabbits (Hoeiuchi et al., 1986). Immunoreactivity studies of these antibodies have demonstrated an interesting observation: both antibodies react with AGE samples obtained from proteins, peptides, lysine derivatives, and monoaminocarboxylic acids, suggesting the presence of a common AGE structures in these AGE preparations. Immunologic studies using 6D12 monoclonal antibodies have disclosed the presence of AGE in several tissues and their potential

involvement in disease processes. Anti-AGE antibodies use as a potential biomarker of AGE depositions during diabetes and its associated secondary complications.

There is increasing evidence of the presence of anti-AGE antibodies in diabetes and its complications. The role of these antibodies and specifically which particular anti-AGE antibodies are involved in the aetiology of diabetic micro- and macrovascular complications is, however, yet to be established. The possibility of effective therapeutic intervention stresses the importance of detecting anti-AGE antibodies, and advancements in measuring anti-AGE antibodies using reliable methods will help determine the role they have in the pathogenesis of many diseases, especially diabetes and its complications.

Antibodies against AGE structures led to the discovery that only a minor proportion of AGE are detectable by autoflourescence and they form to a greater extent in intracellularly than extracellularly because several glucose fragmentation products which occur during the metabolism of glucose in the cell are more reactive than glucose itself (Giardino et al., 1994). It was also found that the non-flourescent CML is the major epitope against which AGE-antibodies are directed (Reddy et al., 1996). Some AGE-antibodies used so far have not been characterized at all. To circumvent this problem researchers applied antibodies directed against the proteins that are abundantly available in blood and more exposed to blood glucose levels in diabetes mellitus as representative markers for the gluco-oxidative pathway. Anti-HSA antibodies have been observed in diabetes (Eilat *et al*., 1981), a fivefold greater occurrence than in nondiabetic persons (Gregor *et al*., 1986). Proteins containing AGEs are highly immunogenic and CML is one of the major epitopes recognised by anti-AGE antibodies (Reddy *et al*., 1995; Ikeda *et al*., 1996). The presence of AGE-antibodies in the serum of streptozotocin-diabetic rats as well as in a small number of diabetic patients have been reported (Shibayama *et al*., 1999) AGE can exert their immunogenicity, demonstrate that presence of AGEs-immune complexes (AGE-IC) in the diabetic patients that may play a role in the artherogenesis (Turk *et al*., 2001). Interactions of AGE autoantibodies with AGEs as a continuously produced antigen result in the formation of AGE-ICs that may play role in diabetic complications (Jakus and Rietbrock, 2004). The analysis of the frequency distribution profile shows that 14% of the diabetic subjects display significant antibody binding to AGE-HSA than the control subjects (Vay *et al*., 2000).

6. Autoantibodies against gluco-oxidative modified proteins in diabetic retinopathy

The autoantibodies have always been important for clinical interest due to their potential role in screening, diagnosis, monitoring treatment of effectiveness and prognosis. Non-enzymatic glycation of proteins can lead to the formation of reactive AGEs, which are thought to be implicated in the formation of micro- and macrovascular complications in diabetes mellitus. Proteins such as serum albumin, collagen, elastin, lens crystalline, are particularly susceptible to glucose modification (Festa et al., 1998). Elevated serum levels of these glycated proteins were detected in diabetic subjects moreover, higher levels of glycated form of proteins or AGEs were found in diabetic patients with secondary complications such as retinopathy, nephropathy and artherosclerosis (Nicoloff et al., 2000; Nicoloff 2001; Ahmed 2005). Previous studies showed reactive AGE can directly alter the physical and structural properties of the extracellular matrix, for instance, by inducing

collagen cross-linking, basement membrane thickening, and covalent trapping of plasma proteins such as LDL and IgG (Bouma et al., 2003). *Ex vivo* HSA was incubated with glucose at the concentration of 50 mM for 5 weeks at 37°C under aerobic conditions (Khan et al., 2007). Biochemical, spectral, electrophoretic, circular dichroism spectropolarimetric, and thermodynamic analyses confirmed that the structure and stability of HSA is significantly affected by glucose induced modification. Recently we showed that gluco-oxidation of proteins alter the structural complexity of the molecule and make them highly immunogenic (Khan et al., 2010). Ex vivo designed gluco-oxidative modified human serum albumin (RG-HSA) were used as an antigen and the titres of antibodies against (RG-HSA-Abs) it were screened in both types of diabetic patients, as well as screening was also done in diabetic patients with complications like retinopathy, nephropathy and artherosclerosis (Table 1). Interestingly, diabetic patients with associated complications (retinopathy, nephropathy and atherosclerosis) generated higher autoantibodies against gluco-oxidative modified HSA than controls and diabetic subjects without secondary complications. This above contention supports that gluco-oxidative proteins are toxic and highly immunogenic. From overall cohort of diabetic patients, the highest recognition of RG-HSA as an antigen by circulatory autoantibodies from diabetic retinopathy as compared to diabetic nephropathy and atherosclerosis (Table 1).

Groups	Sera positive for RG-HSA[1]	Sera positive for N-HSA[2]	Sera positive for both antigens	Carbonyl content (nmol/mg protein)
Type 1 diabetes (n = 30)	21 (52 ± 5.5)	-	1 (43 ± 4.7[1]; 51 ± 5.2[2])	2.9 ± 0.35
Type 2 diabetes (n = 30)	16 (48 ± 4.7)	-	2 (47 ± 4.7[1]; 43 ± 5.2[2], 45 ± 3.3[1]; 41 ± 4.7[2])	2.8 ± 0.4
Diabetes retinopathy (n = 12)	8 (76 ± 4.5)	-	-	3.9 ± 0.5
Diabetes nephropathy (n = 12)	7 (69 ± 3.1)	1 (41 ± 4.7)	-	3.5 ± 0.35
Diabetes atherosclerosis (n = 14)	9 (67 ± 4.0)	-	1 (55 ± 4.1[1]; 55 ± 2.8[2])	3.3 ± 0.55
Controls NH (n = 60)	-	-	-	2.3 ± 0.42

Table 1. Detection of N-HSA-Abs and RG-HSA-Abs and the estimation of carbonyl contents as oxidative stress in the sera of various diabetic groups and control. ELISA plate coated with the respective antigen (20 µg/ml). Sera positive means serum samples which gave inhibition greater than 30%, as less than that may be due to non specific bindings. n denotes the number of sera tested. Values in parentheses are mean ± SD of maximum percent inhibition of positive serum samples at 20 µg/ml of competitor. [1]ROS-glycated and [2]native HSA were used as inhibitor.

Diabetic retinopathic patients also exhibited maximum amount of carbonyl content, which showed a significant correlation between high oxidative stress and presence of anti-RG-HSA antibodies. Clinical and Laboratory examination was also done for 96 diabetic patients (66 males and 30 females) and 60 normal human (41 males and 19 females) serve as controls. According to the data given in Table 2, oxidative stress with chronic hyperglycemia and

advanced age has been considered a potential risk factor in the development of autoantibodies in retinopathy and other diabetic complications as well. AGEs are products of oxidative modifications of glycated proteins, which damage blood proteins. High oxidative stress and toxic blood glucose levels are found to be the common factors behind the generation of high autoantibodies and the progression of disease complications.

Subjects	Number of subjects	Age Years	Duration of disease Years	Fasting blood glucose (mg/dL)	HbA_{1C} (%)
Type 1 diabetic	30	36 ± 14	9 ± 3	254 ± 32	7.4 ± 0.6
Type 2 diabetic	30	44 ± 11	7 ± 3.6	263 ± 28	7.1 ± 0.4
Diabetic retinopathy	12	68 ± 2.9	21 ± 5	434 ± 11	9.0 ± 0.4
Diabetic nephropathy	12	62 ± 4.3	18 ± 3.3	394 ± 13	9.2 ±0.6
Diabetic atherosclerosis	12	59 ± 3.6	17 ± 3.6	390 ± 15	8.8 ± 0.3
Control NH	60	32 ± 9.5	–	88 ± 9.8	5.8 ± 0.4

Table 2. Clinical characterization of the patients and normal control subjects. For the blood glucose estimations, blood was collected in oxalated fluoride containers and the assays were performed immediately. Values are in mean ± SD. NH represents normal human subjects.

Gluco-oxidative modified HSA was immunized in the white New-Zealand rabbit that exhibited high titre of anti-glycated albumin antibodies in the serum of experimental animals (Khan et al., 2010). High titre showed immunogenicity of the gluco-oxidative modified proteins. These antibodies were proven to be a potential probe for the detection of protein lesion in blood proteins during diabetes mellitus. ELISA experiments of these antibodies with the isolated blood proteins such as albumin, IgG and RBC membrane from the diabetic patients showed high recognition. It means that during hyperglycemia there is damage of blood proteins that modifies the normal conformation of and hence generate new-epitopes that share binding specificity with the ex vivo designed gluco-oxidative modified albumin. Moreover, anti-gluco-oxidative modified HSA antibodies showed cross reaction with proteins such as BSA, poly L-lysine, immunoglobulins that were incubated ex vivo with 25 µM of glucose or fructose for 20 days. These findings suggests that paratopes of anti-gluco-oxidative albumin antibodies recognise common epitopes that are present in most gluco-oxidative modified proteins.

During diabetes, persistent hyperglycemia causes increased production of free radicals, especially ROS in all tissues by glucose auto-oxidation, protein glycation and due to decreased destruction by nonenzymic and enzymic catalase , glutathione peroxidase, and superoxide dismutase activity (Kowluru et al., 2001; Baynes & Thorpe 1999; Haskins et al., 2003). The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. Also this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defences (West 2000; Robertson 2004). In diabetes mellitus, alterations in the endogenous free radical scavenging

defence mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury.

Antibodies against glutamic acid decarboxylase-65 (GAD65Abs) are often considered to be an epiphenomenon resulting from the autoimmune destruction of the pancreatic beta cells in type 1 diabetes. Previous studies suggest that they are involved in antigen processing and presentation and thus modulate the immune response (Banga et al., 2004). Because of the high diagnostic sensitivity for autoimmune diabetes, the presence of GAD65Ab is currently used to identify subjects at high risk for the disease. GAD65Abs are detected in about 60% of new-onset cases of type 1 diabetes, and high levels of these autoantibodies were also reported in diabetic patients with secondary complications (such as retinopathy and nephropathy), the leading cause of blindness and renal failure (Falorini et al., 1998; Bonifecio et al., 1995; Jakuc & Reitbrock 2004). The exact aetiology behind these complications is not completely clear. In our recent study; ROS modified GAD65 was found to be more immunogenic in T1D than its native form (Khan et al., 2009). GAD65Abs in T1D are predominantly directed at conformational epitopes located in the middle region of the molecule, whereas they also recognize linear epitopes and epitopes located in the middle, COOH- and NH2-terminuses (Hampe et al., 2000). Shifts in GAD65 epitopes were detected in a subgroup of newly diagnosed children within the first 12 months after disease onset (Hampe et al., 1999). Moreover, epitope spreading has gained credence as a major driver underlying autoimmunity (Cheung & Wong 2007). Growing evidence suggests that ROS plays an important role in the initiation and progression of diabetes and its associated complications. These increased levels of free radicals pose a direct toxic effect on GAD65 and increase its immunogenicity. Specificity of autoantibodies for epitopes on GAD65 and their levels may be a better indicator of impending or actual destruction of islet beta-cells and increasing complications associated with diabetes.

In our 2009 study (Khan et al., 2009), while searching for a potential epitopes, high titre autoantibodies were detected in type 1 diabetes patients. GAD65 was considered a potential marker for type 1 diabetes. GAD65 was exposed to hydroxyl radical (ROS-GAD65), induced structural and conformational alterations were observed and investigated. Presence of autoantibodies against them were found in diabetes patients. Higher titres of autoantibodies were detected against ROS modified GAD65 (ROS-GAD65-Abs) in type 1 diabetic patients as compared to unmodified native GAD65. Increased levels of ROS in type 1 diabetes by molecular pathways or over produced metal catalyzed reactions modified GAD65 and induced biophysical structural alterations that would probably alter immunogenicity leading to induction and elevated levels of autoantibodies in type 1 diabetes. The data demonstrates possible role of ROS in presenting neo-epitopes that may be one of the factors in antigen-driven autoimmune response. Specificity of autoantibodies for epitopes on GAD65 and their levels may be a better indicator of impending or actual destruction of islet beta-cells and increasing complications associated with diabetes. The etiology of ROS-GAD65 in the pathogenesis of diabetic complications was further investigated in patients' diabetic complications in our new study (Khan et al., 2011). In this finding, significantly high levels of circulating ROS-GAD65Abs were detected in complicated diabetic patients especially in retinopathy as compared to uncomplicated type1 diabetic patients (Table 3).

Subjects	Age years	Gender (M:F)	Smoking duration Years	Duration of disease Years	Fasting blood glucose (mg/dl)	HbA_{1C} (%)	ROS-GAD65-Abs (MMPI)	Hyper-tension 140/90 (%)	Carbonyl Content (nmol/mg protein)
Uncomplicated T1D (n=60)	30 ± 09	37:23	*8(5±3.4)	09 ± 5.6	238 ± 27	7.9 ± 0.7	50.6 ± 7.2*	36(60)	3.0 ± 0.22
Complicated T1D Nephropathy (n=20)	37 ± 11	12:8	*14(6±3.8)	14 ± 4.9	311 ± 21	8.8 ± 0.6	70.3 ± 8.2	17(85)	3.4 ± 0.28
Complicated T1D Retinopathy (n=20)	42 ± 14	11:9	*17(8 ± 3.6)	17 ± 4.3	335 ± 17	9.3 ± 0.7	74.5 ± 6.5	16(80)	3.9 ± 0.31
Control (n=50)	32 ± 8	28:22	—	—	96 ± 11.2	5.8 ± 0.4	7.2 ± 3.7	4(8)	2.1 ± 0.17

Table 3. Clinical and laboratory data from complicated and uncomplicated T1D patients; normal human subjects serve as controls. Data are means ± SD. The sign "* "represents number of smokers from given total respective subjects. For blood glucose estimations, blood was collected in oxalated fluoride containers and the assays were performed immediately. Hypertension is defined as sitting systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90 mmHg or the use of antihypertensive medication. Signs * represents 20 number of sera from different patients in the respective group. R-GAD65-Abs (Antibodies against ROS modified GAD65).

This risk of the disease may be enhanced due to acceleration in the formation of free radicals with gradual increase in duration of disease. Smoking and hypertension were also associated with increased antibody production in diabetic retinopathy. Gluco-oxidative stress leads to conformational alterations in native GAD65 protein which could increase or expose cryptic epitopes. Dynamic changes in the GAD65Abs binding pattern suggest subsequent epitopes spreading with disease progression. Concomitantly, these two studies on GAD65 provide us the evidences that hyperglycemia, age, oxidative stress, smoking, and as well as extent of blood protein glycation (HbA1C) participate in etiology of increased GAD65Ab immunogenicity implicated in diabetic retinopathy.

The exact mechanism for the formation of these autoantibodies and progression of diabetic retinopathy is still not well explained. We hypothesized that anti-gluco-oxidative protein autoantibodies bind to soluble glycated proteins and form an intermediary immune complex in the bloodstream that can bind to the basement membranes of the retinal blood vessels. At these sites they can activate complement cascade, resulting in damage to the walls of microvascular cappillaries associated with diabetic retinopathy. This phenomenon results in local necrosis of the vessels. If there is no continuous source of antigen, under conditions of controlled hyperglycemia then gluco-oxidative modified proteins are cleared and the disease can be controlled. However, if there is chronic hyperglycemia that enhances a continuing modification of blood protein, formation of increased immune complexes cause chronic autoimmune pathogenesis of diabetic retinopathy.

Gluco-oxidation associated damage of proteins due to hyperglycemia can be enhanced due to multiple factors such as duration of disease, age, smoking and hypertension and hence

can accelerate production of autoantibodies. This suggests that it is perhaps the rate of accumulation rather than the absolute concentration of gluco-oxidative proteins that is important. The exact mechanism behind the production of these autoantibodies is yet to be elucidated. However it stands to reason that the measurement of serum levels of gluco-oxidative proteins or anti-gluco-oxidative modified protein antibodies is important for estimation of an increased risk for development of diabetic retinopathy.

7. Conclusion

Gluco-oxidation is considered to be an important pathophysiological mechanism in the development of diabetic retinopathy. Gluco-oxidation leads to toxicity of blood proteins in diabetic retinopathic patients. Considerable amounts of AGEs are formed from blood proteins that subsequently develop into immune complexes with anti-AGE antibodies in retinopathic subjects. The ROS and gluco-oxidative modified protein autoantibodies were detectable in high titers in patients suffering from diabetic retinopathy. Chronic hyperglycemia and increased age, that are often seen in such cases, have proven to cause abnormally high production of free radicals with decreased antioxidant defence system. Proteins are damaged by the concomitant effect of glycation and oxidative stress leading to conformational alterations in native structure which could induce neo-epitopes or may increase exposed cryptic epitopes. Dynamic changes in the autoantibody binding patterns suggest subsequent epitope spreading with disease progression. Immune complexes of gluco-oxidative proteins and antibodies against them possibly activate complement cascade system and hence destroy capillaries within the retina. Measurement of these autoantibodies could be useful in assisting the prediction of the development of disease even before non-proliferative diabetic retinopathy. Reduction in the levels of glycation and ROS may lead to decrease in *in vivo* protein modifications, thus delaying the progression of diabetic retinopahy.

8. References

Adams AK, Wermuth EO, McBride PE. (1999) Antioxidant vitamins and the prevention of coronary heart disease. *Am Fam Physc,* 60, 895-904.

Ahmed MU, Thorpe SR, Baynes JW. (1986) Identification of Ne-(carboxymethyl)lysine as a degradation product of fructose lysine in glycated protein. *J Biol Chem,* 261, 4889-4894.

Ahmed N, Babaei-Jadidi R, Howell KS, Beisswenger JP, Thornalley JP. (2005) Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1 diabetes. *Diabetologia,* 48, 1590-1603.

Ahmed N. (2005) Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res Clin Pract*, 67, 3-21.

Armstrong D, Ueda T, Ueda T, et al. (1998) Lipid hydroperoxide stimulates retinal neovascularization in rabbit retina through expression of tumor necrosis factor-alpha, vascular endothelial growth factor and platelet-derived growth factor. *Angiogenesis* 2, 93–104.

Banga JP, Moore JK, Duhindan N, Madec AM, Vanendert PM, Orgiazzi J, Endl J. (2004) Modulation of antigen presentation by autoreactive B cell clonesspecific for GAD65 from a type I diabetic patient. *Clin Exp Immunol*,135, 74-84.

Barcelo A, Aedo C, Rajpathak S, Robles S. (2003) The cost of diabetes in Latin America and the Caribbean. *Bulletin of the World Health Organization*, 81:19–27.

Baydanoff S, Konova E, Ivanova N. (1996). Determination of anti-AGE antibodies in human serum. *Glucoconjugate J*, 13, 335– 339.

Baynes JW, Thorpe SR. (1999) Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*, 48(1), 1–9.

Beisswenger PJ, Howell SK, Nelson RG, Mauer M, Szergold BS (2003a) alpha-Oxoaldehyde metabolism and diabetic complications. *Biochem Soc Trans,* 31, 1358-1363.

Beisswenger PJ, Howell SK, Smith K, Szwergold BS. (2003b) Glyceraldehyde-3-phosphate dehydrogenase activity as an independent modifier of methylglyoxal levels in diabetes. *Biochim Biophys Acta,* 1637: 98-106.

Berg TJ, Bangstad HJ, Torjesen PA, Osterby R, Bucala R, Hanssen KF. (1997a). Advanced glycation end products in serum predict changes in the kidney morphology of patients with insulin-dependent diabetes mellitus. *Metabolism* 46, 661– 665.

Berlett BS, Stadtman ER. (1997) Protein oxidation in aging, disease, and oxidative Stress. *J Biol Chem*, 272, 20313–20316.

Bhavsar AR, Emerson GG, Emerson MV, Browning DJ. (2010) Diabetic Retinopathy. In: Browning DJ. *Epidemiology of Diabetic Retinopathy*. Springer, New York.

Boehm BO, Schilling S, Rosinger S, Land GE, Land GK, Kienthsch-Engel R, Stahl P. (2004) Elevated serum levels of N(epsilon)-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema. *Diabetologia* 47, 1376–1379.

Bonifacio E, Genovese S, Braghi S. (1995) Islet autoantibody markers in IDDM: Risk assessment strategies yielding high sensitivity. *Diabetol*, 38, 816-822.

Bonnardel-Phu E, Wautier JL, Schmidt AM, Avila C, Vicaut E. (1999) Acute modulation of albumin microvascular leakage by advanced glycation end products in microcirculation of diabetic rats in vivo. *Diabetes,*48, 2052–2058.

Boulanger E, Puisieux F, Gaxatte C, Wautier JL. (2007) Aging: role and control of glycation. *Rev Med Interne* 28(12), 832-840.

Boulanger E, Wautier JL, Dequiedt P, Schmidt AM. (2006) Glycation, glycoxidation and diabetes mellitus. *Nephrol Ther,* 2(1), S8-16.

Bouma B, Kroon-Batenbury JML, Wu PY, Brunjes B, Posthuma G, Kranenburg O, DeGroot GP, Voest EE, Gebbink GBFM. (2003) Glycation induces formation of amyloid cross-beta structure in albumin. *J Biol Chem*, 278, 41810-41819.

Bourdon E, Lorea N, Blache D. (1999) Glucose and free radicals impair the antioxidant properties of serum albumin. *FASEB J,* 13, 233-244.

Brownlee M, Cerami A, Vlassara H. (1998) Advanced glycosylation end products in tissue and the biochemical basis of diabetic complication. *N Engl J Med* 318, 1315–21.

Brownlee M. (1995) Advanced protein glycosylation in diabetes and aging. *Annu Rev Med,* 46, 223-234.

Brownlee M. (1996) Advanced glycation end products in diabetic complications. *Curr Opin Endocrinol Diabetes,* 3, 291–97.

Brownlee M. (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature,* 414, 813-820.

Brownlee M. (2005) The pathobiology of diabetic complications – a unifying mechanism. *Diabetes,* 54, 1615-1625.

Bryszewska M, Szosland K. (1988)Association between glycation of erythrocyte membrane fluidity. *Ann Clin Res,* 21, 49–51.

Cai H, Harrison DG. (2000) Endothelial dysfunction in cardiovascular disease: the role of oxidant stress. *Circ Res,* 87, 840–844.

Carter CD, Ho XJ. (1994) Structure of serum albumin. *Adv Prot Chem*, 45, 153-203.

Chakravarthy U, Hayes RG, Stitt AW, McAuley E, Archer DB. (1998) Constitutive nitric oxide synthase expression in retinal vascular endothelial cells is suppressed by high glucose and advanced glycation end products. *Diabetes* 47, 945-952.

Chappey O, Dosquet C, Wautier MP, Wautier JL. (1997) Advanced glycation end products, oxidant stress and vascular lesions. *Eur J Clin Invest* 27, 97–108.

Cheung N, Wong TY. (2007) Obesity and Eye Diseases. *Survey of Ophthalmology,* 52, 180–95.

Clements RS Jr, Robison WG Jr, Cohen MP. (1998) Anti-glycated albumin therapy ameliorates early retinal microvascular pathology in db/db mice. *J Diab Comp* 12, 28-33.

Crawford TN, Alfaro DV 3rd, Kerrison JB, Jablon EP. (2009) Diabetic retinopathy and angiogenesis. *Curr Diabetes Rev,* 5(1), 8-13.

Cribbs DH, Azizeh BY, Cotman CW, LaFerla FM. (2000) Fibril formation and neurotoxicity by a herpes simplex virus glycoprotein B fragment with homology to the Alzheimer's A beta peptide. *Biochemistry,* 39, 5988–5994.

Cui Y, Xu X, Bi H, Zhu Q, Wu J, Xia X, Qiushi R, Ho PC. (2006) Expression modification of uncoupling proteins and MnSOD in retinal endothelial cells and pericytes induced by high glucose: the role of reactive oxygen species in diabetic retinopathy. *Experimental Eye Research*, 83, 807–816.

Cutler P. (1989) Deferoxamine therapy in high-ferritin diabetes. *Diabetes,* 38, 1207-1210.

Dhalla NS, Temsah RM, Netticadan T. (2000) Role of oxidative stress in cardiovascular diseases. *J Hypertens,* 18, 655-673

Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M. (2002) Hyperglycemia-induced mitochondrial superoxide overproduction activates the exosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc NatlAcad Sci USA,* 97, 12222–12226.

Du Y, Miller CM, Kern TS. (2003) "Hyperglycemia increases mitochondrial superoxide in retina and retinal cells," *Free Radical Biology and Medicine*, 35(11), 1491–1499.

Eilat D, Fischel R, Zlotnick A. (1981) Albumin-immunoglobulin complexes in human serum: classification and immunochemical analysis. *Scand J Imminol,* 14, 77-83.

Ellis EA, Guberski DL, Somogyi-Mann M, and Grant MB. (2000) Increased H2O2, vascular endothelial growth factor and receptors in the retina of the BBZ/WOR diabetic rat. *Free Radical Biology and Medicine*, 28(1), 91–101.

Falorni A, Kassi G, Murdolo G, Calcinaro F. (1998) Controversies on humoral immune markers of insulin-dependent diabetes mellitus. *J Ped Endocrinol Metab*, 11, 307-317.

Federman JL, Gouras P, Schubert H. (1994) Systemic diseases. In: Podos SM, Yanoff M, eds., (pp, 7-24) Vol9, *Retina and Vitreous: Textbook of Ophthalmology.*

Festa A, Schmolzer B, Schernthaner G, Menzel EJ. (1998) Differential expression of receptors for advanced glycation end products on monocytes in patients with IDDM. *Diabetologia,* 41, 674– 680.

Ford ES, Mokdad AH, Giles WH, and Brown DW. (2003)The metabolic syndrome and antioxidant concentrations:findings from the Third National Health and Nutrition Examination Survey. *Diabetes,* 52(9), 2346–2352.

Fu MX, Knecht KJ, Thorpe SR, Baynes JW (1992) The role of oxygen in cross-linking and chemical modifications of collagen by glucose. *Diabetes,* 41, 42-48.

Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR. (1996) The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *J Biol Chem* 271, 9982-9986.

Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, Sivitz W, Monnier VM. (2005) Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes,* 54, 3103–3111.

Giardino I, Edelstein D, Brownlee M (1994) Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity. *J Clin Invest,* 94, 110-117

Gilcrease MZ, Hoover RL. (1990) Activated human monocytes exhibit receptor-mediated adhesion to a non-enzymatically glycosylated proteinsubstrate, *Diabetologia,* 33, 329–33.

Giugliano D, Ceriello A, Paolisso G. (1996) Oxidative stress and diabetic vascular complications. *Diabetes Care,* 19, 257–267.

Gregor I, Iberg N, Berger W, Fluckiger R. (1986) Albumin directed antibodies in diabetes; demonstration of human serum albumin-directed IgM autoantibodies. *Diabetologia,* 29, 481-484.

Hammes HP, Alt A, Niwa T, Clausen JT, Bretzel RG, Brownlee M, Schleicher ED. (1999) Differential accumulation of advanced glycation end products in the course of diabetic retinopathy. *Diabetologia,* 42, 728–736.

Hammes HP, Weiss A, Hess S, Araki N, Horiuchi S, Brownlee M, Preissner KT. (1996) Modification of vitronectin by advanced glycation alters functional properties in-vitro and the diabetic retina. *Lab Invest,* 75, 325-338.

Hampe CS, Hammerle LP, Bekris L, Ortqvist E, Kockum I, Rolandsson O,Landin-Olsson M, Torn C, Persson B, Lernmark A. (2000) Recognition of glutamicacid decarboxylase (GAD) by autoantibodies from different GADantibody-positive phenotypes. *J Clin Endocrinol Metab,* 85, 4671-4679.

Hampe CS, Ortqvist E, Persson B, Schranz DB, Lernmark A. (1999) Glutamate decarboxylase (GAD) autoantibody epitope shift during the first year of type 1 diabetes. *Horm Metab Res,* 31, 553-557.

Harhaj NS and Antonetti DA. (2004) Regulation of tight junctions and loss of barrier function in pathophysiology. *Int J of Biochem Cell Biol*, 36(7), 1206–1237.

Haskins K, Bradley B, Powers K, Fadok V, Flores S, Ling X, Pugazhenthi S, Reusch J, Kench J. (2003) Oxidative stress in type 1 diabetes, *Ann NY Acad Sci,* 1005,43–54.

Horiuchi S, Murakami M, Takata K, Morino Y. (1986) Scavenger receptor for aldehyde-modified proteins. *J Biol Chem*, 261, 4962-4966.

Huebschmann G, Regensteiner JG, Vlassara H, Reusch JE. (2006) Diabetes and advanced glycoxidation end products. *Diabetes Care,* 29, 1420-1432.

Hunt JV. (1994) In "Free Radicals in the Environment, Medicine and Toxicology", Nohl H, Esterbauer H, Rice-Evans C. eds., (pp, 137-162), Richeliu Press, London.

Iberg N, Fluckiger R. (1986) Nonenzymatic glycosylation of albumin in vivo. Identification of multiple glycosylated sites. *J Biol Chem*, 261, 13542-13545.

Ienaga K, Nakamura K, Hochi T, Nakazawa Y, Fukunaga Y, Kakita H, Nakano K. (1995) Crosslines, fluorophores in the AGE-related cross-linked proteins. *Contrib Nephrol*, *112*, 42-51.

Ikeda K, Higashi T, Sano H, Jinnouchi Y, Yoshida M, Araki T, Ueda S, Horiuchi S. (1996) *N*-(Carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. *Biochemistry* , 35, 8075-8083.

Imani F, Horii Y, Suthanthiran M, Skolnik EY, Makita Z, Sharma V, Sehaipal P, Vlassara H. (1993) Advanced glycosylation end product-specific receptors on human and rat T-lymphocytes mediates synthesis of interferon gamma: role in tissue remodeling. *J Exp Med,* 178, 2165–72.

Jakus V, Rietbrock N. (2004) Advance glycation end-products and the progress of diabetic vascular complications. *Physiol Res*, 53, 131-142.

Jiang ZY, Woollard AC, Wolff SP. (1990) Hydrogen peroxide production during experimental protein glycation. *FEBS Lett*, 268(1), 69–71.

John WG, Lamb EJ. (1993) the Millard or browning reaction in diabetes. *Eye,* 7, 230-237.

Kameda Y, Makita Z. (2000) Neurotoxicity of advanced glycation end-products for cultured cortical neurons. *J Neuropathol Exp Neurol,* 59, 1094–1105.

Khan MWA, Rasheed Z, Khan WA, Ali R. (2007) Biochemical, biophysical and thermodynamical analysis of i*n vitro* glycated human serum albumin, *Biochemistry (Mosc)*, 72, 146-152.

Khan MWA, Sherwani S, Khan WA, Moinuddin , Ali R. (2009) Characterization ofhydroxyl radical modified GAD65: a potential autoantigen in type 1 diabetes. *Autoimmunity*, 42, 150-158.

Khan MWA., Banga K., Mashal SN., Khan WA. (2011) Detection of autoantibodies against reactive oxygen species modified glutamic acid decarboxylase-65 in type 1 diabetes associated complications. *BMC Immunol,* 12:19-26.

Khan MWA., Qadrie ZL., Khan WA. (2010) Antibodies against gluco-oxidative modified HSA-detected in diabetes associated complications, *Int Arch Allergy Immunol*, 153, 207-214.

Kim W, Hudson BI, Moser B, Guo J, Rong LL, Lu Y, Qu W , Lalla E, Lerner S, Chen Y, Shi Du Yan S, D'Agati V, Naka YU, Ramasamy R, Herold K, Yan SF, Schmidt AM. (2005) Receptor for advanced glycation end products and its ligands: a journey from the complications of diabetes to its pathogenesis. *Ann N Y Acad Sci,* 1043, 553-561.

Kislinger T, Fu C, Huber B, Qu W, Taquchi A, Du Yan S, Hoffmann M, Yan SF, Pischetsrieder M, Stern D, Schmidt AM. (1999) N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J Biol Chem,* 274, 31740–31749.

Koenig RJ, Blobstein SH, Cerami A. (1977) Structure of carbohydrate of hemoglobin Aic. *J Biol Chem,* 252, 2992-2997.

Koga K, Yamagishi S, Okamoto T, Inagaki Y, Amano D, Akeuchi M, Makita Z. (2002) Serum levels of glucose-derived advanced glycation end products are associated with the severity of diabetic retinopathy in type 2 diabetic patients without renal dysfunction. *Int J Clin Pharmacol Res* 22, 13-17.

Kowluru RA, Tang J, and Kern TS. (2001) Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes*, 50(8), 1938–1942.

Koya D, King GL. (1998) Protein kinase C activation and the development of diabetic complications. *Diabetes* 47, 859–66.

Kukreja A, Maclaren KN. (1999) Autoimmunity and diabetes. *J Clin Endocrin Metabol,* 84: 4371-4382.

Leto G, Pricci F, Amadio L, Iacobini C. (2001) Increased retinal endothelial cell monolayer permeability induced by the diabetic milieu: role of advanced non-enzymatic glycation and polyol pathway activation. *Diabetes Metab Res Rev,*17, 448–458.

Liggins J, Furth JA. (1997) Role of protein-bound carbonyl groups in the formation of advanced glycation endproducts. *Biochim Biophys Acta*, 1361: 123-129.

Lou MF (2003) Redox regulation in the lens. Prog Retin Eye Res 22(5), 657-682.

Lyons TJ, Li W, Wojciechowski B, Wells-Knecht MC, Wells-KnechtK. J., and Jenkins, A. J. (2000) Aminoguanidine and the effects of modified LDL on cultured retinal capillary cells. *Invest Ophthalmol Vis Sci,* 41, 1176–1180

Mackay IR. (2000) Tolerance and autoimmunity. *BMJ* 321, 93-96.

Mamputu JC, Renier G. (2002) Advanced glycation end products increase, through a protein kinase C-dependent pathway, vascular endothelial growth factor expression in retinal endothelial cells. Inhibitory effect of gliclazide. *J Diab Comp* 16, 284-293.

ManiKanth SB,Kalishwaralal K, Sriram M, Pandian SBRK, Youn H, Eom S, Gurunathan S. (2010) Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. *J Nanobiotechnology* 8, 16-24.

Mateo MCM, Bustamante JB, Cantalapiedra MAG. (1978) Selinium, zinc, copper and insulin in diabetes millitus. *Biomed,* 29, 56-58.

Matsumoto K, Ikeda K, Horiuchi S, Zhao H, Abraham EC. (1997) Immunochemical evidence for increased formation of advanced glycation end products and inhibition by aminoguanidine in diabetic rat lenses. *Biochem Biophys Res Commun* 241, 352-354.

McDonald MJ, Shapiro R, Bleichman M, Solway J, Bunn HF. (1978) Glycosylated minor components of human adult hemoglobin. *J Biol Chem,* 253, 2327–32.

Meister A. (1988) Glutathione metabolism and its selective modification. *J Biol Chem*, 263(33), 17205–17208.

Monnier VM, Sell DR, Genuth S. (2005) Glycation products as markers and predictors of the progression of diabetic complications. *Ann NY Acad Sci,* 1043, 567-581.

Nakamura, K., Hasegawa, T., Fukunaga, Y., & Ienaga, K. (1992) Crosslines A and B as candidates for the fluorophores in ageand diabetes-related cross-linked proteins, and their diacetates produced by Millard reaction of a-N-acetyl-L-lysine with D glucose. *J Chem Soc Chem Commun,* 14, 992-994.

Nicoloff G, Baydanoff S, Stanimirova N, Petrova CH, Christova P. (2000) Relationship between elastin-derived peptides and the development of diabetic microvascular complications—a longitudinal study in children with Type 1 (insulin-dependent) diabetes mellitus. *Gen Pharmacol,* 35, 59– 64.

Nicoloff G, Baydanoff S, Stanimirova N, Petrova CH, Christova P. (2001) Detection of serum collagen type IV in children with Type 1 (insulin-dependent) diabetes mellitus—a longitudinal study. *Pediatr Diabetes,* 2, 184–190.

Nishikawa T, Edelstein D, Du X-L, Yamagishi S, Matsumura T, Kaneda Y, Yorek M, Beebe D, Oates P, Hammes HP, GiardinoI, Brownlee M. (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature,* 404, 787–790.

Palm F, Cederberg J, Hansell P, Liss P, Carlsson OO. (2003) Reactive oxygen species cause diabetes-induced decrease in renal oxygen tension. *Diabetologia,* 46, 1153–1160.

Peppa M, Uribarri J, Vlassara H. (2002) Advanced glycoxidation: A new risk factor for cardiovascular disease? *Cardiovasc Toxicol* 2, 275-287.

Peppa M, Uribarri J, Vlassara H. (2004) The role of advanced glycation end products in the development of atherosclerosis. *Curr Diab Rep* 4, 31-36.

Peppa M, Vlassara H. (2005) Advanced glycation end products and diabetic complications: A General overview. Hormones 4(1), 28-37.

Poukupec R, Kalauz M, Turk N, Turk Z. (2003) Advanced glycation endproducts in human diabetic and non-diabetic cataractous lenses. *Graefes Arch Clin Exp Ophthalmol,* 241, 378-384.

Prevention of blindness from diabetes mellitus. (2005) Report of a WHO consultation in Geneva, Switzerland 9–11 November 2005.

Rahbar S, Figarola LJ. (2003) Novel inhibitors of advanced glycation endproducts *Arch Biochem Biophys* 419(1), 63-79.

Raj DS, Choudhury D, Welbourne TC, Levi M. (2000) Advanced glycation end products: a Nephrologist's perspective. *Am J Kidney Dis,* 35, 365–380.

Ratnaikes S, Blake D, Shevenan P. (1987) Enzymatic glycation may decrease activity of erythrocyte *S*-aminolevulinate dehydratase in diabetes mellitus. *Clin Chem,* 33, 1807–1810.

Reber F, Geffarth R, Kasper M, Reichenbach A, Schleicher ED, Siegner A, Funk RH. (2003) Graded sensitiveness of the various retinal neuron populations on the glyoxal-mediated formation of advanced glycation end products and ways of protection. *Graefes Arch Clin Exp Ophthalmol* 241, 213-225.

Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. (1995) Nε-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. *Biochemistry,* 34, 10872– 10878.

Resnikoff S, Pascolini D, Etyaale D, Kocur I, Pararajasegaram R, Pokharel GP, Mariotti SP. (2004) Global data on visual impairment in the year 2002. *Bulletin of the World Health Organization,* 82, 844–851.

Robertson, R.P. (2004) Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem*, 279(41), 42351–42354.

Schleicher E, Nerlich A (1996) The role of hyperglycaemia in the development of diabetic complications. *Horm Metab Res,* 28, 367-373.

Schmidt AM, Yan SD, Wautier JL, Stern D. (1999) Activation of receptor for advanced glycation end products – a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res,* 84, 489- 497.

Schoonover LL. (2001) Oxidative stress and the role of antioxidants in cardiovascular risk reduction. *Prog Cardiovasc Nurs,* 16(1), 30-32.

Sebag J, Buckingham B, Charles MA, Reiser K. (1992) Biochemical abnormalities in vitreous of humans with proliferative diabetic retinopathy. *Arch Ophthalmol* 110, 1472-1476.

Sell DR, Monnier VM. (1989) Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. *J Biol Chem,* 264, 21597-21602

Sell DR, Carlson EC, Monnier VM (1993) Differential effects of type 2 (noninsulin-dependent) diabetes mellitus on pentosidine formation in skin and glomerular basement membrane. *Diabetes,* 40, 190-196.

Sell DR, Lapolla A, Odetti P, Fogarty J, Monnier VM (1992) Pentosidine formation in skin correlates with severity of complication in individuals with long-standing IDDM. *Diabetes* 41, 1286-1292.

Sheetz MJ, King GL. (2002) Molecular understanding of hyperglycemia.s adverse effects for diabetic complications. *JAMA* 288, 2579-2588.

Shibayama R, Araki N, Nagai R, Horiuchi S. (1999) Autoantibody against Ne-(carboxymethyl) lysine an advanced glycation end product of the Maillard reaction. *Diabetes,* 48, 1842– 1849.

Singh R, Barden A, Mori T, Beilin L. (2001) Advanced glycation end-products: a review. *Diabetologia,* 44, 129-146.

Skolnik EY, Yang Z, Makita Z, Radoff S, Kirstein M, Vlassara H. (1991) Human and rat mesangial cell receptors for glucose-modified proteins:potential role in kidney tissue remodeling and diabetic nephropathy. *J Exp Med,* 174, 931–39.

Stevens A. (1998) The contribution of glycation to cataract formation in diabetes. *J Am Optom Assoc* 69, 519-530.

Stitt AW, Bhaduri T, McMullen CB, Gardiner TA, Archer DB. (2000) Advanced glycation end products induce blood-retinal barrier dysfunction in normoglycemic rats. *Mol Cell Biol Res Comm,* 3, 380 –388.

Stitt AW, Li YM, Gardiner TA, Bucala R, Archer DB, Vlassara H. (1997) Advanced glycation end products (AGEs) co-localise with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. *Am J Pathol,*150, 523–531.

Stitt AW, Moore JE, Sharkey JA, Murphy G, Simpson DA, Bucala R, Vlassara H, Archer DB. (1998) Advanced glycation end products in vitreous: Structural and functional implications for diabetic vitreopathy. *Invest Ophthalmol Vis Sci* 39, 2517-2523.

Stitt AW. (2001) Advanced glycation, an important pathological event in diabetic and age related ocular disease. *Br J Opthalmol* 85, 746-753.

Sulochana KN, Ramprasad S, Coral K, Lakshmi S, Punitham R, Angayarkanni N, Ramakrishnan S. (2003) Glycation and glycoxidation studies in vitro on isolated human vitreous collagen. *Med Sci Monit* 9(6), 219-224.

Swallow AJ. (1960) In *Radiation Chemistry of Organic Compounds,* Swallow AJ, eds., (pp. 211–224), Pergamon Press, New York.

Takeuchi M, Bucala R, Suzuki T, Ohkubo T, Yamazaki M, Koike T, Kameda Y, Makita Z. (2000) Neurotoxicity of advanced glycation end-products for cultured cortical neurons. *J Neuropathol Exp Neurol*, 59(12), 1094-1105.

Thornalley PJ. (2005) Dicarbonyl intermediates in the Maillard reaction. *Ann NY Acad Sci,* 1043, 111-117.

Thorpe SR, Baynes JW. (1996) Role of the Maillard reaction in diabetes mellitus and diseases of aging. *Drugs Aging* 9, 69-77.

Traverso N, Menini S, Cottalasso D, Odetti P, Marinari MU, Pronzata AM. (1997) Mutual interaction between glycation and oxidation during non-enzymatic protein modification. *Biochim Biophys Acta*, 1336, 409-418.

Turk Z, Ljubik S, Turk N, Benko B. (2001) Detection of autoantibodies against advanced glycation end products and AGE-immune complexes in serum of patients with diabetes mellitus. *Clin Chim Acta,* 303, 105–115.

Turk Z. (2001) Glycation and compications of diabetes. *Diabetol Croat,* 30, 49-54.

Turk Z. (2010) Glycotoxines, Carbonyl Stress and Relevance to Diabetes and Its Complications. *Physiol Res,* 59, 147-156.

Vay D, Vidali M, Allochis G, Cusaro C, Rolla R, Mottaram E, Bellomo G, Albano E. (2000) Antibodies against advanced glycation end product Nepsilon-(carboxymethyl)lysine in healthy controls and diabetic patients. *Diabetologia*, 43, 1385-1388.

Vlassara H, Brownlee M, Cerami A (1985) High-affinityreceptor-mediated uptake and degradation of glucosemodified proteins: a potential mechanism for the removal of senescent macromolecules. *Procl Natl Acad Sci USA,* 82, 5588-5592.

Vlassara H, Li YM, Imani F, Wojciechowicz D, Yang Z, Liu FT, Cerami A. (1995) Identification of Galectin-3 as a high affinity binding protein for advanced glycation end products (AGE): a new member of the AGE receptor complex. *Mol Med,* 1, 634–46.

Vlassara H, Palace MR. (2002) Diabetes and advanced glycation endproducts. *J Intern Med* 251: 87-101.

Vlassara H. (2001) The AGE-receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev* 17, 436-443.

Wautier JL, Guillausseau PJ. (1988) Diabetes, advanced glycation endproducts and vascular disease. *Vasc Med,* 3, 131-137.

West IC. (2000) Radicals and oxidative stress in diabetes. *Diabetic Med*, 17, 171–180.

Wolff SP, Dean RT (1987) Glucose autooxidation and protein modification. The potential role of ìautooxidative glycosylation in diabetes. *Biochem J,* 245, 243-250.

World Health Organization (2011) Diabetes fact sheet N˚312.

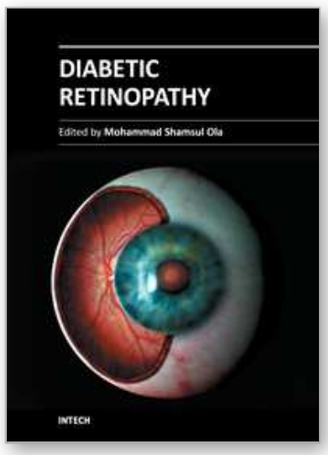
Xu X, Li Z, Luo D. (2003) Exogenous advanced glycosylation end products induce diabetes-like vascular dysfunction in normal rats: a factor in diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 241, 56-62.

Yamagishi S, Inagaki Y, Amano S, Okamoto T, Takeuchi M, Makita Z. (2002) Pigment epithelium-derived factor protects cultured retinal pericytes from advanced glycation end product-induced injury through its antioxidative properties. *Biochem Biophys Res Commun* 296, 877-882.

Yamagishi S, Inagaki Y, Okamoto T, Amano S, Koga K, Takeuchi M, Makita Z. (2002) Advanced glycation end product-induced apoptosis and overexpression of vascular endothelial growth factor and monocyte chemoattractant protein-1 in human-cultured mesangial cells. *J Biol Chem,* 277, 20309–20315

Yang Z, Makita Z, Horii Y, Brunelle S, Cerami A, Sehaipal P, Suthanthiran M, Vlassara H. (1991) Two novel rat liver membrane proteins, that bind advanced glycosylation end products, relationship to macrophage receptor glucose-modified proteins. *J Exp Med,* 174, 515–24.

Zhang X, Saaddine JB, Chou CF, Cotch MF, Cheng YJ, Geiss LS, Gregg EW, Albright AL, Klein BE, Klein R. (2010) Prevalence of diabetic retinopathy in the United States, 2005-2008. *JAMA,* 304(6), 649-656.



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The aim of this book is to provide a comprehensive overview of current concepts in pathogenesis, diagnosis and treatments of diabetic retinopathy. It provides a collection of topics written by excellent authors, covering discussions on advances in understanding of pathophysiology, immunological factors and emerging concepts, relating to clinical aspects and treatment strategies. The contents of the book will not only provide a resource for our knowledge but also improve diagnosis and treatment options for those patients who suffer vision loss due to diabetic retinopathy.

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