Introduction
In 1953, the discovery of the structure of DNA was landmark in the history of DNA biology which sheds light on the inheritance of genetic material, replication, DNA damage, repair, diversity and the evolution of species. Its discovery has united genetics with biochemistry, free radical biology, medicine and physiology. Briefly, the model proposed by James Watson and Francis Crick is based on two paired DNA strands that are complementary in their nucleotide sequence. The model has striking implications for the process of DNA replication and recombination. The DNA in a fully hydrated medium is found in B form (Langridge et al., 1960) and this is the most accepted structure of DNA molecule in solution. X-ray diffraction studies have shown that a number of variations in conformation of this basic structure can occur (Adams et al., 1981). It is well known that the right handed double helical DNA can exist in A, B, C and D conformations, whereas the left handed polynucleotide can adopt the Z structure (Saenger, 1984), so called because phosphate groups in the backbone are zig-zagged (Rich et al., 1984). The importance of DNA lies in its unique property to adopt multiple conformations depending on its bioenvironment. The DNA double helix is both deformed and made deformable by its local base sequence, whereas other regions may be made especially susceptible to a change in conformation when the helix interacts with protein, or an adjacent DNA helix in a crystal lattice.

**Antigenicity of native and modified DNA**

Anti-DNA autoantibodies permanently attract the attention of researchers. In spite of numerous investigations aimed to determine their potential targets and role in the disease, origins of anti-DNA autoantibodies and their pathological role remain to be established. Moreover, taking into account of such newly described features of anti-DNA antibodies as the ability to penetrate into the living cell (Alarcon et al., 1996) and DNA-hydrolyzing activity (Schuster et al., 1992), one may under estimate pathogenic potential of DNA-specific autoantibodies. In general, antibodies that bind DNA do not display strict disease specificity. It is, however, widely accepted that healthy individuals usually express low-affinity antibodies with specificity to single-stranded DNA, while presumably pathogenic high-affinity autoantibodies to double-stranded DNA are frequently overrepresented on the background of systemic autoimmune abnormalities (Paul et al., 1990) and their presence appears a diagnostic criterion for SLE (Bootsma et al., 1996). Some blood tumours and even AIDS are
also characterized by the increase in production of DNA-specific antibodies (Hamblin et al., 1986; Rodriguez et al., 1994). The major known pathogenic process induced by anti-DNA antibodies is the inflammatory response due to their deposition in kidney in form of the immune complexes (Suenanga et al., 1996). However, DNA specific autoantibodies can also trigger apoptosis in cultured mesangial and endothelial cells (Tsai et al., 1993; Lai et al., 1997) and are cytotoxic to the primary cultures of lymphocytes (Shoenfeld et al., 1985). Non-immunogenic status of native DNA has led to the assumption that natural anti-DNA antibodies may be formed against DNA-protein complexes, such as nucleosomes, persisting in blood as a result of disease mediated increase in cellular destruction (Jacob et al., 1992; Mohan et al., 1993). Cross reactivity with proteinaceous nuclear antigens, displayed by anti-DNA antibodies, contributed to this hypothesis and even allowed to specify a pool of anti-nuclear antibodies (ANA) (Tan et al., 1988). Described cross-reactivity of anti-DNA antibodies with membrane antigens (Raz et al., 1993) may potentially result in triggering of certain cellular responses through signal transduction pathways. Polyspecificity of anti-DNA antibodies was also shown to favor to their capacity of penetration into living cell (Avrameas et al., 1998). Entry of anti-DNA autoantibodies into living cell is shown to be accompanied by certain cellular dysfunctions (Tsai et al., 1993; Lai et al., 1997; Yu et al., 1998). In some cases, penetration of antibodies into the cells is connected with either induction (Tsai et al., 1993) or suppression of apoptosis (Yanase et al., 1997), however, precise consequences of antibody entry into the cell and its pathogenic significance remain to be determined. Catalytic activity of anti-DNA autoantibodies (DNA-abzymes) in vitro was described in a number of studies (Schuster et al., 1992; Gololobov et al., 1997), however, physiological essence of antibody DNA-hydrolyzing activity remains elusive. It is evident, that any detrimental effect to the cell that the DNA-abzyme may cause, requires direct contact between DNA-hydrolyzing autoantibodies and chromatin of the living cell.

In 1957, anti-DNA antibodies was identified as a biomarker for systemic lupus erythematosus (SLE) autoantibodies, since then antigenic property of nucleic acids received much attention (Cepellini et al., 1957; Meischer and Strassie, 1957; Robbins et al., 1957). DNA is a complex molecule whose immunologic properties vary with base sequence and encompass both stimulation and inhibition (Messina et al., 1991;
Krieg et al., 1998; Pisetsky and Reich, 2000). The problem of how an individual can immunologically distinguish between self and non self has fascinated and perplexed immunologists. When this distinction cannot be made, autoimmune diseases such as systemic lupus erythematosus (SLE) occur and are associated with the appearance of large amounts of autoantibodies. In the case of lupus, antibodies that react with double-stranded DNA are one of the hallmarks of the disease (Kofler, 1984). In the early 1960s, methods were developed for the experimental induction of antibodies to nucleic acids (Levine et al., 1960; Erlanger and Beiser, 1964; Plescia et al., 1964; Halloran and Parker, 1966) which until then had been considered widely to be non-immunogenic. Studies on the antigenic specificity of anti-DNA antibodies have enforced the view that DNA is immunologically ‘simple and bland’. It has been well established that native DNA in B-conformation is not immunogenic. Nevertheless, various modified forms of DNA, DNA in complexes with DNA-binding proteins are immunogenic and induce antibodies. Origin of anti-ds DNA autoantibodies in cancer patients and tumour-bearing mice has been demonstrated (Zhang et al., 2002). Exogenous native B-DNA has not been found immunogenic in experimental animals. Immunization of experimental animals with denatured DNA, synthetic nucleic acid polymers like poly (dT), poly (dC), poly (dA), poly (A), poly (l), poly (G), dsRNA, left handed Z-DNA, chemically modified DNA and certain helical synthetic polynucleotides with the exception of native B-DNA, induced antibodies that react selectively with the immunogen and do not cross react with native DNA (Stollar, 1986; Anderson et al., 1988).

Mammalian DNA elicit poor responses to single stranded DNA and fails to induce antibodies against native or double stranded DNA, the serologic hallmark of SLE (Madaio et al., 1984). However, it has been shown that DNA complexes with synthetic peptide FUS-I can induce anti-dsDNA response in mice (Desai et al., 1993). Since the antibody reactivity to both single and double stranded form appears independent of DNA species origin, these finding suggest recognition of conserved conformational determinants i.e., helical backbone of B-DNA and a lack of DNA sequence micro heterogeneity on antigenicity (Stollar, 1975; Pisetsky, 1993). Anti-DNA antibodies may also result by autoimmunization with chromatin, rather than native DNA (Theofilopoulos, 1995). The left handed Z-DNA is an example of a helical DNA that is a much more potent immunogen than native B-helical DNA.
Lafer et al., 1981; Madaio et al., 1984). The antibodies induced by Z-DNA have high degree of selectivity and specificity as they react with immunogen but not with B-DNA or ssDNA. The DNA modified by either chemically or physically that differ significantly from B-DNA are much stronger immunologic stimuli than nDNA and most of the antibodies induced by modified nDNA do not react with unmodified DNA (Anderson et al., 1988). Polynucleotides in B-conformation acquired immunogenicity after modification with furocoumarin (Arif and Ali, 1996). DNA modified with drugs, hormones, free radicals etc, has been reported to induce antibodies against the immunogen (Moinuddin and ali, 1994; Dixit et al, 2005; Habib et al., 2005; Khan et al., 2006). Recently, the role of peroxynitrite (ONOO⁻) modified human placental DNA in the induction of circulating cancer autoantibodies has been reported (Habib et al., 2009).

Glycation of biomacromolecules

Glycation is the nonenzymatic addition of reducing sugars (glucose, fructose, mannose, etc. and their phosphate derivatives) as well as compounds related to sugars (e.g. ascorbic acid, methylglyoxal (MG), glyoxal and 3-deoxyglucosone etc.) into biological macromolecules (proteins, DNA and lipids) (Bucala, 1985; Suarez, 1989; Sengupta, 2005). The free carbonyl groups (-C=O) of the sugar and related moieties react with the free amino (-NH₂) residues of the macromolecules in a series of chemical processes known as Maillard reaction. Initiation of glycation occurs by the formation of acid-labile Schiff base adducts which undergoes Amadori or Heyn’s rearrangements into more stable products. The early glycation products undergo slow transformation to yield the irreversible advanced glycation end products (AGEs). These reactions have attracted significant attention in recent days because of their association with the production of free radicals, which play roles in the development of cancer, diabetes, heart disease, cataract, atherosclerosis and neurodegenerative disorder (e.g., Parkinson’s disease, Alzheimer’s disease), etc.

The products of non enzymatic glycation and oxidation of proteins, DNA and lipids, the advanced glycation end products (AGEs), accumulate in a wide variety of environments. AGEs may be generated rapidly or over long times stimulated by a range of distinct triggering mechanisms, thereby accounting for their roles in multiple settings and disease states. A critical property of AGEs is their ability to activate...
receptor for advanced glycation end products (RAGE), a signal transduction receptor of the immunoglobulin superfamily. Due to such interaction, AGEs impart a potent impact in tissues, stimulating processes linked to inflammation and its consequences. Therefore, AGEs cause perturbation in a diverse group of diseases, such as diabetes, inflammation, neurodegeneration and aging (Ramasamy et al., 2005).

A large body of evidence suggests that AGE formation and accumulation are enhanced in diabetes (Brownlee, 1992). AGEs are a heterogeneous class of compounds that are composed of both fluorescent and non-fluorescent species. Glycation adducts of proteins are formed when proteins react with glucose-reactive alpha-oxoaldehydes such as glyoxal, methylglyoxal and 3-deoxyglucosone (3-DG) (Brownlee, 1996). The initial Schiff base adducts formed from glucose and lysine and N-terminal amino acid residues may rearrange to form the key intermediate, fructosamine. Fructosamine degradation and the direct reaction of alpha oxoaldehydes with proteins may form many AGEs. Of the various types of AGEs that may be generated, it has been shown that both cross-linked and noncross-linked AGEs may be generated. In vivo, a diverse array of AGE products has been detected and characterized such as bis(lysyl) imidazolium cross-links, hydroimidazolones and monolysyl adducts (Wautier and Schmidt, 2004). It has been shown that both carboxymethyl lysine (CML) adducts of proteins or lipids, as well as AGEs derived through the generation of hydroimidazolone, species that accumulate in diabetes, are specific ligands for RAGE (Thomalley, 1998; Kislinger et al., 1999). Indeed, evidence indicates that CML-AGEs are highly prevalent in diabetes, as well as in aging and renal failure (Reddy et al., 1995; Ikeda et al., 1996; Schleicher et al., 1997; Tauer et al., 2001).

DNA glycation and AGEs

Glycated DNA has attracted much attention in the past few years and is considered to be a pathogenic factor for diabetes mellitus. During the disorder in the metabolism of reducing sugars, the sugar molecules can initiate glycation of DNA in vivo (Lee and Cerami, 1989; Levi and Werman, 2003; Dutta et al., 2005). Previous investigations by several scientists (Bucala et al., 1985; Lee, 1989; Dutta et al., 2005) using biochemical and molecular biological methods have shown that DNA structure
and function are affected by the addition of sugars, resulting in deleterious modifications and other mutations.

Advanced glycation end-products (AGEs) of DNA are formed spontaneously by the reaction of carbonyl compounds such as sugars, methylglyoxal or dihydroxyacetone in vitro and in vivo. After a complex cascade of dehydration, condensation, fragmentation, oxidation and cyclization reactions, a diverse and largely undefined group of compounds termed AGEs are formed (Schleicher et al., 2001). Only few AGEs have been characterized chemically and identified in tissues, the most investigated of which has been carboxymethyllysine (CML). In renal disease AGE accumulation is ascribed both to the impaired elimination of AGEs and to enhanced formation due to oxidative stress (Heidland et al., 2001). Accumulation of AGEs in tissues is a common phenomenon of normal aging and occurs at accelerated rates in patients with diabetes mellitus (Schleicher et al., 1997; Baynes, 2001). In in vitro studies, mutagenic effects of DNA-AGE such as deletions, insertions and transposon activation were shown in bacterial model systems (Pischetsrieder et al., 1999). Furthermore, it is shown that DNA can be glycated in vitro yielding carboxyethylguanosine as major products. However, little is known about the biological consequences of DNA-AGEs.

In vitro, nucleobases and dsDNA react with sugars in a similar way as proteins (Lee and Cerami, 1987; Knerr and Severin, 1993; Singh et al., 2001). The exocyclic amino group of 2'-deoxyguanosine is particularly prone to glycation reactions, leading to the formation of N^2-carboxyethyl, N^2-carboxymethyl, N^2-(1-carboxy-3-hydroxypropyl), and N^2-(1-carboxy-3,4,5-trihydroxypentyl) modifications, as well as cyclic dicarboxyl adducts (Ochs and Severin, 1994). The two diastereomers of N^2-carboxyethyl- 2'-deoxyguanosine (CEDG A,B) are stable reaction products that are formed from a variety of glycating agents, such as glucose, ascorbic acid, glyceraldehyde, dihydroxyacetone (DHA), or methylglyoxal (Larisch et al., 1998; Frischmann et al., 2005). Recently, carboxyethylated nucleobases were detected in human urine (Schneider et al., 2004) indicating the formation of DNA AGEs in the healthy human organism. A significantly increased number of CEdG positive cells were immunostained in glomeruli of patients with diabetic nephropathy as compared to healthy controls (Li et al., 2006) as well as in glomeruli of diabetic rats (Nakamura
et al., 2007). DNA AGEs are potentially genotoxic compounds because they induce depurination as well as single strand breaks and lead to mutations (Pischetsrieder, 1999) in vitro. In vivo, it was shown, for example, that 3-deoxyglucosone, a glucose degradation product, induces embryonic malformation and teratogenicity, effects that may be related to DNA AGEs (Eriksson et al., 1998). DNA glycation in cultured cells was observed using radioactively labeled glucose (Shires et al., 1990) or a $^{32}$P-postlabeling technique (Vaca et al., 1998). Furthermore, the presence of CEdG$_{A,B}$ was detected in cultured cells by HPLC–diode array detector (DAD) after immunoaffinity chromatography (Schneider et al., 2006).

**Formation of AGEs**

Maillard first described the non-enzymatic reaction of glycine with glucose in 1912 (Maillard, 1912). The reducing sugars and other $\alpha$-dicarbonyl compounds (glyoxal, MG and 3-deoxyglucosone) form a range of heterogeneous AGE adducts on free amine and thiol groups within proteins, lipoproteins and nucleic acids. These adducts alter the structure and function of their target molecules but also form ligands for the receptor for AGEs, RAGE (Nass et al., 2007) and other known AGE receptors (Ahmed and Thornalley, 2007). Structural and inflammatory damage by AGEs is implicated in propagation of diabetes vascular and neuronal complications. MG is a potent AGE precursor, forming adducts on arginine (Arg), lysine (Lys), cysteine (Cys) and deoxyguanosine (dG) residues. The most prevalent MG adduct in proteins is the MG-derived hydroimidazolone (MG-H), but carboxyethyl lysine (CEL), N$\delta$-(4-carboxy-4,6-dimethyl-5,6-dihydroxy-1,4,5,6-tetrahydropyrimidin-2-yl)-ornithine) and argpyrimidine are also formed, as well as the crosslinking dimer MOLD (methylglyoxal lysine dimer). MG-H and CEL equivalents are also found on dG residues within DNA (dG-MG and CEdG), and MG forms a hemithioacetal with thiols, such as that in Cys. MG-AGEs are common in human tissues and are known to play critical roles in diabetes vascular complications (Thomalley, 2008). MG-H is thought to be a ligand for RAGE (Thomalley, 1998), but the role of MG-AGEs in other AGE receptor systems is unclear.

**Detection of AGEs**

AGE detection can be based on the fluorescent properties of AGEs (Monnier and Cerami, 1981). After the excitation at 370 nm, fluorescence emission wavelength is
typically at 440 nm due to the presence of heterocyclic compounds. However, the exact quantitation of AGEs is difficult to achieve due to the lack of proper means to represent the whole diverse AGE family. Candiano et al. (1986) have suggested an alternative method for quantitation involving the formation of a chromophore after reacting AGEs with diazonium salts and subsequently measuring absorbance at 490 nm wavelength. Recently, the use of immunoassays has been incorporated into many AGE investigations, including research on pyrraline (Miyata and Monnier, 1992), lipoprotein AGEs (Doucet et al., 1995), CML (Reddy et al., 1995), pentosidine (Miyata et al., 1995), ribonuclease AGEs, AGE crosslinks (Vasan et al., 1996) and imidazolones (Niwa et al., 1997). However, the antibodies produced may recognize only a limited number of AGEs and leave others undetected.

**Reactive carbonyl species**

Reactive carbonyl species (RCS) are potent mediators of cellular carbonyl stress originating from endogenous chemical processes such as lipid peroxidation and glycation. RCS are a heterogeneous group of small molecular weight carbonyls activated by α, β-unsaturation as in 4-hydroxynonenal and acrolein, α-oxo-substitution as in glyoxal and methylglyoxal, an β-oxo-substitution as in malondialdehyde (Thomalley, 1996). DNA adducts formed by RCS are known to possess strong miscoding potential in vitro and of the DNA adducts formed by MDA, pyrimido(1,2α) purin-10(3H) one (M₁G) is readily detected in many human tissues. Adducts formed by HNE have been detected in healthy human colon tissue (Wacker et al., 2000). Etheno-DNA adducts are formed from the epoxides of enals and elevated levels of this type reflect the extent of oxidative stress (Bartsch and Nair, 2000) Antibodies, both poly- and mono-clonal, have been generated against RCS-modified DNA bases. For example, MDA-deoxyguanosine (Sevilla et al., 1997), deoxycytidine-glyoxal (Mistry et al., 2003). These specific RCS-DNA antibodies have been successfully used to measure carbonyl stress in human and animal studies following oxidative and peroxidative insult (Cooke et al., 2003).

**Carbonyls produced via lipid peroxidation**

The peroxidation of membrane-derived lipid molecules is a well-studied consequence of increased intracellular oxidant levels (Esterbauer et al., 1991). The most
commonly characterized products are aldehydes, derived from ω-6 polyunsaturated fatty acids, such as malondialdehyde (MDA), hexanal, acrolein, glyoxal, crotonaldehyde, trans-2-nonenal, 4-oxo-2-nonenal and 4-hydroxy-2-nonenal (HNE) (Esterbauer et al., 1991). MDA is the most common aldehyde produced, comprising of 70% of the total produced by lipid peroxidation (Esterbauer et al., 1991). Hexanal contributes 15% and HNE contributes 5% of total aldehydes (Benedetti et al., 1980). Acrolein was identified as a lipid peroxidation product more recently through studies that examined the oxidation of low density lipoprotein (LDL) but was previously characterized as an environmental pollutant (Uchida et al., 1998).

**Carbonyls produced via glycoxidation**

Reducing sugars such as glucose can form Schiff bases with amino groups on the amino acids lysine and arginine, through the Maillard reaction. This can, through a series of rearrangements, give rise to advanced glycation endproducts (AGEs) (Munch et al., 1998). Oxidation of these glycation products can release dicarbonyls, such as the α-oxoaldehydes, MG, glyoxal, and 3-deoxyglucosone, as well as short-chain aldehydes, such as diacetyl, acetol, pyruvaldehyde and acrolein (Thoralley, 2005).

**Reactivity of carbonyls**

Many of the carbonyls that are produced as a result of either lipid peroxidation or glycoxidation are extremely reactive alkanals, such as hexanal, are the least reactive and have weaker effects than unsaturated aldehydes. Alkenals containing a C = C unsaturated bond, such as acrolein, are usually an order of magnitude more reactive than the alkanals. This is particularly the case if they contain an α,β- unsaturated (C2-C3) double bond, in addition to the C1 aldehyde. This makes the C3 carbon a strong electrophile that undergoes Michael addition by nucleophilic groups on proteins, DNA and lipids (Marnett et al., 1985), thereby causing damage to these molecules. The aldehyde group is also reactive and can form Schiff bases with amino acids. 4-hydroxy-2-alkenals, such as HNE, are extremely reactive because of the interaction between the electrophilic double bond, the aldehyde moiety and the hydroxyl group (Witz, 1989).

**Cytotoxicity of reactive carbonyls**

Cytotoxicity is generally measured by examining loss of viability. Reactive aldehydes, such as HNE, interact directly with proteins and membranes, causing
significant loss of function to membrane transporters, enzymes, signalling components, transcription factors, microtubules and other proteins, such as tau (Karlhuber et al., 1997, Picklo et al., 2002). Acrolein is also cytotoxic and, in neuronal cells, causes changes in $\text{Ca}^{2+}$ concentrations, altering glucose transport and glutamate uptake (Li et al., 1997, Lovell et al., 2001). As described earlier, other aldehydes, such as MG, can rapidly form Schiff bases with amino acids, which leads to the production of AGE at a much faster rate than from sugars, such as glucose (Thornalley, 1996). MG therefore causes significant toxicity to a range of cell types, including neuronal cells (Suzuki et al., 1998).

**Antibody production against RCS**

DNA adducts formed by RCS are known to possess strong miscoding potential *in vitro* and of the DNA adducts formed by MDA, pyrimido (1,2α) purin-10(3H) one ($\text{M}_1\text{G}$) is readily detected in many human tissues. Other propano-adducts also originate from α, β-unsaturated aldehydes or enals such as acrolein (Acr), crotonaldehyde (Cro), glyoxal (Gly) and 4- hydroxyl-nonenal (HNE). Adducts formed by HNE have been detected in healthy human colon tissue (Wacker et al., 2000). Etheno-DNA adducts are formed from the epoxides of enals and elevated levels of this type reflect the extent of oxidative stress (Bartsch and Nair., 2000). Antibodies, both poly- and monoclonal, have been generated against RCS-modified DNA bases. For e.g., MDA-deoxyguanosine (Sevilla et al., 1997), deoxyctydine-glyoxal (Mistry et al., 2003) deoxyadenosine-acrolein (Kawai et al., 2003), 1, N (6)-ethenodeoxyadenosine (Frank et al., 2004) and ethenodeoxyguanosine (Foiles et al., 1993). These specific RCS-DNA antibodies have been successfully used to measure carbonyl stress in human and animal studies following oxidative and peroxidative insult (Zhang et al., 2002, Cooke et al., 2003).

**Methylglyoxal**

Methylglyoxal (MG) is a metabolite of sugar and is highly reactive α-oxoaldehyde. It is a small molecule with molecular weight of 72. MG has a ketone group and an aldehyde moiety. The aldehyde group is more reactive than the ketone. It is a yellow liquid with characteristic pungent odor. It has 3 forms in aqueous
solution: unhydrated (1%), monohydrate (71%) and dehydrate (28%), which are in rapid equilibrium (Rae, et al., 1990).

Methylglyoxal is inevitably produced in the course of metabolism even under normal conditions. It is formed mainly from the spontaneous transformation of triose phosphates. Therefore, MG is an intrinsic component of the glycolytic pathway in mammalian cells, including vascular smooth muscle cells (VSMCs) (Ekblom, 1998). An increased MG formation may occur because of an increased availability of precursors such as increased plasma glucose or administration of ethanol or threonine (Thornalley and Tisdale, 1988). It is also formed during the non-enzymatic glycation, an early stage of the Maillard reaction (Monnier and Cerami, 1981), which is one of the post-translation modification processes between free reducing sugars and free amino groups of proteins. This compound is more reactive than parent sugar with respect to its ability to react with amino groups of proteins to form cross-links and AGEs (Brownlee et al., 1988). MG has been implicated in secondary diabetic complications promoting formation of AGEs (Uchida et al., 1998). On the other hand, MG is detoxified by the ubiquitous glyoxalase system that highly relies on the cellular level of reduced glutathione (GSH). Reduced availability of GSH also contributes to the increased levels of MG. Numerous studies showed that levels of MG were elevated in patients with diabetes mellitus (Beisswenger et al., 1999). MG levels were correlated with the glycated hemoglobin (HbA1c) (Thornalley et al., 1989) and reflected glycemic fluctuation (Nemet et al., 2005) in diabetic patients.

**Endogenous MG formation**

*Major Pathway*

MG is produced during the metabolism of carbohydrates, lipids and proteins. Several enzymatic or non-enzymatic pathways are involved in the endogenous formation of MG (Fig.1). Endogenous MG is formed from metabolic intermediates of carbohydrates, proteins and fatty acids. The majority of MG is derived from the metabolites of carbohydrate, such as glucose and fructose. MG is formed during glycolysis. Glucose is phosphorylated by glucokinase to form glucose-6-phosphate (G-6-P). This reaction decreases the intracellular glucose levels and promotes continuous transportation of glucose into the cell through the glucose transporter on
Fig. 1  **Formation, metabolism and toxicity of methylglyoxal.**

Source: Adapted from Wang, H., Thesis, University of Saskatchewan, 2009.

AMO : Acetol monooxygenase
GSH : Reduced glutathione
MG : Methylglyoxal
SSAO : Semicarbazide-sensitive amine oxidase
the cell membrane. G-6-P is then converted to fructose-6-phosphate (F-6-P) via glucose phosphate isomerase. This step is reversible but easily driven to F-6-P due to the lower levels of F-6-P. Subsequently, fructose-1, 6- biphosphate (F-1,6-P) is irreversibly formed from F-6-P and G-6-P due to catalysis by phosphofructokinase-1 (PFK-1). This reaction is the key point in the glycolytic process. F-1,6-P, then, is split by aldolase into two triose sugars, dihydroxyacetone phosphate (DHAP), a ketone, and glyceraldehyde-3-phosphate (G-3-P). DHAP and G-3-P can spontaneously convert to MG (Phillips and Thornalley, 1993).

MG is mainly formed nonenzymatically from DHAP and G-3-P, and the non-enzymatic formation of MG occurs in all cells and organisms. For example, MG formation in human red blood cells in vitro under normal glycemic conditions is due to nonenzymatic fragmentation of triosephosphates (Phillips and Thornalley, 1993).

The formation of MG in early glycation was investigated by Thornalley et al. (Thomalley et al., 1999). Glucose (50 mM) degraded slowly at 37 °C to form MG throughout a period of 3 weeks. Therefore, a short period of hyperglycemia may be sufficient to induce MG formation in vivo (Thomalley et al., 1999). Bovine retinal endothelial cells exposed to D-glucose (30 mM) for 7 days produced significantly higher levels of MG than cells cultured with L-glucose or control cells (Padayatti et al., 2001). In addition, high glucose caused increased MG formation and MG modification of the corepressor mSin3A in mouse kidney endothelial cells. Consequently, MG-modified mSin3A mediated high glucose-induced expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in cells and high sensitivity of endothelial cells to tumour necrosis factor-α (TNF-α). It was shown that glucose induced vascular inflammation and disease via the formation of MG (Yao et al., 2006).

Fructose as a precursor for MG has been investigated in vivo (Wang et al., 2008). Increased serum and aortic levels of MG have been observed in fructose-fed rats (Wang et al., 2008). MG induced structural remodeling in mesenteric artery and ROS production in aorta of fructose-fed rats. MG is also responsible for the high blood pressure and hypertriglyceridemia seen in those rats. In addition, MG impairs insulin signaling in adipose tissue of fructose-fed rats through decreasing insulin-induced insulin-receptor substrate-1 (IRS) tyrosine phosphorylation and reducing the
activity of phosphatidylinositol (PI) 3-kinase (Jia and Wu, 2007). MG can also be formed enzymatically from G-3-P and DHAP. Triosephosphate isomerase hydrolyses G-3-P and DHAP and removes phosphate to yield MG (Pompliano et al., 1990).

**Minor pathways**

MG is a byproduct of acetone metabolism. Acetone monoxygenase catalyzes acetone to acetol, and acetol monoxygenase (AMO) converts acetol to MG (Casazza et al., 1984). In addition, formation of MG is also found during the metabolism of aminoacetone, which is a metabolite of proteins. Semicarbazide-sensitive amine oxidase (SSAO) is able to convert aminoacetone into MG (Lyles, 1996). Increased serum SSAO activities have been found in patients with diabetes and vascular disorders, and treatment with selective SSAO inhibitors reduced atherogenesis in diabetic mice fed with a high-cholesterol diet (Yu et al., 2003).

**MG in food and beverages**

MG is formed in food and beverages during the processing, cooking and prolonged storage (Nemet et al., 2006). MG can be formed from carbohydrates by fragmentation of the sugar moiety during retro-aldol condensation and auto-oxidation. In addition, the formation of MG was observed during the heating process of glucose, fructose and maltose, where the amount of MG obtained from monosaccharides was markedly higher than that from disaccharides (Nemet et al., 2006). Moreover, decomposition of different lipids, caused by storage and processing, can also affect the accumulation of MG in food. A broad range of MG levels was obtained during accelerated storage (60°C for 3 and 7 days) or cooking (200°C for 1 h) of oil, depending on oil origin (salmon, cod liver, soybean, olive and corn oils) (Fujioka and Shibamoto, 2006). For instance, the formation of MG ranged from 2.03 ppm in cod liver oil to 2.89 ppm in tuna oil heated at 60 °C for 7 days. However, olive oil is the only vegetable oil that yields MG under the accelerated storage conditions.

Coffee is a widely consumed beverage. It is interesting to know whether MG is present in green and roasted coffee beans. The amount of MG is small in green coffee beans, but increases in the early phases of the roasting process and then declines. Thus, mild or medium toasted coffee beans have the highest amount of MG
content (Daglia et al., 2007). About 100 μg of MG has been determined to be present in one gram of coffee. MG owns the strongest mutagenicity in dicarboxyls in coffee (Nagao et al., 1986). Besides food and beverages, drinking water can also be an exogenous source of MG. Ozonation and chlorination of natural water, the applied process in the treatment of drinking water, can lead to the formation of MG (Matsuda et al., 1992).

**Toxicity of MG**

*MG is inextricably linked to oxidative stress*

Generation of reactive oxygen species (ROS) is associated with MG metabolism. Production of MG from acetone and aminoacetone yields hydrogen peroxide ($\text{H}_2\text{O}_2$) and superoxide radicals ($\text{O}_2^-$) as by products, and degradation of MG by glyoxal oxidase or photolysis produces $\text{H}_2\text{O}_2$ and other radical species (Kalapos, 2008). In addition, treatment of various cell types with MG induces their ROS production (Kalapos, 2008). MG also hinders antioxidant defence by reacting with functional thiol groups of glutathione (GSH) and plasma albumin to reduce their activity (Faure et al., 2005; Kalapos, 2008). MG inhibits the antioxidant enzyme superoxide dismutase by altering its structure (Kang, 2003) and impedes glyoxalase and GSH peroxidase function. Through above mechanisms, MG promotes oxidative stress in cellular systems and encourages its own survival. Oxidative stress is implicated in apoptosis of pancreatic β-cells and impaired insulin signalling in diabetes (Newsholme et al., 2007), as well as in cell and tissue damage. Whereas limited amounts of ROS are used by the immune system for functions, such as signalling and microbicidal activity, unregulated ROS production leads to oxidative protein and DNA damage, which promotes tissue dysfunction, apoptosis and premature cell aging. Cell aging can encourage ill health, as senescence of immune cells is associated with increased incidence of infection and cancer (Martin and Grotewiel, 2006).

**Modification of protein**

Under physiological conditions, more than 90% of MG is bound reversibly with cellular proteins (Lo et al., 1994). MG reacts with arginine, lysine and cysteine
residues of proteins to form advanced glycation endproducts (AGEs). Arginine-derived hydroimidazolone and lysine-derived N-ε-carboxyethyl-lysine (CEL) and N-ε-carboxymethyl-lysine (CML) are products of irreversible reactions of protein residues with MG (Lo et al., 1994). The concentration of AGEs in mammalian tissues, plasma and extracellular matrix in vivo depends on the protein substrate, tissue location and type of AGEs. For instance, the highest concentration of hydroimidazolone was found in the lens of older individuals and CML accumulates on lens, skin and cartilage (Ahmed et al., 1997; Verzijl et al., 2000).

MG-induced AGEs are involved in the pathogenesis of many diseases, such as diabetes, hypertension and neurodegenerative diseases (Desai and Wu, 2007; Munch et al., 2003). AGEs induce cross-linkage of proteins to decrease arterial and myocardial compliance and promote vascular stiffness, leading to the alteration of vascular structure and function, which contributes to the development of hypertension and diabetic vascular complications (Goh and Cooper, 2008). AGEs also have been seen accumulated in diabetic kidney, retina and atherosclerotic plaques (Makita et al., 1994; Bucala and Vlassara, 1995; Hammes et al., 1999), and are closely linked to the development of diabetic complications. In addition, AGEs interact with some receptors, like the receptor for AGEs (RAGE), where they interfere with cell signaling and nuclear factor-κB (NF-κB) mediated pathway, leading to enhanced oxidative stress and generation of proinflammatory cytokines.

MG-induced AGEs formation impairs anti-oxidant enzymes, leading to the excessive accumulation of reactive oxygen species (ROS). Arginine, lysine and cysteine are residues involved in the active sites of enzymes, and the irreversible reaction of MG with residues may alter the activity of those enzymes. For example, activities of glutathione reductase and glutathione peroxidase were reduced significantly, accompanied by the increased MG-induced AGEs formation in aorta from adult SHR (Wang et al., 2005). MG also modifies Cu/Zn-SOD by covalent cross-linking of the proteins, leading to the release of copper ions from the enzyme and the inactivity of the enzyme (Kang, 2003). Furthermore, decreased extracellular SOD activity was due to excessive glycation, not to the impaired synthesis of this enzyme in patients with diabetes (Ciechanowski et al., 2005). Aminoguanidine, a scavenger of MG and AGEs, increased the activities of catalase, glutathione reductase
and glutathione peroxidase in insulin-dependent diabetic rats and prevented the impairment of blood antioxidant systems (Stoppa et al., 2006).

**Modification of nucleic acid**

MG can be a mutagen since it modifies nucleotides poly A, poly G and poly C, but not poly-U (Krymkiewicz, 1973). MG inhibited skin cell proliferation and caused extensive DNA strand cleavage by the extensive formation of DNA-protein cross-links (Roberts et al., 2003). MG-induced cytotoxicity and mutation were concentration dependent. Multi-base deletions were predominant (50%) in MG-induced mutations, followed by base-pair substitutions (35%), in which G:C to C:G and G:C to T:A transversions were predominant (Murata-Kamiya et al., 2000). Furthermore, MG increased point mutations in *Salmonella typhimurium* (Migliore et al., 1990), and the occurrence of point mutations correlated with the glycation rate of DNA (Pischetsrieder et al., 1999). The cross-link formation of protein with DNA by glycation with MG has been investigated. A protein-DNA cross-link was observed after 90 min exposure to MG (1.5 mM) in Chinese hamster ovary cells (Brambilla et al., 1985). In addition, MG cross-linked a guanine residue of the substrate DNA and lysine and cysteine residues near the binding site of the DNA polymerase during DNA synthesis, and that DNA replication was severely inhibited by the MG-induced DNA-DNA polymerase cross-link in *E.coli* (Murata-Kamiya and Kamiya, 2001).

Apart from these modifications, DNA-AGEs are also formed by the reaction of MG and nucleic acids. The major AGE adducts found in DNA are MG-derived dG modifications (Bidmon et al., 2007 and Synold et al., 2008), leading to the formation of carboxyethyl deoxyguanosine (CEdG). CEdG is the major adduct of the glycation reaction of MG and DNA. MG-glycation of dG residues seems to increase the frequency of dG deletion (Seidel and Pischetsrieder, 1998), leading to abasic sites, which are potentially mutagenic. MG treatment of cells leads to reduced DNA replication and increased mutations, the most prominent being multibase deletions and base-pair substitutions (Murata-Kamiya et al., 2000). MG also has the potential to crosslink DNA to proteins, for example between the dG of DNA and Lys or Cys residues close to the DNA polymerase binding site (Murata-Kamiya and Kamiya, 2001). Therefore, any mutations or DNA damage/cross-linking caused by MG could
result in reduced or altered transcription, which might affect genes/proteins involved in immune defence or any other bodily function. Furthermore, because PARP plays a role in DNA repair and is activated by DNA damage, dG glycation or other MG-related DNA damage could also negatively impact glycolysis.

**Scavengers of MG**

To date, specific MG scavengers are not available in the market, but some agents like aminoguanidine, alagebrium and metformin including some AGE inhibitors like D-lysine, D-penicillamine, diclofenac and desferrioxamine are capable of reducing MG levels. In addition to these scavengers, vitamins like pyridoxamine, thiamine and thiamine pyrophosphates, pyridoxal phosphate also play crucial role in inhibiting post amadori glycation cascade and as carbonyl trapping AGE inhibitors. These inhibitors are currently used in different studies, although the mechanism is unclear.

**Physiological and pathological levels of MG**

The levels of MG in plasma of normal human subjects vary from 123 nM to 650 nM depending on different studies (Beisswenger, et al., 1999; Odani, et al., 1999; Lapolla, et al., 2005; Nemet, et al., 2005). The inconsistency of those values seems dependent on different methods used to test the compound.

Elevated levels of MG have been observed in different kinds of diseases, such as hypertension, diabetes and renal failure. MG was significantly elevated in patients with diabetes mellitus versus normal subjects (212 nM vs. 160 nM) (Beisswenger, et al., 1999). The data from another laboratory indicated that plasma MG levels were significantly higher in patients with diabetes mellitus (194 ng/ml) and patients with chronic renal failure (128 ng/ml) than those from normal subjects (59 ng/ml) (Odani et al., 1999). In addition, MG levels were significantly elevated in patients with end-stage renal disease versus normal controls (24 vs. 9 μg/ml) (Lapolla, et al., 2005).

Numerous studies demonstrated that MG played an important role in the pathogenesis of diabetes and diabetic complications. It has been observed that the plasma levels of MG in diabetic patients were significantly increased compared with those in
normal controls (742 ± 141 vs. 520 ± 42 nM). MG was also a parameter reflecting glycemic fluctuation (Nemet, et al., 2005).

**Diabetes mellitus**

Advances in clinical science over a single professional lifetime during the second half of the 20th century have led to improvements in understanding the causes and complications of diabetes, together with alleviation of suffering to an extraordinary degree. Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several types of diabetes mellitus exist and are caused by a complex interaction of genetics and environmental factors, viral infection and autoimmune disease have been implicated (Paik et al., 1982; Kataoka et al., 1983; Sandler et al., 2000; Shewade et al., 2001). While exogenous insulin and other medications can control many aspects of diabetes, numerous complications affecting the vascular system, kidney, retina, lens, peripheral nerves and skin are common and are extremely costly in terms of longevity and quality of life. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Baynes et al., 1999). Diabetes is usually accompanied by increased production of free radicals (Chang et al., 1993; Young et al., 1995; Baynes et al., 1999) or impaired antioxidant defenses (Halliwell and Gutteridge, 1990; McLennan et al., 1991; Saxena et al., 1993). Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs) and protein kinase C. Depending on the etiology of the diabetes mellitus, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes. In the United States, diabetes mellitus is the leading cause of end-stage renal disease (ESRD), non traumatic lower extremity amputations, and adult blindness (Powers, 2008). It also predisposes to cardiovascular diseases. In diabetes mellitus, low insulin levels prevent cells from absorbing glucose, as a result glucose builds up in the blood. When glucose-laden blood passes through kidneys, all the excess glucose cannot be absorbed. This excess
glucose secreted in urine along with water and electrolytes as well as ions required by cells to regulate the electric charge and flow of water molecules across the cell membrane. This causes polyurea, polydipsia and weight loss as classical symptoms of the diabetes. These symptoms together with a random plasma glucose concentration $\geq 11.1$ mmol/L (200mg/dL) is sufficient for the diagnosis of diabetes mellitus although fasting plasma glucose is the most reliable and convenient test for identifying diabetes in asymptomatic individuals (Powers, 2008).

**Classification of diabetes mellitus**

Diabetes mellitus is classified on the basis of the pathogenic process that leads to hyperglycemia (American Diabetes Association, 2007). The two broad categories of diabetes mellitus are designated as type I and type II. Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the pathogenic processes progresses. Type I diabetes is the result of complete or near-total insulin deficiency. Type II is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Distinct genetic and metabolic defects in insulin secretion give rise to the common phenotype of hyperglycemia in type II diabetes mellitus and have important potential therapeutic implications now that pharmacologic agents are available to target specific metabolic derangements. Type II diabetes mellitus is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). The term insulin-dependent diabetes mellitus (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM) are obsolete. Since many individuals with type II diabetes eventually require insulin treatment for control of glycemia, the use of term NIDDM generated considerable confusion. Other etiologies for diabetes mellitus include specific genetic defects in insulin secretion or action, metabolic abnormalities that impair insulin secretion, mitochondrial abnormalities and a host of conditions that impair glucose tolerance. The etiologic classification of diabetes mellitus is illustrated in Table 1. Maturity onset diabetes of the young (MODY) is a subtype of diabetes mellitus characterized by autosomal dominant inheritance, early onset of hyperglycemia (usually $< 25$ years), and impairment in insulin secretion (Powers, 2008).
### TABLE 1

**Etiologic classification of diabetes mellitus**

I. **Type I diabetes** (β-cell destruction, usually leading to absolute insulin deficiency)
   - A. Immune Mediated.
   - B. Idiopathic.

II. **Type II diabetes** (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance).

III. **Other specific types of diabetes**

   A. *Genetic defects of β-cell function characterized by mutations in:*
      1. Hepatocyte nuclear transcription factor (HNF) 4α (MODY 1).
      2. Glucokinase (MODY 2).
      3. HNF-1 α (MODY 3).
      4. Insulin promoter factor-1 (IPF-1; MODY 4).
      5. HNF-1 β (MODY 5).
      6. Neuro D1 (MODY 6).
      7. Mitochondrial DNA
   
   B. *Genetic defects in insulin action*
      1. Type A insulin resistance
      2. Leprechaunism
      3. Rabson-Mendenhall syndrome
   
   C. *Diseases of the exocrine pancreas*
      1. Pancreatitis
      2. Pancreatectomy
      3. Neoplasia
      4. Cystic Fibrosis
      5. Hemochromatosis
      6. Fibrocalculous pancreatopathy
   
   D. *Endocrinopathies*
      1. Acromegaly
      2. Cushing’s syndrome
      3. Glucagonoma
      4. Pheochromocytoma
      5. Hyperthyroidism
      6. Somatostatinoma
   
   E. *Drug- or chemical-induced*
      1. Pentamidine
      2. Nicotinic acid
      3. Glucocorticoids
      4. Thyroid hormone
      5. Diazoxide
      6. β-adrenergic agonists
      7. Phenytoin
      8. α-interferon
      9. Protease inhibitor
   
   F. *Infections*
      1. Congenital rubella
      2. Cytomegalovirus
      3. Coxsackie
   
   G. *Uncommon forms of immune-mediated diabetes*
      1. “Stiff Person” syndrome
      2. Anti-insulin receptor antibodies
   
   H. *Other genetic syndromes sometimes associated with diabetes*
      1. Down’s syndrome
      2. Klinefelter’s syndrome
      3. Turner’s syndrome
      4. Huntington’s chorea
      5. Myotonic dystrophy
      6. Porphyria

IV. **Gestational Diabetes Mellitus (GDM)**

**Source:** Adapted from American Diabetes Association, 2007.
Type I diabetes mellitus

Absolute insulin deficiency caused by autoimmune-mediated destruction of pancreatic β-cells characterizes type I diabetes. This condition is also called “insulin-dependent diabetes” or “juvenile diabetes.” The main cause of the beta cell loss is a T-cell mediated immune attack (Rother, 2007). It is thought to be caused by a combination of environmental factors and viral infection, superimposed on a genetic susceptibility. It accounts for ~10% of those with diabetes in the United States, but the prevalence may be increasing. The disorder may be further sub classified into type IA if autoimmune markers are found, usually at the time of diagnosis (A Report on Diabetes care, 1997) Type IB diabetes is an absolute insulin deficiency in which no autoimmune markers can be identified. Type IB diabetes may be more common in people of Asian heritage (Abiru et al., 2002). Type I diabetes is a multifactorial autoimmune disease thought to arise from a complex interaction between both genetic susceptibility and environmental insult(s). Several autoantibody (Table 2) markers have been detected in autoimmune diabetes including islet cell antibodies (ICA), insulin autoantibodies (IAA) (Atkinson et al., 1992), glutamic acid decarboxylase-65 (GAD-65) autoantibodies (Jun et al., 2002) and antibodies to tyrosine phosphatases IA-2 and IA-2β (Lan et al., 1996; Lu et al., 1996). There is a strong association of type I diabetes with individuals who possess particular HLA haplotypes. HLA DR4-DQ8 and DR3-DQ2 are present in > 90% of children with type IA diabetes (Powers, 2008). Furthermore, 30–50% of patients with type IA diabetes are heterozygotes for HLA DR4-DQ8 and DR3-DQ2, whereas this combination of alleles is only present in ~2.4% of the general population (Devendra and Eisenbarth, 2003). Most people with these HLA alleles do not develop type I diabetes, demonstrating that other factors are involved in the development of the disease. Many triggers have been proposed for the development of type I diabetes in genetically susceptible individuals. Viruses such as enteroviruses, coxackie virus and rubella have been proposed as culprits but have not been definitively shown to induce type I diabetes (Lammi et al., 2005). Food additives or toxins, such as nitrosamines, have also been proposed as a cause of diabetes (Helgason et al., 1981). Some investigators have also implicated cow’s milk as an initiating factor in the development of autoimmunity in type I diabetes (Oute et al., 1999). Whatever initiating mechanism is, the autoimmune destruction of β-cells leads
**TABLE 2**

*Major autoantigens in type I diabetes*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characteristics</th>
<th>GAD65</th>
<th>IA-2</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amino Acid length</td>
<td>585</td>
<td>979</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>Molecular weight (Da)</td>
<td>65,000</td>
<td>106,000</td>
<td>6,000</td>
</tr>
<tr>
<td>3</td>
<td>Chromosome</td>
<td>10p11</td>
<td>2q35</td>
<td>11p15</td>
</tr>
<tr>
<td>4</td>
<td>Cell type in which expressed</td>
<td>Neuroendocrine pancreatic islet cells</td>
<td>Neuroendocrine pancreatic islet cells</td>
<td>Pancreatic islet β cell</td>
</tr>
<tr>
<td>5</td>
<td>Intracellular location</td>
<td>Neuron-like small vesicles</td>
<td>Secretory vesicles</td>
<td>Secretory vesicles</td>
</tr>
<tr>
<td>6</td>
<td>Function</td>
<td>Converts glutamic acid to GABA; inhibitory neurotransmitter</td>
<td>Enzymatically inactive member of PTP family</td>
<td>Ligand for the insulin receptor; regulation of blood glucose</td>
</tr>
</tbody>
</table>

to a progressive decline in the body’s insulin secretory capacity. Eventually, this decline manifests itself in hyperglycemia after a large carbohydrate load, such as a meal or a glucose tolerance test. When ~80% of β-cells have been destroyed, patients develop the first clinical symptoms of diabetes. Interestingly, the rate of β-cell decline can vary based on age, with older patients who develop type I diabetes typically experiencing a much more gradual decline in β-cell mass (Powers, 2008).

**Type II diabetes mellitus**

Type II diabetes is a heterogeneous group of conditions that constitute ~90% of diabetes. Like type I diabetes, type II diabetes also involves both genetic susceptibility and environmental factors, although the genetic component may be greater than in type I diabetes. It is caused by a combination of insulin resistance and relative insulin deficiency with increased hepatic glucose production. It is important to note that some individuals experience predominantly insulin resistance and others insulin deficiency. Insulin resistance is generally thought to precede insulin deficiency. Obesity is associated with increased insulin resistance and may be the reason that, type II diabetes is more common in obese individuals. The precise mechanism by which obesity leads to insulin resistance is not completely described but may be related to several biochemical factors, such as free fatty acids, leptin, tumour necrosis factor-α and other substances. In addition, many genetic polymorphisms may play a part in insulin resistance, possibly through post-insulin receptor signal transduction mechanisms (Powers, 2008). Overweight and obesity are strongly associated with development of type II diabetes and may be responsible for the majority of the growing diabetes pandemic (Wannamethee and Shaper 1999). Furthermore, weight loss is strongly associated in prospective studies with decreased progression from impaired glucose tolerance (IGT) to type II diabetes (Knowler *et al.*, 2002). Insulin resistance alone, however, does not cause diabetes. Most obese people do not develop type 2 diabetes, despite increased insulin resistance (Polansky, 2000). For type II diabetes to emerge, there must also be relative insulin deficiency. Before type II diabetes develops, the pancreatic β-cells increase their production of insulin to compensate for increased insulin resistance. It has been proposed, that there is measurable β-cell hypertrophy present in obese subjects who do not have diabetes. For unclear reasons, β-cell secretory capacity gradually declines in some individuals,
leading to the development of type II diabetes. As β-cell insulin secretory capacity declines, type II diabetes begins to develop. Initially, hyperglycemia is only observed after large meals, as in type II diabetes. As β-cell function declines further, however, hyperglycemia becomes more severe. Studies have suggested that 40% of β-cell mass may be lost in individuals who have glucose intolerance, and ~60% may be lost when clinical type II diabetes develops (Butler et al., 2003). Hepatic insulin resistance and relative insulin deficiency also lead to increased hepatic gluconeogenesis, which further worsens hyperglycemia. Eventually, the degree of hyperglycemia worsens and becomes virtually universal if left untreated (Powers, 2008). The cause of β-cell failure in type II diabetes is unknown. In addition to a genetic predisposition, studies have also demonstrated higher rates of apoptosis and decreased β-cell mass in patients with type II diabetes (Butler et al., 2003). There are also increased amounts of amyloid deposits in the islets of patients with type II diabetes (Khan et al., 1999).

Several authors speculate that increased insulin resistance may be a genetic trait that can be worsened by obesity and that β-cells compensate for this increased resistance. Some individuals, however, cannot maintain this compensation because of β-cell failure, which leads to the development of type II diabetes.

Asian Indians are more prone to type II diabetes and premature coronary artery disease due to “Asian Indian Phenotype”. The phenotype refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity i.e., higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels (Mohan et al., 2007).

**Methylglyoxal and diabetes mellitus**

No matter the cause of diabetes, the result is always hyperglycaemia. This excess glucose metabolism drives several damage pathways and raises concentrations of the reactive dicarbonyl, methylglyoxal (MG). MG can modify the structure and function of target molecules by forming advanced glycation end-products (AGEs) that act through their receptor (RAGE) to perpetuate vascular and neuronal injury responsible for long-term complications of diabetes. Diabetes patients suffer lower resistance to many common infections, although the cause for this lower resistance
remains elusive. It has been suggested that MG could be a missing link between hyperglycaemia and immune suppression in diabetes. The glycolytic by-product methylglyoxal (MG) is a potent modifier of immune components and function, inducing immune damage which might provide a link between hyperglycaemia and diabetes-related infection risk (Claire and Stella, 2009).

MG and metabolic dysfunction in diabetes

The reactive α-oxoaldehyde MG is formed as a natural by-product of several metabolic pathways, mainly from glycolysis but also from lipid peroxidation and threonine catabolism (Kalapos, 1999). To prevent cellular damage, MG is detoxified by defence components, including the specialised glyoxalase system, which converts α-oxoaldehydes to their respective hydroxyacids (Thornalley, 2003). In diabetes, the increased flux of glucose metabolism causes metabolic dysfunction. Higher rates of respiration through the electron transport chain within mitochondria lead to superoxide leakage, increased oxidative stress and activation of the nuclear enzyme, poly (ADP-ribose) polymerase-1 (PARP). PARP activation depletes its substrate NAD⁺ (slowing rate of glycolysis and electron transport) and inhibits glyceraldehyde-3-phosphate (G-3-P) dehydrogenase (GAPDH) (Pacher and Szabo, 2005). By inhibiting the GAPDH conversion of G-3-P, glycolytic intermediates build up, compounded by increased glucose at the head of the chain. Glycolytic intermediates are pushed down their respective metabolic pathways (protein kinase C, polyol and hexosamine), altering cellular balance and causing damage through raised angiogenic factors, reduced nitric oxide and altered gene expression and protein function (Rolo and Palmeira, 2006). This damage, along with that from formation of advanced glycation end-products (AGEs), lies behind the vascular and neuronal complications of diabetes. The glycolytic intermediates fructose-1,6-diphosphate, G-3-P and glycerol phosphate are direct precursors of MG; thus, in diabetes, MG production is vastly increased. Furthermore, the glyoxalase defence against MG becomes overwhelmed, with activity of glyoxalase I decreased by oxidative stress and MG concentrations are able to rise (Thornalley, 2008). Therefore, MG blood levels are 2–6 times higher in diabetes patients compared with controls (McLellan et al., 1994). Because MG initially binds reversibly to tissues in vivo (Lo et al., 1994), actual levels might be far higher (up to 100 times higher) than this measurement suggests and
concentrations probably vary locally depending upon availability of precursors and activity of defences.

**Role of MG in diabetes mellitus**

*MG-induced AGEs in diabetes mellitus*

MG is the most important precursor of AGEs. Numerous studies show that accumulation of intracellular MG and formation of AGEs alter cell function and contribute to the development of type II diabetes mellitus and diabetic complications such as atherosclerosis, nephropathy, and retinopathy.

In cultured endothelial cells, MG accumulated rapidly under hyperglycemic conditions (Shinohara, *et al.*, 1998). In addition, serum levels of AGEs increased in patients with type II diabetes and coronary artery disease (Kilhovd, *et al.*, 1999). AGEs induce diabetic atherosclerosis by multiple ways. Argpyrimidine, the fluorescence product of the reaction of MG with arginine residues in protein, has been demonstrated to localize in atherosclerotic lesions, fatty streaks, lipid containing SMCs and macrophages in diabetic patients (Friedman, 1999; Oya, *et al.*, 1999). A correlation of AGEs and severity of atherosclerotic lesions was also shown. AGEs decrease NO availability by quenching NO, impair LDL removal by trapping LDL in the sub endothelium and decrease LDL 39 receptor recognizing AGEs-modified LDL (Bucala, *et al.*, 1994). Furthermore, AGEs enhanced VCAM-1 expression by activating NF-κB. VCAM-1 stimulates the migration of monocytes through endothelium, which is the first step of atherogenesis (Kunt, *et al.*, 1999).

The kidney is a key target of MG and AGEs mediated damage. Mouse renal damage was found after oral administration of MG. A 5-month treatment with MG resulted in elevated levels of collagen in kidney and increased glomerular basement membrane thickness (Golej, *et al.*, 1998). Diabetic mice have significantly elevated renal AGEs, and these abnormalities have been linked to various structural aspects of diabetic nephropathy, including glomerular basement membrane thickening, glomerulosclerosis, and tubulointerstitial fibrosis (Soulis-Liparota, *et al.*, 1995).
MG-induced hydroimidazolone increased selectively in retinas of streptozotocin-induced diabetic rats (Karachalias, et al., 2003). In addition, MG-modified CML was localized in retinal blood vessels of patients with type II diabetes and was found to correlate with the degree of retinopathy (Stitt, 2001). Furthermore, decreased eNOS expression was observed in retinal vascular endothelial cells exposed to AGEs, which may account for retinal microvascular abnormalities (Chakravarthy, et al., 1998).

**MG-induced oxidative stress in diabetes mellitus**

Growing evidence suggests that MG-induced oxidative damage is responsible for the development of diabetic complications. Type II diabetes mellitus patients without a history of acute cardiovascular events, such as myocardial infarction and unstable angina, during the previous 6 months were recruited. Compared to baseline, MG/AGEs rich, heat-processed food reduced macrovascular flow-mediated dilatation and decreased microvascular reactive hyperemia, indicating macro- and microvascular endothelial dysfunction. The impairment of postprandial flow-mediated dilatation may be the result of a combined effect of reduced NO production and increased NO scavenging, both decreasing NO bioavailability (Stirban, et al., 2006).

Another study of three diabetic populations, the Overt Nephropathy Progressor / Nonprogressor (ONPN) cohort (n = 14), the Natural History of Diabetic Nephropathy study (NHS) cohort (n = 110) and the Pima Indian cohort (n = 45), demonstrated that progression of diabetic nephropathy was significantly correlated with MG levels and oxidative stress (Beisswenger, et al., 2005). The oxidative stress in this study was verified by the reduced GSH levels in red blood cells of diabetic patients. In addition, MG modified renal mitochondrial protein in streptozotocin-treated rats, leading to significantly increased production of mitochondrial $\text{O}_2^{-}$ and oxidative damage (Rosca, et al., 2005). Furthermore, exposure of human neuroblastoma SH-SY5Y cells to MG was associated with increased ROS production, leading to MG-induced cellular damage (de Arriba, et al., 2006). MG also induced diabetic neuropathy through oxidative stress-mediated activation of p38 MAPK.
MG and insulin

Evidence shows that MG destroys pancreatic β-cells, decreases insulin secretion in response to glucose and alters insulin structure and function. MG caused a concentration-dependent increase of apoptotic pancreatic β-cells (Sheader, et al., 2001). Addition of MG (0.5 or 1 mM) to single isolated rat pancreatic β-cells caused a rapid and marked depolarization, and this effect was reversible upon the removal of MG. MG also led to elevated cytosolic calcium concentration and intracellular acidification in intact rat islets (Cook, et al., 1998). Moreover, acute exposure of isolated mouse islets or β-cells to MG resulted in reduced insulin secretion in response to glucose (Pi, et al., 2007).

Immune alterations in diabetes

The damage of the immune system by MG glycation could be a link between diabetes and infection susceptibility (Claire and Stella, 2009). A commonly reported feature of diabetes is reduced production of the proinflammatory cytokine interferon-γ (IFN-γ) (Faresjo et al., 2006). Type I diabetes and Type II diabetes patients also show inhibited tumour necrosis factor-α (TNF-α) production and reduced expression of Th1-associated chemokine receptors on peripheral blood cells (Lohmann et al., 2002), as well as reduced production of the antiviral cytokine IFN-α from dendritic cells (DCs). Type I diabetes patients have lower proportions of natural killer (NK) cells in peripheral blood (important in clearance of virus-infected cells) and a reduced activity of these cells (Rodacki et al., 2006). Diabetes is also associated with raised levels of serum adhesion factors, such as intercellular- and vascular-adhesion molecules (ICAM and VCAM) (Boulbou et al., 2005; Glowinska et al., 2005) and monocytes from type I diabetes patients show increased adhesion but decreased chemotactic migration towards proinflammatory chemokines (Bouma et al., 2005). The chemotactic, phagocytic and microbicidal functions of neutrophils are also hindered (Alba-Loureiro et al., 2007). Increased cell death is associated with hyperglycaemia, with greater apoptosis of lymphocytes and neutrophils reported in type I diabetes and type II diabetes patients (Otton et al., 2004; Glisic-Milosavljevic et al., 2007). Loss of these cell types might hinder both innate and adaptive responses to any infection.
Together, these studies illustrate that hyperglycaemia impacts many different facets of immune function. Any one aspect of these diabetes-associated deficits, including reduced leukocyte migration, impaired killing mechanisms or lower lymphocyte proliferation, might be enough to suppress infection clearance. MG appears to be a potent modifier of immune function in vitro and seems to be able to produce effects which confirm some of the in vivo findings described in diabetes patients. Reduced stimulation of T cells, altered cell phenotype and hindered cytokine responses caused by MG might underlie reduced infection clearance in diabetes.

**Free radicals and diabetes mellitus**

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. 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). Superoxide anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals (Halliwell and Gutteridge, 1990; Hogg et al., 1993). Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Kawamura et al., 1994; Tsai et al., 1994). Another important source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of an Amadori product and then advanced glycation endproducts (AGEs) (Mullarkey et al., 1990; Hori et al., 1996). Similar reactions are also found to occur with nucleic acids and their bases where glucose and other glycating agent reacts with free amino groups of guanine and less common to adenine to form AGEs, hence also called DNA-AGEs. These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions (McCarthy et al., 2001), promote free radical formation (Baynes and Thorpe, 1999), and quench and block anti proliferative effects of nitric oxide (Wautier et al., 1994; Vlassara,
By increasing intracellular oxidative stress, AGEs activate the transcription factor NF-κB, thus promoting up-regulation of various NF-κB controlled target genes (Mohamed et al., 1999). NF-κB enhances production of nitric oxide, which is believed to be a mediator of islet beta cell damage. Considerable evidence also implicates activation of the sorbitol pathway by glucose as a component in the pathogenesis of diabetic complications, for example, in lens cataract formation or peripheral neuropathy (Kador et al., 1984; Greene et al., 1992; Obrosova et al., 1997). Efforts to understand cataract formation have provoked various hypotheses. In the aldose reductase osmotic hypothesis, accumulation of polyols initiates lenticular osmotic changes. In addition, oxidative stress is linked to decreased glutathione levels and depletion of NADPH levels (Cheng and Gonzalez, 1986). Alternatively, increased sorbitol dehydrogenase activity is associated with altered NAD⁺ levels (Williamson et al., 1993), which results in protein modification by nonenzymatic glycosylation of lens proteins (Yano et al., 1989; Ramalho et al., 1996). Mechanisms linking the changes in diabetic neuropathy and induced sorbitol pathway are not well delineated. One possible mechanism, metabolic imbalances in the neural tissues, has been implicated in impaired neurotrophism (Mizisin et al., 1997; Delcroix et al., 1998; Hounsom et al., 1998), neurotransmission changes (Ralevic et al., 1995; Kamei and, Ohsawa, 1996; Stevens et al., 2000), Schwann cell injury (Mizisin et al., 1998; Kalichman et al., 1998) and axonopathy (Chokroverty et al., 1988; Fernyhough et al., 1999).

Epidemiology and prevalence of diabetes mellitus

The worldwide prevalence of diabetes mellitus has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000 (Fig. 2). Based on current trends, > 360 million individuals will have diabetes by the year 2030 (Wild et al., 2004). Estimates which have been produced by the International Diabetes Federation (IDF) is about 194 million individual worldwide to have diabetes in 2003 and is expected to increase to about 333 million by 2025. (International Diabetes Federation, Diabetes Atlas, 2006).

Table 3 shows the 10 countries with the largest numbers of people with diabetes. As might be expected, the countries with the largest populations have
Fig. 2  Worldwide prevalence of diabetes mellitus.

TABLE 3
Top Ten countries for numbers of people aged 20–79 years with diabetes in 2010 and 2030. (Number of diabetic peoples, *in millions*).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Country</th>
<th>Year, 2010</th>
<th>S. No.</th>
<th>Country</th>
<th>Year, 2030</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>50.8</td>
<td>1</td>
<td>India</td>
<td>87.0</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>43.2</td>
<td>2</td>
<td>China</td>
<td>62.6</td>
</tr>
<tr>
<td>3</td>
<td>USA</td>
<td>26.8</td>
<td>3</td>
<td>USA</td>
<td>36.0</td>
</tr>
<tr>
<td>4</td>
<td>Russia</td>
<td>9.6</td>
<td>4</td>
<td>Pakistan</td>
<td>13.8</td>
</tr>
<tr>
<td>5</td>
<td>Brazil</td>
<td>7.6</td>
<td>5</td>
<td>Brazil</td>
<td>12.7</td>
</tr>
<tr>
<td>6</td>
<td>Germany</td>
<td>7.5</td>
<td>6</td>
<td>Indonesia</td>
<td>12.0</td>
</tr>
<tr>
<td>7</td>
<td>Pakistan</td>
<td>7.1</td>
<td>7</td>
<td>Mexico</td>
<td>11.9</td>
</tr>
<tr>
<td>8</td>
<td>Japan</td>
<td>7.1</td>
<td>8</td>
<td>Bangladesh</td>
<td>10.4</td>
</tr>
<tr>
<td>9</td>
<td>Indonesia</td>
<td>7.0</td>
<td>9</td>
<td>Russian</td>
<td>10.3</td>
</tr>
<tr>
<td>10</td>
<td>Mexico</td>
<td>6.8</td>
<td>10</td>
<td>Egypt</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*Source:* Shaw *et al.*, diabetes research and clinical practice 87, 2010
the highest number of persons with diabetes. Only Bangladesh and Nigeria of the world's 10 most populous countries are not among the 10 countries with the highest diabetes numbers (replaced by Germany and Mexico) for 2010. There are marked differences between developed and developing countries. For developing countries, adult diabetes numbers are likely to increase by 69% from 2010 to 2030, compared to 20% for developed countries, whereas total adult populations are expected to increase by 36% and 2% respectively (Shaw et al., 2010).

There is considerable geographic variation in the incidence of both type I and type II diabetes mellitus. Scandinavia (Finland) has the highest incidence of type I diabetes mellitus, whereas in Pacific Rim regions (Japan, China), its prevalence is least. Northern Europe and the United States have an intermediate rate. Much of the increased risk of type I diabetes mellitus is believed to reflect the frequency of high risk HLA alleles among ethnic groups in different geographic locations. The prevalence of type II diabetes mellitus and its harbinger, IGT, is highest in certain pacific islands, intermediate in countries such as India and the United States, and relatively low in Russia. This variability is due to genetic, behavioural and environmental factors.

India is now being termed the "diabetes capital of the world" as it leads the world with largest number of diabetic subjects. WHO report shows that 32 million people in India had diabetes in the year 2000 and is expected to increase about 80 million by 2030. The IDF estimated the total number of diabetes subjects to be around 36 million in India in 2003 and this is further set to rise to 73.4 million by the year 2025. Another report published recently, estimated it to be 50.8 million by the year 2010 and will shoot up to 87.0 million by the end of year 2030 (Shaw et al., 2010).

Diabetes is the leading cause of mortality. A recent estimate suggested that diabetes is the fifth leading cause of death worldwide and is responsible for almost 3 million deaths annually, which is a 1.7-5.2% of deaths worldwide.
Objective

Recently it has been demonstrated that like proteins, DNA is susceptible to nonenzymatic attack by sugar affecting the structure, stability and conformation of DNA molecule. Although the nonenzymatic glycation of biomolecules occurs naturally, in some metabolic disorders such as diabetes, the formation and accumulation of AGEs occurs faster. This rapid formation and accumulation of AGEs is caused not only by D-glucose itself, but also by certain glucose-derived dicarbonyl intermediates such as methylglyoxal (MG), which is a potent precursor of AGEs. The highly reactive electrophile MG, a break down product of carbohydrate is a major environmental mutagen, an off-shoot product of glycolysis which is generated from deprotonation of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. It is also produced from lipid peroxidation and acetone metabolism. Additional endogenous sources include catabolism of threonine and oxidative breakdown of DNA and RNA. MG mutagenicity has been reported in *Escherichia Coli*, wherein it causes mutations at G:C base pairs, as well as in *Saccharomyces cerevisiae*. It has been suggested that MG reacts with free amino groups of proteins and DNA under physiological conditions and forms advanced glycation end products resulting in the generation of hydroxyl (OH) and superoxide (O$_2^-$) ions which play an important role in the pathophysiology of ageing and diabetic complications. Methylglyoxal reacts with 2'-deoxyguanosine via the classic amadori pathway and did not react appreciably with 2'-deoxyadenosine, 2'-deoxythymidine and 2'-deoxycytidine. However, reaction of double stranded DNA or guanine/ 2'-deoxyguanosine with MG *in-vitro* produces primarily N$^\alpha$-carboxyethyl-2'-deoxyguanosine (CEDG), as a major adduct. This adduct (CEDG) might be used as a useful biomarker for monitoring oxoaldehyde-induced stress in response to enhance glycolytic flux or environmental exposure to MG.

In the present study commercially available human placental DNA has been modified by MG and lysine in the presence and absence of Cu$^{2+}$. The modifications on DNA were studied by ultraviolet, fluorescence and circular dichroism spectroscopy, thermal denaturation studies, nuclease-S1 digestibility, HPLC, LC-MS and ESI-MS. Comet assay was performed to detect alkali labile sites, single and double stranded DNA breaks on human lymphocytes exposed to MG alone, lysine alone and in combination of MG + lysine and MG + lysine + Cu$^{2+}$. The hydroxyl
(OH) and superoxide (O$_2^-$) radicals generated in MG + lysine + Cu$^{2+}$ system have been also quantitated. Polyclonal antibodies against native and MG-Lys-Cu$^{2+}$ modified human placental DNA have been generated in experimental animals. The specificity of induced antibodies has been evaluated by competition ELISA and gel retardation assay. These antibodies have been used as an immunochemical probe to detect MG-Lys-Cu$^{2+}$ induced damage in the DNA, isolated from diabetic patients. In order to assess the possible role of MG-Lys-Cu$^{2+}$ modified DNA in the etiology of type I diabetes mellitus, sera from diabetes patients have been assessed for their binding to native and MG-Lys-Cu$^{2+}$ modified human placental DNA. Furthermore, binding of serum antibodies from type II diabetes patients with native and MG-Lys-Cu$^{2+}$ modified human placental DNA has been also evaluated.