METHYLGLYOXAL, DIABETES MELLITUS AND DIABETIC COMPLICATIONS

David L. Vander Jagt*

Department of Biochemistry and Molecular Biology
University of New Mexico School of Medicine
Albuquerque, New Mexico, USA

CONTENTS

* Author for correspondence:
David L. Vander Jagt
Department of Biochemistry and Molecular Biology
University of New Mexico School of Medicine
Albuquerque, New Mexico 87131, USA
dlvanderjagt@salud.unm.edu

SUMMARY

A large literature has developed around methylglyoxal (MG) concerning its role in diabetes mellitus (DM) and in the development of diabetic complications. This is related to the observation that levels of reactive aldehydes, especially 2-oxoaldehydes such as MG, are elevated in DM. There are numerous metabolic origins of MG that are accentuated in DM. MG has effects on insulin secretion from pancreatic beta-cells and is a major precursor of advanced glycation endproducts (AGE). Consequently, MG has a role in primary DM as well in the etiology of long-term complications. There is an extensive literature concerning the enzymes involved in the metabolism of MG, especially the glyoxalase system and aldose reductase. In addition, there is a rapidly developing literature on the direct and indirect effects of MG on signaling pathways that impact DM. This review attempts to integrate this DM-associated literature related to MG.

KEY WORDS

methylglyoxal, glyoxalase, aldose reductase, diabetes mellitus, diabetic complications, advanced glycation endproducts (AGE), oxidative stress, signaling

1. INTRODUCTION

The glyoxalase system and the ability of this enzyme system to metabolize methylglyoxal (MG) have been known for almost a century. Impetus to evaluate glyoxalase and MG in more detail was provided by the suggestion of Albert Szent-Györgyi in the 1960s that MG and glyoxalase are antagonistic growth regulators, which he referred to as the retine (MG)/promine (glyoxalase) theory, in which MG, or a related compound or derivative, is a growth-retarding signal, and glyoxalase is a growth-promoting signal /1-5/. Consequently, introduction of MG and glyoxalase into the cancer biology literature was a logical result of this proposal. Not surprising, development of glyoxalase inhibitors as potential anti-cancer drugs quickly followed and continues to this day /6-8/.

A large literature developed around MG, which is a deceptively simple small molecule. This literature ranges beyond cancer biology to

include, among other areas, a large literature on the role of MG in diabetes mellitus (DM) and especially in the development of diabetic complications. This diabetes-associated literature includes studies of: 1) the effects of MG on insulin secretion from pancreatic beta-cells; 2) the role of MG in the formation of advanced glycation endproducts (AGE); 3) the synthesis of MG by multiple pathways; 4) the catabolism of MG by multiple pathways; 5) the role of MG and MG-derived AGE on cellular oxidative stress; 6) direct and indirect effects of MG on signaling pathways; and 7) the molecular genetics of MG-metabolizing systems. This review attempts to integrate this diabetes-associated literature related to MG, with emphasis on metabolic pathways for formation and elimination of MG, on the enzymes involved, and on more recent studies of the effects of MG and MG-AGE on signaling pathways (see /9-21/ for related reviews).

II. REACTIVE ALDEHYDES THAT ARE ELEVATED IN DIABETES

Numerous carbonyl compounds, especially reactive aldehydes, have been suggested or demonstrated to be elevated in DM (Fig. 1). These include 2-oxoaldehydes, such as glyoxal, MG and 3-deoxyglucosone (3DG), as well as α/β-unsaturated aldehydes such as acrolein and 4-hydroxynonenal (4HNE) /22-24/. Acrolein, malondialdehyde and 4HNE are products of lipid oxidation and are associated with oxidative stress in DM /25-28/. 3DG is a decomposition product of glycated proteins, by way of the Amadori intermediate /29,30/. Glyoxal is a product both of lipid oxidation and of decomposition of glycated proteins as well as oxidation of glucose /31-33/. Glucosone can be derived from glucose under oxidative conditions /33/. Xylosone and deoxyxylosone, which are less prominent than glucosone and 3DG, may be derived from degradation of ascorbic or dehydroascorbic acid or from degradation of Amadori products from glycation of proteins with ascorbic or dehydroascorbic acid /34,35/. Formaldehye is formed from methylamine in a reaction catalyzed by semicarbazide-sensitive amine oxidase (SSAO), an ecto-enzyme that is widely distributed on the surface of endothelial cells and other cells and is highly expressed in DM /36,37/.

Fig. 1: **Diabetes-associated aldehydes and ketones.** Numerous reactive aldehydes and ketones, especially 2-oxoaldehydes and α/β-unsaturated aldehydes, are elevated in diabetes mellitus.

III. SOURCES OF METHYLGLYOXAL ACCENTUATED IN DIABETES

A special case for the importance of MG in the development of diabetic complications compared to the importance of other endogenous aldehydes can be made by considering the multiple sources of MG (Fig. 2). MG has numerous metabolic origins. It is produced non-enzymatically from triose phosphates /38/ and as a byproduct of the triose phosphate isomerase reaction /39/. MG, similar to 3DG and glyoxal, is also produced during decomposition of glycated proteins. MG is also formed by oxidative degradation of carbohydrates and lipids /40-43/ and is formed metabolically from ketone bodies, including oxidation of acetone /44/ catalyzed by liver cytochrome P4502E1 /45/ and oxidation of acetoacetate in neutrophils catalyzed by myeloperoxidase /46/. Catabolism of threonine normally produces glycine and acetylCoA; however, in a low CoA state, such as would exist in diabetic ketoacidosis where much of the CoA is in the form of acetylCoA, threonine is catabolized to aminoacetone /47/. Aminoacetone is the preferred substrate of SSAO which converts aminoacetone into MG /48/. It is noteworthy that all of these sources of MG would be expected to be accentuated in DM. The increased levels of MG in DM /49/ and the identification of a number of AGE that are derived from MG /41-43/ make a strong case for the importance of this reactive 2-oxoaldehyde in the development of diabetic complications. Recent studies suggest that concentrations of MG may be much higher than previously thought, most of it likely existing as reversible carbinolamine adducts of proteins /50/.

The importance of acetol as a precursor of MG is also indicated in Figure 2. Acetol is one of the reduction products of MG, catalyzed by aldo-keto reductases (discussed below). However, acetol can disproportionate into MG and propanediol in the presence of copper ions /51/ and is therefore a potential source of MG. Since acetol is an intermediate product in the P450-catalyzed oxidation of acetone to MG, the conversion of acetol to MG can be enzymatic (via P450) or non-enzymatic (via copper ions). Acetol levels are sometimes elevated in patients with DM, especially during times of ketoacidosis /44/. In addition, dysregulation of metal ion homeostasis, including copper, is associated with DM /52/. Moreover, MG can modify the copper transport protein ceruloplasmin with resulting release of copper ions, thereby increasing the potential for formation of MG from acetol /53/.

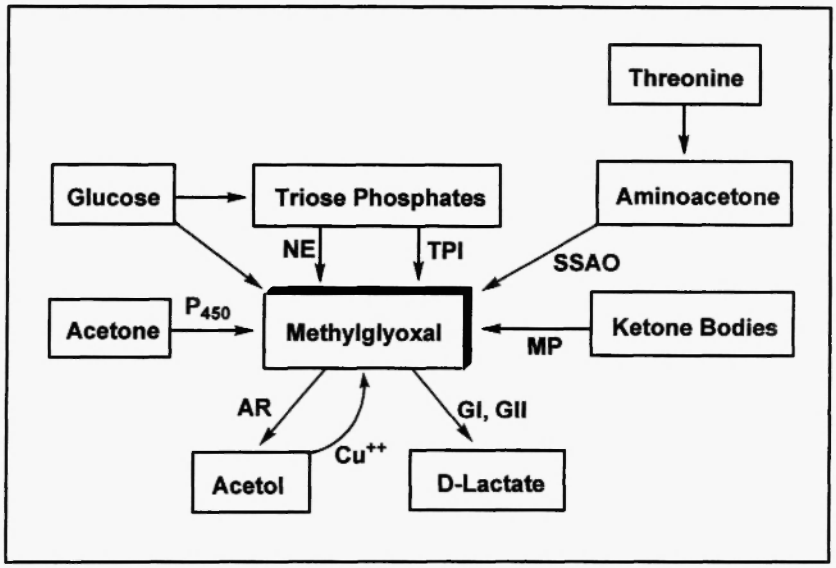

Fig. 2: **Multiple biosynthetic origins of methylglyoxal.** Methylglyoxal, unlike most of the other aldehydes and ketones elevated in diabetes mellitus, can be produced from many sources. NE = non-enzymatic; TPI = triose phosphate isomerase; SSAO = semicarbazide-sensitive amine oxidase; AR = aldose reductase; GI, GII = glyoxalase I and II; MP = myeloperoxidase; P450 = cytochrome P4502E1.

IV. METHYLGLYOXAL AND INSULIN

MG can influence beta-cell function as well as insulin signaling. Exposure of beta-cells to oxidative stressors including MG results in decreased insulin secretion in response to glucose /54/. MG is also toxic to beta-cells and can induce swelling and apoptosis, depending upon conditions /55,56/. Chemical modification of the beta-chain of insulin by MG alters the properties of insulin; the MG-insulin adduct decreases insulin-mediated glucose uptake in adipocytes and muscle cells, and the adduct is unable to provide autocrine control of insulin secretion from beta-cells. In addition, clearance of MG-modified insulin by the liver is impaired /57/. MG also impairs signaling pathways that involve insulin. Short exposure of muscle cells to MG initially increases then decreases insulin-stimulated phosphorylation of

protein kinase B and extracellular-regulated kinase 1/2 (ERK1/2) without affecting phosphorylation of the insulin receptor /58/. This signaling impairment is independent of generation of reactive oxygen species (ROS).

V. METHYLGLYOXAL AND ADVANCED GLYCATION ENDPRODUCTS

A number of AGE produced by modification of proteins by glucose have been identified from either *in vitro* or *in vivo* studies. These are thought to contribute to the matrix protein cross-linking that is associated with the development of long-term diabetic complications. Although it was initially assumed that AGE are formed primarily from glycation of proteins by glucose, it is now clear that AGE can be formed from a variety of compounds besides glucose, including fructose, trioses, ribose, and ascorbate, and even from lipoxidation pathways /29-36,41-43,59-70/. In addition, reactive 2-oxoaldehydes appear to be key intermediates in the formation of many of the AGE identified thus far (Fig. 3). The development of potential therapeutic agents that degrade AGE points to the importance of AGE in the etiology of diabetic complications /71-74/. The known AGE are primarily the products of reactions involving the 2-oxoaldehydes glyoxal, MG and 3DG. The importance of MG in formation of AGE deserves special attention: 1) antibodies against MG-derived AGE cross-react with AGE produced by modification of proteins with glucose, fructose, ribose, glyceraldehyde, glyoxal, ascorbate and ascorbate oxidation products, suggesting that MG may be a common intermediate in AGE formation from a wide variety of glycating agents /43/; 2) MG-derived AGE and glyoxal-derived AGE as well as 3DG-derived AGE are elevated in DM /43,65/; 3) AGE derived from MG and other 2-oxoaldehydes catalyze the production of free radicals /75/; 4) overexpression of glyoxalase I, which acts to increase the detoxification of MG, prevents the accumulation of intracellular AGE in response to high glucose, consistent with the idea that MG-AGE are especially prominent /76/. These observations support the suggestion that 2-oxoaldehydes, especially MG, play an essential role in the chemistry of AGE production.

Fig. 3: **Structures of advanced glycation endproducts (AGE).** Some of the known AGE include crosslinking and non-crosslinking modifications of proteins; the bottom four AGE are derived from methylglyoxal.

VI. ENZYMOLOGY OF METHYLGLYOXAL METABOLISM

In view of the accumulating evidence in support of the roles of 2-oxoaldehydes in the reactions that produce AGE, the question of the metabolic pathways for formation of and for protection against 2-oxoaldehydes, especially MG, is important. One could argue that prevention of the formation of MG or promotion of its detoxification might provide a means to limit the development of long-term diabetic complications by limiting AGE production. There are multiple catabolic pathways for detoxification and elimination of MG (Fig. 4). Removal of MG catalyzed by the glyoxalase system is redox-neutral, since it involves an intramolecular redox reaction to convert MG into D-lactate. Conversion of MG into acetol by NADPH, catalyzed by aldose reductase, represents reductive metabolism of MG. Conversion of MG into pyruvate by NAD or NADP, catalyzed by betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase, respectively, represents oxidative metabolism of MG.

2-Oxoaldehyde dehydrogenase (2-ODH) has received little attention. This dehydrogenase catalyzes the oxidation of MG to pyruvate

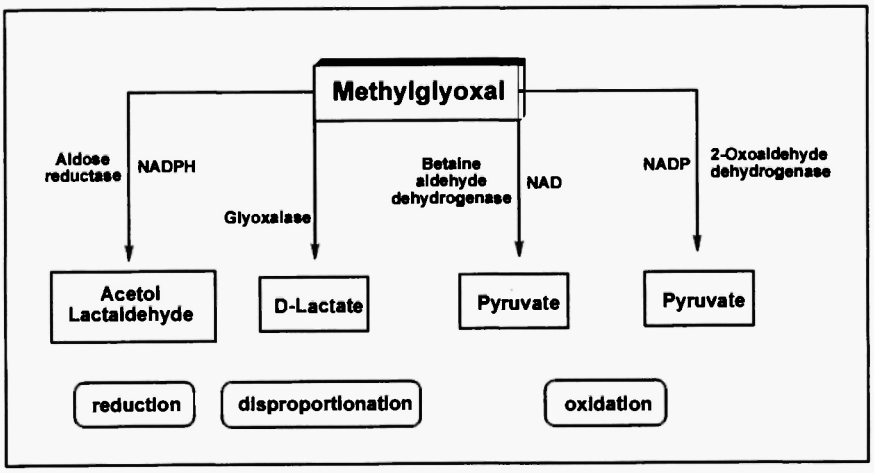

Fig. 4: Detoxification pathways for methylglyoxal. Methylglyoxal can be eliminated through reductive and oxidative as well as disproportionation reactions.

/77/ and 3DG to 3-deoxygluconic acid /78,79/ and therefore is of interest with respect to reactive aldehydes that are precursors to AGE. 2-ODH, which is specific for 2-oxoaldehydes, may represent an important liver detoxification enzyme for protection against these aldehydes, especially MG. 2-ODH is unusual in its requirement for a vicinal amino-alcohol cofactor /80-83/ although, in more recent studies, activation was observed with vicinal amino-thiols as well as with glycine /84/. Nevertheless, the best activators appear to be vicinal amino-alcohols that contain a primary alcohol functional group. The physiological cofactor is unknown. There is some evidence for the existence of multiple forms of 2-ODH /85,86/.

Among the 17 or more functional human aldehyde dehydrogenase genes /87/, at least three human aldehyde dehydrogenases (ALDH 1 ,2 and 3) exhibit broad specificity and carry out the detoxification of numerous aldehydes. ALDH bind unhydrated aldehydes /88/. Most aldehydes are only partially hydrated at physiological pH. 2-Oxo-aldehydes, such as MG, however, are essentially 100% hydrated, which may explain why 2-oxoaldehydes are poor substrates of aldehyde dehydrogenases. However, MG is a fairly good substrate for betaine aldehyde dehydrogenase (ALDH9, E3), although considerably poorer than betaine aldehyde /89/.

The glyoxalase system, consisting of glyoxalase I and II, has been studied extensively and is reviewed elsewhere in this issue /90,91/. The glyoxalase system is an unusual detoxification system. MG first reacts non-enzymatically with glutathione to form a hemi-thioacetal that is the actual substrate for glyoxalase I. Thus, the concentration of glutathione is important in determining the efficiency of the glyoxalase system. Because MG is very reactive, there is little that is free; most MG is distributed between the hydrated form and the hemi-thioacetal of glutathione. At 3 mM concentrations of glutathione, the hydrated form and the hemi-thioacetal are present in a 1:1 ratio (Fig. 5). As the concentration of glutathione falls, less of the hemi-thioacetal is present and the detoxification ability of the glyoxalase system falls rapidly. This means that MG will accumulate whenever there is oxidative stress that impacts the level of glutathione /51/.

Aldose reductase (aldo-keto reductase, AKR1B1; AR2) is part of the polyol pathway, which has been implicated in the development of some diabetic complications, especially cataract formation. The pathway involves two reactions: 1) the NADPH reduction of glucose to the

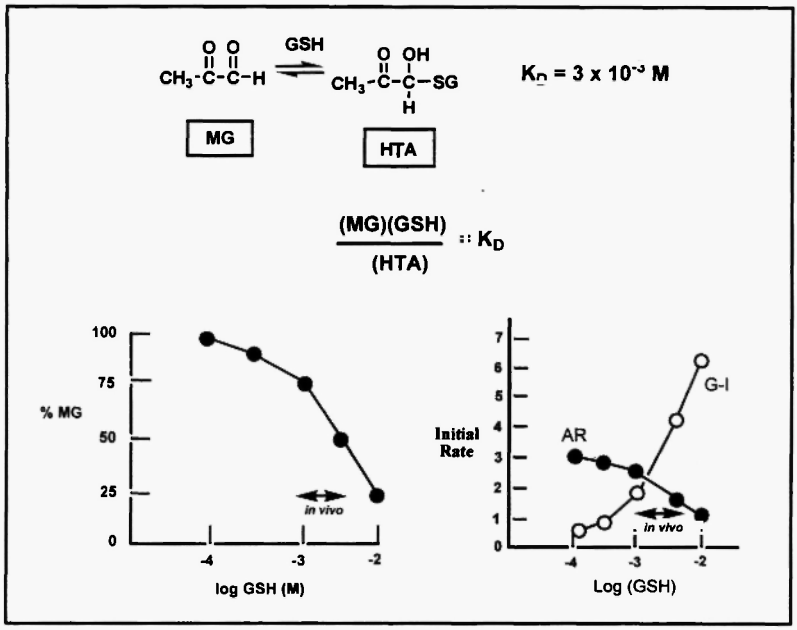

Fig. 5: Disposal of methylglyoxal (MG) through the glyoxalase and aldose reductase pathways. The rates of detoxification of methylglyoxal through the glyoxalase (G-I) pathway and aldose reductase (AR) pathway depend upon the concentration of glutathione (GSH). At 3 mM GSH, methylglyoxal, which exists almost entirely hydrated and likely is present at micromolar concentration, is equally distributed between MG and the hemithioacetal of MG and GSH (HTA). As GSH concentrations decrease, more of the methylglyoxal is available as a substrate for AR. The plot of initial rates through the two pathways (right) assumes GI/AR ratio of ten.

polyol sorbitol, catalyzed by aldose reductase; and 2) the NAD oxidation of sorbitol to fructose, catalyzed by sorbitol dehydrogenase. However, MG is 10^4 times better than glucose as a substrate for aldose reductase /92/. Likewise, all of the aldehydes that are elevated in DM are much better substrates of aldose reductase than glucose /20,21,51, 92-97/, consistent with the idea that the main function of aldose reductase is detoxification of aldehydes. A problem in DM may be the over-production of aldehydes.

In liver, where glutathione levels are highest and where there is little aldose reductase, the glyoxalase system is likely the major catabolic system for detoxification of MG, perhaps in cooperation with 2-ODH. In tissues with significant levels of aldose reductase, which includes those tissues that are associated with the development of diabetic complications, aldose reductasse may assist glyoxalase with the disposal of MG (Fig. 5). The aldose reductase-catalyzed reduction of MG is NADPH-dependent. However, the product distribution is glutathione-dependent (Fig. 6). The non-enzymatic pre-equilibrium reaction between MG and glutathione to form the hemi-thioacetal in equilibrium with MG (free and hydrated) provides two substrates for aldose reductase. Unhydrated MG is converted into acetol through reduction of the aldehyde functional group; the hemi-thioacetol is converted into lactaldehyde through reduction of the ketone functional group, followed by dissociation of the resulting hemi-thioacetal. Both lactaldehyde and acetol can be reduced further to propanediol, catalyzed by aldose reductase /51,92/. Lactaldehyde is a good substrate of aldose reductase. Thus, MG can be efficiently converted first to lactaldehyde and then to propanediol through two aldose reductase-catalyzed reactions. Reduction of MG to acetol, which would become a more important pathway at low glutathione concentrations, produces a much poorer substrate for the second reduction /92/. As mentioned above, acetol has been reported to accumulate to near millimolar levels in some patients with DM /44/. Acetol, however, may not be innocuous. Acetol produces insulin resistance in experimental animals /98/. Whether this involves conversion of acetol back into MG is not known. This is a distinct possibility because acetol can readily undergo disproportionation in the presence of copper to regenerate MG. The reduction of MG to acetol may be an undesirable reaction, and acetol may represent a cryptic form of MG that can be converted back into MG under appropriate conditions, such as in DM.

Aldose reductase is readily oxidized to isoforms with markedly different kinetic properties, including insensitivity to some of the standard aldose reductase inhibitors /92,94/. Given the role of oxidative stress in diabetic complications, the general failure of aldose reductase inhibitors in clinical studies may reflect the presence of oxidized aldose reductase with reduced affinity for these inhibitors. It has been suggested that the potential benefits of aldose reductase

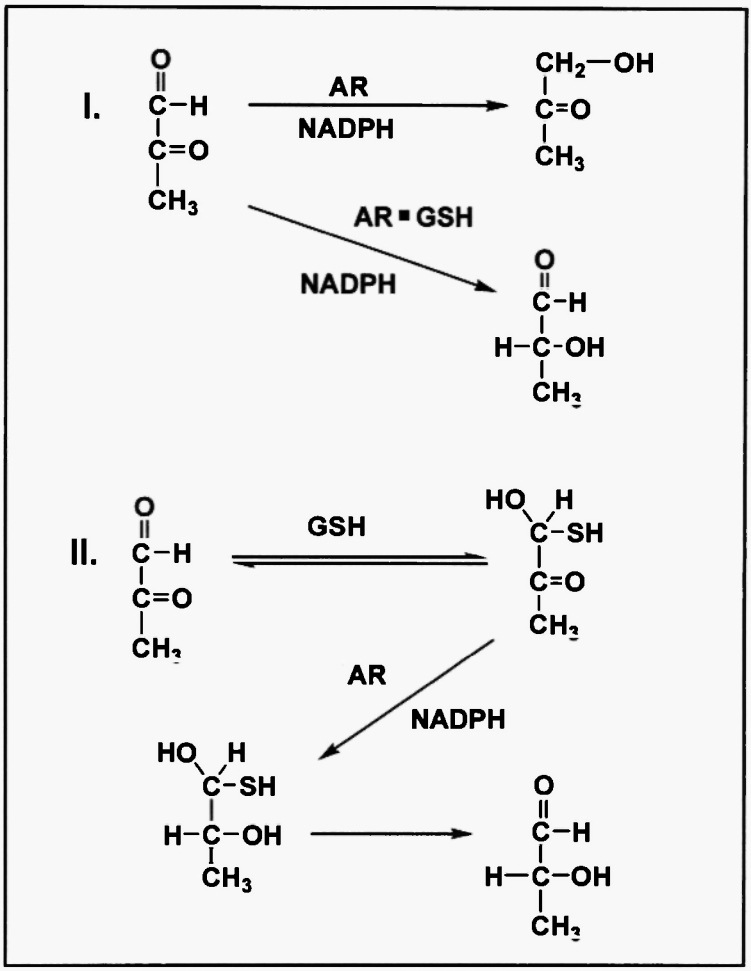

Fig. 6: **Aldose reductase-catalyzed reduction of methylglyoxal.** The reductive metabolism of methylglyoxal (MG), catalyzed by aldose reductase (AR), is more complicated than suggested in Figure 5. The main product from AR-catalyzed reduction of MG by NADPH is acetol. In the presence of glutathione (GSH), lactaldehyde also forms (top). The explanation for this distribution of products, which is dependent on the concentration of GSH, is that AR can also catalyze the reduction of the hemithioacetal (bottom).

inhibitors may be due to protection of NADPH from oxidation by endogenous aldehydes, catalyzed by aldose reductase /92/.

VII. ALDEHYDE (OXIDATIVE) STRESS AND DIABETIC COMPLICATIONS

There are multiple pathways by which MG and other reactive carbonyl compounds, especially aldehydes which are elevated in DM, can contribute to oxidative stress. The chemistry of reactive carbonyl compounds does not need to be redox chemistry /12,16/. Flow of glucose through the polyol pathway may produce an increased NADH/NAD ratio, similar to hypoxia, and therefore termed pseudohypoxia /99/. This results in increased free radical formation and would represent an oxidative stress. In general, DM and diabetic complications are associated with oxidative stress or with aldehyde (or more generally carbonyl) stress /16,92,100-102/.

7a. ROS production

There are a number of ways by which MG can contribute to elevated levels of ROS. Glycation of proteins leading to formation of various AGE or direct modification of proteins by MG to form MG-AGE produce reactive centers that can catalyze production of radicals including superoxide /75,103/. This includes modification by MG of mitochondrial proteins in complex III, resulting in damaged mitochondria that exhibit enhanced formation of superoxide /104/. Some of the production of ROS from MG-AGE may involve copper /105/ or may involve iron-mediated damage to mitochondria as a consequence of oxidative conversion of aminoacetone into MG /106/.

Engagement of RAGE, the receptor for AGE, triggers activation of NADPH oxidase with production of ROS /13,15,107/. NADPH oxidase is a family of oxidases that include five members of the NOX family and two members of the DUOX family. The prototype of the NOX family is NOX2 which is also known as phagocyte NADPH oxidase and is associated with superoxide production by granulocytes and macrophages as part of their anti-microbial activity. However, NOX2 is widely distributed among cells and is found not only as plasma membrane complexes but also intracellularly. NOX2 is regulated by a number of signaling pathways including NFκB (see

below). NOX4 is induced by ischemia and by pro-inflammatory cytokines. A peculiarity of NOX4 is that it can produce both H_2O_2 and superoxide. The two DUOX are novel NOX isoforms that also contain a peroxidase domain /108/.

7b. Impaired detoxification enzymes

Oxidative stress in DM may be elevated as a consequence of reduced ability to detoxify ROS. Glutathione reductase is inactivated by MG and other reactive aldehydes, especially 4HNE /109/. Cu,Zn-superoxide dismutase is inactivated by MG, with release of copper /110/. MG as well as 3DG inactivate glutathione peroxidase /111,112/. In addition, as mentioned above, MG modifies ceruloplasmin with liberation of copper /53/. All of these MG-dependent processes would be expected to contribute to the development of oxidative stress. This may be especially important in beta-cells where detoxification capacity is low /113/.

7c. NADPH and glutathione depletion

Aldose reductase is expressed in a number of tissues including those that are associated with diabetic complications. All of the aldehydes that are elevated in DM, including glucose and MG, are substrates of aldose reductase, as is acetol. Therefore, one source of oxidative stress may be the consumption of NADPH through the aldose reductase-catalyzed reduction of these endogenous aldehydes /20,21,51,92-97/. Consumption of NADPH would also contribute to oxidative stress through loss of ability to maintain glutathione in the reduced form, since glutathione reductase is NADPH-dependent. In addition, as mentioned above, glutathione reductase is inactivated by these endogenous aldehydes including MG. There are a number of reports of depressed levels of glutathione in patients with DM /114-116/. A summary of some of the oxidative stress-producing pathways that directly or indirectly involve MG and other endogenous aldehydes that are elevated in DM is shown in Figure 7.

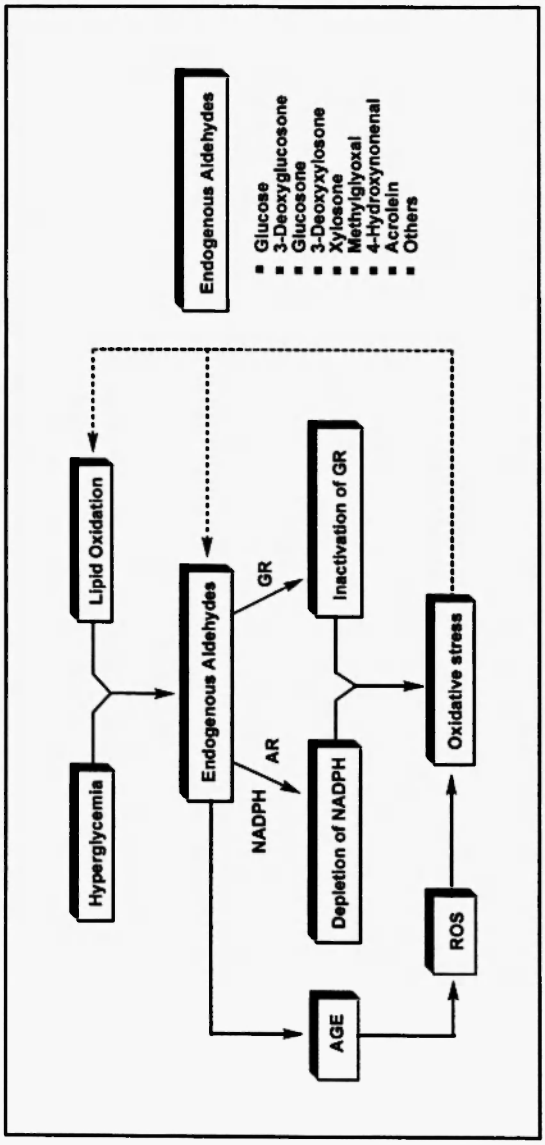

Fig. 7: **Aldehyde (oxidative) stress model of diabetic complications.** The elevation of reactive aldehydes in diabetes mellitus may produce oxidative stress through consumption of NADPH through the aldose reductase (AR) pathway; through inactivation of anti-oxidant enzymes such as glutathione reductase (GR); and through advanced glycation endproducts (AGE)-catalyzed formation of reactive oxygen species (ROS).

VIII. METHYLGLYOXAL AND SIGNALING PATHWAYS

In recent years, there have been numerous studies of the direct or indirect effects of MG on signaling pathways, many of which may pertain to the development of diabetic complications. The physiological significance of these numerous cellular studies is not clear, due to the high concentrations of MG that are often used in order to measure an acute response. This leaves open the question whether these studies are applicable to understanding the etiology of long-term diabetic complications. In cases where the effects are observed rapidly in response to exposure to MG, it is likely that the effect is directly attributable to MG. In cases where the effects of exposure to MG are observed after a significant period, the effects may be indirect, either though initial formation of MG-AGE with subsequent production of ROS or through MG-dependent development of oxidative stress that does not involve MG-AGE. This could include altered redox potential through consumption of NADPH through the aldose reductase-catalyzed reduction of MG or glutathione consumption through the glyoxalase I-catalyzed disproportionation of MG. Given this complexity, the effects of MG on signaling pathways are often difficult to interpret mechanistically.

Some of the effects of MG on signaling pathways impact the metabolism of MG. For example, exposure of smooth muscle cells to MG resulted in a dose-dependent and time-dependent upregulation of aldose reductase at both the mRNA level and the protein level /117/. This effect was blocked by inhibition of p38, suggesting that this signaling is through the MAP kinase pathway. Since ROS production was also increased, the signaling may be through MG-induced oxidative stress. Thus, even when the effects of MG on a signaling pathway are rapid and likely do not involve initial formation of MG-AGE, the interpretation of the effects can be complicated.

When human embryonic kidney cells and mouse fibroblasts were exposed to MG, they became insensitive to the mitogenic effect of insulin-like growth factor-I through activation of the MAP kinase ERK, which was shown to be mediated by upstream activation of MEKK1 /118/. In other studies, MG downregulated Raf-1 protein levels through an ubiquitin-dependent mechanism /119/. Since both Raf-1 and MEKK1 can activate ERK, it is clear that the effects of MG on ERK activation can be opposite, depending on cell type and condi-

tions. Another example of the complexity of MG-induced activation of MAP kinases was the demonstration of distinct pathways in endothelial cells by which MG activated ERK1/2 compared to p38 and JNK. Activation of ERK1/2 was protein tyrosine kinase (PTK)-dependent; by contrast, activation of p38 and JNK was PTK-independent but redox-dependent /120/. In mesenchymal cells, where PDGF is a mitogenic factor, MG treatment resulted in inhibition of the intrinsic tyrosine kinase activity of PDGFR-β, which inhibited subsequent activation of ERK. In this study, MG-AGE were detected on PDGFR-β /121/.

There are numerous examples where treatment of cells with MG generated MG-AGE and oxidative stress, often by way of NADPH oxidase, and led to perturbed cell signaling. MG-modified albumin triggered release of TNFα from macrophages, which may contribute to the chronic inflammatory state associated with both type 1 and type 2 DM. In this study, treatment with MG-modified albumin led to the activation of p38, ERK and NFκB /122/. ROS production also increased. TNFα secretion was suppressed with an inhibitor of NFκB. TNFα is one of the pro-inflammatory genes regulated by NFκB. Moreover, NFκB is a redox-sensitive transcription factor. Therefore, one can speculate that the treatment with MG-modified albumin produced ROS, perhaps through RAGE and NADPH oxidase, and the upregulation of TNFα resulted from ROS-dependent activation of NFκB. ROS derived from MG or MG-AGE were observed in studies of the role of ASK1 in MG-induced apoptosis of Jurkat cells /123/, induction of expression of heparin-binding epidermal growth factor-like growth factor in smooth muscle cells /124/, expression of Mac-1 on neutrophils, leading to platelet-neutrophil aggregation /125/, JNK activation and apoptosis of osteoblasts /126/, genotoxicity of MG-AGE to colon, liver and kidney cells /127/, NFκB activation in smooth muscle cells /128/, NFκB activation and apoptosis in retinal pericytes /129/ and NFκB activation and ICAM-1 expression in vascular smooth muscle cells /130/. Thus the direct or indirect effects of MG on signaling generally involve ROS as intermediates.

Vascular endothelial growth factor (VEGF) is important in physiological and pathological angiogenesis and vascular permeability. Extensive studies link VEGF, diabetic retinopathy and oxidative stress /131,132/. Endothelial cells exposed to MG increased the expression of VEGF /133/. IV administration of AGE to rats increased retinal

vascular permeability through upregulation of VEGF and down-regulation of pigment epithelium-derived factor (PEDF), which is a potent inhibitor of angiogenesis. This involved signaling initiated by AGE that resulted in ras and NFκB activation leading to upregulation of VEGF. PEDF normally works to inhibit this pathway by suppression of NADPH-mediated ROS generation /134/. Related studies demonstrated that exposure of retinal endothelial cells to AGE increased ROS production and VEGF expression, and that the signaling pathway involved PKC-dependent activation of NADPH oxidase /135,136/. In view of the importance of MG as an intermediate in the formation of AGE, these studies provide a likely link between MG and VEGF even when MG was not used directly in the studies.

In type 2 DM, beta-cell dysfunction was linked to ROS formation from AGE-catalyzed reactions /137/. This oxidative stress in beta-cells, and other cells, triggers endoplasmic reticulum (ER) stress that can lead to a number of ER stress responses. ER stress can activate the MAP kinase JNK, one consequence of which is decreased activity of transcription factor PDX-1 which regulates transcription of the insulin gene /138-140/. MG also can activate JNK /120/. The ER can detect a variety of stresses. In retinal pigment epithelial cells, ER stress, triggered by oxidative stress /141/, reductive stress /142/, or unfolded protein stress /143/, in each case resulted in upregulation of expression of VEGF by a pathway that involved activation of transcription factor ATF4.

Potential new roles for MG-AGE, ROS and glyoxalase I are suggested by recent studies of TNF-induced cell death /144/. Increased levels of mitochondria-derived ROS in response to exposure of fibrosarcoma cells to TNF are part of a ROS-dependent cell death pathway. Delineation of the events along this pathway suggested that MG levels increased, along with formation of specific MG-AGE. In addition, there was TNF-induced phosphorylation of glyoxalase I that required PKA. The generality of this pathway for TNF-dependent signaling and its significance in the pro-inflammatory roles that TNF has in numerous disease processes remain to be explored.

New roles for aldose reductase are suggested by recent studies of glucose-induced growth of smooth muscle cells which requires TNFα as an autocrine signal in an NFκB-dependent pathway. Interestingly,

aldose reductase appears to be an essential part of this signaling pathway. It is unclear whether MG or MG-AGE play a role /145/.

IX. MOLECULAR GENETICS OF GLYOXALASE I AND ALDOSE REDUCTASE IN DIABETES

There has been extensive interest in the molecular genetics of both glyoxalase I and aldose reductase in DM. Glyoxalase I and aldose reductase are significantly elevated in mononuclear cells from patients with type 1 DM with complications /146/, consistent with other reports of elevated glyoxalase I and aldose reductase in erythrocytes from symptomatic patients with DM /49,147/. Glyoxalase I is a two allele gene that produces dimers with interchangeable subunits, resulting in the phenotype GLO 1, GLO 1-2, GLO 2-2. This polymorphism has been studied for many years /148/ in many populations, mainly because the *GLO* gene is located on chromosome 6 in the HLA region and has therefore been a useful marker in genetic studies /149/. Glyoxalase I exhibits a single nucleotide polymorphism (A or C) at position 332 of the cDNA, resulting in alanine (C332 allele) or glutamic acid (A332 allele) at protein residue 111 /150,151/. A number of investigators have compared *GLO* genotypes or phenotypes in diabetic populations compared to control populations, but with conflicting results /152-155/.

Aldose reductase has two polymorphic sites in its promoter. There is an $(A\text{-}C)_n$ dinucleotide repeat 2.1 kb upstream that is polymorphic /156,157/. A number of studies, but not all studies, suggest a correlation between the Z-2 allele and the presence of complications /156-160/. This includes studies in which the expression level of aldose reductase was shown to be elevated /159/. There is also a C-106T polymorphism that is associated with diabetic complications, but with conflicting reports as to which allele correlates with increased susceptibility /161-165/. The presence of the Z-2/C-106 combination had the highest transcriptional activity in reporter gene studies /166/. In one study of a highly endogamous Native American population in which both glyoxalase I and aldose reductase genotypes were evaluated, there was an association between type 2 DM and the GLO A332 allele, and between type 2 diabetic nephropathy and the Z-2 allele /155,159/.

X. CONCLUSIONS

In view of much of the recent interest in MG, MG-AGE, ROS, glyoxalase I and aldose reductase in signaling pathways that may be relevant to diabetes mellitus and its complications, there seems to be little slow down in interest in MG. It is therefore appropriate, based upon these recent studies, to restate that MG is a deceptively simple small molecule and that much remains to be learned almost 50 years after Szent-Györgyi initiated his studies of MG and glyoxalase.

REFERENCES

1. Dakin HD, Dudley HW. An enzyme concerned with the formation of hydroxy acids from ketonic aldehydes. J Biol Chem 1913; 14: 155-157.
2. Neuberg C. The destruction of lactic aldehyde and methylglyoxal by animal organs. Biochem Z 1913; 49: 502-506.
3. Szent-Györgyi A. Cell-division and cancer. Science 1965; 149: 34-37.
4. Szent-Györgyi A, Együd LG, McLaughlin JA. Keto-aldehydes and cell division. Science 1967; 155: 539-541.
5. Együd LG, McLaughlin JA, Szent-Györgyi A. Ketone aldehydes in tissues. Proc Natl Acad Sci USA 1967; 57: 1422-1425.
6. Vince R, Wadd WB. Glyoxalase inhibitors as potential anticancer agents. Biochem Biophy Res Comm 1969; 35: 593-598.
7. Creighton DJ, Zheng ZB, Holewinski R, Hamilton DS, Eiseman JL. Glyoxalase I inhibitors in cancer chemotherapy. Biochem Soc Trans 2003; 31: 1378-1382.
8. More SS, Vince R. Design, synthesis, and binding studies of bidentate Zn-chelating peptidic inhibitors of glyoxalase-I. Bioorg Med Chem Lett 2007; 17: 3793-3797.
9. Kalapos MP. On the promine/retine theory of cell division: now and then. Biochim Biophys Acta 1999; 1426: 1-16.
10. Thornalley PJ. Advances in glyoxalase research. Glyoxalase expression in malignancy, anti-proliferative effects of methylglyoxal, glyoxalase I inhibitor diesters and *S*-D-lactoylglutathione, and methylglyoxal-modified protein binding and endocytosis by the advanced glycation endproduct receptor. Crit Rev Oncol Hematol 1995; 20: 99-128.
11. Vander Jagt DL. The glyoxalase system. In: Dolphin D, et al., eds. Glutathione: Chemical, Biochemical, and Medical Aspects-Part A (Coenzymes and Cofactors). New York: J Wiley, 1989; 598-641.
12. Ido Y. Pyridine nucleotide redox abnormalities in diabetes. Antioxid Redox Signal 2007; 9: 931-942.
13. Yan SF, Ramasamy R, Naka Y, Schmidt AM. Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. Circ Res 2003; 93: 1159-1169.

14. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414: 813-820.
15. Tan AL, Forbes JM, Cooper ME. AGE, RAGE, and ROS in diabetic nephropathy. Semin Nephrol 2007; 27: 130-143.
16. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999; 48: 1-19.
17. Chung SS, Chung SK. Aldose reductase in diabetic microvascular complications. Curr Drug Targets 2005; 6: 475-486.
18. Srivastava SK, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. Endocr Rev 2005; 26: 380-392.
19. Uhlmann K, Kovacs P, Boettcher Y, Hammes HP, Paschke R. Genetics of diabetic retinopathy. Exp Clin Endocrinol Diabetes 2006; 114: 275-294.
20. Vander Jagt DL, Hunsaker LA, Deck LM, Chamblee BB, Royer RE. Ketoaldehyde detoxification enzymes and protection against the Maillard reaction. In: Labuza TP, et al., eds. Maillard Reactions in Chemistry (5th International Meeting on the Maillard Reaction). Cambridge: The Royal Society of Chemistry, 1994; 314-318.
21. Vander Jagt DL, Hunsaker LH, Young BS, Brown WM. Aldo-keto reductase-catalyzed detoxication of endogenous aldehydes associated with diabetic complications. American Chemical Society Symposium 2003; Series 865: 23-35.
22. O'Brien PJ, Siraki AG, Shangari N. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. Crit Rev Toxicol 2005; 35: 609-662.
23. Uchida K. Role of reactive aldehydes in cardiovascular disease. Free Radic Biol Med 2000; 28: 1685-1696.
24. Beissenger PJ, Howell SK, Nelson RG, Mauer M, Szwergold BS. Alpha-oxoaldehyde metabolism and diabetic complications. Biochem Soc Trans 2003; 31: 1358-1363.
25. Uchida K, Kanematsu M, Sakai K, et al. Protein-bound acrolien: potential markers for oxidative stress. Proc Natl Acad Sci USA 1998; 95: 4882-4887.
26. Polak M, Zagorski Z. Lipid peroxidation in diabetic retinopathy. Ann Univ Mariae Curie Sklodowska 2004; 59: 434-437.
27. Piconi L, Quagliaro L, Ceriello A. Oxidative stress in diabetes. Clin Chem Lab Med 2003; 41: 1144-1149.
28. VanderJagt DJ, Harrison JM, Ratliff DM, Hunsaker LA, Vander Jagt DL. Oxidative stress indices in IDDM subjects with and without diabetic complications. Clin Biochem 2001; 34: 265-270.
29. Zyzak DV, Richardson KM, Thorpe SR, Baynes JW. Formation of reactive intermediates from Amadori compounds under physiological conditions. Arch Biochem Biophys 1995; 316: 547-554.
30. Niwa T. 3-Deoxyglucosone: metabolism, analysis, biological activity, and clinical implication. J Chromatogr B Biomed Sci Appl 1999; 731: 23-36.
31. Aldini G, Dalle-Donne I, Facino RM, Milzani A, Carini M. Intervention strategies to inhibit protein carbonylation by lipoxidation-derived reactive carbonyls. Med Res Rev 2007; 27: 817-868.

32. Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. N epsilon-(carboxymethyl)lysine is a dominant advanced glycation endproduct (AGE) antigen in tissue proteins. Biochemistry 1995; 34: 10872-10878.
33. Nagai R, Unno Y, Hayashi MC, Masuda S, Hayase F, Kinae N, Horiuchi S. Peroxynitrite induces formation of N(epsilon)-(carboxymethyl)lysine by the cleavage of Amadori product and generation of glucosone and and glyoxal from glucose: novel pathways for protein modification by peroxynitrite. Diabetes 2002; 51: 2833-2839.
34. Shin DB, Feather MS. 3-Deoxy-L-glycero-pentos-2-ulose (3-deoxy-L-xylosone) and L-threo-pentos-2-ulose (L-xylosone) as intermediates in the degradation of L-ascorbic acid. Carbohydr Res 1990; 208: 246-250.
35. Reihl O, Lederer MO, Schwack W. Characterization and detection of lysine-arginine cross-links derived from dehydroascorbic acid. Carbohydr Res 2004; 339: 483-491.
36. Gubisne-Haberle D, Hill W, Kazachkov M, Richardson JS, Yu PH. Protein cross-linkage induced by formaldehyde derived from semicarbazide-sensitive amine oxidase-mediated deamination of methylamine. J Pharmacol Exp Ther 2004; 310: 1125-1132.
37. Obata T. Diabetes and semicarbazide-sensitive amine oxidase (SSAO) activity: a review. Life Sci 2006; 79: 417-422.
38. Ohmori S, Mori M, Shiraha K, Kawase M. Biosynthesis and degradation of methylglyoxal in animals. Prog Clin Biol Res 1989; 290: 397-412.
39. Richard JP. Kinetic parameters for the elimination reaction catalyzed by triosephosphate isomerase and an estimation of the reaction's physiological significance. Biochemistry 1991; 30: 4581-4585.
40. Kalapos MP. Methylglyoxal in living systems: chemistry, biochemistry, toxicology and biological implications. Toxicol Lett 1999; 110: 145-175.
41. Thornalley PJ, Langborg A, Minhas HS. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. Biochem J 1999; 344: 109-116.
42. Degenhardt TP, Thorpe SR, Baynes JW. Chemical modification of proteins by methylglyoxal. Cell Mol Biol (Noisy-le-grand) 1998; 44: 1139-1145.
43. Shamsi FA, Partal A, Sady C, Glomb MA, Nagaraj RH. Immunological evidence for methylglyoxal-derived modifications in vivo. Determination of antigenic epitopes. J Biol Chem 1998; 273: 19338-19345.
44. Reichard GA Jr, Skutches CL, Hoeldtke RD, Owens OE. Acetone metabolism in humans during diabetic ketoacidosis. Diabetes 1986; 35: 668-674.
45. Casazza JP, Felver ME, Veech RL. The metabolism of acetone in rat. J Biol Chem 1984; 259: 231-236.
46. Aleksandrovskii YA. Antithrombin III, CI inhibitor, methylglyoxal, and polymorphonuclear leukocytes in the development of vascular complications in diabetes mellitus. Thromb Res 1992; 67: 179-189.
47. Tressel T, Thompson R Zieske LR, Menendez MITS, Davis L. Interaction between L-threonine dehydrogenase and aminoacetone synthase and mechanism of aminoacetone production. J Biol Chem 1986; 261: 16428-16437.

48. Lyles GA, Singh I. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem Pharmacol 1992; 43: 1409-1414.
49. Thornalley PJ, Hooper NI, Jennings PE, Florkowski CM, Jones AF, Lunec J, Barnett AH. The human red blood cell glyoxalase system in diabetes mellitus. Diabetes Res Clin Pract 1989; 7: 115-120.
50. Chaplen FW, Fahl WE, Cameron DC. Evidence of high levels of methylglyoxal in cultured Chinese hamster ovary cells. Proc Natl Acad Sci USA 1998; 95: 5533-5538.
51. Vander Jagt DL, Hassebrook RK, Hunsaker LA, Brown WM, Royer RE. Metabolism of the 2-oxoaldehyde methyglyoxal by aldose reductase and by glyoxalase-I: roles for glutathione in both enzymes and implications for diabetic complications. Chem Biol Interact 2001; 130-132: 549-562.
52. Zagar AH, Shah NA, Masoodi BA, et al. Copper, zinc and magnesium levels in non-insulin dependent diabetes mellitus. Postgrad Med J 1998; 74: 665-668.
53. Kang JH. Oxidative modification of human ceruloplasmin by methylglyoxal: an in vitro study. J Biochem Mol Biol 2006; 39: 335-338.
54. Pi J, Bai Y, Zhang Q, et al. Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes 2007; 56: 1783-1791.
55. Sheader EA, Benson RS, Best L. Cytotoxic action of methylglyoxal on insulin-secreting cells. Biochem Pharmacol 2001; 61: 1381-1386.
56. Best L, Miley HE, Brown PD, Cook LJ. Methylglyoxal causes swelling and activation of a volume-sensitive anion conductance in rat pancreatic beta-cells. J Membr Biol 1999; 167: 65-71.
57. Jia X, Olson DJ, Ross AR, Wu L. Structural and functional changes in human insulin induced by methylglyoxal. FASEB J 2006; 20: 1555-1557.
58. Riboulet-Chavey A, Pierron A, Durand I, Murdaca J, Giudicelli J, Van Obberghen E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. Diabetes 2006; 55: 1289-1299.
59. Bucala R, Cerami A. Advanced glycosylation: chemistry, biology, and implications for diabetes and aging. Adv Pharmacol 1992; 23: 1-34.
60. Cerami A, Stevens VJ, Monnier VM. Role of nonenzymatic glycosylation in the development of the sequelae of diabetes mellitus. Metabolism 1979; 38: 431-438.
61. Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev Med 1995; 46: 223-234.
62. Fu M-X, Wells-Knecht KJ, Blackledge JA, Lyons TJ, Thorpe SR, Baynes JW. Glycation, glycoxidation, and cross-linking of collagen by glucose. Diabetes 1994; 43: 676-683.
63. Grandee SK, Monnier VM. Mechanism of formation of the Maillard protein cross-link pentosidine. J Biol Chem 1991; 266: 11649-11653.
64. Nagaraj RH, Sell DR, Prabhakaram M, Ortwerth BJ, Monnier VM. High correlation between pentosidine protein crosslinks and pigmentation impli-

cates ascorbate oxidation in human lens senescence and cataractogenesis. Proc Natl Acad Sci USA 1991; 88: 10257-10261.
65. Uchida K, Khor OT, Oya T, Osawa T, Yasuda Y, Miyata T. Protein modification by a Maillard reaction intermediate methylglyoxal. Immunochemical detection of fluorescent 5-methylimidazolone derivatives in vivo. FEBS Lett 1997; 410: 313-318.
66. Hayase F, Konishi Y, Kato H. Identification of the modified structure of arginine residues in proteins with 3-deoxyglucosone, a Maillard reaction intermediate. Biosci Biotech Biochem 1995; 59: 1407-1411.
67. Lo WC, Westwood ME, McLellan AC, Selwood T, Thornalley PJ. Binding and modification of proteins by methylglyoxal under physiological conditions. J Biol Chem 1994; 269: 32299-32305.
68. Nagaraj RH, Shipanova IN, Faust FM. Protein cross-linking by the Maillard reaction. Isolation, characterization, and in vivo detection of a lysine-lysine cross-link derived from methylglyoxal. J Biol Chem 1996; 271: 19338-19345.
69. Ahmed MU, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW. *N*-(Carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem J 1997; 324: 565-570.
70. Yu PH. Deamination of methylamine and angiopathy; toxicity of formaldehyde, oxidative stress and relevance to protein glycoxidation in diabetes. J Neural Transm Suppl 1998; 52: 201-216.
71. Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 1986; 232: 1629-1632.
72. Frank RN, Amin R, Kennedy A, Hohman TC. An aldose reductase inhibitor and aminoguanidine prevent vascular endothelial growth factor expression in rats with long-term galactosemia. Arch Ophthalmol 1997; 115: 1036-1047.
73. Vasan S, Zhang X, Zhang X, et al. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo. Nature 1996; 382: 275-278.
74. Wolffenbuttel BH, Boulanger CM Crijns FR, et al. Breakers of advanced glycation end products restore large artery properties in experimental diabetes. Proc Natl Acad Sci USA 1998; 95: 4630-4634.
75. Lee C, Yim MB, Chock PB, Yim HS, Kang SO. Oxidation-reduction properties of methylglyoxal-modified protein in relation to free radical generation. J Biol Chem 1998; 273: 25272-25278.
76. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, Brownlee M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. J Clin Invest 1998; 101: 1142-1147.
77. Monder C. α-Ketoaldehyde dehydrogenase, an enzyme that catalyzes the enzymic oxidation of methylglyoxal to pyruvate. J Biol Chem 1967; 242: 4603-4609.

78. Fujii E, Iwase H, Ishii-Karakasa I, Yajima, Hotta K. The presence of 2-keto-3-deoxygluconic acid and oxoaldehyde dehydrogenase activity in human erythrocytes. Biochem Biophys Res Commun 1995; 210: 852-857.
79. Oimomi M, Hata F, Igaki N, Nakamichi T, Baba S, Kato H. Purification of α-ketoaldehyde dehydrogenase from the human liver and its possible significance in the control of glycation. Experientia 1989; 45: 463-466.
80. Dunkerton J, James SP. Purification of 2-oxoaldehyde dehydrogenase and its dependence on unusual amines. Biochem J 1975; 149: 609-617.
81. Dunkerton J, James SP. Some observations on the $NADP^+$-linked oxidation of methylglyoxal catalysed by 2-oxoaldehyde dehydrogenase. Biochem J 1976; 153: 503-504.
82. Vander Jagt DL, Davidson LM. Purification and characterization of 2-oxo-aldehyde dehydrogenase from rat liver. Biochim Biophys Acta 1977; 484: 260-267.
83. Vander Jagt DL. 2-Ketoaldehyde dehydrogenase from rat liver. Meth Enzymol 1982; 89: 513-515.
84. Vander Jagt DL, Hunsaker LA. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. Chem Biol Interact 2003; 143-144: 341-351.
85. Ray S, Ray M. Purification and characterization of NAD and NADP-linked α-ketoaldehyde dehydrogenases involved in catalyzing the oxidation of methylglyoxal to pyruvate. J Biol Chem 1982; 257: 10566-10570.
86. Ray M, Ray S. On the interaction of nucleotides and glycolytic intermediates with NAD-linked α-ketoaldehyde dehydrogenase. J Biol Chem 1982; 257: 10571-10574.
87. Sophos NA, Pappa A, Ziegler TL, Vasiliou V. Aldehyde dehydrogenase gene superfamily: the 2000 update. Chem Biol Interact 2001; 130-132: 323-337.
88. Hempel J, Jornvall JH. Aldehyde dehydrogenases–structure. In: Crow KE, Batts RD, eds. Human Metabolism of Alcohol, Vol II. Boca Raton, FL: CRC, 1989; 77.
89. Izaguirre G, Kikonogo A, Pietruszko R. Methylglyoxal as substrate and inhibitor of human aldehyde dehydrogenase: comparison of kinetic properties among the three isozymes. Comp Biochem Physiol Part B 1998; 119: 747-754.
90. Mannervik B. Molecular enzymology of the glyoxalase system. Drug Metab Drug Interact 2008; 23: 13-27.
91. Sukdeo N, Honek JF. Microbial glyoxalase enzymes: metalloenzymes controlling cellular levels of methylglyoxal. Drug Metab Drug Interact 2008; 23: 29-50.
92. Vander Jagt DL, Robinson B, Taylor KK, Hunsaker LA. Reduction of trioses by NADPH-dependent aldo-keto reductases: aldose reductase, methylglyoxal and diabetic complications. J Biol Chem 1992; 267: 4364-4369.
93. Vander Jagt DL, Robinson B, Taylor KK, Hunsaker LA. Aldose reductase from human skeletal and heart muscle. Interconvertible forms related by thiol-disulfide exchange. J Biol Chem 1990; 265: 20982-20987.

94. Vander Jagt DL, Hunsaker LA, Robinson B, Stangebye LA, Deck LM. Aldehyde and aldose reductases from human placenta. Heterogeneous expression of multiple enzyme forms. J Biol Chem 1990; 265: 10912-10918.
95. Vander Jagt DL, Hunsaker LA. Substrate specificity of reduced and oxidized forms of aldose reductase. Adv Exp Med Biol 1993; 328: 279-288.
96. Kolb NS, Hunsaker LA, Vander Jagt DL. Aldose reductase catalyzed reduction of acrolein: implications in cyclophosphamide toxicity. Mol Pharmacol 1994; 45: 797-801.
97. Vander Jagt DL, Kolb NS, Vander Jagt TJ, Chino J, Martinez FJ, Hunsaker LA, Royer RE. Substrate specificity of human aldose reductase: identification of 4-hydroxynonenal as an endogenous substrate. Biochim Biophys Acta 1995; 1249: 117-126.
98. Skutches CL, Owen OE, Reichard GA. Acetone and acetol inhibition of insulin-stimulated glucose oxidation in adipose tissue and isolated adipocytes. Diabetes 1990; 39: 450-455.
99. Williamson JR, Chang K, Frangos M, et al. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 1993; 42: 801-813.
100. Suzuki D, Miyata T. Carbonyl stress in the pathogensis of diabetic nephropathy. Intern Med 1999; 38: 309-314.
101. Miyata T, Ishikawa N, van Ypersele de Strihou C. Carbonyl stress and diabetic complications. Clin Chem Lab Med 2003; 41: 1150-1158.
102. Lyons TJ. Glycation, carbonyl stress. EAGLEs, and vascular complications of diabetes. Semin Vasc Med 2002; 2: 175-189.
103. Ortwerth BJ, James H, Simpson G, Linetsky M. The generation of superoxide anions in glycation reactions with sugars, osones, and 3-deoxyosones. Biochem Biophys Res Commun 1998; 245: 161-165.
104. Rosca MG, Mustata TG, Kinter MT, et al. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. Am J Physiol Renal Physiol 2005; 289: F420-430.
105. Lin J. Pathophysiology of cataracts: copper ion and peroxidation in diabetics. Jpn J Ophthalmol 1997; 41: 130-137.
106. Dutra F, Bechara EJH. Aminoacetone induces iron-mediated oxidative damage to isolated rat liver mitochondria. Arch Biochem Biophys 2004; 430: 284-289.
107. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol Endocrinol Metab 2001; 280: E685-E694.
108. Bedard K, Krause K-H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 2005; 87: 245-313.
109. Vander Jagt DL, Hunsaker LA, Vander Jagt TJ, Gomez MS, Gonzales DM, Deck LM, Royer RE. Inactivation of glutathione reductase by 4-hydroxynonenal and other endogenous aldehydes. Biochem Pharmacol 1997; 53: 1133-1140.
110. Kang JH. Modification and inactivation of human Cu,Zn-superoxide dismutase by methylglyoxal. Mol Cells 2003; 15: 194-199.

111. Niwa T, Tsukushi S. 3-Deoxyglucosone and AGEs in uremic complications: inactivation of glutathione peroxidase by 3-deoxyglucosone. Kidney Int Suppl 2001; 78: S37-S41.
112. Park YS, Koh YH, Takahashi M, et al. Identification of the binding site of methylglyoxal on glutathione peroxidase: methylglyoxal inhibits glutathione peroxidase activity via binding to glutathione binding sites Arg 184 and 185. Free Radic Res 2003; 37: 205-211.
113. Grankvist K, Marklund SL, Taljedal IB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. Biochem J 1981; 199: 393-398.
114. Thornalley PJ, McLellan AC, Lo TW, Benn J, Sonksen PH. Negative association between erythrocyte reduced glutathione concentration and diabetic complications. Clin Sci (Lond) 1996; 91: 575-582.
115. Nwose EU, Jelinek HF, Richards RS, Kerr PG. Changes in the erythrocyte glutathione concentration in the course of diabetes mellitus. Redox Rep 2006; 11: 99-104.
116. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr 2004; 134: 489-492.
117. Chang KC, Paek KS, Kim HJ, Lee YS, Yabe-Nishimura C, Seo HG. Substrate-induced up-regulation of aldose reductase by methylglyoxal, a reactive oxoaldehyde elevated in diabetes. Mol Pharmacol 2002; 61: 1184-1191.
118. Du J, Cai S, Suzuki H, et al. Involvement of MEKK1/ERK/P21Waf1/Cip1 signal transduction pathway in inhibition of IGF-I-mediated cell growth response by methylglyoxal. J Cell Biochem 2003; 88: 1235-1246.
119. Du J, Zeng J, Ou X, Ren X, Cai S. Methylglyoxal downregulates Rat-1 protein through a ubiquitination-mediated mechanism. Int J Biochem Cell Biol 2006; 38: 1084-1091.
120. Akhand AA, Hossain K, Mitsui H, et al. Glyoxal and methylglyoxal trigger distinct signals for map family kinases and caspase activation in human endothelial cells. Free Radic Biol Med 2001; 31: 20-30.
121. Cantero AV, Portero-Otin M, Ayala V, et al. Methylglyoxal induces advanced glycation end product (AGEs) formation and dysfunction of PDGF receptor-beta: implications for diabetic atherosclerosis. FASEB J 2007; 21: 3096-3106.
122. Fan X, Subramaniam R, Weiss MF, Monnier VM. Methylglyoxal-bovine serum albumin stimulates tumor necrosis factor alpha secretion in RAW 264.7 cells through activation of mitogen-activating protein kinase, nuclear factor kappaB and intracellular reactive oxygen species formation. Arch Biochem Biophys 2003; 409: 274-286.
123. Du J, Suzuki H, Nagase F, Akhand AA, Ma XY, Yokoyama T, Miyata T, Nakshima I. Superoxide-mediated early oxidation and activation of ASK1 are important for initiating methylglyoxal-induced apoptosis process. Free Radic Biol Med 2001; 31: 469-478.
124. Che W, Asahi M, Takahashi M, Kaneto H, Okado A, Higashiyama S, Taniguchi N. Selective induction of heparin-binding epidermal growth factor-like growth factor by methylglyoxal and 3-deoxyglucosone in rat aortic

smooth muscle cells. The involvement of reactive oxygen species formation and a possible implication for atherogenesis in diabetes. J Biol Chem 1997; 272: 18453-18459.
125. Gawlowski T, Stratmann B, Stirban AO, Negrean M, Tschoepe D. AGEs and methylglyoxal induce apoptosis and expression of Mac-1 on neutrophils resulting in platelet-neutrophil aggregation. Thromb Res 2007; 121: 117-126.
126. Chan WH, Wu HJ, Shiao NH. Apoptotic signaling in methylglyoxal-treated human osteoblasts involves oxidative stress, c-Jun N-terminal kinase, caspase-3, and p21-activated kinase 2. J Cell Biol 2007; 100: 1056-1069.
127. Schupp N, Schinzel R, Heidland A, Stopper H. Genotoxicity of advanced glycation end products: involvement of oxidative stress and of angiotensin II type 1 receptors. Ann NY Acad Sci 2005; 1043: 685-695.
128. Wu L. The pro-oxidant role of methylglyoxal in mesenteric artery smooth muscle cells. Can J Physiol Pharmacol 2005; 83: 63-68.
129. Kim J, Son JW, Lee JA, Oh YS, Shinn SH. Methylglyoxal induces apoptosis mediated by reactive oxygen species in bovine retinal pericytes. J Korean Med Sci 2004; 19: 95-100.
130. Wu L, Juurlink BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. Hypertension 2002; 39: 809-814.
131. Caldwell RB, Bartoli M, Behzadian MA, et al. Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. Curr Drug Targets 2005; 6: 511-524.
132. Shams N, Ianchulev T. Role of vascular endothelial growth factor in ocular angiogenesis. Ophthalmol Clin North Am 2006, 19: 335-344.
133. Inagi R, Miyata T, Yamamoto T, et al. Glucose degradation product methylglyoxal enhances the production of vascular endothelial growth factor in peritoneal cells. FEBS Lett 1999; 463: 260-264.
134. Yamagishi S, Nakamura K, Matsui T, et al. Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. J Biol Chem 2006; 281: 20213-20220.
135. Li L, Renier G. Activation of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase by advanced glycation endproducts links oxidative stress to altered retinal vascular endothelial growth factor expression. Metabol Clin Exp 2006; 55: 1516-1523.
136. Ido Y, Chang KC, Lejeune WS, Bjercke RJ, Reiser KM, Williamson JR, Tilton RG. Vascular dysfunction induced by AGE is mediated by VEGF via mechanisms involving reactive oxygen species, guanylate cyclase, and protein kinase C. Microcirculation 2001; 8: 251-263.
137. Kaneto H, Fujii J, Myint T, et al. Reducing sugars trigger oxidative modification and apoptosis in pancreatic β-cells by provoking oxidative stress through the glycation reaction. Biochem J 1996; 320: 855-863.
138. Brissova M, Shiota M, Nicholson WE, et al. Reduction in pancreatic transcription factor PDX-1 impairs glucose-stimulated insulin secretion. J Biol Chem 2002; 277: 11225-11232.

139. Kaneto H, Xu G, Fujii N, Kim S, Bonner-Weir S, Weir GC. Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. J Biol Chem 2002; 277: 30010-30018.
140. Kaneto H, Matsuoka T, Nakatani Y, Kawamori D, Miyatsuka T, Matsuhisa M, Yamasaki Y. Oxidative stress, ER stress, and the JNK pathway in type 2 diabetes. J Mol Med 2005; 83: 429-439.
141. Roybal CN, Hunsaker LA, Barbash O, Vander Jagt DL, Abcouwer SF. The oxidative stressor arsenite activates vascular endothelial growth factor mRNA transcription by an ATF4-dependent mechanism. J Biol Chem 2005; 280: 20331-20339.
142. Roybal CN, Yang S, Sun CW, Hurtado D, Vander Jagt DL, Townes TM, Abcouwer SF. Homocysteine increases expression of vascular endothelial growth factor by a mechanism involving endoplasmic reticulum stress and transcription factor ATF4. J Biol Chem 2004; 279: 14844-14852.
143. Roybal CN, Marmorstein LY, Vander Jagt DL, Abcouwer SF. Aberrant accumulation of fiulin-3 in the endoplasmic reticulum leads to activation of the unfolded protein response and VEGF expression. Invest Ophthalmol Vis Sci 2005; 46: 3973-3979.
144. Van Herreweghe F, Mao J, Chaplen FW, Grooten J, Gevaert K, Vandekerckhove J, Vancompernolle K. Tumor necrosis factor-induced modulation of glyoxalase I activities through phosphorylation by PKA results in cell death and is accompanied by the formation of a specific methylglyoxal-derived AGE. Proc Natl Acad Sci USA 2002; 99: 949-954.
145. Ramana KV, Tammali R, Reddy AB, Bhatnagar A, Srivastava SK. Aldose reductase-regulated TNF-α production is essential for high glucose-induced vascular smooth muscle cell growth. Endocrinology 2007; 148: 4371-4384.
146. Ratliff DM, Vander Jagt DJ, Eaton RP, Vander Jagt DL. Increased levels of methylglyoxal-metabolizing enzymes in mononuclear and polymorphonuclear cells from insulin-dependent diabetic patients with diabetic complications: aldose reductase, glyoxalase I, and glyoxalase II. J Clin Endocrinol Metab 1996; 81: 488-492.
147. Oishi N, Kubo E, Takamura Y, Maekawa K, Tanimoto T, Akagi Y. Correlation between erythrocyte aldose reductase level and human diabetic retinopathy. Br J Ophthalmol 2002; 86: 1363-1366.
148. Kompf J, Bissbort S, Gussmann S, Ritter H. Polymorphism of red cell glyoxalase I (EI: 4.4.1.5); a new genetic marker in man. Humangenetik 1975; 27: 141-143.
149. Bender K, Bissbort S, Hiller C, Mayerova A, Wienker TF. On regional mapping of human chromosome 6. Acta Anthropogenet 1983; 7: 85-105.
150. Kim NS, Umezawa Y, Ohmura S, Kato S. Human glyoxalase I: cDNA cloning, expression, and sequence similarity to glyoxalase I from *Pseudomonas putida*. J Biol Chem 1993; 268: 11217-11221.
151. Kim NS, Sekine S, Kiuchi NK, Kato S. cDNA cloning and characterization of human glyoxalase I isoforms from HT-1080 cells. J Biochem 1995; 117: 359-361.

152. Gale CP, Futers TS, Summers LK. Common polymorphisms in the glyoxalase-1 gene and their association with prothrombotic factors. Diab Vasc Dis Res 2004; 1: 34-39.
153. Kirk RL, Ranford PR, Serjeantson SW, et al. HLA, complement C2, C4, properdin factor B and glyoxalase types in South Indian diabetics. Diabetes Res Clin Pract 1985; 1: 41-47.
154. McCann VJ, Davis RE, Welborn TA, Constable IJ, Beale DG. Glyoxalase phenotypes in patients with diabetes mellitus. Aust N Z J Med 1981; 11: 380-382.
155. Degaffe GH, Vander Jagt DL, Bobelu A, et al. Distribution of glyoxalase 1 polymorphism among Zuni Indians. J Diabet Complic 2007; in press.
156. Ko B, Lam K, Wat N, Chung S. An (A-C)n dinucleotide repeat polymorphic marker at the 5′ end of the aldose reductase gene is associated with early onset diabetic retinopathy in diabetic patients. Diabetes 1995; 44: 727.
157. Hessom AE, Hibberd ML, Millward A, Demaine AG. Polymorphism in the 5′-end of the aldose reductase gene is strongly associated with the development of diabetic nephropathy in type 1 diabetes. Diabetes 1997; 46: 287-291.
158. Demaine AG. Polymorphisms of the aldose reductase gene and susceptibility to diabetic microvascular complications. Curr Med Chem 2003; 10: 1389-1398.
159. Shah VO, Scavini M, Nikolic J, et al. Z-2 microsatellite allele is linked to increased expression of the aldose deductase gene in diabetic nephropathy. J Clin Endocrinol Metab 1998; 83: 2886-2891.
160. Park HK, Ahn CW, Lee GT. Et al. $(AC)_n$ polymorphism of aldose reductase gene and diabetic microvascular complications in type 2 diabetes mellitus. Diabetes Res Clin Pract 2002; 55: 151-157.
161. Kao YL, Donaghue K, Chan A, Khight J, Silink M. A novel polymorphism in the aldose reductase gene promoter region is strongly associated with diabetic retinopathy in adolescents with type 1 diabetes. Diabetes 1999; 48: 1338-1340.
162. Demaine A, Cross D, Millward A. Polymorphisms of the aldose reductase gene and susceptibility to retinopathy in type 1 diabetes mellitus. Investig Ophthalmol Vis Sci 2000; 41: 4064-4068.
163. Makiishi T, Araki S, Koya D, Maeda S, Kashiwagi A, Haneda M. C-106T polymorphism of AKR1B1 is associated with diabetic nephropathy and erythrocyte aldose reductase content in Japanese subjects with type 2 diabetes mellitus. Am J Kidney Dis 2003; 42: 943-951.
164. Sivenius K, Niskanen L, Voutilainen-Kaunisto R, Laakso M, Uusitupa M. Aldose reductase gene polymorphisms and susceptibility to microvascular complications in type 2 diabetes. Diabet Med 2004; 21: 1325-1333.
165. Richeti F, Noronha RM, Waetge RTL, et al. Evaluation of AC_n and C(-106)T polymorphisms of the aldose reductase gene in Brazilian patients with DM1 and susceptibility to diabetic retinopathy. Mol Vis 2007; 13: 740-745.
166. Yang B, Millward A, Demaine A. Functional differences between the susceptibility Z-2/C-106 and protective Z+2/T-106 promoter region poly-

morphisms of the aldose reductase gene may account for the association with diabetic microvascular complications. Biochim Biophys Acta 2003; 1636: 1-7.