# Methylglyoxal, a Diabetic Biomarker for Insulin Resistance, Vascular Dysfunction and Neuropathies

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**Abstract:** Diabetes mellitus is a pandemic metabolic disease characterized by chronically elevated blood glucose concentration due to insulin dysfunction. Diabetics are prone to limbs fungal infections and ulcers due to vascular injury and end-organ damage such as nephropathy, retinopathy and neuropathic pain all of which are associated with methylglyoxal elevation. Methylglyoxal is generated through carbohydrate, lipid and protein metabolism which are all found to be exacerbated in diabetes. Moreover, methylglyoxal is highly reactive with various cellular and interstitial molecules such as proteins and phospholipids to form stable adducts and advanced glycation end products. Methylglyoxal induces insulin resistance, pancreatic β-cells cytotoxicity, and induces endothelial dysfunction that accelerates retinopathy, another diabetes complication. Additionally, methylglyoxal induces hyperalgesia and neuronal inflammation associated with neuropathic pain. Therefore, methylglyoxal might represent a potential therapeutic target in diabetes and associated complications.

## 1. Introduction:

Diabetes mellitus (DM) is a pandemic metabolic disease characterized by chronically elevated blood glucose concentration (hyperglycaemia) due to insulin dysfunction which is attributed to insufficient -or blunted- insulin secretion and/or declined tissue sensitivity to insulin[1]. The incidence of diabetes mellitus is increasing throughout the world and numbers are expected to reach 592 million by the year 2035, mainly because of the increase in obesity[2]. Approximately 50% of diabetics show diabetes complications by the time ~~when~~ they are diagnosed[3]. Approximately 5 million people died from diabetes in 2014 globally which accounts for a death every 7 seconds[4]. Despite the differences in aetiology, clinical presentation, and disease prevalence, secondary complications, such as neuropathic pain, occur in both type 1 and 2 diabetes mellitus[5]. Diabetes complications lead to a reduced quality of life posing a huge economic burden to the health system and society[5]. In 2011 in the UK, the NHS spent almost £24 billion on diabetes, 30% of which was spent on managing complications, such as neuropathic pain. By 2035, it is estimated that diabetes will cost the NHS approximately £40 billion, accounting for 17% of total health resource expenditure[6].

Among diabetic patients approximately 10% are diagnosed with type-1-DM (T1DM) which is mainly attributed to autoimmune activity where expressed plasma islet-cells antibodies destroy pancreatic β-cells[1, 7]. Children, under 12 years comprise the majority of T1DM patients who require life-long insulin treatment for survival. However, there are two types of monogenic diabetes which are commonly misdiagnosed as T1DM due to early onset of disease. These are neonatal diabetes (ND) which is diagnosed in the first 6 months of life, whereas the second type; maturity-onset diabetes of the young (MODY) affects individuals younger than 25 years, controlled largely through the use of sulphonylureas such as glibenclamide[7].

The large majority of diabetics, are of type-2-DM (T2DM), which is regarded as a complex disease that embraces genetic factors, lifestyle, age, obesity, pregnancy and gender as risk factors[8]. Unlike T1DM, patients of T2DM usually do not require insulin to survive since insulin secretion is only partially deficient and/or the individual is insulin resistant. Insulin resistance is mainly attributed to chronically elevated levels of insulin reducing sensitivity and further increased by abdominal fat and hence obesity[1]. Reduced insulin secretion may be due to insulin signalling cascade alteration and/or reduced pancreatic β-cells mass,

however, the extent of pancreatic β-cells mass reduction is controversial as some studies stated that 65% loss of pancreatic β-cells is sufficient to induce diabetes[9] while other studies concluded that 10% reduction of pancreatic β-cells mass is also associated with numerous altered insulin signalling components that initiate diabetes[10].

## 2. Methylglyoxal and diabetes:

In addition to the previously mentioned diabetes complications, diabetics suffer from frequent: thirst (polydipsia), urination (polyuria) and hunger (polyphagia)[1] , these common complications are recently found to be associated with plasma/tissue methylglyoxal (MGO) elevation (Table 1).

Chronic hyperglycaemia is the main DM complication where blood glucose concentration exceeds 7mmol/L (125mg/dl). This results in an increasing proportion of glucose metabolism (approximately 0.5% of glycolysis), passing down alternate pathways to generate reactive oxygen species (ROS) such as MGO that is highly reactive with various cellular and interstitial molecules such as proteins and phospholipids to form stable adducts and advanced glycation end products (AGE)[11 12]. Upon forming AGE, MGO is trapped intracellularly and subsequently increases oxidative stress (OS) that disrupts the cellular membrane integrity and thereby allows MGO leakage to the serum from where it can be measured for disease progression and severity[11 13 14]. Physiological human plasma MGO concentration is approximately 150nM and is doubled in T2DM patients' plasma[15]. Moreover, glycolysis-derived MGO interacts with cellular proteins and nucleic acids to accelerate AGE production resulting in pancreatic β-cell cytotoxicity. This exacerbates hyperglycaemia and hence DM complications[11]. However, clinical studies have failed to significantly correlate MGO to blood glucose concentration due to 2 main technical reasons, (i) firstly it is essential to dissociate MGO from protein without causing any DNA damage and/or oxidation to accurately measure total MGO, and (ii) the heterogeneity of the samples due to diverse patient backgrounds[14].

## 2.1. MGO sources:

Being an AGE precursor, MGO levels have been widely studied[14]. The 4 main MGO sources are summarized in the following formula:

$MGO_{total} = MGO_{carbohydrates} + MGO_{lipids} + MGO_{proteins} + MGO_{exogenous}$

As shown in figure 1, three main integrated metabolic pathways are involved in MGO formation:

1. Carbohydrates:

   Reducing sugars are able to react with proteins' amino groups to yield Schiff's base which is structurally rearranged into Amadori product to be subjected to series of reactions that generate AGE[12]. Accordingly, MGO is generated mainly through phosphorylating glycolysis such as triose-phosphate enzymatic metabolism which was found to be increased in hyperglycaemia, the pentose phosphate shunt, sorbitol pathways such as xylitol metabolism and fourthly glucoxidation[14 16]. Moreover, triose-phosphate accumulation is involved in diabetic nephropathy revealing the involvement of carbohydrate generated MGO pathway in diabetes complication and such a pathway was shown to be inhibited through thiamine[17 18] (figure 1).

2. Lipid pathways:

   Lipid peroxidation of polyunsaturated fatty acids yields short chain; 3-9 carbon long fragment molecules of highly reactive aldehyde such as 2-Alkenol, 4-hydroxy-2-alkenal as well as ketoaldehydes from which glyoxal compounds such as MGO is generated from non-enzymatic and enzymatic metabolism of acetoacetate or acetone intermediates, respectively[12 14]. Acetoacetate is a major ketone body (KB) elevated in type 2 diabetics' plasma[19]. Moreover, lipolysis shares a common intermediate with carbohydrate metabolism; triose-phosphate through α-glycero-phosphate dehydrogenase-metabolized glycerol[14]. Previous studies found that lipolysis is increased in diabetes and suppression of lipolysis improves insulin sensitivity and glucose utilization[20 21]. Moreover, isopropyl alcohol (IP) is significantly increased (5mg/dl) in ketoacidosis diabetic plasma[22]. IP is a metabolite of acetone, as

the reaction is governed by alcohol dehydrogenase which favours the production of IP when acetone is increased as in diabetes mellitus where glucose utilization is compromised, and the body adapts the energy supply toward fatty acid oxidation through $NAD^+$ reduction into NADH. Therefore, when NADH/$NAD^+$ ratio is increased, NADH is utilized to produce IP from acetone[22 23].

3. Protein metabolism:

   Numerous in vitro studies demonstrate the vulnerability of tyrosine, serine, threonine and glycine rich proteins toward oxidation as these residues are enzymatically converted to acetone and aminoacetone intermediates which are converted into MGO[12 14]. Moreover, semi-carbazide sensitive amine oxidase (SSAO) which converts aminoacetone into MGO and hydrogen peroxide is the only enzyme found elevated in diabetic plasma[14]. Protein catabolism is increased by approximately 50% in streptozotocin treated rats, and this increased rate of protein catabolism is attributed to insulin resistance and increased glucocorticoids production in normal rats[24].

4. Exogenous MGO:

   AGE are ingested through heated and processed fats, proteins (Maillard reaction) as well as sugars and tobacco[25], whereas previous studies revealed that coffee and whiskies are the main MGO-containing beverages[26 27]. The average daily consumption of AGE is 16000kU AGE (kilo unit AGE per gram of serving size) which is even aggravated by high temperature processing such as oven frying that increases the AGE content of chicken breast to 900kU/g compared to boiled chicken breast (100kU AGE/g)[28]. Chronic consumption of AGE-containing food/beverages causes mild liver inflammation as well as fat disposition on parenchymal cells which alters fasting insulin and thereby reduces glucose tolerance according to previous research conducted on rat hepatocytes [27]. In the Maillard reaction, reducing sugars such as glucose interact with free amino groups found in proteins to form for instance N-substituted glycosylamine which in the presence of water goes through Amadori rearrangement to yield Amadori product, 1-amino-1-deoxy-2-ketose[29]. The

rearranged Amadori product (RAP) is then degraded through 2,3 enolisation to form numerous fission products such as acetol, pyruvaldehyde and diacetyl compounds at pH>7[29]. These carbonyl compounds are highly reactive with amino acids to form aldehydes and α-aminoketones[29]. Previous studies found that Maillard reaction products are significantly increased in diabetics' skin collagen as Nε-carboxymethyl lysine (CML), fructoselysine (FL) and pentosidine which are associated with accelerated aging[30]. Additionally, CML is significantly elevated in diabetic plasma when compared to healthy plasma, and such elevation is exacerbated when purely prepared AGE beverages were ingested[22]. This elevation was associated with altered vascular function through supressing the expression and function of eNOS as well as stimulating the release of vascular cells adhesion molecules (VCAM-1)[25].

## 2.2. MGO metabolism:

Two glutathione (GSH) dependent pathways contribute to MGO metabolism; glyoxalase system (GLO), glyoxalase 1 and glyoxalase 2 (GLO1 & GLO2) and aldose reductase, of which GLO-1 is the major pathway that converts MGO it to non-toxic D-lactate[14 31]. However, as 11% of glucose is metabolized through the sorbitol pathway, the bi-modal aldose reductase acts as aldehyde reductase rather than ketone reductase and thereby preferentially produces acetol which thereafter is converted retrospectively to MGO through CYP2E1 to start a futile cycle that depletes the intracellular GSH and elevates acetol in diabetic plasma[14].

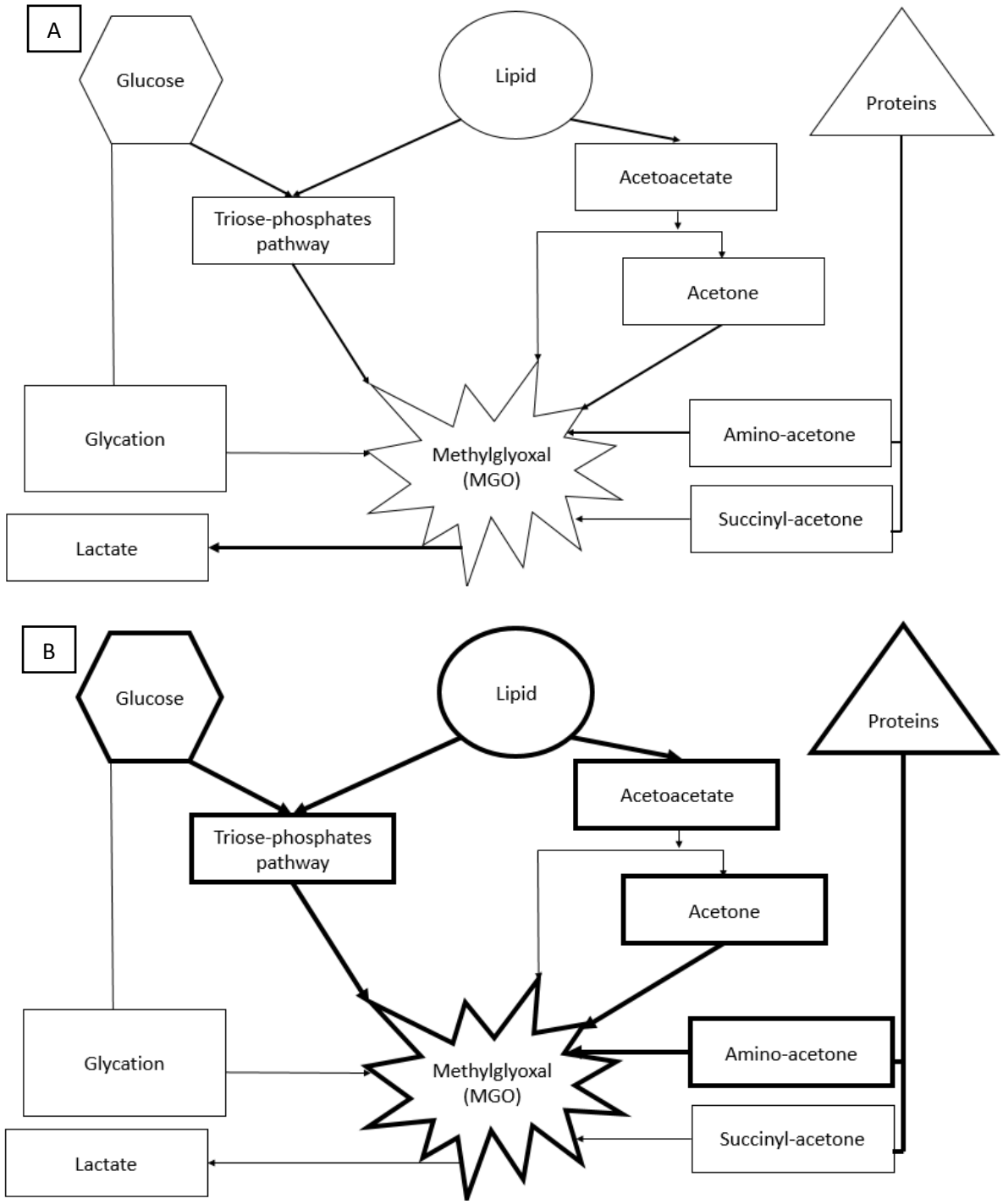


Figure 1 Endogenous sources of methylglyoxal (MGO) from glucose, lipid and protein metabolism. (A) Normal non-diabetic condition shows low MGO production from un-affected glycolysis, lipolysis or proteolysis with major sources represented in bold arrows. (B) Diabetes is associated with increased MGO production from hyperglycaemia, accelerated lipolysis and proteolysis represented with thick borders and bold arrows which are accompanied with compromised glyoxalase activity.

## 2.3. MGO and insulin:

Insulin secretion is a calcium-dependent cascade which starts when pancreatic β-cells glucose-transporter-1 (GLUT-1) take up glucose due to elevated plasma-glucose. This results in ATP synthesis and potassium ATP channel ($K_{ATP}$) closure. Once $K_{ATP}$ are closed, Calcium ions ($Ca^{+2}$) enter through Voltage gated $Ca^{+2}$ channels initiating insulin exocytosis[32]. However, glucose is not the only insulin release stimulator as lipids and proteins are also insulin secretagogues, in addition to other neurotransmitters and hormones such as incretins which stimulate insulin secretion independently from $Ca^{+2}$ as illustrated in figure 2[7].

Insulin resistance is a complex condition where a normal insulin concentration is not sufficient to mediate glucose uptake and utilization due to insulin signalling disruption and hence, more insulin is released to try to maintain glucose homeostasis[33 34]. MGO has been shown to bind with insulin through targeting arginine residues located in chain B and at N-terminus[33]. Subsequently, MGO-modified insulin chain B is heavier than free insulin by an additional 126Da resulting in less glucose uptake and utilization. This has been demonstrated in skeletal muscle L8 cells and 3T3-L1 adipocytes as well as a 50% reduction in metabolism in H4-II-E hepatocytes[33]. This study is further supported by the fact that insulin receptor substrate-1 (IRS-1) phosphorylation and PI3K activity are both suppressed dose dependently following MGO and reversed with MGO scavenger; N-acetylcysteine[34] (Table 1). Moreover, MGO (100μM) induces pancreatic β-cytotoxicity when applied to RINmf5 insulin secreting cells in culture[11]. Such an effect is comparable to a high glucose (16mM) apoptotic effect on rat pancreatic β-cells[11]. These findings suggest that MGO might play a major role in progressive stages of diabetes where chronic hyperglycaemia yields elevated MGO that reduces insulin signalling and pancreatic β-cells numbers leading to a further reduction in insulin secretion[11] (Figure 2). Moreover, when MGO 60mg/kg/day was infused in Sprague-Dawley rats for 28 days, it resulted in a significant reduction in plasma insulin and a significant increase in fasting plasma glucose. Additionally, plasma and pancreatic, muscle and, adipocyte tissues were all characterized by significant MGO elevation associated with significant decrease in glutathione (GSH) and adipocyte plasma membrane glucose transporter-4 (GLUT-4) as well as pancreatic GLUT-2 [35].

Insulin binds to its corresponding tyrosine-kinase coupled endothelial receptor, insulin receptor (IR) which is phosphorylated and provides a docking site for insulin receptor substrate-1 (IRS-1) to bind. As it exerts tyrosine-kinase activity, IR phosphorylates IRS-1 to unveil an interactive sulfhydryl

(SH2) domain which is responsible for activating phosphatidylinositol 3-kinase /protein kinase B (PI-3K/PKB) as well as Ras-mitogen activated protein kinase (MAPK) pathways. These pathways phosphorylate approximately 40 cellular targets including Akt-dependent endothelial nitric oxide synthase (eNOS) that generates nitric oxide (NO) to yield endothelium-dependent vasodilation[34 36 37 38]. Therefore, MGO-induced insulin dysfunction (reduced secretion and increased resistance) might directly cause vascular dysfunction, a common complication in diabetes[37] (Figure 3).

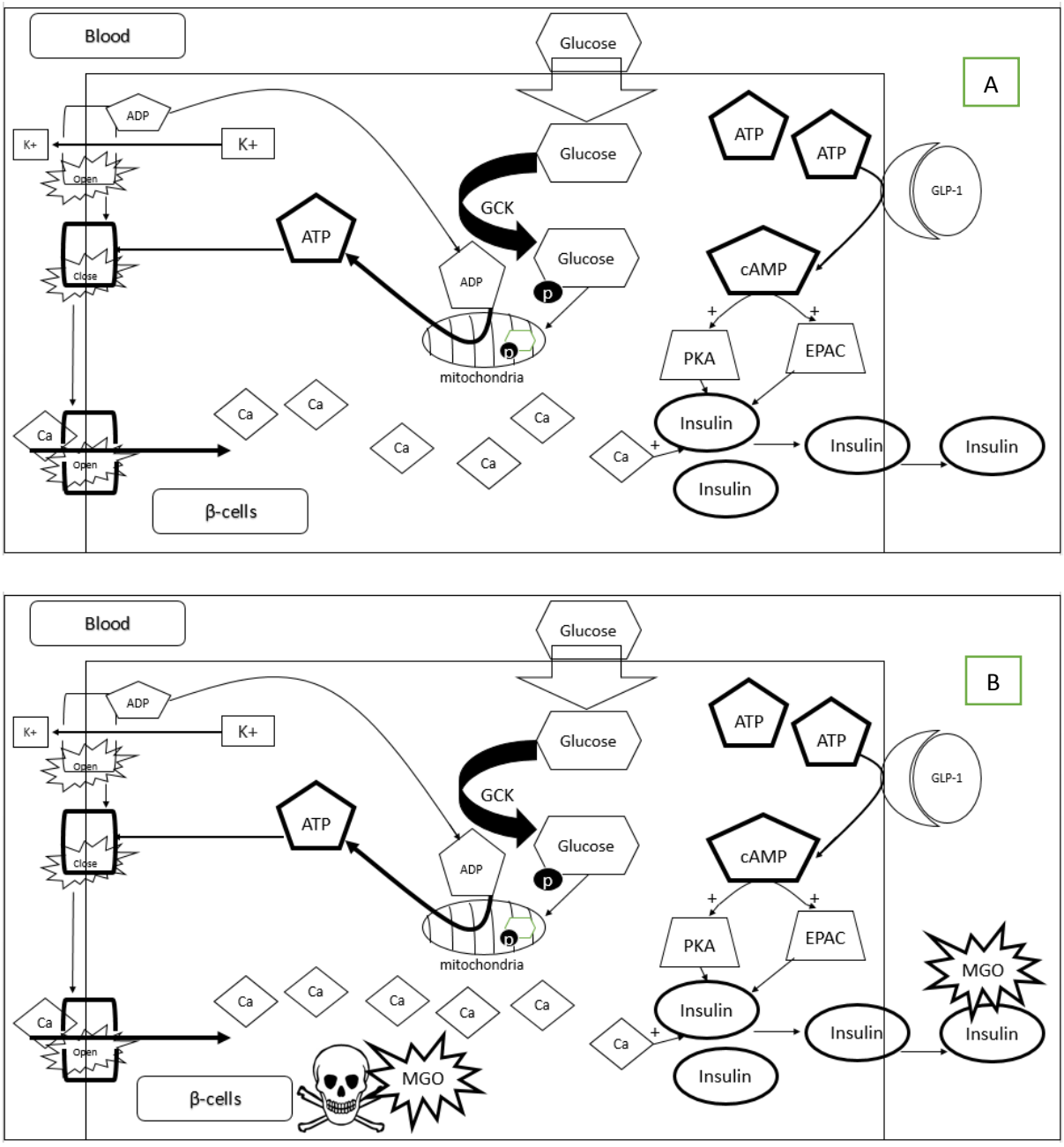


Figure 2 Insulin release from pancreatic β-cells through calcium dependent and independent pathways. GLUT-1 takes up glucose which is metabolised through glucokinase (GCK) to glucose-6-phophate which is metabolised in the mitochondria to generate ATP. A rise in [ATP] stimulates the closure of potassium ATP channels and depolarisation that triggers Ca+2 influx. Whereas glucagon like peptide-1 (GLP-1) stimulates insulin secretion via an exchange protein activated by cAMP (EPAC) and protein kinase A (PKA)-dependent mechanism which are activated through cyclic adenosine monophosphate (cAMP). Plus signs reveal stimulation. (A) normal insulin secretion. (B) Methylglyoxal (MGO) is toxic to pancreatic β-cells and forms insulin-adducts which endows insulin with higher molecular weight and less activity.

## 2.4. MGO and vasculature:

Numerous authors correlate highly accumulated MGO to vascular and other end organ damage such as nephropathy and neuropathy[39]. Vascular dysfunction is a common complication in DM which may culminate with renal failure, blindness and limb amputation[40 41].

As a highly reactive aldehyde MGO forms stable adducts when reacting with multiple macromolecules through preferentially targeted aminoacids as for instance lysine; Nε-carboxyethyl lysine (CEL), MGO-derived lysine-lysine dimer (MOLD), arginine; 5-methylimidazolone, tetra-hydropyrimidine and argpyrimidine as well as sulfhydryl group-containing cysteine which forms stabilized S-lactyl cysteine through keto-enol tautoumerism from which CEL and MOLD are both elevated in DM[12]. Accordingly, MGO inhibits eNOS through inhibiting the phosphorylation of serine 1177, thereby inhibiting NO production and yielding vascular dysfunction[40] (Figure 3). Moreover, rat thoracic aortic smooth muscle cells (ASMC) treated with MGO (100μM) induces NO production and upon further investigations, the researchers found that $H_2O_2$ generation was also enhanced[39]. Furthermore, induced NO reacts with peroxides ($O^{\cdot}_2$) forming the highly reactive oxidant peroxynitrite ($ONOO^-$) so that NO is bound and thereby physiologically non-functional. In addition, $ONOO^-$ itself is considered as an essential atherosclerotic factor[39]. Furthermore, immunohistochemical-based observations in kidneys from diabetic patients show increased argpyrimidine formation in patient arteries suggesting MGO induces arterial injury as a further DM complication[42]. Vascular function restoration in GLO-1-overexpressed STZ treated animals is accompanied by MGO and AGE reduction and another study showed vascular function restoration through MGO scavengers such as diacetyl cysteine[40 41] (Table 1).

MGO is also a redox-based cell signalling regulator[39], which oxidizes GSH to GSSH through irreversible binding to arginine and thereby alters the cellular redox system which pushed the cell toward oxidative stress-induced apoptosis[33].

MGO blocks insulin-stimulated eNOS phosphorylation at serine-1177 and threonine-497 and also inhibited tyrosine phosphorylation of IRS-1 and Akt[37]. GLO-1 inhibition results in elevation of MGO and showed similar inhibitory effects on eNOS, IRS-1 and Akt phosphorylation. Moreover, in vivo studies on mice administered i.p MGO 50-75mg/kg/day for 5 consecutive days/week for 7 weeks showed significant insulin resistance accompanied with compromised endothelial function that was attributed to IRS-1 inhibition through

serine-616 phosphorylation and eNOS signalling suppression[37] (Figure 3). Additionally, endothelial dysfunction is accompanied by an increase in oxidative stress markers such as the vascular monocytes chemoattractant peptide-1 (MCP-1) and RAGE expression in Wistar and Goto-Kakizaki rats[43]. Tetrahydropyrimidine (THP), an AGE product of MGO was elevated in T1DM compared to non-diabetics (115.5U/µl vs 109.8U/µl) and such elevation was strongly associated with an endothelial dysfunction marker, soluble vascular cell adhesion molecule-1 (sVCAM-1) and phospholipase-A2 (sPLA2), a low grade inflammatory marker. This data suggests MGO as a diabetic marker for vascular dysfunction[44].

These diabetic vascular complications are commonly associated with retinopathy. MGO-derived CML, CEL and hydroimidazalone-1 (MG-H1) are increased in diabetic wild type (WT) rat retina but not in diabetic GLO-1 overexpressing transgenic rats or in non-diabetic rats. GLO-1 overexpression prevented the generation of new capillaries in acellular tissues in central and peripheral regions of retina as well as preventing cellular capillary degeneration[45].

In addition to all these vascular complications, the lifespan of erythrocytes (RBC) is reduced in diabetes. The MGO concentration is doubled in diabetics' RBC and elevated by fourfold in diabetics' plasma compared to non-diabetics'[46]. MGO accelerates RBC suicidal death, eryptosis through enhancing phosphatidylserine exposure on the cell surface, a signal that triggers cell death. Further, MGO was shown to reduce ATP and GSH levels in RBCs which would accelerates eryptosis[46 47]. Moreover, ROS increment in haemodialysis patients (HD) reveals that cell injury is a major factor that corresponds to ROS leakage and plasma ROS increment, a fact that is supported by recent studies showing ROS such as methylglyoxal induces RBC injury[14 48].

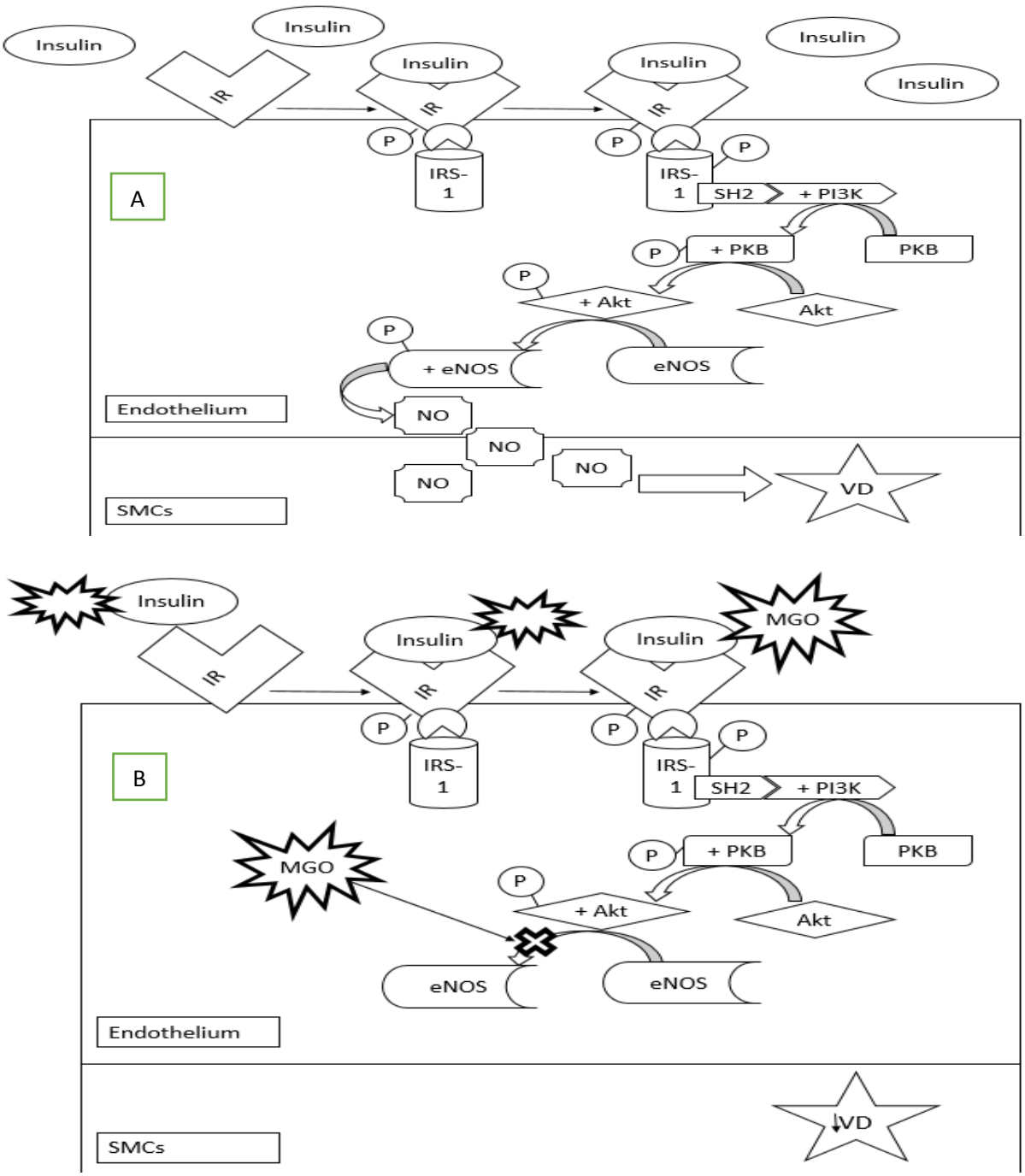


Figure 3 Methylglyoxal (MGO) induces vascular dysfunction by interfering with insulin signalling. (A) Normal endothelial vasodilation stimulated through insulin binding to its insulin receptor (IR) which is phosphorylated to unveil intracellular binding site for insulin receptor substrate-1 (IRS-1) which is phosphorylated upon binding. Phosphorylated IRS-1 provides a sulfhydryl (SH2) residue which is then bound with phosphatidylinositol 3-kinase (PI3K) that phopsphorylates protein kinase B (PKB) which phosphorylates and activates Akt that activates endothelial nitric oxide synthase to generate nitric oxide (NO). NO diffuses into smooth muscle cells (SMCs) causing vasodilation (VD). (B) MGO reduces insulin secretion through pancreatic β-cells toxicity, binds to insulin forming the less active insulin-MGO adduct and inhibiting eNOS phosphorylation which results in less NO production and compromised endothelial vasodilation.

## 2.5. MGO and neuropathic pain:

Diabetes is one of the leading causes of chronic neuropathic pain [5]. Neuropathic pain occurs in both Type 1 and Type 2 diabetics and given the heterogeneity of mechanisms that drives neuropathic pain in patients and over time, it is challenging to identify an optimum treatment strategy[5]. Among the major diabetic complications is diabetic neuropathy where nociception is exacerbated due to diabetes[13]. A recent study found diabetic plasma MGO was approximately doubled and reached 1µM in diabetic individuals with hyperalgesia which was associated with increased COX-2 expression and suppressed GLO-1 activity[31].

~~In mice,~~ MGO induced heat hyperalgesia in a dose dependent manner when administered in WT mice[31]. Moreover, STZ diabetic mice showed GLO suppression ~~which was associated with~~and hyperalgesia, and such complication was reproduced in WT mice when GLO expression was supressed[31]. MGO acutely depolarises the resting membrane potential in sensory neurons increasing their excitability. Such neuropathic events were shown to be NaV1.8 –dependent, as these events were absent in NaV1.8 –KO mice[31]Molecular studies showed MGO triggers changes in NaV1.8 gating through DIII-DIV linker's arginine residue modification in mice dorsal root ganglion (DRG)[31].

Furthermore, MGO increases the release of calcitonin gene related peptide (CGRP) in peripheral nerves and nerve conduction in STZ-diabetic and control mice[31]. These neuronal events are also accompanied by an increase in cerebral blood flow to areas associated with nociception[31].

A further mechanism for exacerbation of diabetic neuropathy is activation by MGO of human ankyrin transient receptor potential TRPA1. An effect which was blocked by the TRPA1 antagonist (HC030031)[13]. TRPA1 is expressed in sensory neurons and mediates nociception through numerous noxious compounds such as the highly reactive electrophile, MGO. Binding of MGO to the cysteine and lysine residues of the channel's N-terminal intracellular domain channel are necessary for channel activation by MGO[13].

Sensory neurons from TRPA1 knockout mouse shows no calcium influx when treated with MGO. MGO facilitates the release of CGRP from vagus and sciatic nerves as well as from the skin that contributes to pain transmission and together with PKA and PLC leads to nerve sensitization. MGO might contribute to neuropathic pain in diabetes[13].

Table 1 Methylglyoxal effects on diabetes induction or diabetic complications

| Model | Diabetes related effect | Significant findings |
|---|---|---|
| Sprague-Dawley rats | MGO 60mg/kg/day for 28 days induces insulin dysfunction and hyperglycaemia and therefore is concluded as diabetogenic | • Fasting plasma glucose elevation<br>• Insulin release, GLUT-4, PI3-K and adipose glucose uptake reduction[35] |
| Human insulin, cell culture studies on 3T3-L1 adipocytes, L8 skeletal muscle cells, H4-II-E cells and INS-1E cells. | MGO 10µM-1mM induces insulin resistance and thus is considered as diabetogenic | • Mass spectrophotometry: additional peaks of MGO-bound insulin<br>• L8-cells showed significant reduction in glucose uptake<br>• MGO binds INS-1E cells and reduces Insulin negative feedback[33] |
| RINmf5 insulin secreting cells | MGO 100µM-10mM induces cells toxicity and thus is considered as diabetogenic | • Fragmented nuclei cells elevation recorded microscopically and with multiparameter flow cytometry[11] |
| Human plasma and erythrocytes | MGO 30-300µM was shown to accelerate eryptosis | • HPLC analysis showed plasma and RBC MGO elevation<br>• MGO reduced GSH and ATP in RBC<br>• MGO dose dependent annexin-V-positive elevation[46] |
| Mouse aortic endothelial cells | MGO 500µM induces endothelial dysfunction through interfering endothelial insulin signalling | • Western blotting: Inhibiting IRS-1, Akt and eNOS phosphorylation[37] |
| Human plasma | MGO derivatives were associated with endothelial dysfunction | • ELISA measurements showed sVCAM-1 and sPLA2 elevation associated with THP in T1DM patients[44] |
| STZ Wistar-Kyoto rats saphenous artery | MGO elevation was associated with vascular function which is a major complication in diabetes | • Mild impairment in cholinergic and sodium nitroprusside (SNP) induced vasodilation[41] |
| Wistar and Goto-Kakizaki rats | MGO induces endothelial dysfunction even when ingested (50-75mg/kg/day for 3 months) | • Cholinergic vasodilation significant impairment<br>• Aortic IHC showed significant reduction in free NO production accompanied with increase in superoxide generation<br>• Western blotting showed significant suppression of phosphorylated and total |

| | | |
|---|---|---|
| | | vasodilator stimulated phosphoprotein<br>• Vascular inflammation through increased monocyte chemoattractant peptide-1 # (MCP-1)[43] |
| Normal human LDL, human BJ fibroblast AND HepG2 cells and Charles River rats | MGO-bound LDL accelerates vascular complications such as atherosclerosis | • LDL particles significantly decreased through MGO binding<br>• Cell free microplate blocked wells showed significant aggregation tendency of MGO-bound LDL<br>• IHC: MGO increased LDL retention in rats aorta<br>• MGO-bound LDL binds significantly more to LDL receptors found on HepG2 and BJ cells[49] |
| Sprague Dawley rat aortic rings, rat aortic and human umbilical veins endothelial cells. | MGO 100µM induces endothelial dysfunction, a common diabetes complication | • Cholinergic endothelium dependent vasodilation significant impairment accompanied with significant decrease in endothelial NO production with suppressed eNOS phosphorylation estimated through western blotting[40] |
| Mice ($TRPA1^{+/-}$) HEK 293t cells and DRG cultures | MGO 10mM induces neuropathic pain, a major diabetic complication | • MGO generates large inward current in HEK 293t cells and depolarizes the membrane from -100 to +100mV<br>• Calcium imaging reveals MGO binding to cysteine preferably to induce calcium entry<br>• TRPA1 or TRPV1 KO DRG showed significant lack of response to MGO[13] |
| Diabetic patients, WT and STZ mice mice ($Glo^{-/+}$), mice ($Scn^{-/-}$), human sciatic nerve, mice DRG, MGO treated Wistar rats | MGO 5µM administered systemically into rats induces hyperalgesia and neuronal inflammation for 3 hours | • MGO elevation was associated with diabetic neuropathy in T2DM patients[31]<br>• MGO depolarizes sensory neurons and modifies NaV1.8 to increase the neuronal excitation and facilitate nociception<br>• MGO slows NaV1.7 inactivation<br>• In mice, MGO reduces nerve conduction and increases calcitonin gene-related peptide (CGRP) and cyclooxygenase-2 (COX-2) expression to promote thermal and mechanical hyperalgesia in mice<br>• MGO increases blood flow to nociceptive cerebral regions[31] |
| Transgenic GLO-1 and normal Wistar rats | Restoring MGO metabolism prevents retinopathy, a common visual complication in diabetes | • Immunoblotting showed significant increase in CEL, CML, MG-H1 in diabetic Wistar rats' retinas suppressed in *GLO1* transgenic rats[45] |

Conclusion:

Diabetes mellitus is a metabolic disorder that is a major health burden in most of the countries around the globe. Although numerous therapeutic options are available to control diabetes, these medications are targeted mainly toward controlling the blood glucose level through supplying insulin, enhancing insulin secretion, enhancing the tissues' sensitivity towards insulin, interfering with glucose absorption or re-absorption. However, diabetic complications such as retinopathy, neuropathic pain, vascular and renal complications are still the main diabetic complications that, in the long term, remain largely resistant to treatments. Numerous studies reviewed in this paper show a correlation between MGO and diabetes as well as diabetes complications which suggest that understanding the actions of MGO might identify therapeutic targets for treating consequences of diabetes in the future.

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