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Methylglyoxal, A Metabolite Increased in Diabetes is Associated with 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 dysfunction in insulin signaling. Diabetics are prone to vascular injury and end-organ damage such as nephropathy, retinopathy and neuropathic pain. Methylglyoxal is generated through carbohydrate, lipid and protein metabolic pathways which are all found to be increased in diabetes. Moreover, methylglyoxal is highly reactive with various cellular and interstitial molecules such as proteins and phospholipids forming stable adducts and advanced glycation end products. Elevated methylglyoxal is associated with insulin resistance, pancreatic β-cell cytotoxicity, and endothelial dysfunction that accelerates retinopathy. Additionally, elevated methylglyoxal is associated with hyperalgesia and neuronal inflammation associated with neuropathic pain. Methylglyoxal might represent a potential therapeutic target in diabetes and associated complications.

Keywords: Diabetes, endothelial dysfunction, glucose, insulin, methylglyoxal, nephropathy, neuropathic pain, retinopathy.

1. INTRODUCTION

Diabetes mellitus (DM) is a pandemic metabolic disease characterized by a chronically elevated blood glucose concentration (hyperglycemia) due to insulin signaling dysfunction. This is attributed to insufficient or blunted insulin secretion and/or reduced 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 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 etiology, 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 and pose 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-cell 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 male gender as risk factors [8, 9]. Unlike T1DM, patients with 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 is further increased by abdominal fat and hence obesity [1]. Reduced insulin secretion may be due to alterations in the insulin signaling cascade and/or reduced pancreatic β-cell mass. However, the extent of pancreatic β-cell mass reduction is controversial as some studies stated that 65% loss of pancreatic β-cells is sufficient to induce diabetes [10] while other studies concluded that as little as 10% reduction of pancreatic β-cell mass is sufficient to initiate diabetes if associated with altered insulin signaling components [11].

1.1. 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 have recently been found to be associated with plasma/tissue methylglyoxal (MGO) elevation (Table **1**).

Chronic hyperglycemia is the main DM complication where blood glucose concentration exceeds 7mmol/L (125mg/dl) [12]. 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 [13]. MGO is highly reactive and forms stable adducts and advanced glycation end products (AGE) with various cellular and interstitial molecules such as proteins and phospholipids [12, 13]. 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 [12, 14, 15]. Physiological human plasma MGO concentration is approximately 150nM and increases ranging from two fold [16] to four fold [46] in T2DM patients' plasma. Moreover, glycolysis-derived MGO interacts with cellular proteins and nucleic acids to accelerate AGE production resulting in pancreatic β-cell cytotoxicity. This exacerbates hyperglycemia and hence DM complications [12]. However, clinical studies have failed to significantly correlate MGO to blood glu-

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cose 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 [17].

1.2. Methylglyoxal Sources

Being an AGE precursor, MGO levels have been widely studied [17]. The 4 main MGO sources are summarized in the following formula:

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

As shown in Fig. (**1**), three main integrated metabolic pathways are involved in MGO formation:

1.2.1. Carbohydrates Metabolism

Reducing sugars are able to react with amino groups on proteins to yield Schiff's base which is structurally rearranged into Amadori product to be subjected to a series of reactions that generate AGE [13]. Accordingly, MGO is generated mainly through phosphorylating glycolysis such as enzymatic metabolism of triose-phosphates which was found to be increased in hyperglycemia, but also the pentose phosphate shunt, sorbitol pathways such as xylitol metabolism and fourthly glucoxidation [17, 18].

The main source of methylglyoxal in physiological systems is the metabolism of triose phosphates, glyceraldehyde 3-phosphate and glycerone phosphate through non-enzymatic and/or enzymatic reactions [19]. In addition, aminoacetone and hydroxyacetone generated from threonine and acetone metabolism respectively are considered as minor endogenous precursors of MGO [20]. In-vitro studies showed first order kinetics of non-enzymatic MGO formation mainly from glyceraldehyde 3-phosphate (90% with catalytic rate (Kcat) = 1.55±0.02 X 10^{-4} S^{-1}), whereas less MGO was formed from glycerone phosphate, also called dihydroxyacetone phosphate (15% with Kcat= 1.94±0.02 X 10^{-5} S^{-1}) with 2 hours incubation at Krebs solution at 37° [19]. The enolisation of glyceraldehyde 3-phosphate and glycerin phosphate is an essential step to form 3-phospho 1, 2-eno-diol, an intermediate which fragments through the removal of phosphate to form methylglyoxal [19]. Therefore, the enolisation rate for glyceraldehyde 3-phosphate was 1.65±0.03x10^{-4} S^{-1}, while it was 1.31±0.05x10^{-5} S^{-1} for glycerone phosphate revealing that glyceraldehyde 3-phosphate is more susceptible to form MGO non-enzymatically than glycerone phosphate [19]. Additionally, when triose phosphate isomerase (10 U/ml) was added to glycerone phosphate (100μM) containing Krebs solution at 37°, the rate of MGO formation was accelerated with the maximum concentration of MGO recorded approximately 3μM in contrast to the formation of MGO in the absence of triose phosphate isomerase which was approximately 2.5μM after 20 minutes [19]. In addition to triose phosphate isomerase, methylglyoxal synthase (MGS) is also involved in MGO generation [21]. MGS is a convergent evolution product of triose phosphate isomerase with high specificity towards dihydroxyacetone phosphate (DHAP) to produce MGO and orthophosphate [21]. MGS isolated from goat liver showed Km= 0.76mM for DHAP which was inhibited by 90% with orthophosphate (40mM) in imidazole-HCl (100μM) buffer pH 7.2 [22]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) oxidizes DHAP into D-glycerate 1, 3-bisphosphate that is converted to pyruvate and then D-lactate [23]. GAPDH compromised activity was shown in diabetics RBC which showed a 3 fold increase in DHAP compared to non-diabetics RBC [24]. Therefore, diabetics' RBC showed DHAP accumulation (29-195nM g^{-1} protein) that was correlated with increased MGO generation (900-2100pM g^{-1} protein) revealing a negative correlation with GAPDH activity [23].

Moreover, triose-phosphate accumulation is associated with diabetic nephropathy suggesting the involvement of the carbohydrate generated MGO pathway in this diabetes complication [25, 26] (Fig. **1**).

1.2.2. Lipid Metabolism

Lipid peroxidation of polyunsaturated fatty acids yields short chain; 3-9 carbon molecules of highly reactive aldehyde such as 2-Alkenol, 4-hydroxy-2-alkenal as well as ketoaldehydes from which glyoxal compounds such as MGO are generated from non-enzymatic and enzymatic metabolism of acetoacetate or acetone intermediates, respectively [13, 17]. Acetoacetate is a major ketone body (KB) elevated in type 2 diabetics' plasma [27]. Acetoacetate decarboxylase generates acetone which is found to be elevated (97mg/dl) in diabetics' plasma [28]. Acetone is metabolized through the monooxygenase CYP2E1 which is mainly found in the liver into acetol and then to MGO through NADPH dependent reactions [20, 29]. Moreover, acetol can be metabolized through CYP2E1 forming 1, 2-propanediol that is then metabolized through 1, 2-propanediol gluconeogenesis to form glucose [30, 31].

Moreover, lipolysis shares a common intermediate with carbohydrate metabolism; triose-phosphate through α-glycero-phosphate dehydrogenase-metabolized glycerol [17]. Previous studies found that lipolysis is increased in diabetes and suppression of lipolysis improves insulin sensitivity and glucose utilization [32, 33].

1.2.3. Protein Metabolism

Numerous *in vitro* studies demonstrated the vulnerability of tyrosine, serine, threonine and glycine rich proteins towards oxidation as these residues are converted through NAD^+ dependent L-threonine dehydrogenase to acetone and aminoacetone intermediates which are then converted into MGO [13, 17, 34]. *In vitro* studies showed that aminoacetone can be converted to MGO through either oxidative deamination (mainly) or oxidative transamination [34]. The conversion of threonine into aminoacetone occurs in two ways. If oxidation occurs before decarboxylation, then the intermediate will be 2-aminoacetate which is spontaneously decarboxylated to aminoacetone and hence this reaction requires only one enzyme, threonine dehydrogenase which is required to convert threonine into aminoacetone [34]. However, if decarboxylation occurs before oxidation, the intermediate will be 1-aminopropan-2-ol which requires threonine decarboxylase and 1-aminopropan-2-ol dehydrogenase to yield aminoacetone [34]. Among these two pathways, the 2-aminoacetate producing reaction is the major pathway [34].

Semi-carbazide sensitive amine oxidase (SSAO), which converts aminoacetone into MGO and hydrogen peroxide, is found elevated in diabetic plasma; T1DM (621±209mU/l), T2DM (619±202mU/l) compared to non-diabetics plasma (352 ± 102mU/l) [17, 35]. 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 STZ treated rats [36].

1.2.4. Exogenous MGO

MGO is ingested in food containing heated and processed fats, proteins (Maillard reaction) as well as sugars and tobacco [37], whereas previous studies revealed that coffee and whiskies are the main MGO-containing beverages [38, 39]. Previous study determined that Nε-carboxymethyl lysine (CML) levels provide a useful marker for total pro-oxidant amounts of AGE [40]. The average daily consumption of AGE in total is 16000kU AGE which is aggravated by high temperature processing such as oven frying that increases the AGE content of chicken breast to 900kU/g (kilo unit AGE per gram of serving size) compared to boiled chicken breast (100kU AGE/g) [40]. Chronic consumption of MGO-containing food/beverages causes mild liver inflammation as well as fat deposition on parenchymal cells which alters fasting insulin and thereby reduces glucose tolerance according to previous research conducted on rat hepatocytes [39]. 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 [41]. The rearranged

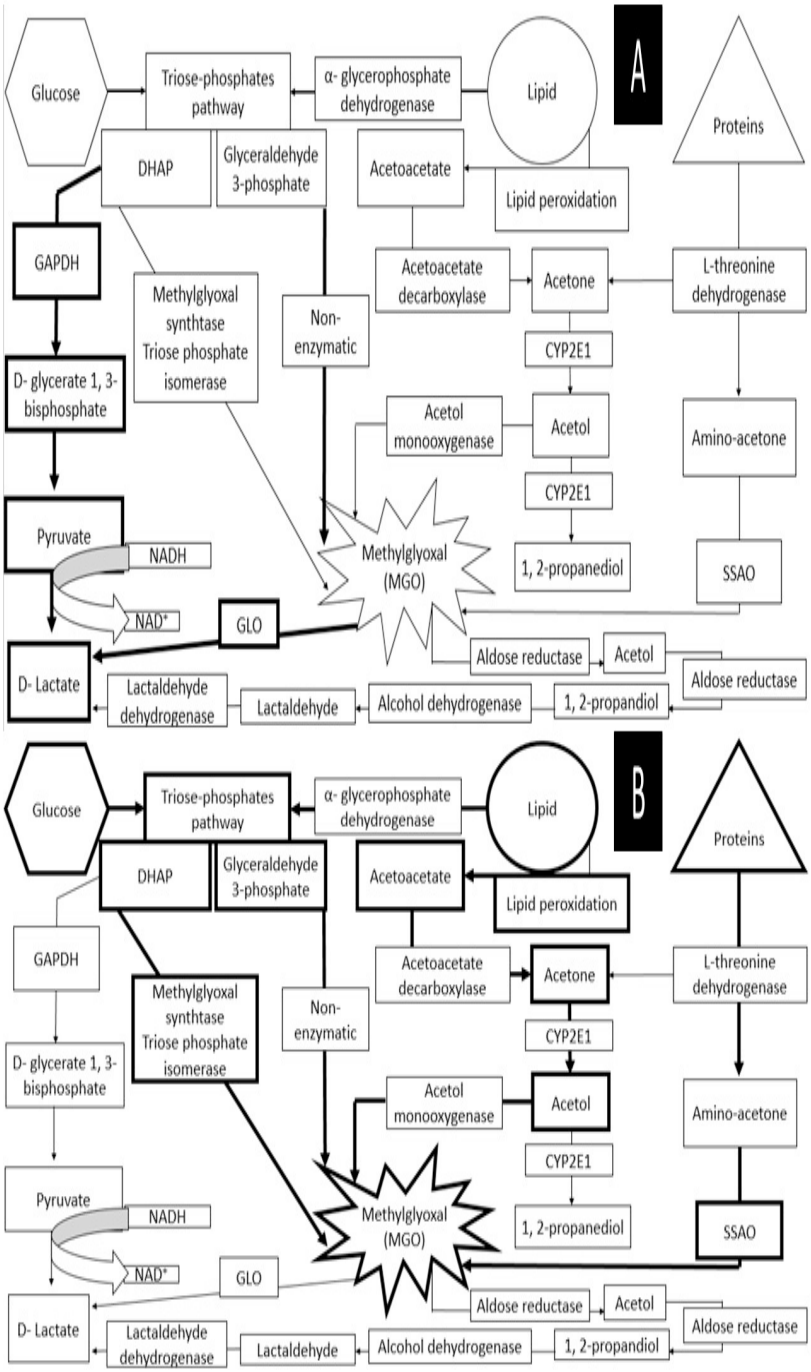

Fig. (1). Endogenous sources of methylglyoxal (MGO) from glucose, lipid and protein metabolism. (**A**) Normal condition shows low MGO production from glycolysis, lipolysis or proteolysis with major sources represented in bold arrows. (**B**) Diabetes is associated with increased MGO production from hyperglycemia, accelerated lipolysis and proteolysis represented with thick borders and bold arrows which are accompanied by compromised glyoxalase activity. Dihydroxyacetone phosphate (DHAP), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), semi-carbazide sensitive amino oxidase (SSAO).

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 [41]. These carbonyl compounds are highly reactive with amino acids to form aldehydes and α-aminoketones [41]. Previous studies found that Maillard reaction products are significantly increased in diabetics' skin collagen as CML, fructoselysine (FL) and pentosidine which are associated with accelerated aging [42]. 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 suppressing the expression and function of eNOS as well as stimulating the release of vascular cells adhesion molecules (VCAM-1) [37].

1.3. Methylglyoxal 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 is the major pathway that converts MGO to non-toxic D-lactate [17,

43]. In the GLO system, the series of reactions is regulated through substrate availability as the substrate of GLO1, hemithioacetal (HTA) inhibits GLO2, and the substrate of GLO2, free GSH inhibits GLO1 [44, 45]. The maximum enzyme velocity of GLO1 Vmax= 70.4±4.7mmol min^{-1} l^{-1} of packed erythrocytes) is approximately 3 fold higher than GLO2 (Vmax= 24±5mmol min^{-1} l^{-1} of packed erythrocytes). Moreover, GLO1 possesses higher affinity (Km= 0.46±0.04mmol) toward MGO than GLO2 (Km= 7.88±0.16mmol). Therefore, the rate limiting step of MGO metabolism is the GLO2 activity [46].

The other route of metabolism of MGO is through aldose reductase. Approximately 11% of glucose is metabolized through the sorbitol pathway through the bi-modal aldose reductase that acts as an aldehyde reductase rather than as a ketone reductase and thereby preferentially produces acetol. Acetol is then converted retrospectively to MGO through CYP2E1 to start a futile cycle that depletes the intracellular GSH and elevates acetol in diabetic plasma [17]. The reduction of MGO through aldose reductase requires NADPH to yield 95% acetol and 5% d-lactate [47]. Acetol is then reduced to L-1, 2-propanediol through aldose reductase in the presence of NADPH, and L-1, 2-propanediol is then metabolized into lactaldehyde and lactate through hepatic alcohol dehydrogenase and lactaldehyde dehydrogenase, respectively [47, 48]. Aldose reductase Kcat toward MGO= $142min^{-1}$ with Kcat/Km= $1.8x10^{7}$ $M^{-1}min^{-1}$ [47].

1.4. Methylglyoxal 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 channels (K_{ATP}) closure. Once K_{ATP} are closed, Calcium ions (Ca^{+2}) enter through voltage gated Ca+2 channels initiating insulin exocytosis [49]. 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 Fig. (**2**) [7]. Insulin resistance is a complex condition where a normal insulin concentration is not sufficient to mediate glucose uptake and utilization. Hence, more insulin is released to try to maintain glucose homeostasis [50, 51]. MGO has been shown to bind to insulin through targeting arginine residues located in chain B and at N-terminus [50]. MGO-modified insulin chain B is heavier than free insulin by an additional 126Da and is less effective in stimulating glucose uptake and utilization. Reduced glucose uptake 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 [50]. Downstream elements of the insulin signaling pathway are also affected. Insulin receptor substrate-1 (IRS-1) phosphorylation and PI3K activity are both suppressed dose dependently following MGO and reversed with MGO scavenger; N-acetylcysteine [51] (Table **1**).

In addition, MGO (100μM) induces cytotoxicity when applied to RINmf5 insulin secreting cells in culture [12]. Such an effect is comparable to the apoptosis inducing effect of high glucose (16mM) on rat pancreatic β-cells [12]. These findings suggest that MGO might play a major role in progressive stages of diabetes where chronic hyperglycemia yields elevated MGO that reduces insulin signaling and pancreatic β-cell numbers leading to a further reduction in insulin secretion [12] (Fig. **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 [52].

Additionally, plasma, 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 [52].

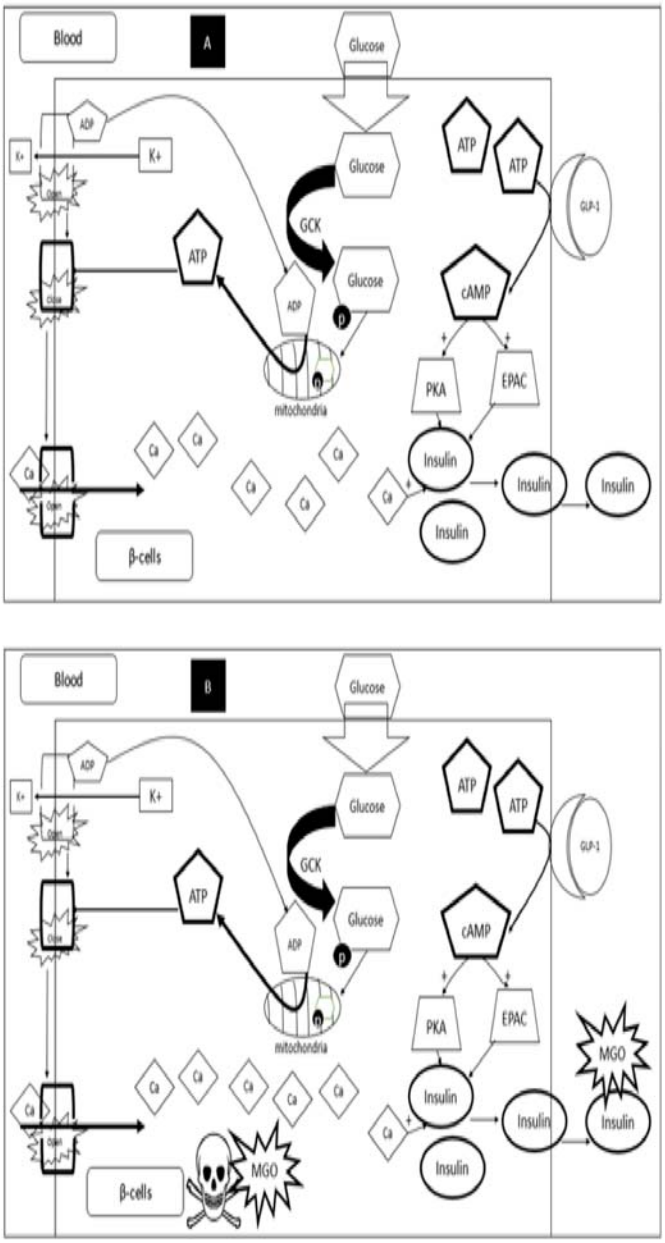

Fig. (2). Insulin release from pancreatic β-cells through calcium dependent and independent pathways. GLUT-1 takes up glucose which is metabolized through glucokinase (GCK) to glucose-6-phophate which is metabolized in the mitochondria to generate ATP. A rise in [ATP] stimulates the closure of potassium ATP channels and depolarization 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 mechanisms which are activated through cyclic adenosine monophosphate (cAMP). Plus signs indicate 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.

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 expose 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 [51, 53, 54, 55]. Therefore, MGO-induced insulin dysfunction (reduced secretion and increased resistance) might directly cause vascular dysfunction, a common complication in diabetes [54] (Fig. **3**).

1.5. Methylglyoxal and Vasculature

Numerous authors have linked elevated MGO levels with vascular and other end organ damage such as nephropathy and neuropathy [56]. Vascular dysfunction is a common complication in DM which may culminate with renal failure, blindness and limb amputation [57, 58]. As a highly reactive aldehyde, MGO forms stable adducts when reacting with multiple macromolecules through preferentially targeted aminoacids such as 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 [13].

MGO inhibits eNOS through inhibiting the phosphorylation of serine 1177, thereby inhibiting NO production and vascular relaxation [57] (Fig. **3**). Moreover, MGO (100μM) induces NO production and H2O2 generation in rat thoracic aortic smooth muscle cells (ASMC) [56]. Furthermore, induced NO reacts with peroxides (O.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 [56]. Immunohistochemical-based observations in kidneys from diabetic patients show increased argpyrimidine formation in arteries from diabetic patients suggesting MGO induces arterial injury as a further DM complication [59].

GLO-1-overexpression or MGO scavengers such as diacetyl cysteine restores Vascular function in STZ treated animals and is accompanied by MGO and AGE reduction [57, 58] (Table **1**). MGO is also a redox-based cell signaling regulator [56], which oxidizes GSH to GSSH through irreversible binding to arginine and thereby alters the cellular redox system pushing cells towards oxidative stress-induced apoptosis [50]. MGO blocks insulin-stimulated eNOS phosphorylation at serine-1177 and threonine-497 and also inhibited tyrosine phosphorylation of IRS-1 and Akt [54]. These effects of exogenous MGO are mimicked by GLO-1 inhibition [54]. Moreover, *in vivo* studies on mice administered i.p MGO 50-75mg/kg/day for 5 consecutive days each week for 7 weeks showed significant insulin resistance accompanied with compromised endothelial function. This was attributed to IRS-1 inhibition through serine-616 phosphorylation and eNOS signaling suppression [54] (Fig. **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 [60]. Tetrahydropyrimidine (THP), an AGE product of MGO was elevated in T1DM compared to non-diabetics (115.5U μl^{-1} vs 109.8U μl^{-1}) 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 elevated MGO is associated with vascular dysfunction [61].

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 [62].

In addition to all these vascular complications, the lifespan of erythrocytes (RBC) is reduced in diabetes. The MGO concentration

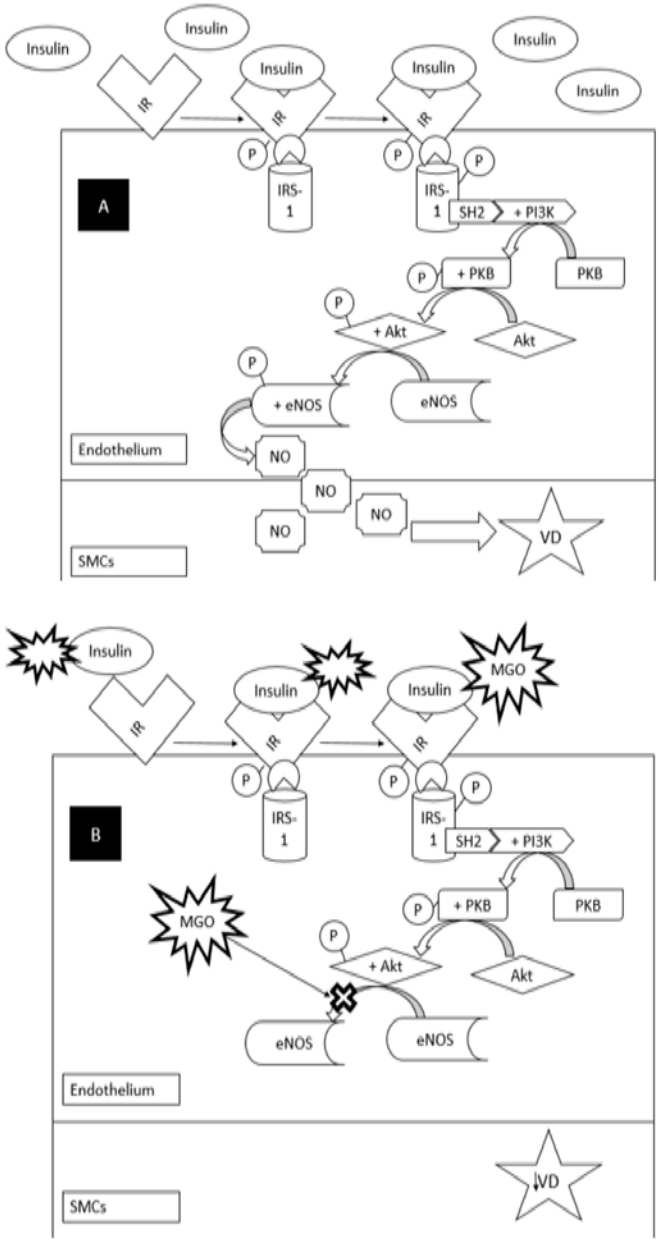

Fig. (3). Methylglyoxal (MGO) induces vascular dysfunction by interfering with insulin signaling. (**A**) Normal endothelial vasodilation stimulated through insulin binding to its insulin receptor (IR) which is phosphorylated to expose an intracellular binding site for insulin receptor substrate-1 (IRS-1) which is phosphorylated upon binding. Phosphorylated IRS-1 provides a sulfhydryl (SH2) residue which is then bound with phosphatidylinositol 3-kinase (PI3K) that phosphorylates protein kinase B (PKB) PKB 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. This results in less NO production and compromised endothelial vasodilation.

is doubled in diabetics' RBC and elevated by fourfold in diabetics' plasma compared to non-diabetics' [63]. 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 accelerate eryptosis [63, 64]. Moreover, ROS elevation

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 • Insulin release, GLUT-4, PI3-K and adipose glucose uptake reduction [52]
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 • L8-cells showed significant reduction in glucose uptake • MGO binds INS-1E cells and reduces Insulin negative feedback [50]
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 [12]
Human plasma and erythrocytes	MGO 30-300μM was shown to accelerate eryptosis	• HPLC analysis showed plasma and RBC MGO elevation • MGO reduced GSH and ATP in RBC • MGO dose dependent annexin-V-positive elevation [63]
Mouse aortic endothelial cells	MGO 500μM induces endothelial dysfunction through interfering endothelial insulin signalling	• Western blotting: Inhibiting IRS-1, Akt and eNOS phosphorylation [54]
Human plasma	MGO derivatives were associated with endothelial dysfunction	• ELISA measurements showed sVCAM-1 and sPLA2 elevation associated with THP in T1DM patients [61]
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 [58]
Wistar and Goto-Kakizaki rats	MGO induces endothelial dysfunction even when ingested (50-75mg/kg/day for 3 months)	• Cholinergic vasodilation significant impairment • Aortic IHC showed significant reduction in free NO production accompanied with increase in superoxide generation • Western blotting showed significant suppression of phosphorylated and total vasodilator stimulated phosphoprotein • Vascular inflammation through increased monocyte chemoattractant peptide-1 • (MCP-1) [60]
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 • Cell free microplate blocked wells showed significant aggregation tendency of MGO-bound LDL • IHC: MGO increased LDL retention in rats aorta • MGO-bound LDL binds significantly more to LDL receptors found on HepG2 and BJ cells [66]
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 [57]
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 • Calcium imaging reveals MGO binding to cysteine preferably to induce calcium entry • TRPA1 or TRPV1 KO DRG showed significant lack of response to MGO [14]

Table (1) contd….

Model	Diabetes related effect	Significant findings
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 • MGO depolarizes sensory neurons and modifies Nav1.8 to increase the neuronal excitation and facilitate nociception • MGO slows Nav1.7 inactivation • 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 • MGO increases blood flow to nociceptive cerebral regions [43]
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 [62]

in hemodialysis patients (HD) suggests that cell injury may be a consequence of methylglyoxal induced RBC injury [17, 65].

1.6. Methylglyoxal 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, 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 [14]. A recent study found plasma MGO was approximately doubled in diabetics and reached 1μM in diabetic individuals with hyperalgesia. |This was associated with increased COX-2 expression and suppressed GLO-1 activity [43]. In mice, MGO induced heat hyperalgesia in a dose dependent manner and this was also seen when GLO expression was suppressed [43]. Moreover, STZ diabetic mice showed GLO suppression and hyperalgesia [43]. MGO acutely depolarizes the resting membrane potential in mouse sensory neurons increasing their excitability. These changes in excitability were shown to be Nav1.8 –dependent, as they were absent in Nav1.8 –KO mice [43]. Molecular studies showed MGO triggers changes in Nav1.8 gating through DIII-DIV linker's arginine residue modification [43]. MGO also increases the release of calcitonin gene related peptide (CGRP) in peripheral nerves in STZ-diabetic and control mice [43]. These neuronal events are also accompanied by an increase in cerebral blood flow to areas associated with nociception [43]. A further molecular 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) [14]. TRPA1 is expressed in sensory neurons and mediates nociception through numerous noxious compounds such as the highly reactive electrophile, MGO. Sensory neurons from TRPA1 knockout mouse shows no calcium influx when treated with MGO Binding of MGO to the cysteine and lysine residues of the channel's N-terminal intracellular domain are necessary for channel activation by MGO [14].

MGO facilitates the release of CGRP from vagus and sciatic nerves as well as from the skin that contributes to nerve sensitization. MGO might contribute to neuropathic pain in diabetes [14].

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 these 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.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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