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# Evolving Evidence of Methylglyoxal and Dicarbonyl Stress Related Diseases from Diabetic to Non-Diabetic Models

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## Abstract

Methylglyoxal (MGO), a byproduct of sugar and lipid metabolic processes, is a major glycating agent. This metabolite reacts with basic residues of proteins and promotes the formation of advanced glycation end products (AGEs). Although MGO and AGEs are widely discussed in the context of diabetes, until recently, MGO was thought to result from insufficient blood sugar control. A new report reveals that plasma MGO, and not blood sugar, distinguishes diabetic patients with no pain from those with pain. This ability brings to MGO a new applicability to disease diagnosis.

Diseases with normal sugar conditions, such as hypertension, sepsis, and renal disease, are increasingly recognized as MGO-related. We review the role of MGO in drug-induced nephropathy, induction of hypertension by oral administration, and as a biomarker of sepsis. We also discuss the measurement of MGO and its stable metabolite d-lactate.

The metabolism and pathogenic mechanisms of MGO need investigation in diverse disease models. Whether MGO can be considered as an individual pathological factor will be an interesting topic.

**Keywords:** Methylglyoxal; Dicarbonyl stress; Oxidative stress; Advanced glycation end products (AGEs); Diagnostic and prognostic biomarkers

## Introduction

Methylglyoxal (MGO) and dicarbonyl stress are widely accepted as pathogenesis factors in diabetes and its related complications. Increasing evidence implicates MGO in other disease models [1]. We already know that diabetes affects a large number of diverse individuals, who have similar disease courses and complications. Methylglyoxal is up to 20,000-fold more reactive than glucose in glycation processes [2]. In diabetic patients, MGO and MGO-related advanced end products (AGEs) are responsible for many diabetes-related complications [3-5]. Recent studies have shown that the impacts of MGO reach far beyond blood sugar level, as we had previously assumed. Plasma MGO, rather than blood sugar, can distinguish between diabetic patients with neuralgia and those without [6]. Moreover, diabetic nephropathy is associated with MGO-related AGEs rather than $HbA1_C$, which reflects the long-term blood sugar level [5,7,8].

High MGO levels are found in diabetic patients, and are thought to be due to excess blood sugar [1,9-11]. Methylglyoxal is formed through non-oxidative mechanisms from triose phosphates during anaerobic glycolysis. This metabolite can modify amino acids, nucleic acids, and proteins [12]. Methylglyoxal reacts with arginine, lysine, and cysteine residues of proteins to form AGEs [12]. Methylglyoxal and its downstream products, AGEs, are together called dicarbonyl stressors. They are well-known contributors to the development of diabetic complications. Recently, dicarbonyl stress has been established as a pathogenesis factor in diseases other than diabetes, such as renal failure [13-17], hypertension [18,19], and sepsis [20]. These diseases present normal blood sugar, yet they share some features with diabetes. A feature common to these carbonyl-stress conditions is systemic damage causing disease progression and complications. Methylglyoxal and AGEs are thought to stimulate chronic low-grade inflammation, lower pain thresholds, and promote oxidative stress [1]. Many reviews discuss the role of MGO in diseases such as nephropathy, hypertension, and atherosclerosis. However, almost all of these reviews rely on studies of diabetes and diabetic complications. Growing evidence shows that MGO and dicarbonyl stress may not only contribute to complications in these systemic diseases, but may also contribute to initial disease pathogenesis. We review MGO-induced stress and dicarbonyl-induced stress in diabetic and non-diabetic disease models.

## Methylglyoxal generation, metabolism and damage

The major physiological sources of MGO are from degradation of the triosephosphates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Methylglyoxal is also produced, to a lesser extent, by gluconeogenesis, glyceroneogenesis, and photosynthesis. Most MGO is metabolized through glyoxalase 1 (GLO1) and 2 (GLO2) into d-lactate; the kidneys can eliminate a small amount of MGO. Methylglyoxal processing consumes glutathione (GSH) [12]. The glyoxalase system is crucial for fighting against dicarbonyl stress. The binding of MGO and GLO1 is the reaction rate-determining step. Most studies of MGO in diabetes and cancer focus on GLO1 function [1,21]. The effect of MGO on GLO1 is two-pronged. At low concentrations of MGO (0.3 mM), the

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glyoxalase system is induced. At high MGO (0.75 mM) concentrations, the glyoxalase system is inhibited and down regulated in nerve cells [7]. When MGO concentration is within a tolerable range, the cell up-regulates GLO1 to hasten detoxification. However, extreme increases in MGO abundance may cause allosteric binding to GLO1 and inhibit this enzyme's function [22]. Different tissues and organs have different quantities of glutathione and GLO1 [23]. The extent to which MGO can cause damage may be distinct in different tissues.

Methylglyoxal reacts with proteins, causes protein dysfunction, and generates AGEs [24]. The major MGO-related AGEs include MG-H1 and $N^{\varepsilon}$-(1-carboxyethyl)lysine (CEL) [25]. In brief, AGEs cause protein dysfunction, and activate the receptor for advanced glycation end products (RAGE). Activation of RAGE causes chronic inflammation and increases oxidative stress [26]. Methylglyoxal also reacts with DNA and forms the glycated DNA derivatives imidazopurinone (MdG) and $N^{2}$-(1-carboxyethyl)deoxyguanosine. Methylglyoxal causes cellular toxicity via reactive oxygen species. Methylglyoxal metabolism depletes intracellular GSH. Induced superoxide production also occurs in a dose dependent fashion [27]. Methylglyoxal induces cell apoptosis [28-30], or direct cellular toxicity with necrosis. The tissue or cellular regulation of MGO metabolism in response to different stress or damage conditions is not fully understood. Facing tissue damage, some diseases exhibit extremely high MGO levels locally, with only minor elevation of systemic MGO levels [13,16].

## Methylglyoxal and nephropathy in diabetic and non-diabetic models

Methylglyoxal is accumulated in chronic renal patients and considered a possible mechanism for renal failure-related complications. Moreover, MGO is widely studied in diabetic nephropathy (DMN), and confirmed as an important factor for progression of renal injury. Recently, a study also revealed that MGO contributes to the pathogenesis of non-diabetic renal diseases, such as aminoglycoside nephropathy (AN) and aristolochic acid nephropathy (AAN).

Extreme carbonyl stress (elevated serum MGO) is observed in uremic patients [31,32], and may be responsible for complications in chronic renal failure. Higher serum MGO is associated with high AGEs, and both may be associated to uremic related vasculopathy and amyloidosis. In addition, the red blood cell MGO level is heightened for renal failure patients without diabetes [31]. Interestingly, levels of AGEs in uremic subjects correlate to levels of serum MGO, but not blood sugar; not all chronic renal failure patients have diabetes [33]. This correlation implies that carbonyl stress from MGO and AGE accumulation can be an important pathway even in non-diabetic diseases. High sugar influx cannot explain the origin of MGO in renal failure subjects.

It is not clear where the dicarbonyl stress originates. In non-diabetic rat models of acute renal failure – achieved with bilateral nephrectomy or ureter ligation – loss of renal function accompanies elevated serum MGO and carbonyl stress-related AGEs [34]. The majority of MGO is metabolized through the glyoxalase system; while the rest is cleared by the kidneys and excreted via urine [24]. Loss of renal clearance is not the cause of high MGO levels in renal failure subjects. Further exploration of the MGO accumulation mechanism and the glyoxalase system in renal failure patients is needed.

The critical role of MGO in diabetes is widely studied. Heightened serum levels of MGO and MGO-related AGEs in DM patients can predict early progression of (DMN), according to a type I DM cohort study of serial renal biopsies [5,9]. Another study focusing on type II DM also reveals serum MGO level is associated with DMN. Compared to the traditional diabetic marker hemoglobin A1C ($HbA1_C$), skin AGEs such as MG-H1 correlate better with diabetic nephropathy parameters. Another study shows that urine d-lactate, a downstream metabolite of MGO, is higher in DMN subjects than healthy subjects [35-37]. These clinical data suggest MGO is crucial for progression of diabetic nephropathy [4,38].

Methylglyoxal is metabolized through the glyoxalase system, and carbonyl stress overwhelms this system in DM patients. The role of MGO in DMN can be demonstrated by administering MGO to rats; this manipulation to the rats' glyoxalase systems interferes with their tissue MGO levels [4,39]. Chronic ingestion of MGO in Wistar-Kyoto rats induces hypertension and renal arterial hyperplasia [40]. In this normal glycemic model, no renal function deterioration, a typical pathology of diabetic nephropathy, or microalbuminuria is identified. In streptozotocin-induced diabetic rats, using mangiferin to up-regulate glyoxalase 1 can prevent diabetic nephropathy progression [41]. Overexpression of glyoxalase 1 mitigates AGE and oxidative stress levels in hyperglycemic streptozotocin-induced diabetic rats [42]. Glyoxalase I knockout mice accumulate MGO and MG-H1 in their kidneys, and show typical DMN pathology, with mesangial expansion and thickened glomerular basement membrane (GBM) [39]. Dicarbonyl stress, combining both MGO accumulation and dysfunction of glyoxalase, explains the pathogenesis of DMN.

The role of dicarbonyl stress in other kidney injury models is not clear; however, initial results are promising. Only recently has its role as a possible mechanism for other kidney injuries been revealed.

Aristolochic acid nephropathy (AAN) is a notorious renal disease caused by ingestion of plant material containing aristolochic acid; usually this plant material is acquired from a herbal supplement. Aristolochic acid nephropathy is characterized by acute to chronic interstitial nephritis, renal failure, and increased risk of urothelial carcinoma [43]. This disease entity is newly discovered, and the definite mechanism is not clear. One study group exhibits elevated MGO levels in renal tissue 2 weeks after AA exposure, accompanied by renal failure (established using blood and urine biochemistry). The AGEs in renal tissue are also present in AAN mice [13]. Low-molecular-weight chitosan is a known MGO-chelating agent, established by *in vitro* and *in vivo* experiments [14]. In an AAN mouse model, administration of low-molecular-weight chitosan after aristolochic acid exposure mitigates renal accumulation of MGO and AGE, reverses glutathione depletion, and prevents further renal failure [15].

Aminoglycosides are antibiotics for treating bacterial infections in humans. Aminoglycoside administration causes acute renal failure in 10-25% of patients [44-46]. The mechanism of renal injury in aminoglycoside nephropathy (AN) is not fully understood. In AN patients, excess aminoglycoside accumulation in renal tubular cells, followed by tubular cell death, results in acute renal failure [44]. A previous study finds accumulation of MGO and AGEs in renal tissue accompanied with overt renal failure within 1 week following aminoglycoside exposure in mice [16]. Using low-molecular-weight chitosan also reverses MGO and AGE increases and prevents further renal dysfunction [14]. Metformin is used as anti-diabetic agent and is approved by US Food and Drug Administration as an MGO lowering agent [9]. Interestingly, one study has shown metformin can prevent AN in mouse, although it does not elucidate the interaction of this drug with carbonyl stress processes [47].

Regarding MGO metabolism in the progression of renal failure, MGO is metabolized into d-lactate by glyoxalase and excreted into urine. Micro-albuminuria can represent progression of DMN and is used as a predictor of DMN related renal failure. Even pre-micro-albuminuria phase diabetic patients (albumin-to-creatinine ratio, ACR, less than 30 mg albumin/mg creatinine) have elevated urine d-lactate levels compared with normal control. As the ACR worsens to 30-299 (micro-albuminuria), the urine d-lactate level increases [37]. In AAN mice models, urine d-lactate is also higher, and can reflect the renal injury [48]. This observation supports the hypothesis that carbonyl stress involves not only DMN, but other models of renal injury as well.

Carbonyl stress is not only involved in diabetes-related nephropathy. Carbonyl stress involves other kinds of nephropathy, such as AN and AAN [13,16]. More studies are required to understand the role of MGO in progression of renal failure in different kind of model. Low-molecular-weight chitosan, a MGO chelating agent, is efficacious in mouse models of diabetes [49], AN, and AAN [14,15]. Study of non-diabetic nephropathy helps us understand carbonyl stress in alternative modes of renal failure and devise potential treatments.

## Methylglyoxal and hypertension

Hypertension is a multi-factorial disease; its exact pathogenesis remains unclear. Hypertension is a crucial component of metabolic syndrome and its numerous complications. These hypertensive complications include different vascular diseases, such as myocardial infarction, stroke, and renal failure. The role of MGO in hypertension has been studied in diabetic subjects. The serum MGO level in diabetes is higher than in normal subjects [9], and the higher serum MGO level is proven as a predictor of hypertension in diabetes [18]. However, there are few clinical results addressing the role of MGO in non-diabetic patients. It is not clear whether MGO-related hypertension is a different clinical entity necessitating a different clinical course. Possible mechanisms of MGO in hypertension onset and complication have been also studied in both animal and cellular non-diabetic models [18,19,35,50-52].

Although lack of solid clinical evidence, MGO is considered an important contributor to the pathogenesis of hypertension without diabetes, based on animal models [19]. In spontaneously hypertensive rat (SHR) models, serum MGO levels are higher than those of control Wistar Kyoto (WKY) rats. Higher MGO, AGEs, and oxidative stress are also found in SHR renal tissue [35] and aortic endothelia [51]. Activated NF-kappaB and enhanced ICAM-1 expression in aortic tissue in SHR model accompany elevated MGO, AGEs, and oxidative stress [52]. Elevated MGO in vascular endothelia, cytosolic calcium and renal vascular hyperplasia are also observed in genetic, fructose-, threonine-, ethanol-, and salt-induced rat models of hypertension [19]. In chronic MGO-treated Wistar-Kyoto (WKY) rats, ingestion of MGO causes hypertension and hyperplasia in renal arterioles; N-acetyl cysteine can prevent MGO related hypertension and vascular damage [40]. Methylglyoxal-treated Wistar rats also exhibit reduction of NO-dependent vasorelaxation [53]. Regarding MGO-related vascular endothelial damage, MGO causes human phospho-ERK dephosphorylation and upregulation of MKP-1 activity in aortic endothelium cells [54]. In humans, rupture-prone atherosclerotic plaque contains more MGO-related AGEs than stable plaque contains; loss of vascular function and heightened vascular resistance contribute to hypertension and its related complications [55]. In non-diabetic hypertension, MGO is a key factor both *in vitro* and animal model. Further clinical study is needed to clarify MGO's role in the pathogenesis of human hypertension.

## Methylglyoxal and sepsis

Compared with routine diagnostic markers, MGO is a more useful marker of sepsis onset, development, and remission [20]. Sepsis is an infection accompanied by a systemic inflammatory response to the infection; this response causes multi-organ dysfunction even in uninfected organ systems; these events frequently lead to death [56]. An observational clinical study uses MGO to promptly identify patients with septic shock more effectively than other indicators, including procalcitonin, C-reactive protein, soluble CD14 subtype, and interleukin-6. Besides, plasma MGO in non-survivors is significantly higher than in survivors ($p=0.018$ for 90 day survival; $p=0.008$ for 28 day survival).

Sepsis is a severe systemic response even to a localized infection. The authors hypothesize that MGO accumulation results from metabolic dysregulation and oxidative stress associated with septic shock. Impairment of MGO detoxification is also proposed as a contributory factor. However, Burke-Gaffney and Creagh-Brown note that sepsis patients typically receive large volumes of intravenous fluids, which may be unaccounted sources of MGO [57]. On the other hand, sepsis patients' plasma MGO levels are 5-fold higher than those of both the general population and post-operational patients; such MGO levels are not easy to attain by administering clinical solutions.

Regarding d-lactate, it was previously used as a marker for bowel ischemia, a special form of lactate acidosis [58-62]. D-lactate was also found as a prognostic marker for septic shock [59]. It also predicts mortality in traumatic or hemorrhagic baboons [63]. In the past, shock induced bowel ischemia with bacterial translocation was hypothesized as the main reason for elevated serum d-lactate levels. Combining previous study on MGO in septic shock patients, whether the d-lactate only reflects the bowel ischemia or excess MGO with carbonyl stress overload, is worth considering. We suggest that the roles of MGO and d-lactate leading carbonyl stress are worth evaluating in sepsis.

## Methylglyoxal in other diseases

Methylglyoxal is postulated as a key cause of systemic complications in diabetic and non-diabetic diseases. These complications occur at macrovascular (e.g., coronary artery disease and stroke) and microvascular (e.g., nephropathy and retinopathy) scales. Clinical studies increasingly seek to explain the unique pathophysiology of dicarbonyl stressor metabolism on a disease-specific basis. An individual's MGO level can increase for three reasons: endogenous MGO formation may increase, metabolism of dicarbonyls may decrease, and exposure to exogenous dicarbonyls may increase.

Higher serum levels of MGO and increasing systemic carbonyl stress are reported for diabetic patients [9]. Increasing serum MGO is reported by many clinical studies [1,9]. Serum MGO can also predict diabetes-related complications [9]. The proposed mechanism of MGO accumulation includes over-production of MGO during hyperglycemia and unmatched MGO metabolism by the glyoxalase system. In an *in vitro* study, inoculating human red blood cells in sugar for 1 hour leads to an elevated cellular MGO level. The activity of glyoxalase remains stable [10]. Red blood cells from diabetic patients also exhibit the same heightened MGO levels and stable glyoxalase activities. The steady-state concentration of intra-cellar MGO increases with the sugar influx [1]. In streptomycin-induced diabetic mice facing hyperglycemic stress, enzyme activity in the glyoxalase system increases in most organs and tissues; however, this system does not overcome the heightened MGO level [23,64]. Failure of MGO normalization is also observed in lens

epithelium cultured in sugar, even when glyoxalase is up-regulated [64]. In the literature on painful diabetic neuropathy, lower glyoxalase activity is observed in disease subjects and leads to heavier carbonyl stress [65,66]. Whether it is up-regulated or down-regulated, the glyoxalase system is overwhelmed by hyperglycemia-induced MGO overproduction.

### Methylglyoxal usage in clinical settings

Dysregulated MGO joins the set of conditions preceding diabetic complications, and marks diabetes progression and prognosis [5,8]. Plasma MGO and MGO-related AGEs can be prognostic markers for diabetic complications. Methylglyoxal lowering agents have been proposed, analogous to sugar lowering medications such as metformin [9]. However, MGO-guided therapy in diabetes is not yet demonstrated by a rigorous clinical trial, and such therapy is not popular. In renal failure patients, there are also efforts to improve dialysis methods using tests of plasma MGO [31,32]. The MGO level in renal tissue in animal models may explain MGO as an important factor. However, direct measurement of tissue MGO requires biopsy or surgical tissue, and is not validated in humans. Plasma MGO levels, determined by HPLC, may prognosticate septic shock [20]. Testing MGO requires more clinical trials exploring its prognostic abilities and potential therapeutic applications.

Most of the analytical methods used in the determination of MGO are based on high performance liquid chromatography (HPLC) using fluorometric detection [67,68]. Methylglyoxal does not exhibit intrinsic fluorescence, so pre-derivatization with a fluorophore is necessary for measurement. However, a disadvantage to fluorometric HPLC-based methods is that they are usually time-consuming and costly. Furthermore, commercially purified MGO typically contains contaminants, and dicarbonyl measurement is a process with many potential sources of interference. Rabbani and Thornally [69] have published protocols to prepare high purity MGO; these protocols improve the reliability of MGO concentration prediction [70]. Other methodologies such as GC-MS and ESI-MS have recently been used to analyze MGO [71,72]. Tissue MGO levels can be determined using the above methods, and MGO accumulation can also be demonstrated with immunohistochemical staining. Methylglyoxal assays are stable and precise, however, they are mostly based on HPLC, which is difficult to commercialize and utilize widely in daily clinical practice.

D-lactate is tested for bowel ischemia, a special type of lactate acidosis; this molecule may be a prognostic predictor for sepsis [58-62]. Urine d-lactate, the metabolite of MGO excreted in urine, may proxy for MGO and carbonyl stress, and it may be considered an early marker of diabetic injury [37]. The analytical methods used in the determination of d-lactate use HPLC [37] and liquid chromatography tandem mass spectrometry [73]. However, a newly developed d-lactate assay system, using d-lactate dehydrogenase to catalyze d-lactate and detect the catalysis with a UV-light-emitting diode, was reported recently [74].

Methylglyoxal-related AGEs may indicate carbonyl stress with greater convenience than MGO; AGEs are accepted as markers of diabetes prognosis and progression [5,38]. The measurement of AGEs is usually done with an enzyme-linked immunosorbent assay. This method can detect many samples at once and does not need expensive equipment. However, most systemic-level AGEs in non-diabetes disease models remain to be studied. The role of AGEs in acute conditions, such as septic shock and acute kidney injury, still needs to be clarified.

## Conclusion

Increasing evidence indicates that MGO-induced and carbonyl-induced stress occurs in non-diabetic normal-glycemic diseases, which include renal failure, hypertension, and sepsis. Methylglyoxal-based prognostic factors and MGO-guided treatments may be the future of inflammatory disease management. Further exploration of MGO-related markers in clinical and experimental studies would be worthwhile.

### Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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