

Commentary

Methylglyoxal and glyoxalase 1—a metabolic stress pathway-linking hyperglycemia to the unfolded protein response and vascular complications of diabetes

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The study of the glyoxalase system by Thornalley and co-workers in clinical diabetes mellitus and correlation with diabetic complications revealed increased exposure of patients with diabetes to the reactive, dicarbonyl metabolite methylglyoxal (MG). Twenty-eight years later, extended and built on by Thornalley and co-workers and others, the glyoxalase system is an important pathway contributing to the development of insulin resistance and vascular complications of diabetes. Other related advances have been: characterization of a new kind of metabolic stress—‘dicarbonyl stress’; identification of the major physiological advanced glycation endproduct (AGE), MG-H1; physiological substrates of the unfolded protein response (UPR); new therapeutic agents—‘glyoxalase 1 (Glo1) inducers’; and a refined mechanism underlying the link of dysglycemia to the development of insulin resistance and vascular complications of diabetes.

Increased formation and accumulation of the glucose-derived reactive metabolite, methylglyoxal (MG), and its metabolism by the glyoxalase pathway is currently viewed as an important contributor to metabolic dysfunction-linking hyperglycemia to the development of vascular complications of diabetes [1,2]. The study by McLellan et al. [3] was the first major study to fully characterize the glyoxalase system in clinical diabetes. It showed that there is a substantial increase in exposure of patients with diabetes to MG. Whole blood concentrations of MG were found to be increased approximately sixfold in patients with type 1 diabetes mellitus (T1DM) and three- to fourfold in patients with type 2 diabetes mellitus (T2DM)—disproportionately high compared with the two- to threefold increase in plasma glucose concentration. This disproportionate increase in MG to increase in glucose concentration was later found to be likely due to synergistic increase in the flux of MG formation and decrease in activity of glyoxalase 1 (Glo1) in tissues suffering metabolic dysfunction in hyperglycemia—reviewed in [4]. The study was preceded by methodological papers by the team of Thornalley who initiated the study, establishing reference methods for assay of metabolites and activity and genotype enzymes of the glyoxalase system, Glo1 and glyoxalase 2—including validation of preanalytic conditions of sample collection and storage (cited in [3]). This provided for robust laboratory protocols, data, and findings that have been confirmed and extended thereafter. Indeed, the study was often the starting point for other investigators in pursuing MG and glyoxalase-related research thereafter [5–11].

At the time of publication in 1994, the study of advanced glycation endproducts (AGEs) in the biomedical research was still emerging. AGEs are stable, end-stage glycation adducts formed on proteins from the degradation of fructosamines and other processes of protein glycation. Increased formation and accumulation of AGEs is linked to the development of vascular complications of diabetes, other disease, and aging [12]. N_ε-Carboxymethyl-lysine (CML) had been identified as the major AGE formed from the

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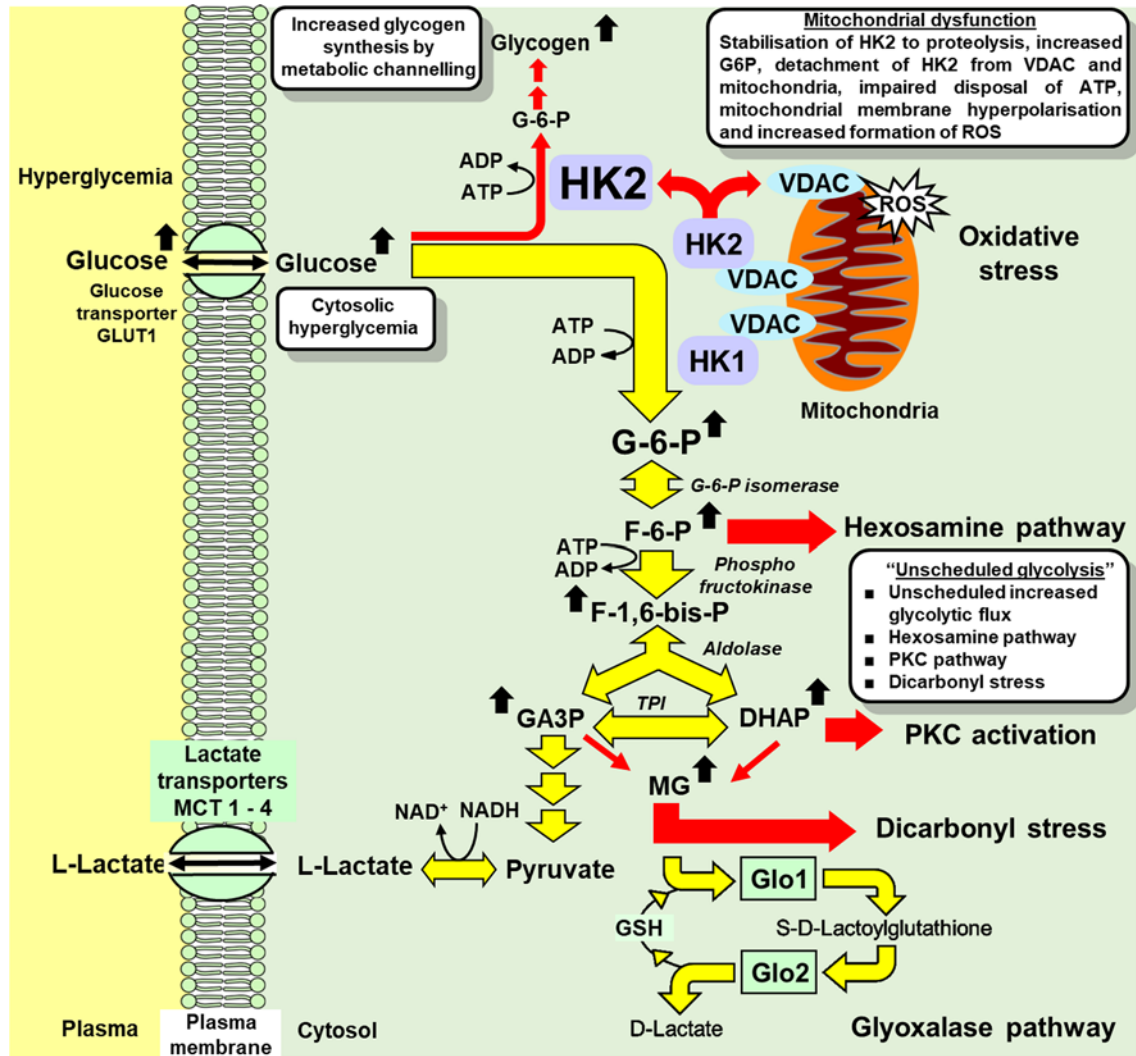


Figure 1. Glycolytic overload and unscheduled glycolysis in hyperglycemia

Key: red arrows—dysfunctional metabolism in unscheduled glycolysis. Metabolic intermediates in glycolysis from GA3P to pyruvate have been omitted for clarity. NB formation of MG and metabolism by the glyoxalase system has a flux of only approximately 0.05–0.1% of the metabolic flux through glycolysis. Abbreviations: DHAP, dihydroxyacetonephosphate; F-1,6-bis-P, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; GA3P, glyceraldehyde-3-phosphate; Glo1, glyoxalase 1; Glo2, glyoxalase 2; GSH, glutathione; HK1, hexokinase-1; HK2, hexokinase-2; MCT 1–4, monocarboxylate transporters 1–4; MG, methylglyoxal; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel. Modified from [41].

fructosamine derivative, N_ε-fructosyl-lysine (FL) [13], and a trace-level AGE with intense fluorescence formed from pentose metabolites called pentosidine had been described [14]. Thornalley and co-workers later showed that MG was the precursor of the major quantitative AGE clinically, MG-derived hydroimidazolone MG-H1 [15]—present in plasma and tissues proteins at approximately ten- and 100-fold higher than CML and pentosidine, respectively [15]. This revealed for the first time that reactive dicarbonyl metabolites such as MG are precursors of AGEs in physiological systems [16]. Indeed, MG-H1 was found to be a major AGE in diabetes, renal failure, cirrhosis, Alzheimer’s disease, arthritis, other disorders, and aging [4].

Protein glycation by MG is now recognized as particularly damaging. This is because the arginine residue target has a high prevalence in functional domains of proteins, enriched 3.8-fold; *cf.* amino acid residues susceptible to oxidative damage, cysteine, methionine, tryptophan, and tyrosine, which are sparse or 0.5- to 0.9-fold enriched in functional domains of proteins [17]. So, proteins are relatively resistant to functional impairment in oxidative stress but susceptible to modification by abnormal increase in MG—a new kind of metabolic stress called ‘dicarbonyl stress’ [4].

Formation of MG-H1 replaces a positively charged arginine residue with an uncharged, hydrophobic MG-H1 residue in the highly structured functional domains of proteins [18,19]. It thereby produces protein inactivation and misfolding. MG is particularly insidious because it also targets chaperonins for modification [18]. So, glycation by MG creates misfolded proteins and inactivates the machinery, which corrects protein misfolding. The consequence is increased of misfolded proteins in dicarbonyl stress and activation of the unfolded protein response (UPR) [18,20]. The UPR is a fundamental part of protein homeostasis, providing for refolding or degradation and clearance of misfolded proteins and activation of low-grade inflammation [21]. Latest research has revealed increased expression of heat shock proteins and ubiquitin ligases targeting removal of MG-modified proteins [18,20]. It now appears that MG-modified proteins are important physiological substrates for the UPR [22] and this provides a further link to the accumulation of MG, vascular inflammation, and development of vascular complications of diabetes.

With this link to pathogenesis in diabetes, MG-derived AGEs may be diagnostic biomarkers of vascular complications of diabetes. In tissues and body fluids, there are MG-modified proteins and MG-modified amino acids—also called protein-bound AGEs and AGE-free adducts, respectively [15]. The latter are formed mainly by cellular proteolysis of MG-modified proteins with a contribution from intestinal absorption from digested glycated proteins in food [23,24]. Protein-bound MG-H1 and N_ε(1-carboxyethyl)lysine (CEL)—a minor MG-derived AGE—contents of renal glomeruli, retina, and sciatic nerve were increased in the streptozotocin-induced diabetic rat model of experimental diabetes during the development of diabetic nephropathy, retinopathy, and peripheral neuropathy [25–27]. There were similar increases in both protein-bound and free adducts of MG-H1 and CEL in plasma [25], indicating that with clinical translation, MG-derived AGEs measured in plasma or serum may be risk markers, and possibly risk predictors of vascular complications of diabetes. Subsequent clinical studies found that plasma MG-H1 and CEL-free adduct concentrations were important features in statistical models for risk prediction of progression of diabetic kidney disease, independent of glycemic control assessed by glycated hemoglobin A1C [28–30]. Protein-bound CEL was associated with increased risk of all microvascular complications in the Joslin Medalist study of patients with very long duration of T1DM (>50 years) [31], a composite plasma AGE score including CEL was associated with risk of CVD events in patients with T2DM [32] and, in cross-sectional studies, increased serum protein-bound MG-H1 was associated diabetic retinopathy and heat pain perception threshold in peripheral diabetic neuropathy [33,34]. In other studies, associations of plasma protein-bound MG-H1 and CEL with progression of vascular complications of diabetes have not been found [35,36]. The diagnostic utility of plasma or serum protein-bound AGEs has potential interferences of increased albuminuria and turnover of albumin, change in the transcapillary escape rate of albumin and tightening of the glomerular filter by angiotensin II receptor blockers (ARBs) and similar therapeutics—as recently reviewed [12]. In all cases, however, increased MG-H1 and CEL adducts are likely reflecting increased severity of dicarbonyl stress sustained in patients and the expected consequence of increased risk of vascular complications. Finally, plasma MG and endogenous flux of formation of MG-H1 (deduced from urinary excretion of MG-H1-free adduct) were companion diagnostic measures to assess effects on target pharmacology in the development of anti-MG therapeutic agents [24].

The role of MG and its metabolism by the glyoxalase system in metabolic stress has been advanced in the intervening years by the discovery of a regulatory antioxidant response element (ARE) in the *GLO1* gene, with Glo1 ARE-linked expression inducible by activation of the cytoprotective transcription factor, nuclear factor-erythroid factor 2-related factor 2 (Nrf2) [37]. Activation of Nrf2 controls both basal and inducible expression of Glo1 in response to dicarbonyl stress and by specific small-molecule inducers of Glo1 or ‘Glo1 inducers’. Indeed, induction of Glo1 expression has emerged as an effective strategy to counter dicarbonyl stress. Initially, MG scavengers were developed as a pharmacological approach to counter dicarbonyl stress but the chemical reactivity required for effective scavenging of MG was associated instability and toxicity of prospective drug candidates. Glo1 inducers benefit from countering diabetes-associated decrease in Glo1 and removing MG catalytically at diffusion-limited rates [38]. A dietary bioactive screen of ARE-linked Glo1 expression led to the development of the optimized Glo1 inducer, *trans*-resveratrol and hesperetin combination (tRES-HESP) [24]. This has the advantage of synergism at the Nrf2 receptor for induction of Glo1 and providing improved bioavailability of *trans*-resveratrol by inhibition of intestinal glucuronosyltransferases by hesperetin [38]. In randomized, placebo-controlled double-blind cross-over clinical trial in overweight and obese subjects—the Healthy Aging Through Functional Food (HATFF or Hats-off) study, treatment with tRES-HESP for 8 weeks corrected insulin resistance and improved dysglycemia and vascular inflammation [24]. The anti-inflammatory effects included decreased expression of the receptor for AGEs, monocyte chemoattractant protein-1, interleukin-8, and cyclo-oxygenase-2. tRES-HESP is now in further development for prevention and early-stage reversal of T2DM and treatment of vascular complications of diabetes where development of a mechanism-based treatment peripheral diabetic neuropathy is a priority [39].

Further investigation of the mechanism of increased cellular concentration of MG in vascular endothelial cells led to the finding that this was produced by increased steady-state levels of triosephosphates, glyceraldehyde-3-phosphate, and dihydroxyacetonephosphate—proportionate to increased flux of glucose metabolism—and increased proteolysis of Glo1 [18]. The Thornalley team reported that low-level spontaneous degradation of triosephosphates was a major source of MG formation in 1993 [40]. This revealed that the increased concentration of triosephosphates and formation of MG is not produced by inhibition of glyceraldehyde-3-phosphate dehydrogenase, as was proposed earlier [1], but rather by increased dysregulated or ‘unscheduled’ early-stage glycolysis arising from increased accumulation and activity of hexokinase-2 (HK2) without similar change in activity of other glycolytic enzymes [18,20]. This produces a wave of increased glycolytic intermediates, accounting for increased formation of MG and also activation of protein kinase C and hexosamine pathways [41]—Figure 1. The cause of the increased HK2 activity was stabilization of HK2 to proteolysis by increased binding of glucose to the C-terminal active site during increased cytosolic hyperglycemia, masking a degradation motif linked to chaperone-mediated autophagy [18]. Increased hexokinase activity under these conditions produces increased glucose-6-phosphate concentration, displacing HK2 from mitochondria, and producing hyperpolarization of the mitochondrial membrane and increased formation of reactive oxygen species (ROS). In HK2-linked unscheduled glycolysis in hyperglycemia, increased formation of ROS is a consequence rather than a cause of metabolic dysfunction and is one of multiple dysfunctional pathways. This may explain why clinical evaluation of antioxidants for therapy of diabetic complications has been disappointing [41]. A better approach emerged when it was found that tRES-HESP corrected increased HK2 protein and activity by off-target effects mediated by Nrf2-mediated increased expression of glucose-6-phosphate dehydrogenase, decreasing cellular concentration of G6P, transcriptional signaling by Mondo A/Mlx/G6P and expression of HK2. tRES-HESP not only countered dicarbonyl stress in endothelial cells in high glucose concentration but also normalized flux of glucose metabolism and corrected metabolic dysfunction [18]. Recent considerations suggest that HK2-linked unscheduled glycolysis is established in skeletal muscle and adipose tissue in fasting hyperglycemia and this may contribute to the development of insulin resistance [41]. This bodes well for further evaluation of tRES-HESP for the prevention and early-stage reversal of T2DM and treatment of vascular complications [39].

So, from initial studies of MG in diabetes published in the *Clinical Science* [3] emerged: key insights into the biochemistry of reactive dicarbonyl metabolites and dicarbonyl stress, hydroimidazolone AGEs and physiological substrates of the UPR, risk factors for the development of insulin resistance and vascular complications of diabetes, and a new strategy for mechanism-based therapeutics through Glo1 inducers. Further research on MG and the glyoxalase system is on-going in diabetes, vascular complications of diabetes, and many other areas of clinical research—with applications in improved clinical diagnostics and therapeutics [4,12,39]. There is now much wider awareness of the clinical importance of MG and the glyoxalase system in health and disease than in 1994. First steps toward Glo1-based treatments have been encouraging [39].

Competing Interests

The author is co-owner of a patent on Glyoxalase 1 inducer mentioned in the text.

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Abbreviations

AGE, advanced glycation endproduct; ARE, antioxidant response element; CEL, N ϵ (1-carboxyethyl)lysine; CML, N ϵ -carboxymethyl-lysine; CVD, cardiovascular disease; FL, N ϵ -fructosyl-lysine; Glo1, glyoxalase 1; HESP, hesperetin; HK2, hexokinase-2; MG, methylglyoxal; MG-H1, methylglyoxal-derived hydroimidazolone; Nrf2, nuclear factor-erythroid factor 2-related factor 2; ROS, reactive oxygen species; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; tRES, *trans*-Resveratrol; UPR, unfolded protein response.

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