Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Opinion

Hexokinase-2 Glycolytic Overload in Diabetes and Ischemia–Reperfusion Injury

Naila Rabbani[1] and Paul J. Thornalley[2,*]

Hexokinase-2 (HK2) was recently found to produce increased metabolic flux through glycolysis in hyperglycemia without concurrent transcriptional or other functional regulation. Rather, stabilization to proteolysis by increased glucose substrate binding produced unscheduled increased glucose metabolism in response to high cytosolic glucose concentration. This produces abnormal increases in glycolytic intermediates or glycolytic overload, driving cell dysfunction and vulnerability to the damaging effects of hyperglycemia in diabetes, explaining tissue-specific pathogenesis. Glycolytic overload is also activated in ischemia–reperfusion injury and cell senescence. A further key feature is HK2 displacement from mitochondria by increased glucose-6-phosphate concentration, inducing mitochondrial dysfunction and oxidative stress. This pathogenic mechanism suggested new targets for therapeutics development that gave promising outcomes in initial clinical evaluation.

Highlights

HK2 produces increased metabolic flux through glycolysis in hyperglycemia through glucose-mediated stabilization of HK2 to proteolysis.

This produces abnormal increases in glycolytic intermediates or glycolytic overload, driving mitochondrial dysfunction and activation of hexosamine, protein kinase C, and dicarbonyl stress pathways.

HK2-linked glycolytic overload explains tissue-specific pathogenesis in diabetes linked to vascular complications and contributes to pathogenesis in ischemia–reperfusion injury and cell senescence.

This new pathogenic mechanism hypothesis suggested new targets for therapeutic development that gave promising outcomes in initial clinical evaluation.

Tight Control of Steady-State Concentrations of Glycolytic Intermediates

The metabolism of glucose involves initial uptake into cells by glucose transporter proteins (GLUTs) [1] and conversion to glucose-6-phosphate (G6P) by one or more hexokinase isozymes [2]. G6P is thereafter further metabolized mainly to pyruvate in glycolysis, with a minor fraction metabolized by the pentose phosphate pathway to ribose-5-phosphate. Pyruvate is further metabolized to carbon dioxide in the tricarboxylic acid cycle. Tissues with the highest *in situ* rates of glucose metabolism, such as brain, heart, and skeletal muscle, have the highest concentrations of glycolytic enzymes. The variation of glycolytic flux in tissues is matched by similar variation of glycolytic enzyme abundance and activity to achieve little change in the steady-state concentration of glycolytic intermediates throughout the body [3]. Within an individual tissue, diurnal variation in the flux of glucose metabolism is also regulated such that in normal metabolic regulation and homeostasis there are only minor increases in glycolytic intermediates with increased rate of glucose metabolism [4]. Why are the steady-state concentrations of glycolytic intermediates so tightly controlled? Here, we describe how abnormal increase of the steady-state concentrations of glycolytic intermediates, from G6P through to the triosephosphates, dihydroxyacetone phosphate (DHAP), and glyceraldehyde-3-phosphate (GA3P), produces abnormal metabolic outflow to multiple pathways which is damaging in diabetes, ischemia, and aging. Wider appreciation and more detailed understanding of this may identify targets for improved drug treatment of disease.

Scheduled and Unscheduled Glycolysis

When is increased flux through glycolysis damaging? We assert that this is when it is unregulated or unscheduled glycolysis. Early-stage glucose metabolism is regulated at four key steps: glucose uptake, hexokinase, phosphofructokinase (PFK), and import and export of lactate [5] (Figure 1). When increased flux of glucose metabolism is required to sustain cellular and tissue processes, the increased metabolism of glucose is regulated. For example, in skeletal muscle

[1]Clinical Sciences Research Laboratories, Warwick Medical School, University of Warwick, University Hospital, Coventry CV2 2DX, UK
[2]Diabetes Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, P.O. Box 34110, Doha, Qatar

*Correspondence:
pthornalley@hbku.edu.qa
(P.J. Thornalley).

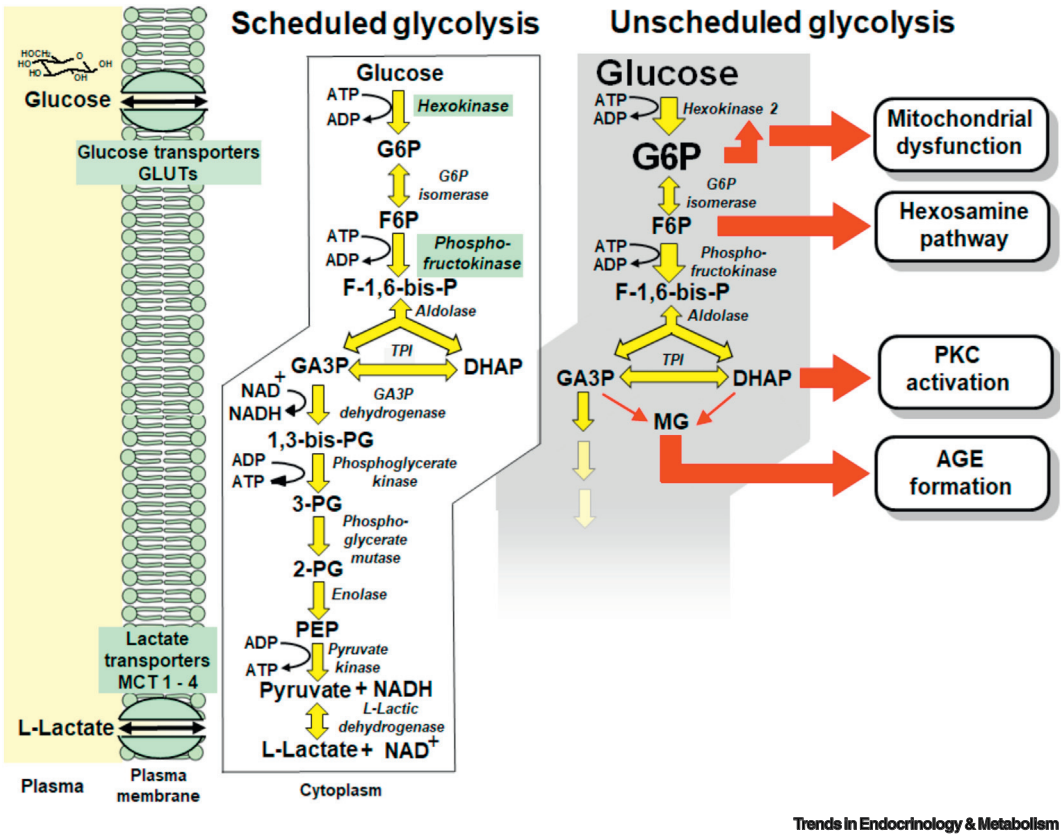

Figure 1. Key Steps of Regulation of Glycolysis and the Shadow of Metabolic Dysfunction in Unscheduled Glycolysis Linked to Increased HK2 and G6P Concentration. Downstream consequences of HK2-linked glycolytic overload. Key: green-filled text, key sites of glycolytic regulation in scheduled glycolysis; red arrows – dysfunctional metabolism in unscheduled glycolysis. Abbreviations: AGE, advanced glycation end-products; 1,3-bis-PG, 1,3-diphosphoglycerate; DHAP, dihydroxyacetone phosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; F-1,6-bis-P, fructose-1,6-bisphosphate; GA3P, glyceraldehyde-3-phosphate; G3P, glycerol-3-phosphate; HK2, hexokinase-2; MG, methylglyoxal; PEP, phosphoenolpyruvate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate.

cells and adipocytes treated with insulin, uptake of glucose is increased through enhanced recruitment of insulin-responsive GLUT4 to the cell surface and coordinated increase in expression and activity of hexokinase-2 (HK2) and activity of PFK [6–8]. Further downstream in glycolysis, expression and activity of glyceraldehyde-3-phosphate dehydrogenase (GA3PD) is increased [9]. With prolonged increase of glucose metabolism, a moderate increase in G6P occurs, which leads to binding and nuclear translocation of the Mondo A/Mlx/G6P transcription factor complex. This regulates expression of a battery of glycolytic and lipogenic genes with a functional carbohydrate response element (ChoRE) [10–12]. This provides a further tier of regulation at the initial stages of glycolytic gene expression. The paralog protein, Mondo B or carbohydrate response element binding protein (ChREBP), is dominant in the liver and adipose tissue [13]. By this regulatory control, increase in flux of glucose uptake and metabolism occurs without marked increase in G6P [4]. This is regulated or scheduled glycolysis where tight control of G6P concentration is a critical part of the regulatory machinery.

Recent research suggests that abnormal increase of HK2 plays a key role when control of glycolysis is impaired, as in unscheduled glycolysis. In this case, abnormal increase in G6P and downstream glycolytic intermediates are key players in the tissue-specific chronic pathogenesis of hyperglycemia associated with diabetes, ischemia–reperfusion injury, and potentially, in cell senescence [14–16]. We call this damaging condition glycolytic overload. The aims of this Opinion

article are: (i) to explain the causes and consequences of unscheduled glycolysis and glycolytic overload; (ii) to describe evidence of this in diabetes, ischemia–reperfusion injury and cell senescence; and (iii) to indicate how the HK2-mediated glycolytic overload hypothesis may be reversed in a new approach to pharmacological therapy.

Involvement of HK2 in Unscheduled Glycolysis and Downstream Glycolytic Overload

HK2 has high expression in skeletal muscle and adipose tissue as part of scheduled glycolysis therein [17]. HK2 expression is also often increased in tumors through promoter methylation and amplification of the HK2 gene [18]. Concomitant increased activity of PFK and expression of glucose-6-phosphate dehydrogenase (G6PD) supports increased flux through glycolysis for rapid tumor growth without increase in G6P concentration [19,20]. HK2 is also expressed in other tissues. It is mostly absent in neurons of the central and peripheral nervous system [17]. Research now suggests that increased HK2 activity is often the initiator of unscheduled glycolysis and downstream metabolic dysfunction driving pathogenesis.

Two unique molecular and functional properties of HK2 (Box 1 and Figure 2), compared with those of other HKs, confer an important role in dysregulation of glycolysis. Downstream consequences are mitochondrial dysfunction and activation of multiple pathways of regulatory dysfunction (Box 2). Indeed, when HK2-linked glycolytic overload occurs in hyperglycemia, there is a surge of increased glycolytic metabolites in early-stage glycolysis starting at G6P and reaching as far as DHAP and GA3P. Outflow to linked signaling pathways creates metabolic and cellular dysfunction where there is usually scheduled glycolysis and homeostasis (Figure 3). This paradigm was demonstrated in human aortal endothelial cells (HAECs) in model hyperglycemia where cell dysfunction was corrected by reversing glycolytic overload through decreasing expression of HK2.

Box 1. Molecular Characteristics of HK2 Supporting Unscheduled Glycolysis

(i) Two Catalytically Active Subunits are Stabilized to Inactivation and Proteolytic Degradation by Increased Substrate Binding in High Cytosolic Glucose Concentration.
This produces increased HK2 protein and activity with corresponding glucose metabolism without concomitant increase in downstream glycolytic enzyme activities or glycolytic overload. HK2 stabilization to proteolysis by high glucose concentration is mediated by glucose binding to the N- and C-terminal domains increasing stability of the enzyme and masking in the C-terminal domain chaperone-mediated autophagy motif $_{712}$QRFEK$_{716}$ from binding to heat shock protein cognate 70 [101,102]. Stabilization of HK2 to degradation was found in response to high cytosolic glucose concentration in HAECs in primary culture [14]. HK2 has a turnover fivefold higher than that of HK1, so change in HK2 protein abundance may have a marked effect on total hexokinase activity; both hexokinases often operate *in situ* under glucose substrate saturation conditions [2].

(ii) Labile Attachment to the Mitochondrial Membrane
This is mediated by binding to voltage-dependent anion channels (VDAC) on the mitochondrial outer membrane under normal metabolic conditions of scheduled glycolysis. VDAC provides a conduit for HK2 to access ATP from the intramembrane space of mitochondria for G6P formation [105]. HK2 displacement by increased concentrations of G6P [14,106], twofold increase in HAECs in hyperglycemia [107], and three- to tenfold increase in myocardial tissue in ischemia [32,33], impairs ATP utilization, and impairs oxygen consumption analogous to that found under conditions of inhibition of ADP recycling. HK2 is only weakly product inhibited by G6P and its activity *in situ* is maintained when G6P concentration is abnormally in high in glycolytic overload; so increased formation of G6P continues, exacerbating and prolonging mitochondrial dysfunction [2]. HK2 displacement from VDAC increases mitochondrial membrane potential, forcing the respiratory chain complexes into a reduced state and increasing electron leakage to oxygen with increased ROS formation [108]. A counter-effect, increased attachment of HK2 to mitochondria, is produced by phosphorylation of HK2 at Thr-473 by protein kinase B (PKB/Akt) [109,110]. Insulin and other growth factor signaling through Akt may, therefore, play a key role in suppressing mitochondrial dysfunction in unscheduled glycolysis. Conversely, when this counter-regulation is diminished, HK2 displacement from mitochondria becomes more facile – as applies to insulin deficiency and insulin resistance in type 1 and type 2 diabetes, respectively [111,112].

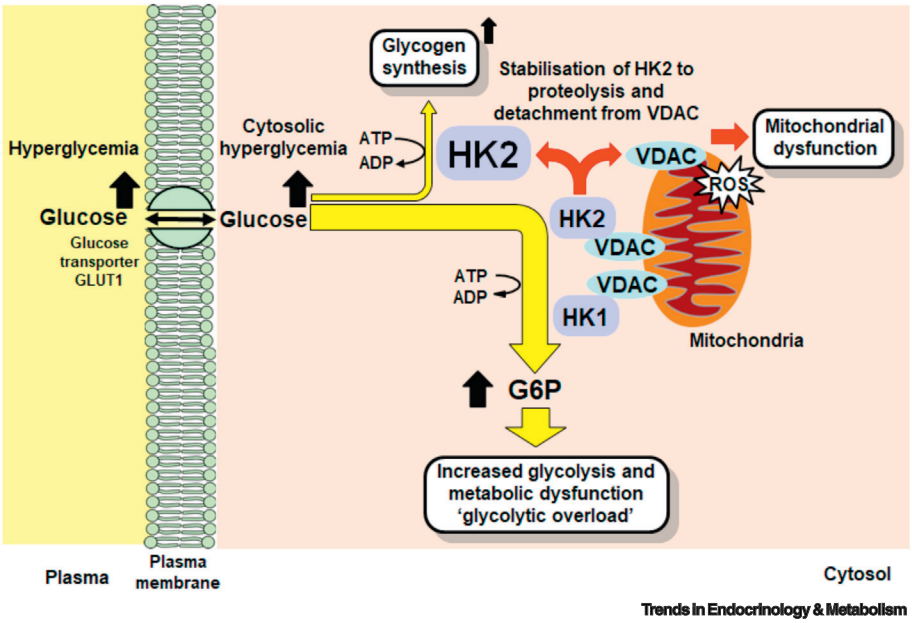

Figure 2. Schematic Diagram of Initiation of Unscheduled Glycolysis and Glycolytic Overload in Cells with GLUT1-Dependent Glucose Uptake and HK2 Expression in Hyperglycemia (Vascular Complications of Diabetes and Diabetic Embryopathy). Glycolytic overload also develops through glycogenolysis in ischemia (cardiac reperfusion injury) and abnormal increase in expression of HK2 (on the approach to cell senescence). Abbreviations: G6P, glucose-6-phospate; GLUT1, glucose transporter 1; HK, hexokinase; MG, methylglyoxal; PKC, protein kinase C; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel. Modified, with permission, from [14].

HAECs incubated in primary culture in high glucose concentration showed selective increase of HK2 protein without change in HK2 mRNA. Selective increase in HK2 was discovered by quantitative characterization of the cytosolic proteome by label-free quantitative proteomics analysis and validated by western blotting. This was associated with increased flux of glucose metabolism [14], corroborating outcomes from previous metabolic tracer studies [21]. A metabolic marker of metabolic dysfunction was increased flux of formation of methylglyoxal (MG) and MG-derived advanced glycation end-products (AGEs). The increased flux of formation of MG was proportional to that of increased glucose consumption, ~0.05%, and was prevented by correction of increased glucose metabolism by siRNA silencing or pharmacologically induced decrease of HK2 expression. The extent of increase of MG formation was that expected by increased glycolytic flux

Box 2. Metabolic Consequences of HK2-Driven Glycolytic Overload

(i) Mitochondrial dysfunction leading to increased formation of ROS and oxidative stress [106].
(ii) Increased fructose-6-phosphate leading to increased formation of glucosamine-6-phosphate and activation of the hexosamine pathway [113].
(iii) Increased DHAP leading to increased metabolic outflow to glycerol-3-phosphate and diacylglycerol *de novo*, activating the protein kinase C pathway [114].
(iv) Increased DHAP and GA3P leading to increased formation and concentration of MG, with related formation of MG-derived AGEs – the dicarbonyl stress pathway [45]. Increased MG-derived AGEs produce increased protein misfolding and thereby activate the UPR in the cytosolic and endoplasmic reticulum, including increasing downstream inflammatory and prothrombotic mediators [14].
(v) Increased glycogen deposition – HK2 displacement from mitochondria increases glycogen synthesis through metabolic channeling of glucose to glycogen synthesis [106]. Stable isotopic labeling studies indicate that there is discrete channeling of G6P into glycolysis and glycogen synthesis [115]. Abnormal increased glycogen synthesis and deposition is a sensitive metabolic indicator or biomarker of HK2 detachment from mitochondria and related metabolic dysfunction [14].

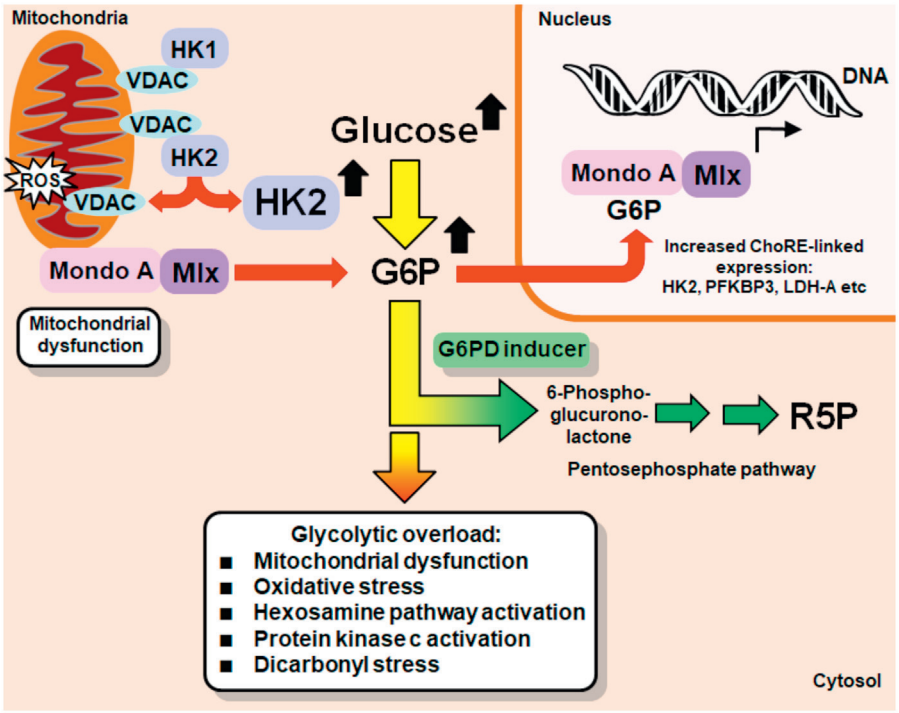

Figure 3. Schematic Diagram of Alleviation of Glycolytic Overload by Induction of G6PD Expression. Decrease of G6P concentration allow reattachment of HK2 and Mondo A/Mlx to mitochondria and decrease of ChoRE-linked expression of HK2. Red tipped arrows: potentially damaging effects; green arrows, target pharmacology for G6PD inducer therapeutics. Abbreviations: ChoRE, carbohydrate response element; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; HK, hexokinase; LDH-A, lactic dehydrognase, isoform A; PFKBP3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3; R5P, ribose-5-phosphate; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel.

and related to increased steady-state concentrations of MG precursors, GA3P and DHAP. Correction of unscheduled glycolysis and glycolytic overload prevented increased glucose metabolism in model hyperglycemia and thereby may correct all metabolic dysfunction [14].

Evidence of HK2-Linked Glycolytic Overload in Diabetes and Ischemia–Reperfusion Injury

Key criteria for HK2-linked glycolytic overload are: (i) expression of HK2; and (ii) susceptibility to cytosolic high glucose concentrations – often produced by high affinity glucose transporter uptake of glucose independent on the activity of insulin (GLUT1 and/or GLUT3) in hyperglycemia. Key biomarkers of this process are: (i) abnormal glycogen deposition; and (ii) one or more of the downstream dysfunction pathways linked to glycolytic overload (Box 2) [14]. Inspection of the literature reveals widespread evidence for HK2-linked glycolytic overload involvement in the development of vascular and other complications of diabetes and cardiac ischemia–reperfusion injury (Table 1).

The strongest evidence of involvement of HK2-linked glycolytic overload in cell dysfunction is for endothelial dysfunction in diabetes, microvascular complications of diabetes – diabetic nephropathy, retinopathy, and neuropathy, and diabetic embryopathy. Increased basal expression of HK2 is also linked to rapid progression of diabetic nephropathy [22]. Constituent cell types exhibiting the criteria for susceptibility to HK2-linked driven glycolytic overload are: vascular endothelial cells; mesangial cells, podocytes and tubular epithelial cells of the kidney; endothelial cells,

Table 1. Evidence for Hexokinase-Driven Glycolytic Overload in Aging and Disease

Pathogenesis	Tissue/cell type	Indications	Refs
Diabetic endothelial dysfunction	Endothelial cells	1. Increased glucose metabolism in hyperglycemia through stabilization of HK2 to proteolysis 2. Glycogen accumulation induced by high glucose concentration *in vitro* and hyperglycemia *in vivo* 3. Downstream metabolic dysfunction (DS, HP, MD, OS, PKC)[a]	[14,80–82]
Diabetic nephropathy	Renal mesangial cells, podocytes, and tubular epithelial cells	1. Increased HK2 protein in human mesangial cells by high glucose concentration *in vitro* 2. Abnormal glycogen deposition in proximal and renal tubules 3. Downstream metabolic dysfunction (DS, HP, MD, OS, PKC)	[83–88]
Diabetic neuropathy	Schwann cells (also dorsal root ganglia and sciatic nerve)	1. Increased HK2 in hyperglycemia 2. Glycogen accumulation in association with demyelination and axonal degeneration in clinical diabetic neuropathy 3. Downstream metabolic dysfunction (DS, MD, OS)	[89–94]
Diabetic retinopathy	Müller cells, endothelial cells, and pericytes (also intact retina)	1. HK2 expression in human retina 2. Abnormal glycogen accumulation 3. Downstream metabolic dysfunction (DS, HP, MD, OS, PKC)	[88,95–99]
Diabetic embryopathy	Early-stage embryo (typically rat embryo, day 9–11 gestation)	1. HK2-dependent glucose metabolism in early stage embryo development. 2. Increased embryo glycogen content after culture in high glucose concentration *in vitro* 3. Downstream metabolic dysfunction (DS, HP, MD, OS, PKC)	[23,26–30]
Cardiac reperfusion injury	Myocardium and cardiomyocytes	1. HK2 expression, high G6P and HK2 detachment from mitochondria. HK2 detachment correlates with infarct size. 2. Downstream metabolic dysfunction (DS, MD, OS)	[33,34,100]
Cell senescence	Dermal and lung fibroblasts	1. Increased HK2 expression and glucose consumption on the approach to senescence 2. Glycogen accumulation in cell senescence 3. Downstream metabolic dysfunction (DS, MD, OS)	[16,52–54]

[a]Abbreviations: DS, dicarbonyl stress; HP, hexosamine pathway; MD, mitochondrial dysfunction; OS, oxidative stress PKC, protein kinase C pathway.

Muller cells and pericytes of the retina; and Schwann cells of the peripheral nervous system. Similar pathogenesis of lower severity is expected in prediabetes.

A further complication of diabetes where HK2-linked glycolytic overload is likely is diabetic embryopathy. Early-stage embryonic cells – up to day 10 postconception for mouse embryos – have mainly glucose uptake by GLUT1 and GLUT3 and HK2-linked glycolysis [23,24]. From days 2 to 10 postconception of embryo development, glucose metabolism is mainly anaerobic glycolysis – predisposing embryo development to dysregulation in the early-stage glycolysis during this period [25]. All biomarkers of HK2-linked glycolytic overload have been recorded for early-stage embryos in high glucose concentration cultures and experimental diabetic embryopathy: glycogen deposition and multiple downstream pathways of metabolic dysfunction [23,26–30]. Moreover, tissue-specific progenitor cells – such as endothelial and cardiac progenitor cells – have similar glucose uptake and HK2 expression. Their dysfunction in diabetes is likely driven by HK2-linked glycolytic overload and also contributes to the development of diabetic complications [31].

A condition associated with high concentrations of G6P with an as yet pathogenic mechanism that is not fully understood and where improvements in therapy are keenly sought is ischemia–reperfusion injury. In the myocardium, cardiac ischemia following myocardial infarction and cardiac arrest activates glycogenosis. This meets the substrate demand for glycolysis, particularly in switching to mainly anaerobic glycolysis in the restricted nutrient and oxygen flow to the myocardium in ischemia. Glycogenosis is considered to be the major cause of a three- to tenfold increase in G6P concentration in the myocardium in ischemia–reperfusion injury. There is concomitant

increase in the cellular glucose concentration and dissociation of HK2 from mitochondria [32,33]. The extent of mitochondrial dissociation of HK2 during ischemia correlated with mitochondrial cytochrome c release and related cell death, reactive oxygen species (ROS) production, and infarct size on reperfusion [34]. A cell-permeable peptide containing the HK2 mitochondrial binding motif that induced displacement of HK2 from mitochondria, TAT-HK2 (TAT peptide, GRKKRRQRRRPQ, is from the transactivator of transcription of HIV), exacerbated cardiac reperfusion injury [15], suggesting that HK2 displacement from mitochondria is a contributing feature to the pathogenesis. HK2 protein was increased and increasingly displaced from mitochondria in ischemia–reperfusion injury of patients with diabetes. For many years the source of increased ROS formation in cardiac ischemia–reperfusion injury was under intense debate where mitochondrial dysfunction was identified as a key source but the initiation mechanism was unclear [35]. Re-evaluation of the pathogenic mechanism of reperfusion injury after cardiac arrest in terms of HK2-linked glycolytic overload may lead to improved treatments.

Similar pathogenesis may apply to reperfusion injury following ischemia in the kidneys – a particular problem in kidneys donated for transplantation after cardiac arrest where there is often long exposure to ischemia. Renal ischemia leads to impairment of tubular epithelial cell function, development of acute kidney injury and poor outcomes post-transplantation [36]. An examination of glycolytic overload remains to be performed but MG-linked dicarbonyl stress appears to be a pathogenic feature [37].

Increased HK2 in Hypoxia May not Produce Glycolytic Overload

There is a hypoxia response element in the gene promoter region of the HK2 gene and hence HK2 expression is increased in hypoxia through activation and binding of hypoxia-inducible factor (HIF)1 [38,39]. HK2 activity in hypoxia is also increased by Tp53-induced glycolysis and apoptosis regulator (TIGAR) [40]. Hypoxia-induced increased expression of HK2 may be considered as a potential driver of glycolytic overload. HIF1 and related hypoxia-activated HIF2, however, also induce expression and activity of downstream glycolytic enzymes – PFK-muscle type (PFK-M), regulatory enzymes 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 and -4, aldolase, triosephosphate isomerase (TPI), and GA3PD [38]. This suggests that, unlike in glycolytic overload where only HK2 activity is increased, in hypoxia the flux of glucose metabolism may increase without an increase in G6P and other glycolytic intermediates because of a coordinated increased onward rate of metabolism of glycolytic intermediates [41,42]. A remaining potentially insidious effect, however, is the down regulation of glyoxalase (Glo)1 in hypoxia [43,44] – the major enzyme that metabolizes and detoxifies MG [45]. The steady-state concentration of MG and related AGE formation may thereby increase in hypoxia and may contribute to increased MG concentration of hypoxic adipose tissue in obesity [46].

HK2-Linked Glycolytic Overload in Cell Senescence

HK2-linked glycolytic overload may be a key mediator of cell senescence. Extended culture of human MRC-5 embryonic lung fibroblasts and human dermal BJ fibroblasts produces a gradual slowing of increase in cumulative population doubling level as replicative senescence is approached [47,48]. It has long been known that there is a progressive increase in glucose consumption with cumulative cell population doubling level, characterized by a marked threefold increase in glucose consumption and sixfold increase in hexokinase activity during the onset of senescence [49]. This was attributed to increased HK2 expression, as judged by increased HK2 mRNA and protein, and total hexokinase activity in senescent human fibroblasts was reported previously [49–51]. The approach to senescence is characterized by mitochondrial dysfunction, oxidative stress, dicarbonyl stress, and deposition of glycogen [16,52–54]. Decrease of HK2 expression on the approach to senescence was achieved by repeated treatment with sulforaphane – an activator of transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2),

which regulates expression of a battery of cytoprotective genes through binding to regulatory antioxidant response element (ARE) in target genes [55,56]. A likely key target was induction of G6PD expression, which has a functional ARE in the promoter region, which in turn decreases G6P by diverting it into the pentose phosphate pathway [20]. This suppressed senescence-associated glycolytic overload, prevented oxidative stress and delayed senescence [16].

HK2-linked glycolytic overload explains increased glycolysis, mitochondrial dysfunction, oxidative stress, and glycogen deposition in senescence and delay of senescence by glucose, caloric restriction, and caloric restriction mimetic compounds [16,52–54]. It thereby adds to current hypotheses of cell senescence, improving explanations for key experimental observations [57]. The relevance of replicative senescence to human aging is claimed through the observation of increased β-galactosidase activity, a biomarker of senescence, in the skin of elderly people [58] and association of cell senescence in human disease [57]. Indications of increased susceptibility to glycolytic overload in aging are: increased steady-state concentrations of G6P and other glycolytic intermediates in skeletal muscle and liver of aging mice [59]; the involvement of mitochondrial dysfunction in senescent features of the aging phenotype [60]; and likely contribution of decreased G6P concentration to the delay of onset of age-associated pathological and physiological changes by experimental caloric restriction [61].

Improved Explanation for Tissue Susceptibility to Damaging Effects of Hyperglycemia, Glycogen Deposition, Circumvention of the 'Hexokinase Block', and Metaflammation

There is evidence that some cell types and related tissues are resistant to HK2-linked glycolytic overload. In skeletal muscle and adipose tissue, glucose uptake and metabolism may be faster than in other tissues damaged by HK2-linked glycolytic overload [17,62]. There are, however, high concentrations of glycolytic enzymes, including hexokinases, TPI, and GA3PDH, in skeletal muscle and adipose tissue, and increased glycolysis is regulated by insulin such that increased G6P and triosephosphate concentrations are avoided [3]. High basal levels of TPI, GA3PDH, and Glo1 [63], may also explain the relative protection of skeletal muscle from dicarbonyl stress in type 2 diabetes where Glo1 expression is decreased [64].

Neurons of the central nervous system (CNS) may be thought potentially susceptible to unscheduled glycolysis since they exhibit relatively high rates of glycolysis and high affinity glucose uptake by GLUT1 and GLUT3. Indeed, consistent with expected increased neuronal glucose concentration of the CNS in diabetes, we found increased fructosyl-lysine content of brain cytosolic protein in experimental diabetes [65]. CNS neurons are mainly protected for glycolytic overload by the absence or low expression of HK2 [17]. The short length of axons in the CNS also confers markedly lower dependence on oligodendrocytes for functional support of axons than the profoundly longer axons of the peripheral nervous system on Schwann cells. The latter are also susceptible to HK2-linked glycolytic overload in hyperglycemia. This may confer susceptibility of the peripheral nervous system to pathogenesis in hyperglycemia and explain the high prevalence of peripheral neuropathy and absence of CNS encephalopathy in diabetes [66].

For the first time, therefore, the HK2-linked glycolytic overload hypothesis explains tissue susceptibility to the damaging effects of hyperglycemia, providing a rational basis for locations in the body where there is susceptibility to the damaging effects of hyperglycemia and development of the complications of diabetes. It also explains the abnormal deposition of glycogen at sites where complications develop, why basal HK2 expression may be a risk predictor of complications progression, the mechanistic basis for resistance of vascular complications of diabetes to antioxidant therapy, and the mechanistic basis for circumvention of the 'hexokinase block' – increased glucose metabolism induced by high glucose concentration when hexokinases present,

HK1 and HK2, are saturated with glucose substrate. The latter effect – a longstanding and apparently intractable problem in diabetes research now resolved – is produced by glucose-induced stabilization of HK2 to proteolysis, increasing turnover number of HK2, and increased hexokinase activity [14]. Related to this, GLUT1 (and/or GLUT3) glucose uptake is a criterion for glycolytic overload but not rate-limiting for glucose metabolism under the normal range of expression. There is evidence for increased GLUT1 expression in endothelial cells in persistent hyperglycemia [67] but this does not exacerbate HK2-linked glycolytic overload because HK2 is the rate-limiting step in this process. HK2-driven glycolytic overload also contributes to immune-metabolic dysregulation of lipotoxicity and metaflammation (Box 3). These features are unexplained by current hypotheses.

Improved Approaches to Therapy by Targeting Hexokinase-2 Driven Glycolytic Overload

A key prediction of the HK2-linked glycolytic overload hypothesis is that the initiating metabolic event – increased HK2-catalyzed glycolysis – is not susceptible to inhibition by antioxidants. This may explain disappointing outcomes of clinical trials with antioxidant intervention for treatment of vascular complications of diabetes [68–70]. The hypothesis implicates increased HK2 protein and G6P concentration as key initiators of pathogenesis. A test of the hypothesis is to suggest and evaluate therapeutic agents designed to counter this pathogenic mechanism.

One approach to therapeutic intervention is prevention of the accumulation of G6P and displacement of HK2 from mitochondria. This may be achieved by inducing increased expression of G6PD and diverting G6P to the pentose phosphate pathway. This corrects increased HK2 protein by decreasing ChoRE-linked expression of HK2 at sites susceptible to glycolytic overload. Mediated by the Mondo A/Mlx/G6P functional complex, decreasing G6P is expected to curb expression of HK2 and other ChoRE-regulated genes [10]. Mondo A/Mlx/G6P also induces expression of thioredoxin interacting protein (TXNIP) – a key mediator of insulin resistance in type 2 diabetes and contributor to vascular inflammation [10,71,72]. Mondo A/Mlx is also bound to the mitochondrial outer membrane and is released for translocation to the nucleus by increased G6P concentration [73], therefore, antiglycolytic and anti-lipogenic gene expression is expected (Figure 3). Induction of G6PD expression was achieved by the combination of *trans*-resveratrol and hesperetin (tRES-HESP), which synergize to activate Nrf2 and induce expression of G6PD via a functional ARE [74]. tRES-HESP normalized HK2 protein levels, glucose consumption, formation of MG, and other dysfunction of HAECs in model hyperglycemia [14]. Importantly for *in vivo* studies, HESP inhibits intestinal glucuronosyl transferases, facilitating uptake and bioavailability of tRES and HESP when coadministered. This circumvents the poor bioavailability of tRES that has stymied previous attempts to translate health benefits of tRES from the experimental to clinical setting [75]. In a recent clinical trial in overweight and obese subjects, tRES-HESP treatment for 8 weeks decreased MG, suppressed vascular inflammation, and reversed insulin resistance; whereas the placebo had no effect [74]. A similar effect of inducing expression of G6PD

Box 3. Contribution of HK2-Driven Glycolytic Overload Hypothesis to Immuno-metabolic Dysregulation of Lipotoxicity and Metaflammation

(i) Explains activation of MondoA/Mlx/G6P transcriptional activity, which has a key role in prolipogenic transcriptional signaling, lipid balance, and lipotoxicity [109,116].
(ii) Provides a mechanism of increased oxidative and MG-mediated lipoprotein modification, contributory drivers of the proatherogenic transformation of low-density lipoprotein and destabilization of high-density lipoprotein in dyslipidemia and 'response to injury' hypotheses of atherosclerosis [103,104,117].
(iii) Provides a contributory driver to immuno-metabolic dysregulation of lipotoxicity through increased cellular MG as a physiological activator of the UPR, producing increased inflammatory response and chronic metabolic inflammation or metaflammation [14,118].

was available in human skin fibroblasts by treatment with the dietary activator of Nrf2, sulforaphane – found in broccoli. Chronic treatment with sulforaphane, once weekly, at concentrations similar to those achieved through dietary broccoli consumption, decreased HK2 expression and glucose consumption and delayed cell senescence [16]. Further optimization of small molecule inducers of G6PD expression may produce improved approaches to treatment of disease where HK2-linked glycolytic overload is implicated in the pathogenic mechanism and provide improved dietary supplements for healthy aging.

Precedent of Damaging Increase of Glycolytic Intermediates: Triosephosphates and Formation of MG

The highest expressed glycolytic enzymes in mammalian tissues are TPI and GA3PD, which catalyze the interconversion and metabolism of reactive triosephosphate glycolytic intermediates, GA3P and DHAP, respectively [3]. Triosephosphates undergo trace level spontaneous degradation to the reactive dicarbonyl metabolite, MG. MG is an endogenous mutagen and impairs proteome integrity, with activation of the unfolded protein response (UPR) or stimulation of proteome repair when proteome damage is excessive [14,76–78]. High expression of TPI and GA3PD keeps steady-state concentrations of GA3P and DHAP at low levels and thereby minimizes the flux of formation of MG. In human cells in primary culture, degradation of triosephosphates to MG accounts for only ~0.05% glucose metabolism [14,79]. When glycolytic flux is increased in response to insulin, G6P/Mondo A/Mlx or HIF signaling, concomitant increased expression of GA3PD serves to maintain low concentrations of triosephosphates and MG formation. This provides a precedent of how regulation of glycolytic enzyme expression serves to minimize an increase in steady-state concentrations of potentially damaging glycolytic intermediates and metabolites derived therefrom.

Concluding Remarks

HK2 expression emerged as a prerequisite for glycolytic overload in cellular metabolism and high G6P concentration a contributing mediator to downstream metabolic dysfunction. G6P is, perhaps, an unlikely insidious metabolite. The tight regulation of enzymes involved in the formation and onward metabolism of G6P, however, suggests that mechanisms of control of metabolite concentrations in glycolysis have evolved to keep G6P concentration below levels that displace HK2 and Mondo A/Mlx from mitochondria and thereby avoid the related potentially damaging consequences. In hyperglycemia, ischemia, and cell senescence, this protective control may be lost and increased HK2 and G6P initiate metabolic dysfunction leading to tissue damage. The hypothesis of HK2-linked glycolytic overload provides an improved explanation of pathogenic mechanisms and experimental observations associated with the development of vascular complications of diabetes and prediabetes, diabetic embryopathy, tissue progenitor cell dysfunction in diabetes, ischemia-linked reperfusion injury, and cell senescence. It also provides a route to new therapeutics through induction of G6PD expression, which may have benefits beyond those previously often-considered limited to antioxidant effects. The hypothesis now deserves further testing through experimental investigation, mathematical modeling of glycolytic metabolism, and G6PD inducer pharmacological agent development and evaluation (see Outstanding Questions). A tractable strategy for the latter is through small molecule activators of Nrf2 and induction of G6PD expression through binding Nrf2 to the functional ARE in the G6PD gene promoter.

Outstanding Questions

Can the hypothesis of HK2-linked glycolytic overload be demonstrated in renal, retinal, Schwann cells, and early-stage embryos suffering metabolic dysfunction in hyperglycemia?

Can HK2 displacement from mitochondria in model hyperglycemia be visualized as it happens by live cell, time-lapse microscopy?

Can further evidence of HK2-linked glycolytic overload be found in clinical diabetes?

Can further evidence of HK2-linked glycolytic overload be found in experimental and clinical myocardial ischemia–reperfusion injury?

Can HK2-linked glycolytic overload be convincingly simulated by mathematical metabolic modeling?

Will small molecule activators of Nrf2, inducers of G6PD expression provide effective treatment for endothelial cell dysfunction and vascular complications of diabetes and effective therapy to limit myocardial damage in post-cardiac arrest reperfusion injury?

Acknowledgments

P.J.T. thanks Qatar Biomedical Research Institute (QBRI) and the Qatar Foundation for support for his research program.

References

1. Thorens, B. and Mueckler, M. (2010) Glucose transporters in the 21st Century. *Am. J. Physiol. Endocrinol. Metab.* 298, E141–E145
2. Traut, T. (2008) Hexokinase. In *Allosteric Regulatory Enzymes*, pp. 179–198, Springer, USA
3. Wiśniewski, J.R. *et al.* (2015) Integrating proteomics and enzyme kinetics reveals tissue-specific types of the glycolytic and gluconeogenic pathways. *J. Proteome Res.* 14, 3263–3273

4. Noguchi, R. *et al.* (2013) The selective control of glycolysis, gluconeogenesis and glycogenesis by temporal insulin patterns. *Mol. Syst. Biol.* 9, 664
5. Tanner, L.B. *et al.* (2018) Four key steps control glycolytic flux in mammalian cells. *Cell Systems* 7, 49–62.e8
6. Printz, R.L. *et al.* (1993) Hexokinase II mRNA and gene structure, regulation by insulin, and evolution. *J. Biol. Chem.* 268, 5209–5219
7. Osawa, H. *et al.* (1996) Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. *J. Biol. Chem.* 271, 16690–16694
8. Probst, I. and Unthan-Fechner, K. (1985) Activation of glycolysis by insulin with a sequential increase of the 6-phosphofructo-2-kinase activity, fructose-2,6-bisphosphate level and pyruvate kinase activity in cultured rat hepatocytes. *Eur. J. Biochem.* 153, 347–353
9. Nasrin, N. *et al.* (1990) An insulin response element in the glyceraldehyde-3-phosphate dehydrogenase gene binds a nuclear protein induced by insulin in cultured cells and by nutritional manipulations *in vivo*. *Proc. Natl. Acad. Sci.* 87, 5273–5277
10. Sans, C.L. *et al.* (2006) MondoA-Mlx heterodimers are candidate sensors of cellular energy status: mitochondrial localization and direct regulation of glycolysis. *Mol. Cell. Biol.* 26, 4863–4871
11. Jeong, Y.S. *et al.* (2011) Integrated expression profiling and genome-wide analysis of ChREBP targets reveals the dual role for ChREBP in glucose-regulated gene expression. *PLoS One* 6, e22544
12. Ma, L. *et al.* (2006) ChREBP•Mlx is the principal mediator of glucose-induced gene expression in the liver. *J. Biol. Chem.* 281, 28721–28730
13. Arden, C. *et al.* (2012) Fructose 2,6-bisphosphate is essential for glucose-regulated gene transcription of glucose-6-phosphatase and other ChREBP target genes in hepatocytes. *Biochem. J.* 443, 111–123
14. Irshad, Z. *et al.* (2019) Activation of the unfolded protein response in high glucose treated endothelial cells is mediated by methylglyoxal. *Sci. Rep.* 9, 7889
15. Nederlof, R. *et al.* (2017) Reducing mitochondrial bound hexokinase II mediates transition from non-injurious into injurious ischemia/reperfusion of the intact heart. *J. Physiol. Biochem.* 73, 323–333
16. Hariton, F. *et al.* (2018) Sulforaphane delays fibroblast senescence by curbing cellular glucose uptake, increased glycolysis, and oxidative damage. *Oxidative Med. Cell. Long.* 2018, 5642148
17. Gardiner, N.J. *et al.* (2007) Expression of hexokinase isoforms in the dorsal root ganglion of the adult rat and effect of experimental diabetes. *Brain Res.* 1175, 143–154
18. Mathupala, S.P. *et al.* (2006) Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene* 25, 4777–4786
19. Yu, L. *et al.* (2017) The glycolytic switch in tumors: how many players are involved? *J. Cancer* 8, 3430–3440
20. Mitsuishi, Y. *et al.* (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* 22, 66–79
21. Quijano, C. *et al.* (2007) Enhanced mitochondrial superoxide in hyperglycemic endothelial cells: direct measurements and formation of hydrogen peroxide and peroxynitrite. *AJ. - Heart Circ. Physiol.* 293, H3404–H3414
22. Iori, E. *et al.* (2008) Glycolytic enzyme expression and pyruvate kinase activity in cultured fibroblasts from type 1 diabetic patients with and without nephropathy. *Biochim. Biophys. Acta (BBA) - Mol. Basis Dis.* 1782, 627–633
23. Wolf, A. *et al.* (2011) Developmental profile and regulation of the glycolytic enzyme hexokinase 2 in normal brain and glioblastoma multiforme. *Neurobiol. Dis.* 44, 84–91
24. Segev, H. *et al.* (2012) The expression of the class 1 glucose transporter isoforms in human embryonic stem cells, and the potential use of GLUT2 as a marker for pancreatic progenitor enrichment. *Stem Cells Dev.* 21, 1653–1661
25. Johnson, M.T. *et al.* (2003) Intermediary metabolism and energetics during murine early embryogenesis. *J. Biol. Chem.* 278, 31457–31460
26. Yang, X. *et al.* (1995) Altered mitochondrial morphology of rat embryos in diabetic pregnancy. *Anat. Rec.* 241, 255–267
27. Eriksson, U.J. *et al.* (1998) Teratogenicity of 3-deoxyglucosone and diabetic embryopathy. *Diabetes* 47, 1960–1966
28. Horal, M. *et al.* (2004) Activation of the hexosamine pathway causes oxidative stress and abnormal embryo gene expression: involvement in diabetic teratogenesis. *Birth Defects Res. A Clin. Mol. Teratol.* 70, 519–527
29. Ellington, S.K.L. (1997) Effects of excess glucose on mammalian post-implantation embryos. *Internat. J. Dev. Biol.* 41, 299–306
30. Hiramatsu, Y. *et al.* (2002) Diacylglycerol production and protein kinase C activity are increased in a mouse model of diabetic embryopathy. *Diabetes* 51, 2804–2810
31. Rodrigues, M. *et al.* (2015) Progenitor cell dysfunctions underlie some diabetic complications. *Am. J. Pathol.* 185, 2607–2618
32. Jennings, R.B. *et al.* (2001) Metabolism of preconditioned myocardium: effect of loss and reinstatement of cardioprotection. *J. Mol. Cell. Cardiol.* 33, 1571–1588
33. Jennings, R.B. *et al.* (1991) Energy metabolism in preconditioned and control myocardium: Effect of total ischemia. *J. Mol. Cell. Cardiol.* 23, 1449–1458
34. Pasdois, P. *et al.* (2013) Extent of mitochondrial hexokinase II dissociation during ischemia correlates with mitochondrial cytochrome c release, reactive oxygen species production, and infarct size on reperfusion. *J. Am. Heart Assoc.* 2, e005645
35. Li, C. and Jackson, R.M. (2002) Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am. J. Phys. Cell Phys.* 282, C227–C241
36. Saat, T.C. *et al.* (2016) Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: lost in translation? *J. Transl. Med.* 14, 20
37. Kumagai, T. *et al.* (2009) Glyoxalase I overexpression ameliorates renal ischemia-reperfusion injury in rats. *AJ. - Renal Physiol.* 296, F912–F921
38. Schödel, J. *et al.* (2011) High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood* 117, e207–e217
39. Lee, H.G. *et al.* (2016) Regulation of HK2 expression through alterations in CpG methylation of the HK2 promoter during progression of hepatocellular carcinoma. *Oncotarget* 7, 41798–41810
40. Cheung, E.C. *et al.* (2012) Mitochondrial localization of TIGAR under hypoxia stimulates HK2 and lowers ROS and cell death. *Proc. Natl. Acad. Sci. U. S. A.* 109, 20491–20496
41. Li, Y. *et al.* (2018) Hexokinase 2-dependent hyperglycolysis driving microglial activation contributes to ischemic brain injury. *J. Neurochem.* 144, 186–200
42. Nyengaard, J.R. *et al.* (2004) interactions between hyperglycemia and hypoxia: implications for diabetic retinopathy. *Diabetes* 53, 2931–2938
43. Shafie, A. *et al.* (2016) Re-appraisal of putative glyoxalase 1 deficient mouse and dicarbonyl stress on embryonic stem cells *in vitro*. *Biochem. J.* 473, 4255–4270
44. Zhang, H. *et al.* (2012) HIF1α is required for survival maintenance of chronic myeloid leukemia stem cells. *Blood* 119, 2595–2607
45. Rabbani, N. *et al.* (2016) Methylglyoxal-induced dicarbonyl stress in aging and disease: first steps towards glyoxalase 1-based treatments. *Clin. Sci.* 130, 1677–1696
46. Masania, J. *et al.* (2016) Dicarbonyl stress in clinical obesity. *Glycoconj. J.* 33, 581–589
47. Wistrom, C. and Villeponteau, B. (1990) Long-term growth of diploid human fibroblasts in low serum media. *Exp. Gerontol.* 25, 97–105
48. Saretzki, G. *et al.* (1998) Similar gene expression pattern in senescent and hyperoxic-treated fibroblasts. *J. Gerontol. a-Biol. Scie. Med. Sci.* 53, B438–B442
49. Zwerschke, W. *et al.* (2003) Metabolic analysis of senescent human fibroblasts reveals a role for AMP in cellular senescence. *Biochem. J.* 376, 403–411
50. Yoon, I.K. *et al.* (2004) Exploration of replicative senescence-associated genes in human dermal fibroblasts by cDNA microarray technology. *Exp. Gerontol.* 39, 1369–1378
51. James, E.L. *et al.* (2015) Senescent human fibroblasts show increased glycolysis and redox homeostasis with extracellular

metabolomes that overlap with those of irreparable DNA damage, aging, and disease. *J. Proteome Res.* 14, 1854–1871
52. Robbins, E. *et al.* (1970) Morphologic changes accompanying senescence of cultured human diploid cells. *J. Exp. Med.* 131, 1211–1222
53. Passos, J.F. and von Zglinicki, T. (2005) Mitochondria, telomeres and cell senescence. *Exp. Gerontol.* 40, 466–472
54. Lawless, C. *et al.* (2010) Quantitative assessment of markers for cell senescence. *Exp. Gerontol.* 45, 772–778
55. Malhotra, D. *et al.* (2010) Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res.* 38, 5718–5734
56. Xue, M. *et al.* (2015) Frequency modulated translocational oscillations of Nrf2 mediate the ARE cytoprotective transcriptional response. *Antioxid. Redox Signal.* 23, 613–629
57. Childs, B.G. *et al.* (2015) Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat. Med.* 21, 1424–1435
58. Dimri, G.P. *et al.* (1995) A biomarker that identifies senescent human-cells in culture and in aging skin *in-vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 92, 9363–9367
59. Houtkooper, R.H. *et al.* (2011) The metabolic footprint of aging in mice. *Sci. Rep.* 1, 134
60. Correia-Melo, C. *et al.* (2016) Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J.* 35, 724–742
61. Hagopian, K. *et al.* (2003) Influence of age and caloric restriction on liver glycolytic enzyme activities and metabolite concentrations in mice. *Exp. Gerontol.* 38, 253–266
62. DeFronzo, R.A. *et al.* (1981) The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30, 1000–1007
63. Xue, M. *et al.* (2014) Measurement of glyoxalase gene expression. *Biochem. Soc. Trans.* 42, 495–499
64. Mey, J.T. *et al.* (2018) Dicarbonyl stress and glyoxalase enzyme system regulation in human skeletal muscle. *Am. J. Phys. Regul. Integr. Comp. Phys.* 314, R181–R190
65. Karachalias, N. *et al.* (2010) Increased protein damage in renal glomeruli, retina, nerve, plasma and urine and its prevention by thiamine and benfotiamine therapy in a rat model of diabetes. *Diabetologia* 53, 1506–1516
66. Feldman, E.L. *et al.* (2017) New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain. *Neuron* 93, 1296–1313
67. Gosmanov, A.R. *et al.* (2006) *De novo* emergence of insulin-stimulated glucose uptake in human aortic endothelial cells incubated with high glucose. *Am. J. Physiol. Endocrinol. Metab.* 290, E516–E522
68. Lonn, E. *et al.* (2002) Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes – results of the HOPE study and MICRO-HOPE substudy. *Diabetes Care* 25, 1919–1927
69. Montero, D. *et al.* (2014) Effect of antioxidant vitamin supplementation on endothelial function in type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *Obes. Rev.* 15, 107–116
70. Han, T. *et al.* (2012) Therapy of endocrine disease: a systematic review and meta-analysis of α-lipoic acid in the treatment of diabetic peripheral neuropathy. *Eur. J. Endocrinol.* 167, 465–471
71. Parikh, H. *et al.* (2007) TXNIP regulates peripheral glucose metabolism in humans. *PLoS Med.* 4, e158
72. Perrone, L. *et al.* (2009) Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capillary endothelial cells under diabetic conditions. *J. Cell. Physiol.* 221, 262–272
73. Stoltzman, C.A. *et al.* (2008) Glucose sensing by MondoA:Mlx complexes: a role for hexokinases and direct regulation of thioredoxin-interacting protein expression. *Proc. Natl. Acad. Sci.* 105, 6912–6917
74. Xue, M. *et al.* (2016) Improved glycemic control and vascular function in overweight and obese subjects by glyoxalase 1 inducer formulation. *Diabetes* 65, 2282–2294
75. Rabbani, N. and Thornalley, P.J. (2019) Glyoxalase 1 modulation in obesity and diabetes. *Antioxid. Redox Signal.* 30, 354–374
76. Phillips, S.A. and Thornalley, P.J. (1993) The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. *Eur. J. Biochem.* 212, 101–105
77. Thornalley, P.J. *et al.* (2010) Imidazopurinones are markers of physiological genomic damage linked to DNA instability and glyoxalase 1-associated tumour multidrug resistance. *Nucleic Acids Res.* 38, 5432–5442
78. Schmidt, R. *et al.* (2015) Specific tandem mass spectrometric detection of AGE-modified arginine residues in peptides. *J. Mass Spectrometry* 50, 613–624
79. Thornalley, P.J. (1988) Modification of the glyoxalase system in human red blood cells by glucose *in vitro*. *Biochem. J.* 254, 751–755
80. Komolafe, O.A. *et al.* (2013) Histological and histochemical studies of the aorta and pulmonary trunk in STZ-induced diabetic Wistar rats treated with *Momordica charantia*. *Int. J. Morphol.* 31, 716–723
81. Nishikawa, T. *et al.* (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemia damage. *Nature* 404, 787–790
82. Xue, M. *et al.* (2008) Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes* 57, 2809–2817
83. Isoe, T. *et al.* (2010) High glucose activates HIF-1-mediated signal transduction in glomerular mesangial cells through a carbohydrate response element binding protein. *Kidney Internat.* 78, 48–59
84. Kang, J. *et al.* (2005) Glycogen accumulation in renal tubules, a key morphological change in the diabetic rat kidney. *Acta Diabetol.* 42, 110–116
85. Geoffrion, M. *et al.* (2014) Differential effects of glyoxalase 1 overexpression on diabetic atherosclerosis and renal dysfunction in streptozotocin-treated, apolipoprotein E-deficient mice. *Physiol. Rep.* 2, e12043
86. Fantus, I.G. *et al.* (2006) The hexosamine biosynthesis pathway. In *The Diabetic Kidney* (Cortes, P. and Mogensen, C.E., eds), pp. 117–133, Humana Press
87. Kiritoshi, S. *et al.* (2003) Reactive oxygen species from mitochondria Induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. *Diabetes* 52, 2570–2577
88. Shiba, T. *et al.* (1993) Correlation of diacylglycerol level and protein kinase C activity in rat retina to retinal circulation. *Amer. J. Physiol.* 265, E783–E793
89. Powell, H.C. *et al.* (1985) Microangiopathy in human diabetic neuropathy. *Acta Neuropathol.* 68, 295–305
90. Kim, E.S. *et al.* (2013) Glucose-induced metabolic memory in Schwann cells: prevention by PPAR agonists. *Endocrinology* 154, 3054–3066
91. Bierhaus, A. *et al.* (2012) Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nature Med.* 18, 926–933
92. Zhang, L. *et al.* (2010) Hyperglycemia alters the Schwann cell mitochondrial proteome and decreases coupled respiration in the absence of superoxide production. *J. Proteome Res.* 9, 458–471
93. Russell, J.W. *et al.* (2008) Oxidative injury and neuropathy in diabetes and impaired glucose tolerance. *Neurobiol. Dis.* 30, 420–429
94. Duran-Jimenez, B. *et al.* (2009) Advanced glycation endproducts in extracellular matrix proteins contribute to the failure of sensory nerve regeneration in diabetes. *Diabetes* 58, 2893–2903
95. Beemer, F.A. *et al.* (1982) Characterization of some glycolytic enzymes from human retina and retinoblastoma. *Cancer Res.* 42, 4228–4232
96. Schellini, S.A. *et al.* (1995) Muller cells and diabetic-retinopathy. *Braz. J. Med. Biol. Res.* 28, 977–980
97. Yao, D. *et al.* (2007) High glucose increases angiopoietin-2 transcription in microvascular endothelial cells through methylglyoxal modification of mSin3A. *J. Biol. Chem.* 282, 31038–31045

98. Tien, T. *et al.* (2017) High glucose induces mitochondrial dysfunction in retinal Müller cells: implications for diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 58, 2915–2921
99. Semba, R.D. *et al.* (2014) The role of O-GlcNAc signaling in the pathogenesis of diabetic retinopathy. *Proteomics Clin. Appl.* 8, 218–231
100. Blackburn, N.J.R. *et al.* (2017) Methylglyoxal-derived advanced glycation end products contribute to negative cardiac remodeling and dysfunction post-myocardial infarction. *Basic Res. Cardiol.* 112, 57
101. Hussain Nawaz, M. *et al.* (2018) The catalytic inactivation of the N-half of human hexokinase 2 and structural and biochemical characterization of its mitochondrial conformation. *Biosci. Rep.* 38, BSR20171666
102. Xia, H.-g. *et al.* (2015) Degradation of HK2 by chaperone-mediated autophagy promotes metabolic catastrophe and cell death. *J. Cell Biol.* 210, 705–716
103. Rabbani, N. *et al.* (2011) Conversion of low density lipoprotein to the pro-atherogenic form by methylglyoxal with increased arterial proteoglycan binding and aortal retention. *Diabetes* 60, 1973–1980
104. Godfrey, L. *et al.* (2014) Arginine-directed glycation and decreased HDL plasma concentration and functionality. *Nutr. diabetes* 4, e134
105. Rostovtseva, T. and Colombini, M. (1997) VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function. *Biophys. J.* 72, 1954–1962
106. John, S. *et al.* (2011) Subcellular localization of hexokinases I and II directs the metabolic fate of glucose. *PLoS One* 6, e17674
107. Kashiwagi, A. *et al.* (1997) Pyruvate Improves deleterious effects of high glucose on activation of pentose phosphate pathway and glutathione redox cycle in endothelial cells. *Diabetes* 46, 2088–2095
108. Viticchiè, G. *et al.* (2015) p63 supports aerobic respiration through hexokinase II. *Proc. Natl. Acad. Sci.* 112, 11577–11582
109. Richards, P. *et al.* (2017) MondoA/ChREBP: The usual suspects of transcriptional glucose sensing; implication in pathophysiology. *Metab. Clin. Exp.* 70, 133–151
110. Roberts, D.J. *et al.* (2013) Akt phosphorylates HK-II at Thr-473 and increases mitochondrial HK-II association to protect cardiomyocytes. *J. Biol. Chem.* 288, 23798–23806
111. Manning, B.D. and Toker, A. (2017) AKT/PKB signaling: navigating the network. *Cell* 169, 381–405
112. Kim, Y.-B. *et al.* (1999) Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. *J. Clin. Invest.* 104, 733–741
113. Buse, M.G. (2006) Hexosamines, insulin resistance, and the complications of diabetes: current status. *AJ. - Endocrinol. Metab.* 290, E1–E8
114. Lee, T.S. *et al.* (1989) Activation of protein kinase C by elevation of glucose concentration: proposal for a mechanism in the development of diabetic vascular complications. *Proc. Natl. Acad. Sci.* 86, 5141–5145
115. Marin de Mas, I. *et al.* (2011) Compartmentation of glycogen metabolism revealed from 13C isotopologue distributions. *BMC Syst. Biol.* 5, 175
116. Ahn, B. *et al.* (2016) MondoA coordinately regulates skeletal myocyte lipid homeostasis and insulin signaling. *J. Clin. Invest.* 126, 3567–3579
117. Steinberg, D. (2005) Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part III: mechanistically defining the role of hyperlipidemia. *J. Lipid Res.* 46, 2037–2051
118. Hotamisligil, G.S. (2017) Inflammation, metaflammation and immunometabolic disorders. *Nature* 542, 177