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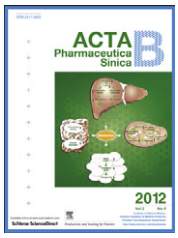

REVIEW

Glycolysis in the control of blood glucose homeostasis

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Abstract Glycolysis, a simple pathway of glucose metabolism, critically regulates insulin secretion and metabolic functions of various cells. Depending on cell types, rates of glycolysis are determined at various steps of glycolysis that are subjected to the control of key metabolic and regulatory enzyme(s), which include glucokinase, 6-phosphofructo-1-kinase, and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. These enzymes are regulated by both nutritional and hormonal signals at the levels of transcription, translation, and post-translational modifications. In hepatocytes, glycolysis is involved in the control of hepatic glucose production. The latter, when excessive, contributes to hyperglycemia in diabetes. In pancreatic β cells, glycolysis couples glucose-stimulated insulin secretion. Absolute or relatively low levels of circulating insulin causes hyperglycemia. In adipocytes, glycolysis generates metabolites for lipogenesis and channels fatty acids from excessive oxidation to triglyceride synthesis, thereby reducing oxidative stress. With increased proinflammatory status, adipocytes produce pro-hyperglycemic factors and bring about hyperglycemia and insulin resistance. In hypothalamic neurons, glycolysis conveys nutrient sensing that is related to feeding control. Dysregulation of glycolysis occurs in conditions of insulin deficiency or resistance, and is attributable to inappropriate amount and/or

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activities of metabolic and regulatory enzymes of glycolysis. Targeting key metabolic and regulatory enzymes to enhance glycolysis may offer viable approaches for treatment of diabetes.

1. Introduction

Glycolysis is the pathway of breakdown of glucose into pyruvate/lactate following glucose uptake by cells and glucose phosphorylation. Glycolysis also provides the substrates for energy production *via* the formation of ATP as well as substrates for storage pathways of glycogenesis and lipogenesis. Depending on types of cells where glycolysis occurs, glycolysis is regulated at several rate-limiting steps such as glucose uptake, glucose phosphorylation, and/or conversion of fructose-6-phosphate (F6P) into fructose-1,6-bisphosphate ($F1,6P_2$). As such, glucose transporter-4 (GLUT4), glucokinase (GK), and 6-phosphofructo-1-kinase (6PFK1) are of essential importance in the regulation of rates of glycolysis. Because 6PFK1 is activated by fructose-2,6-bisphosphate ($F2,6P_2$), the most powerful activator of 6PFK1, $F2,6P_2$ generation is also considered as a regulatory step of glycolysis. A single enzyme, namely 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFK2/FBPase2), is responsible for both the production and breakdown of $F2,6P_2$ in a nutritional status-dependent manner in various cells in particular hepatocytes and pancreatic islet β cells[1]. Thus, 6PFK2/FBPase2 activities tightly control rates of glycolysis (Fig. 1).

Although glycolysis is viewed as the simplest and most well-known pathway of nutrient metabolism, much evidence has increasingly demonstrated the importance of glycolysis in a wide variety of biological functions from the perspective of integrative physiology. As is well documented and reviewed elsewhere, glycolysis is tied closely to functions of glucose production and insulin secretion in the case of the liver and pancreatic islet β cells[2–7], respectively. Additionally, glycolysis couples glycogen synthesis in the liver and muscle[8] and stimulates lipogenesis in the adipose through producing the triglyceride backbone, glycerol phosphate[9]. In the hypothalamus, glycolysis has a role in glucose-sensing that leads to termination of meal feeding[10–12]. With progresses in research involving interdisciplinary approaches, glycolysis has recently been shown to alter inflammatory responses[9]. providing new mechanisms by which glycolysis is critically involved in integrative regulation of glucose homeostasis. These aspects are highlighted in the present review. Discussion on potential therapeutic targets, as well as approaches involving small molecule activators and/or inhibitors pertinent to diabetes treatment is provided. Glycolysis is also of particular importance in the control of cancer cell survival. The pertinent information, however, is not included in this review.

2. Glycolysis in hepatocytes: coordinated regulation of hepatic glucose production

2.1. Glycolysis and the control of hepatic glucose production

The liver plays a central role in the maintenance of glucose homeostasis[2,13–16]. This role is manifested by the ability of the liver to tightly control hepatic glucose production (HGP). During fasting, HGP is elevated, making the liver a main source of glucose production[17] (Fig. 2). After feeding, HGP is suppressed and the liver utilizes and stores glucose (Fig. 2). Using isotopic techniques and nuclear magnetic resonance (NMR) spectroscopy, *in vivo* HGP has been extensively studied[3,18,19]. Generally, gluconeogenesis and glycogenolysis are pathways producing glucose[18–23], whereas glycolysis and glycogenesis are pathways utilizing and storing glucose, respectively. In a given condition, HGP is the sum of these pathways. While controversy exists on the fractional contributions of glycogenolysis and gluconeogenesis to HGP[18–23], glucose out of or into the liver is determined by the fluxes through glycolysis and glycogenesis countering glycogenolysis. In fact, enhancement of glycolysis has shown promising results in lowering the level of plasma glucose[14,24]. This is strong evidence to support the idea that glycolysis plays an important role in the regulation of HGP. In addition, the flux through 6PFK1 may contribute to glycogenesis through the indirect

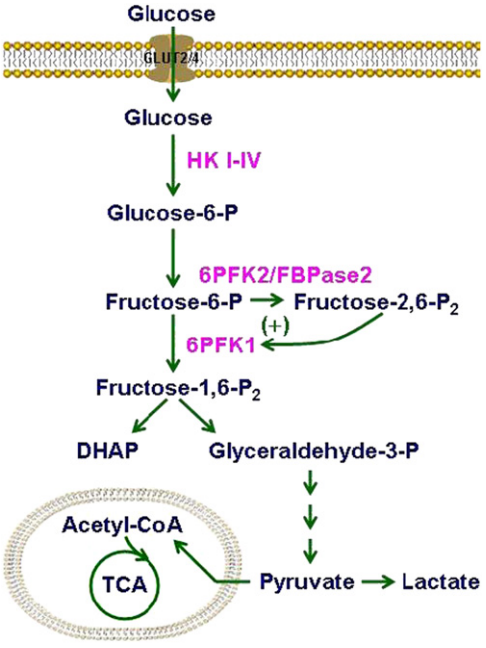

Figure 1 Major steps of glycolysis. Glycolysis is the pathway for the generation of pyruvate/lactate from glucose. Depending on cell types in which glycolysis occurs, glucose uptake is mediated mainly by glucose transporter 2 (GLUT2) or GLUT4. Following glucose uptake, rates of glycolysis are determined at steps of glucose phosphorylation, which is catalyzed by hexokinase II or hexokinase IV (glucokinase, GK), and the generation of fructose-1,6-bisphosphate, which is catalyzed by 6-phosphofructo-1-kinase (6PFK1). The latter is activated by fructose-2,6-bisphosphate ($F2,6P_2$), whose production is controlled by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFK2/FBPase2). DHAP, dihydroxyacetone phosphate; TCA, tricarboxylic acid cycle.

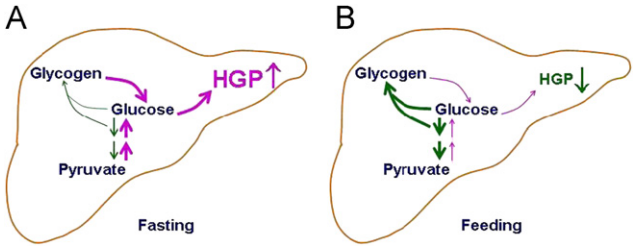

Figure 2 Glycolysis in the control of hepatic glucose production. (A) During fasting, rates of glycolysis in the liver are decreased, which are accompanied by a decrease in rates of glycogenesis and increases in rates of gluconeogenesis and glycogenolysis. The net outcome of these pathways leads to an increase in hepatic glucose production (HGP). (B) In response to feeding, rates of glycolysis are increased, which are accompanied by an increase in rates of glycogenesis and decreases in rates of gluconeogenesis and glycogenolysis. The net outcome of these pathways leads to suppression of HGP. Of importance, increased glycolytic pathway provides substrates that facilitate an increase in glycogenesis through both direct and indirect pathways. Additionally, fructose-2,6-bisphosphate ($F2,6P_2$), whose levels are increased in response to feeding, not only enhances rates of glycolysis, but also inhibits rates of gluconeogenesis, thereby providing the coordinated regulation of HGP.

pathway, which is evident by the synthesis of ^{13}C6-labeled glycogen in the liver after ^{13}C1-labeled glucose infusion[8]. ^{13}C1-labeled glucose is converted to three carbon carbohydrates, which then can be converted back to ^{13}C6-glucose-6-phosphate through gluconeogenic pathway or, strictly, glucose-6-phosphate-genic pathway, and thereby synthesize ^{13}C6-glycogen.

2.2. GK-6PFK2/FBPase2 interaction for coordinated regulation of glycolysis

In hepatocytes, key rate-determining steps of glycolysis occur at glucose phosphorylation and generation of $F1,6P_2$ from F6P. These two steps are catalyzed by GK and 6PFK1, respectively. Due to the powerful effect of $F2,6P_2$ on activation of 6PFK1, increasing $F2,6P_2$ concentrations is also considered a key step to increase glycolysis[1,25]. 6PFK2/FBPase2, as the single enzyme that makes $F2,6P_2$ during feeding and breaks $F2,6P_2$ during fasting, is thus viewed as an essential regulatory enzyme of glycolysis (Fig. 3). Physiologically, the amount and activity of either GK or 6PFK2/FBPase2 are altered by nutritional and hormonal signals in response to fasting and/or feeding, thereby determining rates of glycolysis. Given that glucose-6-phosphate is a powerful activator of glycogen synthase and that $F2,6P_2$, at elevated levels, is a strong inhibitor of gluconeogenic enzyme fructose-1,6-bisphosphatase, altering GK or 6PFK2/FBPase2 amount and/or activity also generates secondary effects on rates of glycogenesis and gluconeogenesis[8,16,26].

As the two key signals that are associated with feeding, insulin and glucose are well documented to stimulate glycolysis. Mechanistically, insulin and glucose act additively or synergistically to stimulate hepatocyte glucokinase dissociation from the inhibitory glucokinase regulatory protein (GKRP) and/or glucokinase translocation from nucleus to cytosol, to increase hepatic expression of GK and 6PFK2/FBPase2, and to maintain the dephosphorylation state of

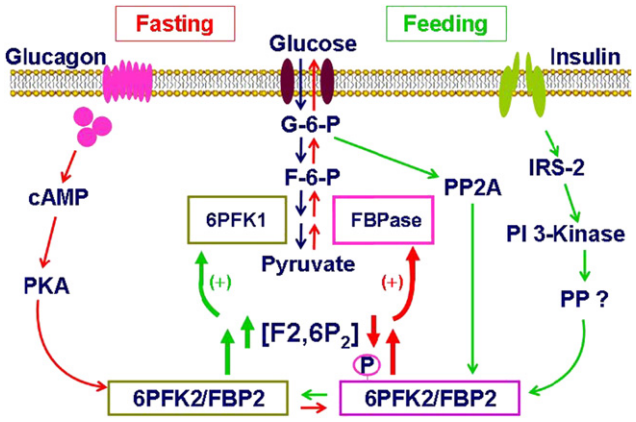

Figure 3 Nutritional and hormonal regulation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Nutritional and hormonal regulation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFK2/FBPase2) is best exemplified by the responses of liver 6PFK2/FBPase to fasting and/or feeding. During fasting, glucagon, at increased levels, activates G protein-coupled receptor signaling cascades to increase protein kinase A (PKA) activity. The latter brings about the phosphorylation of Serine-32 of 6PFK2/FBPase2, thereby decreasing the kinase activity of 6PFK2/FBPase2 and increasing the phosphatase activity of 6PFK2/FBPase2. As a result, the levels of fructose-2,6-bisphosphate ($F2,6P_2$) are decreased, leading to reduction of 6-phosphofructo-1-kinase (6PFK1) activity and elevation of fructose-1,6-bisphosphatase (FBPase) activity. This underlies a decrease in rates of glycolysis and an increase in gluconeogenesis. In response to feeding, the levels of glucose are increased, which are accompanied by an increase in the levels of insulin due to increased glucose-stimulated insulin release (GSIR). Glucose signaling activates protein phosphatase 2A. Additionally, insulin signaling activates other protein phosphatase(s). Through an additive or synergistic manner, glucose and insulin lead to dephosphorylation (Serine32) of 6PFK2/FBPase2, thereby increasing the kinase activity of 6PFK2/FBPase2 and decreasing the phosphatase activity of 6PFK2/FBPase2. As a result, the levels of $F2,6P_2$ are increased, leading to elevation of 6-phosphofructo-1-kinase (6PFK1) activity and reduction of FBPase activity. This underlies an increase in rates of glycolysis and a decrease in rates of gluconeogenesis.

6PFK2/FBPase2[25,27]. Following the finding that GK can bind 6PFK2/FBPase2[28], a new paradigm of glycolysis has proposed. In an acute phase, 6PFK2/FBPase2 promotes GK translocation, which in turn activates GK[27]. Meanwhile, 6PFK2/FBPase2 interacts with GK to form GK:6PFK2/FBPase2 complex, in which GK activity and the kinase activity of 6PFK2/FBPase2 are increased concomitantly[29]. These aspects serve as the first layer of GK-6PFK2/FBPase2 interaction in stimulating glycolysis at both the glucose phosphorylation and $F1,6P_2$ generation steps in a coordinated manner. When the time of feeding is long enough to alter gene expression or in a chronic phase, kinase activity-dominant 6PFK2/FBPase2 stimulates hepatic GK expression[30,31]. This aspect serves as the second layer of GK-6PFK2/FBPase2 interaction in stimulating glycolysis at two individual rate-determining steps. Apparently, this coordinated regulation better explains how hepatocytes react to the elevated levels of glucose to quickly or maximally increase glycolysis.

2.3. Glycolysis in dysregulation of hepatic glucose production

Both metabolic and regulatory enzymes regulate HGP by responding to nutritional and hormonal signals. As such, dysregulation of HGP, referring to failure of insulin to suppress HGP or even excessive HGP, can be attributed to direct defects in the enzymes or impairment of the enzymes to integrate nutritional and hormonal signals. The significance of glycolysis in dysregulated HGP is highlighted by genetic mutations in GK, which cause maturity-onset diabetes of the young (MODY) [32,33]. Similar effects are seen in the liver-specific GK knockout mice, showing an elevated HGP and hyperglycemia[34]. In addition to genetic defects in metabolic enzymes, obesity-associated insulin resistance is accompanied by impairment in the ability of insulin to increase *in vivo* GK activity[35]. Given these observations, decreased glucose phosphorylation as the first step of glycolysis critically contributes to elevated HGP and brings about hyperglycemia.

As mentioned above, HGP is the sum of the four major glucose metabolic pathways. In diabetes where excessive HGP is manifest, decreased or relatively decreased activities of enzymes of glycolysis and glycogenesis, as well as increased activities of enzymes of glycogenolysis and gluconeogenesis are seen and usually occur simultaneously. Considering this, it is necessary to evaluate the role of glycolysis in the dysregulation of HGP relative to the changes in the three other pathways. For instance, streptozotocin (STZ)-induced mouse model of type 1 diabetes manifests dramatic decreases in the amount of hepatic GK and the content of $F2,6P_2$, as well as a significant increase in the amount of hepatic glucose-6-phosphatase (G6Pase)[30]. In contrast, glycolysis may remain unchanged or even slightly increased due to hyperinsulinemia in the insulin resistant state[24]. However, the increase in glycolysis appears to not be able to counter the dramatic elevations in glycogenolysis and gluconeogenesis.

3. Glycolysis in pancreatic islet β cells: GK as a glucose sensor

The physiological relevance of glycolysis in pancreatic β cells has been extensively studied and highlighted by the role of GK in glucose-stimulated insulin secretion (GSIS). In response to feeding, both the amount and activity of GK are increased. This in turn enhances glycolysis primarily at the step of glucose phosphorylation. Because GK activity is positively correlated with glucose concentrations and consequent insulin secretion in β cells[36–38], GK is thus considered as a glucose sensor. Following glucose phosphorylation, the next rate-determining step of glycolysis is the generation of $F1,6P_2$ from F6P. For the same reasons described for hepatocytes, 6PFK2/FBPase2 is another enzyme that critically determines rates of glycolysis. As such, both GK and 6PFK2/FBPase2 tightly control GSIS in response to feeding or an elevation of plasma levels of glucose. Following the identification of GK-6PFK2/FBPase2 interactions in β cells, the significance of 6PFK2/FBPase2 in β cell physiology has since been re-evaluated. The GK-6PFK2/FBPase2 interaction was, in fact, identified initially in β cells, and then extended to hepatocytes[28,29,39]. In terms of enhancing glycolysis, the formation of GK:6PFK2/FBPase2 complex favorably increases activities of both GK and 6PFK2/FBPase2. As such, glycolysis is enhanced at two rate-determining steps simultaneously, resulting in a maximal increase in glycolysis. The latter clearly meets the physiological need of feeding: to quickly increase insulin secretion so that circulating glucose returns to levels within a narrow physiological range in a short time period.

Genetic defects in GK leads to MODY[32,33]. This argues in favor of the importance of GK and glycolysis in the control of glycemia. On the one hand, decreased glycolysis in hepatocytes contributes to excessive HGP as discussed in the previous section. On the other hand, decreased glycolysis in pancreatic β cells accounts for hypoinsulinemia or defects in GSIS. These two events, in combination, demonstrate that decreased glycolysis is the cause of hyperglycemia in MODY patients. In contrast, certain GK mutants are associated with increased GK activity, thereby contributing to familial hyperinsulinemic hypoglycemia[40]. While decreased glycolysis in hepatocytes and β cells may equally contribute to hyperglycemia in MODY, a decrease or a relative decrease in glycolysis in hepatocytes may be more important than a decrease in β cells in type 2 diabetes. This is because most type 2 diabetes patients have hyperinsulinemia. In other words, the inability of insulin to suppress HGP likely makes a greater contribution to hyperglycemia in type 2 diabetes. In this situation, increases in hepatic gluconeogenesis and glycogenolysis also contribute to hyperglycemia. At this point, it is unknown if genetic defects exist in 6PFK2/FBPase2, and the defects, if they exist, contribute to dysfunction in GSIS and to hyperglycemia.

4. Glycolysis in adipocytes: integrative regulation of metabolic and inflammatory responses

4.1. Glycolysis and white adipose tissue physiology

White adipose tissue is an important metabolic organ, which stores energy when energy is in excess and releases energy when energy is required. In the process of storing energy, free fatty acids are generated as the products of triglyceride hydrolysis during delivery of both dietary fats in the form of chylomicrons and endogenous fats in the form of very low density lipoproteins in response to feeding. Free fatty acids and glycerol, products of triglyceride hydrolysis, are then transported into adipocytes *via* a transport complex. Inside adipocytes, free fatty acids are re-esterified and the resultant triglycerides are stored in lipid droplets. However, inside adipocytes, circulation-derived glycerol needs to be activated by phosphorylation, which requires glycerol kinase (GyK). Although a role for GyK has been previously postulated[41], other pathways for the generation of glycerol-3-phosphate exist and appear to play a greater role in addition to glyceroneogenesis[42,43], glycolysis in adipocytes serves a critical way to generate glycerol-3-phosphate. In fact, glucose uptake following insulin-stimulated GLUT4 translocation provides sufficient substrate that can be metabolized through glycolysis to produce glycerol-3-phosphate. Moreover, adipocyte glycolysis also generates pyruvate whose further metabolism in mitochondria provides acetyl-CoA. The latter is used for generation free fatty acid synthesis through lipogenesis, and then to triglycerides. In adipocytes, hexokinase catalyzes glucose phosphorylation. This step, however, is not a rate-limiting step. Instead, generation of $F1,6P_2$ from F6P is the rate-limiting step. Similar to that in hepatocytes and β cells, $F2,6P_2$ also activates 6PFK1 to enhance glycolysis in

adipocytes. However, inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK2), encoded by PFKFB3[9,44], is the enzyme that generates $F2,6P_2$ Given this, glycolysis is key to storing energy in adipocytes. In support of this, iPFK2 is necessary for an increase in fat storage in white adipose tissue and in fat deposition in adipocytes induced by peroxisome proliferator-activated receptor γ (PPARγ) activation[45].

Adipose tissue is also an endocrine organ, whose endocrine functions tightly control systemic metabolic homeostasis. In fact, the balance between the release of pro-hyperglycemic factors such as free fatty acids and resistin and anti-hyperglycemic factors such as adiponectin, as well as the production of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) from both adipocytes and adipose tissue macrophages and other immune cells, determine insulin sensitivity and glucose metabolism locally in adipose tissue and distally in liver and skeletal muscle[46–49]. Of significance, the endocrine function of adipose tissue is tied closely with adipocyte glucose and lipid metabolism, which is detailed in the following sections.

4.2. Integrative regulation of adipocyte metabolic and inflammatory responses

The pathophysiological relevance of iPFK2 to diabetes is evidenced by the fact that the expression of iPFK2 in adipose tissue is decreased in *db*/*db* mice and PPARγ2-disrupted mice[44,45], which all exhibit insulin resistance[50–53]. Under physiological conditions, iPFK2 stimulates adipocyte glycolysis[44]. The resultant increase in glycolysis not only provides lactate and pyruvate (which are converted into acetyl-CoA and used for lipogenesis to provide free fatty acids), but also increases the production of dihydroxyacetone phosphate, which is converted into glycerol-3-phosphate as a required substrate for adipocyte triglyceride synthesis. This role of iPFK2 is supported by the fact that knockdown of iPFK2 in adipocytes leads to a decrease in the incorporation of glucose into lipid. Of importance, the impairment in using glucose as a fuel due to iPFK2 disruption causes a compensatory increase in fatty acid oxidation. The latter contributes to decreased fat accumulation, but more importantly, triggers oxidative stress and increases the adipocyte inflammatory response[9]. Of significance, inhibition of fatty acid oxidation by etomoxir brings about a decrease in the production of reactive oxygen species (ROS) in iPFK2-knockdown adipocytes. As further evidence, overexpression of iPFK2 in adipocytes enhances glycolysis and glycolysis-driven lipogenesis, which are accompanied by decreases in adipocyte inflammatory signaling through the nuclear factor kappa B (NF-κB) pathway and in adipocyte expression of proinflammatory cytokines[54]. Clearly, iPFK2 serves as a coordinator that links adipocyte glycolysis, glycolysis-driven lipogenesis, and the inflammatory response.

The role of iPFK2 in integrative regulation of adipocyte metabolic and inflammatory responses is also highlighted by the fact that iPFK2 is involved in the anti-inflammatory and anti-diabetic effect of PPARγ activation (i.e., treatment with rosiglitazone). In support of this, treatment with rosiglitazone restores euglycemia and reverses high fat diet-induced insulin resistance and glucose intolerance in wild-type mice, but not in iPFK2-disrupted mice[45]. These *in vivo* results are recapitulated in cultured 3T3-L1 adipocytes. Furthermore, inhibiting excessive fatty acid oxidation in iPFK2-knockdown adipocytes rescues the effects of PPARγ activation on suppressing inflammatory signaling and proinflammatory cytokine expression and on improving adipocyte insulin signaling[45].

5. Glycolysis in hypothalamic neurons: glucose-sensing for appetite regulation

Numerous studies have demonstrated the importance of food intake in the control of metabolic homeostasis. As it is well accepted, food intake is controlled by the central nervous system where the hypothalamus plays the most important role. In the hypothalamus, certain neurons express orexic neuropeptide Y (NPY) and agouti-related protein (AgRP), both of which increase food intake. In contrast, certain neurons express anorexic pro-opiomelanocortin (POMC) and cocaine-amphetamine-related transcript (CART), both of which suppress food intake[55–59]. While investigating how nutrients are sensed by hypothalamic neurons, a number of studies have demonstrated that AMP-activated protein kinase (AMPK) is a key cellular energy sensor that responds to peripheral signals, e.g., glucose and leptin, to direct food intake[60–66]. For example, decreased hypothalamic AMPK signaling appears to be responsible for the anorexic effects caused by re-feeding and leptin[61,67,68]. On the other hand, activating hypothalamic AMPK signaling has been shown to abolish the anorexic effect induced by leptin[67]. In the hypothalamus, mammalian target of rapamycin (mTOR) appears to be another cellular sensor involved in nutrient sensing, and likely mediates anorexic effects caused by re-feeding and leptin. In contrast, decreasing mTOR signaling abolishes the anorexic effect of leptin. As such, hypothalamic mTOR plays a key role in the control of food intake with or independent of AMPK.

A role for glucose sensing in the regulation of food intake was initially proposed as glucostatic hypothesis 60 years ago[69–71]. This hypothesis was all but abandoned, but has been recently revisited and revised[72]. New evidence now points to an essential role for glucose metabolism, not the levels of glucose, in the regulation of food intake[72], although a direct link between neuronal glucose sensing and the physiological regulation of food intake has yet to be established[73]. In cultured hypothalamic neurons, glycolysis mediates the effect of glucose on suppressing AgRP expression[74]. This effect, however, appears to be independent of AMPK, although glucose decreases AMPK phosphorylation. While addressing molecular mechanisms underlying glucose sensing, much attention has been paid to the role of GK in the control of neuronal glucose metabolism. In fact, GK in hypothalamic neurons has been proposed as a glucosensor, paralleling GK function in pancreatic β cells and is key to the regulation of food intake[10–12,75–78]. As a key regulatory enzyme of glycolysis, 6PFK2/FBPase2 is present in the brain[79]. However, 6PFK2/FBPase2 has not yet been functionally characterized in neurons of the hypothalamus.

The pathophysiological significance of glucose sensing in glucose homeostasis is evidenced by the fact that disruption of glucose sensing in POMC neurons in mice leads to impairment of whole-body response to a systemic glucose load[80]. Additionally, glucose sensing in POMC neurons is defective in diet-induced obesity, demonstrating a role for loss of glucose

sensing in hypothalamic neurons in the development of diabetes.

6. Anaerobic glycolysis

Anaerobic glycolysis occurs in the absence of oxygen, e.g., in muscle cells during vigorous physical activity, and terminates in the formation of lactate. Of importance, lactate generated in muscle cells circulates to the liver, where lactate is converted back to glucose. In general, anaerobic glycolysis functions to help muscles burn fuels, which is different than aerobic glycolysis. The latter is involved in many biological functions in a wide verity of tissue/cells as discussed above. Anaerobic glycolysis also occurs in erythrocytes, which lack enzymes of the tricarboxylic acid cycle, and in other cells or tissues including brain, gastrointestinal tract, renal medulla, adipose tissue, and skin. Unlike aerobic glycolysis, relatively little is known about changes in anaerobic glycolysis in diabetes. Previous evidence supports the notion that elevated plasma levels of lactate are an independent risk factor for the development of type 2 diabetes[81]. Additionally, lactate- and pyruvate-interconversion rates are greatly enhanced in patients of type 2 diabetes, likely due to concomitant impairment in the oxidative pathway of glucose metabolism[82]. Although anaerobic glycolysis appears to be increased during diabetes, lactate, the end product of anaerobic glycolysis, *per se*, does not induce insulin resistance[83]. Given this, anaerobic glycolysis likely has a limited role in the pathogenesis of diabetes. However, during the development of diabetes complications, ischemia is common and considered as a key factor triggering anaerobic glycolysis through mechanisms involving hypoxia-inducible factor 1 (HIF1). The latter stimulates anaerobic glycolysis[84], which may be a defensive response that is involved in protection against cell/tissue injury. Hypoxia also occurs in adipose tissue during obesity. As such, HIF1 is likely a regulator of chronic inflammation in adipose tissue[85,86]. To date, the exact role for anaerobic glycolysis in regulating adipose tissue inflammation has not been elucidated.

7. Targeting glycolysis for diabetes treatment

7.1. *Mechanisms of actions for potential targets in glycolysis*

As discussed above, glycolysis critically regulates physiological functions of a number of tissues/organs that are essential for glucose metabolic homeostasis in a cell-type-dependent manner. Among those functions, glucose production from hepatocytes/liver and insulin secretion in pancreatic β cells have attracted much attention. Over the past several decades, a few *in vivo* studies have validated reduction of HGP as a potential treatment for diabetes[87–89]. Similarly, increasing insulin secretion from pancreatic β cells is repeatedly shown as a powerful way to restore euglycemia[90]. To be noted, reduction of HGP is indeed a critical consequence secondary to increased insulin secretion. From this perspective, mechanisms of actions (MOA) of glycolysis-based insulin secretagogues, as well as all other insulin-secretagogues, should not be limited to insulin secretion, and should, at least, include suppression of HGP. Similarly, restoring euglycemia upon suppression of HGP lowers hyperinsulinemia due to a decrease in GSIS. In combination, a decrease in levels of glucose and/or insulin has the potential to also bring about a secondary increase in fatty acid oxidation in skeletal muscle, thereby contributing to improvement of overall systemic metabolic homeostasis[15]. Further interpretations pertinent to MOA are detailed in the following sections based on specific targets. Overall, changes in rates of glycolysis in key tissues/cells involved in the control of systemic glucose homeostasis serve as the rationale of targeting glycolysis for treatment of diabetes (Fig. 4).

7.2. *Major targets*

7.2.1. *GK*

GK is abundantly expressed in both hepatocytes and pancreatic β cells. Thus, GK activation is expected to increase glycolysis in hepatocytes and β cells, leading to reduction of HGP and increased insulin secretion, respectively. At the cellular level, 6PFK2/FBPase2 acts as an endogenous activator and GKRP acts as an endogenous inhibitor of GK, thereby critically regulating glucose phosphorylation in both hepatocytes and pancreatic β cells. The potency of GK in terms of reducing HGP has been validated by a few *in vivo* studies *via* adenoviral overexpression of GK[91,92]. Interestingly, a GK activator has been developed and characterized based on its role in stimulating insulin secretion in pancreatic β cells. The activator likely inhibits the binding of GKRP to GK[90]. Following lead optimization, potent preclinical compounds were advanced through phase 2 trials. However, the GK activator project was eventually discontinued[93]. Safety concerns associated with the MOA of these compounds may diminish the ability of targeting GK.

7.2.2. *6PFK1*

6PFK1 catalyzes the generation of F1,6P_2 from F6P as another rate-determining step of glycolysis, and is thus considered as a potential target for glycolysis-based treatment of diabetes. Among known activators, F2,6P_2 is the most powerful activator of 6PFK1. For this reason, 6PFK2/FBP2ase, which both makes and breaks down F2,6P_2, is also a potential target for glycolysis-based treatment of diabetes, and is ranked at a higher priority than 6PFK1 as a potential target for treatment of diabetes.

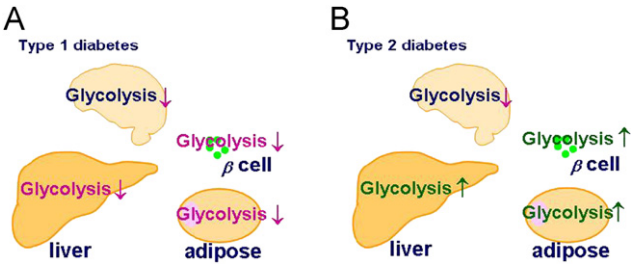

Figure 4 Glycolysis in major tissues in relation to diabetes (A) In type 1 diabetes, insulin insufficiency leads to a decrease in rates of glycolysis in key tissues involved in the regulation of systemic glucose homeostasis. (B) In type 2 diabetes, hyperinsulinemia brings about a compensatory increase in rates of glycolysis in the liver, adipose tissue, and pancreatic β cells. Unlike other tissues, the brain exhibits a decrease in rates of glycolysis as evidenced in rodent models of obesity and type 2 diabetes.

7.2.3. 6PFK2/FBPase2

6PFK2/FBPase2 has been validated as an interesting target for reduction of HGP in mice. Overexpression of a kinase dominant/bisphosphatase-deficient 6PFK2/FBPase2 is shown to increase hepatic content of $F2,6P_2$, which lowers levels of plasma glucose in both type 1 and type 2 diabetic mouse models[14,24]. Of importance, hepatic 6PFK2/FBPase2 overexpression shows the same effects as seen with hepatic GK overexpression through activation of glycolysis, but does not cause fatty liver. The underlying mechanisms for the metabolic differences are due to the ability of the overexpressed 6PFK2/FBP2ase to induce GK expression at a level sufficient to initiate glycolysis but not in excess, which would promote lipogenesis[94]. To be noted, 6PFK2/FBP2ase and $F2,6P_2$ serve as therapeutic targets not only through activation of 6PFK1[95], but also through modification of expression of genes for metabolic enzymes[30]. Since $F2,6P_2$ itself is controlled by the relative activities of the kinase and bisphosphatase domains of 6PFK2/FBP2ase, an activator of 6PFK2 and/or an inhibitor of FBPase2 would be efficacious for reduction of HGP by increasing $F2,6P_2$ content in the liver. As an anti-diabetic reagent, vanadate is shown to increase 6PFK2/FBP2ase. However, vanadate also has broader effects on other enzymes that are not metabolic and/or regulatory enzymes of glycolysis[96]. Identifying new small molecule activators of 6PFK2/FBP2ase is ongoing, but may be challenging.

7.2.4. Inducible 6PFK2 (iPFK2)

On the one hand, PPARγ agonists activate iPFK2. On the other hand, iPFK2 is involved in the anti-diabetic effect of rosiglitazone, one of the two prescribed PPARγ agonists that are powerful anti-diabetes medicines. Due to safety concerns, rosiglitazone has been withdrawn from European markets and pioglitazone use has been suspended in Europe. Compared with PPARγ activation that has broader effects on many biological events, the overall effects of iPFK2 activation are expected to be limited mainly to metabolism in limited number of cell types. If true, iPFK2 activation may have fewer off target effects. To date, small molecular activators of iPFK2 are not available.

7.3. Additional targets and potential approaches

In terms of enhancing glycolysis, a strategy that coordinately affects several targets would provide more pronounced effects. A single effector may accomplish this by either directly interacting with or indirectly modifying metabolic and regulatory enzymes. Unlike activator(s) or inhibitor(s) of metabolic and/or regulatory enzymes of glycolysis, other unclassified approaches may also work through indirect effects to enhance glycolysis. For example, metformin is an anti-diabetic medicine that has been widely used for many decades. While the exact MOA of metformin are not clear, metformin treatment has been repeatedly shown to enhance liver glycolysis, likely through mechanisms involving AMPK activation[97,98]. Berberine is also a powerful anti-diabetic compound that may also act through mechanisms involving AMPK activation to enhance glycolysis[99,100]. However, these compounds have broader effects, and cannot be simply considered as glycolysis-based approaches.

Epigenetic regulation of metabolic and regulatory enzymes of glycolysis is a new field, which is currently being explored[101]. Identifying and characterizing new regulator(s) that stimulate the expression of key enzymes, such as GK, 6PFK1, and 6PFK2/FBPase2, could provide new targets and/or novel approaches for the treatment of diabetes.

8. Conclusions

Glycolysis is of particular importance in the regulation of systemic glucose homeostasis. This review has discussed how glycolysis is regulated in a cell-type-dependent manner, and summarized key glycolysis metabolic and regulatory enzymes that are potential targets for treatment of diabetes. The pertinent mechanisms of actions have also been discussed. While many targets are promising, there still will be a long way to go to develop glycolysis-based therapeutic(s) that are effective enough to restore euglycemia, and more importantly also safe enough to not cause side effects. In future studies, it would be equally important to also find a better way to deliver an effector, which would maintain the same efficacy at a lower dose.

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