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Review

Role of hypoxia-inducible factor 1 in type 1 diabetes

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Type 1 diabetes (T1D) is a common autoimmune disease in which dysregulated glucose metabolism is a key feature. T1D is both poorly understood and in need of improved therapeutics. Hypoxia is frequently encountered in multiple tissues in T1D patients including the pancreas and sites of diabetic complications. Hypoxia-inducible factor (HIF)-1, a ubiquitous master regulator of the adaptive response to hypoxia, promotes glucose metabolism through transcriptional and non-transcriptional mechanisms and alters disease progression in multiple preclinical T1D models. However, how HIF-1 activation in β-cells of the pancreas and immune cells (two key cell types in T1D) ultimately affects disease progression remains controversial. We discuss recent advances in our understanding of the role of hypoxia/HIF-1-induced glycolysis in T1D and explore the possible use of drugs targeting this pathway as potential new therapeutics.

Highlights

The autoimmune destruction of β-cells in type-1 diabetes (T1D) is a multifactorial and poorly understood phenomenon. A major contribution to this autoimmune attack is the generation of autoantigens triggered by β-cell dysfunction.

Under hypoxia, cells shift to anaerobic glycolysis, regulated by hypoxia-inducible factor (HIF)-1α. This adaptation ensures energy production through increased glycolytic flux and decreased oxidative phosphorylation. Dysregulation of glycolysis contributes to β-cell dysfunction in diabetes.

HIF-1α activation in β-cells impacts both *in vivo* and *in vitro* insulin secretion and systemic glucose levels. HIF-1α also modulates immune cell phenotype, potentially influencing autoimmune destruction of β-cells.

Although more studies are needed, prolyl-hydroxylase inhibitors, which stabilize HIF-1α, show promise as T1D therapeutics by protecting β-cells against cellular stress.

Hypoxia and type-1 diabetes

Diabetes is a global disease frequently presented in two forms, T1D and type 2 diabetes (T2D). Worldwide levels of diabetes are currently at epidemic proportions. While there is clearly a significant overlap between the pathology of T1D and T2D, the underlying causes (autoimmunity versus metabolic disease, respectively) differ greatly [1]. T1D is a chronic autoimmune condition, in which **cytotoxic T cells** (see Glossary) inappropriately target pancreatic β-cells. While the exact causes of T1D are not fully understood, it likely involves a combination of genetic predisposition, dysregulated immunity, and exposure to environmental factors. In T1D, β-cells are targeted by autoreactive $CD8^+$ T cells, leading to immune cell infiltration in the **pancreatic islets** and consequent destruction of β-cells [2]. Besides T cells, B cells, neutrophils, natural killer (NK) cells, and macrophages are involved in the destruction of β-cells in T1D [3–5]. β-Cells are metabolically active cells which sense circulating glucose levels and produce insulin in response. In turn, insulin controls the systemic levels of glucose by stimulating glucose uptake in insulin-sensitive cells including adipocytes, myocytes, and hepatocytes. Glucose metabolism and particularly glycolysis play an important role in both β-cell glucose sensing and the induction of insulin production as well as in insulin-dependent glucose uptake in target cells. Attack of β-cells by cytotoxic T cells leads to insulin deficiency with associated **hyperglycemia**.

As with most autoimmune diseases, genetic predisposition, dysregulated immunity, and exposure to environmental factors are implicated in T1D. **Polymorphisms** in genes encoding the HLA/MHC complex account for the strongest T1D susceptibility [6,7]. However, genes involved in β-cell function, apoptosis, and inflammation have also been implicated [3–5,8,9]. Increasing evidence suggests that the β-cells can also contribute to their own destruction in T1D. During early and chronic stages [10] (Box 1) of T1D, β-cell perturbations due to environmental factors activate an adaptive response to endoplasmic reticulum (ER) stress which triggers the expression of alternatively spliced, aberrant translational products generating a pool of neoantigens which can drive **islet autoimmunity** [11–16]. Proposed environmental triggers in T1D include viral infection [17], changes in the microbiome [18], and metabolic dysregulation [19,20].

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Currently, clinical management of T1D typically targets the symptoms rather than the cause by using insulin therapy to enhance glycemic control and reduce the likelihood of diabetes-related complications [21]. Some patients with poor glycemic control may receive pancreas or islet transplantation but hypoxia-induced β-cell dysfunction, cell death, and graft loss may limit the efficacy of islet transplantation [22,23] (Box 2). The effects of hypoxia in T2D have been well investigated and reviewed elsewhere [24], however the role of hypoxia in T1D is less well understood. Hypoxia is a microenvironmental feature of key cell types in T1D including pancreatic β-cells and immune cells as well as cells at sites of diabetic complications. HIF-1α is a master regulator of glycolysis, an essential process for physiological β-cell function [25–27]. The impact of the regulation of glucose metabolism by HIF-1 in diabetes is a controversial field with studies supporting both protective and detrimental roles. Unravelling the controversy is of particular clinical importance as HIF-activating and inhibiting drugs have recently been clinically approved for the treatment of chronic kidney disease (CKD)-associated anemia and renal cancer, respectively, and may potentially be beneficial for diabetes treatment.

Here, we explore recent literature regarding the metabolic response to hypoxia and HIF-1α transcriptional function in T1D and β-cell (dys)function, as well as the involvement of hypoxia and HIF-1 in the development and management of T1D. Furthermore, we will link the extensive literature on the impact of hypoxia on immunity and inflammation with autoimmunity in T1D. Given that HIF has been clearly shown in many hundreds of studies to impact both T and B cell function [28], we will also discuss our limited understanding of the role of hypoxia and the HIF pathway in the mechanisms of autoimmunity.

β-Cell metabolism and glucose-stimulated insulin secretion

Glycolysis is a metabolic pathway which harvests energy from glucose in the cell cytoplasm in an oxygen-independent manner. Pancreatic β-cells rely on glycolysis to perform the initial steps of glucose metabolism that harvest the energy necessary for their functions, including the production and secretion of insulin in a process known as glucose-stimulated insulin secretion (GSIS) [29]. Pyruvate produced during glycolysis is processed in the mitochondria to harvest energy in the form of ATP through aerobic metabolism during oxidative phosphorylation. In β-cells, an increased cytosolic ATP:ADP ratio and other metabolic signals from the mitochondria derived from the tricarboxylic acid (TCA) cycle and oxidative phosphorylation drives the closure of K_{ATP} channels, membrane depolarization and opening of voltage-gated calcium channels (VGCCs) [30–32]. The subsequent increase in cytosolic Ca^{2+} concentration drives the triggering phase of insulin granule exocytosis in response to elevated blood glucose levels (Figure 1). Furthermore, a K_{ATP}-controlling glycolytic signaling complex was recently demonstrated to influence islet glucose sensing and excitability of β-cells of human and rodent pancreatic islets [33].

In T1D, the destruction of β-cells results in a reduction in insulin production that prevents normal glucose uptake and efficient progression of glycolysis. Without sufficient insulin, glucose cannot enter target cells efficiently, resulting in dysregulated blood glucose levels and diabetes. Consequently, cells become deprived of the glucose needed to fuel glycolysis and produce energy. This imbalance in glucose uptake and metabolism leads to increased reliance on alternative metabolic pathways to meet cellular energy demands, such as gluconeogenesis and ketogenesis [34,35]. Therefore, controlled glycolysis is fundamental to maintaining systemic glucose homeostasis. Disruptions or dysregulation in β-cell glycolysis can impair insulin secretion and contribute to the pathophysiology of diabetes [36]. Consequently, understanding the regulation of glycolysis in β-cells is essential for devising strategies to improve insulin secretion and manage blood glucose levels effectively in T1D.

Glossary

Cytotoxic T cells: type of immune cell capable of destroying specific cells, such as foreign cells, cancerous cells, and virus-infected cells. Antigen-specific $CD8^+$ cytotoxic T cells are believed to mediate the direct cytotoxic killing of insulin-producing β-cells [106].
Hyperglycemia: condition characterized by abnormally high level of glucose circulating in the blood plasma.
Islet autoimmunity: defined by the presence of one or several (auto) antibodies against pancreatic islets (auto)antigens, such as insulin, GAD65, IA-2, and ZnT8 [107].
Pancreatic islets: also known as islets of Langerhans, pancreatic islets are clusters of cells within the pancreas that are responsible for producing and releasing hormones into the bloodstream, regulating blood sugar levels. The main four types of cells found in the pancreatic islets are α, β, δ, and pancreatic polypeptide cells.
Peripheral tolerance: refers to the mechanisms that occur outside of the primary lymphoid organs (such as the thymus and bone marrow) to prevent the immune system from attacking the body's own tissues. This is crucial for maintaining immune homeostasis and preventing autoimmune diseases.
Pimonidazole–protein adducts: used as biomarkers for detecting and measuring hypoxia in tissues. Pimonidazole forms covalent bonds with proteins in cells under low oxygen conditions, creating pimonidazole–protein adducts. These adducts can then be detected using specific antibodies.
Polymorphism: occurrence of two or more different forms or alleles of a gene within a population. These genetic variations can lead to diverse physical traits or biological functions.
Streptozotocin (STZ)-induced diabetic mouse model: STZ is an antibiotic known for its ability to destroy pancreatic islet β-cells, and it is commonly used in experiments to create a model of T1D [108].

Box 1. Pathogenesis of T1D

T1D is a multifactorial disease in which patients require constant glycemic control with administration of insulin. Patients with T1D present autoimmune response against insulin-producing β-cells in the pancreas. Contributing factors and schematic representation of T1D pathogenesis are described in Figure I. Genetic predisposition, and epigenetic and environmental factors have been described as contributors to the onset of T1D [15]. The pathogenesis unfolds in three stages [109]. In stage 1, individuals with predisposition are susceptible to immune attack against β-cells, which elicits the observation of two or more autoantibodies (AAbs) in the serum of patients. In stage 2, β-cell function is reduced because of autoimmunity and patients experience dysglycemia, although most people remain asymptomatic. The final stage, stage 3, manifests as clinical symptoms with overt hyperglycemia. As β-cell function and mass diminishes, insulin production declines, necessitating exogenous insulin for glycemic control.

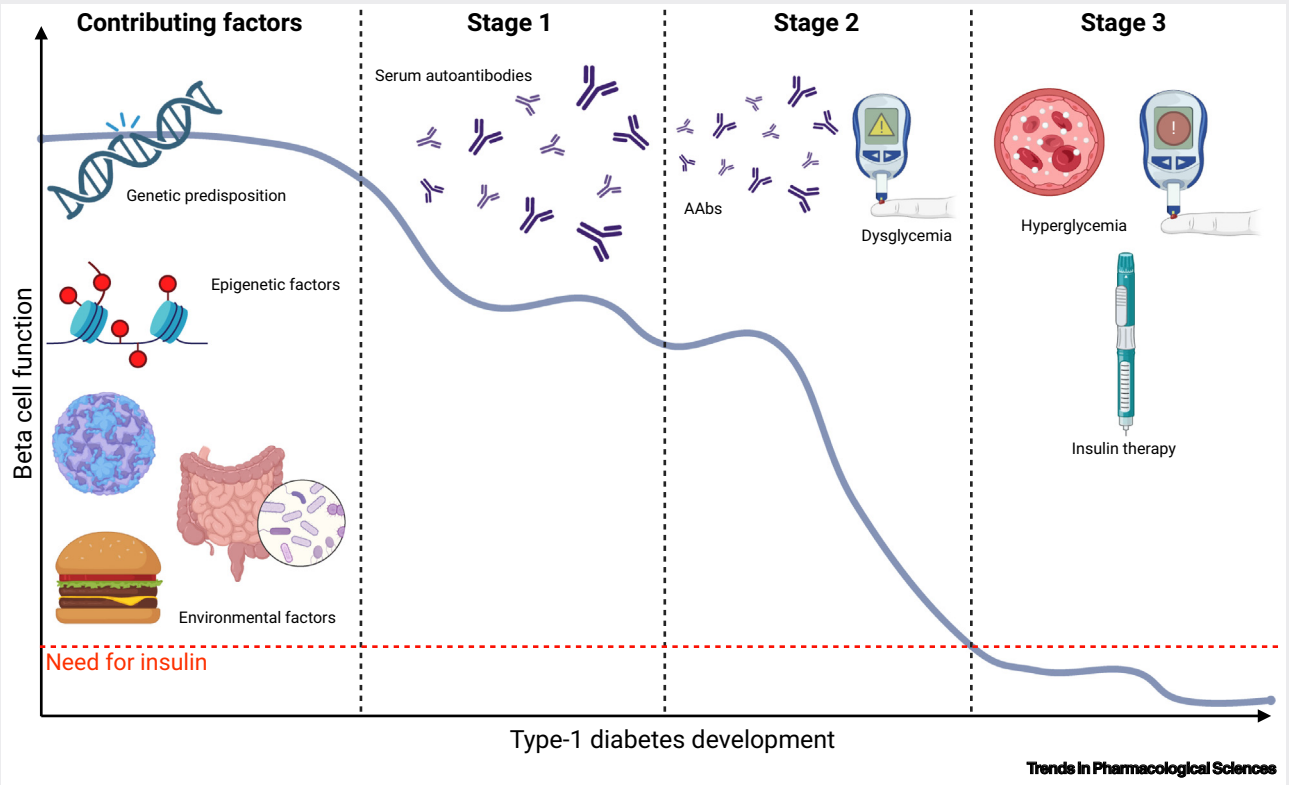

Figure I. Schematic representation of the time-course of type-1 diabetes onset. Figure created with BioRender. Abbreviation: AAbs, autoantibodies.

Regulation of glycolysis by HIF-1

The occurrence of microenvironmental hypoxia in the pancreas and sites of diabetic complications is a key feature of advanced diabetes, which contributes to disease progression [37–41]. Physiologically, levels of molecular oxygen (O_2) vary across tissues. For example, the pulmonary epithelium is normally exposed to almost atmospheric levels of O_2, while the intestinal epithelium is juxtaposed with the anoxic intestinal lumen resulting in these cells experiencing low levels of O_2 even under physiologic conditions [42]. In normoxic conditions, the oxygen supply to individual cells exceeds the requirement to support the level of oxidative phosphorylation for a cell to maintain normal function. Hypoxia is defined at the cellular level as a state where oxygen demand exceeds supply, eliciting molecular signals that trigger the activation of cellular adaptive pathways [43].

HIF-1α is a master regulator of the cellular adaptation to hypoxia [44]. HIF-1α mRNA is constitutively transcribed and ubiquitously expressed. However, its protein stability and transcriptional

Box 2. Hypoxia and pancreatic islet transplantation

Relatively few T1D patients remain insulin independent 5 years after islet transplantation [110]. Among the numerous causes for islet graft loss [111], the ischemic time occurring during organ retrieval (a recurrent problem to all transplants, including the pancreas) and the hypoxia experienced during islet isolation represents a major burden that dramatically affects the islet yield and hampers β-cell function and survival [22,23]. The destruction of the intra-islet endothelium upon enzymatic digestion during islet isolation leaves the islet with insufficient blood supply and oxygen delivery to the graft [112,113]. Although, adaptive transcriptional responses to short-term hypoxia can compensate for oxygen deprivation and enhance glycolysis without detrimental effect on β-cell function [114,115], prolonged hypoxia can lead to cellular stress and formation of reactive oxygen species that may damage cellular component and contribute to cell death [116]. This phenomenon is further amplified by the reduced antioxidant mechanisms in β-cells that make them very sensitive to both ER and oxidative stress [116]. In addition, a pathologically hypoxic environment combined with danger-associated molecular patterns (DAMPs) generated during isolation may, via NF-κB activation, participate in the recruitment of immune cells and to a change in their inflammatory profile [117]. These changes can contribute to an inflammatory environment deleterious for the islet graft function and survival. Overall, the current management of T1D imposes a significant burden in the quality of life of patients and new and improved therapeutics are a real clinical need. The development of encapsulating devices using natural or synthetic biomaterials to protect islets or stem-cell-derived β-cells from immune rejection and limit the need for immunosuppressive drugs raises questions about the vascularization of the implant and long-term graft survival and function. Intensive research aiming at enhancing oxygen delivery using oxygen chambers, different pore size membranes, extracellular matrix based-materials, pre-vascularized capsules or angiogenic releasing materials will likely be necessary to prevent hypoxia-induced β-cell dysfunction, cell death and graft loss. Indeed, this approach may also be useful in other tissue transplantation scenarios.

function are suppressed by a family of oxygen-dependent asparaginyl- and prolyl-hydroxylases (FIH and PHD1–3, respectively). In normoxia, PHD1–3 utilize O_2 as a cofactor for the hydroxylation of HIF-1α, which is then recognized by the von-Hippel–Lindau protein (VHL) and, consequently undergoes proteasomal degradation. FIH-dependent hydroxylation prevents HIF transcriptional activity. In hypoxia, when O_2 demand exceeds supply, sufficient oxygen is not available for the hydroxylation of HIF-1α. Consequently, HIF-1α protein rapidly stabilizes, translocates to the nucleus and drives the transcription of multiple adaptive target genes by binding to hypoxia-responsive elements (HREs) in promoter regions.

Two molecules of pyruvate are formed per molecule of glucose that enters glycolysis in the cytoplasm. When oxygen levels are sufficient, pyruvate is fed to the mitochondria and enters the TCA cycle following conversion to acetyl-CoA, generating intermediate products that feed into the electron transport chain (ETC), and a total of 38 molecules of ATP are harvested in a cell per molecule of glucose consumed in normoxia. However, under conditions of hypoxia, insufficient levels of O_2 reduce mitochondrial aerobic respiration and lead to allosteric and transcriptional upregulation of glycolytic enzymes, increasing the glycolytic flux to harvest energy from glucose in the cytoplasm of a cell [45]. This process produces a total of just two molecules of ATP, along with two H_2O, two NADH, two H^+, and two pyruvate per glucose molecule. Despite the lower energy output during hypoxic conditions compared with aerobic metabolism, the energy harvested by this process allows cells to retain their function to some extent during hypoxic insult.

HIF-1α regulates 200–400 genes which promote the cellular adaptive response to hypoxia. Key among HIF-1α-dependent genes are those encoding all ten enzymes of the glycolytic pathway, as well as multiple glucose transporters [28]. Furthermore, HIF-1α has recently been proposed to promote glycolysis through a non-transcriptional mechanism by facilitating the formation of cytoplasmic glycolytic enzyme complexes [46]. The lack of available intracellular O_2 triggers a transcriptional reprogramming that involves the upregulation of glycolytic enzymes including hexokinases (HK1 and HK2) [47], phosphofructokinases (PFKL and PFKP) [48,49], aldolases (ALDA and ALDC) [48,50], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [51], phosphoglycerate kinase 1 (PGK1) [48], enolases (ENO1 and ENO2) [50], and pyruvate kinase M

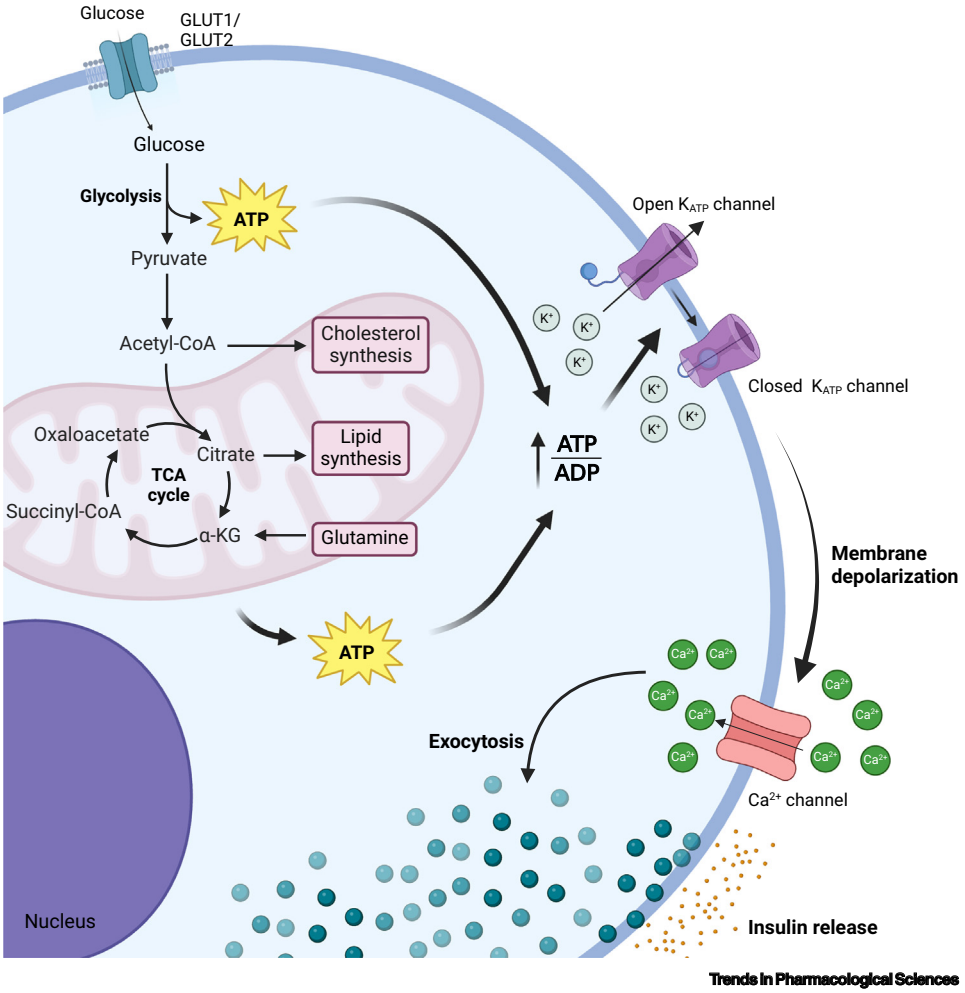

Figure 1. Glucose-stimulated insulin secretion in β-cells. Glucose obtained from food digestion enters β-cells via GLUT1 and GLUT2 transporters. Upon entry, glucose undergoes processing to pyruvate through glycolysis in the cytoplasm, yielding two molecules of ATP per glucose molecule. Subsequently, pyruvate is further converted to acetyl-CoA in the mitochondria, entering the tricarboxylic acid (TCA) cycle. This cycle facilitates the harvesting of a total of 38 ATP molecules per glucose molecule via oxidative phosphorylation. Intermediates generated in this pathway are utilized for the synthesis of various cellular building blocks. For instance, acetyl-CoA contributes to the production of cholesterol, while citrate is involved in the formation of lipids. The elevation in the ATP-to-ADP ratio in the cytosol prompts the closure of K_{ATP} channels, leading to membrane depolarization. This depolarization, in turn, triggers the opening of voltage-gated Ca^{2+} channels. The subsequent increase in intracellular Ca^{2+} levels initiate the post-meal exocytosis of insulin granules. Figure created with BioRender. Abbreviation: α-KG, α-ketoglutarate.

(PKM) [52]. This reprogramming favors glycolytic flux in the cell, allowing the harvest of biomolecular energy in the form of ATP by anaerobic respiration in the most effective manner possible. Besides the upregulation of these glycolytic genes, glucose transporters (e.g., GLUT1 and GLUT3) are also target genes of HIF-1α [53–55]. HIF-1α-dependent upregulation of pyruvate dehydrogenase kinase 1 (PDK1) and lactate dehydrogenase A (LDHA) provides evidence of HIF-1α-mediated suppression of oxidative phosphorylation because of channeling of pyruvate into lactate and repressing its conversion to acetyl-CoA [53,56,57]. Together, these observations point to promotion of an increase in glycolytic flux in cells exposed to hypoxia (Figure 2). The functional consequence of increasing glycolytic activity on behavior of cell types associated with T1D will be considered next.

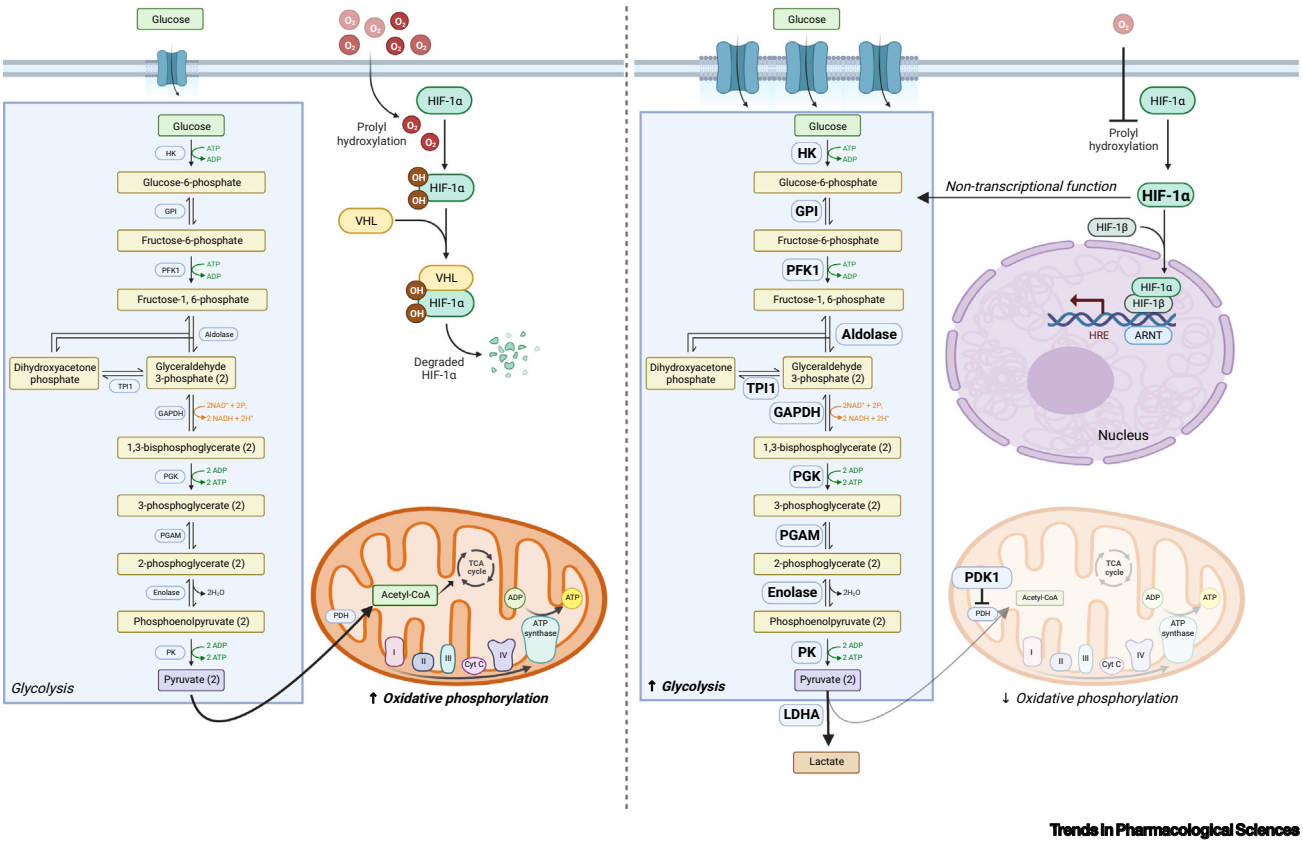

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Figure 2. Impact of hypoxia and HIF-1α activation in cellular metabolism. (A) In normoxia, when intracellular oxygen supply surpasses demand, the HIF-1α protein undergoes degradation by VHL upon enzymatic hydroxylation. The glucose entering the cell is subsequently processed into pyruvate via glycolysis, a coordinated process involving ten glycolytic enzymes in the cytoplasm. Pyruvate produced in the cytoplasm is further metabolized to acetyl-CoA in the mitochondria, contributing to the TCA cycle and facilitating energy harvest in the form of ATP through oxidative phosphorylation. (B) In hypoxia, when oxygen demand exceeds supply, HIF-1α protein stabilizes in the cytosol. It can then orchestrate transcriptional and allosteric upregulation of glycolytic enzymes and glucose transporters, thereby augmenting glycolytic flux within the cell. HIF-1α-dependent upregulation of PDK1 and LDHA reduces pyruvate entry into the mitochondria. Combined with limited oxygen availability, this leads to a decrease in mitochondrial activity and oxidative phosphorylation. Figure created with BioRender. Abbreviations: HIF-1α: hypoxia-inducible factor-1α; HK, hexokinase; GPI, glycosylphosphatidylinositol; HRE, hypoxia response element; LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PFK1, phosphofructokinase 1; PGAM, phosphoglycerate mutase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; TCA, tricarboxylic acid; VHL, von Hippel–Lindau.

Impact of HIF activation in β-cells

Mitochondrial oxidative phosphorylation plays a key role in glucose-sensitive insulin secretion, inferring that oxygen-sensing pathways modulate insulin secretion. In β-cells, exposure to glucose induces ATP production, which increases oxygen consumption by mitochondria [58–60]. These steps are crucial for the initial phase of insulin release after a meal. The current model of the mechanism of insulin secretion in β-cell purports a role for oxidative metabolism. However, this model has recently been challenged by one proposing a stronger role for glycolysis [61,62]. As discussed earlier, HIF-1α activation can promote glycolytic function with downstream consequences for mitochondrial activity. Evidence of the role of HIF-1α in modulating β-cell survival and function exists from multiple studies. However, the impact of this on cell function and disease progression is controversial and appears to be very much context dependent. Despite the difficulty in harvesting human pancreatic material from T1D patients, as well as intrinsic species differences regarding the physiology of the pancreas, a limited number of studies have attempted to investigate the impact of hypoxia and activation of hypoxia-sensitive pathways in β-cells. This is an important area of research in need of further investigation.

Studies to date report both deleterious and protective effects of HIF-1α activation in β-cells (depending on the model and approach used) indicating a dual role for HIF-1α, which appears to be dependent upon whether the β-cells are under stressed or homeostatic conditions when exposed to hypoxia. We will discuss these two scenarios later.

Role of HIF-1 in healthy β-cells

Mice lacking pVHL, a pivotal protein in the oxygen-dependent degradation of HIF protein, exhibit constitutive, oxygen-independent activation of HIF pathways. Mice with pancreatic or β-cell-specific knockout of pVHL present with diabetes, impaired insulin secretion, and altered expression of genes crucial for β-cell function, including those related to glucose transport and glycolysis [63]. In addition, islets isolated from these mice displayed compromised glucose uptake, defective glucose metabolism, and impaired GSIS. This is consistent with a deleterious role for HIF-1 in diabetes. However, it should be remembered that regulation by pVHL is not restricted to HIF and many other pathways are likely affected in these mice. However, the restoration of GSIS was achieved by selective deletion of *HIF-1* in VHL-deficient β-cells [63]. In a separate study, combined conditional deletion of pVHL and HIF-1α rescued pVHL-knockout mice from compromised insulin secretion [64]. Overexpression of pancreatic HIF-2α did not result in significant differences in systemic glucose levels or β-cell area compared with wild-type littermates, even in metabolic stress conditions [65]. These data point to a specific role of HIF-1, rather than HIF-2, in modulating glucose and energy homeostasis in β-cells lacking pVHL, which is directly linked to insulin secretion and, therefore, regulation of systemic glucose levels.

Consistent with a deleterious role for hypoxia in T1D, hypoxia was shown to activate the insulin transcriptional repressor basic helix–loop–helix family member e40 (*BHLHE40*) in murine and human β-cells [66]. *In vitro* and *ex vivo BHLHE40* silencing reversed hypoxia-induced defects in insulin secretion, demonstrating an important role of hypoxia as regulator of the β-cell function. The authors identified that hypoxia inducible *BHLHE40* expression inhibited insulin secretion in β-cells via downregulation of the musculoaponeurotic fibrosarcoma oncogene family A (MAFA) transcriptional factor. Importantly, in this study, the effects were not shown to be HIF-1-dependent. Interestingly, rodent β-cell specific knockout of *Hif1a* or *Arnt* (the gene encoding the HIF-1β protein) triggered a global downregulation of glycolytic enzymes, reducing GSIS *in vivo* and *in vitro* [67,68]. This underscores the critical role of hypoxia and HIF-1α activation in glycolytic enzyme regulation, impacting both *in vivo* and *in vitro* β-cell response to glucose stimulation as well as insulin secretion in homeostatic (healthy) β-cells.

The aforementioned studies suggest that modulation of HIF-1α can contribute to dysregulation of the metabolic network required for effective β-cell function and insulin secretion, although whether this is protective or detrimental remains unclear and is likely context- and model-dependent. Because of the requirement for mitochondrial activity for appropriate insulin secretion in β-cells (as outlined earlier), activation of HIF-1α may be predicted to reduce GSIS as HIF-1 has been shown to reduce oxidative phosphorylation. This concept aligns with the established notion that insulin secretion is intricately tied to mitochondrial oxidative phosphorylation activity that is regulated by the HIF-1α pathway. Although this evidence in homeostatic β-cells indicates a deleterious effect of HIF-1α activation, other studies have demonstrated a protective role for the HIF-1 pathway under stressed conditions in β-cells that model T1D (or T2D). This issue related to differential effects of HIF-1 in healthy or stressed β-cells is elaborated later.

Role of HIF-1 in stressed β-cells

A previous study has described the onset of hypoxia in β-cells in a glucose concentration-dependent manner, as assessed by the formation of **pimonidazole–protein adducts** in rat islets

and insulin-producing cell line (INS-1 832/13) treated with glucose [69]. In the same study, glucose treatment upregulated HIF-1α and HIF-2α nuclear translocation, along with the expression of HIF-target genes. This effect was blunted by small interfering RNA-mediated knockdown of *Hif1α* and *Hif2α*, alone or in combination. The authors hypothesize that glucose-induced O_2 consumption generates intracellular hypoxia that activates the HIF pathway in rat β-cells. This effect has also been described in other cellular models, such as intestinal epithelial cells [70]. Furthermore, the accumulation of pimonidazole has also been demonstrated upon glucose stimulation in *ob/ob* mice and mice under high-fat diet, compared with lean and chow-fed mice, respectively [71]. In these lines, evidence points to the activation of hypoxia-sensitive pathways in β-cells as part of an adaptive response to metabolic stress. Metabolically induced hypoxia contributes to the upregulation of glycolytic enzymes, along with other hypoxia-responsive genes. In fact, studies using islets from hyperglycemic rats have shown the upregulation of the glycolytic enzyme hexokinase (Hk1), and Ldha, Mct1 and 4, all of which are involved in pyruvate escape from mitochondrial metabolism [72–75]. This process is similarly noted in islets from diabetic mice. In summary, these studies indicate that hyperglycemia instigates hypoxia in β-cells *in vivo*.

The activation of the HIF-1α pathway by hypermetabolism in pancreatic islets was also demonstrated by exposing non-diabetic mouse islets to the glucokinase activator GKA50 [71]. In this model, chemical inhibition of HIF-1α by PX-478 restored islet basal insulin secretion to near control values, although it was unable to restore the high insulin release observed in hyperglycemic conditions. Also, inhibition of HIF-1α increased cell death by apoptosis in a disease model of T2D via upregulation of the human islet amyloid polypeptide (hIAPP) in the rat β-cell line INS-1 832/13. Alongside its impact on cell viability, the authors noted that HIF-1α activation in stressed β-cells is protective, albeit at the cost of limiting β-cell function [76]. Therefore, while there are limited pharmacological studies on the role of HIF altering drugs in T1D, these studies indicate it may be protective. Of note, while this may contrast with some of the genetic knockout studies suggesting a protective role for HIF (outlined earlier), it should be considered that genetic studies are often poor predictors of pharmacological studies.

There are limited data describing the role of HIF-1α in the pathogenesis of T1D. To date, most studies have focused on the effect of hypoxia and β-cell function in experimental models of T2D [24] and, due to its more abundance occurrence, more attention has been given to T2D. However, recent evidence in a population-based study point to overweight and obesity as a potential contributor to development of autoimmunity against β-cells and T1D, somewhat blurring the lines between T1D and T2D [20]. In specific experimental models of T1D, β-cell specific deletion of *Hif1α* was shown to render these cells more vulnerable to the β-cell toxin streptozotocin (STZ) or infection with coxsackieviruses [27], pointing to a protective role of HIF-1α pathway in stressed β-cells during T1D disease development. In this study, ablation of *Hif1α* increased viral replication, triggered autoimmunity, and increased the susceptibility of male nonobese diabetic (NOD) mice to develop T1D. Similarly, *in vitro* treatment with the HIF-1α-antagonist KC7F2 increased proinflammatory cytokine-induced apoptosis in the INS-1 832/13 rat insulinoma cell line [37]. Pathophysiological activation of HIF-1α in β-cells of pre-T1D and T1D patients point to an adaptive, and likely protective, role of HIF-1 in stressed islets. Finally, polymorphism in the *HIF1A* gene, which enhances the activation of HIF-1α in response to stress, is observed less frequently in both T1D and T2D patients compared with healthy nondiabetic individuals [25]. Overall, these studies implicate a protective role for HIF-1 in stressed β-cells during T1D, however clearly this is an area in need of further research, particularly *in vivo* and clinical studies.

These stress conditions in pancreatic islets promote the occurrence of metabolic, transcriptional, and translational errors in β-cells, all of which are linked to their targeted autoimmune destruction.

Consequently, the interplay of HIF-1α in stressed islets, along with the observed polymorphism in the *HIF1A* gene, underscores the adaptive nature of this pathway, shedding light on potential therapeutic avenues to halt the development of T1D in *de novo* patients. Based on these studies we propose the possibility that activation of HIF-1α pathway may be protective both for β-cell recovery in stress conditions and to prevent the generation of neoepitopes and subsequent targeted autoimmune response. Nonetheless, the conflicting evidence regarding the role of HIFs in homeostatic and stressed islets necessitates further investigation, particularly to determine the impact of pharmacologic manipulation of the HIF-1α pathway in the development of T1D. Importantly, while thousands of patients have been treated for CKD-associated anemia with HIF-1 promoting prolyl-hydroxylase inhibitors (PHIs) [77], deleterious effects in terms of the induction of diabetes has not been extensively described arguing against a prodiabetic role for HIF in patients. In a recent meta study of patients treated with PHIs, kidney or cardiac-related side effects were not reported, however hyperkalemia and risk of hypertension were reported in some groups [78]. In separate meta-analyses, no increase in all-cause mortality was reported and PHIs were deemed to have a good safety profile [79–81]. Of interest, in the context of diabetes, treatment with the PHI roxadustat improved glucose metabolism in human primary myotubes from diabetic patients as well as healthy controls [82].

In summary, emerging evidence suggests a significant impact of HIF-1α activation in the context of β-cell vulnerability, viral infections, and inflammatory and autoimmune responses. However, whether HIF is protective or deleterious is complex and likely context dependent and in need of further study.

Impact of HIF-1α activation on immune cells

Besides the impact on β-cell function described earlier, HIF-1α activation modulates the phenotype and activity of T lymphocytes (and other immune cells) that drive the autoimmune destruction of β-cells in T1D. *In vitro*, low oxygen levels improve cell expansion, effector phenotype, and cytotoxic capacities of CD4 and CD8 T cells. This is evident by increased expression of CD25, EOMES, TOX, and increased granzyme B (GZMB) and interferon-γ (IFN-γ) production [83–86]. In autoimmune diseases, HIF expression has been associated with the cytotoxic signature of skin infiltrating CD4 and CD8 cells [87] suggesting a role for hypoxia as regulator of autoimmunity. Similarly, during the course of T1D, the hypoxic environment in β-cells may contribute to the loss of **peripheral tolerance** and accelerate β-cell destruction by controlling the T helper type 17 (Th17)/regulatory T cell (Treg) axis, tilting cells towards a proinflammatory phenotype via FOXP3 degradation and by increasing cytotoxic capacity of autoreactive T cells, preventing T cell exhaustion via LAG3 inhibition [88,89]. Control of this exhaustion program within autoantigen specific CD8 T cells appeared critical for disease development and allowed the discrimination between slow and rapid disease progressors [90]. Considering the scope of this review, we refer the reader to a more extensive overview on the impact of HIF-1α modulation on different immune cell phenotypes [28]. In summary of this extensive work, multiple studies over many years have identified HIF-1α as a key regulator of immune cell survival, phenotype, and function. Of note, pharmacological activation of HIF-1α effectively reduces inflammatory processes in a variety of chronic experimental disease models including inflammatory bowel disease, arthritis, and *Helicobacter pylori*-induced gastric injury [91–93]. The immunomodulatory and cytoprotective effects of HIF-1α activation shed light on new therapeutic approaches against the development of T1D. The therapeutic potential of pharmacologically activating HIF-1α during the onset of T1D is discussed next.

Pharmacological modulation of the HIF-1α pathway in T1D

In this review, based on the literature discussed, we propose that HIF-1 through its complex and context-specific regulation of β-cell and immune cell function likely has an important role to play in

disease progression in T1D. However, the *in vivo* pharmacological studies of treating T1D with drugs that alter the HIF pathway (either positively or negatively) remain to be done. Therefore, the pharmacological modulation of the HIF-1α pathway may represent a new and exciting approach to T1D therapy. PHIs target PHDs enzymes that sense intracellular oxygen levels to elicit adaptive responses to hypoxia. Thus, blocking of PHD enzyme function leads to activation of the HIF-1α pathway. It would be of interest to investigate these drugs in the treatment of T1D. By contrast, a number of studies have described HIF-1-inhibiting drugs although these have yet to be introduced clinically [94].

Interestingly, a protective effect of PHIs has been described in models of T2D, improving symptoms related to obesity, metabolic dysfunction, and glycemic control [95–97]. There is, however, limited evidence of the role these enzymes in β-cell metabolism and function in the context of T1D. Treatment of the rat β-cell line INS-1 832/13 with the PHI ethyl-3,4-dihydroxybenzoate (EDHB, 1000 μM) decreased insulin secretion and glucose utilization in INS-1 cells, both in low and high glucose conditions [98]. Treatment with EDHB in high glucose conditions decreased the abundance of TCA cycle intermediates and increased lactic acid production in these cells. Similarly, *ex vivo* cultures of human pancreatic islets with EDHB also decreased insulin production in both low and high glucose exposure. Importantly, INS-1 cells treated with increasing doses of EDHB retained their cell viability comparable with control conditions. The authors point to a role of PHD3 inhibition as the target for the metabolic reprogramming observed in β-cells exposed to EDHB [98]. *In vivo* treatment with the pan-hydroxylase inhibitor dimethyloxalylglycine (DMOG) decreased insulin secretion upon glucose challenge in C57BL/6N mice [99]. These findings are consistent with the current literature, in which activation of the HIF-1α pathway decreases mitochondrial function, which is directly linked to the ability of β-cells to secrete insulin.

The use of HIF-1α-activating drugs, such as PHIs, may initially appear counterintuitive in the context of β-cell function. Notably, inhibition of HIF-1α with PX-478 demonstrated enhanced islet insulin production and improved glycemic control in a **STZ-induced diabetic mouse model** [71]. It is crucial to highlight that in this study, exposure of mice to STZ was concluded at the start of PX-478 treatment, meaning that mice were already diabetic when treatment with HIF-1α inhibitor was initiated. By contrast, others have demonstrated increased susceptibility to STZ-induced T1D in NOD mice with β-cell-specific deletion of HIF-1α [27], pointing to a cytoprotective role of a functional HIF-1α pathway in stressed β-cells. These results suggest that treatment with PHIs, and therefore pharmacological activation of the HIF-1α pathway, is beneficial to β-cells that are undergoing stress before the onset of diabetes (see stages 1 and 2 in Figure I in Box 1), rather than attempting to reverse established diabetes. Activation of HIF-1α pathway may convey a protective effect on β-cells with regards to mitochondrial dysfunction, viral replication, and exposure to proinflammatory cytokines. Consequently, treatment with PHIs may prevent the accumulation of metabolic, transcriptional, and translational errors that trigger the autoimmune attack of β-cells in the early stages of T1D. Cleary, this is an area in need of further investigation.

There are numerous studying reporting the therapeutic impact of PHIs in extra pancreatic complications of diabetes in patients and experimental models that include diabetic kidney disease [96,100], peripheral neuropathy [101], atherosclerosis [102], and wound healing [103,104]. Roxadustat, a PHI that induces the stabilization and activation of HIF-1α, has been approved for clinical use in the treatment of anemia associated with CKD disease [105], signaling their potential efficacy in the management of T1D. While there are PHIs approved for other medical conditions, further research is needed to elucidate the intricate interplay of oxygen-sensitive pathways in the pathogenesis of T1D, including the role of PHD enzymes in β-cell metabolism and function. These findings collectively provide avenues for novel therapeutic interventions to

prevent β-cell destruction in T1D. Importantly, strategies to overcome oxygen deprivation during transplantation programs also represent a technical and economical challenge for patients with established T1D (Box 2). In summary, the exploration of HIF-related strategies offers an exciting prospect for advancing our understanding and treatment options in the complex metabolic landscape of T1D.

Concluding remarks and future perspectives

In conclusion, in this review, we discussed evidence for intricate connections between hypoxia, HIF activation, and glycolysis, unraveling their pivotal roles in β-cell function and likely the pathogenesis of T1D (see Outstanding questions). We explored the current literature regarding the impact of HIF-1α on insulin secretion, glucose metabolism, and immune cell phenotype, shedding light on its multifaceted contributions to T1D onset and progression. Emphasizing recent advances, we have elucidated how metabolically induced hypoxia upregulates glycolytic enzymes, impacting both *in vivo* and *in vitro* insulin secretion and glucose tolerance. The intricate network involving VHL, HIFs, PHDs, MAFA, and BHLHE40 adds complexity to the understanding of β-cell responses under varying physiological conditions, which is relevant to the pathogenesis of T1D. In this context, sufficient evidence exists for HIF-altering drugs such as PHIs or indeed the HIF-1 inhibitor PX-478 to be further investigated in pre-clinical studies as potential novel T1D therapeutics. In conclusion, the studies outlined in this review suggest the potential of pharmacologic manipulation of the HIF pathway as a new therapeutic option for the treatment or prevention of T1D disease.

Outstanding questions

What are the key factors influencing the impact of HIF-1α activation on β-cell function, and how does this vary across different stages of T1D onset and progression?

What is the specific role of HIF-1α, compared with HIF-2α, in modulating glucose and energy homeostasis in β-cells, and how does this relate to insulin secretion and systemic glucose uptake?

What are the mechanisms underlying the role of HIF-1α in modulating the phenotype of immune cells and its potential influence on the auto-immune destruction of β-cells?

Can pharmacological manipulation of the HIF pathway serve as a viable therapeutic option in T1D?

How can strategies to overcome oxygen deprivation during transplantation of pancreatic islets be developed, and what technical and economic challenges might arise in implementing encapsulating devices or enhancing oxygen delivery?

Can the role of HIFs and glycolytic enzymes provide a more comprehensive understanding of the multifactorial nature of T1D pathogenesis?

Declaration of interests

The authors declare no competing interests.

References

1. Skyler, J.S. *et al.* (2017) Differentiation of diabetes by pathophysiology, natural history, and prognosis. *Diabetes* 66, 241–255
2. Roep, B.O. (2003) The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. *Diabetologia* 46, 305–321
3. Zirpel, H. and Roep, B.O. (2021) Islet-resident dendritic cells and macrophages in type 1 diabetes: in search of bigfoot's print. *Front. Endocrinol. (Lausanne)* 12, 666795
4. Smith, M.J. *et al.* (2017) B cells in type 1 diabetes mellitus and diabetic kidney disease. *Nat. Rev. Nephrol.* 13, 712
5. Sabetkam, S. *et al.* (2023) The dysfunction of natural killer cells is essential for the development of type 1 diabetes. *Pathol. Res. Pract.* 247, 154556
6. van Lummel, M. *et al.* (2016) Dendritic cells guide islet autoimmunity through a restricted and uniquely processed peptidome presented by high-risk HLA-DR. *J. Immunol.* 196, 3253–3263
7. Barrett, J.C. *et al.* (2009) Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat. Genet.* 41, 703–707
8. Ramos-Rodríguez, M. *et al.* (2021) The β-cell genomic landscape in T1D: implications for disease pathogenesis. *Curr. Diab. Rep.* 21, 1
9. Eizirik, D.L. *et al.* (2021) From pancreatic β-cell gene networks to novel therapies for type 1 diabetes. *Diabetes* 70, 1915–1925
10. Quattrin, T. *et al.* (2023) Type 1 diabetes. *Lancet* 401, P2149–P2162
11. Marhfour, I. *et al.* (2012) Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. *Diabetologia* 55, 2417–2420
12. Thomaidou, S. *et al.* (2021) Long RNA sequencing and ribosome profiling of inflamed β-cells reveal an extensive translatome landscape. *Diabetes* 70, 2299–2312
13. Kracht, M.J.L. *et al.* (2016) Neoantigens and microenvironment in type 1 diabetes: lessons from antitumor immunity. *Trends Endocrinol. Metab.* 27, 353–362
14. Kracht, M.J.L. *et al.* (2017) Autoimmunity against a defective ribosomal insulin gene product in type 1 diabetes. *Nat. Med.* 23, 501–507
15. Roep, B.O. *et al.* (2021) Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). *Nat. Rev. Endocrinol.* 17, 150–161
16. Marré, M.L. *et al.* (2016) Inherent ER stress in pancreatic islet β cells causes self-recognition by autoreactive T cells in type 1 diabetes. *J. Autoimmun.* 72, 33–46
17. De Beeck, A.O. and Eizirik, D.L. (2016) Viral infections in type 1 diabetes mellitus–why the β cells? *Nat. Rev. Endocrinol.* 12, 263–273
18. Zheng, P. *et al.* (2018) Gut microbiome in type 1 diabetes: a comprehensive review. *Diabetes Metab. Res. Rev.* 34, e3043
19. Warncke, K. *et al.* (2022) Elevations in blood glucose before and after the appearance of islet autoantibodies in children. *J. Clin. Invest.* 132, e162123
20. Zucker, I. *et al.* (2022) Obesity in late adolescence and incident type 1 diabetes in young adulthood. *Diabetologia* 65, 1473–1482
21. DM, N. *et al.* (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 329, 977–986
22. Komatsu, H. *et al.* (2018) Impact of oxygen on pancreatic islet survival. *Pancreas* 47, 533–543
23. Wassmer, C.H. *et al.* (2021) Impact of ischemia time on islet isolation success and posttransplantation outcomes: a retrospective study of 452 pancreas isolations. *Am. J. Transplant.* 21, 1493–1502
24. Yamagata, K. *et al.* (2024) Roles of β-cell hypoxia in the progression of type 2 diabetes. *Int. J. Mol. Sci.* 25, 4186
25. Geza, N. *et al.* (2009) Association of hypoxia inducible factor-1 alpha gene polymorphism with both type 1 and type 2 diabetes in a Caucasian (Hungarian) sample. *BMC Med. Genet.* 10, 79
26. Krishan, P. *et al.* (2017) Carbohydrate restriction ameliorates nephropathy by reducing oxidative stress and upregulating HIF-1α levels in type-1 diabetic rats. *J. Diabetes Metab. Disord.* 16, 47
27. Lalwani, A. *et al.* (2019) β Cell hypoxia-inducible factor-1α is required for the prevention of type 1 diabetes. *Cell Rep* 27, 2370–2384.e6

28. Taylor, C.T. and Scholz, C.C. (2022) The effect of HIF on metabolism and immunity. *Nat. Rev. Nephrol.* 18, 573–587
29. MacDonald, P.E. *et al.* (2005) Glucose-sensing mechanisms in pancreatic β-cells. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (360), pp. 2211–2225
30. Ashcroft, S.J.H. and Ashcroft, F.M. (1990) Properties and functions of ATP-sensitive K-channels. *Cell. Signal.* 2, 197–214
31. Rorsman, P. and Ashcroft, F.M. (2018) Pancreatic β-cell electrical activity and insulin secretion: of mice and men. *Physiol. Rev.* 98, 117–214
32. Campbell, J.E. and Newgard, C.B. (2021) Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nat. Rev. Mol. Cell Biol.* 22, 142
33. Ho, T. *et al.* (2023) A plasma membrane-associated glycolytic metabolon is functionally coupled to KATP channels in pancreatic α and β cells from humans and mice. *Cell Rep.* 42, 112394
34. Jiang, S. *et al.* (2020) Diabetic-induced alterations in hepatic glucose and lipid metabolism: the role of type 1 and type 2 diabetes mellitus. *Mol. Med. Rep.* 22, 603–611
35. Veneti, S. *et al.* (2023) Ketone bodies in diabetes mellitus: friend or foe? *Nutrients* 15, 4383
36. Haythorne, E. *et al.* (2022) Altered glycolysis triggers impaired mitochondrial metabolism and mTORC1 activation in diabetic β-cells. *Nat. Commun.* 13, 6754
37. Nomoto, H. *et al.* (2020) Activation of the HIF1α/PFKFB3 stress response pathway in beta cells in type 1 diabetes. *Diabetologia* 63, 149–161
38. Zhang, D. *et al.* (2018) Increased mitochondrial fission is critical for hypoxia-induced pancreatic beta cell death. *PLoS One* 13, e0197266
39. Bernardi, L. *et al.* (2017) Oxygen-induced impairment in arterial function is corrected by slow breathing in patients with type 1 diabetes. *Sci. Rep.* 7, 6001
40. Catrina, S.B. and Zheng, X. (2021) Hypoxia and hypoxia-inducible factors in diabetes and its complications. *Diabetologia* 64, 709–716
41. Zheng, X. *et al.* (2022) Repression of hypoxia-inducible factor-1 contributes to increased mitochondrial reactive oxygen species production in diabetes. *Elife* 11, e70714
42. Fagundes, R.R. and Taylor, C.T. (2017) Determinants of hypoxia-inducible factor activity in the intestinal mucosa. *J. Appl. Physiol.* 123, 1328–1334
43. Semenza, G.L. (2011) Oxygen sensing, homeostasis, and disease. *N. Engl. J. Med.* 365, 537–547
44. Wang, G.L. *et al.* (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl. Acad. Sci. U. S. A.* 92, 5510–5514
45. Kierans, S.J. and Taylor, C.T. (2021) Regulation of glycolysis by the hypoxia-inducible factor (HIF): implications for cellular physiology. *J. Physiol.* 599, 23–37
46. Kierans, S.J. *et al.* (2023) Hypoxia induces a glycolytic complex in intestinal epithelial cells independent of HIF-1-driven glycolytic gene expression. *Proc. Natl. Acad. Sci. U. S. A.* 120, e2208117120
47. Mathupala, S.P. *et al.* (2001) Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. *J. Biol. Chem.* 276, 43407–43412
48. Semenza, G.L. *et al.* (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J. Biol. Chem.* 269, 23757–23763
49. Minchenko, O. *et al.* (2003) Hypoxic regulation of the 6-phosphofructo-2-kinase/fructose-2,6- bisphosphatase gene family (PFKFB-1-4) expression in vivo. *FEBS Lett.* 554, 264–270
50. Semenza, G.L. *et al.* (1996) Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J. Biol. Chem.* 271, 32529–32537
51. Graven, K.K. *et al.* (1999) Identification of an oxygen responsive enhancer element in the glyceraldehyde-3-phosphate dehydrogenase gene. *Biochim. Biophys. Acta* 1447, 208–218
52. Luo, W. *et al.* (2011) Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* 145, 732–744
53. O'Rourke, J.F. *et al.* (1996) Identification of hypoxically inducible mRNAs in HeLa cells using differential-display PCR. Role of hypoxia-inducible factor-1. *Eur. J. Biochem.* 241, 403–410
54. Maltepe, E. *et al.* (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* 386, 403–407
55. Iyer, N.V. *et al.* (1998) Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev.* 12, 149–162
56. Papandreou, I. *et al.* (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab.* 3, 187–197
57. Kim, J.W. *et al.* (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* 3, 177–185
58. Wang, W. *et al.* (2005) Increased oxygen consumption rates in response to high glucose detected by a novel oxygen biosensor system in non-human primate and human islets. *J. Endocrinol.* 185, 445–455
59. Longot, E.A. *et al.* (1991) Oscillations in cytosolic free Ca2+, oxygen consumption, and insulin secretion in glucose-stimulated rat pancreatic islets. *J. Biol. Chem.* 266, 9314–9319
60. Hutton, J.C. and Malaisse, W.J. (1980) Dynamics of O2 consumption in rat pancreatic islets. *Diabetologia* 18, 395–405
61. Merrins, M.J. *et al.* (2022) Metabolic cycles and signals for insulin secretion. *Cell Metab.* 34, 947–968
62. Merrins, M.J. and Kibbey, R.G. (2024) Glucose regulation of β-cell KATP channels: it is time for a new model! *Diabetes* 73, 856–863
63. Cantley, J. *et al.* (2009) Deletion of the von Hippel-Lindau gene in pancreatic beta cells impairs glucose homeostasis in mice. *J. Clin. Invest.* 119, 125–135
64. Zehetner, J. *et al.* (2008) PVHL is a regulator of glucose metabolism and insulin secretion in pancreatic beta cells. *Genes Dev.* 22, 3135–3146
65. Brunt, J.J. *et al.* (2014) Overexpression of HIF-2α in pancreatic β cells does not alter glucose homeostasis. *Islets* 6, e1006075
66. Tsuyama, T. *et al.* (2023) Hypoxia causes pancreatic β-cell dysfunction and impairs insulin secretion by activating the transcriptional repressor BHLHE40. *EMBO Rep.* 24, e56227
67. Cheng, K. *et al.* (2010) Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. *J. Clin. Invest.* 120, 2171–2183
68. Gunton, J.E. *et al.* (2005) Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. *Cell* 122, 337–349
69. Bensellam, M. *et al.* (2012) Glucose-induced O consumption activates hypoxia inducible factors 1 and 2 in rat insulin-secreting pancreatic beta-cells. *PLoS One* 7, e29807
70. Kelly, C.J. *et al.* (2015) Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 17, 662–671
71. Ilegems, E. *et al.* (2022) HIF-1 inhibitor PX-478 preserves pancreatic β cell function in diabetes. *Sci. Transl. Med.* 14, eaba9112
72. Li, X. *et al.* (2006) Islet microvasculature in islet hyperplasia and failure in a model of type 2 diabetes. *Diabetes* 55, 2965–2973
73. Ross Laybutt, D. *et al.* (2002) Genetic regulation of metabolic pathways in beta-cells disrupted by hyperglycemia. *J. Biol. Chem.* 277, 10912–10921
74. Jonas, J.C. *et al.* (1999) Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. *J. Biol. Chem.* 274, 14112–14121
75. Hosokawa, H. *et al.* (1995) Upregulated hexokinase activity in isolated islets from diabetic 90% pancreatectomized rats. *Diabetes* 44, 1328–1333
76. Montemurro, C. *et al.* (2019) IAPP toxicity activates HIF1α/PFKFB3 signaling delaying β-cell loss at the expense of β-cell function. *Nat. Commun.* 10, 2679
77. Minutolo, R. *et al.* (2024) Efficacy and safety of hypoxia-inducible factor prolyl hydroxylase inhibitors in patients with chronic kidney disease: meta-analysis of phase 3 randomized controlled trials. *Clin. Kidney J.* 17, sfad143
78. Tian, L. *et al.* (2024) Cardiovascular and renal safety outcomes of hypoxia-inducible factor prolyl-hydroxylase inhibitor roxadustat for anemia patients with chronic kidney disease: a systematic review and meta-analysis. *Ren. Fail.* 46, 2313864
79. Li, M. *et al.* (2021) Effectiveness of hypoxia-induced factor prolyl hydroxylase inhibitor for managing anemia in chronic kidney

disease: a systematic review and meta-analysis. *Eur. J. Clin. Pharmacol.* 77, 491–507
80. Zheng, Q. *et al.* (2020) Efficacy and safety of HIF prolyl-hydroxylase inhibitor vs epoetin and darbepoetin for anemia in chronic kidney disease patients not undergoing dialysis: a network meta-analysis. *Pharmacol. Res.* 159, 105020
81. Zhao, H. *et al.* (2023) An updated meta-analysis on the efficacy and safety of hypoxia-inducible factor prolyl hydroxylase inhibitor treatment of anemia in nondialysis-dependent chronic kidney disease. *Ren. Fail.* 45, 2258986
82. Mäkinen, S. *et al.* (2024) Activation of the hypoxia-inducible factor pathway by roxadustat improves glucose metabolism in human primary myotubes from men. *Diabetologia*, Published online May 30, 2024. https://doi.org/10.1007/S00125-024-06185-6
83. Liikanen, I. *et al.* (2021) Hypoxia-inducible factor activity promotes antitumor effector function and tissue residency by CD8+ T cells. *J. Clin. Invest.* 131, e143729
84. Gropper, Y. *et al.* (2017) Culturing CTLs under hypoxic conditions enhances their cytolysis and improves their anti-tumor function. *Cell Rep.* 20, 2547–2555
85. Cunha, P.P. *et al.* (2023) Oxygen levels at the time of activation determine T cell persistence and immunotherapeutic efficacy. *Elife* 12, e84280
86. Veliça, P. *et al.* (2021) Modified hypoxia-inducible factor expression in CD8+ T cells increases antitumor efficacy. *Cancer Immunol. Res.* 9, 401–414
87. Little, A.J. *et al.* (2023) HIF-1 regulates pathogenic cytotoxic T cells in lupus skin disease. *JCI Insight* 8, e166076
88. Grebinoski, S. *et al.* (2022) Autoreactive CD8+ T cells are restrained by an exhaustion-like program that is maintained by LAG3. *Nat. Immunol.* 23, 868–877
89. Liu, X. *et al.* (2021) N-Acetylcysteine improves inflammatory response in COPD patients by regulating Th17/Treg balance through hypoxia inducible factor-1 α pathway. *Biomed. Res. Int.* 2021, 6372128
90. Wiedeman, A.E. *et al.* (2020) Autoreactive CD8+ T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. *J. Clin. Invest.* 130, 480–490
91. Noto, J.M. *et al.* (2023) Targeting hypoxia-inducible factor-1 alpha suppresses Helicobacter pylori-induced gastric injury via attenuation of both cag-mediated microbial virulence and proinflammatory host responses. *Gut Microbes* 15, 2263936
92. Muz, B. *et al.* (2012) Prolyl hydroxylase domain enzyme 2 is the major player in regulating hypoxic responses in rheumatoid arthritis. *Arthritis Rheum.* 64, 2856–2867
93. Tambuwala, M.M. *et al.* (2015) Targeted delivery of the hydroxylase inhibitor DMOG provides enhanced efficacy with reduced systemic exposure in a murine model of colitis. *J. Control. Release* 217, 221–227
94. Bui, B.P. *et al.* (2022) Hypoxia-inducible factor-1: a novel therapeutic target for the management of cancer, drug resistance, and cancer-related pain. *Cancers (Basel)* 14, 6054
95. Riopel, M. *et al.* (2020) Inhibition of prolyl hydroxylases increases hepatic insulin and decreases glucagon sensitivity by an HIF-2α-dependent mechanism. *Mol. Metab.* 41, 101039
96. Sugahara, M. *et al.* (2020) Prolyl hydroxylase domain inhibitor protects against metabolic disorders and associated kidney disease in obese type 2 diabetic mice. *J. Am. Soc. Nephrol.* 31, 560–577
97. Rahtu-Korpela, L. *et al.* (2014) HIF prolyl 4-hydroxylase-2 inhibition improves glucose and lipid metabolism and protects against obesity and metabolic dysfunction. *Diabetes* 63, 3324–3333
98. Huang, M. *et al.* (2016) Role of prolyl hydroxylase domain proteins in the regulation of insulin secretion. *Physiol. Rep.* 4, e12722
99. Hoang, M. *et al.* (2022) Isoform-specific roles of prolyl hydroxylases in the regulation of pancreatic β-cell function. *Endocrinology* 163, bqab226
100. Zhang, L. *et al.* (2022) Effect of Roxadustat versus erythropoietin (EPO) for treating anemia in patients with diabetic kidney disease: a retrospective cohort study. *Ann. Transl. Med.* 10, 1224
101. Rojas, D.R. *et al.* (2018) Hypoxia-inducible factor 1α protects peripheral sensory neurons from diabetic peripheral neuropathy by suppressing accumulation of reactive oxygen species. *J. Mol. Med.* 96, 1395–1405
102. Rahtu-Korpela, L. *et al.* (2016) Hypoxia-inducible factor prolyl 4-hydroxylase-2 inhibition protects against development of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 36, 608–617
103. Zhu, Y. *et al.* (2019) Roxadustat promotes angiogenesis through HIF-1α/VEGF/VEGFR2 signaling and accelerates cutaneous wound healing in diabetic rats. *Wound Repair Regen.* 27, 324–334
104. Botusan, I.R. *et al.* (2008) Stabilization of HIF-1α is critical to improve wound healing in diabetic mice. *Proc. Natl. Acad. Sci. U. S. A.* 105, 19426
105. Chen, N. *et al.* (2019) Roxadustat for anemia in patients with kidney disease not receiving dialysis. *N. Eng. J. Med.* 381, 1001–1010
106. Peters, L.D. *et al.* (2023) Modeling cell-mediated immunity in human type 1 diabetes by engineering autoreactive CD8+ T cells. *Front. Immunol.* 14, 1142648
107. Eringsmark Regnéll, S. and Lernmark, Å. (2013) The environment and the origins of islet autoimmunity and Type 1 diabetes. *Diabet. Med.* 30, 155
108. Furman, B.L. (2021) Streptozotocin-induced diabetic models in mice and rats. *Curr. Protoc.* 1, e78
109. Insel, R.A. *et al.* (2015) Staging presymptomatic type 1 diabetes: a scientific Statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 38, 1964
110. Shapiro, A.M.J. *et al.* (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* 343, 230–238
111. Harlan, D.M. *et al.* (2009) Current advances and travails in islet transplantation. *Diabetes* 58, 2175–2184
112. Narayanan, S. *et al.* (2017) Intra-islet endothelial cell and β-cell crosstalk: IMPLICATION for islet cell transplantation. *World J. Transplant.* 7, 117
113. Konstantinova, I. and Lammert, E. (2004) Microvascular development: learning from pancreatic islets. *Bioessays* 26, 1069–1075
114. Gunton, J.E. (2020) Hypoxia-inducible factors and diabetes. *J. Clin. Invest.* 130, 5063–5073
115. Lum, J.J. *et al.* (2007) The transcription factor HIF-1alpha plays a critical role in the growth factor-dependent regulation of both aerobic and anaerobic glycolysis. *Genes Dev.* 21, 1037–1049
116. Lenzen, S. (2008) Oxidative stress: the vulnerable beta-cell. *Biochem. Soc. Trans.* 36, 343–347
117. Paredes-Juarez, G.A. *et al.* (2015) DAMP production by human islets under low oxygen and nutrients in the presence or absence of an immunoisolating-capsule and necrostatin-1. *Sci. Rep.* 5, 14623