Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Review

Gluconeogenic Enzymes in β-Cells: Pharmacological Targets for Improving Insulin Secretion

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Pancreatic β-cells express the gluconeogenic enzymes glucose 6-phosphatase (G6Pase), fructose 1,6-bisphosphatase (FBP), and phosphoenolpyruvate (PEP) carboxykinase (PCK), which modulate glucose-stimulated insulin secretion (GSIS) through their ability to reverse otherwise irreversible glycolytic steps. Here, we review current knowledge about the expression and regulation of these enzymes in the context of manipulating them to improve insulin secretion in diabetics. Because the regulation of gluconeogenic enzymes in β-cells is so poorly understood, we propose novel research avenues to study these enzymes as modulators of insulin secretion and β-cell dysfunction, with especial attention to FBP, which constitutes an attractive target with an inhibitor under clinical evaluation at present.

Highlights

Glucose must be metabolized into pyruvate through the glycolytic pathway in β-cells for proper oscillatory and biphasic insulin secretion.

Chronic impairment of glycolysis negatively impacts β-cell function, leading to hyperglycemia and diabetes.

Functional gluconeogenic enzymes that reverse the glycolytic pathway are expressed in β-cells and modulate glucose-stimulated insulin secretion (GSIS).

Dysregulation of gluconeogenic enzymes in β-cells is associated with impaired GSIS, so they constitute interesting pharmacological targets for intervention in insulin secretion.

Deeper understanding of the regulation of gluconeogenic enzymes in β-cells is critical to unravel their physiological and pathological role and to discover new points of intervention for antidiabetic therapeutics.

GSIS by β-Cells: Biochemical and Clinical Features

Glucose itself is the most potent **physiological insulin secretagogue** (see Glossary) and there is a well-characterized mechanism for **GSIS** by **β-cells**, which is dependent on glucose metabolism [1] (Figure 1). Glucose enters β-cells through glucose facilitative transporters (GLUTs), and glucokinase (GCK), the pacemaker for GSIS, phosphorylates it to yield glucose 6-phosphate (Glc-6-P). Glc-6-P is metabolized through **glycolysis** into pyruvate and enters the mitochondrion where it is further metabolized through the tricarboxylic acid (TCA) cycle and ultimately leads to an increase in the ATP:ADP ratio. A high ATP:ADP ratio closes ATP-dependent potassium channels, causing membrane depolarization and the opening of voltage-gated calcium channels, leading to hormone secretion [1]. This process is referred to as the triggering pathway [1]. In addition, there is an amplifying pathway that increases the efficacy of insulin exocytosis once the triggering pathway has induced an elevation of intracellular calcium. Although it remains largely uncharacterized, the amplifying pathway also depends on glucose metabolism, and several metabolites/factors from the mitochondria and pentose phosphate pathway are implicated as potential coupling agents [1–3]. As a result, the complexity of the intrinsic regulation of GSIS is not fully characterized.

Insulin secretion displays an oscillatory behavior that helps in maintaining blood glucose within a narrow range, especially by increasing hepatic insulin sensitivity [4]. **Oscillatory insulin secretion** is the consequence of fluctuations in levels of metabolites/factors that impact the ATP:ADP ratio, calcium mobilization, and exocytosis, allowing glycolytic flux to be self-regulatory [5]. Among many factors, fructose 1,6-bisphosphate (Fru-1,6-P_2) is one main source of glycolytic oscillations in β-cells, through autocatalytic activation of the muscle isoform of 6-phosphofructo-1-kinase (PFK) (PFKM), the key glycolytic enzyme catalyzing the phosphorylation of fructose 6-phosphate (Fru-6-P) into Fru-1,6-P_2, and through allosteric activation of muscle isoform 2 of

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 https://doi.org/10.1016/j.tem.2019.05.004

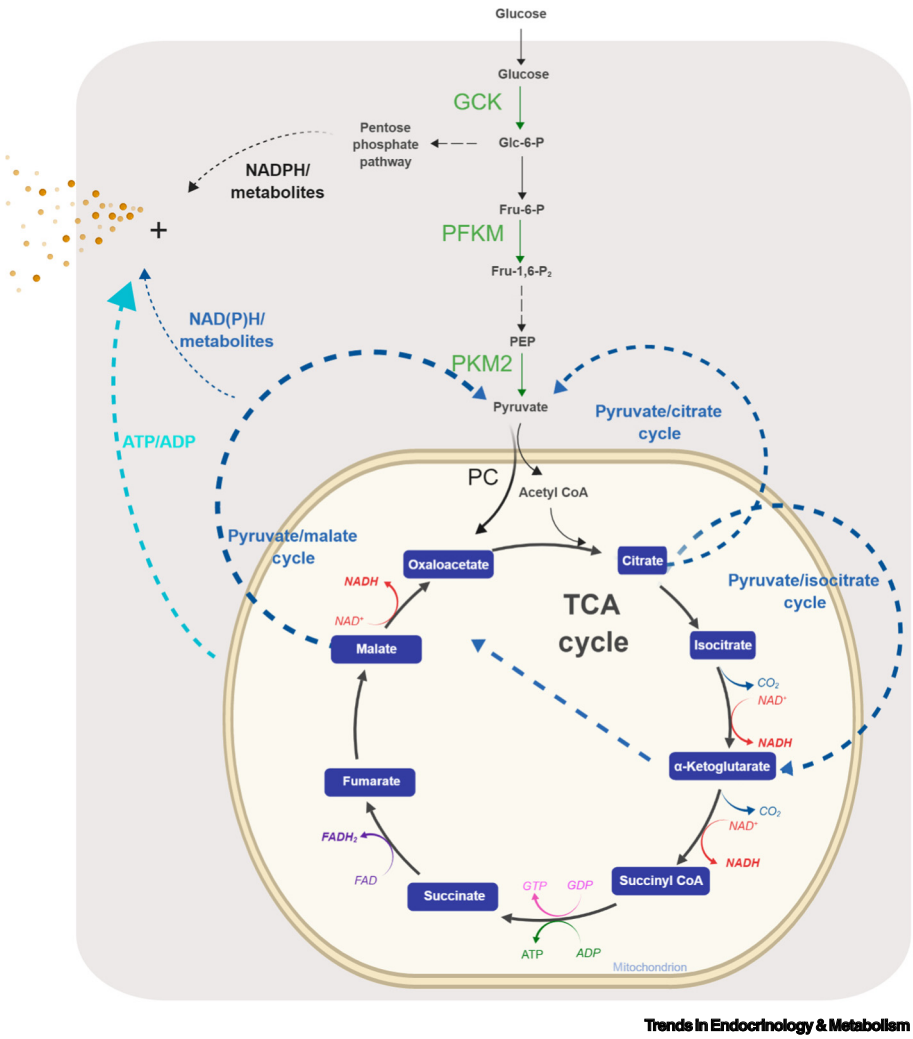

Figure 1. Model of Glucose-Stimulated Insulin Secretion by Pancreatic β-Cells. Glucose phosphorylation into glucose 6-phosphate (Glc-6-P) is proportional to glycemia due to expression of the glucokinase (GCK) isoenzyme. Glc-6-P is mainly metabolized though the glycolytic pathway to produce pyruvate, which is incorporated into the mitochondrion to feed the tricarboxylic acid (TCA) cycle for oxidative phosphorylation and the production of ATP. Increased ATP/ADP levels stimulate secretion of insulin through the triggering pathway. In parallel, other pathways (referred to as the amplifying pathway) are also activated to increase the levels of signals that magnify the efficacy of insulin secretion. Among them are metabolites/factors produced through pyruvate cycling (blue) and in the pentose phosphate pathway (black). GCK, the muscle isoform of phosphofructo-6-kinase (PFKM), and the M2 isoform of pyruvate kinase (PKM2) are the key enzymes catalyzing rate-limiting steps in the glycolytic pathway (green) in β-cells.

pyruvate kinase (PK) (PKM2), the enzyme that converts PEP into pyruvate and ATP [5] (Figure 1). This feedforward loop allows an increased glycolytic rate and avoids the accumulation of glycolytic intermediates. β-Cells also express other isoforms of PFK [6,7] and PK [8,9], which have different sensitivities towards Fru-1,6-P_2 [7,10]. Consequently, changes in PFK and PK isoform ratios affect enzymatic properties and activities, leading to altered glycolytic flux and metabolic oscillations [7]. Hence, Fru-1,6-P_2 is a pivotal metabolite for GSIS, modulating oscillatory insulin secretion, which is critical to promote hepatic insulin sensitivity to efficiently reduce hepatic glucose production [4]. Accordingly, individuals with diabetes display abnormal oscillatory insulin secretion [11]. In parallel, **biphasic insulin secretion** (first and second phase) occurs in

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response to an intravenous glucose bolus in individuals with normal glucose tolerance [12] (Figure 2). First-phase insulin secretion is necessary to efficiently suppress hepatic glucose production, which is one main contributor to hyperglycemia in diabetes [4,13]. Given the essential role of glycolysis in GSIS, interfering with its normal flow is expected to have a negative impact on the oscillatory and biphasic pattern of insulin secretion and therefore on glucose homeostasis. Impairment of first-phase insulin secretion is an early marker of prediabetic states [12,13] (Figure 2). It is therefore an obvious conclusion that further knowledge of the cellular mechanisms that modulate glycolysis and the proper pattern of insulin secretion is imperative to define how to correct the alterations present in prediabetic states, which in turn would delay the development of diabetes.

Gluconeogenic Enzymes in Pancreas

For many years, the most important assumption in the study of GSIS in β-cells was its total dependence on glycolysis to feed the TCA cycle, due to the absence of the **gluconeogenic**

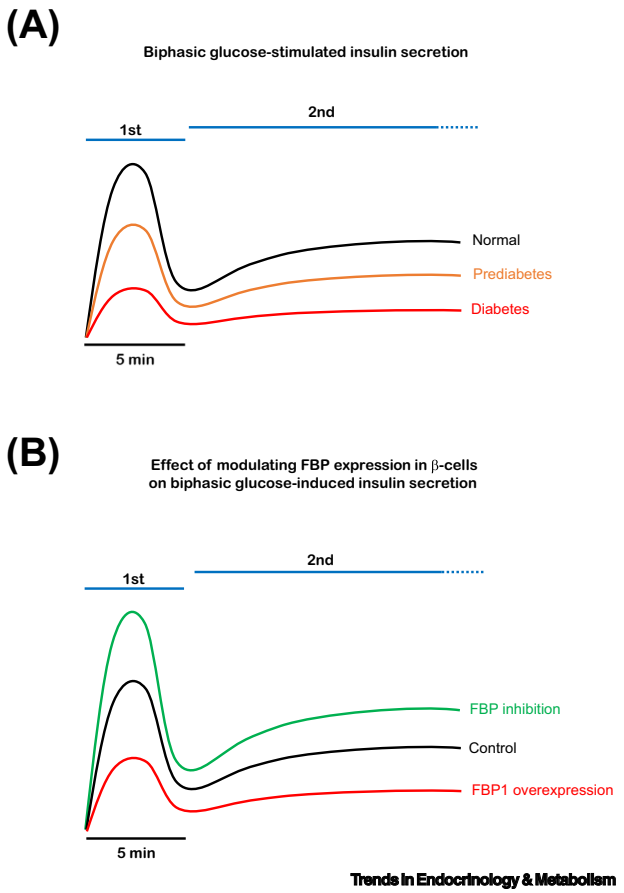

Figure 2. Schematic Representation of the Biphasic Pattern of Glucose-Stimulated Insulin Secretion and the Effect of Fructose 1,6-Bisphosphatase (FBP) Manipulation. (A) Insulin secretion occurs in a biphasic manner in individuals with normal glucose tolerance after intravenous glucose administration, with a rapid first phase lasting few minutes and a gradual, long-lasting second phase that lasts until the glucose concentration returns to basal levels (black). This pattern is altered in prediabetic (orange) and diabetic (red) states. (B) Biphasic insulin secretion in normal mice (black) is improved by oral administration of the FBP inhibitor MB06322 (green), whereas specific FBP1 overexpression in β-cells causes the opposite effect (red).

Glossary

β-Cells: specialized endocrine cells located in the pancreatic islets of Langerhans that produce the hormone insulin and secrete it into the circulation for reduction of blood glucose levels. Loss or dysfunction of β-cells is critical for the development of diabetes, resulting in chronic hyperglycemia.
Biphasic insulin secretion: comprises a rapid first phase lasting a few minutes and a gradual, long-lasting second phase, which lasts until the glucose concentration returns to basal levels. Although it does not occur under physiological conditions *in vivo*, it is an important clinical tool to study the response of β-cells to a glucose load.
Cataplerotic enzyme: an enzyme that removes an intermediate metabolite from a given pathway to be used in another pathway.
Global knockout mouse: animal model that has lost the expression of a given gene in all tissues.
Gluconeogenic enzymes: specialized enzymes whose expression is restricted to a few tissues and which reverse key glycolytic steps for the production of glucose during fasting. Generally, these enzymes are increased in diabetes, contributing to hyperglycemia.
Glucose-stimulated insulin secretion (GSIS): cellular metabolism of glucose is required for the stimulation of insulin secretion from pancreatic β-cells.
Glucotoxicity: structural and functional alterations caused by chronic hyperglycemia.
Glycogen: polymeric form of glucose for efficient storage in liver and muscle, although its accumulation in other tissues is associated with altered metabolism.
Glycolysis: metabolic pathway for the conversion of glucose into pyruvate and energy production.
Multienzyme complexes: specific and reversible associations between sequential enzymes of a metabolic pathway promoting channeling of intermediates to provide tight regulation of catalysis, which avoids dilution of substrates, intermediates, and products.
Oscillatory insulin secretion: inherent ability of β-cells to secrete insulin in pulses, with periods of 5–10 min, that depends on oscillation of metabolism.
Physiological insulin secretagogue: endogenous stimulus that induces

enzymes G6Pase, FBP, and PCK, that could interfere with the conversion of glucose into pyruvate [14–16]. Together, these enzymes are expressed at high levels in gluconeogenic tissues, such as liver and kidney, and play major roles in endogenous glucose production to maintain euglycemia during fasting [17–19]. However, increased expression/activity of gluconeogenic enzymes in these tissues is associated with hyperglycemia and diabetes [20,21]. G6Pase, FBP, and PCK bypass the respective rate-limiting steps catalyzed by GCK, PFK, and PK in glycolysis to form the so-called futile cycles of glycolysis/gluconeogenesis that are now considered key metabolic crossroads for exquisite short-term regulation [22,23]. A limited view of these enzymes has long hampered their study, as they are mainly associated with gluconeogenesis [24], which obviously has the effect of underestimating their real contribution to metabolism. These enzymes are essential in other pathways (e.g., glycerol synthesis in adipose tissue [17], **glycogen** synthesis in muscle [25]).

insulin secretion in the normal state of an animal.

Polygenic diabetic patients: the most common form of diabetes, where hyperglycemia is the consequence of β-cell dysfunction and peripheral insulin resistance due to mutations in several different genes.

Single-nucleotide polymorphism (SNP): the variation of a single nucleotide in a gene, which may or may not affect the gene product.

The Original Idea That β-Cells Do Not Express Gluconeogenic Enzymes, or That These Enzymes Do Not Affect GSIS, Is Now Known to Be False

G6Pase

G6Pase catalyzes the hydrolysis of Glc-6-P into free glucose and inorganic phosphate. The enzyme, comprising catalytic (G6PC) and transporter (G6PT) subunits, is located in the endoplasmic reticulum [18]. The enzymatic activity is dependent on the expression of one of three G6PC subunits described to date, namely G6PC1–3 [18], which in turn identifies the whole complex. G6PC1 is higher in gluconeogenic tissues [18]. Human and mouse β-cells express G6PC2 with lower activity than G6PC1 [26,27]. The study of a G6PC2 **global knockout mouse** was critical to unequivocally demonstrate that G6PC2 is active in pancreatic islets and that it is involved in GSIS, as these mice displayed a mild metabolic phenotype with ~15% decrease in fasting blood glucose [28–30]. This phenotype agrees with the idea that the specific expression of G6PC2 in β-cells allows Glc-6-P consumption through a substrate cycle with GCK [29] (Figure 3). Therefore, ablation of G6PC2 increases Glc-6-P availability and GSIS, resulting in the observed reduced glycemia [28,29]. G6PC2 upregulation has also been associated with endoplasmic reticulum stress, deficient Ca^{2+} mobilization, and impaired GSIS in β-cells on lipotoxic treatment [31].

Interestingly, **SNPs** that affect G6PC2 expression/activity have been mapped to the G6PC2 gene and are important determinants of variations in fasting glycemia in humans [26,32]. However, the effect of G6PC2 on GSIS is complex. Downregulation of G6PC2 in islets from diabetic subjects [33] and hyperglycemic mice [34] is associated with glycogen accumulation, which is proposed to be toxic for β-cells [34]. Also, G6PC2 SNPs associated with increased fasting blood glucose are in turn associated with enhanced insulin secretion [32]. A possible explanation is that G6PC2 affects oscillations of insulin secretion by retaining calcium in the endoplasmic reticulum [26,28,31]. G6PC3 has been assigned a similar function in glucose uptake by human astrocytes [35]. Taking these findings together, specific expression of G6PC2 in β-cells adds a new level of control to GSIS.

FBP

FBP catalyzes the hydrolysis of Fru-1,6-P_2 into Fru-6-P and inorganic phosphate [36]. There are two well-characterized isoforms encoded by separate genes: liver FBP (FBP1), present in gluconeogenic tissues and considered a key player contributing to glucose homeostasis [19], and muscle FBP (FBP2) that is predominantly expressed in muscle tissue where it is proposed to regulate indirect glycogen synthesis from lactate [25]. Despite the original evidence that pancreatic islets lack FBP activity [14], FBP1 is functionally expressed in rat pancreas and was also detected in human pancreatic islets [37] (Figure 3). FBP2 mRNA has also been detected in murine pancreas [34]. FBP1 activity is inhibited by AMP, Fru-2,6-P_2, and Fru-1,6-P_2 [36], being a key

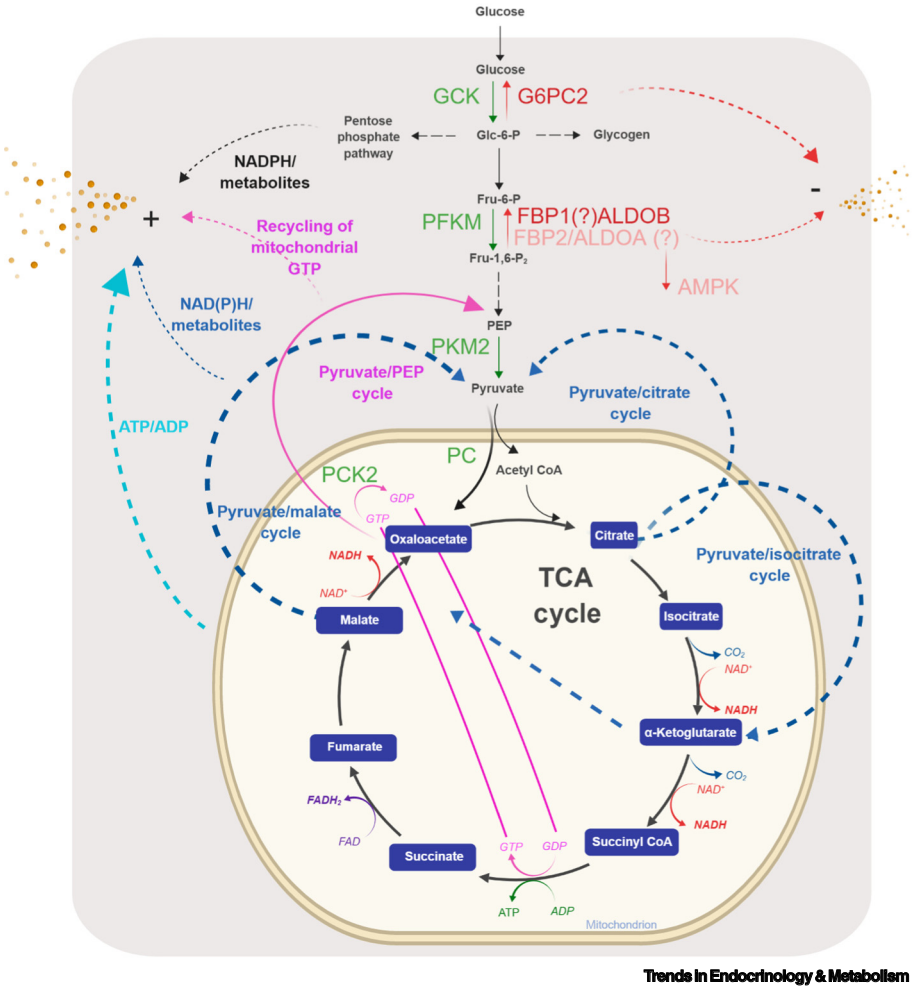

Figure 3. Gluconeogenic Enzymes in Glucose-Stimulated Insulin Secretion by Pancreatic β-Cells. Based on the model in Figure 1, the gluconeogenic enzymes glucose 6-phosphatase catalytic subunit 2 (G6PC2), liver fructose 1,6-bisphosphatase (FBP1), and mitochondrial phosphoenolpyruvate carboxykinase (PCK2) are introduced into the picture. These enzymes catalyze rate-limiting steps opposing the reactions of the respective glycolytic enzymes glucokinase (GCK), 6-phosphofructo-1-kinase (PFKM), and pyruvate kinase (PKM2). At first glance, due to their activities opposing key glycolytic steps, gluconeogenic enzymes are expected to inhibit glucose-stimulated insulin secretion by interfering with glycolysis. However, this is true only for G6PC2 and FBP1 (red), whereas PCK2 (note the different color representation) potentiates glucose-stimulated insulin secretion through pyruvate cycling and the regeneration of mitochondrial GDP (pink). Understanding the participation of gluconeogenic enzymes in β-cell metabolism under both physiological and pathological conditions is critical to advance the full characterization of glucose-stimulated insulin secretion and the development of new antidiabetic therapies. Some new regulatory mechanisms have been identified that are interesting targets for research on β-cell metabolism in relation to gluconeogenic enzymes and their effect on glucose-stimulated insulin secretion, particularly the interaction between FBP1 and ALDOB, the function of FBP2–ALDOA, and the consequent putative activation of AMPK.

sensor of intracellular energy status. By contrast, AMP, Fru-2,6-P_2, and Fru-1,6-P_2 activate PFKM [38], one candidate for the oscillatory behavior of insulin secretion. Interestingly, the specific pair 'FBP1/PFKM' has also been detected in spermatogenic cells, where it is proposed to play a key role in the generation of metabolic signals [39].

FBP1 plays a prominent role in GSIS by β-cells. As expected, due to hydrolysis of Fru-1,6-P_2, FBP1 negatively regulates insulin secretion. Downregulation/inhibition of FBP1 in β-cells

enhances both first- and second-phase insulin secretion in mice [40]. By contrast, biphasic insulin secretion is inversely proportional to the increase in FBP1 expression [41] (Figure 2). Accordingly, FBP1 is upregulated in pancreatic islets from hyperglycemic rats [42], diabetic mice [34], and diabetic subjects [41]. FBP2 is also upregulated in islets from hyperglycemic diabetic animal models [34], although its involvement in GSIS remains unknown. PFKM expression is also reduced in islets from diabetic patients [43], indicating that the combined opposing alterations in the expression of these enzymes is a hallmark of β-cell failure, resulting in further impairment of normal glycolytic flux and GSIS.

PCK

Although widely studied in the context of gluconeogenesis, PCK should be considered a **cataplerotic enzyme** involved in the removal of TCA cycle intermediates [44]. Although the cytosolic (PCK1) and mitochondrial (PCK2) isoforms are homologous proteins (~71% identity) and depend on GTP to convert oxaloacetate into PEP, PCK1 has long eclipsed the contribution of PCK2 [45]. After the first reports on the absence of PCK1 in β-cells [16], it was demonstrated that murine β-cells do express PCK2 [46] (Figure 3). Notably, PCK2 does not play a role that is in any way the same as that of the other two gluconeogenic enzymes, FBP1 and G6PC2, as, rather, it stimulates GSIS [46]. Although the stimulatory effect of PCK2 over GSIS is somehow contradictory to its pre-established 'gluconeogenic' function, its presence explains the connection between GSIS and pyruvate cycling [46]. Cycling of pyruvate metabolism in β-cells, which participates in the amplifying pathway of GSIS by translating mitochondrial metabolism status into cytosolic signals, occurs through at least four different cycles; namely, the pyruvate/malate, pyruvate/citrate, pyruvate/isocitrate, and pyruvate/PEP cycles [2,3,46]. PEP is a glycolytic intermediate that is converted into pyruvate by PK reaction. Then, pyruvate enters the mitochondrion and is converted into oxaloacetate via pyruvate carboxylase (PC), which is expressed at high levels in murine β-cells [47]. PCK2 converts oxaloacetate back into PEP, which leaves the mitochondrion and mixes with the cytosolic pool, completing the cycle [46] (Figure 3). Whereas the other pyruvate cycles are proposed to couple mitochondrial metabolism to GSIS by the generation of cytosolic NADPH (see [2,3] for detailed reviews of pyruvate cycling in β-cells), hydrolysis of mitochondrial GTP by PCK2 to yield PEP is the proposed connection for pyruvate/PEP cycle [46]. GTP is produced by succinyl-CoA synthetase in proportion to TCA cycle activity and is critical for GSIS [48]. In addition, PEP regeneration by PCK2 could add another point of regulation to oscillations in GSIS by negatively controlling PFKM activity [38]. No definitive information regarding altered expression of PCK2 in diabetes has been reported as yet, although both downregulation in mouse β-cell line MIN-6 [49] and progressive upregulation in rat islets [50] were observed in the presence of glucose.

The expression of PCK2 has not been addressed in human β-cells. However, expression of PC, the mitochondrial enzyme that provides the bulk of the substrate oxaloacetate for the proposed pyruvate cycling through PCK2, is lower in human than murine β-cells [6,47]. Therefore, the relevance of PCK2 in GSIS by human β-cells remains to be fully defined.

Pharmacological Intervention in GSIS by Targeting Gluconeogenic Enzymes

G6Pase

The inorganic salt sodium tungstate, widely studied due to its potent antidiabetic properties and lack of hypoglycemic effect [51], acts as a G6PC1 and G6PC2 inhibitor *in vitro* [27] (Table 1), although it does not influence FBP1 activity [52]. Tungstate has shown to induce renal glycogen accumulation in IRS2 knockout mice, which has been attributed in part to inhibition of G6PC1 activity [53]. Tungstate has further been shown to enhance basal insulin secretion and GSIS in the rat BRIN-BD11 β-cell line [54] and perfused rat pancreas [55] (Table 1). Nevertheless, therapeutic use of tungstate remains controversial due to the potential for significant undesirable

Table 1. Known Inhibitors of Gluconeogenic Enzyme Activity and Their Effect on Insulin Secretion[a]

Compound	Effect on target activity	Insulin secretion	Model	Refs
Sodium tungstate	↓ G6PC	NA	Overexpressed human G6PC1 and G6PC2 in insect SF-9[b] cells	[27]
		↑	BRIN-BD11[c] β-cell line	[54]
		↑	Perfused rat pancreas	[55]
MB05032	↓ FBP	NA	Human and rat FBP1; human FBP2	[59]
		↑	MIN6[d] β-cell line	[40]
		↑	Isolated mouse islets	[40]
MB06322 (MB05032 prodrug)	↓ FBP	↑	Normal mice	[40]
3-Mercaptopicolinic acid	↓ PCK	↓	Hepatic vagotomized and sham-vagotomized rats	[65]

NA, not applicable.
[a]Refer to text for other inhibitors that have not been assessed for insulin secretion activity.
[b]SF-9, *Spodoptera frugiperda* insect cell line for recombinant protein production using baculovirus.
[c]BRIN-BD11, electrofusion of RINm5F β-cell line with NEDH rat pancreatic islet cells.
[d]MIN6, mouse insulinoma 6 β-cell line.

effects [56,57]. Interestingly, a selective inhibitor of G6PC2 has been reported that does not inhibit G6PC1 [27], suggesting that specific inhibitors against G6PC2 can be designed. However, contrary to intuition, G6PC2 SNPs are not associated with an increased risk of development of diabetes, which is probably due to the dependence of their effects on glycemia, as higher blood glucose levels have been shown to abolish the negative impact [32]. This may be explained by the downregulation of G6PC2 observed during chronic hyperglycemia [33,34], suggesting that its contribution becomes less important during the progression of the disease. Inhibition of G6PC2 might reduce fasting glycemia in prediabetic states when this isoform still contributes to disease pathology.

FBP

Many FBP inhibitors with different modes of action have been designed, although few have moved forward into further development [58]. In particular, the FBP inhibitor MB05032 exhibits high potency and specificity for human FBP1 and FBP2 [59] (Table 1). *In vitro*, MB05032 stimulated both basal insulin secretion and GSIS in the MIN6 β-cell line and isolated mouse islets, whereas administration of MB05032's prodrug, MB06322 (CS-917), improved first- and second-phase insulin secretion in mice [40] (Table 1 and Figure 2). First-generation MB06322 reached a Phase I and IIb trial but was discontinued [58]. Second-generation MB07803 is currently being assessed for its clinical value, having successfully completed five Phase I trials in healthy volunteers, one Phase Ib trial in poorly-controlled diabetic patients, and a Phase IIa trial as an initial proof-of-concept study in diabetic patients, showing statistically and clinically significant reductions of fasting blood glucose without hypoglycemic effects [58,60]. Therefore, FBP inhibitors are attractive therapeutics for the improvement of GSIS.

PCK

Although PCK2 was the first isoform described [61], its metabolic function remains largely unresolved. Ideally, PCK inhibitors are attractive antidiabetic therapeutics, but this role is largely dependent on their specificity against PCK1 in insulin-resistant gluconeogenic tissues to improve glycemic control [62]. Given the high degree of sequence identity between PCK1 and PCK2, no isoform-specific inhibitor has yet been identified [63]. Although a potent competitive inhibitor

against human PCK1 has been reported [64], it is expected to inhibit PCK2 as well due to the relatively identical nature of the active site architectures. Another PCK inhibitor, 3-mercaptopicolinic acid, exhibits mixed inhibition, binding to the active site and to a novel allosteric site of PCK1 [63]. It is theoretically possible that analogs could be developed to differentially inhibit PCK1 and PCK2 through binding to this allosteric pocket [63]. Selective inhibition of PCK1 is clearly important if PCK activity is to be targeted in a therapeutic capacity, as PCK2 has a positive effect on GSIS [46]. In agreement, insulin secretion was reduced in rats treated with 3-mercaptopicolinic acid [65] (Table 1), suggesting that overall PCK inhibition is negative for GSIS.

Physiological and Pathophysiological Relevance of Gluconeogenic Enzymes in β-Cells

β-Cells sense blood glucose levels and secrete insulin in response. This glucose-sensing machinery is so specific that β-cells possess a strict repression program to prevent the activation of metabolic pathways that may interfere with it [66]. Then, expression of gluconeogenic enzymes in β-cells must provide some advantage, despite their dysregulation during diabetes finally contributing to impaired GSIS.

β-Cells accumulate glycogen under hyperglycemic conditions [34,66–69], which is a potential pathway for detoxification from excess glucose [69]. Glycogen accumulates linearly with at least up to 25 mM glucose exposure for 1 h in the INS-1 (832/13) β-cell line [69]. In agreement, inhibition of glycogen phosphorylase, the enzyme that catalyzes the breakdown of glycogen, improves β-cell function [70]. Glycogen accumulation has also been suggested as an adaptive mechanism for enhanced glucose sensitivity in hyperglycemia, during the progression of diabetes, and preceding the loss of GSIS due to **glucotoxicity** [71,72]. Given that the magnitude of glycogen accumulation depends on the extent of exposure and the glucose concentration [34,67,68], it is proposed that transient glycogen storage may be a protective mechanism during short-term hyperglycemia but that chronic glycogen accumulation is detrimental, reflecting the metabolic alterations through which hyperglycemia finally leads to β-cell dysfunction [34,68,73].

The first step in the β-cell's glucose metabolism is its phosphorylation to yield Glc-6-P catalyzed by GCK, the pacemaker for GSIS. Glc-6-P is both the precursor of and an allosteric activator for glycogen synthesis [74]. However, GCK expression/activity is reduced both in patients with a monogenic form of diabetes linked to mutations of the GCK gene that negatively affect its activity [75] and in animal models of diabetes [76]. Even when GCK expression may not be affected in **polygenic diabetic patients** [6,43], it is proposed that GCK's full potential is decreased due to alteration of other regulatory mechanisms that in turn modulate GCK activity [77,78]. Given that GCK exerts most control over the β-cell's glucose-phosphorylating capacity, even small reductions in GCK activity will parallel reductions in Glc-6-P production. Can chronic hyperglycemia induce glycogen accumulation in the case of reduced activity of GCK in the β-cell? One possibility is that glycogen is indirectly synthesized from Glc-6-P provided by gluconeogenic activity [34]. As mentioned above, FBP1 expression is upregulated in islets from hyperglycemic mice and rats [34,42] and might be involved in the reduction of Fru-1,6-P_2 and glycolysis, redirecting glycolytic intermediates back to Glc-6-P for glycogen synthesis. It is noteworthy that the FBP inhibitor MB06322 stimulated GSIS in control nondiabetic mice [40], indicating that the expression and activity of FBP are high enough in normal β-cells *in vivo* so its inhibition influences GSIS (Figure 2). In other words, FBP may have a physiological role in GSIS.

Interestingly, FBP interacts with Fru-1,6-P_2 aldolase (ALDO), which catalyzes the reversible conversion of Fru-1,6-P_2 to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [79–81]. This protein–protein interaction reduces sensitivity to inhibitors and increases substrate channeling [79–81]. Protein–protein interaction between sequential enzymes has become a popular drug

target for the disruption or stabilization of **multienzyme complexes**, which are increasingly recognized as key regulatory mechanisms [82]. The FBP/ALDO complex is isoform specific: FBP2 interacts with muscle ALDO (ALDOA) and FBP1 interacts with liver ALDO (ALDOB) [79–81]. The FBP1/ALDOB association constant depends on the metabolic status of the cell: FBP1 inhibitors decrease it while metabolites that stimulate gluconeogenesis increase it [79]. Moreover, similar to FBP1, ALDOB is upregulated in β-cells of diabetic subjects [6,83] and by short-term hyperglycemia *in vitro* [50], which indicates that both the increased amounts of these enzymes and the altered metabolic context contribute to enhanced activity of FBP1/ALDOB complex (Figure 3). This evidence indicates that disruption of protein–protein interactions between FBP1 and ALDOB enzymes might help to restore glucose homeostasis in diabetics.

Recently, Fru-1,6-P_2 has been shown to regulate AMP-activated protein kinase (AMPK) [84]. AMPK is activated by glucose deprivation and plays a key role in reducing GSIS and insulin gene expression [85]. However, it has been determined that reduced Fru-1,6-P_2 production (by knocking down PFKM or overexpression of FBP1) induced AMPK activation even in the presence of high glucose, with this ability being dependent on the presence of ALDO for sensing Fru-1,6-P_2 levels [84] (Figure 3). Although this sensing mechanism has not been demonstrated in β-cells yet, it may add another explanation for how chronic hyperglycemia causes glucotoxicity in β-cells. It is noteworthy that cytoplasmic rate-limiting enzymes involved in glycolysis/gluconeogenesis (PFK, FBP, PK, and PCK) are compartmentalized in clusters in human cell lines [86]. A similar multienzyme complex formed by PFKM, FBP1, ALDOB, and PKM2, all of them regulated by Fru-1,6-P_2, might be present in β-cells, allowing tight regulation of glucose flux and AMPK activity, which in turn regulates GSIS.

Of note, FBP2 is also upregulated in islets from hyperglycemic diabetic animals [34]. Although FBP2 is proposed to participate in indirect glycogen synthesis [25], it has been recently reported that increased activity of FBP2 does not induce glycogen synthesis but rather it increases insulin-stimulated glucose uptake in skeletal muscle, suggesting that inhibitors against FBP may have a negative impact on muscle metabolism if not specific enough to differentiate between the isoforms [87]. Activity of FBP2 is strongly dependent on the expression of ALDOA for desensitization towards AMP and Ca^{2+} inhibition [80]. ALDOA expression is downregulated in islets from diabetic subjects [43]. In summary, the involvement of FBP2/ALDOA in β-cell metabolism needs further study (Figure 3).

Increased expression of glycolytic enzymes after short-term glucose stimulation has been associated with higher glucose responsiveness of β-cells [88] but also with glucotoxicity [50], whereas chronic exposure decreased the expression of glycolytic enzymes and is associated with impaired GSIS [43,89,90]. Forty-eight hours of hyperglycemia induces an increase of all glycolytic intermediate levels in β-cells, with particularly greater levels of Glc-6-P and Fru-6-P [91]. Accumulation of these metabolites could be the consequence of downstream blockage of metabolism [91], which may be attributed to reduced expression of G6PC2 and increased expression/activity of FBP1/ALDOB, respectively. FBP1 induction has been detected already at 24 h in islets from hyperglycemic diabetic mice [34] and ALDOB at 18 h in rat islets cultured at high glucose [50], whereas G6PC2 mRNA shows a tendency towards reduction at 24 h and significant downregulation after 4 weeks in hyperglycemic diabetic mice [34] and also in diabetic subjects [33]. Moreover, FBP1 induction was reversed when β-cells cultured at high glucose for 24 h were cultured back at low glucose [34]. However, after 4 weeks of hyperglycemia FBP1 upregulation was not normalized when euglycemia was restored by pharmacological treatment [34], indicating a derangement of its normal regulation. This might be also true for ALDOB. Therefore, increased but reversible expression of FBP1/ALDOB in the context of unaltered or slightly reduced expression of G6PC2 during short-term hyperglycemia may counter-regulate excess glucose

metabolism by redirecting glycolytic intermediates into glycogen synthesis, as further supported by acute upregulation of PPP1R3C in response to short-term hyperglycemia [34,50], which strongly activates glycogen synthesis [92]. By contrast, increased and dysregulated expression of FBP1/ALDOB in the context of reduced expression of G6PC2 during chronic hyperglycemia may contribute to greater glycogen accumulation as a desperate way for β-cells to deal with excess Glc-6-P once glycolytic flux is reduced as one of many mechanisms mediating glucotoxicity.

Concluding Remarks and Future Directions

β-Cell dysfunction is partially accounted for by alterations in the expression/activity of many enzymes of glucose metabolism, affecting glucose sensing and insulin secretion. Contrary to the previous idea that β-cells do not express 'gluconeogenic' enzymes because they interfere with the unidirectional metabolism of glucose into pyruvate, we now unequivocally know that these enzymes are present and play important roles in β-cell metabolism. These enzymes – namely, G6PC2, FBP1, and PCK2 – are postulated to allow the fine-tuning of GSIS by modulating key substrate cycles. However, given the critical role of glycolysis on GSIS, chronically interfering with its normal flow has a negative impact on insulin secretion and its oscillatory pattern. This is the problem with increased activity of gluconeogenic enzymes in the β-cell, especially during prediabetes. Further studies of these enzymes will be of great relevance to understand both normal and altered GSIS (see Outstanding Questions), especially because two of them, G6PC2 and FBP1, are known modulators of human β-cell metabolism and constitute attractive targets for pharmacological intervention in insulin secretion.

Outstanding Questions

Is G6PC2 inhibition a viable therapeutic approach to reduce fasting blood glucose levels, at least in prediabetic subjects?

Could the specific interaction between FBP1 and ALDOB be targeted as a new therapeutic alternative to reduce FBP activity and improve GSIS?

Does the FBP–ALDOB interaction promote AMPK activation and the β-cell refractory response to hyperglycemia?

Do FBP2 and ALDOA play important roles in β-cell metabolism?

Is PCK2 present in and relevant to human β-cell metabolism and therefore targetable for intervention in GSIS?

Acknowledgments

This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) from Chilean State, grant 11160854 to R.B. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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