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Review

Glucokinase activity in diabetes: too much of a good thing?

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Type 2 diabetes (T2D) is a global health problem characterised by chronic hyperglycaemia due to inadequate insulin secretion. Because glucose must be metabolised to stimulate insulin release it was initially argued that drugs that stimulate glucokinase (the first enzyme in glucose metabolism) would enhance insulin secretion in diabetes. However, in the long term, glucokinase activators have been largely disappointing. Recent studies show it is hyperactivation of glucose metabolism, not glucose itself, that underlies the progressive decline in beta-cell function in diabetes. This perspective discusses if glucokinase activators exacerbate this decline (by promoting glucose metabolism) and, counterintuitively, if glucokinase inhibitors might be a better therapeutic strategy for preserving beta-cell function in T2D.

Highlights

Glucokinase catalyses the first step in glucose metabolism. It functions as a glucose sensor in the insulin-secreting pancreatic beta-cells.

Activating mutations in glucokinase cause too much insulin secretion (hyperinsulinism), whereas inactivating mutations cause too little insulin release and varying degrees of diabetes. Glucokinase activators have been trialled as antidiabetic drugs but generally fail to enhance insulin secretion in the long term.

Recent studies show it is a glucose metabolite and not glucose itself that causes the decline in beta-cell function in response to chronic hyperglycaemia or diabetes. This argues that glucokinase activation would exacerbate beta-cell decline in diabetes.

Partial glucokinase inhibition prevents the hyperglycaemia-induced decline in beta-cell function in beta-cell lines and animal models of diabetes.

The enzyme glucokinase is essential for glucose homeostasis

Glucokinase (GCK or hexokinase IV) (see Glossary) plays a critical role in glucose homeostasis. In pancreatic beta-cells it ensures that insulin secretion is matched to the circulating blood glucose level, in the liver it facilitates glycogen storage and the post-prandial clearance of glucose from the bloodstream, and in certain neurones and neuroendocrine cells it mediates glucose sensing. Its critical importance for insulin secretion is convincingly demonstrated by the fact that inactivating mutations in the glucokinase gene cause diabetes, whereas activating mutations result in **congenital hyperinsulinism**. Given that insulin secretion is impaired in **type 2 diabetes (T2D)**, it was initially assumed that drugs that stimulate glucokinase activity might enhance insulin secretion. Unfortunately, although preliminary results were promising, ultimately glucokinase activators have not been successful. This review suggests reasons why this might be the case. It also postulates that, paradoxically, glucokinase inhibitors might be a better therapeutic strategy for preserving beta-cell function in T2D. Evidence from patients with heterozygous inactivating mutations in glucokinase supports this idea.

Glucokinase serves as the beta-cell glucose sensor

Glucose metabolism plays a central role in glucose-stimulated insulin secretion from the pancreatic beta-cell [1] (Figure 1). Glucose enters the beta-cell via transporters that are insulin independent and high capacity, so that intracellular and extracellular glucose concentrations rapidly equilibrate. It is then metabolised via **glycolysis** and mitochondrial metabolism, leading to an increase in cytosolic ATP and a decrease in cytosolic ADP. These changes in adenine nucleotides cause inhibition of the ATP-sensitive potassium **(K_{ATP}) channel**, which triggers membrane depolarisation and leads to opening of voltage-gated calcium channels, calcium influx, and exocytosis of insulin granules [2]. In addition, glucose metabolism has effects downstream on K_{ATP} channel inhibition and calcium influx that amplify insulin secretion. It is also required for insulin synthesis.

The phosphorylation of glucose, the initial step in glucose metabolism, is catalysed by the enzyme hexokinase, of which there are several isoforms (HK1–4). In most cells, the hexokinases

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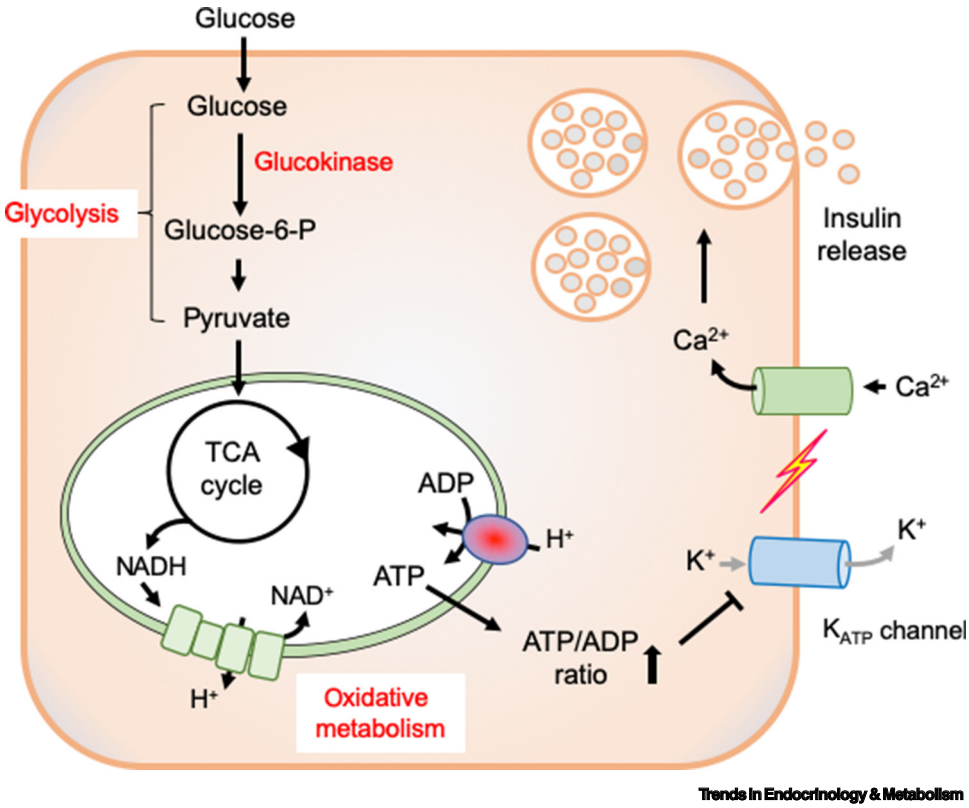

Figure 1. Glucokinase serves as the beta-cell glucose sensor. Glucose is taken up and converted to glucose 6-phosphate by glucokinase. It is then metabolised via glycolysis and oxidative metabolism to produce ATP, which closes the ATP-sensitive potassium (K_{ATP}) channels and triggers membrane depolarization, calcium influx, and insulin secretion.

expressed (HK1–3) have a high affinity for glucose, their capacity to phosphorylate glucose is saturated at normal fasting plasma glucose levels (~5 mM) and they are allosterically inhibited by their end product, glucose-6-phosphate (G6P). Consequently, their capacity for glucose metabolism is limited and they are insensitive to changes in plasma glucose within the physiological range (Figure 2). By contrast, glucokinase (HK4), the only hexokinase expressed at any significant level in the beta-cell, has several unique properties that make it an ideal glucose sensor [3]. It has a sigmoidal glucose dependency, with an inflexion point at ~4 mM glucose, close to the threshold for insulin secretion (~5 mM in human beta-cells and slightly higher in mice). It also has a low affinity for glucose (EC_{50} ~8–10 mM) and it does not saturate at physiological glucose concentrations (V_{max} > 20 mM). Unlike other hexokinase isoforms, it is also not inhibited by G6P. These properties ensure that the rate of glucose phosphorylation varies across the entire physiological range (3–15 mM) and is proportional to the extracellular glucose concentration, thus enabling glucokinase to serve as the beta-cell glucose sensor.

Glucokinase is present in other tissues

Glucokinase is not confined to the beta-cell. It also serves as a glucose sensor in a number of other neuroendocrine cells, some entero-endocrine cells, and certain glucose-sensing neurones in the brain [4]. For example, in pancreatic alpha-cells, glucokinase is required for glucose-dependent regulation of glucagon secretion [5–8], while in arcuate nucleus neurones it promotes neuropeptide Y secretion [9]. In the liver, glucokinase plays an important role in glucose homeostasis by ensuring glucose is rapidly cleared from the bloodstream following a carbohydrate meal

Glossary

Apoptosis: the process of programmed cell death.
Congenital hyperinsulinism: disease in which insulin is persistently released in an uncontrolled fashion, leading to dangerously low blood glucose levels. Can be caused by mutations in several different genes, including gain-of-function mutations in the glucokinase gene (GCK-HI).
Counter-regulatory response: the physiological changes that act to elevate blood glucose when blood glucose falls.
Euglycaemia: the normal blood glucose level.
Glucokinase (GCK or hexokinase 4): enzyme that catalyses glucose phosphorylation, the first step in glucose metabolism. Found only in selected tissues such as the pancreatic beta-cell and the liver. Also known as hexokinase 4 (HK4).
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH): a glycolytic enzyme that catalyses the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate.
Glycolysis: the metabolic process in which glucose is converted into pyruvate.
Hepatic steatosis: the accumulation of fat in the liver (>5% of liver weight). Initially harmless but may progress to cirrhosis.
Hyperglycaemia: high blood glucose level (or high extracellular glucose).
Hypoglycaemia: low blood glucose level (or low extracellular glucose).
K_{ATP} channel: ion channel that plays a key role in glucose-stimulated insulin secretion as it links glucose metabolism to beta-cell electrical activity and insulin secretion. Regulated by metabolically generated changes in the ATP/ADP ratio.
PDH: pyruvate dehydrogenase, the mitochondrial enzyme complex that converts pyruvate to acetyl-CoA.
PDK1: pyruvate dehydrogenase kinase 1, an enzyme that phosphorylates and thereby inactivates pyruvate dehydrogenase.
Streptozotocin: toxin that causes beta-cell death.
Sulphonylurea: class of drugs that binds to and inhibits the K_{ATP} channel, thereby stimulating insulin secretion. Used to treat neonatal and type 2 diabetes.
Type 2 diabetes (T2D): disease of elevated blood glucose caused by an

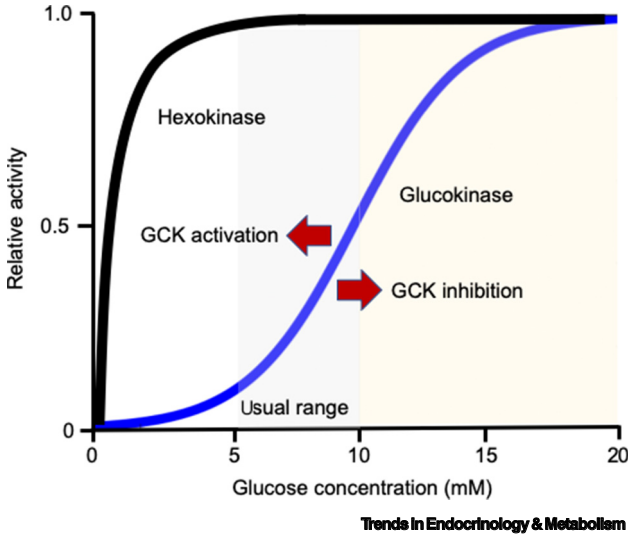

Figure 2. Glucokinase (GCK) versus hexokinase. Cartoon showing the relationship between glucose concentration and enzyme activity for hexokinase I and glucokinase (hexokinase 4). Hexokinase I has a high affinity for glucose and is already fully saturated at fasting blood glucose concentrations (~4–5 mM). Glucokinase has a lower affinity for glucose and is only active at higher glucose concentrations such as those reached post-prandially or in diabetes. Activation of glucokinase (genetically or by drugs) shifts the dose-response curve to the left, while inhibition of glucokinase shifts it to the right.

insufficiency of insulin. Pancreatic beta-cells are present but fail to release sufficient insulin in response to a glucose challenge. Often accompanied by insulin resistance.

and by maintaining blood glucose levels between meals [10]. It facilitates glucose uptake (by reducing cellular concentrations of the unphosphorylated sugar) and is required for glycogen synthesis and fat storage.

Glucokinase exists as two different isoforms, which have the same kinetic properties but different functions [10,11]. These isoforms are encoded by the same gene, but separate promoters lead to different splicing patterns, producing different variants of the enzyme. The downstream promoter drives hepatic mRNA expression, while the upstream (neuroendocrine) promoter drives mRNA synthesis in all other GCK-expressing tissues, including beta-cells [12]. Although the different promoters result in a very short difference in the N-terminal sequence, this has no known functional effect.

The regulation of GCK expression and activity differs between tissues [10]. In beta-cells, GCK is constitutively expressed, enabling the cell to respond instantly to changes in blood glucose levels. In contrast, liver GCK is regulated at both transcriptional and post-translational levels to ensure its activity is inhibited when blood glucose levels are low. Its expression is dependent on insulin and inhibited by glucagon. It is also regulated at the post-translational level by the glucokinase regulatory protein GCKR. In the fasting state GCKR binds to GCK, which inactivates the enzyme and causes its translocation to the nucleus [13]. Following a carbohydrate meal, GCK dissociates from GCKR and translocates back to the cytoplasm, where it stimulates glycolysis, glycogen synthesis, and *de novo* lipogenesis. This shuttling does not happen in beta-cells, which lack GCKR.

Genetic activation of glucokinase

Given that glucokinase sets the rate of metabolic flux in the beta-cell, it was initially predicted that glucokinase activation would enhance beta-cell metabolism and promote insulin secretion. However, this turned out not to be quite so straightforward.

Instead, overexpression of GCK in INS1 cells suppressed glucose utilisation at high glucose, without affecting it at low glucose [14,15]. Levels of glycolytic metabolites upstream of **glyceraldehyde 3-phosphate dehydrogenase (GAPDH)** were markedly elevated, consistent with limited flux through this enzyme [15]. This was found to result from a failure to regenerate sufficient NAD^+, an essential cofactor for GAPDH.

Consistent with these studies, activation of a homozygous gain-of-function GCK mutation (Y214C) in adult mice rapidly triggered moderate fasting **hypoglycaemia**, beta-cell replication, and increased oxygen consumption at 2 mM glucose, where GCK activity is normally negligible [16]. Surprisingly, however, the hypoglycaemia was transient, reaching a nadir after 4 days, and it was subsequently followed by restoration of blood glucose levels. This was due to enhanced **apoptosis** and a loss of beta-cells caused by double-stranded DNA breaks. GCK activation also resulted in numerous gene changes [17], which resembled those found in islets from humans and mice with diabetes, and in beta-cell lines cultured at high glucose [18–20]. These changes were evident during the period of greatest hypoglycaemia, suggesting they were due to enhanced GCK activity and not an elevated blood glucose concentration.

Whether human patients with activating glucokinase mutations similarly progress from hypoglycaemia to hypoglycaemia remission remains unclear. Heterozygous activating GCK mutations cause congenital hyperinsulinism (GCK-HI), characterised by persistent and unregulated insulin secretion leading to life-threatening hypoglycaemia that usually manifests at, or shortly after, birth [21–26]. The phenotype can vary, even in families with the same mutation, and some people are not diagnosed until adulthood [21,27]. Some patients with GCK-HI mutations do not need treatment and can manage their condition with diet alone. Those who are unable to do so usually respond to diazoxide, which opens K_{ATP} channels, hyperpolarising the beta-cells and switching off the excessive insulin secretion [25].

As for mice with the Y214C mutation, a greater incidence of apoptotic beta-cells is found in patients with GCK-HI mutations [16,28]. Thus, one might predict that the hypoglycaemia of GCK-HI patients would gradually remit and might even progress to diabetes. Because of the rarity of the disorder, it is hard to be completely confident that this is the case. Nevertheless, some mutation carriers have indeed shown hypoglycaemia remission, or developed diabetes, with age [16,21]. This raises the interesting possibility that long-term overactivity of GCK leads to reduced beta-cell functional mass in humans as well as mice (Figure 3). However, a greater number of observations are needed to be certain this is the case.

Glucokinase activators have failed to live up to their promise

Numerous small molecule GCK activators have been developed, based on the premise that they will stimulate insulin secretion and enhance hepatic glucose uptake in diabetes [29,30]. These drugs activate GCK by binding to its allosteric activator site and increasing the glucose affinity and/or V_{max} of the enzyme. Initial studies were promising. A single oral dose increased plasma insulin and reduced blood glucose in both normal and diabetic rodent models [29]. Other studies showed beneficial effects on glycaemia and diabetes progression in animal models [30–32]. Furthermore, in a small Phase 1 clinical trial a GCK activator lowered blood glucose levels in patients with mild T2D [33].

However, right from the start it was recognised that there were potential risks to GCK activation. These included hypoglycaemia due to an excess of insulin release at low glucose, beta-cell stress due to overactivation, and **hepatic steatosis** (fatty liver) [34]. These warnings were justified when hypoglycaemic episodes were reported in both animal models and patients with T2D treated with GCK activators [33,35]. Furthermore, GCK activation enhanced lipid synthesis in the liver. Significant hepatic fat accumulation was reported in *db/db* mice, an animal model of diabetes, which were treated with GCK activators [36]. Most seriously, in a large 54-week long Phase 2 trial, patients with T2D developed hyperlipidaemia, hepatic steatosis, and vascular hypertension. Blood pressure and plasma triglycerides were elevated and there was only a modest effect on glucose regulation, which was not sustained and disappeared after 3–4 months [35].

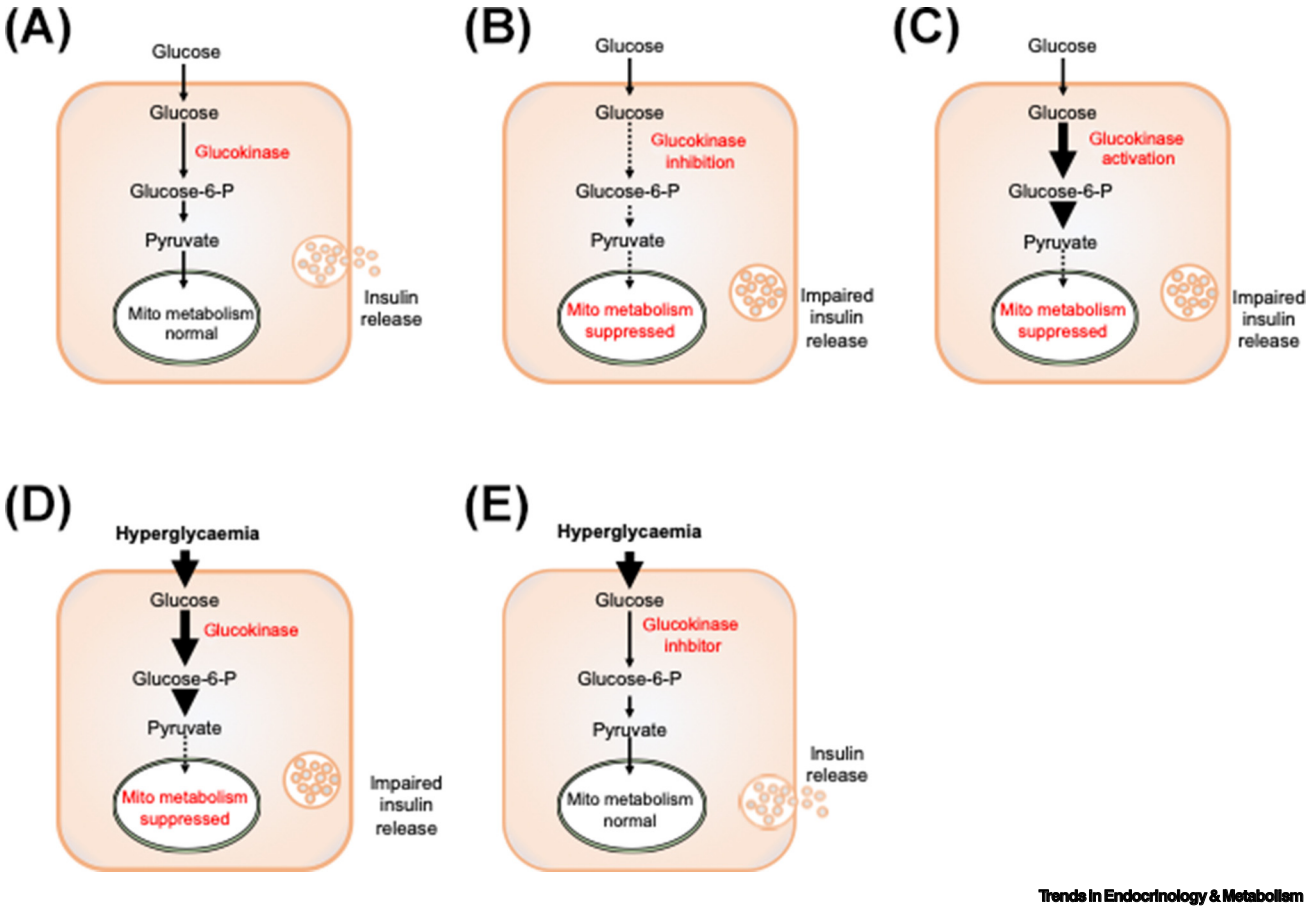

Figure 3. Effects of glucokinase (GCK) activation and inhibition. (A) Under control conditions, glucose flux through GCK increases in response to acute glucose elevation, leading to stimulation of insulin release. (B) When GCK activity is severely reduced (as in permanent neonatal diabetes caused by GCK mutations) metabolic flux and insulin secretion are impaired. (C) Severe GCK activation (as seen with some GCK mutations that cause congenital hyperinsulinism) initially stimulates metabolism and insulin secretion but subsequently leads to impaired metabolism and insulin release. (D) In diabetes, plasma glucose levels are chronically elevated. This eventually leads to suppression of mitochondrial metabolism and insulin release. (E) Partial inhibition of GCK in diabetes restores normal levels of metabolic flux and insulin secretion, despite the elevated plasma glucose levels.

Subsequently, other trials failed to show long-term improvements in glycaemia [37]. This loss of efficacy has been a major reason for why GCK activators have fallen out of favour.

More recent studies have revived the idea that GCK activators may be useful in T2D. The SEED trial showed that newly diagnosed T2D patients treated with the GCK activator dorzagliatin had lower $HbA1_c$ levels after 24 weeks than those treated with placebo [38]. Interestingly, however, when the placebo-treated patients were subsequently treated with dorzagliatin for a further 28 weeks, their $HbA1_c$ was significantly less than that of patients treated with dorzagliatin for the full year. This raises the possibility that the drug may compromise beta-cell function in the long term and suggests its efficacy is primarily due to its action on the liver [39]. Further, and longer term, studies are now needed.

Such mixed results have led many to question the use of GCK activators to treat impaired insulin secretion in T2D [30,37,40,41]. Indeed, is the rationale for the use of GCK activators flawed? Given that recent studies suggest enhanced glucose metabolism, rather than glucose *per se*, underlies the progressive deterioration of beta-cell function in diabetes [42,43], might we, in fact, be looking at the problem the wrong way around?

Beta-cell metabolism is impaired in T2D

T2D is characterised by defective insulin secretion from the pancreatic beta-cells, which results in chronically elevated blood glucose. The disease is progressive, beginning with impaired glucose tolerance and progressing to overt diabetes as beta-cell function gradually fails. By the time of diagnosis, it is estimated only 50% of beta-cell function remains. The precise trigger for T2D is still contested and it appears to be both phenotypically and genetically diverse [44]. However, there is good evidence that once chronic **hyperglycaemia** is established it contributes to beta-cell decline. Both diabetes and chronic exposure of beta-cells to hyperglycaemia experimentally have deleterious effects on beta-cell function, including a dramatic loss of insulin content, marked changes in metabolic and other gene expression, decreased glucose metabolism, and, eventually, a partial loss of beta-cells [18–20,43,45–47]. Importantly, genes involved in mitochondrial metabolism are downregulated, while **PDK1**, which inhibits **PDH** and thereby pyruvate entry into the TCA cycle, is upregulated [43]. These changes further impair glucose-stimulated insulin secretion and lead to increasing hyperglycaemia, thus promoting a vicious spiral that underlies the progression from impaired glucose tolerance to overt diabetes [20].

There is substantial evidence that lowering blood glucose can cause T2D remission. First, intensive insulin therapy is able to reverse newly established diabetes, at least for diabetes of short duration [48]. Second, a very low-calorie diet normalises plasma glucose levels within a week, restores first-phase insulin secretion within 8 weeks, and results in diabetes remission [49,50]. Third, patients who undergo bariatric surgery, which leads to caloric restriction, often show remission of diabetes long before there is any substantial weight loss [51]. Furthermore, patients with neonatal diabetes due to gain-of-function K_{ATP} channel mutations have normal insulin secretion when given **sulphonylurea** drugs, even after many years of diabetes [52,53]. These data tell us that reversing chronic hyperglycaemia in diabetes restores beta-cell function. However, compliance is a problem with very low calorie diets and bariatric surgery and intensive insulin therapy are not available to all. Furthermore, remission is dependent on the capacity for beta-cell recovery and is primarily effective in individuals with diabetes of short duration [50]. Thus additional methods to slow or reverse beta-cell decline in T2D are needed.

There is accumulating evidence that the detrimental effects of chronic hyperglycaemia are mediated not by glucose itself, but by a metabolite downstream of glucokinase and upstream of GAPDH [42,43]. This raises the possibility that reducing glucokinase activity might prevent the deleterious effects of high glucose (Figure 3). Mannoheptulose is a 7-carbon sugar found at high concentrations in unripe avocado, which acts as a competitive inhibitor of glucokinase [54]. It has been shown both to prevent, and to reverse, the deleterious effects of chronic hyperglycaemia in beta-cell lines and islets [42,43,55,56].

Genetic reduction of glucokinase activity in diabetic mice can also partially alleviate hyperglycaemia and the consequential reduction in beta-cell mass [56–58]. Mice carrying a heterozygous gain-of-function K_{ATP} channel mutation develop neonatal diabetes, as do mice expressing a GCK inactivating mutation. However, mice that simultaneously expressed both heterozygous mutations showed lower blood glucose levels, improved glucose tolerance, and greater insulin content [58]. This is likely because the GCK mutation reduces glycolytic flux and, thereby, the accumulation of glycolytic metabolites and gene expression changes that impair beta-cell metabolism and insulin release.

Partial GCK inactivation also ameliorated glucose intolerance in *db/db* mice [57]. Mice that were simultaneously homozygous for the *db* mutation and heterozygous for a null GCK mutation ($gck^{+/-}$*db/db* mice) were moderately more glucose tolerant than *db/db* or $gck^{+/-}$ *mice* at

24 weeks of age, although notably this difference only presented at about 12 weeks of age. Heterozygous *gck*$^{+/-}$*db/db* mice also had more beta-cell mass than *db/db* mice, as assessed by insulin staining, and their islets secreted more insulin. Furthermore, metabolic genes that are upregulated in islets from diabetic rodent models and human patients with T2D [18–20] were also lower in *gck*$^{+/-}$*db/db* mice [57].

Overnight treatment of isolated *db/db* islets with the GCK inhibitor mannoheptulose enhanced insulin secretion and restored pulsatile release [56]. Paradoxically, in contrast to control islets, mannoheptulose also enhanced ATP content and restored NAD(P)H flux. Likewise, GCK inhibition reversed the impaired insulin secretion found in islets isolated from mice carrying a loss-of-function mutation in the TCA cycle enzyme fumarase [55], and prevented the changes in gene expression, and insulin secretion produced by chronic hyperglycaemia in INS-1 cells [42,43].

Taken together, these studies suggest that what drives progressive beta-cell failure in T2D is excessive glucose metabolism and that reducing metabolic flux by decreasing glucokinase activity is beneficial. Importantly, they also explain why glucokinase activation is not a useful therapeutic strategy in T2D; in the long term it merely exacerbates the problem. This raises the question of whether reducing glucokinase activity may provide a better approach to diabetes therapy.

Consequences of total loss of glucokinase activity

Given that GCK acts as the beta-cell glucose sensor, it is unsurprising that a total loss of glucokinase activity in either humans or mice results in severe diabetes. Mice homozygous for either a global or beta-cell-specific deletion of *gck* died of diabetes within a week of birth due to severe diabetes [59,60]. Likewise, homozygous inactivating mutations in the human glucokinase gene cause permanent neonatal diabetes (GCK-PNDM) [61]. Fortunately, they are extremely rare. All GCK mutations act by decreasing glucose-dependent ATP production, which results in impaired K_{ATP} closure. Consequently, insulin secretion is reduced, leading to diabetes (Figure 3).

Nevertheless, some species lack GCK completely, with no adverse effects (Box 1).

Heterozygous inactivating GCK mutations cause mild diabetes

Mice expressing a heterozygous inactivating GCK mutation, which possess only ~50% Gck activity in their beta-cells, exhibit early onset mild diabetes, with slightly elevated fasting blood glucose levels and mild glucose intolerance. Insulin secretion from isolated islets is normal at 3 and 20 mM glucose, but secretion in response to 10 mM glucose is significantly impaired, consistent with a shift in the glucose dose-response curve to higher glucose concentrations. This is associated with a similar shift in the sensitivity of the K_{ATP} channel to glucose inhibition [65], which underlies the lower insulin release. Likewise, the I336F mutation in GCK, which reduced

Box 1. Hummingbirds do without

Many vertebrate species that have a limited carbohydrate diet, including ruminants and some carnivores, lack all GCK activity in their liver [62]. This is because liver GCK activity is only needed when blood glucose levels are elevated above the homeostatic set-point; for example, due to dietary carbohydrate or excess gluconeogenesis. However, while liver GCK activity is not required in these species, they still need beta-cell GCK for insulin secretion and glycaemic control. By contrast, the ruby-throated hummingbird entirely lacks glucokinase in its genome [63]. Hummingbirds feed entirely on nectar, which is very high in glucose, fructose, and sucrose. They maintain very high levels of plasma glucose in their bloodstream, which can be as much as 17 mM in the fasted state and rises to ~40 mM after feeding [64]. This may be an adaptation to enable the high metabolic rate demanded by rapid hovering flight. The lack of global glucokinase expression may account for the elevated blood glucose. Despite their severe chronic hyperglycaemia, hummingbirds are not known to experience any of the complications associated with diabetes. Why this is the case is unclear, but one possibility is that the lack of glucokinase prevents the deleterious effects of glucose on their beta-cells, and on other glucose-sensing cells, by limiting excessive glycolytic flux.

enzyme activity to ~70% of normal in the heterozygous state, is associated with an increase in fasting blood glucose and impaired glucose tolerance [66]. Liver-specific homozygous *gck* null mice exhibit mild hyperglycaemia due to defective glycogen synthesis [60]. Insulin secretion is also reduced, likely due to the deleterious effects of the resulting hyperglycaemia on the beta-cell.

Heterozygous loss-of-function mutations in humans cause a subtype of maturity onset diabetes of the young (GCK-MODY) [67,68]. This is characterised by mild stable fasting hyperglycaemia (typically 5.5–8 mM compared with 4–6 mM for nondiabetic subjects) which often goes undiagnosed [69]. The threshold for glucose-stimulated insulin secretion is only moderately elevated (to ~7 mM) and glycated haemoglobin ($HbA1_c$) is increased to around 7%. Unlike T2D, hyperglycaemia is detectable at birth and deteriorates little with age: the increase in fasting blood glucose is only about 5% over 70 years, which resembles that seen in nondiabetic family members [70]. GCK-MODY patients also show only a relatively small increment in glucose during an oral glucose tolerance test [70]. The 2-h concentration is around 9 mM and does not change with age [70]. Glycogen synthesis in the liver is mildly reduced, as is the suppression of hepatic glucose production produced by insulin [71,72].

Numerous inactivating human *GCK* mutations have been identified [22]. Most decrease the affinity of GCK for glucose and/or reduce the V_{max} of the enzyme. Those that affect the EC_{50} increase the threshold for glucose stimulation of insulin secretion and shift the glucose dose-response to higher glucose concentrations. In essence, blood glucose is simply regulated around a new set point.

GCK-MODY patients do not require therapy

The prevalence of GCK-MODY is around 1.1 in 1000 but the disease is underdiagnosed as it is asymptomatic and in many people goes unrecognised or is misdiagnosed as type 1 diabetes (T1D) or T2D [69]. It is often only picked up in later life during routine health screening [73]. These patients do not need medication and there is no evidence for progressive deterioration of beta-cell function [74]. Importantly, their prevalence of diabetic complications is low, despite their lifelong hyperglycaemia [69,75]. In marked contrast to patients with T2D, individuals with GCK-MODY are not at increased risk of microvascular and macrovascular complications (stroke and ischemic heart disease). Neuropathy and nephropathy are rare. A slightly higher prevalence of background retinopathy has been detected, compared with controls, but the rate of proliferative retinopathy is very low. Crucially, despite the mild hyperglycaemia, there appears to be no progressive deterioration of beta-cell function.

Hypoglycaemia is a common side-effect of insulin therapy in diabetes. Because glucose is the main fuel supply for the brain, hypoglycaemia can be fatal or result in lasting neurological damage. Fear of hypoglycaemia means that most T1D and T2D patients are treated less aggressively with insulin than would be optimal for glycaemic control, which enhances the risk of diabetic complications. Normally, robust counter-regulatory mechanisms ensure that hypoglycaemia does not occur. These switch off endogenous insulin secretion when blood glucose levels fall and stimulate the release of counter-regulatory hormones such as glucagon and adrenalin. GCK plays an important role in the **counter-regulatory response**: beta-cell GCK is responsible for suppression of insulin release when blood glucose levels fall, alpha-cell GCK is responsible for the increase in glucagon secretion, and neuronal GCK contributes to the adrenalin response.

Patients with heterozygous activating GCK mutations show enhanced counter-regulatory responses as their lower beta-cell GCK activity reduces glycolytic flux and triggers an earlier reduction in insulin secretion [76]. Their glucagon response also has an earlier onset, and a larger peak amplitude, than that of either T2D patients or healthy controls. Similar results were seen in

heterozygous mice with a loss-of-function GCK mutation (I336F) [76]. In these mice, the improved response persisted after beta-cell ablation with **streptozotocin**, suggesting it does not involve paracrine signalling in islets. It was also unaffected by knockout of brain GCK. This argues that GCK-mediated sensing by alpha-cells plays an important role in the glucagon counter-regulatory response. The adrenalin response was also increased in GCK-MODY patients, in beta-cell ablated GCK-mutant mice, and in mice in which GCK was ablated in brain neurones. Thus, brain GCK appears to be involved in triggering adrenaline release when blood glucose levels fall. These data raise the possibility that GCK activators may increase the risk of hypoglycaemia (by reducing the counter-regulatory response) and, conversely, that partial GCK inhibition may enhance counter-regulatory defences to hypoglycaemia in both T2D and T1D [76].

In summary, studies of the effects of heterozygous inactivation of GCK in human and mouse demonstrate that a significant reduction in GCK activity produces a small rise in fasting blood glucose but, critically, no progressive decline of beta-cell function or increased risk of diabetic complications. In T2D, a target $HbA1_c$ level of <7% is recommended to reduce the risk of diabetic complications. This target is, in part, based on the observation that GCK-MODY patients have no complications. Similarly, it is widely assumed that the failure of diabetes to progress in GCK-MODY patients is because their hyperglycaemia is relatively mild. However, these arguments may be invalid if it is the reduced GCK activity itself that protects beta-cell function from the deleterious effects of chronic hyperglycaemia.

A new therapeutic approach?

Current therapeutic strategies for T2D are directed at lowering blood glucose levels. However, there is evidence from reconstituted human islets that even a very moderate chronic increase in extracellular glucose (to 8 mM) is sufficient to impair insulin secretion [77]. Such tight glycaemic control is difficult to achieve in T2D because of the risk of fatal hypoglycaemia. However, given the accumulating evidence (reviewed earlier) that it is excess glucose metabolism, rather than excess glucose, that causes beta-cell failure, an alternative strategy might be to reduce glucose metabolism to the same level as that found normally under **euglycemic** conditions. One way to do this would be by partial GCK inhibition. Although at first sight it may seem counterintuitive to suggest inhibition of GCK activity may be therapeutic in T2D, several lines of evidence support this idea.

As discussed earlier, these include: (i) the ability of mannoheptulose to prevent, and reverse, the effects of chronic hyperglycaemia *in vitro*; (ii) recent studies which report that, paradoxically, partial glucokinase inhibition can help preserve β-cell function and mass in mouse models of diabetes; and (iii) perhaps most importantly, the effects of inactivating glucokinase mutations in humans suggest partial reduction of GCK activity will not be harmful. Despite mild fasting hyperglycaemia (~6.5 mM), these patients require no medication, their hyperglycaemia does not progress, and their prevalence of diabetic complications is not increased.

A key question is whether GCK inhibition in T2D might have unwanted side-effects. Most cell types use other hexokinases (HK1–3) for glucose phosphorylation and their metabolism would therefore be unaffected. Moreover, in the very few other tissues in which GCK is expressed, GCK inhibition appears to be beneficial. Selective GCK deletion in the liver results in very mild hyperglycaemia in mice [60], so it seems likely that adverse effects on the liver would be limited. Inhibition of liver GK activity may even be beneficial in the context of T2D, as it will reduce fatty liver disease. As the counter-regulatory response is increased by GCK inhibition [76], better control of blood glucose without increasing the risk of hypoglycaemia may even be possible.

Numerous questions remain to be addressed. What might happen in obese T2D individuals? GCK-MODY patients are not obese or insulin-resistant and reduced GCK activity might have different effects in an obese T2D patient. Nevertheless, the improvement in first phase insulin secretion in patients on a low calorie diet suggests reducing blood glucose is effective in diabetes of short duration [50]. How much must GCK be lowered to prevent diabetes progression? What further lessons can be learnt from patients with inactivating GCK mutations? Is it possible to design a drug that inhibits glucokinase but not all other hexokinases? Would such a drug affect endogenous GCK expression? What side-effects might it have? Is it in fact a practical approach (Box 2)?

Concluding remarks and future perspectives

Glucokinase is a key beta-cell glucose-sensor that confers the unique ability to respond to plasma glucose levels over a wide range and is essential for insulin secretion. However, this very ability may be detrimental in chronic hyperglycaemia, as the beta-cell has no way to limit glucose metabolism. This leads to detrimental changes in the beta-cell that cause the progressive decline in beta-cell function found in T2D. As a consequence, it can be expected that GCK activators will inevitably fail as a T2D therapeutic strategy for preserving beta-cell function as they will compound the problem by boosting beta-cell metabolism further. The finding that many GCK activators cease controlling glycaemia in patients with T2D after a few months supports this view. Evidence instead favours the idea that the opposite approach might be more valuable, because reducing glycolytic flux in diabetes would help prevent further decline in beta-cell function, and facilitate the gradual reversal of changes caused by chronic hyperglycaemia. The example of patients with inactivating GCK mutations argues it should also prevent diabetic complications, which

Box 2. Is GCK inhibition a practical therapeutic diabetes strategy?

Clinical use of GCK inhibitors is challenging because their therapeutic value depends on the extent of inhibition: too much inhibition would have adverse effects (as shown in GCK-PNDM). However, this is also true for most drugs, including both insulin and sulphonylureas. Clearly, the aim would be to develop a glucokinase inhibitor with a wide therapeutic window, that would enable GCK activity in diabetes to be safely reduced to levels found at normal blood glucose levels in nondiabetic individuals. Indeed, as GCK activity varies with its substrate (glucose) it may be necessary to reduce the drug dose as beta-cell recovery progresses and hyperglycaemia is better controlled. The reduction in GCK activity may have beneficial effects in liver and the fall in blood glucose will also benefit peripheral tissues.

As use of mannoheptulose is impractical for clinical use, a high-affinity, small-molecule GCK inhibitor is required. This needs to be selective for GCK and not target other hexokinases, it should have a wide therapeutic window, and it should not alter the EC_{50} for GCK. Development of such a drug should be aided by the fact that the atomic structure of GCK is known [78,79] and structure–activity relationships have been characterised for some GCK mutations [21,22,80].

Which patients would a GCK inhibitor be useful for? As reducing GCK activity should be effective at reducing the decline in beta-cell function associated with elevated blood glucose, GCK inhibition might be most appropriate in individuals with glucose intolerance or newly diagnosed diabetes. Improvements in beta-cell function could be examined by monitoring urinary C-peptide/creatinine levels, which can easily be carried out in the clinic [81]. The GCK dose could be adjusted to prevent an increase in fasting blood glucose while enhancing C-peptide levels. It has been postulated that the hyperinsulinaemia found early in diabetes development may contribute to obesity and ectopic fat deposition [82]. Thus, reducing insulin secretion in patients with impaired glucose tolerance might benefit peripheral tissues, in addition to protecting the beta-cells. GCK inhibition might also be useful in T1D, to help preserve existing beta-cell function, as a small number of functional beta-cells are known to remain in T1D [83].

As with any therapeutic strategy, there are also caveats. The use of GCK inhibitors in pregnant women is best avoided because of the risk of the drug affecting fetal growth if it crosses the placenta. Insulin enhances fetal growth and neonates with inactivating GCK mutations are generally small due to their reduced insulin secretion [84]. Furthermore, while it is likely that GCK inhibitors might slow the decline in beta-cell function, the extent to which they will be able to reverse changes found in long-established T2D is less clear. Nevertheless, at least in the short term, it is possible to reverse beta-cell changes induced by severe hyperglycaemia in mice [46,47]. And the fact that T2D can be reversed by bariatric surgery and low calorie diets offers hope this is also the case in humans [49–51].

Outstanding questions

Does enhancing glucokinase activity in human beta-cells promote their functional decline and eventually lead to beta-cell death?

Do patients with activating glucokinase mutations eventually develop glucose intolerance and diabetes?

How high and for how long must blood glucose be chronically raised to initiate beta-cell decline?

How reversible are the effects of chronic hyperglycaemia?

How much suppression of glucokinase activity is needed to prevent the deleterious effects of chronic hyperglycaemia on beta-cell function?

Does partial inhibition of glucokinase maintain beta-cell function in the face of chronic hyperglycaemia in human as well as animal islets?

Is it possible to design a drug that selectively inhibits glucokinase activity with no effect on other hexokinases?

Would a drug that causes partial glucokinase inhibition slow or prevent beta-cell decline in diabetes and which patients might benefit most from this?

How long might such a drug protect the beta-cells for and what might be the potential side-effects?

have devastating effects on patients' lives. It seems that an exploration of the use of GK inhibitors for diabetes therapy is ripe for investigation (see Outstanding questions).

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Declaration of interests

No interests are declared.

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