

Glucose Mediated Regulation of Beta Cell Proliferation

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Abstract: The pancreatic beta cell, as the sole source of circulating insulin, is indispensable for normal glucose metabolism. All forms of diabetes are characterized by insufficient insulin to meet metabolic needs, and inadequate beta cell mass is present in most forms of diabetes. Beta cell regenerative therapies provide one potential avenue for the prevention or cure of diabetes. Study of the pathways and mechanisms involved in endogenous beta cell proliferation can provide important clues to how regeneration occurs. Glucose is one important regulator of beta cell replication. Here we review the published literature investigating the effect of glucose on beta cell proliferation, and then describe the data supporting various known mechanisms of action. First, *in vitro* studies on fetal and adult beta cells from rodents and humans are reviewed. Next, we review evidence that circulating blood glucose regulates beta cell proliferation in living animals. These studies encompass a variety of *in vivo* models, including dietary manipulation, partial pancreatectomy, intravenous infusion of glucose, islet transplantation, and studies on diabetic hyperglycemia. Finally, mechanistic studies, both *in vitro* and *in vivo*, point to several different mitogenic pathways activated by glucose. Especially prominent are insulin signaling pathway members, glucose metabolism, and glucose impact on signaling by other growth factors. Multiple unanswered questions remain, and are highlighted throughout. With today's technologies we can now address questions first raised in the early 1900s, and hopefully develop new approaches to assuage the devastation of clinical diabetes.

Keywords: Glucose, pancreatic beta cell, proliferation, insulin, diabetes.

INTRODUCTION

Historically, beta cells of adult animals were thought to be largely post-mitotic. This dogma persisted for many years, in part because techniques for studying replication in whole animals were not as easy to apply as they are today, and in part because the actual replication rate in steady-state, unstressed adult animals is indeed quite low [1]. However, in times of increased metabolic need, the rodent endocrine pancreas is an organ with impressive plasticity. Concomitant with an increase in insulin requirement in the body, there is an enhancement in beta cell mass. Conversely, when insulin requirements are lowered, a prompt reduction of beta cell mass takes place. This increase in islet mass has been observed in pregnancy, partial pancreatectomy, and obesity-induced insulin resistance [2-5]. The reverse scenario with reduction of islet mass occurs following parturition, glucose level normalization and sustained improved insulin sensitivity. Studies show that similar compensatory mechanisms may be active in human conditions characterized by increased insulin requirements [6, 7]. Although the origin of the new beta cells in these situations remains controversial and seems to depend on the pathophysiological model under study, beta cell proliferation is widely accepted to play a critical role in all of them. Thus, during normal physiologic and pathologic metabolic alterations, the rate of beta cell proliferation changes over time depending on the metabolic

requirements and the local environment in and around the beta cell.

Intuitively, blood glucose level is a logical primary actor influencing beta cell proliferation in situations of beta cell compensation due to increased metabolic demand. An important effect of insulin is to increase flux of glucose out of the bloodstream and into tissues such as liver, muscle and fat. As glucose is the primary regulator of insulin secretion, the beta cell is exquisitely sensitive to glucose concentrations [8]. Elevated glucose concentration in the blood is an early indicator of insulin insufficiency. Therefore, elevated glucose seems a reasonable candidate to mediate need-driven beta cell expansion.

In this review we will first examine the *in vitro* evidence showing the mitogenic effects of glucose on rodent and human pancreatic beta cells. Next, we will describe *in vivo* studies that have demonstrated the relevance of glucose as a physiological regulator of beta cell replication. Finally, although the mechanisms involved in beta cell compensation to glycemic stress *in vivo* remain largely unidentified, we will explore the mechanistic insight provided by *in vitro* experimentation.

EVIDENCE THAT GLUCOSE INDUCES BETA CELL PROLIFERATION *IN VITRO*

From the earliest work in culturing beta cells, glucose was known to be an important requirement for optimal beta cell growth. Many culture model systems, including fetal and neonatal beta cells [9], continuous insulinoma cell lines [10, 11], and primary islets [12-14] have all demonstrated a growth advantage in response to glucose. Thus, cultured beta

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cells have been shown in multiple different laboratories, in culture systems ranging from continuous insulinoma cell lines to primary freshly isolated islets, to proliferate in response to elevated glucose.

Rodent Beta Cells

In the early eighties, Swenne and colleagues were pioneers in analyzing the effect of glucose on DNA synthesis in rat beta cells [9, 13, 15-17]. Islets of fetal, neonatal, and adult rats were isolated and cultured at different glucose concentrations and DNA synthesis rates were calculated by pulse labeling with ^3H -thymidine. When the glucose concentration of the culture medium was raised from 2.7 to 16.7 mM the rate of beta cell proliferation increased 2.5-fold in all age groups, but there was no further increase in proliferation at 33.3 mM. Although the fold increase was equal, the rate of beta cell replication at each of the glucose concentrations decreased with increasing donor age. These results were the first to demonstrate that normal rodent beta cells replicate in response to glucose and that aging decreases the capacity of beta cells to proliferate in absolute terms in the presence of high glucose concentrations (16.7mM). These data were also the first to suggest an interesting parallel to the development of type 2 diabetes in humans, in which an inherited low capacity for beta cell regeneration in the presence of increasing blood glucose may participate in the development of the disease.

Other research groups confirmed these initial results by labeling fetal and adult rat islets *in vitro* using a thymidine analog that cannot be reutilized by the cell, 5-bromo-2-deoxyuridine (BrdU) or by fluorescence activated cell sorting (FACS) analysis of dispersed adult rat islet cells [18, 19]. Furthermore, the proliferative effects of glucose in adult beta cells have also been observed in mouse beta cells as well as several insulinoma beta cell lines [10, 11, 18, 20-22].

However, the involvement of glucose in fetal beta cell proliferation seems more controversial. In contrast to the studies mentioned above, recent studies by the Scharfmann group have shown that 10 mM glucose increases 20-fold the area of insulin-positive cells in embryonic pancreases in culture (compared with 0 mM) without increasing beta cell proliferation, but rather enhancing the differentiation of early progenitors into beta cells by regulating the transition between *Ngn3* and *NeuroD* [23]. However, basal (low glucose) beta cell proliferation was not measured in this study. These studies are important since they may uncover a role for glucose in beta cell allotment in intrauterine growth retardation or maternal gestational diabetes, with possible repercussions on glucose homeostasis and beta cell mass in adulthood.

Human Beta Cells

Encouraged by the mitogenic effects of glucose in rodent beta cells, several groups have analyzed the dose- and time-dependent effects of glucose on human beta cell replication *in vitro*. Tyrberg *et al.* reported in 1996 that the human beta cell labeling index (LI; i.e. % of labeled beta-cells over total number of beta-cells) was increased when islets were cultured for 48h at 5.6 mM glucose compared with 2.8 mM glucose. Interestingly, and in contrast to the results observed in adult rat islets, 11 mM glucose failed to further increase

beta-cell proliferation [24]. More recently, the Donath group has performed a more detailed analysis of the temporal effects of glucose in human beta cells employing the cell cycle progression marker, Ki67, and insulin staining as means to detect beta cell proliferation in cultured human islets [14, 25, 26]. Short-term (1–3 days) exposure of cultured human islets to 33.3 mM glucose increased the number of proliferating (Ki67-positive) beta cells, whereas prolonged (>4-day) exposure resulted in an inhibition of proliferation, relative to islets at 5.5 mM glucose. This decrease in human beta cell proliferation induced by chronic incubation with glucose was independent of the age of the islet donor. On the other hand, 33 mM glucose increased human beta cell apoptosis after the first day of incubation. These studies suggest that the reduced glucose-mediated proliferative response coupled with increased beta cell death in response to extremely high-glucose concentrations *in vitro* might represent a similar sequence of events observed *in vivo* during the evolution of diabetes. Unfortunately, in these later studies, lower concentrations of glucose representing milder hyperglycemia that could potentially trigger beta cell compensatory expansion were not used.

EVIDENCE THAT GLUCOSE INDUCES BETA CELL PROLIFERATION *IN VIVO*

It is well documented that pancreatic beta cells perform quite differently in a petri dish than they do when embedded in the living organism, although the reasons for this remain speculative [8]. Thus, for the purposes of developing treatments for diabetes, investigating the signals that drive beta cell replication must be performed or confirmed *in vivo*. Several different *in vivo* approaches have suggested that glucose may regulate beta cell replication. Manipulation of food macronutrients has been used to raise the blood glucose. Partial pancreatectomy, or surgical removal of a portion of the pancreas, induces hyperglycemia to a variable degree. Glucose infusion is a more direct approach to raising the blood glucose, not subject to the myriad local changes in the pancreas caused by surgery and healing. Infusion protocols, however, are technically challenging and require specialized equipment and expertise. Islet cell transplant, while also challenging, is a flexible technique, allowing study of islets from different species including human, and pre-transplant manipulation of islets as well. The challenge in using transplantation to study the role of glucose in beta cell replication is developing tools to allow titration of circulating host blood glucose. Mouse genetic models have also been used to address the question of glucose as a driver of beta cell replication. Finally, diabetes itself is characterized by hyperglycemia, and this has been used to investigate the role of glucose on beta cell replication. In this section we will review the data suggesting that glucose induces beta cell proliferation *in vivo*.

Manipulation of Orally-Administered Macronutrients

Oral administration of sucrose is a technically simple means to increase the metabolic load on the pancreas and, in some cases, increase blood glucose. Rats allowed ad libitum access to a sucrose-rich diet for thirty weeks [27] developed significant hyperglycemia (8.1 mM in sucrose-treated rats versus 6.6 mM in controls), which correlated with a robust increase in beta cell mass (15 mg versus 6 mg) and a large

6.8x increase in beta cell replication 30 weeks into the treatment (0.27% versus 0.04% PCNA positive beta cells). Similarly, feeding hamsters excessive sucrose *via* the drinking water caused a robust increase in beta cell replication [28]; however, blood glucose did not increase (7.1 mM), and in this case the changes were attributed to sucrose-driven insulin resistance.

In *Psammomys obesus*, researchers independently manipulated the energy content of the diet and the blood glucose (using phlorizin), and found that beta cell replication increased dramatically after 2 and 5 days of high blood glucose (approximately 15 mM and 20 mM, respectively) [29]. After 22 days of high blood glucose (approximately 21 mM) beta cell replication was still elevated over baseline, although the rate declined from the high seen at 2-5 days. Lowering blood glucose with phlorizin (approximately 7 mM) promptly reduced beta cell replication to baseline levels, suggesting that the beta cell replication was due to hyperglycemia.

Recovery from Hypoglycemia

In an interesting reverse experiment exploring the effects of hypoglycemia on islet morphology and function, female Wistar rats received continuous infusion of insulin *via* miniosmotic pump for six days [30]. During hypoglycemia (approximately 2mM glucose), beta cell replication was suppressed, and during the rebound hyperglycemia following pump removal (approximately 11 mM) beta cell replication increased. These data suggest that hyperglycemia stimulates beta cell replication, but also that hypoglycemia suppresses basal beta cell replication. Furthermore, this is one piece of evidence that insulin itself may not be the glucose-dependent driver of mitogenesis (see below), although peripherally administered insulin may not come close to reaching the intra-islet insulin concentration that occurs during normal beta cell insulin release.

Pancreatectomy: Does Glucose Signaling Contribute to Beta Cell Regeneration?

In rodents, partial pancreatectomy is a well characterized injury model of beta cell regeneration. The degree of post-operative hyperglycemia, if present, is dependent on the volume of pancreas removed [31-33]. A role for glucose in post-operative beta cell regeneration has been debated, largely due to the fact that a commonly used pancreatectomy procedure, 60% pancreatectomy, which leads to robust beta cell regeneration, does not lead to elevated circulating blood glucose [34]. Certainly, other signals besides glucose, such as local surgery-stimulated growth factors and cytokines, may contribute to beta cell regrowth. Hyperglycemia, however, may contribute to post-pancreatectomy regeneration; when drinking water was replaced with sucrose water post-operatively, blood glucose increased (9.2 mM), and beta cell mass increased dramatically relative to pancreatectomy controls drinking untreated water (8.3 mM) [35]. However, hyperglycemia after pancreatectomy has also been shown to negatively impact insulin secretion [34, 35].

Intriguingly, even in the setting of normoglycemia post-pancreatectomy, glucose metabolism appears to be increased in the islet. One study found that glucose utilization increased and glucokinase activity doubled in islets four

weeks after 60% pancreatectomy [34]. A follow-up study determined that glucose metabolism was markedly increased five days after pancreatectomy, with a tripling of glucose phosphorylation, a doubling of glucose usage, and a six-fold increase in glucose-6-phosphate metabolism through the pentose phosphate shunt [36]. At four weeks, these metabolic changes had mostly returned to normal. Beta cell replication correlated exactly with the changes in glucose metabolism, being highest at five days and essentially back to baseline by four weeks. In another related work, increased flux through the pyruvate carboxylase pathway after pancreatectomy in rats was found to be important for beta cell regeneration [37].

Therefore, in the 60% pancreatectomy model of beta cell regeneration, circulating blood glucose is not increased, but beta cell replication is robustly induced. Interestingly, increased flux through glucose metabolic pathways in the beta cell may represent an equivalent signal to hyperglycemia. Experiments such as these may give insight into downstream mechanisms of glucose signaling in beta cell regeneration.

Glucose Infusion Models: Rat and Mouse

Intravenous infusion of glucose is a direct method of increasing circulating blood glucose, without attendant changes due to surgery on the pancreas, or the endocrine/hormonal changes of obesity, diabetes, or pregnancy. As early as 1938, in a tour de force experiment starting with fashioning a catheter out of rubber tubing and devising a continuous pumping apparatus, continuous infusion of glucose into the external jugular vein of guinea pigs was reported to increase blood glucose slightly (approximately 5.6 mM basal, 8.2 mM during infusion), without glycosuria [38]. In these guinea pigs, they found an increase in islet tissue at four to five days of infusion, which was associated with mitotic figures in beta cells of larger islets at four and seven days. In another remarkable study, wild Argentine toads were captured and infused with glucose for five days, which resulted in hyperglycemia (28 mM *vs* 1.3 mM) and increased beta cell replication as measured by BrdU incorporation [39].

In the 1980s, the continuous glucose infusion experiment was resurrected and optimized as a tool to study regulators of beta cell regeneration [40]. In rats, glucose was infused through the jugular vein at a rate that resulted in severe hyperglycemia (approximately 23 mM) on the first day, but declined significantly on the second day (approximately 18 mM), and normalized by the fourth day (7.8 mM). Beta cell replication was analyzed by counting mitotic figures after a four hour colchicine arrest. After four days of infusion, beta cell replication increased five fold. Additionally, individual beta cell volume increased 50%, and overall beta cell mass increased 50%. These findings have been supported [41] and contested [42]. A longer glucose infusion study in rats, extending the infusion duration to six days, found that beta cell replication was decreased on day 1, but increased by days 3-6; beta cell mass linearly increased with time [43]. Glucose infusion studies in which the duration of infusion was less than 72 hours have not reported an increase in beta cell replication [44-46]. The majority of rat glucose infusion studies have utilized infusion protocols that result in severe

hyperglycemia on the first 1-2 days (>20 mM). One study infused a lower quantity of glucose, resulting in a subtle increase in blood glucose levels on days 1-2 (approximately 6.7 mM), with no hyperglycemia on days 3-4 (approximately 5.6 mM). Data from this experiment showed no glucose-related increase in beta cell replication, but the blood glucose was only slightly elevated for two days, and the control rate of replication was high (5% Ki67-positive beta cells), which may represent adolescence-related overall (and beta cell) growth [42].

Rat glucose infusion studies have been quite sophisticated, including combination interventions such as glucose infusion after streptozotocin [45, 46] or hypothalamic ablation [47], or in combination with euglycemic hyperinsulinemic clamps [41, 44, 46, 48]. Illuminating post-intervention analyses such as immunostaining [42], RNA quantification [41, 49], or immunoblot [42] have been performed. However, one of the most powerful tools to dissect mechanistic pathways, transgenic and knockout technology, is not widely developed in the rat. In order to apply the power of mouse genetics to the question of how glucose induces beta cell proliferation, we adapted the rat glucose infusion procedure for use in mice [50]. In mice, four day infusion of glucose results in mild persistent hyperglycemia (7.6 mM; 25% increase over baseline) and hyperinsulinemia (125% increase), and a large (500%) increase in beta cell replication (Fig. 1). These data confirm, in a second rodent model, that hyperglycemia induces beta cell proliferation. Our model shows interesting differences with the rat glucose infusion work. In rats, the blood glucose surges to extremely high levels for 1-2 days, returning to normal by 96 hours. In mice, the blood glucose increase is quite moderate, but is sustained for as long as the infusion continues, up to four days. Infusion of greater quantities of glucose in the mouse, to attempt to achieve higher blood glucose, is not tolerated (personal observation). Plasma insulin levels in both mouse and rat increase greatly during the first 1-2 days, with subsequent return to near-baseline levels by day 4. In mice, no increase in beta cell size or mass was detected after four days of infusion, whereas both these parameters increase in rats infused with glucose.

Peripheral insulin resistance increases beta cell replication (e.g. [51]). It has been hypothesized that glucose infusion increases beta cell replication by increasing insulin resistance. However, studies do not support this hypothesis. In the rat, in a euglycemic clamp after four days of continuous glucose infusion, an increased glucose infusion rate was required to sustain the same blood glucose, suggesting increased insulin sensitivity [41]. In a different study protocol, cyclically infused glucose (four hours on, four hours off) did not alter insulin sensitivity [48]. Four day infusion of glucose in rats, clamping blood glucose at 9.4 mM, required increasing glucose infusion rate to maintain the same degree of hyperglycemia, and insulin sensitivity was increased two hours after termination of the infusion [52]. In the mouse glucose infusion model, insulin resistance does not increase (personal communication, Dr. Christopher O'Donnell, University of Pittsburgh). Therefore, alterations in peripheral insulin sensitivity do not explain the increase in beta cell replication resulting from glucose infusion in the rodent, suggesting that glucose may be acting at the level of the beta cell.

Beta Cell Transplant Models

Islet transplantation is under intense investigation as a potential therapy for both type 1 and type 2 diabetes. Transplantation is also a useful model to study beta cell physiology, allowing experimental manipulation of the islet outside of its normal pancreatic environment, yet in an *in vivo* setting. Combining islet transplantation with chemical ablation of the endogenous beta cells allows independent control of beta cell mass and blood glucose. The islet transplantation technique has been applied to the question of whether glucose stimulates beta cell replication *in vivo*, in a transplant setting. In one early study, streptozotocin-diabetic mice received syngeneic islet transplants under the kidney capsule, of either an insufficient number of islets to achieve normoglycemia, or of a full replacement dose of islets [53]. The former mice remained severely hyperglycemic (20 mM), whereas the latter were normoglycemic. Beta cell replication was increased in the hyperglycemic mice ten days after transplant, although beta cell graft mass progressively declined and the beta cell replication rate at 18 or 30 days was no longer elevated, suggesting graft failure due to severe hyperglycemia.

In a complicated rat experiment, involving 95% pancreatectomy and/or islet transplantation in various combinations and sequences, it was noted that rats transplanted before pancreatectomy showed an increase in beta cell replication after pancreatectomy, in both the graft and the pancreas, whereas rats transplanted after pancreatectomy did not [4]. Blood glucose was elevated (18.8 mM) in the latter group but not the former (6.1 mM). It is not clear that these groups are comparable, however, since the duration of pancreatectomy-induced metabolic stress on the beta cell was 14 days in the former group but 28 days in the latter group, perhaps implicating a time-dependence of the beta cell replication response (see below).

Studies have confirmed the hyperglycemia-related increase in beta cell replication in islet grafts [54, 55]. Streptozotocin-diabetic mice received an insufficient mass of syngeneic islets to restore normoglycemia, either with or without insulin therapy to normalize blood glucose. Normoglycemic mice showed no increase in beta cell replication in the graft, whereas hyperglycemic (25 mM) mice showed increased graft beta cell replication at 14 days after transplant but not at 60 days after transplant. This again confirms that severe hyperglycemia increases beta cell replication early, but the replication response is not sustained. A follow up study by this group again showed that glucose-related beta cell proliferation occurred at 14 days but was lost at 60 days. Interestingly, the glucose-induced replication that was lost after long term hyperglycemia was recoverable following a period of normoglycemia induced by a second islet transplant [56]. In another follow up study, with a shorter time course, graft beta cell replication was increased after 10 days of hyperglycemia (24 mM) but not after 3 or 30 days; replication was suppressed by insulin therapy [57]. In one final mouse study, a carefully performed double transplant protocol was developed, in which streptozotocin-diabetic mice received a therapeutic transplant in the epididymal fat pad, then a minimal transplant (62 islet equivalents) under the kidney capsule [58]. When the therapeutic graft was removed, the mice developed

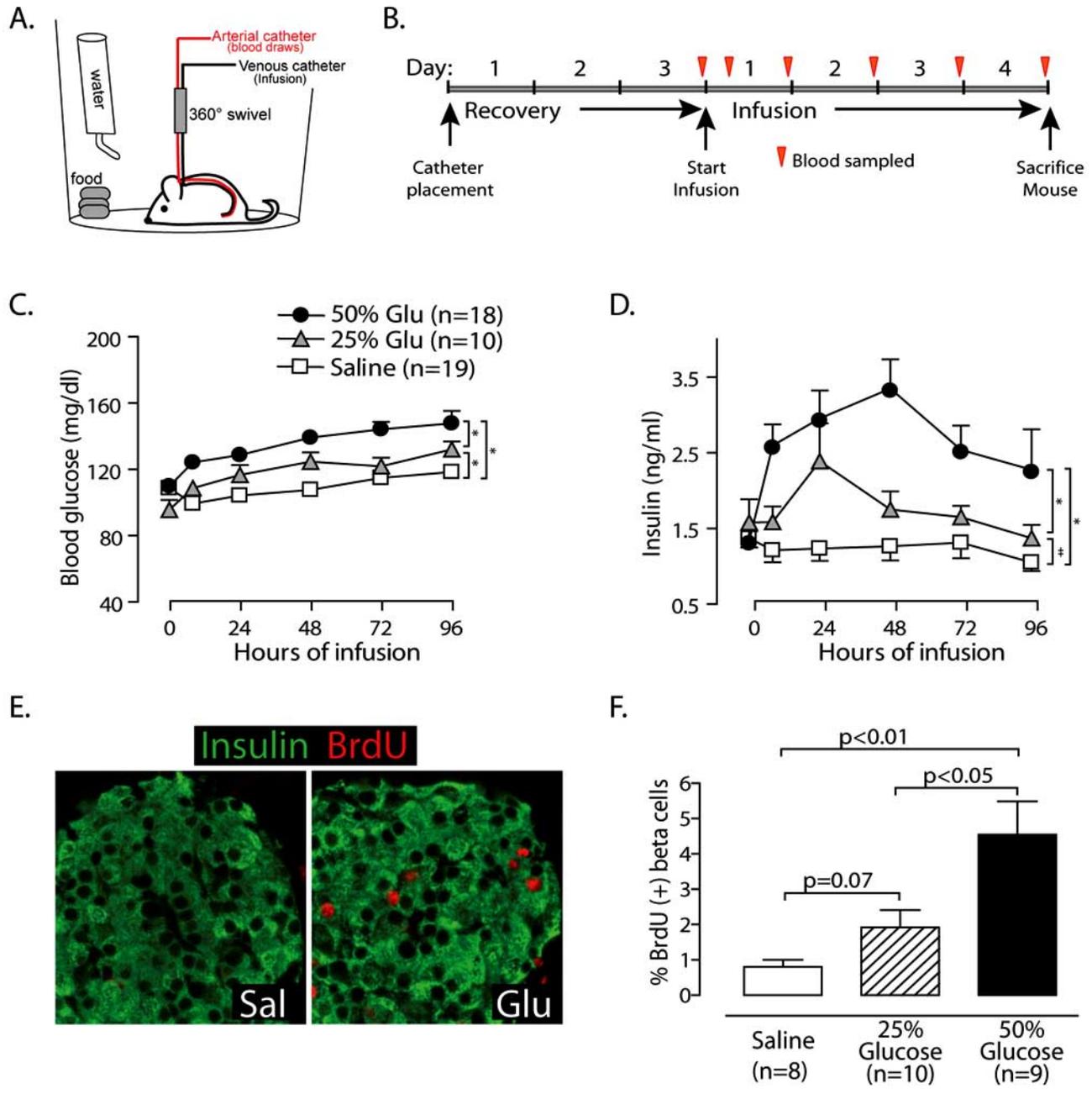


Fig. (1). Glucose infusion induces beta cell replication *in vivo* in mice. Mice with catheters in the femoral artery and vein are individually housed with ad libitum access to food and water (A). After a three day recovery period, four day infusion with saline, 25% glucose, or 50% glucose (B), results in moderate, sustained hyperglycemia (C) and hyperinsulinemia (D). Glucose infusion causes a dose-dependent increase in replication (E-F). Copyright 2007 American Diabetes Association, from [50], modified with permission.

hyperglycemia (13.5 mM); beta cell replication in the minimal graft increased fifteen-fold, as measured by both BrdU incorporation and Ki67 immunodetection. In contradiction to much published literature, they found similar proliferation rates in islet grafts from young (3 months) and old (20-24 months) mice.

Human islets transplanted into immune-compromised mice have been evaluated for glucose-induced beta cell replication. In one study, human beta cells exposed to severe hyperglycemia (24 mM) had a similar rate of replication to

those in normoglycemic conditions, fourteen days after transplant [24]. In a different study, human islets were transplanted into Ob/Ob mice with immunosuppression. Human beta cell replication doubled in the context of Ob/Ob relative to lean controls [59]. Although glucose was mildly increased in the Ob/Ob (9 mM vs 6 mM), other obesity-related differences (body mass, hyperinsulinemia, hyperlipidemia, inflammatory changes, etc) could also have induced beta cell replication.

In summary, islet transplant experiments confirm a role for hyperglycemia in stimulating beta cell proliferation, and suggest that transplanted islets respond similarly to islets in their usual pancreatic environment. Glucose-stimulated beta cell replication in these experiments was time-dependent, with replication response declining after several weeks' exposure to hyperglycemia, but the capacity of beta cells to mount a proliferative response to hyperglycemia (25 mM) was restored after a period of normoglycemia.

Hyperglycemia of Diabetes

One could argue that determining whether beta cells replicate during the hyperglycemia associated with the onset of diabetes is the most important question, because this gives insight into the endogenous beta cell regeneration process that is the therapeutic goal. If regeneration is ongoing, but overwhelmed by autoimmune destruction (type 1) or insulin requirement and toxic environment (type 2), regenerative therapies are more likely to be effective than if a *de novo* beta cell regeneration process has to be created.

One group has looked carefully at beta cell proliferation rates in early murine autoimmune diabetes [60]. Across several different genetic backgrounds, using multiple models of diabetes and modulation of blood glucose using insulin pellets and islet transplants, they found a direct correlation between beta cell proliferation rate and blood glucose across a wide range of glucose concentrations (6-22 mM).

In many rodent models of type 2 diabetes, beta cell replication has been shown to be increased; this increase has been attributed to insulin resistance. A role for glucose or glucose signaling has never been demonstrated, and in some cases, the onset of beta cell proliferation occurs prior to the onset of frank hyperglycemia [61, 62]. However, given the exquisite sensitivity of the beta cell for minor changes in blood glucose, potential unexamined effects of intermittent postprandial glucose excursion, and the stress-related increase in blood glucose inherent in various methods of sample collection (tail snip, retro-orbital bleed, submandibular bleed), it remains possible that a role for glucose in this process has been missed.

Evidence for glucose-driven beta cell proliferation in diabetic humans is sparse [63]. One study found increased beta cell replication and apoptosis in a man requiring pancreatectomy early in the course of type 1 diabetes [64]. Another study found ongoing beta cell apoptosis in people with longstanding type 1 diabetes, which indirectly suggests ongoing replication [65]. Neither study directly associates hyperglycemia with these findings. Pancreatectomy in humans leads to diabetes, without overt evidence for any regeneration [66, 67]. Clearly, more investigation is warranted to determine whether glucose regulates human beta cell replication, and if so, what mechanisms are involved.

MECHANISTIC INSIGHTS INTO HOW GLUCOSE INDUCES BETA CELL PROLIFERATION

Glucose influences extracellular mitogens, internal receptor-related signaling, downstream signaling pathways, pro- or anti-mitogenic transcription factors, and the cell cycle (Fig. 2). However, whether these glucose-mediated alterations in the intracellular network are involved in

glucose-induced beta cell proliferation *in vivo* awaits further studies. The generation of a chronic glucose infusion model in mice (Fig. 1) and the presence of myriad mouse genetic models in which genes encoding intracellular molecules have been altered specifically in beta cells will provide new clues to glucose-regulated beta cell proliferation and expansion *in vivo* in the near future. The elucidation of glucose-mediated growth-regulating pathways may give rise to novel pharmacological means of expanding the remaining endogenous beta cell population in diabetes.

Insulin Signaling Pathway

The most obvious potentially mitogenic effect of glucose is the local secretion of insulin, a robust mitogen for some cell types. Insulin signaling promotes beta cell replication [68, 69]. Knockout of the insulin receptor (IR) in beta cells results in normal beta cell mass, at least initially, suggesting that insulin is not essential for beta cell proliferation [70]. However, *in vitro* studies have shown that glucose modulates well-documented intracellular signaling molecules of the insulin signaling pathway including insulin receptor substrate 2 (IRS2), phosphatidylinositol 3'-kinase (PI3K) [11, 71, 72], protein kinase B (PKB), glycogen synthase kinase-3 (GSK-3), extracellular signal-regulated kinase (ERK)1/2, and mammalian target of rapamycin (mTOR) [11, 20, 71-78]. Interestingly, the major effects of glucose on beta cells, activation of PI3K, PKB, GSK3, ERK, S6K and mTOR, are also the principal downstream effects of activation of IRS2.

IRS2 is a central factor that mediates signals from several known beta cell mitogens. One of the best physiologic models of beta cell compensation comes from generation of peripheral insulin resistance through targeted deletion of insulin signaling components in mice. IRS1 knockout mice have increased insulin requirement due to peripheral resistance, and to compensate develop a markedly increased beta cell mass [79, 80]. Insulin secretion remains adequate, and the mice do not become diabetic. Mice lacking IRS2, on the other hand, have a quite different phenotype [51, 79, 81]. Peripheral insulin resistance is increased like in the IRS1 knockout mouse, but beta cells are unable to effectively compensate. Beta cell proliferation is reduced, beta cell mass declines, and the mice become diabetic. In a different strain, a similar but milder phenotype was observed [82]. Thus, IRS2 appears to be essential for beta cell compensation for increased insulin demand due to peripheral insulin resistance. Conversely, increased IRS2 expression promotes beta cell replication and prevents diabetes [83, 84]. Remarkably, even mice lacking only one allele of IRS2, with one normal allele present, have reduced beta cell compensatory reserve. IRS2^{+/-} mice have normal body weight, blood glucose, beta cell mass and plasma insulin [79-81, 85]. However, glucose tolerance worsens in IRS2^{+/-} mice that have only one allele of IGF1R [81] or Pdx1 [85] compared to the glucose tolerance in IRS2^{+/+} mice that are IGF1R^{+/-} or Pdx1^{+/-}. Thus, the presence of both alleles of IRS2 in Pdx1 or IGF1R haploinsufficient mice is necessary to preserve their glucose tolerance. Recent studies by Kulkarni's group have shown that beta cells from IR or IRS2 knockout mice display reduced DNA synthesis in response to glucose stimulation *in vitro* [20]. Intriguingly, in insulinoma cells overexpressing IRS2, glucose (15 mM vs 3

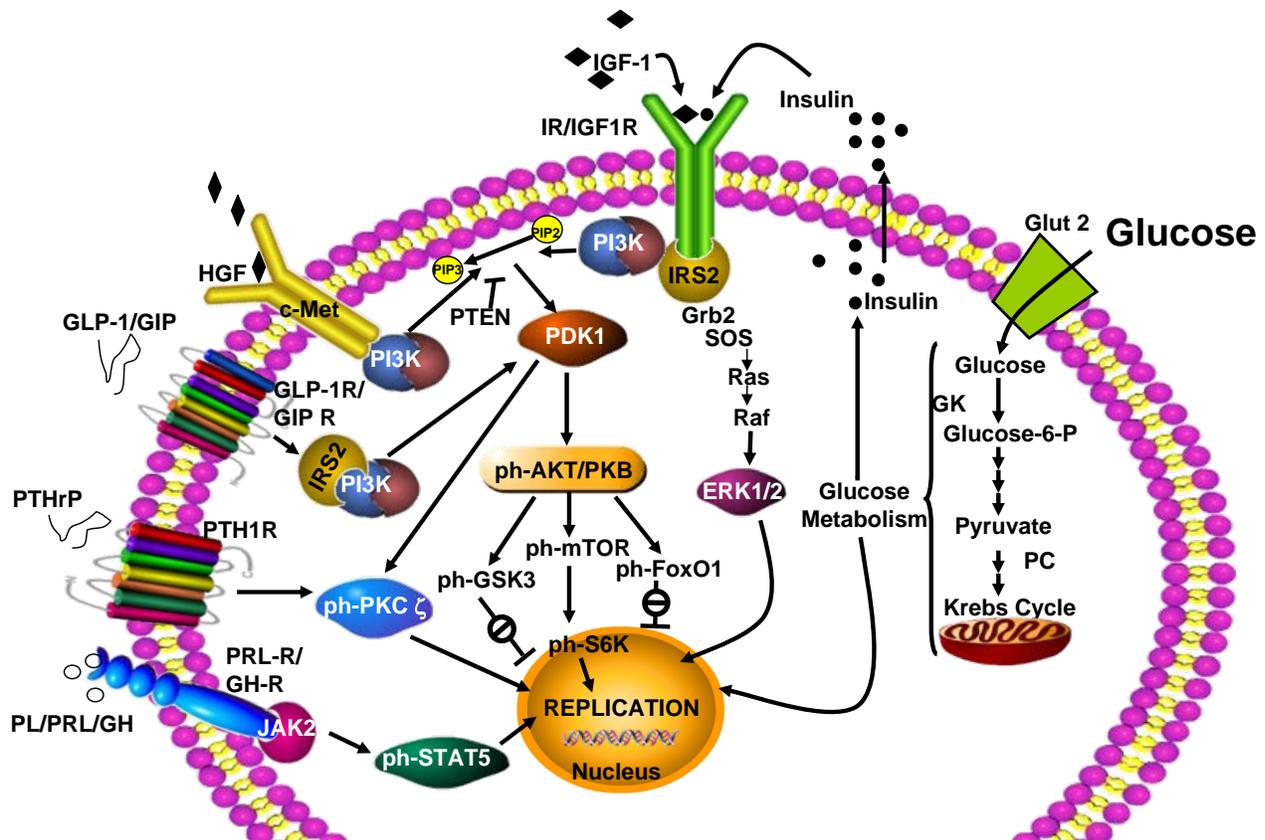


Fig. (2). Schematic representation of the signaling elements involved in glucose-mediated beta cell proliferation *in vitro*. Since intracellular pathways downstream of the insulin receptor (IR) and IGF1-R are similar, only one receptor is depicted here. Similarly, one receptor is depicted for lactogenic hormones placental lactogen (PL), prolactin (PRL), and growth hormone (GH). ph=phosphorylated; PTEN=phosphatase and tensin homolog; PDK1=phosphoinositide-dependent kinase 1; PIP2=Phosphatidylinositol (4,5)-bisphosphate; PIP3=Phosphatidylinositol (3,4,5)-triphosphate; Grb2=growth factor receptor-bound protein 2; SOS=Son of Sevenless; ⊖ =blockade of inhibitory action.

mM) stimulation of beta cell proliferation is synergistic with IRS2 [71]. Taken together, these studies highlight a potential key role of IRS2 in glucose-induced beta cell proliferation *in vivo*.

Inhibition of PI3K with wortmannin blocks glucose-mediated INS-1 cell proliferation, suggesting a role for PI3K in beta cell proliferation induced by 15 mM glucose [11]. Studies also suggested that there is a requirement for activation of both ERK1/ERK2 and mTOR/p70S6K signaling pathways for a full commitment to 15 mM glucose-induced pancreatic beta cell mitogenesis [11, 71]. In this regard, McDaniel's group has shown that inhibition of mTOR with rapamycin blocks glucose-stimulated DNA synthesis in rat islets (25 mM vs 3 mM) [77]. More recently the same group has reported that a combination of glycogen synthase kinase-3 (GSK-3) inhibition and glucose activation of mTOR (8 mM vs 5 mM) contributes to enhanced DNA synthesis, cell cycle progression, and proliferation of human beta-cells [78]. Glucose- and growth factor-mediated inactivation of FoxO transcription factors, which are PI3K/AKT targets, leads to downregulation of the transcriptional repressor BCL-6 and increased cyclin D2

expression and beta cell proliferation, implicating FoxO as a key mediator in glucose-induced beta cell replication [21].

In summary, while it is known that glucose acts in the beta cell to promote insulin secretion and to generate important signals which help to regulate gene expression and protein synthesis, the precise involvement of the insulin signaling pathway in glucose-mediated beta cell proliferation *in vivo* is unknown. Of note, insulin signaling has also been shown to increase glucose sensing in the beta cell, through upregulation of Glut2 and glucokinase expression [86, 87]. Chronic glucose infusion experiments in IRS2^{+/-} mice might be an interesting model to test for IRS2 involvement in glucose-induced beta cell compensatory mechanisms, because these mice are metabolically normal at baseline but decompensate if challenged.

Glucose Metabolic Pathways

Glucokinase (GK) phosphorylates glucose to form glucose 6-phosphate and is the glucose sensor that regulates insulin secretion in beta cells [88]. Haploinsufficiency of beta cell-specific GK (GK^{+/-}) causes impaired insulin secretion to glucose, although the animals have a normal beta cell mass [89]. When fed a high-fat (HF) diet, GK^{+/-}

mice display decreased beta cell replication and insufficient beta cell hyperplasia despite having a normal degree of insulin resistance. Islets from HF diet-fed GK+/- mice display reduced expression of IRS2 compared with those of HF diet-fed wild-type mice. Overexpression of IRS2 in beta cells of HF diet-fed GK+/- mice partially prevented diabetes by increasing beta cell mass. These results suggest that GK is critical for beta cell hyperplasia in response to insulin resistance. More recently, the effect of various GK activators (Compound A, GKA50 and LY2121260) (GKA) that trigger GK *via* binding to an allosteric site of the enzyme have been tested for their capacity to induce beta cell proliferation *in vitro* and *in vivo* [90, 91]. GKA was able to increase both beta cell replication and beta cell number *in vitro* in INS-1 cells. Furthermore, 3-day administration of GKA in mice also enhances beta cell replication [91]. However, chronic GKA administration failed to cause a further increase in β -cell mass *in vivo*. The authors suggest that a chronic reduction in ambient blood glucose levels by GKA treatment could be responsible for this absence of chronic effects. However, the studies *in vitro* demonstrating an effect in INS-1 cells were performed at 3mM glucose. The authors did not test higher glucose concentrations to determine whether the effect could be amplified by a further increase in glucose phosphorylation. Finally, GK activators also induced a concomitant upregulation of IRS2 and activation of Akt/PKB phosphorylation.

In type 2 diabetes, the failure of beta cell adaptation coincides with reduced activities of pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDH), two enzymes required for pyruvate conversion to the Krebs cycle intermediates oxaloacetate and acetyl-CoA, respectively [92-95]. The PC inhibitor phenylacetic acid reduces beta cell proliferation in 60% pancreatectomized rats and in Zucker fatty rats, indicating the importance of this enzyme in the adaptive beta cell response to both insulin resistance and diminished beta cell mass *in vivo* [37, 96]. Downregulation of PC with siRNA decreases DNA synthesis in INS-1 cells [97]. On the other hand, overexpression of PC resulted in increased INS-1 cell replication. Taken together, these studies suggest that glucose metabolism by both glycolysis and the Krebs cycle is necessary for glucose-mediated beta cell proliferation, at least *in vitro*.

Glucose as a Facilitator of Growth Factor-Mediated Beta Cell Proliferation

Glucose has also been shown to increase beta cell proliferation indirectly through synergistic actions with growth factors. Glucose (range, 0-24 mM) enhances rodent beta cell replication induced by insulin-like growth factor-I (IGF-I) through a mechanism facilitated by ERK1/2 activation and dependent on IRS2/PI3K/p70S6 kinase intracellular pathway induction [11]. Similar synergistic effects on beta cell proliferation and immediate-early response genes coding for transcription factors involved in beta cell replication such as c-fos, JunB, nur-268 have been observed in the combination of glucose (range, 2.5-25 mM) and the gluco-incretin peptides glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide I (GLP-1) [98, 99]. A potential role for Ca²⁺ and IRS2/PI3K signaling pathways are suggested in these cases. Another important set of hormones that display synergistic effects

with glucose on beta cell proliferation are the lactogen hormones [100-102]. Growth hormone (GH) and prolactin (PRL) stimulate beta cell proliferation through activation of the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 5 (STAT 5). Glucose (15 mM) does not activate JAK2/STAT 5 and the synergistic effect of glucose and GH on beta cell proliferation does not appear to involve crosstalk among the signaling pathways. On the other hand, hepatocyte growth factor (HGF) and parathyroid-hormone related protein (PTHrP), two well known beta cell mitogens, do not display synergistic or additive effects with glucose (range, 0-24 mM) suggesting potential similar intracellular targets for inducing their mitogenic effects [10, 22].

Effect of FFA in Glucose-Mediated Beta Cell Proliferation

In conditions of insulin resistance, such as obesity, an increased demand for insulin is compensated by an increase in insulin production and an enhancement in beta cell mass. However, when beta cell mass does not increase as a compensation for insulin resistance, obesity-linked type 2 diabetes develops. In addition to genetic susceptibilities implicated in the pathogenesis of type 2 diabetes, the mechanisms involved in beta cell failure are unknown. It is postulated that prolonged hyperglycemia and hyperlipidemia are contributing factors detrimental to beta cell mass and function [103, 104]. *In vitro* studies by the Rhodes group have clearly established that long-chain FFA inhibit glucose-mediated (3 mM vs 15 mM) and glucose-dependent IGF-1 induced beta cell DNA synthesis [105, 106]. This is in part due to interference in IRS2-mediated signaling pathway and activation of novel protein kinase C (PKC) such as PKC δ . Mice expressing a kinase-dead PKC δ isoform in beta cells are protected from HF diet-induced beta cell failure and display enhanced islet size in this setting [107]. Taken together these results suggest that FFA might limit beta cell replication and expansion *in vivo*, and increase peripheral insulin resistance that would contribute to beta cell compensatory failure and type 2 diabetes.

CONCLUSIONS

Physiologically speaking, glucose is a logical regulator of beta cell replication. Since a primary function of the beta cell is to lower blood glucose levels through insulin secretion, elevated blood glucose is an indicator of insufficient insulin and need for more insulin secretory capacity. The beta cell is also exquisitely sensitive to glucose concentrations, and is thus equipped to respond to small changes in glucose. To summarize decades of work in a few sentences, beta cells across widely varying species, from toad to rodent, gerbil to human, replicate in response to elevated glucose *in vitro* and *in vivo*. *In vivo*, the replication response to glucose is observable starting after several days' exposure to high glucose, and lasting for several weeks. Prolonged hyperglycemia results in toxicity and lower beta cell replication rate; however, restoration of normoglycemia for a period of time allows recovery of glucose stimulated replication upon re-challenge. Data *in vivo* in human beta cells is sparse. Mechanistically, insulin signaling pathway members such as PI3K and downstream molecules seem critically important; a direct role for secreted insulin is still debated. Glucose metabolism in the beta cell may also play a

role, and glucose signaling also interacts with other growth factor signaling pathways in the beta cell. In summary, although much has been learned, this remains a rich territory for understanding the biology of diabetes as well as finding new therapeutic approaches to achieve beta cell regeneration.

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