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## Review

## Islet Biology During COVID-19: Progress and Perspectives

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## Key Messages

- Key new targets in beta cell function.
- Novel technologies in islet research.
- Perspectives of people living with diabetes on islet research during COVID-19.

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## ABSTRACT

The coronavirus-2019 (COVID-19) pandemic has had significant impact on research directions and productivity in the past 2 years. Despite these challenges, since 2020, more than 2,500 peer-reviewed articles have been published on pancreatic islet biology. These include updates on the roles of isocitrate dehydrogenase, pyruvate kinase and incretin hormones in insulin secretion, as well as the discovery of inceptor and signalling by circulating RNAs. The year 2020 also brought advancements in *in vivo* and *in vitro* models, including a new transgenic mouse for assessing beta-cell proliferation, a “pancreas-on-a-chip” to study glucose-stimulated insulin secretion and successful genetic editing of primary human islet cells. Islet biologists evaluated the functionality of stem-cell-derived islet-like cells coated with semipermeable biomaterials to prevent autoimmune attack, revealing the importance of cell maturation after transplantation. Prompted by observations that COVID-19 symptoms can worsen for people with obesity or diabetes, researchers examined how islets are directly affected by severe acute respiratory syndrome coronavirus 2. Herein, we highlight novel functional insights, technologies and therapeutic approaches that emerged between March 2020 and July 2021, written for both scientific and lay audiences. We also include a response to these advancements from patient stakeholders, to help lend a broader perspective to developments and challenges in islet research.

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## R É S U M É

La pandémie de la maladie à coronavirus 2019 (COVID-19) a eu des répercussions importantes sur les orientations et la productivité de la recherche au cours de la dernière année. En dépit de ces enjeux, depuis 2020, plus de 2500 articles revus par les pairs sur la biologie des îlots pancréatiques ont été publiés, y compris des articles qui portent sur les rôles de l'isocitrate-déshydrogénase, de la pyruvate kinase et des incrétines dans la sécrétion de l'insuline, ainsi que sur la découverte de l'« inceptor » et de la signalisation par les ARN circulants. L'année 2020 a aussi donné lieu à des avancées des modèles *in vivo* et *in vitro*, notamment la nouvelle souris transgénique pour permettre l'évaluation de la prolifération de cellules bêta, le « pancréas sur puce » pour étudier la sécrétion d'insuline stimulée par le glucose et la réussite de l'édition génomique des principales cellules humaines d'îlots pancréatiques. Les biologistes spécialisés dans le domaine des îlots pancréatiques ont évalué la fonctionnalité des cellules de type îlots pancréatiques issues de cellules souches recouvertes de biomatériaux semi-perméables pour prévenir les attaques auto-immunes, révélatrices de l'importance de la maturation cellulaire après la transplantation. Du fait que l'aggravation des symptômes de la COVID-19 est observée chez les personnes obèses ou diabétiques, les chercheurs ont examiné la façon avec laquelle les îlots sont directement affectés par le coronavirus 2 du syndrome respiratoire aigu. Dans le présent article, nous présentons les nouvelles perspectives fonctionnelles, les technologies et les approches thérapeutiques qui sont apparues entre mars 2020 et juillet 2021, et destinées aux scientifiques et aux non-scientifiques. Nous proposons aussi une réponse à ces avancées issues des groupes de patients pour permettre de donner une perspective plus large aux développements et aux enjeux de la recherche sur les îlots pancréatiques.

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## Introduction

The year 2021 marked the 100th anniversary of the discovery of insulin and, to this day, we continue to gain new insight into the synthesis, secretion and mechanism of action of this essential hormone. Although the regulation of insulin secretion from beta cells in response to nutrient intake has been studied for decades, new molecular clues that contribute to pathologic pancreatic islet dysfunction underlying type 1 diabetes (T1D) and type 2 diabetes (T2D) are continually being identified. In this review, we discuss recent advances addressing significant gaps in knowledge surrounding islet biology. In our opinion, the studies highlighted in this review represent research that shifts our fundamental understanding of islet biology or represents significant technological advances in the islet field, which would have relevance to Patient Partners with lived experience of diabetes.

We first focus on the central player in insulin homeostasis, the beta ( $\beta$ ) cell, identifying recent developments for mitochondrial control of insulin exocytosis, and addressing a long-standing paradox involving how  $\beta$  cells mitigate the effects of their own insulin secretion. We also explore new research showing how alpha cells may be both a target of incretin hormone signalling and, controversially, a source of incretin release. Next, we elaborate on how extracellular vesicles from adipocytes and noncoding RNAs can influence  $\beta$  cells and insulin secretion. We also highlight recent *in vitro* and *in vivo* technological advancements, including a new animal model to quantify  $\beta$ -cell proliferation, applications of CRISPR-Cas9 gene editing and innovations to create a “pancreas-on-a-chip” (PoC). We discuss the current state of  $\beta$ -cell transplantation as a treatment for diabetes, integrating new research on stem-cell generation and protective encapsulation of  $\beta$  cells. Last, we discuss recent findings on the effects of COVID-19 on patients living with diabetes and potential links between the infection and cases of new-onset diabetes. To ensure that these new research findings reached both the science community and important stakeholders, we include a lay description of our review and a summary of patient perspectives on this research from people living with diabetes.

## Novel Insights Into the Mechanism of Insulin Secretion

Paul Langerhans's discovery of pancreatic islets in 1869 was pivotal in diabetes research history, initiating over 150 years of work in islet biology, insulin production and glucose homeostasis (1). However, the details of insulin exocytosis are still being unraveled. Zhang et al provided new insight into the activation of insulin granule exocytosis, via ancillary signals produced by the “counterclockwise” flux of signalling molecules to and from the tricarboxylic acid (TCA) cycle (2). These key signals are generated from citrate/isocitrate metabolism by isoforms of isocitrate dehydrogenase in mitochondria (IDH2) and the cytosol (IDH1). Metabolite flux via IDH1 and IDH2 generates cytosolic signals that trigger insulin granule exocytosis, which small-molecule antagonists of IDH2 can block. The authors also show that IDH1-mediated insulin secretion is enhanced by the presence of glutamate, an amino acid that leads to citrate generation via reversal of the TCA cycle. Although such counterclockwise metabolite flux in the TCA cycle has been implicated in cancer cell growth (3,4) and in  $\beta$ -cell function (5–7), here the authors provide additional evidence for this important mechanism in regulating insulin secretion and islet function.

Glucose sensing in  $\beta$  cells describes how changing ambient glucose levels during the transition between fasting and fed states leads to glucose oxidation and coupling to regulate insulin exocytosis (1,2,8). However, like Zhang and colleagues, Lewandowski et al recently challenged this classic model, showing coordinated roles for electrophysiologic oscillations and oxidative phosphorylation in  $\beta$ -cell glucose-stimulated insulin secretion (GSIS) (8). In this revised model, pyruvate kinase (PK) directs metabolites between the phosphoenolpyruvate cycle and oxidative phosphorylation to modulate adenosine diphosphate (ADP) production. Therefore, PK mediates insulin secretion by reducing ADP availability in mitochondria, altering cytosolic adenosine triphosphate (ATP)/ADP ratios and causing the closure of ATP-sensitive  $K^+$  channels (8). They also showed that pharmacologic activation of PK depolarizes the cell membrane, leading to enhanced insulin secretion independent of glucokinase activity (i.e. glucose sensing), in contrast to current canonical models. In a companion study, the authors used

mouse models to show that PK activators can amplify insulin secretion, regulate gluconeogenesis and improve insulin sensitivity *in vivo*, further illustrating the importance of this pathway (9).

A defining feature of insulin action is glucose uptake in peripheral tissues such as muscle and fat. One of the hallmarks of prediabetes is insulin resistance (10), a phenomenon whereby insulin-sensitive tissues downregulate or inactivate components of the insulin signalling pathway in the presence of consistently elevated insulin levels (10). Insulin also induces the growth and proliferation of target cells, including  $\beta$  cells (11). Thus, a long-standing mystery in islet biology is what prevents  $\beta$  cells from autocrine/paracrine-induced insulin resistance and hyperplasia in a healthy individual (11–13). Key insight into this mystery came from the exciting discovery of a new protein expressed in  $\beta$  cells, termed inceptor, for insulin-inhibitory receptor/endosome-lysosome-associated apoptosis and autophagy regulator 1 (14). Using  $\beta$ -cell lines and mice, Ansarullah et al showed that inceptor interacts with the receptors for insulin and insulin growth factor 1 to promote their internalization via clathrin-mediated endocytosis, protecting  $\beta$  cells from autocrine/paracrine stimulation by their own insulin (14). Knockout of the inceptor gene in mice enhances  $\beta$ -cell proliferation and mass—a critical finding that could help to increase  $\beta$ -cell numbers for diabetes therapy (15). In addition, if inceptor is found in other insulin-sensitive tissues, blocking it could facilitate a reversal of insulin resistance.

### Islet Communication: Incretin Signalling From Within

A hallmark of T2D is dysfunction of both alpha ( $\alpha$ ) and  $\beta$  cells (16,17). Although the  $\alpha$  cell is often the primary focus in studies of islet dysfunction, people living with type 2 diabetes also exhibit inappropriate glucagon release from  $\beta$  cells. Although mechanisms of  $\alpha$ -cell dysfunction in diabetes remain unclear, numerous studies suggest that complex crosstalk between  $\alpha$  and  $\beta$  cells influences this phenomenon.

It is well established that incretin hormones released by intestinal cells during feeding amplify  $\beta$ -cell insulin secretion in a stimulatory endocrine process known as the “incretin effect” (18,19). Activation of incretin receptors on  $\beta$  cells triggers insulin release only in high glucose, which has made incretin mimetics invaluable therapeutic agents (20–23). However, signalling from intestinal cells is not the only pathway that can stimulate insulin release from  $\beta$  cells. Recent work from several groups has revealed a pivotal paracrine role for  $\alpha$  cells in stimulating  $\beta$ -cell insulin secretion during postprandial hyperglycemia (24–26). Until now, a link between the intestinal incretin effect and  $\alpha$ -cell paracrine effects had not been discerned, suggesting that both pathways stimulate  $\beta$ -cell insulin release independently of each other. However, findings by El et al show that part of the incretin effect stems from the action of gut hormone glucose-dependent insulinotropic polypeptide (GIP) directly on  $\alpha$  cells, causing glucagon release to enhance insulin secretion from the  $\beta$  cell (27). Without this  $\alpha$ -to- $\beta$ -cell communication, less insulin was secreted by  $\beta$  cells in response to a mixed meal tolerance test in mice (27). These recent findings demonstrate previously unappreciated pathways that work together to enhance  $\beta$ -cell insulin release.

Another intriguing, yet controversial, mediator of intra-islet communication involves the production of the incretin hormone glucagon-like peptide 1 (GLP-1) from  $\alpha$  cells. Generally believed to be synthesized and secreted by intestinal L cells during feeding, recent studies have provided new insight into the idea that GLP-1 may also be synthesized locally within the islet (28–30). Although most previous studies used either  $\alpha$ -cell lines or rodent islets, Campbell et al identified an  $\alpha$ -cell subpopulation that secretes GLP-1 in islets from human donors (31). They found that approximately 40% of  $\alpha$  cells of healthy donors

express GLP-1, and this proportion increases to 60% in T2D. Treatment with a GLP-1 receptor (GLP-1R) antagonist significantly blunts the insulin response, and the authors postulated that this increase in local GLP-1 contributes to glucose-stimulated insulin secretion, especially in the early stages of  $\beta$ -cell exhaustion in T2D.

Although different stimuli are proposed to increase pancreatic GLP-1 production, mechanisms remain poorly defined. Saikia et al found that increased  $\beta$ -cell GLP-1 expression occurs after activation of its receptor on  $\beta$  cells (32). Specifically, pharmacologic activation of the GLP-1R on  $\beta$  cells stimulates  $\alpha$ -cell expression of prohormone convertase 1/3, which cleaves proglucagon to create GLP-1 in the  $\alpha$  cell. Interestingly, activation of GLP-1R also promotes the expression of additional  $\beta$  cell-like genes in  $\alpha$  cells, such as *INS*, *MAFA* and *IAPP*, pointing to a possible interconversion of these 2 islet cell types (26). Together, these new studies with incretin hormones suggest that  $\alpha$  cells do not simply respond to hypoglycemia but also contribute to an integrated islet-cell response to hyperglycemia, highlighting the therapeutic potential of targeting the  $\alpha$  cell and this beneficial crosstalk.

### Islet Communication: Small RNAs Enter the Chat

There is also increasing evidence supporting roles for noncoding RNAs in modulating paracrine communication between different islet cell types. Stoll et al reported that insulin secretion is regulated by a conserved intronic circular RNA derived from insulin (*INS*) gene transcripts, dubbed *ci-Ins2* in rodents and *ci-INS* in humans (33). Deficiency in this circular RNA alters the expression of multiple genes involved in calcium signalling and insulin exocytosis, including reducing calcium-channel subunit *Cacna1d*, 2 calcium sensors *Syt4*, *Syt7* and *Pclo*, as well as the insulin granule recruitment factor, *Unc13a*. In  $\alpha$  cells, *ci-Ins2* is only marginally detected; however, new data suggest that noncoding RNAs can travel between islets cells via extracellular vesicles EVs (34–36). Taken together, these discoveries reshape our fundamental understanding of the complexity of regulatory mechanisms for signalling events in islet function, dysfunction and diabetes.

Islets also receive numerous signals from peripheral tissues to coordinate insulin release. Gesmundo et al have raised the possibility of targeting extra-islet tissues to improve insulin secretion. They showed that EVs derived from healthy human adipocytes can have beneficial effects on  $\beta$ -cell function and survival (37). In contrast, EVs derived from inflamed adipocytes collected from obese individuals exacerbate insulin resistance and  $\beta$ -cell dysfunction. By comparing the contents of EVs from healthy vs inflamed adipocytes, the authors pinpointed microRNAs as key signalling molecules that impact several key inflammatory genes in rodent (*INS-1E*) and human (*EndoC- $\beta$ H3*)  $\beta$ -cell lines, such as tumor necrosis factor-alpha, interferon-gamma, interleukin-1beta, adiponectin and immune system complement factors. The authors highlighted an unappreciated role for regulatory crosstalk between adipocytes and  $\beta$  cells.

### Tools for Understanding Islet Biology

The period from March 2020 to July 2021 also brought advancements in the development of *in vivo* and *in vitro* models to study the islet. The recently developed RIP-Cre; R26Fucci2aR mouse model promises to improve our ability to quantify  $\beta$ -cell proliferation (38). Traditionally, measurement of proliferation relies on immunohistochemical methods, which lack specificity for  $\beta$  cells and are subject to high variability between studies (39). The R26Fucci2aR mouse addresses these caveats by expressing a fluorescent Fucci2a reporter in  $\beta$  cells driven by the rat insulin promoter

(RIP), specific to  $\beta$  cells. When activated, the fluorophore emits red light in the G1 phase and green light during the S/G<sub>2</sub>/M phases of the cell cycle, permitting clear definition and isolation of  $\beta$  cells undergoing active proliferation. This new tool allows for more reliable and specific quantification and characterization of replicating  $\beta$  cells, which is very useful when testing novel therapeutics claiming to expand  $\beta$ -cell mass (38).

The PoC was developed due to the need for an in vitro model of islet function recapitulating the in vivo microenvironment of human islets (40). One recent model described by Zbinden et al involves entrapping islet-like structures, or “pseudo-islets,” composed of aggregated immortalized human EndoC- $\beta$ H3  $\beta$  cells, into individual compartments of a microfluidic chip (40). Unique to this PoC model is the incorporation of Raman microspectroscopy, a chemical analysis technique that assists in real-time monitoring of insulin release (40). Experimental modelling has demonstrated that PoC pseudo-islets are highly glucose responsive and exhibit the biphasic glucose-stimulated insulin secretory responses characteristic of primary human islets (40). Thus, the PoC model provides a new tool to study insulin secretion kinetics in a multitude of cell models, including primary human islets and nonimmortalized human  $\beta$ -cell cultures, including stem cell (SC)-derived  $\beta$  cells (40).

Representing a major advancement, the genome-editing technology CRISPR-Cas9 was used to generate the first genetically modified primary human islets (41). As a proof of concept, Bevacqua et al deleted pancreatic and duodenal homeobox 1 (*PDX1*), a transcription factor important for maintaining  $\beta$ -cell identity and function, impairing calcium-channel activity, reducing total insulin content and glucose-stimulated insulin secretion (41). CRISPR-Cas9 constructs delivered by lentivirus were also used to delete the *KCNJ11* gene encoding  $K_{ATP}$  channel subunit *KIR6.2*, or to introduce noncoding genetic variation at the *ABCC8-KCNJ11* locus, both of which caused impaired  $K_{ATP}$  channel activity in human islets (41,42). Successful editing of the human islet genome via CRISPR-Cas9 provides proof of principle for generating a wide variety of human primary islet knock-in and knock-out models, which will help bridge the gap between in vitro models and human islet biology.

### Developments in Cell-based Therapies

Because we cannot yet correct islet defects in vivo, pancreatic islet transplantation is an effective therapy to restore physiologic glycemic control. However, several barriers still exist, such as a shortage of organ donors and the requirement for lifelong immunosuppression. Alternative islet sources, such as embryonic or induced pluripotent SC-derived islet-like cells (ESCs and iPSCs), are at the forefront, whereas generating  $\beta$ -like cells from these SCs that respond appropriately to glucose has been difficult (43,44). Millman et al recently addressed this challenge by investigating key maturation changes that occur throughout differentiation of iPSC or ESC stage 6 SC islet cells before and after transplantation (45). Single-cell RNA sequencing determined that SC-derived  $\beta$  cells 6 months after transplantation more closely resemble primary human  $\beta$  cells than before transplant, and genes associated with  $\beta$ -cell maturation, such as *INS*, *IAPP*, *MAFA*, *SIX2*, *CHGB*, *MX1*, *SHI-SAL2B* and *G6PC2*, were increased. One could imagine that the newly integrated approaches combining transcriptomic, metabolic and functional analyses, like those described by Lien et al and Balboa et al, could help to provide an abundance of information about the biology and function of these SC-derived islet-cell populations, bringing us even closer to islet cells that more closely mimic primary islets (46,47).

Even as safe and effective sources of  $\beta$  cells become available, cell replacement therapy still requires immunosuppression (48). Biocompatible encapsulation devices, which allow efficient blood

glucose regulation while protecting the islet mass from immune attack, are a major focus in islet transplantation (49). Pancreatic islet encapsulation involves loading donor islets into a protective device or matrix that allows the exchange of glucose, insulin, oxygen and other metabolic products while providing a physical barrier against immune-cell infiltration (50). In their recent study, Stock et al used cryorecovered stage 6 SC-derived islet cells encapsulated with a 5% poly(ethylene glycol)-maleimide hydrogel to show that the function of coated SC-derived islet cells is similar to that of noncoated controls in vitro (51). When transplanted into the fat pads of immunocompromised mice, coated and noncoated SCs were equally effective in re-establishing glycemic control. Investigating this novel coating on islets in immunocompetent humanized mice and evaluating the safety of coated islet cells in larger species are important next steps before clinical trials.

### COVID-19: Does Severe Acute Respiratory Syndrome Coronavirus 2 Impact the Islet?

In the early days of the current pandemic, media reports claimed that COVID-19, the disease resulting from infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), may be more severe in people who are living with diabetes, and may even cause new-onset diabetes (52,53). Indeed, people living with diabetes were eventually prioritized for early vaccination in Canada (54). Excellent reviews on the impact of SARS-CoV-2 on clinical outcomes in diabetes are available elsewhere (55,56). However, the pathophysiologic mechanisms underlying this observation remain unknown (57). Once it became clear that people living with diabetes showed substandard outcomes during COVID-19, there was great interest in exploring possible underlying mechanistic connections. For example, it was unknown whether the SARS-CoV-2 virus could access or infect the pancreas and/or islets. Herein we review the literature on SARS-CoV-2 and the islet.

Literature on COVID-19 and the islet appeared slowly, starting in early 2020, but, due to the omnipresent nature of the COVID-19 pandemic, accelerated significantly in 2021. SARS-CoV-2 infects host cells via docking to the cell surface protein angiotensin-converting enzyme 2 (ACE2), expressed in several human organs, including lungs, stomach, small intestine, liver and spleen (58). Multiple labs examined ACE2 expression in pancreatic islets to determine how SARS-CoV-2 may affect pancreatic islet function and potentially impact diabetes. Fignani et al discovered that only the short ACE2 isoform is expressed in human pancreatic  $\beta$  cells, whereas both short and long isoforms are found in pancreatic microvasculature (59). Induction of pro-inflammatory cytokines from macrophages and natural killer cells (e.g. interleukin-1 $\beta$ , interferon-gamma) increases ACE2 receptor expression in both the microvasculature and  $\beta$  cells, likely due to the anti-inflammatory role of ACE2. Consequently, increased ACE2 receptor expression in the pancreas raises the possibility that SARS-CoV-2 could also infect islet cells, with anticipated detrimental effects on islet function.

Along the same lines, Taneera et al demonstrated that ACE2 expression was upregulated in islets from donors with T2D compared with nondiabetic islets, suggesting that individuals with T2D may be more susceptible to pancreatic consequences of SARS-CoV-2 infection (60). Researchers also reported that islets from female donors (with or without diabetes) have higher ACE2 receptor levels than male donors. Androgenic hormone signalling may cause the heterogeneity of ACE2 expression between sexes. In contrast, Coate et al used single-cell RNA sequencing and found expression of ACE2 in the microvasculature and duct cells of the human pancreas, with <1.5% of  $\beta$  cells expressing ACE2 or transmembrane serine protease 2 (TMPRSS2), another SARS-CoV-2 entry factor (61).

The first report that SARS-CoV-2 can infect and replicate in primary human pancreatic islet cells was done by Müller et al. Infected islets have impaired insulin secretion and fewer insulin granules, but no increase in apoptosis (62). These factors may contribute to the acute metabolic dysfunction observed in patients with COVID-19, as hyperglycemia is seen in individuals with and without diabetes (53). Müller et al also detected the SARS-CoV-2 nucleocapsid protein (N-protein) in endocrine and exocrine pancreatic cells of 4 deceased patients with COVID-19, showing that the virus can infect the pancreas. Although the authors reported that pancreatic cells co-stain for the  $\beta$ -cell marker NKX6.1 and the SARS-CoV-2 N-protein, very few nucleocapsid-positive cells stained positive for insulin. Müller et al also suggested that infected cells may lose their hormone content via de-differentiation, but this hypothesis requires further exploration. Yang et al were able to infect human SC-derived  $\beta$  cells with the SARS-CoV-2 virus, and Wu et al found that primary human islets infected with SARS-CoV-2 have reduced glucose-stimulated insulin secretion and insulin granule content (63,64). They also reported SARS-CoV-2 N-protein in the  $\beta$  cells of deceased patients with COVID-19 concurrent with increased islet-cell apoptosis that may be due to the viral spike protein (64).

Together, these studies provide evidence that  $\beta$ -cell infection may be involved in COVID-19 pathogenesis or, alternatively, that pancreatic infection may impact  $\beta$  cells by changing their local microenvironment. This evidence raises the possibility that SARS-CoV-2 infection may contribute to pancreatic endocrine and/or exocrine dysfunction. Although those studies have all provided evidence supporting a possible mechanistic link between COVID-19 and new-onset diabetes, it is important to note that recent data suggest that the incidence of T1D has, so far, not increased after SARS-CoV-2 infection (65). It is important to note that viral entry is not limited to mature pancreatic  $\beta$  cells, but also occurs in iPSC-derived  $\beta$ -like cells. Examining how SARS-CoV-2 affects acute and chronic islet cell function will be of future interest, particularly the pathophysiology underlying new-onset cases of diabetes after SARS-CoV-2 infection.

## Conclusions

Despite the adversity faced over the past 2 years due to the COVID-19 pandemic, our understanding of islet biology has advanced, with the design of new model systems and fundamental discoveries regarding the control of insulin secretion, islet dysfunction and the impact of SARS-CoV-2 infection (Figure 1). Technological advances in protection of SC-derived islets are reflective of a renewed interest in using cell therapy to achieve better control of glucose levels. With the pandemic continuing into 2022, mechanistic questions continue to arise underlying the vulnerability of people living with diabetes to worse COVID-19 outcomes. The direct impacts of viral infection on islets and the causal association between infection and new diabetes onset remain incompletely defined. Although the pandemic has temporarily slowed research progress, the advances discussed herein provide new insights and tools to kick-start islet and diabetes research as labs work toward again reaching full operating capacity.

## Lay Review

Paul Langerhans's discovery of the pancreatic islets in 1869 helped initiate over 150 years of research on insulin and blood sugar (glucose) control (1). Islets are clusters of hormone-secreting cells within the pancreas responsible for releasing insulin (from  $\beta$  cells, which lowers blood glucose) and glucagon (from  $\alpha$  cells,

which increases blood glucose) to maintain blood glucose levels at a healthy level. Our understanding of insulin secretion and how it is controlled is constantly evolving. The period from March 2020 to July 2021 gave us many advances in islet biology, including novel molecular mechanisms, tools and cell proteins that could target new drugs. Although the pandemic has dramatically slowed research, studies on COVID-19 unexpectedly expanded our understanding of how the islet may be impacted by the virus that causes COVID-19, while also revealing new challenges that must be faced by those living with diabetes.

## New Mechanisms That Control Insulin Secretion

It is generally appreciated that glucose is the signal to  $\beta$  cells to secrete insulin (1,2,66). In back-to-back articles, researchers revealed that pyruvate kinase, the enzyme that catalyzes the final step in glucose breakdown, is critical for controlling insulin release at the cell surface (9,66). The researchers went on to show that drugs targeting pyruvate kinase can enhance insulin secretion (even in the absence of glucose) and increase the effectiveness of insulin in mice. Along the same lines, Zhang et al recently showed that other signals from mitochondria, parts of a cell that use pyruvate to make energy, also help  $\beta$  cells secrete insulin (2). They demonstrated that these signals, sent back and forth between mitochondria, help to amplify insulin release.

In this past year, an emerging theme is that insulin secretion from  $\beta$  cells can be controlled by other islet cell types. El et al revealed that, while eating a meal, an intestinal hormone called glucose-dependent insulinotropic polypeptide, or GIP, is secreted from the intestine and causes  $\beta$  cells to release glucagon (27). Glucagon is usually secreted when blood sugar is low, but it was revealed that, in this context, glucagon promotes insulin secretion from neighbouring  $\alpha$  cells. Although the role of  $\alpha$  cells in regulating insulin secretion has received much attention this past year, El et al are the first to demonstrate involvement of GIP in this  $\alpha$ - and  $\beta$ -cell relationship (24–26). Their study has also highlighted the importance of mixed meals—those consisting of protein, carbohydrate and fat, as opposed to carbohydrates alone—in insulin control, emphasizing that the focus should not be on sugar intake alone.

Another hallmark of diabetes is “insulin resistance,” where cells stop responding to insulin partially because of overstimulation. This is common in tissues such as muscle and fat and leads to poor glucose absorption. A long-standing puzzle is why  $\beta$  cells do not develop insulin resistance, despite being bathed in the large amounts of insulin they secrete (11,12). An important work by Ansarullah et al identified a new protein the authors named insulin inhibitory receptor, or “inceptor” (14), that hides the insulin receptor inside  $\beta$  cells, preventing insulin overstimulation and the generation of new  $\beta$  cells. Not only does this finding solve a mystery, it also suggests that interfering with inceptor function could increase insulin levels and allow more  $\beta$  cells to form, which could be an added advantage for regenerative and stem cell (SC)–based therapies.

## Disrupted Communication Impacts Islet Function

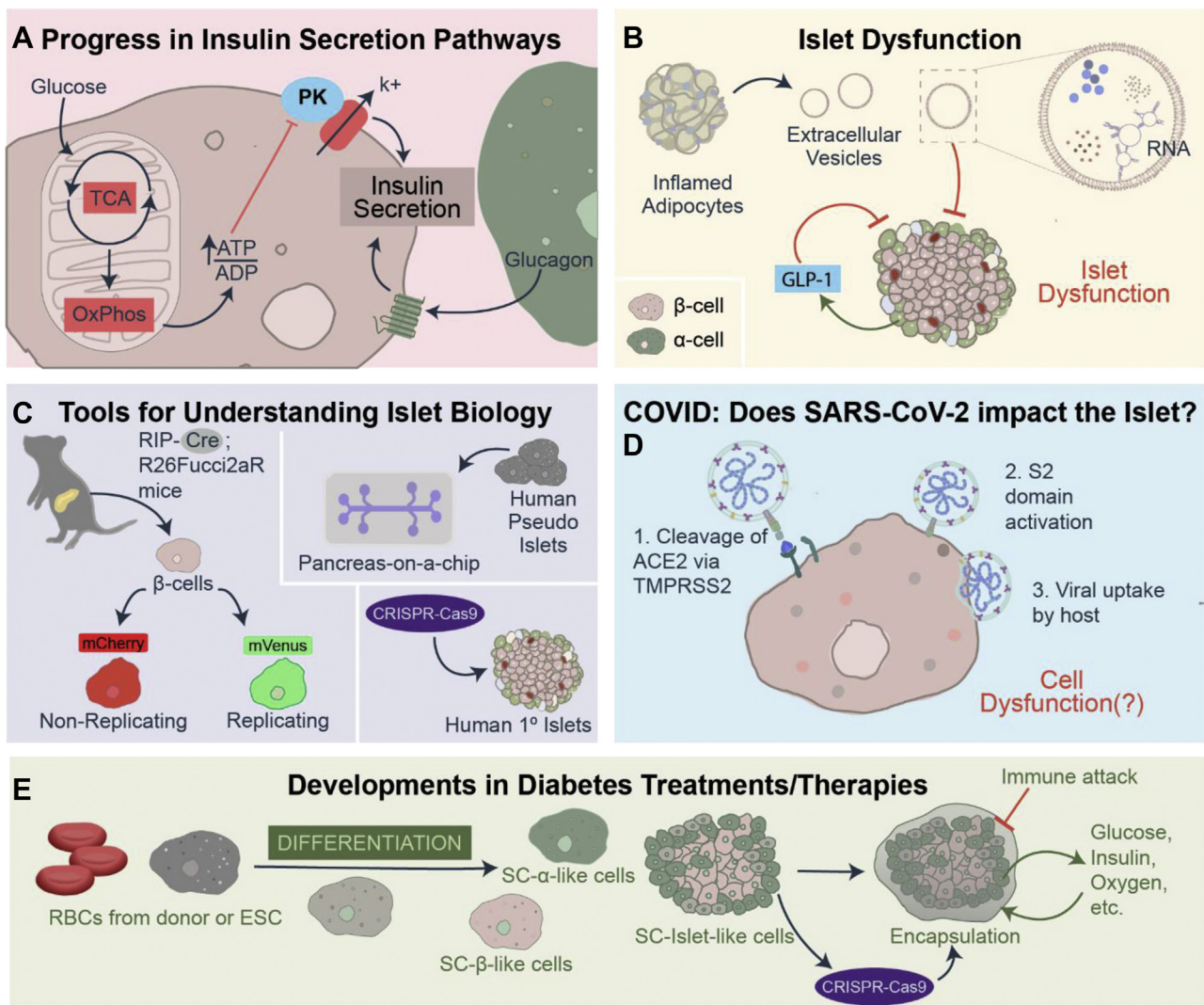
A growing body of evidence suggests that impaired islet function can result from signals originating within the pancreas as well as from other organs (16,17,36,67). Gesmundo et al discovered that signals involving small pieces of ribonucleic acid, known as microRNA, are sent between the fat cells and  $\beta$  cells (37). Interestingly, when fat cells come from lean patients, the signals can promote insulin secretion and increase  $\beta$ -cell health, whereas fat

cells from obese individuals send signals that can impair  $\beta$ -cell function and even cause cell death. Similarly, Stoll et al found a new signal from a microRNA called *ci-INS* that is essential for insulin to be properly secreted by  $\beta$  cells (33).

It was also shown this year that human  $\alpha$  cells not only secrete glucagon but also glucagon-like peptide 1 (GLP-1), a hormone that promotes insulin secretion and is thought to mainly come from the intestine. Intestinal GLP-1 lowers blood sugar levels by increasing insulin and lowering glucagon secretion. Campbell et al reported that  $\alpha$  cells in cadaveric and living human donors secrete GLP-1 and that there are more  $\alpha$  cells containing GLP-1 in donors with T2D (31). Recently, Saikia et al revealed that treatment with a drug that turns on the GLP-1 receptor stimulates  $\beta$  cells to produce more GLP-1 (32). Thus, a continuing theme of recent findings is that  $\alpha$  cells seem to work together with  $\beta$  cells to control high blood sugar levels.

## New Tools for Studying Islets

This past year has brought new and exciting research tools to improve our understanding of how pancreatic islets work in the laboratory. It has always been a challenge to detect when new  $\beta$  cells are being made. This year, a particular genetically modified mouse model was developed, in which  $\beta$  cells appear green when they are dividing to create a new  $\beta$  cell and red when not dividing. This allows researchers to better detect and count the  $\beta$  cells that are making new copies—a critical step toward testing novel therapies designed to increase the number of  $\beta$  cells (38). Another promising tool, “pancreas-on-a-chip,” can trap and grow human pancreatic cells on a small device, or “chip,” while also mimicking the natural environment of islets in the body (40). This small chip has a set of microchannels etched or molded into it, which are connected to allow blood flow. Islet cells can then detect changes



**Figure 1.** (A) A pool of pyruvate kinase at the plasma membrane regulates membrane depolarization and insulin secretion. GLP-1 from  $\alpha$  cells (right) governs insulin output by neighbouring  $\beta$ -cells. (B) Complex crosstalk between  $\beta$  cells,  $\alpha$  cells and adipocytes. EVs released from inflamed adipocytes contain small RNA molecules that inhibit insulin exocytosis. Levels of GLP-1 produced locally within the islet increase in type 2 diabetes and contribute to increased insulin secretion. (C) The R26Fucci2aR mouse model allows quantification of  $\beta$ -cell proliferation by marking  $\beta$ -cell pools with green (replicating) or red (nonreplicating) fluorophores. The “pancreas-on-a-chip” model allows for assessment of human islet function in vitro. CRISPR-Cas9 technology opens new avenues for genetic modification of islets that could help restore normal function. (D) A model for SARS-CoV-2 infection of a  $\beta$  cell in the islet. The spike protein of the SARS-CoV-2 virus, primed by TMPRSS2, docks to the cell-surface protein ACE2. (E) A macroencapsulation device for islet-cell transplants protects islet cells from immune-cell infiltration. Alternative sources of  $\beta$  cells, differentiated to closely resemble mature  $\beta$  cells at a genetic level, can be genetically modified using CRISPR-Cas9 technology. ACE2, angiotensin-converting enzyme 2; ADP, adenosine triphosphate; ATP, adenosine triphosphate; ESC, embryonic stem cell; EV, extracellular vesicle; GLP-1, glucagon-like peptide 1; RBC, red blood cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SC, stem cell; TCA, tricarboxylic acid.

in nutrients and release the necessary hormones to maintain normal blood sugar levels. The pancreas-on-a-chip technology is not new, and has been modified this year to incorporate a technique called Raman imaging, which can measure insulin release on the chip in real time (40). This development allows the study of how these islets function in an environment that mimics the body.

For the first time, researchers have used a Nobel Prize–winning technique called CRISPR-Cas9–mediated gene editing (see CRISPR-Cas9 explained for an easy-to-follow primer on how this technology works) to modify genes in pancreatic islets from human donors (41). This allows islet biologists to quickly modify specific genes in human islet cells to understand their importance. This technology offers the potential to allow scientists to improve human donor-derived pancreatic islets, such as by repairing harmful mutations, enhancing function or improving survival (41). Although this represents a major advancement, we are again reminded of the continual need to revisit and discuss ethical and socioeconomic concerns that underlie gene therapy.

### Development in Diabetes Treatments/Therapies

Pancreatic islet transplantation can restore normal blood sugar regulation in patients with T1D (68). However, barriers to using this therapy include a global shortage of organ donors and the need for immunosuppressants to prevent transplant rejection. To address the organ shortage, scientists look to other islet sources, such as pig islets and human stem cells.

Generating islet cells from embryonic stem cells has been possible since the early 2000s (69,70). Induced pluripotent stem-cell (iPSC) technology, which involves generating SCs from mature cells taken directly from the patient involved, has further opened the door to using one's own cells to make islets, avoiding problems of rejection. However, generating new cells that function like normal  $\beta$  cells has been challenging (43,44). This past year, the Millman group identified many new genes that are associated with maturation of SC-derived  $\beta$  cells, and Lien et al and Balboa et al discovered several new aspects controlling how these cells behave in their environment, advancing our understanding of how these cells work and how best to generate them (45–47).

As effective and safe sources of replacement  $\beta$  cells become available, we will still need to address transplant rejection. Immunosuppressive drugs help prevent the body from attacking foreign islet transplants but come with significant negative side effects. Recently, scientists investigated how to “coat” transplanted islet cells to prevent immune attack, while still allowing the islet to sense sugar and release insulin (50,71). One recent study showed that precoating transplanted  $\beta$  cells with a new protective gel did not prevent reversal of diabetes in mice, a critical first step in addressing transplant rejection (51). Researchers must now determine whether this coating also guards  $\beta$  cells against immune attack—the next step in advancing this technology from bench to bedside.

### COVID-19 and Diabetes

SARS-CoV-2 has had a global impact on all aspects of society. It quickly became clear that living with diabetes made an individual more likely to have severe COVID-19 symptoms and experience worse blood glucose control (72). In addition, SARS-CoV-2 infection has been associated with new-onset diabetes, yet it remains unclear whether this results directly from infection (54).

SARS-CoV-2 infects a host cell by attaching to the cell surface protein called angiotensin-converting enzyme 2, or ACE2. This protein is found in cells of the lung, stomach, small intestine, liver and spleen. Over the past year, multiple studies revealed that the virus

can enter cells within the pancreas, likely using the ACE2 protein (59,60,62–64). Fignani et al also found that inflammation caused by the coronavirus increases the amount of ACE2 in the pancreas, thereby leading to more detrimental consequences for those infected (59). Müller et al and Wu et al provided evidence that coronavirus can replicate itself in pancreatic cells and that the virus decreases the capacity of the cells to secrete insulin (62,64). The authors also detected the virus in pancreatic islets of patients who died from COVID-19. Finally, Taneera et al found that people living with T2D are more likely to have increased levels of ACE2, raising the possibility that more virus could enter their cells, which may underlie the more severe health consequences in this population (60).

The research has shed light on why those with diabetes are more susceptible to COVID-19. Broadly speaking, the past year has also revealed the role of ACE2 as a potential avenue for viruses to enter the pancreas, and we may see an emergence of work on the role of viral infection in the development and severity of diabetes.

### Patient Partner Response to Advancements in Islet Biology From March 2020 to July 2021

The following section features responses to the lay review from a diverse group of 7 Patient Partners living with type 1 or type 2 diabetes. The group response synthesizes commonly held perspectives of all, whereas individual comments reflect unique personal perspectives. This effort was coordinated through Diabetes Action Canada.

*What are you most excited about?*

*Group response:* Several areas in this review piqued our interest—any strides toward applicable changes for diabetes are really exciting. We feel enthusiastic about advancements in understanding how SC-islet cells function and how to best generate them. The possibility for immune attack after islet cell transplants is an area of concern for us, so utilizing encapsulation technologies that avoid the need for immunosuppressants after cell replacement therapy is a topic of keen interest. Targeted gene therapy and cell therapy using CRISPR-Cas9 are also very intriguing and exciting:

*The potential for iPSCs to create new  $\beta$  cells that the body would recognize as its own sounds like pretty close to the holy grail of this area of research. (Tom Weisz)*

*I'm excited by the amount of research on how COVID affects those with diabetes. (Matt Larson)*

*I'm excited to see research make some real-life changes. Firstly, the process and struggles the team may face and how patient partners could help will really engage me with better diabetes practices. (Kiano Vafaeian)*

*What are your worries or fears?*

*Group response:* Collectively, we are concerned about the costs related to diabetes and innovative treatments. The financial accessibility of diabetes care advancements, funding for innovative ideas and costs of SC islet-cell treatments were the areas that stuck out. COVID-19–related complications were also a concern for the group. We are worried about future complications for diabetics caused by COVID-19 and the potential for the virus to trigger the onset of other long-term chronic conditions (such as diabetes).

There was also concern around the responsible use and safety regarding some of these novel treatments, such as the idea of genetic modification. The need to ensure that safeguards are in place to avoid triggering uncontrolled cell proliferation with mitochondrial pathways for insulin secretion was also a fear:



*I am worried that these advancements may not be available to patients for a long time. I also worry that if these treatments do become available to people living with diabetes, they will be really commodified because it's a novel treatment. (Megan Patton)*  
*After living through a pandemic, I am now concerned that COVID-19 may trigger the onset of long-term chronic conditions such as diabetes. (Christina Marie Mulchandani)*

#### What New Discoveries/Advancements Would You Like to See in the Next 3 to 5 Years?

**Group response:** Coauthoring this review made evident how critical collaboration between researchers and people living with diabetes is. To ensure new research is meeting the diverse needs of those living with the condition, as a group, we are hopeful to see more patient engagement occurring within the field. We are hopeful for the continued development of novel treatments mentioned throughout this review. The affordability of diabetes management supplies is a large contributing factor to proper care. We are hopeful that current supplies and future care strategies will be made accessible to all socioeconomic groups and allow everyone to adequately care for their condition. Establishing a reliable process for SC islet cells to mimic human islet cells, deeper understanding around how GLP-1 is produced, further development in genetic modification to treat diabetes and treatments that target insulin resistance were areas that the group was particularly interested in seeing further advancements. We would like to see more data around the correlation between viral infiltration in pancreatic islets and COVID-19 severe complications and deaths. Understanding how COVID-19 can affect the cells in the pancreas is also an area we would like to see be discovered more. Finally, we are increasingly hopeful that the novel treatments outlined in this review will put us on the path towards a cure for diabetes:

*I hope that the collaboration integrated into this commentary will highlight the value of patient partnership and encourage other researchers to work alongside people with diabetes in the future. (Marley Greenberg)*

*I would love to see that this disease is eradicated for me and for the generations that come, that research in this field continues to be inclusive of the voices and concerns of socioculturally diverse and marginalized patients, and that cures and treatments are accessible and affordable for all. (Seeta Ramdass)*

#### Author Disclosures

Conflicts of interest: None.

#### Author Contributions

J.L.E., E.E.M. and R.A.S. conceptualized this study and participated in project administration. All authors participated in writing the manuscript.

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#### References

- Campbell JE, Newgard CB. Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nat Rev Mol Cell Biol* 2021;22:142–58.

- Zhang G-F, Jensen MV, Gray SM, et al. Reductive TCA cycle metabolism fuels glutamine- and glucose-stimulated insulin secretion. *Cell Metabol* 2021;33:804–817.e5.
- Metallo CM, Gameiro PA, Bell EL, et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* 2012;481:380–4.
- Jiang L, Shestov AA, Swain P, et al. Reductive carboxylation supports redox homeostasis during anchorage-independent growth. *Nature* 2016;532:255–8.
- Fu A, Robitaille K, Faubert B, et al. LKB1 couples glucose metabolism to insulin secretion in mice. *Diabetologia* 2015;58:1513–22.
- Swisa A, Granot Z, Tamarina N, et al. Loss of liver kinase B1 (LKB1) in beta cells enhances glucose-stimulated insulin secretion despite profound mitochondrial defects. *J Biol Chem* 2015;290:20934–46.
- Ferdaoussi M, MacDonald PE. Toward connecting metabolism to the exocytotic site. *Trends Cell Biol* 2017;27:163–71.
- Lewandowski SL, Cardone RL, Foster HR, et al. Pyruvate kinase controls signal strength in the insulin secretory pathway. *Cell Metab* 2020;32(5):736–50. <https://doi.org/10.1016/j.cmet.2020.10.007>.
- Abulizi A, Cardone RL, Stark R, et al. Multi-tissue acceleration of the mitochondrial phosphoenolpyruvate cycle improves whole-body metabolic health. *Cell Metab* 2020;32:751–766.e11.
- Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev* 2018;98:2133–223.
- Zick Y. Ser/Thr phosphorylation of IRS proteins: A molecular basis for insulin resistance. *Sci STKE* 2005;2005:pe4.
- Goldfine AB, Kulkarni RN. Modulation of  $\beta$ -cell function: A translational journey from the bench to the bedside. *Diabetes Obes Metab* 2012;14(Suppl. 3):152–60.
- Rhodes CJ, White MF, Leahy JL, Kahn SE. Direct autocrine action of insulin on beta-cells: Does it make physiological sense? *Diabetes* 2013;62:2157–63.
- Ansarullah, Jain C, Far FF, et al. Inceptor counteracts insulin signalling in  $\beta$ -cells to control glycaemia. *Nature* 2021;590:326–31.
- Oakie A, Nostro MC. Harnessing proliferation for the expansion of stem cell-derived pancreatic cells: Advantages and limitations. *Front Endocrinol* 2021;12:636182. <https://www.frontiersin.org/articles/10.3389/fendo.2021.636182/full>. Accessed February 11, 2022.
- Szabat M, Page MM, Panzhinskiy E, et al. Reduced insulin production relieves endoplasmic reticulum stress and induces  $\beta$  cell proliferation. *Cell Metab* 2016;23:179–93.
- Wang Z, York NW, Nichols CG, Remedi MS. Pancreatic  $\beta$  cell dedifferentiation in diabetes and redifferentiation following insulin therapy. *Cell Metab* 2014;19:872–82.
- Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab* 2013;17:819–37.
- Holst JJ. The incretin system in healthy humans: The role of GIP and GLP-1. *Metabolism* 2019;96:46–55.
- Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007;132:2131–57.
- Gilbert MP, Pratley RE. GLP-1 analogs and DPP-4 inhibitors in type 2 diabetes therapy: Review of head-to-head clinical trials. *Front Endocrinol (Lausanne)* 2020;11:178.
- Htike ZZ, Zaccardi F, Papamargaritis D, Webb DR, Khunti K, Davies MJ. Efficacy and safety of glucagon-like peptide-1 receptor agonists in type 2 diabetes: A systematic review and mixed-treatment comparison analysis. *Diabetes Obes Metab* 2017;19:524–36.
- Aroda VR. A review of GLP-1 receptor agonists: Evolution and advancement, through the lens of randomised controlled trials. *Diabetes Obes Metab* 2018;20(Suppl. 1):22–33.
- El K, Capozzi ME, Campbell JE. Repositioning the alpha cell in postprandial metabolism. *Endocrinology* 2020;(11):1–11.
- Rodriguez-Diaz R, Tamayo A, Hara M, Caicedo A. The local paracrine actions of the pancreatic  $\alpha$ -cell. *Diabetes* 2020;69:550–8.
- Moede T, Leibiger IB, Berggren P-O. Alpha cell regulation of beta cell function. *Diabetologia* 2020;63:2064–75.
- El K, Gray SM, Capozzi ME, et al. GIP mediates the incretin effect and glucose tolerance by dual actions on  $\alpha$  cells and  $\beta$  cells. *Sci Adv* 2021;7:eabf1948.
- Traub S, Meier DT, Schulze F, et al. Pancreatic alpha cell-derived glucagon-related peptides are required for beta cell adaptation and glucose homeostasis. *Cell Rep* 2017;18:3192–203.
- Lin HV, Wang J, Wang J, et al. GPR142 prompts glucagon-like peptide-1 release from islets to improve beta cell function. *Mol Metab* 2018;11:205–11.
- Muller TD, Finan B, Bloom SR, et al. Glucagon-like peptide 1 (GLP-1). *Mol Metab* 2019;30:72–130.
- Campbell SA, Golec DP, Hubert M, et al. Human islets contain a subpopulation of glucagon-like peptide-1 secreting  $\alpha$  cells that is increased in type 2 diabetes. *Mol Metabol* 2020;39:101014.
- Saikia M, Holter MM, Donahue LR, et al. GLP-1 receptor signaling increases PCSK1 and  $\beta$  cell features in human  $\alpha$  cells. *JCI Insight* 2021;6.
- Stoll L, Rodríguez-Trejo A, Guay C, et al. A circular RNA generated from an intron of the insulin gene controls insulin secretion. *Nat Commun* 2020;11:5611.
- Pomatto MAC, Gai C, Deregibus MC, Tetta C, Camussi G. Noncoding RNAs carried by extracellular vesicles in endocrine diseases. *Int J Endocrinol* 2018;2018:4302096. <https://www.hindawi.com/journals/ije/2018/4302096/>. Accessed February 11, 2022.
- Abramowicz A, Story MD. The long and short of it: The emerging roles of non-coding RNA in small extracellular vesicles. *Cancers* 2020;12:1445.

36. Chidester S, Livinski AA, Fish AF, Joseph PV. The role of extracellular vesicles in  $\beta$ -cell function and viability: A scoping review. *Front Endocrinol* 2020;11. <https://www.frontiersin.org/articles/10.3389/fendo.2020.00375/full>. Accessed February 11, 2022.
37. Gesmundo I, Pardini B, Gargantini E, et al. Adipocyte-derived extracellular vesicles regulate survival and function of pancreatic  $\beta$  cells. *JCI Insight* 2021;6.
38. Tokumoto S, Yabe D, Tatsuoka H, et al. Generation and characterization of a novel mouse model that allows spatiotemporal quantification of pancreatic  $\beta$ -cell proliferation. *Diabetes* 2020;69:2340–51.
39. Cox AR, Barrandon O, Cai EP, et al. Resolving discrepant findings on ANGPTL8 in  $\beta$ -cell proliferation: A collaborative approach to resolving the betatrophin controversy. *PLoS One* 2016;11:e0159276.
40. Zbinden A, Marzi J, Schlünder K, et al. Non-invasive marker-independent high content analysis of a microphysiological human pancreas-on-a-chip model. *Matrix Biol* 2020;85–86:205–20.
41. Bevacqua RJ, Dai X, Lam JY, et al. CRISPR-based genome editing in primary human pancreatic islet cells. *Nat Commun* 2021;12:2397.
42. Yuan F, Guo D, Gao G, et al. Generation of a KCNJ11 homozygous knockout human embryonic stem cell line WAe001-A-12 using CRISPR/Cas9. *Stem Cell Res* 2017;24:89–93.
43. Nair GG, Tzanakakis ES, Hebrok M. Emerging routes to the generation of functional  $\beta$ -cells for diabetes mellitus cell therapy. *Nat Rev Endocrinol* 2020;16:506–18.
44. Velazco-Cruz L, Goedegebuure MM, Millman JR. Advances toward engineering functionally mature human pluripotent stem cell-derived beta cells. *Front Bioeng Biotechnol* 2020;8:786.
45. Hogrebe NJ, Augsornworawat P, Maxwell KG, Velazco-Cruz L, Millman JR. Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem cells. *Nat Biotechnol* 2020;38:460–70.
46. Lien Y-C, Won K-J, Simmons RA. Transcriptomic and quantitative proteomic profiling reveals signaling pathways critical for pancreatic islet maturation. *Endocrinology* 2020;161(12):1. <https://academic.oup.com/endo/article-abstract/161/12/bqaa187/5923720?redirectedFrom=fulltext>. Accessed February 11, 2022.
47. Balboa D, Barsby T, Lithovius V, et al. Functional, metabolic and transcriptional maturation of stem cell derived beta cells. *bioRxiv* 2021:2021.03.31.437748. <https://www.biorxiv.org/content/10.1101/2021.03.31.437748v1>. Accessed February 11, 2022.
48. Vantyghem MC, de Koning EJP, Pattou F, Rickels MR. Advances in beta-cell replacement therapy for the treatment of type 1 diabetes. *Lancet* 2019;394:1274–85.
49. Desai T, Shea LD. Advances in islet encapsulation technologies. *Nat Rev Drug Discov* 2017;16:367.
50. Desai TA, Tang Q. Islet encapsulation therapy—racing towards the finish line? *Nat Rev Endocrinol* 2018;14:630–2.
51. Stock AA, Manzoli V, De Toni T, et al. Conformal coating of stem cell-derived islets for  $\beta$  cell replacement in type 1 diabetes. *Stem Cell Rep* 2020;14:91–104.
52. Rubino F, Amiel SA, Zimmet P, et al. New-onset diabetes in Covid-19. *N Engl J Med* 2020;383:789–90.
53. Zhu L, She ZG, Cheng X, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. *Cell Metab* 2020;31:1068–1077.e3.
54. Boddu SK, Aurangabadkar G, Kuchay MS. New onset diabetes, type 1 diabetes and COVID-19. *Diabetes Metab Syndr* 2020;14:2211–7.
55. Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: From pathophysiology to clinical management. *Nat Rev Endocrinol* 2021;17:11–30.
56. Apicella M, Campopiano MC, Mantuano M, Mazoni L, Coppelli A, Del Prato S. COVID-19 in people with diabetes: Understanding the reasons for worse outcomes. *Lancet Diabetes Endocrinol* 2020;8:782–92.
57. Hollstein T, Schulte DM, Schulz J, et al. Autoantibody-negative insulin-dependent diabetes mellitus after SARS-CoV-2 infection: A case report. *Nat Metab* 2020;2:1021–4.
58. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004;203:631–7.
59. Fignani D, Licata G, Brusco N, et al. SARS-CoV-2 receptor angiotensin I-converting enzyme 2 (ACE2) is expressed in human pancreatic  $\beta$ -cells and in the human pancreas microvasculature. *Front Endocrinol (Lausanne)* 2020;11:596898.
60. Taneera J, El-Huneidi W, Hamad M, Mohammed AK, Elaraby E, Hachim MY. Expression profile of SARS-CoV-2 host receptors in human pancreatic islets revealed upregulation of ACE2 in diabetic donors. *Biology (Basel)* 2020;9:215.
61. Coate KC, Cha J, Shrestha S, et al. SARS-CoV-2 cell entry factors ACE2 and TMPRSS2 are expressed in the microvasculature and ducts of human pancreas but are not enriched in  $\beta$  cells. *Cell Metab* 2020;32:1028–1040.e4.
62. Müller JA, Grosse R, Conzelmann C, et al. SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. *Nat Metab* 2021;3:149–65.
63. Yang L, Han Y, Nilsson-Payant BE, et al. A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. *Cell Stem Cell* 2020;27:125–136.e7.
64. Wu C-T, Lidsky PV, Xiao Y, et al. SARS-CoV-2 infects human pancreatic  $\beta$ -cells and elicits  $\beta$ -cell impairment. *Cell Metabol* 2021;33:1565–76.
65. Scheen AJ, Marre M, Thivolet C. Prognostic factors in patients with diabetes hospitalized for COVID-19: Findings from the CORONADO study and other recent reports. *Diabetes Metab* 2020;46:265–71.
66. Lewandowski SL, Cardone RL, Foster HR, et al. pyruvate kinase controls signal strength in the insulin secretory pathway. *Cell Metab* 2020;32:736–750.e5.
67. Wong WKM, Sørensen AE, Joglekar MV, Hardikar AA, Dalgaard LT. Non-coding RNA in pancreas and  $\beta$ -cell development. *Non-Coding RNA* 2018;4:41.
68. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343:230–8.
69. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes* 2001;50:1691–7.
70. Liu X, Huang J, Chen T, et al. Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. *Cell Res* 2008;18:1177–89.
71. Fath-Bayati L, Ai J. Assessment of mesenchymal stem cell effect on foreign body response induced by intraperitoneally implanted alginate spheres. *J Biomed Mater Res A* 2020;108:94–102.
72. Liao Y-H, Zheng J-Q, Zheng C-M, Lu K-C, Chao Y-C. Novel molecular evidence related to COVID-19 in patients with diabetes mellitus. *J Clin Med* 2020;9:3962.