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Review Article

Mitochondrial metabolism and dynamics in pancreatic beta cell glucose sensing

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Glucose-regulated insulin secretion becomes defective in all forms of diabetes. The signaling mechanisms through which the sugar acts on the ensemble of beta cells within the islet remain a vigorous area of research after more than 60 years. Here, we focus firstly on the role that the privileged oxidative metabolism of glucose plays in glucose detection, discussing the importance of 'disallowing' in the beta cell the expression of genes including *Lactate dehydrogenase* (*Ldha*) and the lactate transporter *Mct1/Slc16a1* to restrict other metabolic fates for glucose. We next explore the regulation of mitochondrial metabolism by Ca^{2+} and its possible role in sustaining glucose signaling towards insulin secretion. Finally, we discuss in depth the importance of mitochondrial structure and dynamics in the beta cell, and their potential for therapeutic targeting by incretin hormones or direct regulators of mitochondrial fusion. This review, and the 2023 Sir Philip Randle Lecture which GAR will give at the Islet Study Group meeting in Vancouver, Canada in June 2023, honor the foundational, and sometimes under-appreciated, contributions made by Professor Randle and his colleagues towards our understanding of the regulation of insulin secretion.

Background

Type 2 diabetes (T2D) affects almost 1 in 10 adults worldwide, with the total number of individuals affected by the disease predicted to increase to >700m by 2045 (IDF.org). As revealed in the 1990s by the U.K. prospective diabetes study [1], a fall in insulin sensitivity during pre-diabetes is initially compensated by increased insulin production. When the pancreas fails, hyperglycemia and frank diabetes ensue. Other data suggest that functional beta cell loss occurs early in the disease process [2]. Further highlighting the critical role of beta cell deficiency, the majority of genetic variants associated with disease risk identified in genome-wide association studies affect insulin production rather than action [3,4]. Although decreases in beta cell mass may occur in T2D (as reported in cross-sectional studies [5]), other work [6] suggests that these changes are relatively small (but might nevertheless be consequential if they affect subsets of beta cells with critical roles) [7–9]. Defective glucose-stimulated insulin secretion [10], and possible loss of cellular identity [11,12], are now thought to be the more important drivers of disease development and progression.

Here, we discuss firstly the evidence that mitochondrial oxidative metabolism is essential for beta cell glucose sensing, explaining how these organelles integrate both nutritional and receptor-derived signals. We next explore how mitochondrial structure and dynamics contribute to both the activity of individual beta cells and, potentially, to the ensemble behavior of these cells within the islet microorgan. Finally, we discuss the possibility that mitochondrial dynamics may represent a therapeutic target in T2D.

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An unusual enzymatic configuration favors the oxidative metabolism of glucose carbons by mitochondria in beta cells

Studies by Coore and Randle [13] in Cambridge and by Grodsky et al. [14] in San Francisco, established the 'substrate site hypothesis' for glucose control of insulin secretion, in which metabolic signaling by the sugar plays the key role. Amongst the first evidence supporting this theory were the observations that the dose response for glucose metabolism closely matched that of insulin secretion. Furthermore, the stimulatory effects of glucose were mimicked by metabolizable (mannose), but not by non-metabolizable (galactose) sugars. It was later concluded that the rapid equilibration of glucose across the beta cell plasma membrane (catalyzed by glucose transporters GLUT2 [SLC2A2] in rodents and GLUT1–3 [SLC2A1–SLC2A3] in human beta cells) [15] is followed by glucose phosphorylation: both glucose phosphorylation and insulin secretion are inhibited by mannoheptulose [16]. Glucose phosphorylation, catalyzed by glucokinase (see below), thus represents the flux generating and 'rate limiting' step (i.e. that with the highest control strength) [17] for glucose metabolism, and hence in the triggering of insulin secretion [18,19]. Metabolism through glycolysis and the citrate cycle, and increases in cytosolic ATP/ADP ratio, then impact plasma membrane potential and Ca^{2+} influx to trigger the fusion of secretory granules at the plasma membrane, and ultimately insulin release [20,21] (Figure 1). Additional, and less well-defined pathways (dubbed 'amplifying') [22,23] further potentiate the effects of glucose and the actions of Ca^{2+} on insulin exocytosis (see later sections). Of note, and as described by Ashcroft et al. [24], reductive metabolism of glucose via the pentose phosphate shunt does not appear to be involved in the stimulation of insulin secretion. However, more recent studies [25], using time resolved metabolomics, have questioned this conclusion.

Hormones and neurotransmitters, usually acting through G protein-coupled receptors and including the 'incretins' released from the gut during food transit [26], further modulate insulin release via cAMP generation and activation of beta-arrestins [27] (see later sections).

Mitochondria generate 36 ATP per glucose molecule oxidized versus the two generated by anaerobic glycolysis, suggesting an important contribution of mitochondrial bioenergetics to the triggering of secretion by glucose, as indicated by early observations of Randle, Grodsky and co-workers. Supporting such a role are the inhibitory effects on secretion of mitochondrial uncouplers, as observed by Coore and Randle [13], and subsequent findings from Panten et al. [28], and Hutton et al. [29], showing that extracellular fuels metabolized only (or primarily) by mitochondria e.g. leucine and ketoisocaproate, are efficient stimulators of secretion [30]. Studies by Ashcroft et al. [31] and by Cook and Hales [32], later revealed the critical importance of closure of ATP-sensitive K^+ (K_{ATP}) channels (Figure 1). As such, the latter findings described a molecular target both for alterations in ATP/ADP ratio and for the sulphonylurea class of anti-hyperglycemic drugs which bind to the SUR subunit of K_{ATP} [13,33]. Whilst other targets for ATP/ADP have been proposed, including AMP-activated protein kinase (AMPK) [34], current evidence suggests that the role of the latter enzyme in the control of insulin secretion is complex, and time-dependent [35]. Interestingly, the upstream AMPK kinase, liver kinase B1 (LKB1/STK11), is also required for normal mitochondrial function in beta cells [36]. However, and in contrast with the effects of AMPK inactivation, deletion of LKB1 lowers the requirement of glucose-stimulated insulin secretion for mitochondrial function, through as-yet poorly understood mechanisms that involve the activation of 'amplifying' pathways [36].

Critical roles for mitochondrial metabolism in glucose-regulated insulin secretion were also inferred from human genetics. Thus, a mutation in the transfer RNA tRNAleu, required for the efficient translation of mitochondrial DNA (mtDNA), leads to maternally inherited diabetes and deafness [37,38]. Likewise, variants in the *TFB1M* gene, encoding another mitochondrial transcription factor, are associated with increased risk of T2D [39]. Corroborating these findings, deletion of the mitochondrial transcription factor and mtDNA stabilizing factor TFAM caused diabetes in mice [40].

Further studies revealed that the beta cell has an unusual enzymatic configuration which strongly favors oxidative versus non-oxidative metabolism of glucose. Thus, in a report published by G.A.R. with Sekine et al. [41], we demonstrated that the extractable enzymatic activity of lactate dehydrogenase (LDH) in fluorescence-activated cell-sorted (FACS) primary beta cells from the rat was remarkably low, and some 100-fold less than in the liver. This result was unexpected given the generally accepted view that LDH (encoded by the *LDHA-D* genes in rodents and humans) was a 'housekeeping' enzyme in mammalian cells, responsible

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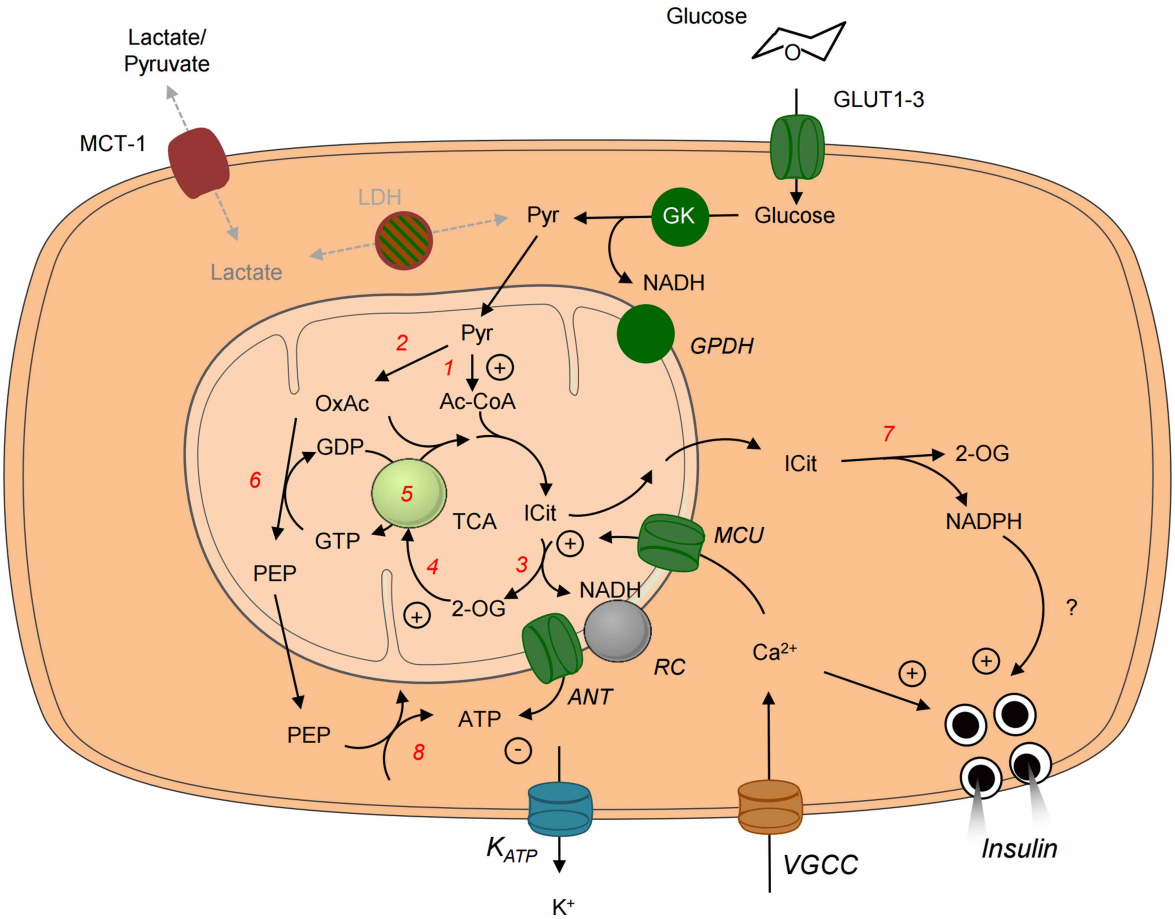

Figure 1. Simplified overview of the intracellular signaling pathways involved in glucose-stimulated insulin secretion.
See the text for further details. 'Disallowed' enzymes or transporters are illustrated in red/grey, favored pathways in green (red/green hatching for LDH reflects species differences). *1*. Pyruvate dehydrogenase (PDH); *2*. Pyruvate carboxylase, PC; *3*. NAD^+-isocitrate dehydrogenase, IDH2; *4*. 2-oxoglutarate dehydrogenase, OGDH; *5*. Succinyl-CoA synthetase; *6*. Phospho*enol*pyruvate carboxykinase 2, PCK2; *7*. $NADP^+$-dependent isocitrate dehydrogenase, IDH1; *8*. pyruvate kinase, PK. *GK*, glucokinase; *RC*, respiratory chain, *MCU*, mitochondrial uniporter, *ANT*, adenine nucleotide translocase; *VGCC*, voltage-gated Ca^{2+} channels (L-type). '+' indicates stimulation by Ca^{2+} ions, either by activation of the associated protein phosphatase (PDH) or by direct binding to the enzymes (IDH2 and OGDH).

for ensuring rapid equilibration of pyruvate and lactate and required for lactate efflux in times of O_2 deprivation (as observed, for example, in exercising muscle). Whilst the above findings in primary beta cells were also true for the most well-differentiated rodent beta cell lines at the time, INS1 cells [42], less glucose-responsive lines (RINm5f, HIT-T15) showed dramatically higher LDH activities (Figure 2a). These findings suggested that, in the beta cell, pyruvate derived from glucose has one principal metabolic fate, i.e. decarboxylation by the pyruvate dehydrogenase (PDH) complex and oxidative degradation via the citrate cycle. Other measurements performed in the same study [41], and independently by Schuit et al. [43], demonstrated that >80% of glucose carbon is converted to CO_2 and H_2O in isolated rat beta cells, a remarkable feature, matched in the animal kingdom only by insect flight muscle [44]. Strikingly, inhibition of the respiratory chain in beta cells also inhibits glucose utilization, indicating that glycolysis and oxidative metabolism are obligatorily linked in these cells. Thus, neither the Pasteur effect (enhanced glucose utilization in the absence of O_2) nor the Crabtree effect (whereby high levels of ATP suppress oxidative metabolism and favor fermentation) [45], are observed in beta cells. Instead, high glycerol phosphate dehydrogenase (GPD2) [41,46,47], and to a lesser extent malate–aspartate shuttle [48] activities, provide efficient mechanisms to allow electrons derived from cytosolic NADH to enter the respiratory chain (Figure 1).

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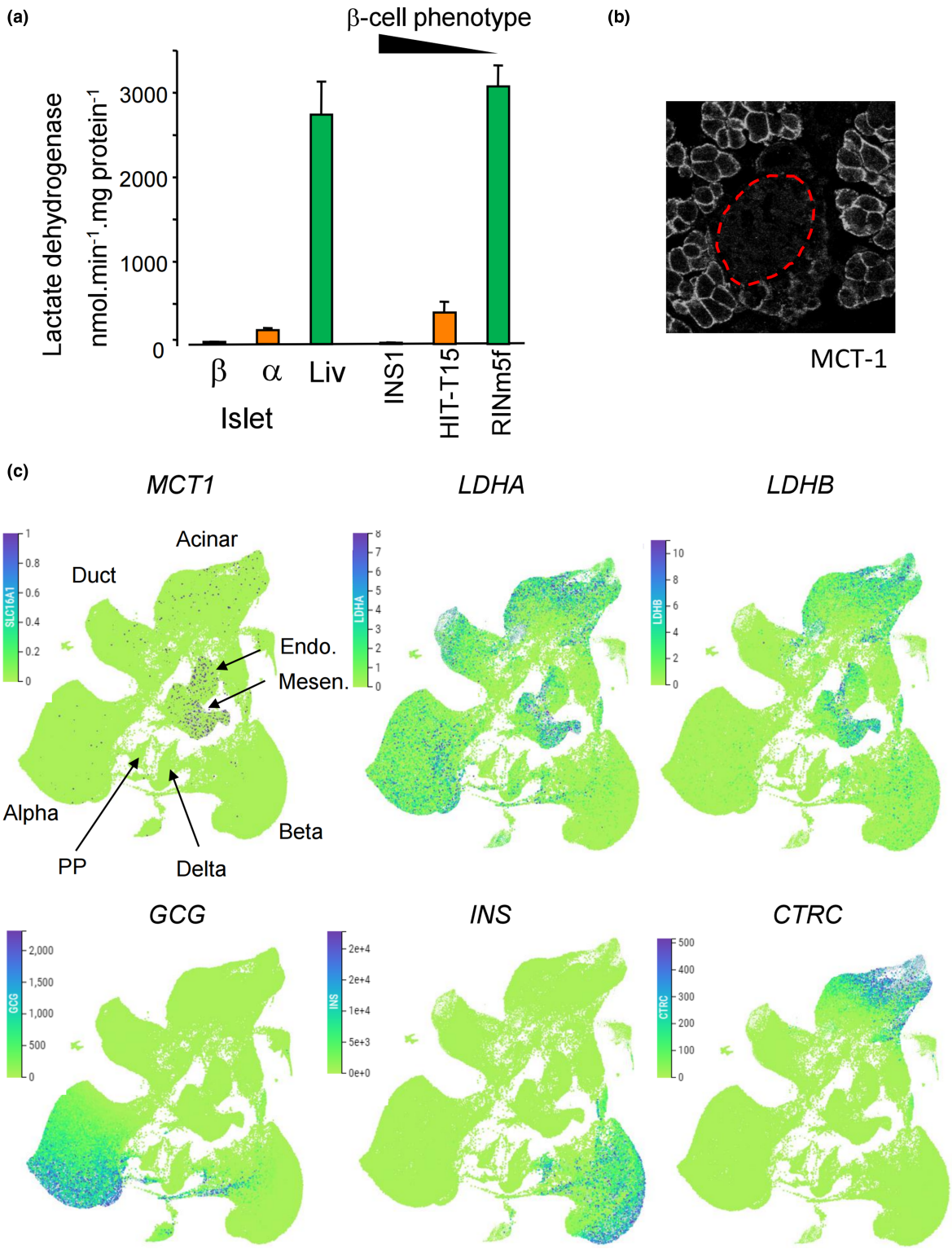

Figure 2. "Disallowance" of lactate/pyruvate transporters and metabolizing enzymes in islet endocrine cells.
Expression of lactate dehydrogenase isoforms and lactate transporters in rat (**a**,**b**) and human (**c**) tissues. (**a**) Total extracted LDH activities were measured spectrophotometrically in the indicated cell types, as described [41]. (**b**) Immnocytochemistry for MCT1 (SLC16A1) in fixed rat pancreas was performed as described [51]. Red circle indicates a morphologically identified islet. (**c**) IUMPs derived from human scRNA-Seq data provided by the PANC-DB database (https://hpap.pmacs.upenn.edu/), based on 107 087 single cells from 27 normoglycemic subjects using the display tool cellxgene (https://faryabi16.pmacs.upenn.edu/view/T1D_T2D_public.h5ad/).

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In an apparent challenge to these findings, working with Randle and others, Ashcroft et al. [16] reported glucose concentration-dependent lactate output from mouse islets. However, lactate production was completely refractory to the glucokinase inhibitor mannoheptulose (which blocks glucose oxidation completely). Malaisse et al. [49] also reported significant lactate output from whole intact rat islets, as did Tamarit-Rodriguez et al. [50] from beta cell-enriched *ob/ob* mouse islets. Tellingly, however, lactate output in this case did not increase at glucose 20 versus 3 mM glucose, despite a 2.5-fold and ∼4 fold increase in glucose utilization and oxidation, respectively. Even more strikingly, lactate and pyruvate transport activity [41], and expression of the monocarboxylate transporter MCT1 (Slc16a1) [51], were later found to be essentially absent from purified rat beta cells (Figure 2b). Taken together, these findings argue for a non-endocrine cell source of lactate from rodent islets. Correspondingly, lactate production was not detected from INS1 beta cells [41], nor the clonal sub-line, INS1 832/13 [48]. *Ldha* and *Slc16a1* became the founder members of the so-called 'disallowed' gene group in beta cells [52,53].

Recent findings suggest that the above scenario might be subtly different in human islets. LDHA is essentially absent from human beta cells (but expressed in alpha cells) [54,55]. On the other hand, and as first noted by Lawlor et al. [56], LDHB mRNA is clearly detectable in human beta (but not alpha) cells (Figure 2c) [55]. Accordingly, lactate generation from glucose, assessed using radiotracers and highly sensitive gas chromatography–mass spectrometry approaches is more substantial in human than in rodent islets [55]. However, and as for rodent islets [49], the interpretation of these experiments is complicated, since beta cells typically comprise only ∼35% of the total volume of human islet preparations (Figure 2c) [57] and LDHA (and B) levels are higher in contaminating ductal, endothelial, mesenchymal and other non-islet cells (Figure 2c). Moreover, in a recent study from Bilbao et al. [58], embryonic stem cell-derived beta cells, but not primary human islets, produced significant lactate from glucose. Nevertheless, the presence of LDHB in human beta cells at levels sufficient to equilibrate lactate and pyruvate may provide a 'buffer' against extensive changes in redox potential and might limit the accumulation of reactive oxygen species (ROS). Interestingly, the latter, as well as contributing to the acute stimulation of insulin release by glucose (at least in rodents) [59], have been suggested recently [60] as promoters of rodent beta cell proliferation. On the other hand, lactate has recently been proposed to regulate cell division in immortalized cell lines by inhibiting the SUMO protease SENP1, thus stabilizing the anaphase-promoting complex (APC/C) to stimulate mitosis [61]. Although these studies have yet to be extended to the islet, the balance between intracellular lactate and ROS levels may conceivably contribute to inter-species differences in the potential for beta cell expansion.

In addition to influencing the metabolic fate of glucose, extinction of MCT1 in beta cells is also important to prevent pyruvate (or lactate) -induced insulin secretion during exercise [62,63]. This feature is unmasked in individuals carrying activating mutations in the *SLC16A1* gene who, as a result, suffer from exercise-induced hyperinsulinemia and hypoglycemia. In rodents, the expression of LDHA is insufficient to allow significant flux from intracellular lactate, via pyruvate, towards mitochondrial oxidation even when MCT1 is up-regulated. Thus, lactate-induced secretion in rat islets is only evident after adenovirus-mediated overexpression of *both Mct1* and *Ldha* [64]. Correspondingly, transgenic overexpression of *Slc16a1* selectively in the beta cell facilitated pyruvate, but not lactate-induced, insulin secretion from mouse islets [63].

Anaplerosis and mitochondrially derived coupling factors

Although flux through PDH likely represents the chief metabolic fate for pyruvate in beta cells, other fluxes, notably 'anaplerotic' input into the citrate cycle via pyruvate carboxylation (catalyzed by pyruvate carboxylase, PC) are also significant, and may represent as much as 40% of carbon flux from glucose into mitochondria [43,65]. The cataplerotic output of intermediates may then generate additional signaling molecules [23] — such as citrate or glutamate [66] (but see also [67]) — or reducing equivalents such a NADPH [68] generated via malate–pyruvate or isocitrate shuttles (Figure 1). Lipid coupling factors, notably monoacylglycerol [69], may also regulate the exocytotic machinery. Some, or all, of these molecules, may then contribute to the 'amplification' of the effects of K_{ATP} channel closure on Ca^{2+}-dependent insulin secretion [70]. Exploration of this possibility is, nevertheless, complicated, and inhibitors of PC, such as phenylacetic acid, also lower the cytosolic ATP/ADP ratio [71], indicating the importance of anaplerosis for the triggering (K_{ATP} channel-dependent) insulin secretion. Of note, the overall fluxes of mitochondrially derived metabolites are likely to be minor, given that the net efflux of glucose-derived carbons from beta cells other than as CO_2 (and possibly lactate) is limited (see above). Readers are referred to Prentki et al. [72], Maechler and Wollheim [66] and Merrins et al. [23] for a comprehensive survey of candidate signaling molecules for glucose-stimulated insulin secretion.

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Ca^{2+} stimulates mitochondrial oxidative metabolism in beta cells

Activated Ca^{2+} influx, and oscillatory changes in cytosolic Ca^{2+}, are fundamental features of glucose-stimulated insulin secretion (see above) [73,74]. Using the photoprotein aequorin [75], targeted to the mitochondrial matrix by in-frame fusion with the leader sequence of CoxVIII [76], we [77] demonstrated that increases in cytosolic calcium led to ∼10-fold higher increases in mitochondrial free calcium in rat-insulinoma-derived (INS-1 beta cells). Similarly, exaggerated mitochondrial (versus cytosolic) Ca^{2+} changes were later observed at high glucose [78]. These findings indicated that mitochondria are likely to serve as a *sink*, rather than a *source* of Ca^{2+} when beta cells are challenged at high glucose. As such, they were in line with the proposals of Denton (a former student of Randle) and McCormack [79], as discussed by Hansford [80], that stimulation of intramitochondrial dehydrogenases (pyruvate dehydrogenase, [PDH], NAD^+-isocitrate dehydrogenase, NAD-ICDH [IDH2] and 2-oxoglutarate dehydrogenase [OGDH]) in mammalian cells enhances NADH production by the citrate cycle, ultimately driving increased ATP synthesis by the respiratory chain under circumstances where cytosolic ATP demand is increased [81].

To further explore a role for Ca^{2+} regulation of the intramitochondrial dehydrogenases in primary mouse beta cells, we [82,83] used a genetically-encoded ATP sensor based on the amino acid transporter GlnK1 (from the deep sea bacteria *Methanococcus jannaschii*), fused to GFP, alongside a red cytosolic Ca^{2+} probe. Simultaneously measuring plasma membrane potential using the cell-attached perforated patch, this approach also revealed that, after glucose challenge, an initial increase in ATP/ADP ratio was followed by transient decreases following action potential-induced Ca^{2+} spikes, likely reflecting an increase in ATP demand (for ion pumping, etc.) which transiently exceeded mitochondrial ATP synthesis, even after dehydrogenase activation by Ca^{2+}. Cytosolic ATP/ADP then recovered gradually, possibly reflecting time-dependent activation of mitochondrial respiratory chain activity [84,85] (Figure 3). We were also able to show that Ca^{2+} transport converts a binary signal (cytosolic Ca^{2+} oscillations of near-unvarying amplitude) into a time-averaged change in amplitude which can be 'read' by the TCA cycle [83].

Consistent with this view, silencing of the mitochondrial calcium uniporter MCU [86] in beta cells inhibited intramitochondrial Ca^{2+} increases and cytosolic ATP/ADP rises [82,87]. Inactivation of the *Mcu* gene selectively in the beta cell in mice by deletion of *floxed* alleles correspondingly impaired glucose-stimulated insulin secretion from primary islets [88]. Surprisingly, however, these animals remained normoglycemic, hinting at alternative pathways for mitochondrial Ca^{2+} uptake *in vivo*, or robust compensatory mechanisms after *Mcu* deletion.

The control of mitochondrial O_2 consumption and ATP synthesis in the beta cell thus appears, at different stages following stimulation by glucose, to be achieved by a combination of (1) 'push' (i.e. increased substrate supply

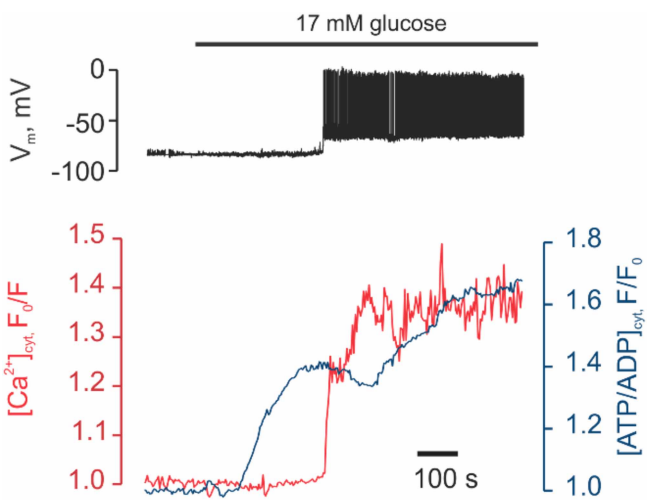

Figure 3. Sequential activation by high glucose of oxidative metabolism, plasma membrane depolarization and Ca^{2+} influx.
Changes in ATP/ADP ratio (blue) membrane potential (black), and cytosolic Ca^{2+} (red) in a single mouse beta cell following glucose (17 vs 3 mM) challenge. See the text and [82] for further details.

provided by GPD2 and citrate cycle-derived NADH), enhanced by Ca^{2+} activation of the intramitochondrial dehydrogenases and (2) 'pull' (from lowered ATP/ADP ratios). The interplay between these is discussed below.

In displaying headroom for increases in ATP/ADP ratio as glucose concentrations rise, the beta cell differs from most other cell types since, in the latter, this ratio is usually held at high levels. In these cases, ATP synthesis is increased to meet cellular *requirements*, matching the demands of a given workload (e.g. for contraction, motility, ion pumping, biosynthesis, etc.). In contrast, the beta cell represents a special case, as a professional fuel sensor [20]. At fasting glucose concentrations (<5 mM), where the glycolytic generation of pyruvate from glucose is limited by the kinetic properties of the low affinity hexokinase, glucokinase (Michaelis constant, $K_M > 10$ mM), cytosolic ADP levels are higher (and ATP/ADP ratio lower, ~3) in beta cells than most cell types, rising to ~15 at high glucose [89]. Thus, at low glucose, mitochondria are in 'state 3.5' [90], rather than state 4, such that substrate supply, rather than ADP availability, is limiting for respiratory chain activity. Hence, elevated glucose concentrations can enhance O_2 consumption and ATP synthesis, in the face of concomitant increases in cytosolic ATP/ADP (Figure 3, phase 1) [89,91–93]. Supporting this view, in recent elegant experiments using a microfluidic device, Schulze et al. [94] demonstrated that a step increase in glucose promotes a coincident increase in NAD(P)H, O_2 consumption and insulin secretion.

Interplay between the control of oxidative phosphorylation by Ca^{2+} and ADP during metabolic and electrical oscillations in the beta cell

As suggested above, the bioenergetic situation in the beta cell is likely to be complex during glucose-induced oscillations in cytosolic ATP/ADP and Ca^{2+}. Under these conditions, altered ADP supply to mitochondria, accelerated ATP consumption and an intrinsic glycolytic oscillator [95], may all contribute to fluctuations in ATP/ADP and O_2 consumption. In an important study, Luciani et al. [96] noted that Ca^{2+} appeared to shape NAD(P)H oscillations in islets, with forced Ca^{2+} increases elevating NAD(P)H at low glucose (via dehydrogenase activation) whilst lowering this parameter at high glucose, as inferred from changes in mitochondrial membrane depolarization. G.A.R.'s laboratory identified similar relationship based on cytosolic ATP/ADP measurements with cytosolic luciferase. Here, KCl-induced Ca^{2+} influx increased cytosolic ATP/ADP at low glucose, but decreased ATP/ADP at high glucose [97]. Changes in the opposite direction were observed when extracellular EGTA was used to suppress Ca^{2+} influx.

Li et al. [98] reported similar findings, including antiparallel changes in cytosolic Ca^{2+} and ATP at 20 mM glucose, providing further evidence that increased Ca^{2+} may stimulate O_2 consumption at Ca^{2+} peaks (ATP/ADP troughs). In contrast, Tanaka et al. [99], observed glucose-induced cytosolic ATP increases which preceded Ca^{2+} increases, whilst cytosolic ATP subsequently failed to oscillate in the face of Ca^{2+} oscillations. Whether these differences versus other reports may reflect the fact that the probe used measured ATP, rather than ADP, or that recordings were made over the whole islet, is unclear. For further discussion on the above the reader is referred to the excellent review by Bertram et al. [100].

Whether incretins affect mitochondrial metabolism directly is still contested. In studies on beta cell lines, Tsuboi et al. [101] demonstrated that glucagon-like peptide-1, (GLP-1) stimulated intracellular Ca^{2+} mobilization via protein kinase A (PKA)-stimulated phosphorylation of inositol-1,4,5 *tris*phosphate receptors, subsequently stimulating mitochondrial ATP synthesis and K_{ATP} channel closure. In contrast, Peyot et al. [102] observed no effects on intracellular ATP levels nor on O_2 consumption of treatment with GLP-1 or the analog exendin-4.

Finally, it should be noted that metabolic oscillations — however controlled — may represent a transitioning between two states: catabolic (O_2 consumption and ATP generation) and anaplorosis (output of signaling intermediates) [23].

Potential importance of Ca^{2+}-insensitive 2-oxoglutatrate dehydrogenase variants

Following the identification of multiple isoforms of the decarboxylase (E1) subunit of OGDH [103], to which Ca^{2+} binds [104], we demonstrated differential expression of these isoforms in different tissues with ~30% of OGDH E1 mRNA encoding a Ca^{2+} insensitive form in islets (and brain) versus <5% in skeletal muscle and heart [105]. An important feature of the regulation of the citrate cycle dehydrogenases is that Ca^{2+} (synergistically with ADP and NAD^+) lowers the K_M for substrate [105,106]. Thus, Ca^{2+} activation may be important to

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regulate substrate and product *concentrations*, as well as fluxes. In any case, where the Ca^{2+}-insensitive variants predominate, accelerated conversion of 2-oxoglurarate to succinyl-CoA will be largely resistant to intramitochondrial Ca^{2+} increases. Consequently, isocitrate (and citrate) will be conserved for cataplerotic *efflux* from mitochondria, and thus the generation of potential coupling factors in the cytosol [23] (Figure 1).

Role and regulation of mitochondrial dynamics in the beta cell

Changes in mitochondrial shape and connectedness are also likely to impact the function of these organelles, and ultimately that of the 'host' beta cell. Mitochondria exist as both threads (from the Greek, *mitos*) and grain- (*chondros*) like structures. The existence of the latter form is required for efficient turnover of mitochondria (mitophagy) and maintenance of a healthy mitochondrial population.

While mitochondrial fission precedes efficient turnover of aged or unhealthy mitochondria by mitophagy [107], maintaining a well-connected mitochondrial network is essential for metabolite and substrate exchange and thus optimal metabolic activity [108]. The balance between the two morphological configurations (fission and fusion) is tightly controlled depending on cell type, nutrient demand and extracellular/extraorganelle stimuli [109,110]. Abnormalities in this intricate balance have been implicated as an underlying cause for reduced mitochondrial function in metabolic diseases such as diabetes and obesity [108]. Indeed, beta cells from human islet donors with T2D display abnormal mitochondrial morphology with dilated or swollen mitochondria with reduced cristae and accompanied by diminished glucose-stimulated ATP/ADP ratio [111]. Moreover, and potentially complementing the actions of Ca^{2+} on intramitochondrial oxidative metabolism discussed above, Ca^{2+} also controls mitochondrial dynamics, at least in non-beta cells [112].

Given their role in integrating fuel metabolism, and the spatial requirements of both Ca^{2+} signals [113,114] and of ATP production [91] one might suspect that mitochondrial shape would be important for normal beta cell fuel sensing. These requirements may include allowing the formation of localized subcellular domains e.g. of ATP generation at the plasma membrane and the efficient operation of a 'phophoenolpyruvate (PEP) cycle' [115] (Figure 1), allowing transfer energetic equivalents from GTP generated in the mitochondrial matrix towards ATP microdomains close to K_{ATP} channels at the plasma membrane [116].

Mitochondrial dynamics are orchestrated by the co-ordinated actions of membrane-associated dynamin-related proteins (DRPs), capable of oligomerization at the mitochondrial membrane and subsequent membrane remodeling [117]. Mitochondrial fission is mediated by dynamin-related protein 1 (DRP1) which forms a helix around the mitochondria, leading to membrane constriction and eventual scission. Mitochondrial fusion is mediated by mitofusins 1 and 2 (MFN1 and 2) and optic atrophy 1 (OPA1) localized on the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM), respectively. By dimerizing with their counterparts on neighboring mitochondria, MFN1 and 2 facilitate fusion by bringing the OMM into close proximity. This is followed by the interactions of long and short forms of OPA1 on the IMM of the opposing mitochondria leading to their fusion. The co-ordinated activity of these mitochondrial DRPs is mediated by cyclic GTP binding and hydrolysis that facilitates their oligomerization and disassembly [118]. Given the contribution of these events in controlling mitochondrial shape and network, the net balance between fission and fusion controls multiple aspects of mitochondrial physiology and quality control [119–121]. Mitochondrial fission by DRP1 allows for the segregation of depolarized mitochondria for clearance by mitophagy [107]. On the other hand, mitochondrial fusion allows for the maintenance of a homogenous mitochondrial network by ensuring uniform content distribution and exchange across the mitochondrial population [122–126]. Of note, the intermixing of mtDNA molecules with the mtDNA replication machinery allows for the maintenance of mtDNA integrity, while preventing mtDNA damage and accumulation of mutations [123,125].

Whereas the creation of a more highly fused network (achieved by inactivation of *Drp1*) leads to deficiencies in insulin secretion [127], we [128,129] have recently shown that fragmentation of mitochondria selectively in beta cells induced by disruption of the mitofusin genes (*Mfn1* and *Mfn2*) leads to changes in beta cell function and to severe hyperglycemia in mice. Loss of MFN1/2 either during development [128] or in adult mice [129] was associated with a less highly interconnected mitochondrial network and also significant changes in the structure of the cristae. While deletion of *Mfn1*- of *Mfn2*- alone did not lead to mitochondrial defects, depletion of both *Mfn1* and *2* caused significant hyperglycemia and a drastic reduction in mtDNA. In complementary studies, we [128] also showed that loss of mitofusin function in beta cells, accomplished by deleting the *Mfn* alleles one by one, was more closely correlated to mtDNA content than to network fragmentation *per se*.

In our gene dosage studies in mice, animals bearing only a single functional allele of *Mfn2* displayed mitochondrial fragmentation while retaining normal glycaemic control, glucose-stimulated insulin release, and islet mtDNA content (Figure 4). On the other hand, mice bearing only a single allele of *Mfn1* displayed mitochondrial network instability, mtDNA depletion, and significant hyperglycemia. These two distinct results implied that depletion of mtDNA content, rather than impaired mitochondrial architecture, leads to beta cell dysfunction. Moreover, our results clarified a long-standing challenge in the field of mitochondrial biology, distinguishing the relative roles for mitochondrial architecture and mtDNA copy number control by MFN1/2, which had previously been difficult to separate from one another. Thus, our studies established that the primary physiological importance of MFN1/2 in cellular metabolism *in vivo* is via regulation of mitochondrial genome stability, rather than mitochondrial structure.

Beta cell mass was only modestly affected in *Mfn1–Mfn2* double knockout (DKO) mice [128,104] (and not detectably after deletion in adults [129,105]), though a slight increase in apoptosis was evident in both models. Beta cell identity, assessed through the expression of *Slc2a2* and other signature genes, was only marginally affected, and we did not see an increase in the canonical dedifferentiation marker *Aldh1a3* [130], or other 'disallowed' genes [53]. Altered expression of genes in the autophagic pathway was, though, apparent [128].

Much more striking were alterations to the bioenergetic function of mitochondria. These included a drastic lowering in glucose-induced mitochondrial membrane potential changes and ATP synthesis after PdxCreER-mediated deletion in adult mice. Both cytosolic and, to an even greater degree, mitochondrial Ca^{2+} changes, were severely compromised in MFN1/2 DKO islets [129].

By encoding 13 essential subunits of the OXPHOS machinery, mtDNA enables individual mitochondria to regulate their own function depending on nutrient availability [131]. Consistent with reduced mtDNA, mtRNA, and protein levels of mitochondrially expressed proteins were markedly reduced in the MFN1/2 DKO islet. These findings suggested that the impact of *Mfn1* and *Mfn2* deletion on mtDNA content was due to decreased expression of the mitochondrial transcription factor, TFAM, and deleterious effects of mitofusin deletion changes were rescued in part by *Tfam* overexpression. Transcriptomic analysis suggested a post-transcriptional regulation of Tfam levels by Mfn1 and 2, as *Mfn1/2* deletion did not affect *Tfam* mRNA levels. Also evident from our transcriptomic profiling of MFN1/2 DKO islets was an increase in the expression of *Lonp1*, a mitochondrially localized protease involved in the quality control of mitochondrial proteins [132]. Aberrant activation of LonP1 function, however, has been reported to cause a constellation of mitochondrial defects that include degradation of TFAM and destabilization of mtDNA nucleoids [133–135]. While it remains unclear how LonP1 regulates mitochondrial function in beta cells, the above results implicate *Mfn1* and *Mfn2* preservation of mtDNA content, in part through maintenance of TFAM.

Our studies add new and previously uncharacterized roles for mitofusins in beta cells. Together with previous work demonstrating beta cell dysfunction and glucose intolerance *in vivo* following loss of DRP1 or OPA1, these results demonstrate that the mitochondrial network balance is vital to promote beta cell health and function. However, several key questions remain. Our results suggest that MFN1/2 are essential for maintaining mtDNA content to promote mitochondrial respiration and insulin secretion. Furthermore, we find that GLP1R agonists can bypass mitochondrial defects to restore insulin secretion in MFN1/2-deficient islets, suggesting beta cells can activate alternative metabolic pathways to maintain insulin secretion during mitochondrial dysfunction (see next section). Notably, mice bearing Opa1-deficiency selectively in beta cells develop glucose intolerance and ultimately impaired beta cell replication [136], an effect not seen in MFN1/2-deficient islets. Moreover, mice with Drp1-deficiency in beta cells develop glucose intolerance that is speculated to be secondary to abnormalities in the amplifying pathway of insulin secretion and thought to be independent of mitochondrial respiration [127]. Taken together, these studies suggest the potential for a spectrum of cellular effects of altering the mitochondrial dynamics machinery in beta cells (both within and outside mitochondria) that remain to be harmonized. Additional clarity into the shared and distinct functions of the mitochondrial dynamics network will hopefully provide opportunities to better understand the linkages between beta cell mitochondrial structural and functional defects in T2D.

Mitochondrial dynamics: a therapeutic target for incretins and other drugs?

An exciting aspect of the above studies [129] was that the impact of deleting mitofusins on oral glucose tolerance (OGT) was greater than that on intraperitoneal tolerance (IGT). Given that the latter are driven by the

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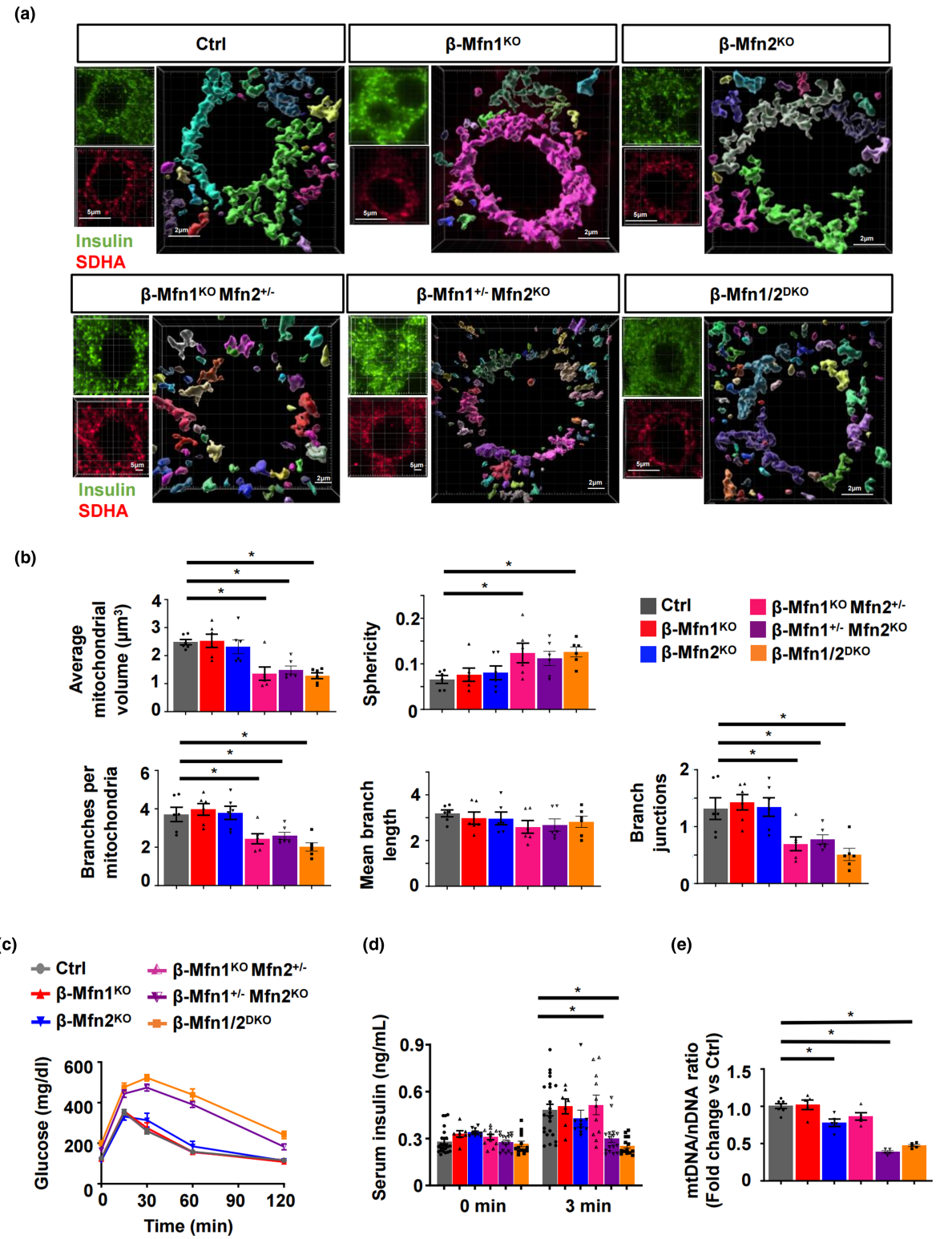

Figure 4. Impact of mitofusin gene deletion selectively in beta cells on mitochondrial structure, mtDNA content and glucose metabolism.

Part 1 of 2

(**a**) Three-dimensional renderings of β-cell mitochondrial networks generated on Imaris®, (**b**) mitochondrial morphology and network analysis performed using MitoAnalyzer of deconvolution immunofluorescence Z-stack images from pancreatic sections stained for insulin and SDHA, as previously described. (**c**) Blood glucose concentrations measured during IP-glucose

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Figure 4. Impact of mitofusin gene deletion selectively in beta cells on mitochondrial structure, mtDNA content and glucose metabolism. Part 2 of 2

tolerance tests; (**d**) serum insulin concentrations measured during *in vivo* glucose-stimulated insulin release testing, and (**e**) relative mtDNA content normalized to nuclear DNA expression as measured by qPCR. *$P < 0.05$ by ANOVA. Reproduced with permission from [128].

release of incretin (e.g. GLP-1 and glucose-dependent insulinotropic peptide, GIP, and others) [26], we explored the effects of an incretin analog after mitofusin deletion. Exendin-4 almost completely rescued the abnormal glucose tolerance *in vivo*, and rescued defective glucose-induced insulin secretion *in vitro*. These changes were associated with normalized Ca^{2+} dynamics, including beta-cell beta cell connectivity defects. Pharmacological analysis suggested that rescue was due to engagement by cAMP of the Exchange Protein regulated (EPAC2) pathway, since it was still observed when PKA was inhibited, and was mimicked using an EPAC activator (Figure 5).

A potential role for mitochondria in determining beta cell heterogeneity and connectivity

In addition to mitochondrial heterogeneity within *individual* beta cells [137], we have suggested [20] that differences in mitochondrial function or dynamics may contribute to the observed heterogeneity and connectivity between beta cells. Several recent reviews [138,139] have described these differences and we refer readers to those for a comprehensive discussion of this topic. Importantly, at the functional level, populations of both 'leaders' from which Ca^{2+} waves appear to emanate [8,140–142], and highly connected 'hub' [7,9] cells, each of which comprises ~5% of all beta cells, play important and complementary roles in controlling islet wide Ca^{2+} dynamics and oscillatory insulin secretion. Johnston et al. [7] showed that mitochondrial hyper-polarisation in response to glucose was more marked in hubs versus the beta cell population as a whole. However, no differences between the structure of mitochondria (sphericity and mean length) were detected between the two populations. Differences in mitochondrial structure and function in 'leader' versus other beta cells have not yet been explored. Likewise, Lu et al. [143] described an epigenomically discrete subset of beta cells in which mitochondrial genes were over-represented. Finally, Chabosseau et al. [140] revealed that several genes involved in mitochondrial function are differentially expressed between leader and follower beta cells.

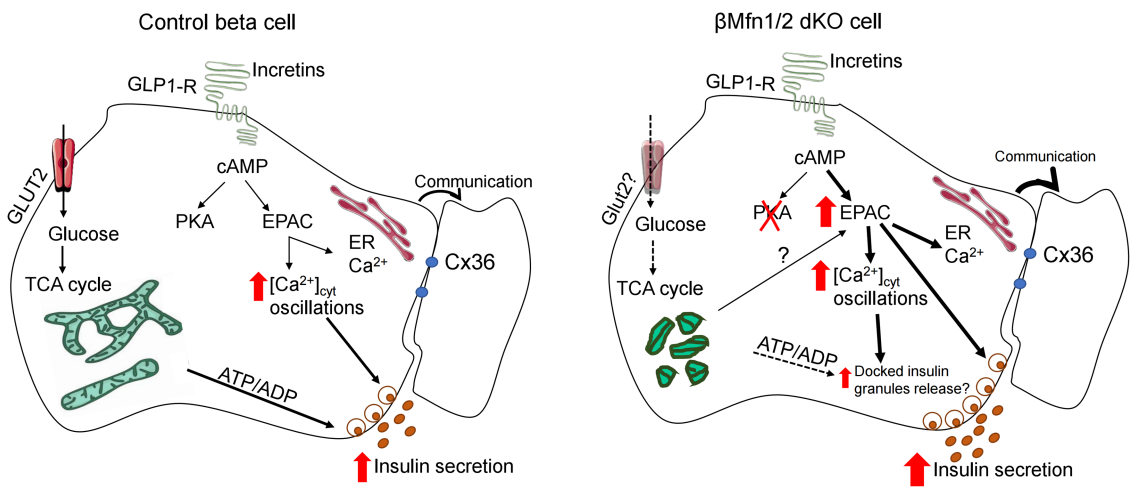

Figure 5. Impact of mitochondrial structure on signaling by glucose and incretins in the beta cell.

Left panel: Glucose-dependent changes in cytosolic ATP/ADP ratio are potentiated by GLP-1R signaling, acting through PKA, EPAC2 and altered Ca^{2+} changes. Right: Mitochondrial fragmentation and disruption of cristae structure after *Mfn1*, *Mfn2* disruption enhances EPAC signaling, compensating for impaired mitochondrial oxidative metabolism under these conditions.

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Conclusions and translational perspectives

In the healthy islet, glucose enhances mitochondrial oxidative metabolism, which is critical for the normal stimulation of insulin secretion. Incretins, including GLP1, potentiate these effects by only partly understood mechanisms. When mitochondria are fragmented as mitofusin levels are lowered, and conceivably in diabetes, they become dysfunctional as a result of being unable to maintain an adequate mtDNA content and hence respiratory chain function. GLP1R agonism rescues or bypasses these effects, ensuring normalized K_{ATP} channel-dependent calcium dynamics, beta-cell beta cell connectivity and insulin secretion. The impact of these changes on amplifying pathways is as yet undefined.

Whether differences may exist between the effects of GLP1R agonists which are biased for signaling via either cAMP-dependent pathways (including EPAC2) and through beta-arrestins [144] is also unexplored, but might represent a way of favoring the effects of these drugs to rescue deficiencies resulting from altered mitochondrial structure and function in T2D. Direct agonism of MFN2 [145] may provide a complementary therapeutic strategy.

Finally, the importance of mitochondrial function in the control of the secretion of other islet hormones (glucagon, somatostatin, etc.) represents an emerging area of interest, especially since changes in the expression of genes associated with mitochondrial metabolism in alpha cells are observed in both type 1 and type 2 diabetes [146].

Competing Interests

G.A.R. has received grant funding from, and is a consultant for, Sun Pharmaceuticals Industries. S.A.S. has received grant funding from Ono Pharmaceutical Co., Ltd. and is a consultant for Novo Nordisk.

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CRediT Author Contribution

Guy A. Rutter: Conceptualization, Funding acquisition, Methodology, Writing — original draft. **Vaibhav Sidarala**: Writing — original draft, Writing — review and editing. **Brett A. Kaufman**: Writing — review and editing. **Scott A. Soleimanpour**: Writing — original draft, Writing — review and editing.

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Abbreviations

AMPK, AMP-activated protein kinase; DKO, double knockout; DRPs, dynamin-related proteins; GLP-1, glucagon-like peptide-1; GPD2, glycerol phosphate dehydrogenase; IMM, inner mitochondrial membrane; LDH, lactate dehydrogenase; mtDNA, mitochondrial DNA; OGDH, 2-oxoglutarate dehydrogenase; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PKA, protein kinase A; ROS, reactive oxygen species.

References

1 Turner, R.C., Cull, C.A., Frighi, V. and Holman, R.R. (1999) Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *J. Am. Med. Assoc.* **281**, 2005–2012 https://doi.org/10.1001/jama.281.21.2005

2 Kahn, S.E., Zraika, S., Utzschneider, K.M. and Hull, R.L. (2009) The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. *Diabetologia* **52**, 1003–1012 https://doi.org/10.1007/s00125-009-1321-z

3 Fuchsberger, C., Flannick, J., Teslovich, T.M., Mahajan, A., Agarwala, V., Gaulton, K.J. et al. (2016) The genetic architecture of type 2 diabetes. *Nature* **536**, 41–47 https://doi.org/10.1038/nature18642

4 Mahajan, A., Taliun, D., Thurner, M., Robertson, N.R., Torres, J.M., Rayner, N.W. et al. (2018) Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat. Genet.* **50**, 1505–1513 https://doi.org/10.1038/s41588-018-0241-6
5 Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A. and Butler, P.C. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102–110 https://doi.org/10.2337/diabetes.52.1.102
6 Marselli, L., Suleiman, M., Masini, M., Campani, D., Bugliani, M., Syed, F. et al. (2014) Are we overestimating the loss of beta cells in type 2 diabetes? *Diabetologia* **57**, 362–365 https://doi.org/10.1007/s00125-013-3098-3
7 Johnston, N.R., Mitchell, R.K., Haythorne, E., Pessoa, M.P., Semplici, F., Ferrer, J. et al. (2016) Beta cell hubs dictate pancreatic islet responses to glucose. *Cell Metab.* **24**, 389–401 https://doi.org/10.1016/j.cmet.2016.06.020
8 Salem, V., Silva, L.S., Suba, S., Georgiadou, E., Mousavy Gharavy, S.N., Khtar, N. et al. (2019) Leader beta cells coordinate Ca^{2+} dynamics across pancreatic islets in vivo. *Nat. Metab.* **1**, 615–629 https://doi.org/10.1038/s42255-019-0075-2
9 Gosak, M., Yan-Do, R., Lin, H., MacDonald, P.E. and Stožer, A. (2022) Ca^{2+} oscillations, waves, and networks in islets from human donors with and without type 2 diabetes. *Diabetes* **71**, 2584–2596 https://doi.org/10.2337/db22-0004
10 Del Guerra, S., Lupi, R., Marselli, L., Masini, M., Bugliani, M., Sbrana, S. et al. (2005) Functional and molecular defects of pancreatic islets in human type 2 diabetes. *Diabetes* **54**, 727–735 https://doi.org/10.2337/diabetes.54.3.727
11 Talchai, C., Xuan, S., Lin, H.V., Sussel, L. and Accili, D. (2012) Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell* **150**, 1223–1234 https://doi.org/10.1016/j.cell.2012.07.029
12 Rutter, G.A., Pullen, T.J., Hodson, D.J. and Martinez-Sanchez, A. (2015) Pancreatic beta cell identity, glucose sensing and the control of insulin secretion. *Biochem. J.* **466**, 202–218 https://doi.org/10.1042/BJ20141384
13 Coore, H.G. and Randle, P.J. (1964) Inhibition of glucose phosphorylation by mannoheptulose. *Biochem. J.* **91**, 56–59 https://doi.org/10.1042/bj0910056
14 Grodsky, G.M., Batts, A.A., Bennett, L.L., Vcella, C., McWilliams, N.B. and Smith, D.F. (1963) Effects of carbohydrates on secretion of insulin from isolated rat pancreas. *Am. J. Physiol.* **205**, 638–644 https://doi.org/10.1152/ajplegacy.1963.205.4.638
15 Thorens, B. (2015) GLUT2, glucose sensing and glucose homeostasis. *Diabetologia* **58**, 221–232 https://doi.org/10.1007/s00125-014-3451-1
16 Ashcroft, S.J., Hedeskov, C.J. and Randle, P.J. (1970) Glucose metabolism in mouse pancreatic islets. *Biochem. J.* **118**, 143–154 https://doi.org/10.1042/bj1180143
17 Kacser, H. and Burns, J.A. (1973) The control of flux. *Symp. Soc. Exp. Biol.* **27**, 65–104 PMID:4148886
18 Iynedjian, P.B. (1993) Mammalian glucokinase and its gene. *Biochem. J.* **293**, 1–13 https://doi.org/10.1042/bj2930001
19 Matschinsky, F.M. (1990) Glucokinase as glucose sensor and metabolic signal generator in pancreatic á-cells and hepatocytes. *Diabetes* **39**, 647–652 https://doi.org/10.2337/diab.39.6.647
20 Rutter, G.A., Georgiadou, E., Martinez-Sanchez, A. and Pullen, T.J. (2020) Metabolic and functional specialisations of the pancreatic beta cell: gene disallowance, mitochondrial metabolism and intercellular connectivity. *Diabetologia* **63**, 1990–1998 https://doi.org/10.1007/s00125-020-05205-5
21 Rorsman, P. and Ashcroft, F.M. (2018) Pancreatic beta-cell electrical activity and insulin secretion: of mice and men. *Physiol. Rev.* **98**, 117–214 https://doi.org/10.1152/physrev.00008.2017
22 Henquin, J.C. and Rahier, J. (2011) Pancreatic alpha cell mass in European subjects with type 2 diabetes. *Diabetologia* **54**, 1720–1725 https://doi.org/10.1007/s00125-011-2118-4
23 Merrins, M.J., Corkey, B.E., Kibbey, R.G. and Prentki, M. (2022) Metabolic cycles and signals for insulin secretion. *Cell Metab.* **34**, 947–968 https://doi.org/10.1016/j.cmet.2022.06.003
24 Ashcroft, S.J., Weerasinghe, L.C., Bassett, J.M. and Randle, P.J. (1972) The pentose cycle and insulin release in mouse pancreatic islets. *Biochem. J.* **126**, 525–532 https://doi.org/10.1042/bj1260525
25 Spégel, P., Sharoyko, V.V., Goehring, I., Danielsson, A.P., Malmgren, S., Nagorny, C.L. et al. (2013) Time-resolved metabolomics analysis of β-cells implicates the pentose phosphate pathway in the control of insulin release. *Biochem. J.* **450**, 595–605 https://doi.org/10.1042/bj20121349
26 Nauck, M.A., Quast, D.R., Wefers, J. and Meier, J.J. (2021) GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. *Mol. Metab.* **46**, 101102 https://doi.org/10.1016/j.molmet.2020.101102
27 Jones, B., Bloom, S.R., Buenaventura, T., Tomas, A. and Rutter, G.A. (2018) Control of insulin secretion by GLP-1. *Peptides* **100**, 75–84 https://doi.org/10.1016/j.peptides.2017.12.013
28 Panten, U., VonKriegstein, E., Poser, W., Schönborn, J. and Hasselblatt, A. (1972) Effects of l-leucine and alpha-ketoisocaproic acid upon insulin secretion and metabolism of isolated pancreatic islets. *FEBS Lett.* **20**, 225–228 https://doi.org/10.1016/0014-5793(72)80801-9
29 Hutton, J.C., Sener, A., Herchuelz, A., Atwater, I., Kawazu, S., Boschero, A.C. et al. (1980) Similarities in the stimulus-secretion coupling mechanisms of glucose- and 2-keto acid-induced insulin release. *Endocrinology* **106**, 203–219 https://doi.org/10.1210/endo-106-1-203
30 Matschinsky, F.M., Meglasson, M., Ghosh, A., Appel, M., Bedoya, F., Prentki, M. et al. (1986) Biochemical design features of the pancreatic islet cell glucose- sensory system. *Adv. Exp. Med. Biol.* **211**, 459–469 https://doi.org/10.1007/978-1-4684-5314-0_46
31 Ashcroft, F.M., Harrison, D.E. and Ashcroft, S.J.H. (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic [beta]-cells. *Nature* **312**, 446–448 https://doi.org/10.1038/312446a0
32 Cook, D.L. and Hales, C.N. (1984) Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. *Nature* **311**, 271–273 https://doi.org/10.1038/311271a0
33 Ashcroft, F.M., Kakei, M., Gibson, J.S., Gray, D.W. and Sutton, R. (1989) The ATP- and tolbutamide-sensitivity of the ATP-sensitive K-channel from human pancreatic B cells. *Diabetologia* **32**, 591–598 https://doi.org/10.1007/BF00285333
34 Rutter, G.A., da Silva Xavier, G. and Leclerc, I. (2003) Roles of 5'-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. *Biochem. J.* **375**, 1–16 https://doi.org/10.1042/BJ20030048
35 Nguyen-Tu, M.S., Harris, J., Martinez-Sanchez, A., Chabosseau, P., Hu, M., Georgiadou, E. et al. (2022) Opposing effects on regulated insulin secretion of acute vs chronic stimulation of AMP-activated protein kinase. *Diabetologia* **65**, 997–1011 https://doi.org/10.1007/s00125-022-05673-x
36 Swisa, A., Granot, Z., Tamarina, N., Sayers, S., Bardeesy, N., Philipson, L. et al. (2015) Loss of liver kinase B1 (LKB1) in beta cells enhances glucose-stimulated insulin secretion despite profound mitochondrial defects. *J. Biol. Chem.* **290**, 20934–20946 https://doi.org/10.1074/jbc.M115.639237

Biochemical Journal (2023) **480** 773–789
https://doi.org/10.1042/BCJ20230167

37 van den Ouweland, J.M., Lemkes, H.H., Ruitenbeek, W., Sandkuijl, L.A., de Vijlder, M.F., Struyvenberg, P.A. et al. (1992) Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nat. Genet.* **1**, 368–371 https://doi.org/10.1038/ng0892-368

38 Reardon, W., Ross, R.J., Sweeney, M.G., Luxon, L.M., Pembrey, M.E., Harding, A.E. et al. (1992) Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. *Lancet* **340**, 1376–1379 https://doi.org/10.1016/0140-6736(92)92560-3

39 Koeck, T., Olsson, A.H., Nitert, M.D., Sharoyko, V.V., Ladenvall, C., Kotova, O. et al. (2011) A common variant in TFB1M is associated with reduced insulin secretion and increased future risk of type 2 diabetes. *Cell Metab.* **13**, 80–91 https://doi.org/10.1016/j.cmet.2010.12.007

40 Silva, J.P., Kohler, M., Graff, C., Oldfors, A., Magnuson, M.A., Berggren, P.O. et al. (2000) Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. *Nat. Genet.* **26**, 336–340 https://doi.org/10.1038/81649

41 Sekine, N., Cirulli, V., Regazzi, R., Brown, L.J., Gine, E., Tamarit-Rodriguez, J. et al. (1994) Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogease in pancreatic á-cell. Potential role in nutrient sensing. *J. Biol. Chem.* **269**, 4895–4902 https://doi.org/10.1016/S0021-9258(17)37629-9

42 Asfari, M., Janjic, D., Meda, P., Li, G., Halban, P.A. and Wollheim, C.B. (1992) Establishment of 2-mercaptoethanol-dependent differentiated insulin secreting cell lines. *Regul. Pept.* **130**, 167–178 https://doi.org/10.1210/endo.130.1.1370150

43 Schuit, F., DeVos, A., Farfari, S., Moens, K., Pipeleers, D., Brun, T. et al. (1997) Metabolic fate of glucose in purified islet cells - glucose-regulated anaplerosis in beta cells. *J. Biol. Chem.* **272**, 18572–18579 https://doi.org/10.1074/jbc.272.30.18572

44 Keilin, D. (1925) On cytochrome, a respiratory pigment, common to anials, yeast and higher plants. *Proc. R. Soc. Lond.(Biol)* **98**, 312–319 https://doi.org/10.1098/rspb.1925.0039

45 Crabtree, H.G. (1929) Observations on the carbohydrate metabolism of tumours. *Biochem. J.* **23**, 536–545 https://doi.org/10.1042/bj0230536

46 MacDonald, M.J. (1981) High content of mitochondrial glycerol-3-phosphate dehydrogenase in pancreatic islets and its inhibition by diazoxide. *J. Biol. Chem.* **256**, 8287–8290 https://doi.org/10.1016/S0021-9258(19)68840-X

47 Rutter, G.A., Pralong, W.F. and Wollheim, C.B. (1992) Regulation of mitochondrial glycerol phosphate dehydrogenase by Ca^{2+} within electropermeabilized insulin secreting cells (INS-1). *Biochim. Biophys. Acta* **1175**, 107–113 https://doi.org/10.1016/0167-4889(92)90016-5

48 Stamenkovic, J.A., Andersson, L.E., Adriaenssens, A.E., Bagge, A., Sharoyko, V.V., Gribble, F. et al. (2015) Inhibition of the malate-aspartate shuttle in mouse pancreatic islets abolishes glucagon secretion without affecting insulin secretion. *Biochem. J.* **468**, 49–63 https://doi.org/10.1042/bj20140697

49 Malaisse, W.J., Maggetto, C., Leclercq-Meyer, V. and Sener, A. (1993) Interference of glycogenolysis with glycolysis in pancreatic islets from glucose-infused rats. *J. Clin. Invest.* **91**, 432–436 https://doi.org/10.1172/jci116219

50 TamaritRodriguez, J., Idahl, L.A., Gine, E., Alcazar, O. and Sehlin, J. (1998) Lactate production in pancreatic islets. *Diabetes* **47**, 1219–1223 https://doi.org/10.2337/diab.47.8.1219

51 Zhao, C., Wilson, C.M., Schuit, F., Halestrap, A.P. and Rutter, G.A. (2001) Expression and distribution of lactate/monocarboxylate transporter (MCT) isoforms in pancreatic islets and the exocrine pancreas. *Diabetes* **50**, 361–366 https://doi.org/10.2337/diabetes.50.2.361

52 Lemaire, K., Thorrez, L. and Schuit, F. (2016) Disallowed and allowed gene expression: two faces of mature islet beta cells. *Annu. Rev. Nutr.* **36**, 45–71 https://doi.org/10.1146/annurev-nutr-071715-050808

53 Pullen, T.J., Huising, M.O. and Rutter, G.A. (2017) Analysis of purified pancreatic islet beta and alpha cell transcriptomes reveals 11beta-hydroxysteroid dehydrogenase (Hsd11b1) as a novel disallowed gene. *Front. Genet.* **8**, 41 https://doi.org/10.3389/fgene.2017.00041

54 Sanchez, P.K.M., Khazaei, M., Gatineau, E., Geravandi, S., Lupse, B., Liu, H. et al. (2021) LDHA is enriched in human islet alpha cells and upregulated in type 2 diabetes. *Biochem. Biophys. Res. Commun.* **568**, 158–166 https://doi.org/10.1016/j.bbrc.2021.06.065

55 Cuozzo, F., Nasteska, D., Jiao, Z., Smith, H.R., Bonner, C., Kerr-Conte, J. et al. (2022) High-resolution glucose fate-mapping reveals *LDHB*-dependent lactate production by human pancreatic β cells. *bioRxiv* 2022.2012.2021.521364 https://doi.org/10.1101/2022.12.21.521364

56 Lawlor, N., George, J., Bolisetty, M., Kursawe, R., Sun, L., Sivakamasundari, V. et al. (2017) Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. *Genome Res.* **27**, 208–222 https://doi.org/10.1101/gr.212720.116

57 Pisania, A., Weir, G.C., O'Neil, J.J., Omer, A., Tchipashvili, V., Lei, J. et al. (2010) Quantitative analysis of cell composition and purity of human pancreatic islet preparations. *Lab. Invest.* **90**, 1661–1675 https://doi.org/10.1038/labinvest.2010.124

58 Balboa, D., Barsby, T., Lithovius, V., Saarimäki-Vire, J., Omar-Hmeadi, M., Dyachok, O. et al. (2022) Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. *Nat. Biotechnol.* **40**, 1042–1055 https://doi.org/10.1038/s41587-022-01219-z

59 Leloup, C., Tourrel-Cuzin, C., Magnan, C., Karaca, M., Castel, J., Carneiro, L. et al. (2009) Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. *Diabetes* **58**, 673–681 https://doi.org/10.2337/db07-1056

60 Vivoli, A., Ghislain, J., Filali-Mouhim, A., Angeles, Z.E., Castell, A.L., Sladek, R. et al. (2023) Single-cell RNA sequencing reveals a role for reactive oxygen species and peroxiredoxins in fatty acid-induced rat β-cell proliferation. *Diabetes* **72**, 45–58 https://doi.org/10.2337/db22-0121

61 Liu, W., Wang, Y., Bozi, L.H.M., Fischer, P.D., Jedrychowski, M.P., Xiao, H. et al. (2023) Lactate regulates cell cycle by remodelling the anaphase promoting complex. *Nature* **616**, 790–797 https://doi.org/10.1038/s41586-023-05939-3

62 Otonkoski, T., Jiao, H., Kaminen-Ahola, N., Tapia-Piaz, I., Ullah, M.S., Parton, L.E. et al. (2007) Physical exercise-induced hyperinsulinemic hypoglycemia caused by failure of monocarboxylate transporter 1 silencing in pancreatic beta cells. *Am. J. Hum. Genet.* **81**, 467–474 https://doi.org/10.1086/520960

63 Pullen, T.J., Sylow, L., Sun, G., Halestrap, A.P., Richter, E.A. and Rutter, G.A. (2012) Over-expression of monocarboxylate transporter-1 (Slc16a1) in the pancreatic *ƒ*-cell leads to relative hyperinsulinism during exercise. *Diabetes* **61**, 1719–1725 https://doi.org/10.2337/db11-1531

64 Ishihara, H., Wang, H., Drewes, L.R. and Wollheim, C.B. (1999) Overexpression of monocarboxylate transporter and lactate dehydrogenase alters insulin secretory responses to pyruvate and lactate in beta cells. *J. Clin. Invest.* **104**, 1621–1629 https://doi.org/10.1172/JCI7515

65 Khan, A., Ling, Z.C. and Landau, B.R. (1996) Quantifying the carboxylation of pyruvate in pancreatic islets. *J. Biol. Chem.* **271**, 2539–2542 https://doi.org/10.1074/jbc.271.5.2539

66 Maechler, P. and Wollheim, C.B. (1999) Mitochondrial glutamate acts as a messenger in glucose- induced insulin exocytosis. *Nature* **402**, 685–689 https://doi.org/10.1038/45280

67 MacDonald, M.J. (2003) Export of metabolites from pancreatic islet mitochondria as a means to study anaplerosis in insulin secretion. *Metabolism* **52**, 993–998 https://doi.org/10.1016/s0026-0495(03)00149-5

68 Ferdaoussi, M., Dai, X., Jensen, M.V., Wang, R., Peterson, B.S., Huang, C. et al. (2015) Isocitrate-to-SENP1 signaling amplifies insulin secretion and rescues dysfunctional beta cells. *J. Clin. Invest.* **125**, 3847–3860 https://doi.org/10.1172/JCI82498
69 Zhao, S., Mugabo, Y., Iglesias, J., Xie, L., Delghingaro-Augusto, V., Lussier, R. et al. (2014) A/β-Hydrolase domain-6-accessible monoacylglycerol controls glucose-stimulated insulin secretion. *Cell Metab.* **19**, 993–1007 https://doi.org/10.1016/j.cmet.2014.04.003
70 Henquin, J.C. (2000) Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* **49**, 1751–1760 https://doi.org/10.2337/diabetes.49.11.1751
71 Fransson, U., Rosengren, A.H., Schuit, F.C., Renström, E. and Mulder, H. (2006) Anaplerosis via pyruvate carboxylase is required for the fuel-induced rise in the ATP:ADP ratio in rat pancreatic islets. *Diabetologia* **49**, 1578–1586 https://doi.org/10.1007/s00125-006-0263-y
72 Prentki, M., Matschinsky, F.M. and Madiraju, S.R. (2013) Metabolic signaling in fuel-induced insulin secretion. *Cell Metab.* **18**, 162–185 https://doi.org/10.1016/j.cmet.2013.05.018
73 Gylfe, E. (1988) Nutrient secretagogues induce bimodal early changes in cytoplasmic calcium of insulin-releasing ob/ob mouse beta-cells. *J. Biol. Chem.* **263**, 13750–13754 https://doi.org/10.1016/S0021-9258(18)68305-X
74 Pralong, W.F., Bartley, C. and Wollheim, C.B. (1990) Single islet beta-cell stimulation by nutrients: relationship between pyridine nucleotides, cytosolic Ca^{2+} and secretion. *EMBO J.* **9**, 53–60 https://doi.org/10.1002/j.1460-2075.1990.tb08079.x
75 Shimomura, O., Johnson, F.H. and Saiga, Y. (1962) Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J. Cell Comp. Physiol.* **59**, 223–239 https://doi.org/10.1002/jcp.1030590302
76 Rizzuto, R., Simpson, A.W.M., Brini, M. and Pozzan, T. (1992) Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. *Nature* **358**, 325–327 https://doi.org/10.1038/358325a0
77 Rutter, G.A., Theler, J.M., Murta, M., Wollheim, C.B., Pozzan, T. and Rizzuto, R. (1993) Stimulated Ca^{2+} influx raises mitochondrial free Ca^{2+} to supramicromolar levels in a pancreatic á-cell line: possible role in glucose and agonist-induced insulin secretion. *J. Biol. Chem.* **268**, 22385–22390 https://doi.org/10.1016/S0021-9258(18)41540-2
78 Kennedy, E.D., Rizzuto, R., Theler, J.M., Pralong, W.F., Bastianutto, C., Pozzan, T. et al. (1996) Glucose-stimulated insulin secretion correlates with changes in mitochondrial and cytosolic Ca^{2+} in aequorin- expressing INS-1 cells. *J. Clin. Invest.* **98**, 2524–2538 https://doi.org/10.1172/JCI119071
79 Denton, R.M. and McCormack, J.G. (1980) On the role of the calcium transport cycle in the heart and other mammalian mitochondria. *FEBS Lett.* **119**, 1–8 https://doi.org/10.1016/0014-5793(80)80986-0
80 Hansford, R.G. (1985) Relation between mitochondrial calcium transport and control of energy metabolism. *Rev. Physiol. Biochem. Pharmacol.* **102**, 1–72 https://doi.org/10.1007/BFb0034084
81 Griffiths, E.J. and Rutter, G.A. (2009) Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. *Biochim. Biophys. Acta* **1787**, 1324–1333 https://doi.org/10.1016/j.bbabio.2009.01.019
82 Tarasov, A.I., Semplici, F., Ravier, M.A., Bellomo, E.A., Pullen, T.J., Gilon, P. et al. (2012) The mitochondrial Ca^{2+} uniporter MCU is essential for glucose-induced ATP increases in pancreatic ƒ-cells. *PLoS One* **7**, e39722 https://doi.org/10.1371/journal.pone.0039722
83 Tarasov, A.I., Semplici, F., Li, D., Rizzuto, R., Ravier, M.A., Gilon, P. et al. (2013) Frequency-dependent mitochondrial Ca(2+) accumulation regulates ATP synthesis in pancreatic beta cells. *Pflugers Arch.* **465**, 543–554 https://doi.org/10.1007/s00424-012-1177-9
84 Jouaville, L.S., Pinton, P., Bastianutto, C., Rutter, G.A. and Rizzuto, R. (1999) Regulation of mitochondrial ATP synthesis by calcium: evidence for a long term, Ca^{2+} triggered, mitochondrial activation. *Proc. Natl Acad. Sci. U.S.A.* **96**, 13807–13812 https://doi.org/10.1073/pnas.96.24.13807
85 Ainscow, E.K. and Rutter, G.A. (2001) Mitochondrial priming modifies Ca^{2+} oscillations and insulin secretion in pancreatic islets. *Biochem. J.* **353**, 175–180 https://doi.org/10.1042/0264-6021:3530175
86 De Stefani, D., Raffaello, A., Teardo, E., Szabo, I. and Rizzuto, R. (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* **476**, 336–340 https://doi.org/10.1038/nature10230
87 Alam, M.R., Groschner, L.N., Parichatikanond, W., Kuo, L., Bondarenko, A.I., Rost, R. et al. (2012) Mitochondrial Ca^{2+} uptake 1 (MICU1) and mitochondrial Ca^{2+} uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic beta-cells. *J. Biol. Chem.* **287**, 34445–34454 https://doi.org/10.1074/jbc.M112.392084
88 Georgiadou, E., Haythorne, E., Dickerson, K., Lopez-Noriega, L., Pullen, T.J., DaSilva Xavier, G. et al. (2020) The pore-forming subunit MCU of the mitochondrial Ca^{2+} uniporter is required for normal glucose-stimulated insulin secretion in vitro and in vivo in mice. *Diabetologia* **63**, 1368–1381 https://doi.org/10.1007/s00125-020-05148-x
89 Detimary, P., Dejonghe, S., Ling, Z.D., Pipeleers, D., Schuit, F. and Henquin, J.C. (1998) The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in beta cells but not in a cells and are also observed in human islets. *J Biol. Chem.* **273**, 33905–33908 https://doi.org/10.1074/jbc.273.51.33905
90 Nicholls, D.G. (1982) *Bioenergetics. An Introduction to the Chemiosmotic Theory*, Academic Press, London
91 Kennedy, H.J., Pouli, A.E., Ainscow, E.K., Jouaville, L.S., Rizzuto, R. and Rutter, G.A. (1999) Glucose generates sub-plasma membrane ATP microdomains in single islet beta-cells. Potential role for strategically located mitochondria. *J. Biol. Chem.* **274**, 13281–13291 https://doi.org/10.1074/jbc.274.19.13281
92 Maechler, P., Wang, H. and Wollheim, C.B. (1998) Continuous monitoring of ATP levels in living insulin secreting cells expressing cytosolic firefly luciferase. *FEBS Lett.* **422**, 328–332 https://doi.org/10.1016/s0014-5793(97)01618-9
93 Kohler, M., Norgren, S., Berggren, P.O., Fredholm, B.B., Larsson, O., Rhodes, C.J. et al. (1998) Changes in cytoplasmic ATP concentration parallels changes in ATP-regulated K+-channel activity in insulin-secreting cells. *FEBS Lett.* **441**, 97–102 https://doi.org/10.1016/s0014-5793(98)01539-7
94 Schulze, T., Mattern, K., Früh, E., Hecht, L., Rustenbeck, I. and Dietzel, A. (2017) A 3D microfluidic perfusion system made from glass for multiparametric analysis of stimulus-secretioncoupling in pancreatic islets. *Biomed. Microdevices* **19**, 47 https://doi.org/10.1007/s10544-017-0186-z
95 Merrins, M.J., Van Dyke, A.R., Mapp, A.K., Rizzo, M.A. and Satin, L.S. (2013) Direct measurements of oscillatory glycolysis in pancreatic islet β-cells using novel fluorescence resonance energy transfer (FRET) biosensors for pyruvate kinase M2 activity. *J. Biol. Chem.* **288**, 33312–33322 https://doi.org/10.1074/jbc.M113.508127
96 Luciani, D.S., Misler, S. and Polonsky, K.S. (2006) Ca^{2+} controls slow NAD(P)H oscillations in glucose-stimulated mouse pancreatic islets. *J. Physiol.* **572**, 379–392 https://doi.org/10.1113/jphysiol.2005.101766

Biochemical Journal (2023) **480** 773–789
https://doi.org/10.1042/BCJ20230167

97 Ainscow, E.K. and Rutter, G.A. (2002) Glucose-stimulated oscillations in free cytosolic ATP concentration imaged in single islet á cells: evidnece for a Ca^{2+}-dependent mechanism. *Diabetes* **51**, S162–S170 https://doi.org/10.2337/diabetes.51.2007.s162
98 Li, J., Shuai, H.Y., Gylfe, E. and Tengholm, A. (2013) Oscillations of sub-membrane ATP in glucose-stimulated beta cells depend on negative feedback from Ca(2+). *Diabetologia* **56**, 1577–1586 https://doi.org/10.1007/s00125-013-2894-0
99 Tanaka, T., Nagashima, K., Inagaki, N., Kioka, H., Takashima, S., Fukuoka, H. et al. (2014) Glucose-stimulated single pancreatic islets sustain increased cytosolic ATP levels during initial Ca^{2+} influx and subsequent Ca^{2+} oscillations. *J. Biol. Chem.* **289**, 2205–2216 https://doi.org/10.1074/jbc.M113.499111
100 Bertram, R., Satin, L.S. and Sherman, A.S. (2018) Closing in on the mechanisms of pulsatile insulin secretion. *Diabetes* **67**, 351–359 https://doi.org/10.2337/dbi17-0004
101 Tsuboi, T., da Silva Xavier, G., Holz, G.G., Jouaville, L.S., Thomas, A.P. and Rutter, G.A. (2003) Glucagon-like peptide-1 mobilizes intracellular Ca^{2+} and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells. *Biochem. J.* **369**, 287–299 https://doi.org/10.1042/BJ20021288
102 Peyot, M.L., Gray, J.P., Lamontagne, J., Smith, P.J., Holz, G.G., Madiraju, S.R. et al. (2009) Glucagon-like peptide-1 induced signaling and insulin secretion do not drive fuel and energy metabolism in primary rodent pancreatic beta-cells. *PLoS One* **4**, e6221 https://doi.org/10.1371/journal.pone.0006221
103 Armstrong, C.T., Anderson, J.L. and Denton, R.M. (2014) Studies on the regulation of the human E1 subunit of the 2-oxoglutarate dehydrogenase complex, including the identification of a novel calcium-binding site. *Biochem. J.* **459**, 369–381 https://doi.org/10.1042/BJ20131664
104 Rutter, G.A. and Denton, R.M. (1989) The binding of Ca^{2+} ions to pig heart NAD+-isocitrate dehydrogenase and the 2-oxoglutarate dehydrogenase complex. *Biochem. J.* **263**, 453–462 https://doi.org/10.1042/bj2630453
105 Denton, R.M., Pullen, T.J., Armstrong, C.T., Heesom, K.J. and Rutter, G.A. (2016) Calcium-insensitive splice variants of mammalian E1 subunit of 2-oxoglutarate dehydrogenase complex with tissue-specific patterns of expression. *Biochem. J.* **473**, 1165–1178 https://doi.org/10.1042/BCJ20160135
106 Rutter, G.A. and Denton, R.M. (1988) Regulation of NAD^+-linked isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase by Ca^{2+} ions within toluene- permeabilized rat heart mitochondria. Interactions with regulation by adenine nucleotides and NADH/NAD^+ ratios. *Biochem. J.* **252**, 181–189 https://doi.org/10.1042/bj2520181
107 Twig, G., Elorza, A., Molina, A.J., Mohamed, H., Wikstrom, J.D., Walzer, G. et al. (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J.* **27**, 433–446 https://doi.org/10.1038/sj.emboj.7601963
108 Liesa, M., Palacín, M. and Zorzano, A. (2009) Mitochondrial dynamics in mammalian health and disease. *Physiol. Rev.* **89**, 799–845 https://doi.org/10.1152/physrev.00030.2008
109 Glancy, B., Kim, Y., Katti, P. and Willingham, T.B. (2020) The functional impact of mitochondrial structure across subcellular scales. *Front. Physiol.* **11**, 541040 https://doi.org/10.3389/fphys.2020.541040
110 Ngo, J., Choi, D.W., Stanley, I.A., Stiles, L., Molina, A.J.A., Chen, P.H. et al. (2023) Mitochondrial morphology controls fatty acid utilization by changing CPT1 sensitivity to malonyl-CoA. *EMBO J.* e111901 https://doi.org/10.15252/embj.2022111901
111 Anello, M., Lupi, R., Spampinato, D., Piro, S., Masini, M., Boggi, U. et al. (2005) Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia* **48**, 282–289 https://doi.org/10.1007/s00125-004-1627-9
112 Saotome, M., Safiulina, D., Szabadkai, G., Das, S., Fransson, A., Aspenstrom, P. et al. (2008) Bidirectional Ca2+-dependent control of mitochondrial dynamics by the Miro GTPase. *Proc. Natl Acad. Sci. U.S.A.* **105**, 20728–20733 https://doi.org/10.1073/pnas.0808953105
113 Rutter, G.A. and Rizzuto, R. (2000) Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection. *Trends Biochem. Sci.* **25**, 215–221 https://doi.org/10.1016/s0968-0004(00)01585-1
114 Gilon, P., Chae, H.Y., Rutter, G.A. and Ravier, M.A. (2014) Calcium signaling in pancreatic beta-cells in health and in Type 2 diabetes. *Cell Calcium* **56**, 21 https://doi.org/10.1016/j.ceca.2014.09.001
115 Sugden, M.C. and Ashcroft, S.J. (1977) Phosphoenolpyruvate in rat pancreatic islets: a possible intracellular trigger of insulin release? *Diabetologia* **13**, 481–486 https://doi.org/10.1007/BF01234500
116 Foster, H.R., Ho, T., Potapenko, E., Sdao, S.M., Huang, S.M., Lewandowski, S.L. et al. (2022) β-cell deletion of the PKm1 and PKm2 isoforms of pyruvate kinase in mice reveals their essential role as nutrient sensors for the K(ATP) channel. *Elife* **11**, e79422 https://doi.org/10.7554/eLife.79422
117 Giacomello, M., Pyakurel, A., Glytsou, C. and Scorrano, L. (2020) The cell biology of mitochondrial membrane dynamics. *Nat. Rev. Mol. Cell Biol.* **21**, 204–224 https://doi.org/10.1038/s41580-020-0210-7
118 Tilokani, L., Nagashima, S., Paupe, V. and Prudent, J. (2018) Mitochondrial dynamics: overview of molecular mechanisms. *Essays Biochem.* **62**, 341–360 https://doi.org/10.1042/ebc20170104
119 Chan, D.C. (2020) Mitochondrial dynamics and its involvement in disease. *Annu. Rev. Pathol.* **15**, 235–259 https://doi.org/10.1146/annurev-pathmechdis-012419-032711
120 Liesa, M. and Shirihai, O.S. (2013) Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. *Cell Metab.* **17**, 491–506 https://doi.org/10.1016/j.cmet.2013.03.002
121 Youle, R.J. and van der Bliek, A.M. (2012) Mitochondrial fission, fusion, and stress. *Science* **337**, 1062–1065 https://doi.org/10.1126/science.1219855
122 Chen, H., McCaffery, J.M. and Chan, D.C. (2007) Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell* **130**, 548–562 https://doi.org/10.1016/j.cell.2007.06.026
123 Chen, H., Vermulst, M., Wang, Y.E., Chomyn, A., Prolla, T.A., McCaffery, J.M. et al. (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell* **141**, 280–289 https://doi.org/10.1016/j.cell.2010.02.026
124 Sato, A., Nakada, K. and Hayashi, J. (2009) Mitochondrial complementation preventing respiratory dysfunction caused by mutant mtDNA. *Biofactors* **35**, 130–137 https://doi.org/10.1002/biof.14
125 Ramos, S., Motori, E., Brüser, E., Kühl, C., Yeroslaviz, I., Ruzzenente, A. (2019) Mitochondrial fusion is required for regulation of mitochondrial DNA replication. *PLoS Genet.* **15**, e1008085 https://doi.org/10.1371/journal.pgen.1008085
126 Chen, H., Chomyn, A. and Chan, D.C. (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J. Biol. Chem.* **280**, 26185–26192 https://doi.org/10.1074/jbc.M503062200

127 Hennings, T.G., Chopra, D.G., DeLeon, E.R., VanDeusen, H.R., Sesaki, H., Merrins, M.J. et al. (2018) In vivo deletion of beta-cell Drp1 impairs insulin secretion without affecting islet oxygen consumption. *Endocrinology* **159**, 3245–3256 https://doi.org/10.1210/en.2018-00445
128 Sidarala, V., Zhu, J., Levi-D'Ancona, E., Pearson, G.L., Reck, E.C., Walker, E.M. et al. (2022) Mitofusin 1 and 2 regulation of mitochondrial DNA content is a critical determinant of glucose homeostasis. *Nat. Commun.* **13**, 2340 https://doi.org/10.1038/s41467-022-29945-7
129 Georgiadou, E., Muralidharan, C., Martinez, M., Chabosseau, P., Akalestou, E., Tomas, A. et al. (2022) Mitofusins Mfn1 and Mfn2 are required to preserve glucose- but not incretin-stimulated β-cell connectivity and insulin secretion. *Diabetes* **71**, 1472–1489 https://doi.org/10.2337/db21-0800
130 Cinti, F., Bouchi, R., Kim-Muller, J.Y., Ohmura, Y., Sandoval, P.R., Masini, M. et al. (2016) Evidence of beta-cell dedifferentiation in human type 2 diabetes. *J. Clin. Endocrinol. Metab.* **101**, 1044–1054 https://doi.org/10.1210/jc.2015-2860
131 Picard, M. and Shirihai, O.S. (2022) Mitochondrial signal transduction. *Cell Metab.* **34**, 1620–1653 https://doi.org/10.1016/j.cmet.2022.10.008
132 Pollecker, K., Sylvester, M. and Voos, W. (2021) Proteomic analysis demonstrates the role of the quality control protease LONP1 in mitochondrial protein aggregation. *J. Biol. Chem.* **297**, 101134 https://doi.org/10.1016/j.jbc.2021.101134
133 Matsushima, Y., Goto, Y. and Kaguni, L.S. (2010) Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). *Proc. Natl Acad. Sci. U.S.A.* **107**, 18410–18415 https://doi.org/10.1073/pnas.1008924107
134 Lu, B., Lee, J., Nie, X., Li, M., Morozov, Y.I., Venkatesh, S. et al. (2013) Phosphorylation of human TFAM in mitochondria impairs DNA binding and promotes degradation by the AAA+ Lon protease. *Mol. Cell* **49**, 121–132 https://doi.org/10.1016/j.molcel.2012.10.023
135 Peter, B., Waddington, C.L., Oláhová, M., Sommerville, E.W., Hopton, S., Pyle, A. et al. (2018) Defective mitochondrial protease LonP1 can cause classical mitochondrial disease. *Hum. Mol. Genet.* **27**, 1743–1753 https://doi.org/10.1093/hmg/ddy080
136 Zhang, Z., Wakabayashi, N., Wakabayashi, J., Tamura, Y., Song, W.J., Sereda, S. et al. (2011) The dynamin-related GTPase Opa1 is required for glucose-stimulated ATP production in pancreatic beta cells. *Mol. Biol. Cell* **22**, 2235–2245 https://doi.org/10.1091/mbc.E10-12-0933
137 Wikstrom, J.D., Katzman, S.M., Mohamed, H., Twig, G., Graf, S.A., Heart, E. et al. (2007) Beta-cell mitochondria exhibit membrane potential heterogeneity that can be altered by stimulatory or toxic fuel levels. *Diabetes* **56**, 2569–2578 https://doi.org/10.2337/db06-0757
138 Chabosseau, P., Rutter, G.A. and Millership, S.J. (2021) Importance of both imprinted genes and functional heterogeneity in pancreatic beta cells: is there a link? *Int. J. Mol. Sci* **22**, 1000 https://doi.org/10.3390/ijms22031000
139 Benninger, R.K.P. and Kravets, V. (2022) The physiological role of β-cell heterogeneity in pancreatic islet function. *Nat. Rev. Endocrinol.* **18**, 9–22 https://doi.org/10.1038/s41574-021-00568-0
140 Chabosseau, P., Yong, F., Delgadillo-Silva, L.F., Lee, E.Y., Melhem, R., Li, S. et al. (2023) Molecular phenotyping of single pancreatic islet leader beta cells by "Flash-Seq". *Life Sci.* **316**, 121436 https://doi.org/10.1016/j.lfs.2023.121436
141 Kravets, V., Dwulet, J.M., Schleicher, W.E., Hodson, D.J., Davis, A.M., Pyle, L. et al. (2022) Functional architecture of pancreatic islets identifies a population of first responder cells that drive the first-phase calcium response. *PLoS Biol.* **20**, e3001761 https://doi.org/10.1371/journal.pbio.3001761
142 Šterk, M., Dolenšek, J., Skelin Klemen, M., Križančić Bombek, L., Paradiž Leitgeb, E., Kerčmar, J., et al. (2023) Functional characteristics of hub and wave-initiator cells in β cell networks. *Biophys. J.* **22**, 784–801 https://doi.org/10.1016/j.bpj.2023.01.039
143 Lu, T.T., Heyne, S., Dror, E., Casas, E., Leonhardt, L., Boenke, T. et al. (2018) The polycomb-dependent epigenome controls beta cell dysfunction, dedifferentiation, and diabetes. *Cell Metab.* **27**, 1294–1308 https://doi.org/10.1016/j.cmet.2018.04.013
144 Jones, B., Buenaventura, T., Kanda, N., Chabosseau, P., Owen, B.M., Scott, R. et al. (2018) Targeting GLP-1 receptor trafficking to improve agonist efficacy. *Nat. Commun.* **9**, 1602–03941 https://doi.org/10.1038/s41467-018-03941-2
145 Dang, X., Williams, S.B., Devanathan, S., Franco, A., Fu, L., Bernstein, P.R. et al. (2021) Pharmacophore-based design of phenyl-[hydroxycyclohexyl] cycloalkyl-carboxamide mitofusin activators with improved neuronal activity. *J. Med. Chem.* **64**, 12506–12524 https://doi.org/10.1021/acs.jmedchem.1c00163
146 Bosi, E., Marchetti, P., Rutter, G.A. and Eizirik, D.L. (2022) Human alpha cell transcriptomic signatures of types 1 and 2 diabetes highlight disease-specific dysfunction pathways. *iScience* **25**, 105056 https://doi.org/10.1016/j.isci.2022.105056