

REVIEW

The pathogenetic role of β -cell mitochondria in type 2 diabetes

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Abstract

Mitochondrial metabolism is a major determinant of insulin secretion from pancreatic β -cells. Type 2 diabetes evolves when β -cells fail to release appropriate amounts of insulin in response to glucose. This results in hyperglycemia and metabolic dysregulation. Evidence has recently been mounting that mitochondrial dysfunction plays an important role in these processes. Monogenic dysfunction of mitochondria is a rare condition but causes a type 2 diabetes-like syndrome owing to β -cell failure. Here, we describe novel advances in research on mitochondrial dysfunction in the β -cell in type 2 diabetes, with a focus on human studies. Relevant studies in animal and cell models of the disease are described. Transcriptional and translational regulation in mitochondria are particularly emphasized. The role of metabolic enzymes and pathways and their impact on β -cell function in type 2 diabetes pathophysiology are discussed. The role of genetic variation in mitochondrial function leading to type 2 diabetes is highlighted. We argue that alterations in mitochondria may be a culprit in the pathogenetic processes culminating in type 2 diabetes.

Key Words

- ▶ TCA cycle
- ▶ coupling signal
- ▶ oxidative phosphorylation
- ▶ mitochondrial transcription
- ▶ genetic variation

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Introduction

Seminal work in the 1960s and 1970s established that insulin secretion is deficient in type 2 diabetes (T2D) (Bagdade *et al.* 1967, Simpson *et al.* 1968). In the last decade, a number of genome-wide association studies (GWAS) of patients with T2D and metabolic traits have been published (Saxena *et al.* 2007, Scott *et al.* 2007, Sladek *et al.* 2007, Zeggini *et al.* 2007, Gaulton *et al.* 2015, Fuchsberger *et al.* 2016). The number of gene loci conferring increased risk for T2D that have been identified now surpasses one hundred. Those genes, which are most strongly associated with T2D, all seem highly relevant for β -cell function and development (Prasad & Groop 2015). Therefore, there is currently very strong evidence

to support the notion that failure of insulin secretion is the defining event in the pathogenesis of T2D (Kasuga 2006, Muoio & Newgard 2008, Ashcroft & Rorsman 2012, Prasad & Groop 2015).

Accordingly, this breakthrough has changed the focus of much of the research on the pathogenesis of T2D. It has prompted research on genomic processes, e.g., genetic polymorphisms, epigenetic mechanisms, micro and linker RNAs and chromatin structure, which are robustly linked to the disease and centering on the pancreatic β -cell. This notwithstanding, information about how functional and structural abnormalities, resulting from genomic alterations in islets, lead to the perturbation of insulin

secretion, which underlies the disease in humans, is still scant for most candidate genes described thus far (Ng & Gloyn 2013).

Whether deficient β -cell mass plays a pathogenetic role in T2D has been debated for decades (Ahrén 2005). Available data suggest that there is loss of β -cell mass in T2D due to increased apoptosis (Butler *et al.* 2003). However, such reduction cannot by itself account for the impairment of insulin secretion in T2D; β -cell loss in obese and lean individuals is ~60 and 40%, respectively, compared with obese and lean non-diabetic individuals (Butler *et al.* 2003). Thus, a functional defect is still required, in addition to the observed loss of β -cell mass, for insulin secretion to be impaired.

There is consensus that insulin secretion is mainly controlled by metabolism of fuels, foremost glucose, in the pancreatic β -cell (Muoio & Newgard 2008, Ashcroft & Rorsman 2012, Wiederkehr & Wollheim 2012). Stimulation by glucose, and hence β -cell metabolism, is required for effective control of insulin secretion by circulating hormones, paracrine and autocrine mechanisms and neuronal (autonomic and sensory) activity. All these mechanisms combine to enhance insulin secretion in an efficacious fashion (Ahrén 2000). Here, mitochondria play a key role (Wollheim 2000 Mulder & Ling 2009, Nicholls 2016); oxidation of most cellular fuels produces reducing equivalents, driving the electron transport chain and subsequently ATP production via oxidative phosphorylation (OXPHOS). A rise in ATP:ADP ratio closes the ATP-sensitive K^+ channel (K_{ATP}), depolarizes the plasma membrane, opens voltage-gated Ca^{2+} channels and subsequently triggers exocytosis of insulin-containing granules. This pathway is also known as the K_{ATP} -dependent or triggering pathway (Henquin 2000). In addition, mitochondria play an essential role in the K_{ATP} -independent or amplifying pathway of insulin secretion (Maechler *et al.* 2006). Metabolites from mitochondria, reducing equivalents or metabolite fluxes, account for the sustained phase of insulin secretion (Prentki & Corkey 1996), which cannot be upheld by raised intracellular Ca^{2+} alone (Jonas *et al.* 1994), i.e., the result of the triggering pathway. Mitochondria hereby play an essential role in the control of insulin secretion, the deciding pathogenetic event in T2D.

Mitochondria also serve a critical role in control of β -cell mass. Available data suggest that increased apoptosis underlies the loss of β -cell mass observed in islets from patients with T2D (Butler *et al.* 2003). In fact, a loss of β -cell mass by 64% in pancreatectomized patients is associated with diabetes (Meier *et al.* 2012). Apoptotic

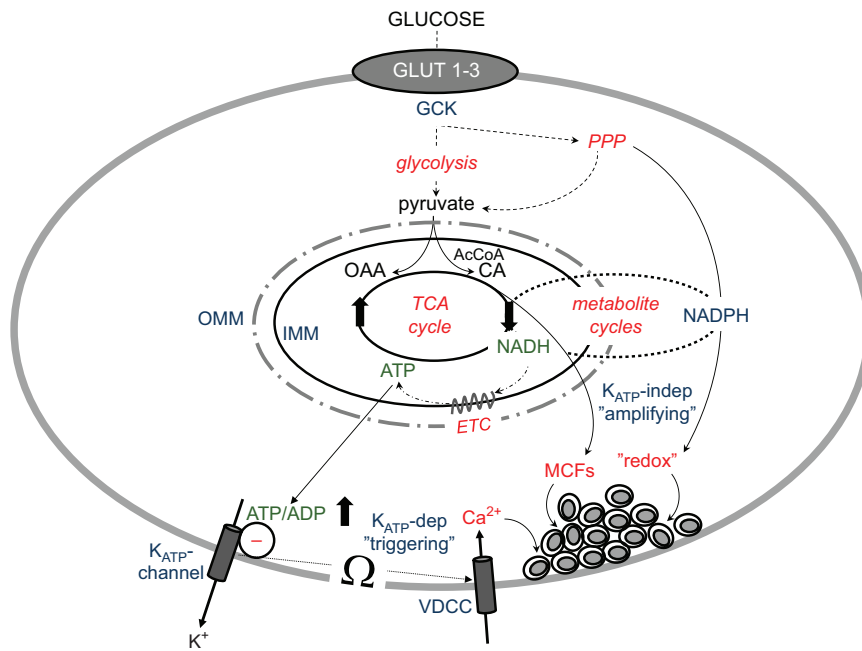
pathways converge in mitochondria, where a cascade of caspase activation by cytochrome C, after being exported from mitochondria, is required for triggering of apoptosis. Interestingly, reduction of cytochrome C levels and translocation have also been proposed as coupling signals in glucose-stimulated insulin secretion (GSIS) (Jung *et al.* 2011). Ageing is another component of the pathogenesis of T2D, again implicating mitochondrial metabolism in β -cell dysfunction (Ling *et al.* 2004, Lee *et al.* 2007, Ling *et al.* 2007, Trifunovic & Larsson 2008). For all these reasons, mitochondria are main players in the pathogenesis of T2D. Here, we will describe the role of mitochondria in β -cells during the events leading to T2D. We will focus on human studies, but where relevant, also include observations from experimental *in vitro* and *in vivo* models.

The consensus model of fuel-stimulated insulin secretion

The manner by which glucose and other fuels control insulin secretion has been extensively reviewed elsewhere (Ashcroft & Rorsman 2012, Wiederkehr & Wollheim 2012, Mulder 2017). Thus, only some of the main aspects will be summarized here for context, as well as some recent work, performed by us, to deepen the understanding of how mitochondria and glycolysis co-operate to control insulin release and how oscillations in metabolism may contribute to this (Fig. 1).

Coupling of mitochondrial and glycolytic metabolism in pancreatic β -cells

Glucose taken up by the β -cell is rapidly phosphorylated by the low-affinity enzyme glucokinase (GCK). Loss-of-function mutations in *GCK* cause a monogenic form of diabetes (maturity onset diabetes of the young 2; MODY2) that result in a right-shift in the dose–response curves of glucose and insulin secretion; however, this does not result in progressive β -cell dysfunction and hyperglycemia does not increase over time (Fajans *et al.* 2001). On the contrary, activating (gain-of-function) mutations in *GCK* cause familial hyperinsulinism (Glaser *et al.* 1998). The end product of glycolysis, the triose pyruvate, is then destined for mitochondrial aerobic metabolism as expression of lactate dehydrogenase A (LDHA) is low in the β -cell; it is in fact considered a ‘disallowed/forbidden β -cell gene’ (Pullen *et al.* 2010, Thorrez *et al.* 2011). To compensate for the lack of LDH, the β -cell is equipped

**Figure 1**

Stimulus-secretion coupling in the pancreatic β -cell. Glucose is taken up into the β -cell in a process termed facilitated diffusion; this occurs in proportion to the extracellular concentration of the hexose. Next, glucose is phosphorylated by glucokinase (GCK), a rate-limiting step in glucose metabolism, followed by glycolysis and further metabolism in the tricarboxylic acid (TCA) cycle, resulting in a rise in the ATP/ADP ratio. This closes an ATP-dependent K⁺ channel (K_{ATP}) in the plasma membrane. As a consequence, voltage-dependent Ca²⁺ channels (VDCC) open and the intracellular surge of Ca²⁺ triggers exocytosis of the hormone. Metabolic coupling factors (MCF) cannot induce insulin secretion by themselves but are thought to amplify insulin secretion. They are supposedly generated by metabolite cycles, which are associated with the TCA cycle, as well as the pentose phosphate pathway (PPP). Examples of MCFs are NADPH, mediating its effect via cellular redox, glutamate or lipid moieties. AcCoa, acetyl-CoA; CA, citrate; ETC, electron transport chain; GLUT, glucose transporters; IMM, inner mitochondrial membrane; OAA, oxaloacetate; OMM, outer mitochondrial membrane; Ω, membrane polarization.

with both the malate-aspartate shuttle and the glycerolphosphate shuttle (Eto *et al.* 1999). Thereby, replenishment of cytosolic NAD⁺ is efficiently accomplished and mitochondrial glucose oxidation maximized. Hence, glycolysis in the β -cell is designed to shuttle glucose, via pyruvate, to the mitochondria, to fuel ATP production. This is further supported by the fact that more than 80% of supplied glucose is completely oxidized to CO₂ and water in the β -cell (Schuit *et al.* 1997). The importance of glucose-dependent pyruvate supply to the mitochondria is further underscored by the fact that reduced expression of GLUT2 is associated with lower glucose oxidation in islets from donors with T2D (Del Guerra *et al.* 2005). On the contrary, expression of the monocarboxylate transporter 1, another 'disallowed β -cell gene' (Pullen *et al.* 2010, Thorrez *et al.* 2011), in the β -cell results in exercise-induced hypoglycemia, as pyruvate released from exercised muscle activates mitochondrial metabolism, resulting in hyperinsulinism (Otonkoski *et al.* 2007).

Hence, glycolytic metabolism is tightly coupled to mitochondrial metabolism. We showed that glucose responsiveness in INS-1-derived clonal cell lines is tightly linked to expression of *LDHA* and that expression of this 'disallowed β -cell gene' in human islets correlates positively with HbA1c levels (Malmgren *et al.* 2009). In a follow-up study, we demonstrated that this circumstance is likely linked to increased stabilization of hypoxia-inducible

factor 1 α , targets of which exhibit increased expression in islets from donors with T2D (Spiegel *et al.* 2014).

In addition to production of ATP – the main trigger of insulin secretion – multiple coupling factors, mainly emanating from mitochondrial metabolism, have been suggested to amplify secretion of the hormone (Wiederkehr & Wollheim 2012, Mulder 2017). Although the significance of many of these factors has been questioned, studies of them have clearly shown that mitochondrial enzymes catalyzing production of putative coupling factors are essential for a robust GSIS. One of the most extensively studied coupling factors is glutamate (Maechler & Wollheim 1999), which also is an important component of the malate-aspartate shuttle. Indeed, activating mutations in the gene encoding glutamate dehydrogenase, result in increased flux from glutamate to the TCA cycle intermediate α -ketoglutarate, likely underlying hyperinsulinism (Stanley *et al.* 1998). However, there are also reports refuting the role of glutamate as a metabolic coupling factor (Bertrand *et al.* 2002).

Although the majority of publications highlights the mitochondria as the source of factors that trigger and amplify secretion of insulin, studies have also implicated purely cytosolic processes in β -cell stimulus-secretion coupling. To exemplify, we reported that the pentose phosphate pathway is highly active in rodent-derived insulin-secreting cells and that inhibition of this pathway

perturbs GSIS from islets (Spégel *et al.* 2013). This pathway may signal to insulin secretion from human β -cells via formation of NADPH and altered redox potential (Ivarsson *et al.* 2005), but potentially also via production of adenylosuccinate, as was recently suggested (Gooding *et al.* 2015). Clearly, GSIS is a complex process, which is influenced by multiple metabolic pathways. Mitochondrial metabolism is indispensable in this machinery, but relies on a close interplay with cytosolic metabolic events. There has been a tendency for metabolic and electrophysiological investigations of β -cells to proceed along parallel tracks with limited interaction. Thus, the crucial importance of plasma membrane potential oscillations, central to the electrophysiology of the intact islet and the control of insulin secretion, may have been overlooked in metabolic investigations.

Transient opening of voltage-activated Ca^{2+} channels (Goehring *et al.* 2012) and exocytosis (Gerencser *et al.* 2015) are tightly coupled to depolarizing spikes in the plasma membrane potential. While 'Spiking' requires inhibition of K_{ATP} -channels by mitochondrial bioenergetics, no associated oscillations in ATP levels or mitochondrial membrane potential were detected (Goehring *et al.* 2012). Glycolytic oscillation could also be eliminated, since exogenous provision of pyruvate fully supports plasma membrane potential oscillations. A critical, but poorly understood, determinant of GSIS, at least in INS-1 832/13 cells, would appear to be the factor that controls spike frequency and amplitude.

Changes in mitochondrial metabolism in type 2 diabetes

Mitochondrial metabolism in β -cells of insulin-resistant mice

At this point, there is little information on how mitochondria in human β -cells adapt when an individual becomes insulin resistant. In contrast, there are data available on how hyperinsulinemia evolves as a compensatory process to account for insulin resistance in skeletal muscle and adipose tissue (Abdul-Ghani *et al.* 2006). Concurrently, the remarkable plasticity of the mitochondria as an organelle has become increasingly appreciated (Detmer & Chan 2007). Mitochondria alter shape and volume in response to changes in cellular function and demands, a plasticity which serves to optimize mitochondrial function and metabolism for every specific situation.

A number of mouse models have been used to study mitochondrial dysfunction in metabolic diseases (Supale *et al.* 2012). To study β -cell mitochondrial metabolism in insulin resistance, we fed C57BL/6J mice a high-fat diet. These mice become severely insulin resistant but do not develop frank diabetes (Ahrén *et al.* 1997), thus allowing studies of the normal processes in pancreatic β -cells that strive to adapt to the diabetogenic metabolic perturbations associated with reduced insulin sensitivity. High-fat diet-fed C57BL/6J mice exhibit basal hyperinsulinemia but retarded glucose disposal. *Ex vivo*, GSIS is blunted, while responses to fuels directly mediating their effects via mitochondrial metabolism are exaggerated (Fex *et al.* 2007a). This metabolic switch from glucose to other mitochondrial fuels parallels enhanced oxidation of palmitate and glutamine in the face of reduced glucose oxidation. The dissociation of effects provoked by glucose and mitochondrial fuels is associated with perturbed GLUT2 localization, suggesting a disruption of glucose metabolism and confirming previous findings (Reimer & Ahrén 2002). The number of mitochondria is unchanged, while mass is increased by ~60% (Fex *et al.* 2007a). Exaggerated deposition of neutral lipids suggests altered lipid flux. Despite these marked metabolic changes, alterations in gene expression of transcription factors and co-activators involved in mitochondrial biogenesis and control of metabolic enzymes and respiratory chain proteins are largely absent; this includes peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α), PGC-1 β , nuclear respiratory factor-1 and mitochondrial transcription factor A (TFAM). Expression levels of the nuclear-encoded mitochondrial protein cytochrome C and the mitochondria-encoded mitochondrial protein cytochrome c oxidase IV (COXIV) remain unaltered during or after 12 weeks of high-fat feeding (Fex *et al.* 2007a).

Mitochondrial bioenergetics in glucotoxicity

Another main pathogenetic paradigm in T2D is the adverse effects of hyperglycemia, termed glucotoxicity. A plethora of studies has addressed it and will not be reviewed here for the sake of space (Poitout *et al.* 2010). We have used INS-1 832/13 cells to understand mitochondrial bioenergetics in glucotoxicity; these cells respond to chronic incubation under hyperglycemic conditions (16.7 mM glucose for 48h, followed by 2h recovery in 2.8 mM glucose) with decreased responsiveness, relative to cells cultured in 2.8 mM glucose, to acute glucose or exogenous pyruvate with respect to mitochondrial

inner membrane hyperpolarization, plasma membrane depolarization and insulin secretion (Goehring *et al.* 2012, 2014, Gerencser *et al.* 2015). 'Bioenergetic overload' or chronic provision of saturating mitochondrial substrate could be eliminated, since cells cultured in low glucose plus pyruvate show no deficit, and do not retain elevated levels of glycolytic or mitochondrial substrates after the 2-h preincubation. Indeed, the 'high glucose' cells display enhanced glycolysis and respiration. We concluded that extra-mitochondrial metabolism upstream of pyruvate, rather than excess mitochondrial substrate provision, underlies glucotoxicity in these insulin-secreting cells (Goehring *et al.* 2012, 2014, Gerencser *et al.* 2015).

Mitochondrial dysfunction in C57BL/6J mice

A strain of C57BL/6J mice exhibits impaired glucose disposal on a normal diet, which could be attributed to impaired insulin secretion (Toye *et al.* 2005). The glucose-unresponsive islets exhibit a normal rise in intracellular Ca^{2+} when stimulated by tolbutamide, and K_{ATP} -channels close in response to ATP. Collectively, these findings point towards a metabolic defect in pancreatic β -cells. Indeed, this strain was found to harbour a 5-exon deletion in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene. This enzyme catalyzes the reversible conversion of NADP^+ and NADH into NADPH and NAD^+ . It is associated with the respiratory chain and has been suggested to protect mitochondria from an overload of reactive oxygen species (ROS), which could impair their function (Jonas *et al.* 2009). Glutathione reductase requires NADPH to reduce glutathione, a component of the detoxifying mechanism.

Recent studies in this substrain of C57BL/6J show that NNT is required for the acute glucose-induced rise in islet NADPH/ NADP^+ ratio, while decreasing the mitochondrial glutathione oxidation (Santos *et al.* 2017). This action depends on a reduction in NADPH consumption by NNT, which relies on glucose and appears to operate in reverse mode rather than from enhancing its forward mode of operation. Interestingly, the insulinotropic effect is accounted for by an enhancement of Ca^{2+} -induced exocytosis rather than influx of Ca^{2+} and mitochondrial events.

Not all strains of C57BL/6J mice harbour the *Nnt* mutation. The 5-exon deletion is lacking from the C57BL/6N strain that we use (Fex *et al.* 2007b), which was derived from the one kept at the NIH Animal Genetic Resource, and embryo-derived into Taconic's facility; C57BL/6J mice are also available from the Jackson

Laboratory, and these were used in the studies on NNT (Toye *et al.* 2005).

Mitochondrial changes in human β -cells in T2D

Development of international centers for islet transplantation has allowed access to human islets for research purposes. As a consequence, there is now information about islet function and mitochondrial alterations in human T2D. Metabolites enhancing mitochondrial metabolism evoke insulin release from T2D islets (Fernandez-Alvarez *et al.* 1994), while GSIS is impaired. Moreover, GSIS, but not arginine- and glibenclamide-stimulated insulin release, is impaired in islets from patients with T2D (Del Guerra *et al.* 2005). Mitochondrial fuels were not tested. Islets, which were obtained from 14 cadaveric donors with T2D, are smaller and contain a reduced proportion of β -cells (Deng *et al.* 2004). In addition, GSIS is impaired while the maximal secretory response, elicited by KCl, is unchanged (Deng *et al.* 2004). These results suggest that islets from donors with T2D have a specific metabolic impairment, since the secretory dysfunction is restricted to glucose stimulation.

Analyses of insulin secretion and mitochondrial function in islets from seven donors with T2D showed that glucose-stimulated, but not arginine-stimulated, insulin secretion is impaired (Anello *et al.* 2005), suggesting the existence of a specific metabolic impairment in β -cells in T2D. Basal ATP levels are elevated in T2D islets, and they fail to respond with a rise in the ATP:ADP ratio, an event known to trigger insulin secretion. Hyperpolarization of the inner mitochondrial membrane upon glucose stimulation is reduced in the diabetic islets. A moderate increase in uncoupling protein-2 (UCP-2) levels is evident, which could uncouple the respiratory chain from ATP production (Anello *et al.* 2005). Interestingly, changes in mitochondrial structure paralleled these functional alterations: there is no increase in mitochondrial number, but their volume is increased, a finding similar to that we reported in C57BL/6J mice on a high-fat diet (Fex *et al.* 2007a). Also, expression of complex I and V is increased in the islets from patients with T2D (Anello *et al.* 2005).

Reduced utilization (Fernandez-Alvarez *et al.* 1994) and oxidation (Lupi *et al.* 2004) of glucose also support that there is metabolic dysfunction in islets from T2D; there is increased oxidative stress (Del Guerra *et al.* 2005), as well as nitrotyrosine levels, in islets from donors with T2D (Anello *et al.* 2005). β -cells from patients with T2D and from non-diabetic donors possess similar numbers of mitochondria but the mitochondrial volume density is

significantly higher in T2D (Anello *et al.* 2005). Activities of metabolic enzymes such as glycerol phosphate dehydrogenase, pyruvate carboxylase and succinyl-CoA:3-ketoacid-CoA transferase are reduced in islets from patients with T2D (MacDonald *et al.* 2009), as is expression of the FAD-linked glycerol phosphate dehydrogenase (Lupi *et al.* 2004), an enzyme-linking glycolysis with TCA cycle metabolism.

These data clearly point towards a role for mitochondria in the pathology of islets in T2D. Nevertheless, while some data support the role of UCP-2 in the pathogenesis of diabetes (Zhang *et al.* 2001), others have raised concerns about whether the protein is indeed an uncoupler (Nicholls 2006, Pi *et al.* 2009). It is unclear whether the modest increase of UCP-2 expression that was observed can account for such a profound effect on ATP production (Anello *et al.* 2005); clearly, much greater increases in UCP-2 expression were employed in experimental studies, where the protein was found to impair insulin secretion (Chan *et al.* 1999). Moreover, changes in metabolism were not analyzed, and such changes could also have exerted the observed effects on ATP production.

Structural dynamics, biogenesis of mitochondria and mitophagy

Mitochondrial structure and number are subject to plasticity (Mannella 2006, Detmer & Chan 2007). Observations we have made in β -cells from insulin-resistant mice (Fex *et al.* 2007a) and in mice where mitochondrial transcription factors have been targeted (Koeck *et al.* 2011), and others have made in human islets from donors with T2D (Anello *et al.* 2005) underscore

the importance of flexibility in mitochondrial number and volume density. These events are highly regulated, providing the cell with organelles that are optimally functional in any given cellular state. The process by which mitochondria are formed is termed mitochondrial biogenesis. It is the result of a balance of fission and fusion of pre-existing mitochondria. In addition to these inter-mitochondrial alterations, changes within mitochondria are also observed (Mannella 2006). Fusion and fission of the inner mitochondrial membrane seem to be the critical events and are supposedly regulatory processes in the organelle; these structural changes may affect diffusion of protons as well as proteins (e.g., cytochrome C) and ATP production.

Removal of damaged mitochondria occurs via mitophagy, a process serving to clear cells of harmful and dysfunctional components (Fig. 2). Here, mitochondria are specifically targeted and selectively removed when damaged or dysfunctional. Mitophagy was first identified in mammalian cells by electron microscopy that identified mitochondrial sequestration in lysosomal compartments following stimulation of hepatocytes with glucagon (De Duve & Wattiaux 1966). Based on this model, and a starvation model, the term mitophagy was coined to describe the engulfment of mitochondria into double-membrane vesicles coated with the autophagy marker LC3 (Kim *et al.* 2007). Indeed, activation of resident mitochondrial proteins that contain LC3-interacting regions is thought to attract autophagosomes to dysfunctional mitochondria. FUNDC1 and the BH3-only family protein BNIP and NIX are such proteins; they are implicated in mitophagy induced by hypoxia and metabolic stress (Zhang & Ney 2009, Liu *et al.* 2012). Additional proteins known to play a major role in mediating mitophagy are PINK1 and Parkin (Park2); they

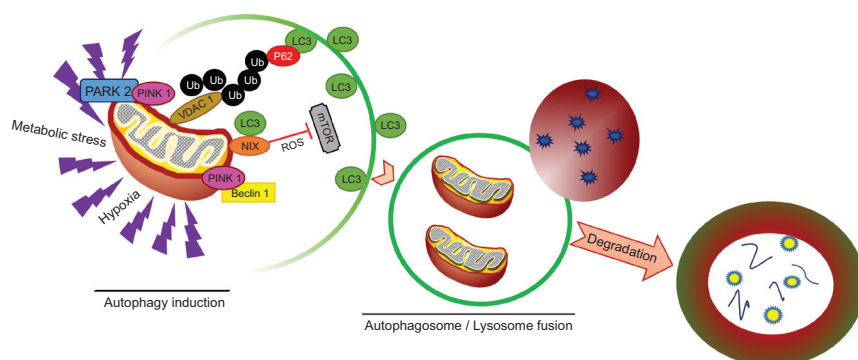


Figure 2

Mitophagy in β -cells. Mitophagy is an autophagic process, whereby dysfunctional mitochondria are eliminated and cellular material reutilized. Mitochondria are thus engulfed into double-membrane vesicles coated with the autophagy marker LC3, which attract autophagosomes to dysfunctional mitochondria. PINK1 and Parkin (Park2) play a major role in mediating mitophagy, as they are involved in processes upstream of autophagosome formation. Ubiquitination (Ub) is key to salvage proteins into amino acids in the proteasome, which may refuel cellular metabolism. Also, pathways controlled by the mammalian target of rapamycin (mTOR), including formation of reactive oxygen species (ROS), play a decisive role in the processes culminating in mitophagy.

are both involved in processes upstream of autophagosome formation and are also expressed in β -cells (Jin & Youle 2012). Interestingly, expression of PDX1, a β -cell master-regulator, was recently implicated in control of mitophagy in β -cells (Soleimanpour *et al.* 2015). Indeed, we observed reduced expression of PDX1 in β -cells from mice lacking transcription factor B2 mitochondrial (TFB2M), where both mitophagy and autophagy were evident (Nicholas *et al.* 2017).

The heterogeneity and subsequent plasticity of mitochondria in β -cells have also recently been demonstrated functionally. Under normal condition, exposure to glucose leads to hyperpolarization of the inner mitochondrial membrane. However, it has been shown that a subpopulation of mitochondria exhibits a lower level of polarization – these mitochondria may even become depolarized (Wikström *et al.* 2007). Glucose and methyl-succinate, a mitochondrial fuel, recruit mitochondria from this pool into a hyperpolarized one. Under glucolipotoxic conditions, the proportion of mitochondria in the hyperpolarized population increases. This has been proposed as a potential mechanism for how glucolipotoxicity arises (Liesa & Shirihai 2013). Moreover, depolarized mitochondria may be more amenable to mitophagy (Twig *et al.* 2008).

Mitofusin 1 and 2 are localized to the outer mitochondrial membrane, together with the optic atrophy 1 protein (OPA1), which is localized to the inner mitochondrial membrane. These proteins are the key regulators of mitochondrial fusion events (Cipolat *et al.* 2004, Frezza *et al.* 2006). The cytosolic dynamin-related protein 1 oligomerizes when mitochondrial fission occurs; it is recruited to the mitochondrion via an interaction with the outer membrane protein FIS1 (Lee *et al.* 2004, Zhang & Chan 2007). This causes constriction and severing of mitochondria. MtDNA copy number in β -cells lacking *Opa1* is unchanged, but the activity of complex IV is significantly decreased, perturbing glucose-stimulated ATP production and GSIS (Zhang *et al.* 2011). It was found that glucose-unresponsive cells exhibit poorer mitochondrial dynamics than glucose-responsive cells (Schultz *et al.* 2016). Overexpression of *FIS1* in glucose-unresponsive cells restores GSIS, and the mitochondrial network becomes more homogeneous. In contrast, silencing of *Fis1* in murine insulin-secreting cells lessens glucose responsiveness and a higher frequency of elongated, likely dysfunctional, mitochondria is encountered (Schultz *et al.* 2016). The prohibitin proteins have also been suggested to play important roles in maintaining mitochondrial integrity and function. Indeed, targeting *Phb2* in mouse

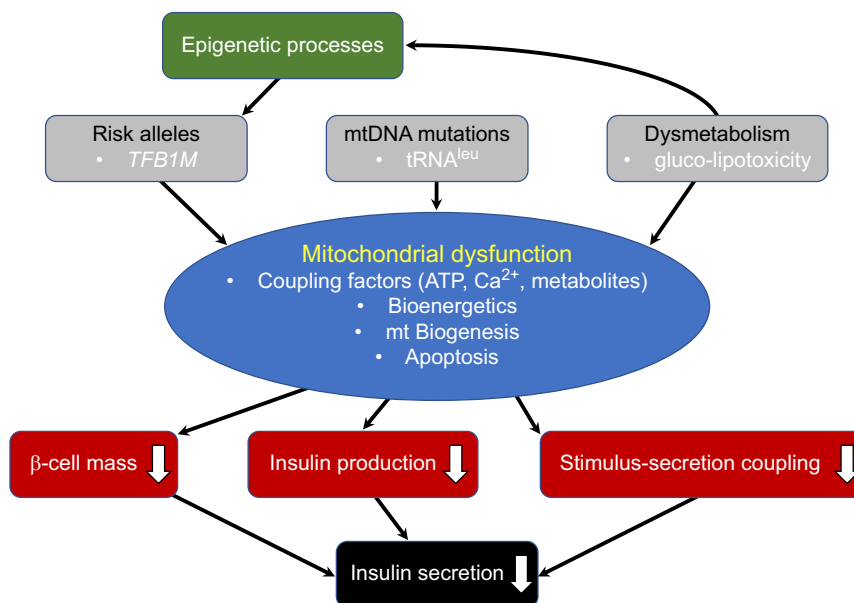


Figure 3

Mechanisms of β -cell mitochondrial dysfunction in type 2 diabetes (T2D). Mitochondrial dysfunction in pancreatic β -cells leading to impaired insulin secretion and T2D may be accounted for by several processes, alone or in combination. A number of mutations in mitochondrial DNA (mtDNA) cause dysfunction of mitochondria in pancreatic β -cells; this is known as mitochondrial diabetes, which is distinct from T2D, since it has a distinct etiology. At least one of the identified risk alleles for T2D, i.e., *TFB1M*, which encodes a factor (transcription factor B1 mitochondrial, TFB1M) that is required for translation of proteins in mitochondria, has been implicated in mitochondrial dysfunction in T2D. Epigenetic processes, influenced by chronic metabolic changes, may interact with gene variants. More generally, metabolic abnormalities, e.g., elevations of circulating lipids and/or glucose, known as lipo- and glucotoxicity, have also been shown to impact negatively on mitochondrial function in β -cells. Mitochondrial dysfunction may negatively affect formation of coupling factors, dynamics of cellular Ca²⁺, and the rise in the ATP/ADP ratio, as well as bioenergetics and biogenesis and induce apoptosis. These processes will hamper production and release of insulin but may also lead to loss of β -cell mass.

β -cells impairs mitochondrial function, leading to a loss of β -cell mass and GSIS (Supale *et al.* 2013); the deficits can be attributed to both loss of function and number of β -cells.

PGC-1 α appears to be a master switch in mitochondrial biogenesis via co-activation of many nuclear receptors and other factors (Handschin & Spiegelman 2006). Its control is mainly exerted by activation of nuclear respiratory factor 1 and 2. TFAM and TFB2M are essential for transcription of the mitochondrial DNA (Ekstrand *et al.* 2004), a prerequisite for mitochondrial biogenesis. Interestingly, a conditional knock out of *Tfam* or *Tfb2m* in β -cells results in impaired fuel-stimulated insulin secretion and diabetes in mice (Silva *et al.* 2000, Nicholas *et al.* 2017).

Genetic regulation of mitochondria and associations with T2D

The mitochondrial genome and diabetes

Mitochondrial ATP production is a pre-requisite for fuel-stimulated insulin secretion (Henquin 2000, Wollheim 2000, Muoio & Newgard 2008). OXPHOS and ATP production occur in a system consisting of five multiprotein complexes with 90 known protein subunits (Scarpulla 2008). The vast majority of these subunits is encoded by the nuclear genome, while 13 subunits are encoded by mitochondrial DNA (mtDNA; Table 1), a 16.6-kb circular double-stranded DNA molecule. Depletion of mtDNA in β -cells results in perturbed mitochondrial metabolism and abrogated fuel-stimulated insulin secretion (Kennedy *et al.* 1996).

Heteroplasmy, i.e., the fact that cells contain multiple mitochondria, which may differ with respect to mtDNA sequence, may explain that work in this area is often contradictory. This notwithstanding, rare mutations in mtDNA in humans have been shown to cause mitochondrial dysfunction, and this may lead to diabetes due to β -cell dysfunction (Maassen *et al.* 2005). These mutations mostly target mitochondrial tRNA genes; for instance, a mutation of 3243 in tRNA^{Leu} causes the syndromes of maternally inherited diabetes and deafness and mitochondrial encephalopathy, lactic acidosis and stroke (Maassen *et al.* 1996). It is also likely that a mutation in the sequence for tRNA^{Leu} causes mitochondrial dysfunction via disrupted expression and/or function of other mitochondrial genes, owing to disturbed protein synthesis. Hybrid cells, containing mitochondria with mutated DNA, exhibit metabolic impairments that may lead to defective β -cell stimulus-secretion coupling

Table 1 The mitochondrial DNA encodes 13 mitochondrial proteins, which constitute critical parts of the electron transport chain.

Mitochondrial genes encoding proteins			
Gene name	Gene bank ID	Protein	Complex
<i>MT-ND1</i>	4535	NADH dehydrogenase 1	I
<i>MT-ND2</i>	4536	NADH dehydrogenase 2	I
<i>MT-ND3</i>	4537	NADH dehydrogenase 3	I
<i>MT-ND4</i>	4538	NADH dehydrogenase 4	I
<i>MT-ND4L</i>	4539	NADH 4L dehydrogenase	I
<i>MT-ND5</i>	4540	NADH dehydrogenase 5	I
<i>MT-ND6</i>	4541	NADH dehydrogenase 6	I
<i>MT-CYB</i>	4519	Cytochrome b	III
<i>MT-CO1</i>	4512	Cytochrome c oxidase I (COX1)	IV
<i>MT-CO2</i>	4513	Cytochrome c oxidase II (COX2)	IV
<i>MT-CO3</i>	4514	Cytochrome c oxidase III (COX3)	IV
<i>MT-ATP6</i>	4508	ATP synthase 6	V
<i>MT-ATP8</i>	4509	ATP synthase 8	V

The remainder of the 37 genes in mitochondrial DNA encodes tRNAs and rRNAs.

(de Andrade *et al.* 2006). This is likely to explain perturbed GSIS in patients harbouring the mutations.

Previous studies have investigated whether common variations in mtDNA are associated with impaired insulin secretion and T2D. A variant at position 16189 has been shown to be associated with elevated fasting insulin concentrations (Poulton *et al.* 1998) and T2D in a population-based case-control study (Poulton *et al.* 2002). Other studies, however, have been unable to replicate this association (Chinnery *et al.* 2005, Mohlke *et al.* 2005, Das *et al.* 2007). Moreover, another study aimed to capture the entire common variation (except the hypervariable D-loop) in mtDNA and examine whether any of these variants are associated with T2D (Saxena *et al.* 2006). No significant associations between common mtDNA variants and T2D or related phenotypes, e.g., insulin secretion, were observed. In contrast, when polymorphisms in the coding region of mtDNA were genotyped in Asian case-control cohorts, carriers of a haplogroup N9a display a significantly reduced risk of T2D (Fuku *et al.* 2007). On balance, it seems that common variation in mtDNA is not a major pathogenetic factor of impaired β -cell function and increased risk of T2D.

Transcriptional and translational control of mitochondrial function - TFB1/2M

Transcription of mtDNA requires a specialized machinery, including factors necessary for promoter recognition,

such as TFAM and TFB2M (Table 2). Mitochondrial transcription factor B1 (TFB1M) is alternatively known in protein databases as S-adenosylmethionine-6-N⁷,N⁷-adenosyl(rRNA)-dimethyltransferase. TFB1M was originally thought to serve as a transcription factor along with its paralog TFB2M; however, it is now established that it mainly functions as a dimethyl transferase. It dimethylates two highly conserved adjacent adenines in a stem-loop structure at the 3' end of the 12S rRNA (Metodiev *et al.* 2009). The covalent modification of 12S rRNA is crucial for the stability of the small mitochondrial ribosomal subunit; mitochondrial translation is severely impaired in its absence.

We have identified a common variant (rs950994) in the human *TFB1M* gene (Koeck *et al.* 2011). It is associated with reduced insulin secretion, elevated postprandial glucose levels and future risk of T2D in females. Carriers of the risk allele exhibit decreased complex I activity and protein level in pancreatic islets (Koeck *et al.* 2011). Because islet *TFB1M* mRNA levels were lower in carriers of the risk allele and correlated with insulin secretion, we examined mice heterozygous for *Tfb1m* deficiency – homozygous *Tfb1m* knockout mice are embryonically lethal (Metodiev *et al.* 2009). Heterozygous *Tfb1m* mice display lower expression of TFB1M in islets and impaired mitochondrial function; they release less insulin in response to glucose both *in vivo* and *in vitro* (Koeck *et al.* 2011). Moreover, silencing of *TFB1M* in clonal insulin-secreting cells impairs complexes of the mitochondrial OXPHOS system. Consequently, nutrient-stimulated ATP generation is reduced, leading to perturbed insulin secretion (Koeck *et al.* 2011).

Table 2 The mitochondrial transcriptional unit consists of the transcription factor A and B2, both of which are required for efficient transcription of the DNA.

Mitochondrial transcriptional machinery			
Gene name	Gene bank ID	Protein	Function
<i>TFAM</i>	7019	Transcription factor A	Controls mitochondrial transcription
<i>TFB1M</i>	51106	Transcription factor B1	Stabilizes mitochondrial 12S rRNA
<i>TFB2M</i>	64216	Transcription factor B2	Controls mitochondrial transcription

TFB1M, on the other hand, is not a bona fide transcription factor. It functions as a methyl transferase, targeting two adjacent adenines in the 3'-end of the mitochondrial 12S ribosomal RNA. If this methylation is lost, ribosomes are destabilized and protein synthesis is abrogated.

These findings suggest that mitochondrial dysfunction is a causal pathogenetic process in the common form of human T2D. However, the underlying mechanisms and the precise role of TFB1M in pancreatic β -cells, however, remained unclear. To further understand the function of TFB1M and how it is associated with T2D, we created a mouse model with a β -cell-specific knockout of *Tfb1m* (β -*Tfb1m*^{-/-}), a mouse which gradually develops diabetes (Sharoyko *et al.* 2014). Prior to the onset of diabetes, β -*Tfb1m*^{-/-} mice exhibit retarded elimination of glucose owing to impaired insulin secretion. β -*Tfb1m*^{-/-} islets secrete less insulin in response to fuels, contain less insulin and secretory granules, as well as showing reduced β -cell mass (Sharoyko *et al.* 2014). Moreover, mitochondria in *Tfb1m*-deficient β -cells are more abundant, displaying a dysmorphic shape. We found that the levels of TFB1M and mitochondrial-encoded proteins, mitochondrial 12S rRNA methylation, ATP production and oxygen consumption rate are all reduced in β -*Tfb1m*^{-/-} islets. Furthermore, ROS levels in response to cellular stress are increased, whereas activation of defense mechanisms is attenuated. We also found signs of apoptosis and necrosis, as well as infiltration of macrophages and CD4⁺ cells in β -*Tfb1m*^{-/-} islets. Our findings of cell death may also be relevant for human T2D, where β -cell loss and apoptosis in islets have been reported (Butler *et al.* 2003).

In summary, our findings demonstrated that *Tfb1m* deficiency in β -cells leads to mitochondrial dysfunction and subsequently diabetes owing to combined loss of β -cell function and mass. These observations reflect pathogenetic processes in human islets: using RNA sequencing, we found that the *TFB1M* risk variant exhibits a negative gene-dosage effect on islet *TFB1M* mRNA levels, as well as insulin secretion (Sharoyko *et al.* 2014). Thus, our studies highlight a pathogenetic and clinically relevant role of TFB1M in T2D.

Whilst the role of TFAM in pancreatic β -cells has been well characterised (Silva *et al.* 2000), it is only recently that we have elucidated the importance of TFB2M in mitochondrial and cellular function of pancreatic β -cells, using a mouse model of a β -cell specific homozygous and heterozygous knockout of *Tfb2m*, as well as in rat clonal insulin-producing cells (Nicholas *et al.* 2017). A striking result of this study was how rapidly these perturbations evolve in *Tfb2m*-deficient mice compared to those previously seen in mice with a β -cell-specific loss of either *Tfam* (Silva *et al.* 2000) or *Tfb1m* (Sharoyko *et al.* 2014). Loss of *Tfb2m* results in decreased expression of mitochondrial-encoded genes, as well as reduced mtDNA content. This leads to severe mitochondrial dysfunction,

characterised by diminished hyperpolarisation of the inner mitochondrial membrane, impaired oxygen consumption and reduced ATP production. Importantly, we found that, in some instances, perturbation of β -cell metabolism leads to activation of compensatory mechanisms, limiting cellular dysfunction and damage. Ultimately, however, widespread mitochondrial dysfunction overwhelms cell-protective systems, such as mitochondrial unfolded protein response, leading to β -cell dysfunction as well as increased apoptosis and loss of β -cell mass. The rapid onset and dramatic consequences of TFB2M deficiency suggest an unforeseen critical role for this mitochondrial transcription factor (Nicholas *et al.* 2017).

Loss of Tfb2m in β -cells from 18-day-old β -*Tfb2m*^{-/-} mice and in INS1 832/13 cells also activates both autophagy and mitophagy (Nicholas *et al.* 2017). An increased number of mitochondria targeted to LC3-positive vesicles are observed, which implies either impaired autophagosome–lysosome fusion or increased autophagy flux through mitophagy. The lack of any major changes in expression of genes and proteins involved in mitophagy, however, supports the notion that mitophagic flux in *Tfb2m*-deficient β -cells is impaired (Nicholas *et al.* 2017). Autophagy is also activated in response to ER stress. DDIT3, an ER stress marker, was increased in β -*Tfb2m*^{-/-} islets and in TFB2M-deficient clonal β -cells (Nicholas *et al.* 2017). DDIT3 also regulates the mitochondrial unfolded protein response, activated in response to mitochondrial stress upstream of the mitophagy pathway. Importantly, if cellular homeostasis is not kept by the autophagosomal machinery, cells could undergo cell death, either by activation of apoptosis or as a result of the inability to survive degradation of large amounts of cytoplasmic contents (De Duve & Wattiaux 1966).

Nuclear-encoded genes and control of mitochondrial function

Mitochondria have arisen due to engulfment of an ancestral α -proteobacterium by archaeobacterium in an endosymbiotic event postulated to have occurred during early evolution (Lang *et al.* 1999). Thus, mtDNA is likely a remnant of primitive bacterial DNA. However, most of these genes have over time transmigrated into the nucleus and exert control over mitochondria as nuclear genes. Therefore, in addition to studies of the possible association of common variation in mtDNA and risk of T2D, it is warranted to establish whether polymorphisms in nuclear-encoded genes with control over mitochondria

or directly involved in mitochondrial function are associated with T2D and associated traits.

Impaired expression of the protein frataxin is responsible for the neurodegenerative disease Friedreich's ataxia. It is caused by an intronic GAA-triplet expansion. Frataxin is thought to play a role in the assembly of iron–sulphur complexes (Puccio & Koenig 2000), which are found in respiratory chain proteins, as well as in the TCA cycle enzymes, such as aconitase. These patients develop a fatal neurological disorder: one-third of patients also develop diabetes (Ristow *et al.* 2003, Ristow 2004, Cnop *et al.* 2013). Moreover, several studies have shown linkage of T2D with the *FRDA* locus (9q13) (Lindgren *et al.* 2002). β -cell-specific knockout mice for frataxin develop insulin-dependent diabetes with loss of β -cell mass, due to cellular growth arrest and increased apoptosis, which is paralleled by an increase of ROS in islets (Ristow *et al.* 2003). Friedreich's ataxia patients exhibit insulin resistance, which is not sufficiently compensated for by increased insulin secretion, evident from a reduced disposition index (Cnop *et al.* 2012). Moreover, silencing of frataxin in insulin-producing cells leads to impaired GSIS and increased rates of apoptosis (Cnop *et al.* 2012). Deficient ATP production in skeletal muscle has also been observed in Friedreich's ataxia patients (Lodi *et al.* 1999), which could contribute to impaired insulin sensitivity in the disease.

NDUFB6 encodes a protein, which is part of complex 1 of the respiratory chain; it is significantly downregulated in skeletal muscle from diabetic patients (Mootha *et al.* 2003). Carriers of A/A at rs540467 in *NDUFB6* display a nominally increased risk of T2D in Scandinavian cohorts (Ling *et al.* 2007). Moreover, a polymorphism at rs629566 in the *NDUFB6* promoter region creates a putative DNA methylation site; it is associated with an age-related decline in muscle *NDUFB6* expression (Ling *et al.* 2007).

The gene encoding PGC-1 α is mapped to chromosomal region 4p15.1. This region has previously been linked to fasting serum insulin concentrations in Pima Indians (Pratley *et al.* 1998). Furthermore, variants, such as Gly482Ser, of *PPARG1A* are associated with T2D and with indices of β -cell function in some, but not all, studies (Ek *et al.* 2001, Lacquemant *et al.* 2002, Muller *et al.* 2003, Oberkofler *et al.* 2004, Vimalleswaran *et al.* 2005, Barroso *et al.* 2006, Sun *et al.* 2006). In isolated rodent islets and clonal β -cells, overexpression of PGC-1 α suppresses insulin secretion (Yoon *et al.* 2003). It has been shown that the *PPARG1A* Gly482Ser variant influences the expression of this gene in human pancreatic islets (Ling *et al.* 2008) and in human skeletal muscle

(Ling *et al.* 2004). It was also demonstrated that expression of *PPARG* is reduced in islets from patients with T2D and that the expression level correlates with GSIS. Moreover, DNA methylation of the *PPARG* promoter is increased in T2D islets (Ling *et al.* 2008). At this point, the experimental *in vitro* work in some rodent models (Yoon *et al.* 2003), and observations on genetic and epigenetic regulation of *PPARG1A* in human pancreatic islets and its correlation with β -cell function are at odds. Clearly, more work in this important area needs to be performed.

Genetic variations in *NRF1* are associated with T2D in a Korean case–control cohort (Cho *et al.* 2005). *UCP-2* has been suggested to influence insulin secretion (Zhang *et al.* 2001, Joseph *et al.* 2002), and carriers of a *UCP2* variant are at reduced risk of developing T2D (Bulotta *et al.* 2005). There is also a study suggesting that the mitochondrial leucyl tRNA synthase (*LARS2*) gene represents a T2D susceptibility gene (Maassen *et al.* 2005). Together, these studies highlight the important, but as of yet not completely resolved, role of genetic variation for mitochondrial function in the pathogenesis of T2D.

Concluding remarks

In this review, we have highlighted a number of factors and circumstances that underscore the pathogenetic role of mitochondrial perturbations in T2D (Fig. 3). Seemingly, metabolic disruption of β -cell function, which is a likely culprit in T2D, can be attributed to changes in mitochondrial enzymes and structural proteins as well as mitochondrial mass and morphology. These mitochondrial functions are likely subject to control by genetic and epigenetic mechanisms. The future will tell if these processes have a utility as therapeutic targets in T2D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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