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VIEWPOINT

Mitochondrial Dysfunction and Type 2 Diabetes

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Maintenance of normal blood glucose levels depends on a complex interplay between the insulin responsiveness of skeletal muscle and liver and glucose-stimulated insulin secretion by pancreatic β cells. Defects in the former are responsible for insulin resistance, and defects in the latter are responsible for progression to hyperglycemia. Emerging evidence supports the potentially unifying hypothesis that both of these prominent features of type 2 diabetes are caused by mitochondrial dysfunction.

Type 2 diabetes is the most common metabolic disease in the world. In the United States, it is the leading cause of blindness, end-stage renal disease, and nontraumatic loss of limb, with associated health care costs estimated to exceed $130 billion per year (*1*). Of even greater concern, type 2 diabetes is rapidly becoming a global pandemic and is projected to afflict more than 300 million individuals worldwide by the year 2025, with most of the increase occurring in India and Asia (*2*). Although the primary cause of this disease is unknown, it is clear that insulin resistance plays an early role in its pathogenesis and that defects in insulin secretion by pancreatic β cells are instrumental in the progression to hyperglycemia. Here, we explore the potentially unifying hypothesis that these two prominent features of type 2 diabetes are both attributable to defects in mitochondria, the organelles that provide energy to the cell.

Role of Intracellular Fatty Acid Metabolites in Insulin Resistance

Several lines of evidence indicate that insulin resistance is an early feature of type 2 diabetes. First, virtually all patients with type 2 diabetes are insulin-resistant, and prospective studies have shown that this insulin-resistant state develops 1 to 2 decades before the onset of the disease (*3–5*). Second, insulin resistance in the offspring of parents with type 2 diabetes is the best predictor for later development of the disease (*6*). Lastly, perturbations that reduce insulin resistance prevent the development of diabetes (*7*).

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Skeletal muscle and liver are the two key insulin-responsive organs responsible for maintaining normal glucose homeostasis, and their transition to an insulin-resistant state accounts for most of the alterations in glucose metabolism seen in patients with type 2 diabetes. Before considering whether mitochondrial dysfunction contributes to the development of insulin resistance in these organs, it is first important to understand the cellular mechanisms responsible for insulin resistance. As discussed by Lazar (*8*), there is growing evidence that circulating cytokines secreted by fat tissue can modulate the insulin responsiveness of liver and muscle. However, fatty acids (*9*) and/or intracellular fatty acid metabolites such as fatty acyl coenzyme As (fatty acyl CoAs) (*10, 11*), diacylglycerol (*10, 11*), or ceramides (*12*) are also thought to play a critical role.

Over 40 years ago, Randle *et al.* demonstrated that fatty acids caused insulin resistance in an in vitro rat muscle preparation, and they hypothesized that this occurred by a substrate competition mechanism (*13*). According to his model, increased oxidation of muscle fatty acids would produce increased levels of intracellular acetyl CoA and citrate, which in turn would inhibit, respectively, two enzymes involved in glucose utilization, pyruvate dehydrogenase and phosphofructokinase. Inhibition of the glycolytic pathway at these steps would increase intracellular glucose and glucose-6-phosphate concentrations, ultimately resulting in reduced insulin-stimulated glucose uptake.

More recent studies using ^{13}C and ^{31}P magnetic resonance spectroscopy (MRS) have shown that this mechanism for fatty acid–induced insulin resistance is untenable in human skeletal muscle (*14*); rather, fatty acids appear to cause insulin resistance by directly inhibiting insulin-stimulated glucose transport activity (*15*). This inhibition is likely because of the accumulation of intracellular

fatty acyl CoAs and diacylglycerol, which then activate critical signal transduction pathways that ultimately lead to suppression of insulin signaling (Fig. 1). One might therefore predict that any metabolic perturbation that promotes the accumulation of fatty acids in liver and/or muscle and/or any defect in the ability of these organs to metabolize fatty acids might result in insulin resistance (*10*). Indeed, defects in adipocyte metabolism, which occur in conditions such as severe lipodystrophy (*16*), can result in the former, and it has become increasingly evident that defects in mitochondrial fatty acid oxidation can result in the latter and may be responsible for the more common forms of insulin resistance.

Mitochondrial Dysfunction, Intracellular Fatty Acids, and Insulin Resistance

It is well established that mitochondrial function is required for normal glucose-stimulated insulin secretion from pancreatic β cells. In addition, maternally inherited defects in mitochondrial DNA that disrupt mitochondrial function are known to cause an insulin-deficient form of diabetes resembling type 1 diabetes (*17*). However, recent MRS studies of humans suggest that more subtle defects in mitochondrial function might also play a role in the pathogenesis of insulin resistance and type 2 diabetes. Petersen *et al.* found that in comparison with matched young controls, healthy lean elderly subjects had severe insulin resistance in muscle, as well as significantly higher levels of triglycerides in both muscle and liver (*18*). These changes were accompanied by decreases in both mitochondrial oxidative activity and mitochondrial adenosine triphosphate (ATP) synthesis. These data support the hypothesis that insulin resistance in humans arises from defects in mitochondrial fatty acid oxidation, which in turn lead to increases in intracellular fatty acid metabolites (fatty acyl CoA and diacylglyerol) that disrupt insulin signaling (Fig. 1).

Alterations in mitochondrial DNA (MtDNA) have been correlated with human aging in several previous studies, and a recent study of genetically manipulated mice provided evidence that such alterations may play a causal role in aging (*19*). Whether the mitochondrial

dysfunction detected in the elderly subjects studied by Petersen et al. (18) is related to age-associated accumulation of MtDNA mutations is not yet clear.

Other studies using the MRS technique have revealed similar decreases in mitochondrial activity and increases in intramyocellular fat content in young insulin-resistant offspring of parents with type 2 diabetes, a group that has a strong tendency to develop diabetes later in life (20). In addition, in comparison with insulin-sensitive controls, the insulin-resistant subjects were found to have a lower ratio of type 1 to type 2 muscle fibers. Type 1 fibers are mostly oxidative and contain more mitochondria than type 2 muscle fibers, which are more glycolytic. Conceivably, these individuals may have fewer muscle mitochondria, possibly because of decreased expression of nuclear-encoded genes that regulate mitochondrial biogenesis, such as peroxisome proliferator-activated receptory coactivator 1α [PGC-1α (21) and PGC-1β (22)]. Microarray studies support this idea: PGC-1α–responsive genes are down-regulated in obese Caucasians with impaired glucose tolerance and type 2 diabetes (23), and PGC-1α and PGC-1β are themselves down-regulated in both obese diabetic and overweight nondiabetic Mexican-Americans (24).

Alternatively, the reduction in mitochondrial oxidative-phosphorylation activity in insulin-resistant individuals could be due not to mitochondrial loss but rather to a defect in mitochondrial function. This hypothesis is supported by muscle biopsy studies. In one study, the activity of mitochondrial oxidative enzymes was found to be lower in type 2 diabetic subjects (25), and in another, the activity of mitochondrial rotenone-sensitive nicotinamide adenine dinucleotide oxidoreductase [NADH:O(2)] was found to be lower (26). However, in contrast to the MRS studies, these studies were performed with isolated mitochondria obtained from diabetic subjects who were also obese. Because obese individuals have also been shown to have smaller mitochondria with reduced bioenergetic capacity compared with lean controls (26), the mitochondrial abnormalities in these subjects might be related to obesity rather than to insulin resistance. The role of the obese state in the down-regulated expression of the PGC-1α and PGC-1β

genes discussed above (23, 24) is an important question that remains to be answered.

Mitochondrial Dysfunction and Insulin Secretion by Pancreatic β Cells

Many obese individuals with marked insulin resistance do not develop frank diabetes. In these individuals, the pancreatic β cells adapt to meet the body's markedly increased demand for insulin. This adaptation involves expansion of β cell mass, as well as maintenance of normal responsiveness of β cells to glucose. Conversely, in obese individuals destined to develop type 2 diabetes, β cells do not secrete enough insulin to compensate for the increased demand. This β cell failure is likely caused by inadequate expansion of the β cell mass and/or failure of the existing β cell mass to respond to glucose (27).

β cell mass is governed by several factors, including β cell size, the rate of β cell replication and/or differentiation, and the rate of β cell apoptotic cell death. Although difficult to quantify, β cell mass appears to be decreased

in individuals with type 2 diabetes relative to matched individuals with similar degrees of insulin resistance (28, 29). Although the cause of this relative decrease in β cell mass is unknown, increased rates of apoptosis may play an important role (27, 28, 30). The signals to and from mitochondria that regulate apoptosis in β cells and the effect of the prediabetic milieu on these signals are incompletely understood (31, 32) but are likely to be a fertile area of future investigation.

Numerous studies have documented that, in individuals with type 2 diabetes, β cells do not sense glucose properly and therefore do not release appropriate amounts of insulin (33). Glucose sensing requires oxidative mitochondrial metabolism, leading to the generation of ATP (34). This increases the ratio of ATP to adenosine diphosphate (ADP) in the β cell, which then initiates the following chain of events: inhibition of the cell's ATP/ADP-regulated potassium channel (K_{ATP}), plasma membrane depolarization, opening of a voltage-gated calcium channel, calcium
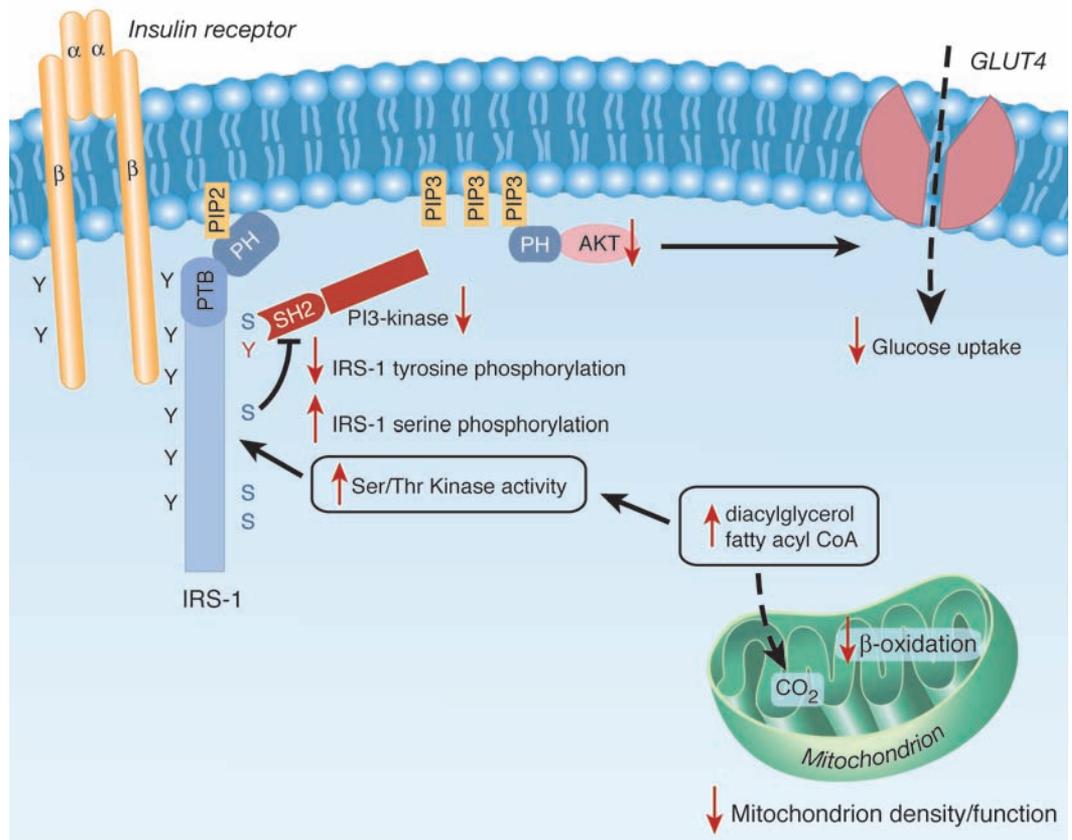

Fig. 1. Potential mechanism by which mitochondrial dysfunction induces insulin resistance in skeletal muscle. In the depicted model, a decrease in mitochondrial fatty acid oxidation, caused by mitochondrial dysfunction and/or reduced mitochondrial content, produces increased levels of intracellular fatty acyl CoA and diacylglycerol. These molecules activate novel protein kinase C, which in turn activates a serine kinase cascade [possibly involving inhibitor of nuclear factor κB kinase (IKK) and c-Jun N-terminal kinase–1], leading to increased serine phosphorylation (pS) of insulin receptor substrate–1 (IRS-1). Increased serine phosphorylation of IRS-1 on critical sites (e.g., IRS-1 Ser[307]) blocks IRS-1 tyrosine (Y) phosphorylation by the insulin receptor, which in turn inhibits the activity of phosphatidyl inositol 3-kinase (PI 3-kinase). This inhibition results in suppression of insulin-stimulated glucose transport, the process by which glucose is removed from the blood. PIP3 indicates phosphatidylinositol 3,4,5-trisphosphate; PTB, phosphotyrosine binding domain; PH, pleckstrin homology domain; SH2, src homology domain.

influx, and secretion of insulin (Fig. 2). Although insulin secretion is also modulated by a number of stimuli that operate outside this pathway, it is clear that oxidative mitochondrial metabolism is central to glucose-stimulated insulin secretion (34).

The critical role of mitochondria is evident from the rare hereditary disorders in which diabetes with β cell dysfunction have been traced to specific mutations in the mitochondrial genome (34, 35). Given the central role of mitochondria in glucose sensing, it is possible that

decreased mitochondrial function in β cells, analogous to that observed in skeletal muscle (described above), might predispose individuals to develop β cell dysfunction and type 2 diabetes. However, because of the difficulties in obtaining β cell samples for analyses, this
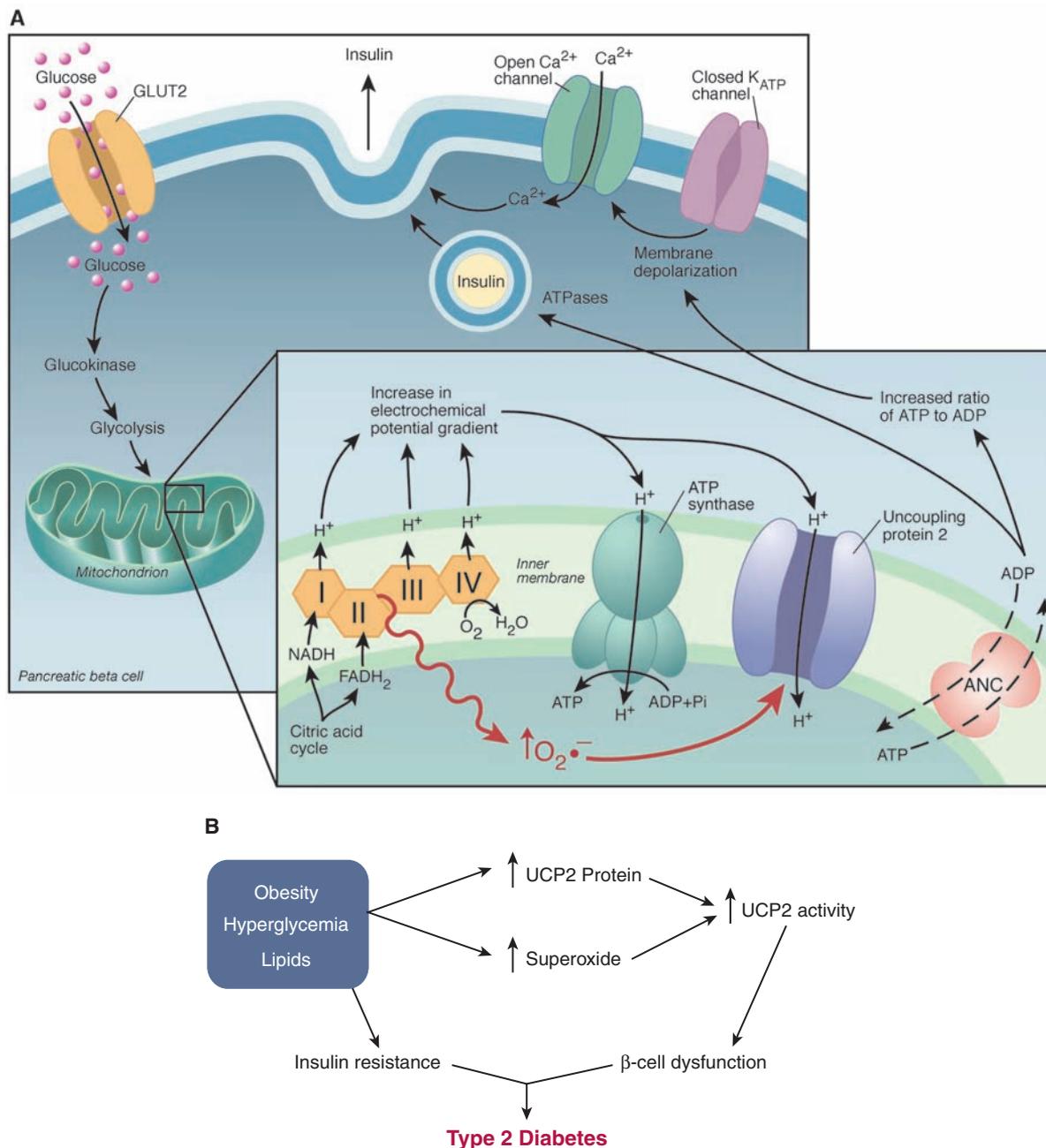

Fig. 2. Potential mechanism by which UCP2-mediated mitochondrial dysfunction disrupts insulin secretion from pancreatic β cells. (**A**) UCP2, superoxide, and glucose-stimulated insulin secretion. Insulin secretion is coupled to glucose metabolism by the subsequent increase in the ATP/ADP ratio arising from glucose oxidation, which closes K^{ATP} channels. This depolarizes the plasma membrane, opening voltage-gated Ca^{2+} channels with the influx of Ca^{2+} stimulating secretion of insulin. UCP2 decreases glucose-stimulated insulin secretion by increasing proton leak across the mitochondrial inner membrane, diverting energy stored within electrochemical potential gradient away from ATP synthase, thereby decreasing the yield of ATP from glucose. Superoxide generated by the electron transport chain stimulates proton leak activity of UCP2 protein, thereby decreasing glucose-stimulated insulin secretion. [Figure adapted with permission from (58). Copyright 2001, Massachusetts Medical Society. All rights reserved.] (**B**) The effects of obesity, hyperglycemia, and lipids on UCP2. In the obese state, hyperglycemia, and high lipid levels each induce expression of UCP2 protein in pancreatic β cells. These stimuli also increase production of superoxide by the electron transport chain. As a result, UCP2 is activated, leading to a marked increase in UCP2-mediated proton leak. This proton leak impairs glucose-stimulated insulin secretion, resulting in β cell dysfunction. β cell dysfunction and insulin resistance in muscle, liver, and fat are characteristic features of type 2 diabetes.

interesting hypothesis has not yet been directly tested.

β cell dysfunction in type 2 diabetes is thought to be secondary to increased exposure of β cells to glucose (glucotoxicity) and/or lipids (lipotoxicity), frequently associated with the obese, insulin-resistant state (36–38). A number of hypotheses have been proposed to explain how these conditions induce β cell dysfunction (36–38). One of these hypotheses, discussed below, focuses on changes in the expression and function of a mitochondrial inner membrane protein called uncoupling protein–2 (UCP2) (39–42). To understand the role of UCP2, it is first necessary to review relevant aspects of mitochondrial oxidative metabolism.

Oxidative metabolism of glucose involves the transfer of energy stored within the carbon bonds of glucose to the third phosphate bond of ATP (Fig. 2A). This complex reaction begins as electrons within the carbon bonds are transferred to the dinucleotide electron carriers, NADH and flavin adenine dinucleotide ($FADH_2$). These in turn donate electrons to the mitochondrial electron transport chain, a multiprotein unit grouped into four complexes (I to IV), all located within the mitochondrial inner membrane. Ultimately, the electrons are funneled to their final destination, reduction of oxygen to water. Complexes I, III, and IV are reduction- and oxidation-driven proton pumps that use energy carried by the electrons to pump protons out of the matrix, creating a proton electrochemical potential gradient across the mitochondrial inner membrane (Fig. 2A). These protons then reenter the mitochondrial matrix via ATP synthase with the use of energy stored within the electrochemical gradient to drive synthesis of ATP from ADP. UCP2 is an integral membrane protein that, when activated, leaks protons across the inner membrane (43), hence uncoupling glucose oxidative metabolism from ATP production. Because it decreases the amount of ATP generated from glucose, UCP2 is predicted to negatively regulate glucose-stimulated insulin secretion. Experimental evidence has shown that this is indeed the case. Forced overexpression of UCP2 in β cells in cell culture decreases glucose-stimulated insulin secretion (40), whereas targeted inactivation of the UCP2 gene in mice has the opposite effect (39). Importantly, heterozygosity for a null UCP2 allele produces an effect that is intermediate between those observed in wild-type and homozygous mice, indicating that relatively small changes in UCP2 expression have meaningful effects on glucose-stimulated insulin secretion (39). Thus, UCP2 exerts substantial negative control over glucose-stimulated insulin secretion.

Does increased expression of UCP2 have a causal role in β cell dysfunction in type 2 diabetes? This idea is supported by the finding that UCP2 expression is stimulated, in vitro and in vivo, by hyperglycemia (glucotoxicity) and lipid fuels (lipotoxicity), and in animal models with type 2 diabetes (39, 41, 42, 44–47). Moreover, genetic deficiency of UCP2 has been found to greatly improve β cell function in rodent models of obesity/diabetes (38, 40). Similarly, genetic deficiency of UCP2 prevents β cell dysfunction in in vitro models of glucotoxicity and lipotoxicity (42, 48, 49). Together, these data from experimental models suggest that UCP2 plays an important pathogenic role. A similar role seems likely in human type 2 diabetes, because UCP2 is expressed in human β cells and its expression is increased by hyperglycemia (50). Additionally, a polymorphism in the promoter of the human UCP2 gene that appears to increase UCP2 expression has been linked to reduced insulin secretion and higher frequency and/or earlier onset of type 2 diabetes (51–53).

It was recently discovered that superoxide, a byproduct of electron transport chain activity, stimulates the proton leak activity of UCP2 when added exogenously to isolated mitochondria (54) or when generated in situ within intact β cells (42) (Fig. 2A). The mechanism by which superoxide activates UCP2 is unknown but may involve the generation of free radical intermediates (55). Stimulation of UCP2 activity by superoxide is relevant to the development of β cell dysfunction, because superoxide production is increased in β cells of rodents with type 2 diabetes (42, 56) and in cultured β cells exposed to hyperglycemia and elevated levels of lipids (42, 56). This increase in superoxide, coupled with the increase in UCP2 protein, results in a large stimulation of proton leak, ultimately leading to β cell dysfunction (Fig. 2B). Indeed, removal of endogenous superoxide in β cells that are unresponsive to glucose, either because of in vitro exposure to hyperglycemia or because of the in vivo obese or diabetic state, acutely inhibits UCP2 activity and restores glucose-stimulated insulin secretion (42). Thus, the superoxide-UCP2 proton leak pathway is an important contributor to β cell dysfunction and may play an important role in the pathogenesis of type 2 diabetes (Fig. 2B). These findings raise the possibility that UCP2 inhibitors could be used to prevent or treat type 2 diabetes.

Conclusions

A series of diverse experiments support the proposal that mitochondrial defects play a critical role in two prominent features of type 2 diabetes: insulin resistance and pancreatic β cell dysfunction. Several important questions remain to be answered: (i) Is the reduction in mitochondrial function in vivo due to mitochondrial loss, functional defects in the mitochondria, or both? (ii) Is the down-regulation of PGC-1α/PGC-1β responsive genes a primary or secondary event in the pathogenesis of type 2 diabetes? If it is a primary event, what are the upstream genes responsible for their altered expression? (iii) Does UCP-2 play an important role in β cell dysfunction in patients with type 2 diabetes? Answers to these questions may provide new pharmacologic targets for the prevention and treatment of the world's most common metabolic disease.

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