

## Mitochondrial Dysfunction in Obesity and Diabetes

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### Abstract

Mitochondrial abnormalities have been reported in both insulin-deficient and insulin-resistant states and in the related condition of obesity. The phrase 'mitochondrial dysfunction' is often used in this regard. However, beyond dysfunction, there is evidence for defects in mitochondrial biogenesis, number, morphology, and dynamics (fusion and fission). Diabetes and obesity are also associated with the overproduction of mitochondrial reactive oxygen species (ROS), leading to mitochondrial and cellular oxidative damage. This, in turn, contributes to the development and progression of diabetic complications and to worsening of the diabetic state *per se*. Here we will review the evidence for mitochondrial abnormalities in type 2 diabetes and obesity and consider underlying mechanisms. We will also discuss potential therapeutic interventions targeted at mitochondria.

### Keywords

Diabetes, obesity, mitochondria, superoxide, reactive oxygen species, respiration

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The pathogenesis of type 2 diabetes includes pancreatic  $\beta$ -cell dysfunction and insulin resistance; most importantly in hepatocytes, myocytes, and adipocytes. Type 2 diabetes is well known to be a progressive disorder<sup>1</sup> characterized by deteriorating capacity for insulin release and action. Both defects are recognizable early on and present even in non-diabetic offspring of patients with type 2 diabetes.<sup>2-4</sup> However, there is general consensus that insulin sensitivity is impaired early, whereas worsening of hyperglycemia over time is related to  $\beta$ -cell dysfunction. Hence, insulin resistance in obesity is strongly associated with type 2 diabetes; the major reasons include fatty acid delivery to the liver (especially from intra-abdominal fat) and other organs and adipose tissue release of inflammatory cytokines and peptides that impair insulin signaling and islet insulin secretion. At cellular and molecular levels the pathogenesis of diabetes becomes far more complex. Here, the focus will be on the role of mitochondria and mitochondrial reactive oxygen species (ROS) in mediating the general mechanisms.

### Mitochondrial Function by Cell Type

Our approach will be to consider mitochondrial function within the most relevant cell types including myocytes, hepatocytes, adipocytes, and islet  $\beta$ -cells as well as non-insulin-sensitive cells representing targets for complications. We will attempt to integrate defects in a way consistent with the pathophysiology of diabetes and its complications.

### Muscle

Impaired oxidative phosphorylation by muscle mitochondria is associated with insulin resistance. Nicotinamide adenine dinucleotide (NADH) oxidoreductase and citrate synthase activity were noted to be reduced in mitochondria isolated from human muscle biopsy specimens obtained from diabetic and obese subjects compared with lean subjects.<sup>5</sup> Mitochondrial oxidative phosphorylation has also been assessed in human muscle *in vivo* using nuclear magnetic resonance (NMR) spectroscopy. In this way, Szendroedi et al.<sup>6</sup> demonstrated defective muscle adenosine triphosphate (ATP) synthetic flux in subjects with type 2 diabetes, even under hyperinsulinemic, hyperglycemic conditions.

Studies using <sup>13</sup>C NMR to assess tricarboxylic acid (TCA) flux rates along with <sup>31</sup>P NMR to assess phosphorylation of adenosine diphosphate (ADP) demonstrated impaired skeletal muscle oxidative phosphorylation, increased intra-myocellular lipid, and decreased TCA cycle substrate oxidation in insulin-resistant offspring of individuals with type 2 diabetes.<sup>7-9</sup> Similar findings were reported in the muscle of elderly subjects with insulin resistance compared with young controls.<sup>10</sup> In another study, type 2 diabetes was characterized by increased lipid content in myocytes as well as a relative decrease in the proportion of enzymes regulating oxidative, as opposed to glycolytic, metabolism.<sup>11</sup>

Furthermore, exercise tolerance and recovery of intracellular phosphocreatine post-exercise are impaired in subjects with type 2 diabetes consistent with mitochondrial dysfunction.<sup>12,13</sup>

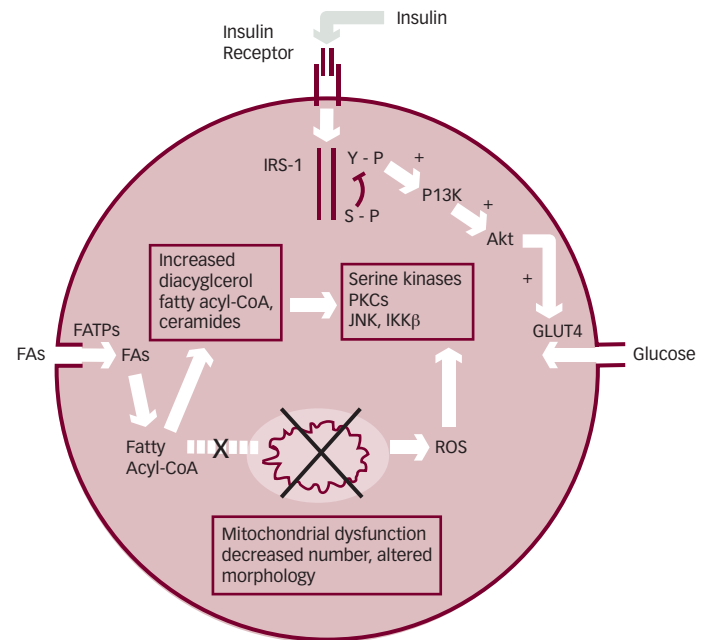
Other studies of mitochondria or saponin-permeabilized muscle fibers isolated from humans with type 2 diabetes showed impairments in oxygen consumption<sup>14,15</sup> even when normalized for mitochondrial content.<sup>15</sup> However, another report showed that mitochondrial respiration was normal when expressed per DNA content, suggesting that the impairment was not in function but in number of mitochondria.<sup>16</sup> In a recent study of mitochondria isolated from insulin-resistant, obese, but not diabetic humans compared with insulin-sensitive lean subjects, maximal respiration rates were increased in the obese subjects<sup>17</sup> associated with increased H<sub>2</sub>O<sub>2</sub> production. That study also showed that the mitochondria of the insulin-resistant subjects maintained a higher extra-mitochondrial ATP free energy suggesting a higher thermodynamic driving force thought responsible for the increase in ROS production.

Perturbed mitochondrial biogenesis may be a cause of reduced mitochondrial number as well as reduced capacity for oxidative phosphorylation in diabetes. An important factor driving mitochondrial biogenesis at the molecular level is the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) coactivator (PGC-1 $\alpha$ ), representing a coactivator of nuclear transcription factors, PPAR $\gamma$  and PPAR $\alpha$ , and several other genes involved in energy homeostasis.<sup>18–20</sup> Muscle biopsy studies showed that PGC-1 $\alpha$  is reduced in patients with type 2 diabetes<sup>21–23</sup> as well as in family members of individuals with type 2 diabetes.<sup>23</sup>

There have been recent studies of intrinsic respiration by heart and skeletal muscle mitochondria isolated from tissues of type 2 diabetic models. Boudina et al.<sup>24</sup> examined heart mitochondrial function in saponin-permeabilized heart muscle fibers obtained from insulin-resistant, diabetic, leptin-receptor-deficient db/db mice. These investigators reported decreased respiration on complex I substrates and on palmitoyl-carnitine, associated with proportionally reduced ATP production and decreased content of the F1 alpha-subunit of ATP synthase. Our laboratory recently examined respiratory function in heart and skeletal muscle mitochondria isolated from high-fat-fed rats also subject to low-dose streptozotocin (STZ) to mimic impaired glucose tolerance.<sup>25</sup> Our data revealed no change in respiration in these mildly hyperglycemic rats compared with high-fat-fed controls.

High-fat feeding associated with insulin resistance<sup>26</sup> results in downregulation of several human genes involved in oxidative phosphorylation and mitochondrial biogenesis.<sup>27</sup> Metabolomic studies in rodents suggest that enhanced fat metabolism seen with high fat feeding overloads muscle mitochondria with oxidation products in a way that restricts their ability to completely metabolize these products to CO<sub>2</sub>. Koves et al.<sup>28</sup> showed that high-fat feeding increased acylcarnitines representing products of incomplete  $\beta$ -oxidation of fatty acids. This was associated with decreased TCA intermediates and an inability of mitochondria to switch from using fat-derived substrates to the glucose-derived metabolite, pyruvate. These perturbations could be prevented by restricting the mitochondrial entry of fatty acids by knock-out of malonyl coenzyme-A (malonyl-CoA) decarboxylase (MCD). These data were interpreted to imply that the mitochondria of

**Figure 1: Inhibition of Insulin Signaling by Mitochondrial Dysfunction**



The schematic diagram depicts the consequences of excess fatty acyl-coenzyme A (acyl-CoA) and reactive oxygen species (ROS) production on the insulin signaling pathway leading to the insulin responsive glucose transporter, GLUT4. In response to an insulin-induced conformational change in the internal or  $\beta$ -subunits of the insulin receptor (IR), tyrosine residues undergo autophosphorylation and the IR acquires tyrosine kinase activity, leading to phosphorylation of the insulin receptor substrate-1 (IRS-1). This initiates a signaling cascade activating serine/threonine kinase protein kinase B (Akt) and translocation of the GLUT4 to the cell membrane. GLUT4 fusion with the membrane results in glucose uptake by facilitated diffusion. Mitochondrial dysfunction opposes insulin signaling in two ways: (i) by interfering with oxidation of fatty acyl-CoA and consequent accumulation of intracellular lipid and diacylglycerol and (ii) by generation of ROS. Both processes activate serine kinase reactions leading to serine phosphorylation of IRS-1 and interference with insulin signal transduction. FA = fatty acid; FATPs refers to various transport proteins active in fatty acid uptake.

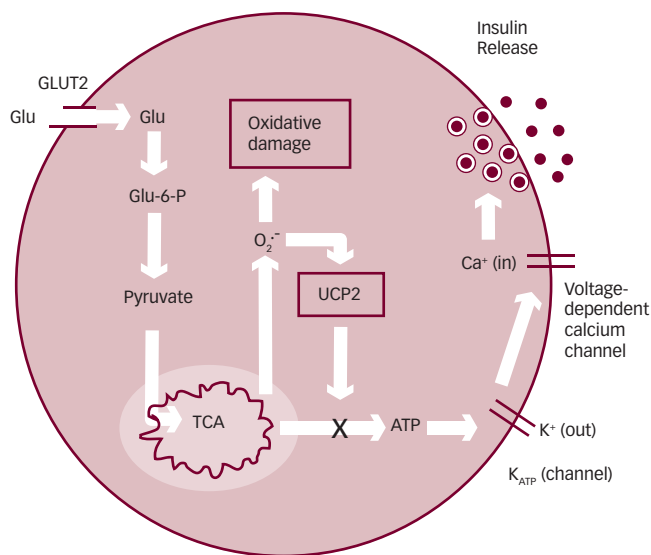
high-fat-fed rodents are exposed to increased, rather than decreased, rates of  $\beta$ -oxidation, but become impaired and unable to handle the high rate of flux.

How do the above findings translate to insulin resistance? As indicated in Figure 1, there is a rationale whereby mitochondrial dysfunction (or reduced mitochondrial density) might impair insulin signaling.<sup>29</sup> Mitochondrial dysfunction should lead to impaired fatty acid oxidation, resulting in increased intracellular fatty acyl-CoA and diacylglycerol content with consequent activation of protein kinase C.<sup>30,31</sup> This, in turn, triggers a serine kinase cascade ultimately resulting in serine phosphorylation of insulin receptor substrate type 1 (IRS-1). This has the consequence of blocking the tyrosine kinase activity of the insulin receptor on IRS-1, thereby blocking the insulin signaling pathway.

### Liver

Any mitochondrial-mediated alteration in energy hemostasis in hepatocytes would impact the balance between gluconeogenesis, glycolysis, and glycogen storage/breakdown, thereby impacting glycemia in diabetic states. In hepatocytes, PGC-1 $\alpha$  regulates gluconeogenesis and fat oxidation.<sup>32</sup> The NAD<sup>+</sup>-dependent histone deacetylase, SIRT1, increases gluconeogenesis in liver cells through its effects on PGC-1.<sup>33</sup> Consistent with the above, mice deficient in PGC-1

**Figure 2: Mitochondria and Insulin Secretion**



Glucose-induced insulin release is dependent on mitochondrial adenosine triphosphate (ATP) generation and affected by both mitochondrial reactive oxygen species (ROS) and uncoupling protein-2 (UCP2). ATP is required to open potassium ATP channels, thereby inducing entry of calcium and insulin release from storage granules. If hyperglycemia induces excess superoxide (O<sub>2</sub><sup>-</sup>) and consequent oxidative damage, the result would be progressively impaired insulin secretion and worsening of the diabetic state. Glu = glucose; Glu-6-p = glucose 6-phosphate; GLUT2 = glucose transporter 2; TCA = tricarboxylic acid.

develop hepatic steatosis and are prone to hypoglycemia,<sup>34,35</sup> among several other multisystem abnormalities.

In hepatocytes, the forkhead transcription factor Foxa2 activates transcription of genes regulating lipid metabolism and ketogenesis. In insulin-resistant or hyperinsulinemic mice, Foxa2 is inactive and confined to the cytoplasm of hepatocytes,<sup>36</sup> promoting lipid accumulation as opposed to oxidation, thereby encouraging export of fat, ketones, and glucose. Indeed, degradation of malonyl-CoA in liver by overexpression of the degrading enzyme malonyl-CoA-decarboxylase favors mitochondrial fat oxidation and reduces circulating free fatty acids and ketones, improving insulin sensitivity.<sup>37</sup> Mice deficient in acetyl-CoA carboxylase 2 (ACC2) manifest reduced malonyl-CoA levels and a higher rate of fatty acid oxidation and resist diet-induced obesity and diabetes.<sup>38</sup>

Recent studies of mice with selective hepatic insulin resistance due to deletion of IRS-1 and IRS-2 revealed that target genes of forkhead box O1 (Foxo1) were upregulated.<sup>39</sup> These target genes included heme oxygenase-1, which disrupts mitochondrial complexes III and IV causing mitochondrial dysfunction. In addition, PGC-1 $\alpha$ , although upregulated, was acetylated and therefore inactive towards mitochondrial biogenesis. Mitochondrial oxidative metabolism was impaired in these mice but ameliorated by deletion of hepatic Foxo1, suggesting an important role for Foxo1 in integrating insulin signaling and mitochondrial function.

## Adipose Tissue

There is also evidence for altered mitochondrial function of adipocytes in type 2 diabetes. Mitochondrial respiration, mitochondrial numbers, and fatty acid oxidation, were reported to be decreased in db/db mice, a leptin-receptor-deficient obese model of type 2 diabetes.<sup>40</sup> Other

studies revealed an attenuated activation of mammalian target of rapamycin (mTOR) signaling in adipose tissue obtained at surgery from patients with type 2 diabetes compared with controls.<sup>41</sup> Downstream effects included mitochondrial dysfunction and increased autophagy. Exposing 3T3 adipocytes to high concentrations of glucose or free fatty acids resulted in decreased mitochondrial potential, morphologic changes wherein mitochondria became smaller and more compact, and downregulation of PGC-1.<sup>42</sup>

The above findings appear applicable to the pathogenesis of human type 2 diabetes, since reduced mitochondrial function in adipose tissue would result in net lipolysis. The consequent increase in fatty acid release could contribute to the insulin resistance of type 2 diabetes, since fatty acids impair muscle and liver insulin sensitivity.<sup>29</sup> This could be further compounded by adipocyte release of inflammatory cytokines associated with increased fat mass. The insulin-sensitizing thiazolidinedione drugs reportedly improve adipose mitochondrial function,<sup>43</sup> possibly a mechanism for improved whole body insulin sensitivity.

## Islet $\beta$ -cells

Beyond actions on insulin-sensitive target cells, mitochondria are critical in modulating  $\beta$ -cell insulin secretion. As depicted in Figure 2, any alteration in mitochondrial function that could change ATP production would have a major impact on the capacity of glucose to trigger insulin secretion. In particular, altered activity of uncoupling protein 2 (UCP2), the UCP subtype expressed in islets, would be important given its effect of reducing ATP production at any given level of fuel oxidation. Indeed, Zhang et al.<sup>44</sup> reported that mice genetically deficient in UCP2 manifest higher islet ATP levels and increased glucose-stimulated insulin release. Further, the defect in first-phase insulin release, known to be present in leptin-deficient obese ob/ob mice, could be restored by UCP2 knock-out.<sup>44</sup> UCP2 knockout also protected insulin release in high-fat-fed rodents and in islets exposed to lipid *in vitro*.<sup>45,46</sup> Interestingly, a kinetic analysis<sup>47</sup> revealed that the ATP-ADP ratio was much more regulated by mitochondria in islet  $\beta$ -cells (modeled by insulinoma cells) than by mitochondria of skeletal muscle, underscoring the importance of mitochondria in regulating islet insulin secretion. As opposed to UCP knock-down, overexpression of UCP2 inhibits glucose-induced insulin release as demonstrated by our laboratory using INS-1 cells<sup>48</sup> and by Chan et al.<sup>49</sup> in cultured pancreatic islets.

UCP2 may mediate a link between mitochondrial superoxide production and impaired insulin release, possibly explaining the progressive nature of type 2 diabetes. In this paradigm, islets exposed to high concentrations of glucose or fatty acids may generate more superoxide (see below). Superoxide is known to activate UCPs, possibly as a feedback means of protection from further radical generation through reduction of membrane potential.<sup>50</sup> However, this would also decrease ATP formation and reduce insulin secretion. In fact, Krauss et al.<sup>51</sup> showed that induction of UCP2 by endogenous superoxide-impaired insulin secretion from isolated islets in wildtype but not UCP2 knock-out mice.

In past years, glycolysis and glucokinase have been considered the major factors regulating glucose-induced insulin secretion.<sup>52</sup> However, the above considerations now direct attention to mitochondria with a major role for UCP2 in modulating mitochondrial potential, ATP

production, and therefore insulin release.<sup>53</sup> The interrelations of mitochondrial ATP formation, mitochondrial uncoupling, and insulin release are depicted in *Figure 2*.

Interestingly, insulin resistance at the level of skeletal muscle may induce islet  $\beta$ -cell mitochondrial dysfunction and progression to diabetes. Evidence for this comes from the MKR mouse, which has a dominant-negative IGF-I receptor mutation specifically in skeletal muscle leading to insulin resistance and hyperglycemia.<sup>54</sup> These mice manifest defective  $\beta$ -cell mitochondrial membrane polarization and impaired calcium signaling and differential expression of mitochondrial proteins, including membrane proteins and proteins involved in electron transport.<sup>54</sup>

### Mitochondrial Reactive Oxygen Species, Diabetes, and Diabetic Complications

Mitochondrial ROS are believed to be important in the pathogenesis, progression, and long-term complications of diabetes. This follows from evidence that elevated glucose and/or free fatty acids drive the formation of ROS,<sup>55–57</sup> impairing both  $\beta$ -cell insulin release and insulin sensitivity. Moreover, oxidative damage to non-insulin-sensitive cells chronically exposed to high glucose and fatty acids likely contributes to the complications of diabetes.<sup>55,58,59</sup> How this happens still needs more detailed resolution. The general supposition is that mitochondrial metabolism in the presence of excess nutrients generates high levels of substrate flux to mitochondria resulting in high mitochondrial NADH/NAD and flavin adenine dinucleotide (FADH<sub>2</sub>)/FAD ratios and high potential at low respiration rates (closer to state 4 conditions) and, thereby, more electron leak.<sup>59,60</sup> In particular, this would apply to the classic sites of diabetic complications including retina, kidney, neurons, and vascular endothelium; in other words, cells that take up glucose by facilitated diffusion unregulated by insulin.<sup>61</sup> Hence, ROS may be involved in a vicious self-perpetuating process favoring the development and worsening of the diabetic state and induction of complications.

On the other hand, the above explanation has been criticized as over-simplistic<sup>59</sup> and is not supported by all studies. For example, cultured hepatocytes exposed to high glucose generate more glycogen rather than increase respiration, potential, or reducing equivalents<sup>62</sup> and some studies do not support an effect of glucose to induce ROS at the cell level.<sup>63,64</sup> Differences may be due to methodology, including specific cell type(s) examined, antecedent cell nutrition, and the particular means of detecting ROS. In this author's view, data on nutrient-induced ROS production need to be viewed in critical fashion as there are many pitfalls and potential for non-specific findings.<sup>65</sup> A recent report<sup>66</sup> demonstrated several respiratory abnormalities and downregulation of proteins, but without excess ROS production, in dorsal root ganglia of streptozotocin diabetic rats (a model more reflective of type 1 diabetes).

Beyond ROS production, there is evidence for oxidative damage in animals and humans with diabetes. Plasma levels of markers of lipid peroxides such as 8-iso-prostaglandin F<sub>2</sub> $\alpha$ ,<sup>67</sup> conjugated dienes, and lipid hydroperoxides<sup>68</sup> are elevated, at least in type 1 diabetes, while antioxidant capacity assayed as total plasma antioxidant capacity (TRAP) is reduced.<sup>68</sup> Moreover, DNA damage is detectable in circulating lymphocytes of subjects with insulin-dependent diabetes and correlates

to the extent of glucose elevation.<sup>69</sup> Furthermore, the extent of urinary 8-OHdG excretion, a marker of DNA damage, correlates with the extent of renal damage in subjects with type 2 diabetes.<sup>70</sup> A recent study showed that mice fed a high-fat diet manifest reduced peak exercise oxygen consumption along with reduced ADP-stimulated mitochondrial respiration, mitochondrial content, and complex I and III activities.<sup>71</sup> These defects were ameliorated by feeding apocynin, an inhibitor of NAD(P) oxidase, suggesting that cytoplasmic ROS had secondary adverse effects on mitochondrial function. Of additional note is that oxidative stress is well known to trigger the formation of advanced glycation end-products such as carboxymethyl lysine (CML).<sup>72,73</sup> CML is known to induce protein cross-linking contributing to diabetic complications. Moreover, higher levels of oxidized low-density lipoprotein (LDL) have been observed in type 2 diabetes and contribute to macrovascular disease.<sup>74</sup>

### Mitochondrial Morphology, Fission, and Fusion

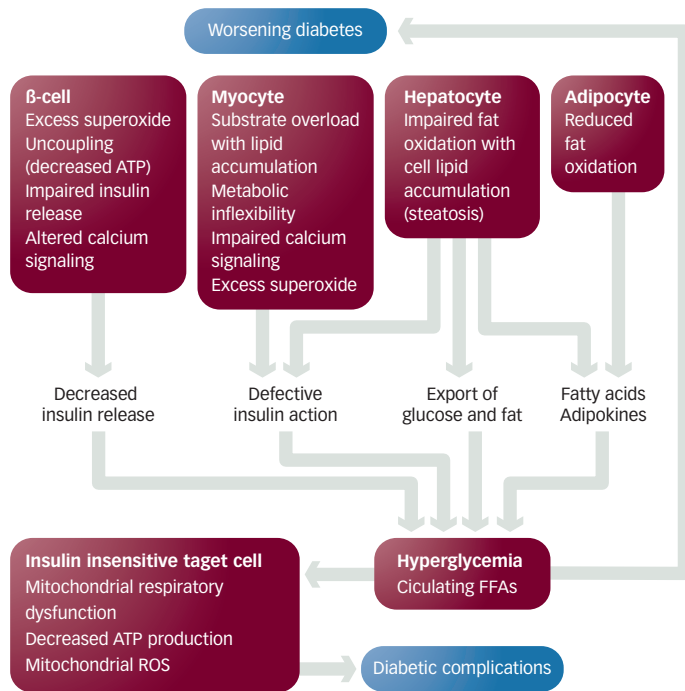
Beyond mitochondrial function, type 2 diabetes is associated with changes in the size, number, and morphology of muscle mitochondria. Biopsies of skeletal muscle from subjects with type 2 diabetes and obesity reveal lower density of mitochondria and smaller size; size correlating to whole body insulin sensitivity.<sup>5,75</sup> There is also mitochondrial subtype selectivity in muscle. Skeletal myocytes and cardiomyocytes contain two populations of mitochondria: subsarcolemmal (SLM) and intermyofibrillar (IMFM). Electron microscopy revealed reduced numbers of SLM mitochondria in skeletal muscle of type 2 diabetic and obese subjects associated with reduced electron transport activity per unit mitochondrial DNA, suggesting functional impairment as well.<sup>75</sup> It is believed that the SLM contribute energy for membrane and transport processes while the IMFM contribute more to contractile function. Interestingly, type 2 diabetes is also associated with increased SLM lipid accumulation compared with obese controls.<sup>76</sup>

Mitochondrial networking in the form of frequent fusion and fission events may play a role in regulating  $\beta$ -cell function and sensitivity to apoptosis. There is evidence that high nutrient exposure of islet  $\beta$ -cells *in vitro* leads to arrest of fusion activity and fragmentation. Shifting the dynamics to fusion by inhibiting fission seems to prevent  $\beta$ -cell apoptosis.<sup>77</sup> Fusion and fission depend on certain proteins including two isoforms of mitofusin, which are involved in docking, and the presenilin-associated rhomboid-like (PARL) protein, important for morphologic integrity.<sup>78</sup> There is now evidence that obesity in both humans and rodents is associated with reduced mitofusin (MFN).<sup>79</sup> Moreover, polymorphisms of PARL in humans are associated with insulin resistance.<sup>80</sup>

### Mitochondrial Calcium and the Pathogenesis of Diabetes

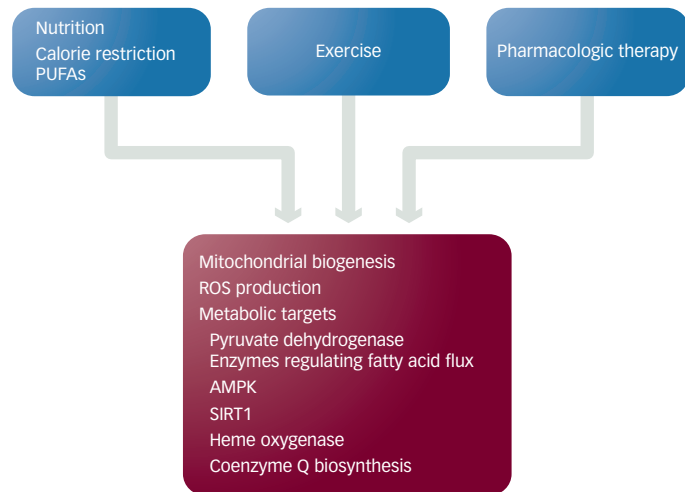
A large volume of literature links calcium flux to mitochondrial function. Ionic calcium influx increases respiration and ATP formation, probably by enhancing the activity of mitochondrial dehydrogenase enzymes and stimulation of ATP synthase.<sup>81</sup> However, whether alterations in calcium handling are of primary importance in the pathology associated with diabetes is not clear. Oliveira et al.<sup>82</sup> reported that heart mitochondria isolated from 21-day STZ-diabetic rats with severe hyperglycemia demonstrated increased sensitivity to calcium-triggered reduction in

**Figure 3: Detrimental Events Whereby Mitochondrial Dysfunction in Islet  $\beta$ -cells and in Insulin-responsive Tissues Leads to Deterioration of Insulin Secretion and Insulin Action**



Concurrently, hyperglycemia and elevated free fatty acids (FFAs) lead to worsening diabetic complications. ATP = adenosine triphosphate; ROS = reactive oxygen species.

**Figure 4: Molecular Targets for Mitochondrial Therapy Directed at Diabetes, Insulin Resistance, and Obesity**



AMPK = adenosine monophosphate-activated protein kinase; PUFAs = polyunsaturated fatty acids; ROS = reactive oxygen species; SIRT1 = sirtuin (silent mating type information regulation 2 homolog) 1.

membrane potential. Prevention of this by cyclosporin suggested that this was due to greater susceptibility of these mitochondria to opening of the mitochondrial permeability transition pore.<sup>82</sup> There is evidence for leakage of calcium from muscle sarcoplasmic reticulum stores in db/db mice<sup>83</sup> and impaired mitochondrial calcium transients in ob/ob mice.<sup>84,85</sup>

In islet  $\beta$ -cells, calcium is a critical mediator for respiration and consequent ATP formation and for insulin release by a direct effect on extrusion of the stored hormone from intracellular granules. As noted above, impaired calcium signaling has been noted in islets of hyperglycemic insulin-resistant MKR mice.<sup>84</sup>

## Mitochondria of Multiple Cell Types and Type 2 Diabetes

Given the above considerations, we can ask how mitochondrial dysfunction within different cell and tissue types might lead to type 2 diabetes or, if not directly causative, how mitochondrial dysfunction could contribute to the progressive nature of diabetes and its complications. *Figure 3* represents a simplistic and hypothetical overview of this process. Obviously, there is considerable detail to be resolved. Hopefully, further understanding will lead to approaches that effectively target mitochondria within multiple tissues in a way that mitigates the pathophysiology involved in the onset and progression of type 2 diabetes.

## Therapeutic Considerations

Based on the above, therapy directed at mitochondria could prove an effective way to prevent, treat and/or to minimize the complications of diabetes (see *Figure 4*). Exercise increases mitochondrial biogenesis through effects on PGC-1<sup>86,87</sup> and activates adenosine monophosphate (AMP)-activated protein kinase (AMPK), which improves both glucose and fat oxidation.<sup>86</sup> A recent study revealed that aerobic exercise training increased insulin sensitivity, maximal oxygen consumption, and mitochondrial respiration in both type 2 diabetic subjects and obese controls matched for age and body mass index (BMI).<sup>88</sup> However, there was no difference in these parameters between these groups. Calorie restriction favors mitochondrial biogenesis, oxygen use, ATP formation, and expression of SIRT1, which activates PGC1- $\alpha$ .<sup>89,90</sup> There is also evidence that n-3 polyunsaturated fatty acids activate AMPK, favoring mitochondrial biogenesis and enhance lipid catabolism in adipose tissue and liver, suppressing lipogenesis.<sup>91</sup>

Pharmacologic efforts to improve mitochondrial function go back to the 1930s when attempts were made to treat human obesity with the mitochondrial chemical uncoupler dinitrophenol.<sup>92</sup> Although quite effective, this treatment was abandoned following cases of fulminant liver failure. On the other hand, recent research has uncovered additional targets that may prove amenable to therapies directed at mitochondrial function. The thiazolidinedione pioglitazone induces mitochondrial biogenesis in adipose tissue as well as expression of PGC-1 $\alpha$  and genes in the fatty acid oxidation pathway.<sup>43</sup> However, somewhat paradoxically, thiazolidinediones are limited by a tendency for weight gain due to increased fat,<sup>93</sup> fluid retention, and heart failure.<sup>94</sup> Metformin, most often used in the initial pharmacologic management of type 2 diabetes, has mitigating effects on ROS production, activates AMPK, and favors mitochondrial proliferation.<sup>95,96</sup> In addition, there is evidence that angiotensin receptor blockers or inhibitors of angiotensin-converting enzyme enhance mitochondrial biogenesis.<sup>97</sup>

Newer approaches may soon be available. Resveratrol, an ingredient in red wines, is a polyphenolic SIRT1 activator which, at least in rodents, improves insulin resistance, protects against diet-induced obesity,

induces genes for oxidative phosphorylation, and activates PGC-1 $\alpha$ .<sup>98–100</sup> Recently, it has been reported that the adipokine apelin enhances mitochondrial content in muscle by means independent of AMPK and PGC-1 $\alpha$ .<sup>101</sup> It may also be possible to improve glucose utilization through measures that inhibit mitochondrial uptake of long-chain acyl-CoA molecules. Lipid suppression of glucose utilization is mitigated by etomoxir, an inhibitor of carnitine palmitoyltransferase 1, or by knockdown of malonyl-CoA decarboxylase, an enzyme that promotes mitochondrial  $\beta$ -oxidation by preventing malonyl-CoA-induced inhibition of carnitine palmitoyl transferase 1 (CPT-1).<sup>28,102</sup> Other targets potentially amenable to pharmacologic manipulation include AMPK, which enhances both glucose and fat oxidation<sup>103,104</sup> and increases PGC-1 $\alpha$  favoring mitochondrial biogenesis,<sup>105,106</sup> pyruvate dehydrogenase,<sup>107</sup> or the various shuttle mechanisms regulating uptake of TCA intermediates.<sup>108</sup>

Ubiquinone (coenzyme Q or CoQ) has antioxidant properties and is widely available as a health supplement. Unfortunately, however, this compound does not easily enter mitochondria. The endogenous mitochondrial CoQ is localized to these organelles by virtue of its synthesis within mitochondrial membranes. This problem has led to the development of compounds linking agents such as redox forms of coenzyme Q (ubiquinol and ubiquinone) or vitamin E to alkylated triphenylphosphonium compounds. These are lipophilic cations avidly taken up into the relatively negative mitochondrial matrix.<sup>109</sup> Of note is that ubiquinol, the reduced form of CoQ, does not directly scavenge oxygen radicals but acts as an antioxidant in mitochondria both by regeneration of vitamin E and by reacting with peroxy radicals, serving as a chain-breaking agent towards lipid peroxidation.<sup>110</sup> In fact, we and others<sup>111,112</sup> have shown that mitochondrial-targeted coenzyme Q (mitoQ) actually increases superoxide production when added directly to isolated mitochondria, an effect that is mediated by the semiquinone

form of mitoQ generated during redox cycling of the compound. MitoQ also has metabolic effects when added to mitochondria, including uncoupling properties, so one can speculate that mitochondrial-targeted agents like coenzyme Q might be potentially useful in treating obesity.<sup>113</sup> Other approaches to mitochondrial-targeted antioxidant therapy are under investigation. One approach involves synthetic peptides with antioxidant properties. Certain peptides containing tyrosine residues have been found to effectively scavenge oxygen radicals and peroxynitrite and inhibit lipid peroxidation.<sup>114,115</sup>

## Summary

Mitochondria have an important role in the pathophysiology of diabetes. Mitochondrial perturbations involve function, number, morphology, and dynamics. Altered mitochondrial metabolism in part explains the decrease in insulin sensitivity within muscle, liver, and adipose tissue as well as defective  $\beta$ -cell insulin release, thus contributing to the progressive nature of type 2 diabetes. Moreover, ROS appear important in mediating oxidative damage to non-insulin-sensitive target cells, contributing to the long-term complications of diabetes. New treatment strategies directed at mitochondrial function and ROS production should benefit type 2 diabetes and obesity. ■



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