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Mitochondrial Oxidative Phosphorylation, Obesity and Diabetes

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Mitochondria are essential for ATP synthesis via oxidative phosphorylation (OXPHOS) and their dysfunction may cause energy deficiency in cells resulting in metabolic disorders: obesity and diabetes. Obesity or excessive bodyweight with elevated free fatty acids in the blood stream affects 2.1 billion people worldwide, and one of its adverse consequences is type 2 diabetes mellitus (T2DM). T2DM is characterized by hyperglycemia resulting from insufficient production of insulin by pancreatic β-cells, and insulin resistance in target tissues (muscle, liver and fat). Lipotoxicity and glucotoxicity in obesity and T2DM induce the β-cell overexpression of uncoupling protein 2 which increases proton leakage across the mitochondrial inner membrane and decreases ATP synthesis leading to insufficient secretion of insulin. Insulin resistance in the target tissues has been related to decreased mitochondrial content, reduced fatty acid oxidation, defective OXPHOS, and poor ATP production. This review focuses on the cellular and molecular mechanisms underlying defective mitochondrial OXPHOS in obesity and T2DM.

Introduction

Mitochondria are viewed as the ‘power houses’ of the cell in the sense that they convert the chemical energy stored within food into adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS). In addition, mitochondria play fundamental roles in many metabolic pathways, such as the tricarboxylic acid cycle, β-oxidation, and urea cycle, the synthesis of steroid hormones and heme, and calcium signaling. Mitochondria are the only cytoplasmic organelles that carry circular mitochondrial DNA (mtDNA), incorporating nuclear and mitochondrial gene products, and possessing the machinery necessary for replication, transcription and translation (Margulis, 1996). Abnormalities in mitochondrial structure and function are increasingly recognized in common diseases such as obesity, diabetes,
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cardiomyopathy, neurodegeneration, cancer and aging (Welch, 1998; Enns, 2003; Wallace, 2005; Boudina & Abel, 2006; Lin & Beal, 2006).

Obesity is a condition in which the reserved fat tissue in the body is increased to an excessive degree, that is to say men present with more than 25% body fat by weight, and women with over 30%. Excessive bodyweight is the sixth most important risk factor contributing to the overall burden of human disease, and the number of obese individuals has reached 2.1 billion (1.1 billion adults and 10% of all children) worldwide. The main adverse consequences of obesity are various diseases, including type 2 diabetes mellitus (T2DM), musculoskeletal disorders, cardiovascular diseases, pulmonary disorders and cancers. Together these account for a significant percentage of health care costs and are strongly associated with increased morbidity and mortality (Haslam & James, 2005; Li et al., 2005; Olshansky, 2005).

Diabetes (i.e. diabetes mellitus) is a metabolic disorder, characterized by hyperglycemia, that results either from an inability of the pancreatic β-cells to produce sufficient insulin, or else an acquired or inherited resistance of target tissues to the insulin released coupled with insufficient plasma levels of insulin (Rother, 2007). Insulin resistance is defined as the impaired ability of insulin to inhibit glucose output from liver and to promote glucose uptake in fat and muscle tissues (Saltiel & Kahn, 2001). The characteristic symptoms are polyuria, polydipsia, and blurred vision. Diabetes forms include chronic type 1 and type 2 diabetes, and also gestational diabetes. Type 1 disease is usually due to the autoimmune destruction of pancreatic β-cells. Type 2 is characterized by insulin resistance in target tissues and decreased insulin secretion by the β-cells, a form which has become epidemic worldwide (Zimmet et al., 2001). Gestational diabetes is associated with pregnancy. Although all three types may be treated with insulin, dietary and lifestyle adjustments, diabetes may result in many complications, including hypoglycemia, ketoacidosis, cardiovascular disease, chronic renal failure, retinal damage and blindness, nerve and microvascular damage, and poor wound healing.

Obesity-associated insulin resistance is a major risk factor for T2DM (Qatanani & Lazar, 2007) and is extensively associated with mitochondrial dysfunction (Enns, 2003). Because insulin resistance is observed in virtually all patients with T2DM (Lillioja et al., 1988; Lillioja et al., 1993), and exists one to two decades before the onset of T2DM, it is the best predictor for the development of this disease (Warram et al., 1990). The association between obesity and insulin resistance is likely a cause-and-effect relationship because human and animal studies indicate that weight loss/gain correlates closely with increasing/decreasing insulin sensitivity, respectively (Sims et al., 1973; Freidenberg et al., 1988; Bak et al., 1992). Mitochondrial dysfunction, including defective OXPHOS, poor ATP production, reduced fatty acid oxidation and decreased mitochondrial content, has been directly associated with the development of insulin resistance in T2DM (Ling et al., 2007; Scheele et al., 2007; Handschin et al., 2007). This brief review will focus on recent progress that sheds light upon the cellular and molecular mechanisms underlying defective mitochondrial OXPHOS in obesity and T2DM.

**Mitochondrial structure, genome and proteins**
Mitochondria are double-membrane-enclosed organelles which possess unique structural and functional features typically found within prokaryotic cells. The number of mitochondria in a cell varies widely, from a single mitochondrion to thousands per cell (Clayton, 1991; Graff et al., 1999). The structure of a mitochondrion includes an outer membrane, intermembranal space, inner membrane, cristae and the matrix. Mitochondria are the only cytoplasmic organelles which have genomic DNA and possess replicative, transcriptional and translational machinery (Margulis, 1996). Mitochondrial DNA (mtDNA) is exclusively maternally inherited (Giles et al., 1980).

![Human Mitochondrial DNA Map and OXPHOS Subunits](image)

**Figure 1. Human mitochondrial DNA and OXPHOS components.** Human mitochondrial DNA (16,569 bp) encodes 37 genes including 13 polypeptides, 22 tRNAs and 2 ribosomal RNAs. The poly peptides are constitutes of the structural components of OXPHOS: 7 complex I subunits (encoded by ND1, 2, 3, 4, 4L, 5,
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6), 1 complex III subunit (cytochrome b, encoded by CYTB), 3 complex IV subunits (encoded by COX1, 2, 3) and 2 complex V subunits (encoded by ATP6 and 8). The positions of genes are presented as green (H-strand) or blue (L-strand) bars with arrowheads indicating transcription directions. H and L represent H- and L-strand promoters. H and L represent the origin of replication, and D-loop is the control region. The map is adapted from Mitomap (www.mitomap.org/mitoseq.html). A total number of components in each OXPHOS complex and those encoded by mtDNA are indicated.

mtDNA was first identified in the 1960s (Nass & Nass, 1963), and the human mtDNA sequence was first determined in 1981 (Anderson et al., 1981). It is a circular molecule 16,569 base pairs (bp) in length (Figure 1). The mitochondrial genome is symmetrically transcribed from two promoters, one for the G-rich heavy (H) strand and the other for the C-rich light (L) strand (Fernandez-Silva et al., 2003; Brandon et al., 2005; sin-Cayuela & Gustafsson, 2007). The 1,121-bp control region D-loop encompasses the H-strand origin of replication (OH), and the H- and L-strand promoters (P and L) for binding of mitochondrial transcription factor A (TFAM) and one of the two mitochondrial transcription factor B paralogues (TFB1M and TFB2M). The L-strand origin of replication (OL) is 5.3-kb apart from OH in a counterclockwise direction. Transcription is initiated at P or L and progresses around the DNA, generating polycistronic messages. The primary transcripts are then processed to mature mRNAs, rRNAs, and tRNAs via a series of enzymatic steps requiring tRNA-processing endonucleases RNase P and tRNase Z. mtDNA uses modified genetic codes for translation, for example, UGA encodes tryptophan rather than a stop codon as is the case in nuclear DNA (nDNA).

mtDNA-encoded genes differ from those encoded by nDNA, in that a structural gene has no 5’- or 3’- noncoding sequences, no introns, and no spacers between genes. The 37 mtDNA-encoded gene products include 12S (RNR1) and 16S (RNR2) ribosomal RNAs, 22 transfer RNAs (tRNAs), and 13 subunits encoding mitochondrial OXPHOS enzymes. The subunits include seven (ND1, ND2, ND3, ND4L, ND4, ND5, ND6) of 46 polypeptides which constitute the complex I, one (CYTB) of 11 proteins of complex III, three (COX1, COX2, COX3) of 13 proteins of complex IV, and two (ATP6 and ATP8) of 16 proteins present within complex V (Wallace, 2005). Other OXPHOS subunits are encoded by nDNA (Figure 1).

Although the mitochondrial genome is essential for function, the vast majority of mitochondrial proteins (~1000 proteins) are encoded by nDNA (Wallace, 2005; Bai et al., 2007; Bohnert et al., 2007). The nDNA-encoded mitochondrial proteins may be categorized into the groups including OXPHOS (e.g., all 4 subunits of complex II), replication (e.g., DNA polymerase γ subunit), transcription (e.g., transcription factor TFAM), translation (e.g., mitochondrial translation initiation factor 3), metabolic enzymes (e.g., pyruvate dehydrogenase 1), mitochondrial transport (e.g., the translocase of inner mitochondrial membrane 17 homolog A), responsivity to oxidative stress (e.g., glutathione peroxidase 1), and the regulation of apoptosis (e.g., B-cell CLL/lymphoma 2; Alesci S et al., 2004; Bai et al., 2007). These genes are transcribed within the nucleus and are translated on the cytosolic ribosomes. The protein products are imported into the mitochondria via various mitochondrial protein import systems (Bohnert et al., 2007). nDNA-encoded gene products control expression of mtDNA through regulatory proteins such as mitochondrial transcription factors (TFAM, TFB1M and TFB2M; Dairaghi et al., 1995; sin-Cayuela & Gustafsson, 2007).
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Mitochondrial function, OXPHOS, and insulin secretion of pancreatic β-cells

Their structural characteristics allow mitochondria to play a key role within cellular processes. OXPHOS is a major function of mitochondria, namely producing ATP as a source of chemical energy (Sherratt & Turnbull, 1990). The latter drives virtually all forms of work in the body, from muscle contraction to protein synthesis. The total quantity of ATP in the human body is about 0.1 moles. The majority of ATP is not usually synthesized de novo, but instead is generated from ADP. The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily which is approximately 65 kg every day (Buono & Kolkhorst, 2001). Because ATP cannot be stored, its consumption is closely coupled to its synthesis. In addition, mitochondria play a key role in other diverse cellular processes, including metabolism, signaling, cellular differentiation, cell survival and death, as well as in the control of the cell cycle and cellular growth (McBride et al., 2006).

Mitochondrial OXPHOS (also referred to as the electron transport chain [ETC], or respiratory chain) generates ATP by coupling electron transfer to the phosphorylation of ADP. The OXPHOS machinery is located on the inner mitochondrial membrane and consists of 5 transmembrane complexes, including NADH CoQ reductase (complex I), succinate dehydrogenase (complex II), ubiquinol-cytochrome c reductase (complex III), cytochrome c oxidase (complex IV), and ATP synthase (complex V; Figure 2). Catabolic biochemical processes, such as glycolysis, tricarboxylic acid (TCA) cycle and β-oxidation, produce the reduced coenzyme NADH and flavin adenine dinucleotide (FADH2). Electrons of NADH and FADH2 can enter the ETC at the level of the complex I and complex II, respectively. Electrons are passed down the complexes, and protons are pumped into the mitochondrial intermembrane space by complexes I, III and IV. This generates an electrochemical gradient across the membrane. When protons re-enter the mitochondrial matrix via the ATP synthase complex, they release energy to catalyze the condensation of ADP with inorganic phosphate, yielding ATP. Finally, ATP is transported to the cytosol in exchange for ADP by the adenine nucleotide translocator (ANT). The entire bioenergetic process is regulated by substrate and/or Ca2+ flux, which increases the activities of several mitochondrial dehydrogenases. For example, glucose induces activation of pancreatic β-cells, leading to increases in free cytosolic Ca2+. A uniporter transports Ca2+ into the mitochondrial matrix, resulting in the activation of ATP synthesis (McCormack et al., 1990; Boitier et al., 1999).
Figure 2. Mitochondrial OXPHOS and ATP production. Lipids and carbohydrates fuel the respiratory chain (complex I to V) on the mitochondrial inner membrane. Acetyl-CoA from degradation of glucose or fatty acids is further oxidized in the TCA cycle, producing CO2 and reducing equivalents such as NADH and FADH2. These reducing equivalents provide electrons to the respiratory chain upon their reoxidation. An electronchemical gradient is established by pumping protons from the matrix across the inner membrane through complex I, III and IV, and is used for synthesis of ATP from ADP and Pi by complex V (ATP synthase). Reactive oxygen species (ROS) is by-products of ATP generation and induces mtDNA mutations and protein damage. High ROS may induce apoptosis. UCP2, uncoupling protein 2; ANT, adenine nucleotide translocator.

The level of blood glucose is regulated by insulin which is secreted by pancreatic β-cells. Insulin acts upon target tissues such as muscle, liver and adipocytes. In the healthy human, β-cells secrete insulin in a manner which is positively correlated with the concentration of glucose in blood. Glucose is transported into β-cells where it is phosphorylated to glucose-6-phosphate by glucokinase, the initial rate-limiting step in both glycogen synthesis and glycolysis (Newgard & McGarry, 1995). Pyruvate as an end product of glycolysis is transported into the mitochondria where it becomes the substrate of pyruvate dehydrogenase and pyruvate carboxylase to yield acetyl-CoA and oxaloacetate, respectively, precursors of the TCA cycle. The irreversible conversion of pyruvate into acetyl-CoA is catalyzed by the pyruvate dehydrogenase complex which provides the primary link between glycolysis and the TCA cycle (Schuit et al., 1997). Through the TCA cycle, acetyl-CoA is converted into
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metabolites including NADH and FADH₂ for OXPHOS. The resulting ATP molecules are transported into the cytosol, leading to an increase in the ATP/ADP ratio (Figure 2 & Figure 3). This change causes a depolarization of the plasma membrane by opening the voltage-gated Ca²⁺ channels (Ashcroft et al., 1994) and closing ATP-sensitive K⁺ channels (Rorsman, 1997). It is at this step that glucose stimulates insulin secretion (Figure 3), as the increase in cytosolic calcium ions is the main trigger for exocytosis, the process by which the insulin-containing granules fuse with the plasma membrane and release insulin (Lang, 1999). OXPHOS is therefore central to glucose-stimulated insulin secretion (Maechler & Wollheim, 2001).

Figure 3. Mechanism of glucose-stimulated insulin release in pancreatic β-cells. Under normal conditions, glucose is transported into β-cell, phosphorylated by glucokinase and converted to pyruvate via glycolysis. Pyruvate is shuttled into mitochondria and activates the TCA cycle, resulting in the transfer of reducing equivalents to the respiratory chain and ATP generation. An increase in the ATP/ADP ratio leads to closing of ATP-controlled potassium channels (KATP), cell membrane depolarization and opening of voltage-gated calcium (Ca²⁺) channel. The raised cytosolic Ca²⁺ concentration triggers insulin exocytosis and therefore, secretion. In obesity, free fatty acids (FFA) and hyperglycemia induce overexpression of uncoupling protein 2 (UCP2) and superoxide (O₂⁻). The former increases the proton leak across mitochondrial inner membrane, and diverts electrochemical potential away from ATP synthesis. The latter activates UPC2 activity, worsens ATP production, promotes apoptosis and therefore, impaired insulin secretion by β-cells.

High mutation rate and heteroplasmy of mtDNA

The mtDNA mutation rate is 10 to 20 times higher than that of nDNA (Shoffner & Wallace,
Mitochondria contain no chromatin protection such as histones, and lack an effective DNA repair system. Consequently, mtDNA is more sensitive than nDNA to agents that induce DNA damage. In addition, mtDNA is exposed to a high level of agents which cause DNA damage, for example reactive oxygen species (ROS). About 90% of all oxygen consumption in the cell occurs in the mitochondria and, as a result, there can be extensive oxidative stress in mitochondria due to the generation of ROS, an inevitable by-product of OXPHOS (Richter et al., 1988). ROS may be increased when the ETC harbors excess electrons as a result of genetic OXPHOS inhibition or excessive caloric intake. The resulting free radicals can cause oxidative damage that varies among cell types depending upon the ROS and antioxidants involved. Oxidative damage includes the accumulation of defective protein, increased mutation rates within mtDNA and the release of cytochrome c, leading to apoptosis (Wallace, 2005). Oxidatively damaged proteins may become resistant to degradation, leading to an accumulation of defective proteins which further impair mitochondrial function (Newgard & McGarry, 1995). A comparison of the evolutionary mutation rate of the mtDNA-encoded OXPHOS genes with a number of nDNA-encoded genes revealed significant differences. For example, the mutation rate of ATPases 6 and 8 was 12 times greater than that of the nDNA-encoded ATPase. An average mutation rate of a mitochondrial gene has been calculated to be 17-fold higher than that of an equivalent nuclear gene (Wallace et al., 1987). High mutation rates of mtDNA explain a disproportionately large number of human hereditary diseases due to alterations in OXPHOS.

Mutations in mtDNA are the most important cause of known inherited and acquired deficiencies in OXPHOS, resulting in various diseases (Schapira & Cock, 1999). The vast majority of patients suffering from mtDNA disorders exhibit heteroplasmy, that is, more than one genotype is exhibited (mutated vs. wild-type) of mtDNA in individuals. This is partly due to the high mutation rate and partly due to the presence of thousand copies of mtDNA per cell. Each mitochondrion contains 2-10 copies of mtDNA, and each cell may carry as many as a thousand mitochondria (Clayton, 1991). Following the first observation that heteroplasmy was associated with disease (Holt et al., 1988), mounting evidence has demonstrated that point mutations and rearrangements within mtDNA are involved in diverse clinical abnormalities (Johns, 1995). The high metabolic requirement of the tissues/cells involved and the level of mtDNA heteroplasmy in the tissue/cell are important variants which explain why mitochondrial diseases are clinically and genetically heterogeneous (Chinnery et al., 1997).

**mtDNA mutations in diabetes**

The critical role of mitochondria in diabetes with β-cell dysfunction has been traced to specific mutations within the mitochondrial genome (Maechler & Wollheim, 2001; Maassen et al., 2004). mtDNA molecules harboring mutations tend to accumulate in non-dividing cells, such as nerve cells and skeletal/cardiac muscles, as well as pancreatic β-cells. Table 1 lists mtDNA deletions (Ballinger et al., 1992; Hinokio et al., 1995; Suzuki et al., 1999; Vielhaber et al., 2002) and mutations of mtDNA-encoded tRNA\textsubscript{Leu} (van den Ouweland et al., 1992; Velho et al., 1996; Vielhaber et al., 2002), tRNA\textsubscript{Glu} (Perucca-Lostanlen et al., 2002), tRNA\textsubscript{Ser} (Lynn et al., 1998) and tRNA\textsubscript{Lys} (Suzuki et al., 1994). These genetic alterations cause maternally inherited
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Evidence that mtDNA defects are a common factor in the etiology of diabetes comes from the observation that as the age-of-onset of the proband increases, the probability that the mother was the affected parent also increases, reaching a ratio of 3:1 for patients with a mean age at onset of 46 years old (Wallace & Lott 2002).

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Gene Affected</th>
<th>Disease</th>
<th>Typical Feature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3243G*</td>
<td>tRNA&lt;sub&gt;Leu&lt;/sub&gt; (tRNA leucine 1 [UUA/G])</td>
<td>Maternally inherited diabetes and deafness (MIDD)</td>
<td>Pancreatic β cell failure; Sensorineural hearing loss</td>
<td>(van den Ouwehand, Lemkes et al. 1992; Kadowaki, Kadowaki et al. 1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T2DM</td>
<td>Hyperglycaemia,</td>
<td>(van den Ouwehand, Lemkes et al. 1992; Velho, Byrne et al. 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type 1 diabetes mellitus</td>
<td>Pancreatic β cell failure</td>
<td>(Kadowaki, Kadowaki et al. 1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kearn–Sayre syndrome (KSS)</td>
<td>Diabetes mellitus</td>
<td>(Vielhaber, Varlamov et al. 2002)</td>
</tr>
<tr>
<td>T14709C*</td>
<td>tRNA&lt;sub&gt;Glu&lt;/sub&gt;</td>
<td>Malnutrition-modulated diabetes mellitus (MMDM); MIDD</td>
<td>Diabetes mellitus</td>
<td>(Hao, Bonilla et al. 1995; Vialettes, Paquis-Flucklinger et al. 1997; Perucca-Lostenlen, Taylor et al. 2002)</td>
</tr>
<tr>
<td>C12258A*</td>
<td>tRNA&lt;sub&gt;ser&lt;/sub&gt;</td>
<td>MIDD</td>
<td>Pancreatic β cell failure</td>
<td>(Lynn, Wardell et al. 1998)</td>
</tr>
<tr>
<td>A8344G*</td>
<td>tRNA&lt;sub&gt;lys&lt;/sub&gt;</td>
<td>Diabetes and myoclonus with epilepsy with ragged red fibers</td>
<td>Diabetes mellitus, Pancreatic β cell failure</td>
<td>(Suzuki, Hinokio et al. 1994)</td>
</tr>
<tr>
<td>Deletion* (10.4 kb)</td>
<td>28 genes from tRNA&lt;sub&gt;Glu&lt;/sub&gt; (tRNA glutamine) to CYTB (cytochrome b)</td>
<td>MIDD</td>
<td>Diabetes mellitus Premature deafness</td>
<td>(Ballinger, Shoffner et al. 1992; Ballinger, Shoffner et al. 1994)</td>
</tr>
<tr>
<td>Deletion (5778 bp)</td>
<td>14 genes from COX2 to ND5*</td>
<td>Diabetic amyotrophy; IDDM and diabetic fatty liver</td>
<td>Pancreatic β cell failure; Diabetic amyotrophy; Myoatrophy with diabetic nephropathy, chronic renal failure, diabetic fatty liver</td>
<td>(Hinokio et al., 1995)</td>
</tr>
<tr>
<td>Deletion* (4977 bp)</td>
<td>12 genes from ATP8 to ND5*</td>
<td>T2DM</td>
<td>Diabetic nephropathy; Diabetes mellitus; Retinopathy</td>
<td>(Suzuki, Hinokio et al. 1999)</td>
</tr>
<tr>
<td>G866A**</td>
<td>UCP2</td>
<td>T2DM</td>
<td>Higher promoter activity, obesity, diabetes</td>
<td>(Esterbauer, Schneiter et al. 2001; Sasahara, Nishi et al. 2004)</td>
</tr>
</tbody>
</table>

* Mitochondrial DNA. The number of nucleotides was based on the original articles (see Figure 1 for all genes deleted). ** nuclear DNA

Table 1. Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) abnormalities associated with diabetes
The β-cell with mtDNA mutations exhibits a defective mitochondrial inner membrane potential and induces diabetes. Maternally inherited diabetes and deafness (MIDD) is a rare genetic disease found only in 1-2% of individuals with diabetes (Maassen et al., 2004). The most frequent mutation encountered is the A3243G mutation in the gene for tRNA^{Leu} (bearing the anticodon UUR). This mutation causes MIDD (van den Ouweland et al., 1992) in most carriers, and is also associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS; Jacobs, 2003). Heteroplasmy varies between cells and tissues. Defective mitochondrial function does not become apparent until the mutant molecules become a large part of the total mtDNA population. Diabetic patients with A3243G mutation showed a 63% heteroplasmy (Kobayashi et al., 1997) and reduction in the pancreatic islet mass including β-cells (Otabe et al., 1999). The extent of heteroplasmy contributes to the degree to which ATP production is lowered and signaling requirements for glucose-stimulated insulin secretion, a concept supported by clinical observations (Suzuki et al., 1997). The A3243G mutation is examined most often by using peripheral leucocytes because of easy access to the cells. However, the mutant heteroplasmy in leucocytes is known to be 7.8-fold lower than that in pancreatic islet cells (Kobayashi et al., 1997). It is possible that the mutation rate in β-cells is underestimated due to the low incidence of heteroplasmy in leucocytes. In addition, over 200 point mutations have been found to date in mitochondrial diseases. Assuming that all these mutations are potentially a cause for the disease, some researchers propose that up to 20% of patients with diabetes mellitus may be mtDNA-related (Kang & Hamasaki, 2005).

mtDNA with large deletions has been detected in patients with diabetes associated with deafness (Table 1). The enzyme activities of <5% of the tolerance levels of complexes I, I+III, & IV have been detected in skeletal muscle biopsies (Ballinger et al., 1992). The mutation with 10.4 kb deletion of mtDNA is inherited and associated with MIDD, and this mutation causes defective OXPHOS and an inability to respond to hyperglycemia (Ballinger et al., 1994). Suzuki et al. investigated the correlation of a mtDNA deletion of 4977 bp with diabetes, and found that accumulation of this mtDNA deletion increases the severity of diabetic complications such as nephropathy and retinopathy (Suzuki, Hinokio et al. 1999).

Effects of defective OXPHOS on insulin secretion of pancreatic β-cells

In patients with T2DM, β-cells do not properly respond to glucose and are unable to secrete sufficient amounts of insulin (Gerich, 2003). Molecular mechanisms underlying T2DM appear due to defective mitochondrial OXPHOS and insufficient ATP production in β-cells. This view is supported by lowering oxygen supply to the pancreatic β-cell (Malaisse et al., 1979), and by suppressing production of the mtDNA-encoded OXPHOS subunits via creation of the ρ0 cell lines which lack mtDNA (King & Attardi, 1989; Soejima et al., 1996; Kennedy et al., 1998; Tsuruzoe et al., 1998), by hindering mitochondrial transcription (Hayakawa et al., 1998), and by uncoupling protons from ATP synthesis (Zhang et al., 2001). For example, long-term exposure of β-cells to ethidium bromide resulted in a depletion of mtDNA with preservation of insulin biosynthesis and cell viability, albeit with reduced proliferation. In such cell lines, glucose-induced insulin release was absent as a result of the loss of mtDNA and defective
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Oxidative phosphorylation (OXPHOS); whereas a secretory response to Ca\(^{2+}\)-raising agents was still present. Convincingly, the glucose-induced insulin secretion can be restored by replenishing \(\rho^0\) \(\beta\)-cells with normal mitochondria.

The nDNA defects that impair OXPHOS also abolish the glucose-responsive component of insulin secretion. Expression of mtDNA-encoded genes is controlled by the nDNA-encoded mitochondrial transcription factor (TFAM; Dairaghi et al., 1995; sin-Cayuela & Gustafsson, 2007). TFAM is essential for expression and maintenance of the mitochondrial genome. A homozygous defect of TFAM in mice is lethal, and its heterozygous knockout displays abolished OXPHOS (Larsson et al., 1998). The deletion of TFAM in pancreatic \(\beta\)-cells results in a diabetic phenotype (Silva et al., 2000), that is, the mouse mutants developed diabetes from the age of 5 weeks. At 7-9 weeks, these mice displayed severe mtDNA depletion, deficient OXPHOS, and altered mitochondrial morphology in the pancreatic islets. Thus, both in vitro and in vivo studies highlight a pivotal role of mitochondrial OXPHOS in insulin secretion by pancreatic \(\beta\)-cells.

Diabetic hyperglycemia per se is a major factor in pancreatic \(\beta\)-cell dysfunction and a variety of pathologic changes, as a consequence of hyperglycemia-induced overproduction of ROS (Nishikawa et al., 2000; Brownlee, 2001). In addition, \(\beta\)-cells are vulnerable to damage caused by ROS at least partly because of very low expression of antioxidant enzyme genes (Tiedge et al., 1997). To explain how lipids (lipotoxicity) and/or hyperglycemia (glucotoxicity) induce insufficient secretion of insulin by \(\beta\)-cells, uncoupling protein-2 (UCP2) emerges as a convincing biomarker as both its expression and activity are negatively correlated to glucose-stimulated insulin secretion (Zhang et al., 2001; Chan et al., 2001; Joseph et al., 2002; Krauss et al., 2002). Hyperglycemia in obese or T2DM individuals induced upregulation of UCP2 within \(\beta\)-cells (Patane et al., 2002; Laybutt et al., 2002), and an increase in the production of ROS (Tanaka et al., 2002; Bindokas et al., 2003). The increased ROS or superoxide promotes not only apoptosis, but also activates UCP2; and both changes lead to the observed decrease in insulin secretion (Joseph et al., 2002; Echtay et al., 2002; Krauss et al., 2003). The UCP2 polymorphism, G866A, has been associated with obesity due to an increase in associated promoter activity and transcriptional levels of UCP2 within adipose tissue (Esterbauer et al., 2001), and also with T2DM due to the decrease in insulin secretion by \(\beta\)-cells (Krempler et al., 2002; Sesti et al., 2003; Sasahara et al., 2004; Table 1). In contrast, a genetic deficiency in UCP2 prevents \(\beta\)-cell dysfunction within in vitro models of glucotoxicity and lipotoxicity (Joseph et al., 2004; Yamashita et al., 2004). Heterozygosity with a null UCP2 allele produces an intermediate phenotype, indicating that relatively small changes in UCP2 expression exert meaningful effects on glucose-stimulated insulin secretion (Zhang et al., 2001). UCP2 is an nDNA-encoded integral membrane protein found within the mitochondrial inner membrane. The increase in UCP-2 protein levels promotes the pumping of protons from the mitochondrial intermembranal space to the matrix. The protons bypass the mitochondrial ATP synthase leading to a decrease in the ATP/ADP ratio within the cytoplasm of \(\beta\)-cells. This change results in the opening of K\(^+\) channels and the closure of Ca\(^{2+}\) channels and, in turn, a reduction in insulin secretion (Figure 3). Therefore, the hyperglycemia-superoxide-UCP2 pathway mediates a proton leak, which provides us with a molecular mechanism underlying \(\beta\)-cell dysfunction.
dysfunction and insufficient insulin secretion in obesity and T2DM.

**Plasma free fatty acids (FFA) and pancreatic β-cells in obesity and T2DM**

Obesity is one of the major causative factors in peripheral insulin resistance and mitochondrial dysfunction (Stark & Roden, 2007). Within obese individuals, the pancreatic β-cells adapt to meet the body’s markedly increased demand for insulin through an expansion of β-cell mass to maintain the glucose response. Failure in this adaptation leads to the development of T2DM (Butler et al., 2003; Rhodes, 2005). The β-cell mass is determined by cellular size and a combined rate of cell proliferation, differentiation and apoptosis. Using Zucker diabetic fatty (ZDF) rats, age-matched insulin-resistant fatty (ZF) rats, and lean control (ZLC) rats, Pick and his colleagues (Pick et al., 1998) demonstrated a significant difference in β-cell mass in the order ZF > ZDF > ZLC, despite a proliferation rate of ZDF β-cells being higher than that of ZF or ZLC. In addition, the ZF and ZDF β-cells display morphological evidence of apoptosis, suggesting that apoptosis plays an important role in decreased insulin secretion within β-cells.

**FFA and insulin resistance of skeletal muscle and liver cells in obesity and T2DM**

Insulin resistance in skeletal muscle is characterized by impaired glucose oxidation and reduced glycogen synthesis during insulin stimulation. Insulin resistant subjects also exhibit a blunted suppression of lipolysis under insulin stimulated conditions. The diminished glucose oxidation could either result from a decreased glucose influx into the myocyte, or from an impaired oxidative capacity of mitochondria. In the fasted state with basal insulin concentrations, insulin resistant obese subjects feature reduced efficiency of lipid oxidation despite increased lipid availability, as reflected by elevated plasma FFA. This phenomenon has been termed metabolic inflexibility and implies mitochondrial dysfunction (Kelley, 2005).

*In vivo* studies using 31P magnetic resonance (MRS) spectroscopy in several populations have associated mitochondrial function and intramyocellular lipids (IMCL) with insulin resistance (Petersen et al., 2003; Morino et al., 2006). Glucose transporter 4 (GLUT4) is a protein that acts as an insulin-regulated glucose transporter. In the absence of insulin, this integral membrane protein is sequestered within the cells of muscle and adipose tissue. Within minutes of insulin stimulation, the protein moves to the cell surface and begins to transport glucose across the cell membrane. In healthy individuals, GLUT4 in skeletal muscle is activated by a pathway which includes insulin, insulin receptor substrate (IRS)-1, and phosphatidylinositol-3 (PI-3) kinase. The binding of insulin to its receptor induces the phosphorylation of the cytosolic substrate IRS1 by the insulin receptor tyrosine kinase. Tyrosine-phosphorylated IRS-1 binds and activates PI-3 kinase, which in turn activates GLUT4 to transport glucose into the cell (Dresner et al., 1999) and hexokinase II to generate glucose-6-phosphate (Roden et al., 1999), which commits glucose to glycolysis and ultimately to oxidation. It has been shown that an increase in plasma FFA levels in humans abolishes the insulin-mediated GLUT4 activation (Dresner et al., 1999).

In subjects with obesity and/or T2DM, skeletal muscle is exposed to elevated levels of plasma
FFA, which are likely derived from the excessive hydrolysis of triglycerides due to a reduced insulin-mediated inhibition of lipolysis in adipocytes, and also from an increased triglyceride production by the liver (Roden, 2006). The elevated FFA in the circulation leads to increases in IMCL and intracellular long-chain fatty acyl-CoA (LCFA-CoA), which promotes the intracellular deposition of triglycerides (Figure 4). FFA, IMCL and LCFA-CoA have all been correlated with insulin resistance (Roden, 2005). LCFA-CoA activates a nuclear factor kappa B (NFκB) and protein kinase C theta (PKC-θ), which in turn activate a serine/threonine kinase cascade. This cascade leads to the phosphorylation of serine/threonine residues of IRS-1 within skeletal muscle. In contrast to insulin-induced tyrosine phosphorylation, the serine/threonine phosphorylation of IRS-1 inhibits PI-3 kinase and may result in a 50% reduction in insulin stimulated PI-3 kinase activity (Griffin et al., 1999). Consistent with these findings, PKC-θ knockout mice are protected from lipid-induced insulin resistance within mouse skeletal muscle (Kim et al., 2004). Inhibition of PI-3 kinase activity leads to reduced GLUT4 and hexokinase activities for the uptake and phosphorylation of glucose, respectively (Figure 4).

Figure 4. Flow diagrams of the molecular mechanisms underlying insulin resistance and mitochondrial dysfunction in muscle (liver) cells of obesity or insufficient treated T2DM. In obese or T2DM individuals, the elevated plasma free fatty acids (FFA) will primarily increase intracellular long-chain fatty acyl-CoA (LCFA-CoA) and triacylglyceride, which mediate three pathways. They would activate serine/threonine kinases such as protein kinase C-θ (PKC-θ [PKC-ε in liver]), which inhibit insulin signaling by phosphorylation of serine residue (S) through insulin receptor substrate-1 (IRS-1 [IRS-2 in liver]). The serine phosphorylation, in turn, inhibits phosphatidylinositol-3 (PI-3) kinase for activation of insulin-dependent glucose transport, phosphorylation and glycogen synthesis, resulting in the muscular (or liver) insulin resistance. In addition, the increased intracellular LCFA-CoA and triacylglyceride inhibit PGC1α.
expression, which is unable to stimulate expression of mitochondrial transcriptional factors (NRFs and TFAM). Downregulation of NRFs and TFAM result in reduction of mitochondrial biogenesis and transcription, leading to decreases in mitochondrial density, β oxidation, TCA cycle, OXPHOS and ATP production. Furthermore, increased intracellular LCFA-CoA and triacylglyceride upregulate expression of UCP2, resulting in decrease in ATP production and increase in ROS generation. The latter enhances UCP2 activity and worsens ATP production, promotes apoptosis, and decreases mitochondrial density, β oxidation, TCA cycle, OXPHOS and ATP production. The black lines and arrows indicate pathways in skeletal muscle and liver cells, the red in pancreatic β-cells, and the blue are common in all these three types of cells.

GLUT4: glucose transporter 4; G6P: glucose-6-phosphate; PI-3 kinase: phosphatidylinosital-3 kinase; IRS: insulin receptor substrate; UCP2: uncoupling protein 2; ROS: reactive oxygen species; PGC1α: peroxisome proliferator-activated receptor gamma coactivator 1α; NRFs: nuclear respiratory factors; TFAM: transcriptional factor A, mitochondrial; IMCL: intramyocellular lipids.

A similar molecular mechanism of fat-induced insulin resistance likely occurs within the liver of subjects with obesity and/or T2DM. Accumulation of intracellular lipid metabolites in the liver activates a serine kinase cascade involving PKC-ε, leading to increased serine phosphorylation of IRS-2, a key mediator of insulin action in liver (Previs et al., 2000; Samuel et al., 2004). The serine phosphorylation of IRS-2 inhibits PI-3 kinase. One of the metabolites found by animal studies using knockout mice is mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 (mtGPAT1), a known activator of PKC-ε (Neschen et al., 2005). Inhibition of PI-3 kinase leads to reduced GLUT4 and hexokinase activities for glucose uptake and phosphorylation, respectively (Figure 4). Thus, glucose is unable to enter glycogen synthesis in liver cells under these conditions.

FFA and mitochondrial dysfunction of skeletal muscle in obesity and T2DM

Maternally inherited defective mtDNA is known to cause diabetes (Goto et al., 1990; Rotig et al., 1992). In comparison with body fat-matched youth controls, lean elderly subjects display not only elevated triglycerides and insulin resistance in muscle and liver, but also decreased mitochondrial OXPHOS and ATP production (Petersen et al., 2003). The increase in IMCL and decrease in mitochondrial function have also been observed in young insulin-resistant offspring of parents with T2DM (Petersen et al., 2004). The decrease in mitochondrial function further impairs OXPHOS and ATP production, which in turn worsens the accumulation of IMCL (Figure 4).

Skeletal muscle adapts its oxidative capacity to the prevailing pattern of bioenergetic demand, which is largely determined by physical activity (Larson-Meyer et al., 2000; Kim et al., 2000). In obese individuals, skeletal muscle displays a reduced oxidative capacity, a metabolic characteristic associated with insulin resistance (Simoneau et al., 1995; Sun et al., 2002). In previously sedentary obese adults, a combined intervention of weight loss with physical activity is associated with the enlargement of mitochondria, an increase in mitochondrial content within skeletal muscle, and an improvement in insulin sensitivity (Toledo et al., 2006). This type of exercise has been shown to increases oxidative enzyme activity probably through an increase in the number of mitochondrial cristae (Menshikova et al., 2005).
Genes involved in OXPHOS have been reported to be down-regulated in skeletal muscle from patients with T2DM. Dysregulated intra-myocellular fatty acid metabolites have been associated with insulin resistance within skeletal muscle derived from the offspring of patients with T2DM, possibly due to an inherited defect in OXPHOS (Petersen et al., 2003; Petersen et al., 2004). Moreover, genes involved in OXPHOS are downregulated within skeletal muscle. Peroxisome proliferator-activated receptor-γ (PPARG) coactivator-1α (PPARGC1A, also known as PGC1α) has been identified as a master gene in the control of expression of the OXPHOS genes, and downregulation of both PGC1α and PGC1β, as well as PGC1α-responsive genes has been observed in both obesity and diabetes (Patti et al., 2003; Mootha et al., 2003). PGC1α, a cold-inducible coactivator of nuclear receptors, stimulates mitochondrial biogenesis and respiration in muscle cells through regulation of the nuclear respiratory factors (NRFs). PGC1α induces expression of NRF-1 and NRF-2 genes, in addition to the binding and coactivation of the transcriptional activity of NRF-1 on the promoter for TFAM, a direct activator of mtDNA replication/transcription. These data identify a pathway that directly links external physiological stimuli to the regulation of mitochondrial biogenesis and function (St-Pierre et al., 2003). Furthermore, 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 typically contain more mitochondria (oxidation) than type 2 (glycolysis).

**Mitochondria as drug targets for treating diabetes and obesity**

Because mitochondria play a key role in the control of the production of ATP and ROS, and the secretion of insulin, drugs targeting mitochondrial functions may facilitate treatment of diabetes and obesity. Potential therapeutic strategies include a delivery of antioxidants to mitochondria to prevent oxidative damage and the modulation of uncoupling proteins (UCPs) for ATP production in diabetes and obesity. Other potential therapeutic approaches include the inhibition of cell death and somatic mitochondrial gene therapy (Wallace, 2005). Several antioxidants have been applied in the treatment of diabetes. Coenzyme Q10 (CoQ or ubiquinone) has potent antioxidant actions and is an important intermediate in the mitochondrial electron transport chain. CoQ is believed to reduce oxidative stress by ‘recoupling’ mitochondrial OXPHOS, thereby reducing superoxide production (Chew & Watts, 2004). Supplementation with CoQ in T2DM has improved endothelial function of conduit arteries of the peripheral circulation (Watts et al., 2002). MitoQ is a mitochondrial-targeted antioxidant, which comprises a lipophilic triphenyl-phosphonium (TPP) cation covalently attached to an ubiquinol antioxidant. The high concentration of MitoQ protects mitochondria from oxidative damage, serving as a promising new therapeutic strategy in diabetes (Adlam et al., 2005). Overexpression of Mn superoxide dismutase (MnSOD) led to a decrease in ROS (Nishikawa et al., 2000), suggesting that MnSOD might serve as a drug to reduce mitochondrial oxidative damage.

Overexpression of UCP1 has been shown to reduce the glucose-induced proton gradient and superoxide production in endothelial cells (Nishikawa et al., 2000). While UCP overexpression reduces ROS levels as well as diabetic signaling in the endothelium, it could reduce ATP production in pancreatic β-cells, which in turn inhibits ATP-dependent insulin secretion.
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(Lowell & Shulman, 2005). This suggests that modulation of UCP function for therapeutic purposes must be done in a tissue-specific manner (Mattiasson & Sullivan, 2006). UCPs are also targets for obesity treatment. Animal studies have shown that the genetically engineered mice overexpressing different UCP homologues are lean and resistant to diet-induced obesity with features of mitochondrial uncoupling in adipocytes and increased energy expenditure (Kopecky et al., 2001; Dalgaard & Pedersen, 2001).

Other potential therapeutic approaches targeting mitochondrial diseases include the inhibition of cell death and somatic mitochondrial gene therapy (Wallace, 2005). The mitochondrial permeability transition pore (mtPTP) is an important component in mediating mitochondrial apoptosis, and thus may be regarded as a potential drug target. One strategy used to treat mtDNA mutations has been to clone the wild type version of the mutant gene, converting the mitochondrial genetic code to the universal genetic code via site-directed mutagenesis, adding a mitochondrial targeting peptide, and transforming the nucleus with the resulting construct. This approach aims to import functional proteins into the mitochondrion to correct defective function, and has been used to experimentally treat other mitochondrial diseases (Guy et al., 2002; Manfredi et al., 2002), although to date no attempt has been made for correction of mtDNA mutations in diabetes.

Conclusions

Defective mitochondrial OXPHOS plays a critical role in the pathogenesis of obesity and diabetes. Two prominent features of individuals presenting with obesity and T2DM include insufficient secretion of insulin by pancreatic β-cells and insulin resistance within muscle and liver cells. Mitochondrial dysfunction is correlated with the development of pancreatic β-cell dysfunction, and insulin resistance in the target tissues by four mechanisms as summarized in Figure 4. (1) UCP2 pathway: elevated FFA in obesity are transported to pancreatic β-cells and induce the upregulation of UCP2. High UCP2 protein mediates proton leaks, and therefore reduces ATP synthesis and increases ROS that activate UCP2 and apoptosis, resulting in the suppression of insulin secretion by the β-cells. (2) GLUT4 pathway: elevated FFA are transported to skeletal muscle and liver cells whereupon they become long-chain fatty acyl-CoA and TAG, activating PKCs and a serine/threonine kinase cascade, leading to the inhibition of GLUT4 and hexokinase activities for glucose uptake and oxidation. (3) PGC1α pathway: intracellular fatty acid metabolites inhibit PGC1α-mediated mitochondrial biogenesis and transcription, leading to a reduction in glucose oxidation. (4) Mitochondrial genetic dysfunction as a causative factor for diabetics. Despite progress, several questions remain to be answered. For example, what is the molecular mechanism linking mitochondrial tRNA mutation to diabetics? How can we effectively prevent T2DM by educating the young generation to regulate overeating? Can we identify natural products to overcome insulin resistance in muscle and liver cells? Future studies should focus upon mitochondrial OXPHOS dysfunction, obesity and diabetics for improved understanding, prevention and treatment.
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