Minireview

The contribution of mitochondria to common disorders

Gregory M. Enns*

Department of Pediatrics, Division of Medical Genetics, Stanford University, 300 Pasteur Drive, H-315, Stanford, CA 94305-5208, USA

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Abstract

Mitochondrial dysfunction secondary to mitochondrial and nuclear DNA mutations has been associated with energy deficiency in multiple organ systems and a variety of severe, often fatal, clinical syndromes. Although the production of energy is indeed the primary function of mitochondria, attention has also been directed toward their role producing reactive oxygen and nitrogen species and the subsequent widespread deleterious effects of these intermediates. The generation of toxic reactive intermediates has been implicated in a number of relatively common disorders, including neurodegenerative diseases, diabetes, and cancer. Understanding the role mitochondrial dysfunction plays in the pathogenesis of common disorders has provided unique insights into a number of diseases and offers hope for potential new therapies.

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Introduction

We all require oxygen to live, but only because mitochondria reduce oxygen to form water in the process of generating energy. Mitochondrial oxidative phosphorylation (OXPHOS) produces the majority of energy that drives eukaryotic cellular reactions. Because of the critical role the mitochondrial electron transport chain (ETC) plays in generating cellular energy, disorders that affect respiratory chain activity can cause dysfunction of any organ system, with the neuromuscular system often being compromised to a significant extent. The ETC is composed of five multimeric complexes consisting of >80 subunits in total. Electron transport between ETC complexes I–IV is coupled to the extrusion of protons across the inner mitochondrial membrane by proton pump components of the respiratory chain (complexes I, III, and IV). This movement of protons creates an electrochemical gradient (ΔΨ) across the inner mitochondrial membrane, which is alkaline and negative on the side of the mitochondrial matrix and acidic and positive externally. Protons return to the mitochondrial matrix by flowing through ATP synthase (complex V, EC 3.6.3.14), which utilizes the energy thus produced to synthesize ATP from adenosine diphosphate (ADP) and inorganic phosphate (P). ATP from the mitochondrial matrix is then exchanged for cytosolic ADP by the adenine nucleotide translocator [1].

The mitochondrial ETC is formed and maintained by the coordinate interaction of both the mitochondrial and nuclear genomes. Mitochondria contain between 2 and 10 copies mitochondrial DNA (mtDNA) and each cell contains at least several to >100 mitochondria, depending on the energy needs of a given tissue. Mitochondrial DNA is a double-stranded circular genome of approximately 16.5 kb that encodes 13 OXPHOS subunits, 22 tRNAs, and 2 rRNAs. The nuclear genome encodes the remaining majority of the respiratory chain subunits, as well as proteins responsible for the assembly and maintenance of the respiratory chain, ribosomal proteins, mtDNA regulatory factors, and DNA and RNA polymerases. Because disorders that affect the function of the respiratory chain can be caused by mutations in either the nuclear or mitochondrial genomes, inheritance of mitochondrial disease can be either Mendelian or maternal (mitochondrial). Although the main function of mitochondria is the production of...
ATP, these organelles have other significant roles in cellular metabolism such as the production of reactive oxygen species (ROS), regulation of apoptosis, and glucose and calcium homeostasis [1,2]. This review primarily focuses on the effects of these “alternative” roles of mitochondria on the pathogenesis of diseases typically associated with aging, including neurodegenerative disorders, diabetes mellitus (DM), and cancer.

Free radicals: reactive oxygen and nitrogen species

Toxic ROS and reactive nitrogen species (RNS) are byproducts of OXPHOS. Up to an estimated 1% of all oxygen consumption may result in the formation of superoxide (O$_2^-$) or hydrogen peroxide (H$_2$O$_2$) [3]. The transfer of one electron from the ETC to molecular oxygen generates superoxide anion and subsequent conversion to hydrogen peroxide is accomplished by mitochondrial manganese superoxide dismutase (MnSOD, EC 1.15.1.1) or cytosolic copper–zinc superoxide dismutase (Cu/ZnSOD, EC 1.15.1.1). Catalase or glutathione peroxidase (GPx, EC 1.11.1.9) reduce hydrogen peroxide to water and molecular oxygen. The production of superoxide and hydrogen peroxide is highest in state IV respiration, when mitochondrial ADP is lacking and the utilization of oxygen is determined by the slow leak of protons through the mitochondrial inner membrane. When the ETC is damaged or inhibited, the production of ROS may be increased. Uncoupling proteins-2 and -3 (UCP-2 and UCP-3) play a role in modulating ROS production. UCP-1 is considered to be thermogenic [4–6]. Superoxide may interact with UCPs to decrease intramitochondrial concentrations of ROS [7]. The iron–sulphur (Fe–S) centers of the ETC and tricarboxylic acid cycle (TCA) are especially sensitive to oxidative stress [8]. Hydrogen peroxide, by interacting with Fe$^{2+}$ via the Fenton reaction, can form the highly cytotoxic and reactive hydroxyl radical (·OH) [9]. Acute exposure to ROS leads to inactivation of Fe–S centers present in complexes I, II, III and the TCA cycle enzyme aconitate. Chronic ROS exposure leads to oxidative damage to mitochondrial and cellular proteins, lipids, and nucleic acids.

Mitochondrial ETC dysfunction has also been implicated in the generation of RNS. Defective mitochondrial Ca$^{2+}$ homeostasis secondary to mitochondrial dysfunction activates nitric oxide synthetase (NOS, EC 1.14.13.39), with subsequent increases in superoxide and nitric oxide (·NO) [10]. Nitric oxide can react with superoxide to form the highly reactive peroxynitrite (ONOO$^-$), a compound that has the ability to traverse biological membranes and react with tyrosine residues, forming 3-nitrotyrosine [11]. Peroxynitrite can irreversibly inhibit cytochrome c oxidase (COX, complex IV, EC 1.9.3.1), leading to further generation of superoxide and peroxynitrite and further damage to the ETC [11].

Antioxidants (e.g., protein thiols, reduced glutathione, α-tocopherol) and scavenging enzymes (e.g., catalase, GPx, MnSOD, Cu/ZnSOD) offer protection against the deleterious accumulation of ROS [12]. Although cellular antioxidant defenses limit potential damage from the generation of ROS and RNS, if these toxic compounds are generated continuously, the cellular defenses may be overwhelmed, leading to a downward spiral of further damage and eventual cellular death by apoptosis or necrosis.

The glutathione system

The concentration of intracellular reduced glutathione (GSH) appears to be of central importance in protecting cells from oxidant damage [12]. Loss of GSH and oxidative damage have been implicated in progressive cellular damage and apoptosis [9]. Mitochondria contain approximately 15% of total cellular GSH, but lack enzymes for GSH synthesis. Therefore, GSH is synthesized in the cytoplasm through the action of the γ-glutamyl cycle and enters the mitochondria via high and low affinity transport systems. Inner mitochondrial membrane dicarboxylate and 2-oxoglutarate carriers may also be involved in GSH uptake [12]. Mitochondrial and cytosolic hydrogen peroxide are inactivated primarily by Gpx in a reaction that is dependent on GSH. In addition, GSH reacts with NO$^-$ and NO$^+$ to form hydroxylamine and S-nitrosoglutathione respectively. GSH also reacts with ONOO$^-$ to form a thyl radical (GS$^\cdot$) that can initiate an oxygen dependent chain reaction that leads to further depletion of intracellular GSH [12].

Neurodegenerative disorders

Neurons are highly susceptible to oxidant stress. Oxidation and nitration of proteins, lipids, and DNA have been found to be associated with neurodegeneration in postmortem tissues [3]. Established mitochondrial disorders with identified mutations in either mtDNA (e.g., MERRF, MELAS, and NARP) or nDNA (e.g., COX deficiency secondary to SURF1 mutations) are well-known, albeit relatively rare, causes of neurological dysfunction. Although the causal link may not be as clearly established as in the prototypical mitochondrial disorders, substantial evidence exists for implicating mitochondria in the pathogenesis of some of the more common neurodegenerative disorders, including Alzheimer disease, Parkinson disease, and Huntington disease. Secondary mitochondrial ETC dysfunction is also important in the pathogenesis in other, more rare neurological disorders, including Friedreich ataxia and amyotrophic lateral sclerosis. The common unifying theme is the production of reactive
intermediates, which cause further cellular damage in a worsening metabolic spiral. Similarly, the production of ROS, RNS, abnormal Ca\(^{2+}\) homeostasis, and induction of apoptosis via the mitochondrial permeability transition pore may play a role in neuronal damage following brief and prolonged seizures or stroke in individuals without mitochondrial disease per se [13,14]. Much attention has also been given to diminished mitochondrial function associated with accumulation of mtDNA mutations in normal aging; mitochondrial dysfunction has become one model to explain the slow decline in mental, and other organ system, function associated with the passage of time.

**Normal aging**

Somatic mtDNA rearrangements and point mutations may accumulate with time and contribute to the neurologic impairment associated with aging [15,16]. Heart muscle, skeletal muscle and brain tissue from normal adults contain a low level of the ‘common’ 4977 bp mtDNA deletion that has been associated with mitochondrial disorders such as Kearns-Sayre syndrome, Pearson marrow-pancreas syndrome and chronic external ophthalmoplegia [16–19]. In heart muscle from individuals with coronary artery disease, the level of the ‘common’ mtDNA deletion was elevated 7–220 times when compared with normal age-matched controls [19]. The 4977 bp deletion was also present in cerebral cortex, putamen and cerebellum in elderly brains [16]. Human skeletal muscle shows a progressive decline in ETC activity associated with the accumulation of the ‘common’ mtDNA deletion [18]. Point mutations in mtDNA have also been found to accumulate progressively with age [1,20]. The most likely cause of these somatic mtDNA mutations is damage from ROS [1]. Normally only about 0.1–1% of total O\(_2\) consumption is directed toward the formation of ROS, but this percentage increases with age [3,21]. Overall, mtDNA has been estimated to be approximately 10 times more sensitive to oxidative damage than nDNA [22]. The mutations appear to arise at random within a given cell [23] and are correlated with a decline in OXPHOS, further accumulation of mtDNA mutations, and activation of genes important in oxidative stress response [1,24,25].

**Alzheimer disease**

Alzheimer disease (AD) is a common form of late-onset dementia, characterized by memory loss and progressive deterioration, affecting between 5 to 15% of individuals over 65 years [26]. Most cases are sporadic, although mutations in amyloid precursor protein or presenilin genes have been found in patients who demonstrate a clear family history. Activities of brain pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and COX are also decreased in AD [27]. Circumstantial evidence has implicated ROS in the pathogenesis of AD, but the exact mechanism of pathogenesis still remains unclear. Advanced glycation end-products capable of generating ROS and peroxynitrite have been found in neurofibrillary tangles and neuritic plaques, the pathologic hallmarks of AD [28–31]. The activity of the mitochondrial ETC may be impaired by β-amyloid [32,33]. In addition, β-amyloid may induce the production of ROS and therefore cause further oxidative damage [34–36]. AD appears to be associated with multiple respiratory chain defects [26]. The hippocampal formation of AD patients showed reduced immunoreactivity to antibodies directed against two mitochondrial COX subunits (COI and COII) as well as one nuclear-encoded subunit of complex III. Mitochondrial complex I subunit and COX mRNAs, as well as mRNA for nuclear COX and ATPase genes, are reduced in the temporal cortex of AD patients [37], without decrease of non-ETC proteins. Overall, there appears to be a global down-regulation of all ETC subunits, but the role in disease pathogenesis is uncertain. This down-regulation may simply represent a physiological response to reduced neuronal activity [26]. On the other hand, cybrid experiments using platelet mitochondria in patients with AD have shown that focal defects of ETC function can be reproduced. In addition, AD cybrids display increased oxidative stress and alterations in Ca\(^{2+}\) homeostasis [38]. Measurement of 8-hydroxy-2\’-deoxyguanosine (8OHdG), a marker of oxidative damage to DNA, in postmortem brains of 13 patients with AD showed a 3-fold increase in 8OHdG levels in mtDNA from the parietal cortex [39]. Enhanced oxidative damage has been associated with increased levels of the ‘common’ mtDNA deletion as well, although this finding has not been corroborated [26,40]. Although several candidate mtDNA mutations have been reported, the evidence for supporting a causative role in these alterations is sparse or contradictory. Some possible mtDNA mutations in AD patients are likely to be polymorphisms and others have been shown to be artifacts from PCR amplification of nDNA [26]. An A4336G alteration has been found in a higher frequency in AD by some groups, but not confirmed by others [41–44]. When the mtDNA control region was analyzed in brains from 35 individuals with AD, 47 with dementia with Lewy bodies and 31 normal elderly individuals, there was no evidence for somatic accumulation of mtDNA point mutations [45]. Therefore, while there is evidence for altered OXPHOS in AD, a specific mtDNA mutation has not yet been identified.

**Parkinson disease**

Parkinson disease (PD) is caused by degeneration of substantia nigra dopaminergic neurons. Surviving
neurons often contain intracytoplasmic inclusions (Lewy bodies), which contain α-synuclein, neurofilaments and ubiquitin [46]. PD is the second most common neurodegenerative disease after AD, with a prevalence of about 1 in 350 [47]. Bradykinesia, tremor and rigidity occur usually between the fifth and seventh decade. As in AD, no specific mtDNA pathologial mutations have been identified in PD, although there is good evidence for compromise of the mitochondrial ETC [10]. Complex I (EC 1.6.5.3) deficiency has been found in the substantia nigra of PD postmortem specimens [10]; other brain areas have shown normal OXPHOS activity. Complex I deficiency has also been found in other tissues, including skeletal muscle, platelets and lymphoblasts, although normal ETC activities have also been reported [10]. Lymphocytes from PD patients have shown significantly lower complexes I, III and IV activities [48]. Supportive evidence of ETC compromise in PD is also provided by cybrid experiments; platelets from PD patients with complex I deficiency were fused with ρ0 cells and found to have a 25% reduction of complex I activity [10,49]. Cybrids from PD patients also have been shown to have increased markers of oxidative stress and abnormal Ca2+ homeostasis [38].

Several lines of evidence suggest that oxidative stress and the induction of apoptosis are also important in disease pathogenesis. Postmortem substantia nigra isolates show findings consistent with free radical exposure, including lipid peroxidation, protein nitration, and DNA damage [10]. Reduced levels of GSH, GPx1, and catalase and increased oxidized glutathione (GSSG) also point to the effects of ROS imbalance [10]. Additional evidence of oxidative and nitritative damage in PD and other neurodegenerative diseases comes from the finding of nitrated α-synuclein in signature lesions from brains of individuals with PD, dementia with Lewy bodies, Lewy body variant of AD, and multiple system atrophy [50]. Furthermore, in rat brain mitochondrial isolates, exposure to dopamine quinone led to increased state 4 respiration and swelling of brain and liver mitochondria, an effect that can be prevented by addition of GSH [51]. Dopamine is metabolized by monoamine oxidase (MAO, EC 1.4.3.4) to 3,4-dihydroxyphenylacetaldehyde, hydrogen peroxide, and ammonia, which results in suppression of rat brain mitochondrial ETC activity. MAO inhibitors prevent this ETC suppression, suggesting that an increase in dopamine metabolism may play a role in the observed ETC deficiencies observed in PD [52]. The complex I inhibitor and apoptosis inducing toxin N-methyl-4-phenylpyridinium (MPP+) causes a parkinsonian syndrome in humans and primates after uptake into dopaminergic neurons. In addition to inhibition of complex I, MPP+ acts by opening the mitochondrial permeability transition pore (MPTP), which results in subsequent release of cytochrome c and apoptosis. The addition of rotenone, a non-competitive inhibitor of complex I, ameliorates this MPP+ effect. The MPP+-induced opening of the MPTP is synergistic with nitric oxide and atracysolide, an inhibitor of adenosine nucleotide transporter [53]. MPP+ also causes expression of the stress activated c-Jun N-terminal kinase (JNK) and transcription factor nuclear factor-κB (NF-κB) in SH-SY5Y neuroblastoma cells. The activation of NF-κB appears to be dependent, in part, on the function of the mitochondrial ETC; increased levels of NF-κB were found in PD cybrids and may represent a response to oxidative stress [54].

Fascinating, albeit indirect, evidence of the involvement of mitochondrial impairment in PD comes from a recent study that genotyped 10 single-nucleotide polymorphisms (SNPs) that define the European mtDNA haplogroups in 609 PD patients and 340 unaffected controls [55]. Individuals classified as haplogroup J had a significantly decreased risk of developing PD compared to the most common haplogroup. A specific SNP (10398G) was found to be strongly associated with the protective effect. This SNP causes a nonconservative amino acid change within the nuclear ND3 subunit of complex I. In addition, a SNP present in complex V subunit ATP6 (9055A) was shown to have a protective effect in women [55].

Huntington disease

Huntington disease is a progressive autosomal dominant disorder characterized by ataxia, chorea and dementia, with onset typically in adulthood. Although abnormal CAG expansion in the gene encoding huntington protein has been established as the etiology of HD, the exact mechanisms of disease pathogenesis remain obscure. The caudate nucleus is primarily involved. Glutamate excitation of N-methyl-D-aspartate (NMDA) receptors, with subsequent influx of Ca2+, activation of NOS and production of NO, has been implicated in disease pathogenesis [15]. Deficiencies in complexes II and III and the TCA cycle enzyme aconitase have been documented [56], raising the possibility of oxidative and nitritative stress causing damage, because these enzymes contain highly susceptible Fe-S complexes [57]. Various other ETC deficiencies have been reported in platelets and postmortem brain samples from HD patients, although findings have not been consistent [15]. There appears to be deficient ETC function in the striatum, but such deficiency has not been conclusively demonstrated in other tissues. However, lymphoblast mitochondria from HD patients have recently been found to have lower ATP and depolarize at lower Ca2+ levels than controls [58]. A similar defect was present in transgenic mice expressing full-length mutant huntington. When normal mitochondria were incubated with a fusion protein containing an abnormally long polyglutamine repeat, the mitochondrial Ca2+ defect
seen in HD patients and transgenic mice was replicated [58]. Therefore, abnormalities in mitochondrial Ca\(^{2+}\) metabolism may occur early in disease and may represent a direct effect of mutant huntington protein on mitochondria [58].

**Amyotrophic lateral sclerosis**

Individuals with amyotrophic lateral sclerosis (ALS) have progressive degeneration of motor neurons starting in mid-to-late life. Most cases are sporadic (90–95%), although familial cases occur and have been associated with lesions in SOD1, the gene encoding Cu/ZnSOD [59,60]. Cu/ZnSOD catalyzes the conversion of superoxide to hydrogen peroxide and is localized to the cytosol, nucleus, and mitochondrial intermembrane space [61]. Two main theories of disease pathogenesis have arisen: the oligomerization hypothesis and the oxidative damage hypothesis [61]. Misfolding of mutant Cu/ZnSOD proteins and subsequent oligomerization into high molecular weight complexes that aggregate and become part of abnormal, toxic protein deposits underlies the former hypothesis. Oxidative damage may occur if mutant Cu/ZnSOD catalyzes reactions with superoxide or peroxynitrite that lead to damage of intracellular lipids, protein and DNA. Oxidative stress appears to play some role in the damage observed in ALS transgenic mice [62]. Hydrogen peroxide has been shown to react with a redox-active metal ion to generate a hydroxyl radical, which then oxidizes an amino acid residue adjacent to the Cu/ZnSOD redox metal ion-binding site [63]. In addition, ALS transgenic mice demonstrate morphological mitochondrial abnormalities and decreased mitochondrial ETC activity [64,65].

**Other neurodegenerative disorders**

**Friedreich ataxia (FA)** is an autosomal recessive condition and the most common form of inherited ataxia, with a birth prevalence of 1 in 50,000. Clinical features include onset in early childhood of progressive gait and limb ataxia, upper motor neuron dysfunction, cardiomyopathy, and diabetes mellitus [66]. Abnormal GAA expansion (a few patients with X25 point mutations have been reported) in the X25 gene results in a severe reduction in the levels of frataxin, a protein targeted to the mitochondrial matrix. Frataxin is associated with intramitochondrial iron homeostasis and plays a role in the formation of Fe-S clusters. Impaired in vivo mitochondrial respiration in skeletal muscle has been detected in FA patients using phosphorus magnetic resonance spectroscopy [67]. Lymphoblasts from FA patients are more sensitive to oxidative stress when challenged with free iron and/or hydrogen peroxide [68]. Although the total mitochondrial iron concentration remained unchanged in FA cells, filterable mitochondrial iron was increased [68]. Therefore, altered intramitochondrial iron homeostasis may lead to mitochondrial dysfunction in FA. Total and free glutathione levels in blood in patients with FA are significantly lower than controls, implicating oxidative stress in disease pathogenesis as well [69].

**X-linked sideroblastic anemia and ataxia and vitamin E binding protein deficiency** are other spinocerebellar ataxias in which defective mitochondrial metabolism has been established [70], the common link being the production of ROS and decreased levels of antioxidants.

**Autism**, although not a neurodegenerative disorder, has also been suggested to be caused in part by mitochondrial dysfunction, although evidence for mitochondrial involvement is quite sparse. Scattered reports of lactic acidemia, carnitine deficiency, and increased urine TCA cycle intermediates are unconvincing [71]. Recently, a mutation in mtDNA (G8363A tRNA\(^{\text{Lys}}\)) has been reported in 12 children who, in addition to autism, had other findings more typically associated with mitochondrial disease [72].

**Diabetes mellitus**

Diabetes mellitus (DM) affects >150 million individuals worldwide and is divided into two main types: type 1 DM, or insulin-dependent DM (IDDM) and type 2 DM, or non-insulin dependent DM (NIDDM) [73]. Microvascular and macrovascular complications of DM cause considerable morbidity and mortality worldwide. DM is the main cause of blindness in middle age and a major cause of heart disease [73]. Approximately 20% of individuals with DM develop significant renal disease and may require hemodialysis or transplantation [73]. NIDDM affects more than 3 per 1000 individuals in the United States and is the most common type of DM, being present in 80% of cases [6]. NIDDM is transmitted 2–3 times more frequently from the mother and may be inherited through multiple maternal generations [74]. On the other hand, the risk of a child who has a father with IDDM to develop IDDM is more than 2-fold elevated when compared to the risk if the mother has IDDM [73]. Mitochondrial disorders commonly have DM as a significant part of their multi-system involvement and multiple mtDNA mutations have been associated with DM (Table 1, see 75 for a review). However, more subtle alterations in cellular metabolism involving toxic effects of hyperglycemia and secondary mitochondrial dysfunction in individuals with DM without mtDNA mutations have been the focus of intensive research.

**MIDD (Maternally Inherited Diabetes and Deafness) and mtDNA depletion**

In 1992, three landmark reports documented the presence of mtDNA mutations in familial DM
[74,76,77]. A 10.4 kb deletion involving the mtDNA light strand origin of replication (O_L) was found in a family that demonstrated maternal transmission of IDDM and/or deafness over three generations [74]. Unlike other mtDNA deletion syndromes, ophthalmoplegia and myopathic features were absent. Two groups reported the presence of the A3243G mutation typically associated with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) in large pedigrees with individuals affected by NIDDM and deafness [76,77]. The proband in one pedigree also had a cardiomyopathy and died relatively young [77]. The A3243G mutation has been estimated to occur in 0.3–2% of DM [78] and may be associated with either IDDM or NIDDM. A carrier mother transmits the A3243G mutation to nearly all of her children and 2% of DM [78] and may be associated with either IDDM or deafness over three generations [74].

In addition to qualitative changes in mtDNA, the mean mtDNA quantity in peripheral blood leukocytes may be decreased in NIDDM patients [82,83]. Mean mtDNA quantity in peripheral blood leukocytes was 35% lower in NIDDM patients than controls, but did not correlate with age, body mass index, duration of symptoms, or HbA1c levels [82]. A reduction in mtDNA in presymptomatic individuals who converted to NIDDM within 2 years was also found [82]. In healthy young men, mtDNA content in peripheral leukocytes correlated negatively with indices of insulin resistance and insulin secretion [84]. In skeletal muscle from NIDDM patients, a ~50% decrease in mtDNA copy number was found, but four genes encoded by the mtDNA (COI, COIII, ND4, and 12s rRNA) had 1.5–2.2-fold increased expression. No change in expression of the nDNA encoded proteins COX VIIaM or mitochondrial trans-acting factor 1 was present [85].

Increased mtDNA content has also been found in animal models of DM [86,87]. Content of mtDNA is decreased in the Goto-Kakizaki rat, a model of NIDDM [86]. A mouse model of mitochondrial diabetes was created by tissue-specific knock out of the nuclear gene encoding mitochondrial transcription factor A (Tfam) in pancreatic β-cells. Tfam-mutant mice developed DM at approximately 5 weeks and had severe mtDNA depletion, OXPHOS abnormalities, and abnormal mitochondrial ultrastructure [87]. Therefore, quantitative changes in mtDNA have been postulated to underlie insulin resistance in NIDDM [83].

Using 8OHdG as a marker of oxidative damage, several reports have documented the increased presence of oxidative stress in NIDDM patients [88–91]. Levels of 8OHdG were 6–23-fold higher in mtDNA than nDNA, showing the especially high sensitivity of mtDNA to oxidative damage [89]. Higher levels of 8OHdG also correlate with HbA1c levels and degree of diabetic complications [90,91].

Overall, there is unequivocal evidence of mitochondrial involvement in DM, but how does such involvement translate into impaired insulin secretion? 

Glucose induced stimulus-secretion coupling (glucose-stimulated insulin secretion)

Mitochondria are directly linked to glucose-stimulated insulin secretion (GSIS). Inhibitors of mitochondrial ETC function, including rotenone, antimycin A, sodium azide, and cyanide, also inhibit insulin release [6]. In pancreatic islet β-cells, glucose equilibrates across the plasma membrane through the high capacity, low affinity transporters GLUT1 and GLUT2 [92]. GLUT2 has a study of 124 individuals with NIDDM, single stranded conformational polymorphism analysis did not detect any heteroplasmic mutations, although several homoplasmic variants were found [81].
capacity greater than 100 times the maximal rate of glycolysis to utilize glucose and sufficient carrying capacity to assure equilibration of extra- and intracellular glucose within milliseconds [93]. Glucose is then phosphorylated to glucose-6-phosphate by cytosolic glucokinase, the enzyme that determines the rate of flux through glycolysis and the rate of pyruvate generation [75,92], and, as such, functions effectively as a cellular “glucose sensor” [93]. Pyruvate generated from glycolysis then enters the mitochondrial TCA cycle. A high proportion of glucose-derived carbon enters the β-cell and is converted to CO₂ in the mitochondria [94]. Mitochondrial pyruvate can be metabolized by either pyruvate dehydrogenase, generating acetyl-CoA, or pyruvate carboxylase, forming oxaloacetate and ensuring anaplerosis to the TCA cycle [75]. Pyruvate carboxylase is expressed at very high levels in rat islet and insulinoma (INS-1) β-cells; anaplerosis may be crucial in activating the pyruvate/malate shuttle with subsequent increase in cytosolic NADPH [94]. Reducing equivalents in the form of NADH or FADH₂ generated by the TCA cycle are transferred to the mitochondrial ETC with subsequent production of ATP. ATP enters the cytoplasm leads to flux through glucokinas, the production of ATP/ADP ratio, with resultant depolarization of the plasma membrane secondary to closure of ATP-sensitive K⁺ channels. Upregulation of β-cell UCP-2 results in increased ROS production, decreased ATP, closure of ATP-sensitive K⁺ channels and impaired insulin secretion [4]. Conversely, knockout of UCP-2 activity in mice leads to enhanced β-cell ATP production and insulin secretion during glucose stimulation [5]. Plasma membrane depolarization, in turn, results in the opening of voltage-sensitive Ca²⁺ channels and rapid increase in cytosolic Ca²⁺, the triggering event for stimulation of insulin secretion by exocytosis of secretory granules (Fig. 1). In addition to directly stimulating exocytosis, Ca²⁺ is a co-factor for rate limiting steps of the TCA cycle and, therefore, flux through the cycle and ultimately OXPHOS are enhanced by increased intramitochondrial Ca²⁺ [95]. Glucose induced stimulus-secretion coupling may be facilitated by the close proximity of mitochondria to insulin-containing secretory granules [75].

In contrast to glucose, leucine directly enters mitochondria and causes generation of acetyl-CoA, which then enters the TCA cycle, starting the biochemical process that ultimately results in insulin secretion [92]. Glucose and the leucine deamination product 2-oxoisocaprate raise intracellular Ca²⁺ concentration in a dose-dependent manner in cultured rat islet β-cells, leading to insulin secretion [96].

Animal models have provided further evidence of the importance of intact mitochondrial ETC function for insulin release. In Tfm-deficient mice, total mtDNA is decreased and mutant β-cells display an initial decrease in stimulus-secretion coupling, followed by β-cell loss [87]. The importance of OXPHOS integrity to insulin secretion is illustrated by the abolishment of glucose-induced insulin release by depleting β-cells of mtDNA [78]. Mitochondrial DNA is required for glucose-induced stimulus secretion coupling in a mouse pancreatic cell line (MIN6) [97]. Depletion of MIN6 cells of mitochondria resulted in inhibition of the glucose-stimulated Ca²⁺ influx and subsequent insulin secretion. This ability was restored when β⁰ MIN6 cells were repopulated with foreign mtDNA [97].

Other factors, including GTP, malonyl-CoA, long-chain acyl-CoA esters, pyridine nucleotides, and glutamate have been implicated in stimulus-secretion coupling, but their exact role has not been completely elucidated [96,98–103]. A GTP stable analogue (GTP₆S) caused insulin secretion in permeabilized RINm5F cells at extremely low levels of intracellular Ca²⁺ [98]. GTP or GTP₆S also produces a concentration dependent increase in cell capacitance in the absence of intracellular Ca²⁺ [100]. Malonyl-CoA (a physiologic inhibitor of carnitine palmitoyl transferase-1) and long-chain acyl-CoA esters may serve as metabolic coupling factors in insulin secretion when β-cells are stimulated by glucose or other secretogogues [99], although the ability for these compounds to stimulate exocytosis is controversial. An adenoviral vector expressing malonyl-CoA decarboxylase (converts malonyl-CoA to acetyl-CoA) was targeted to INS-1 cells. Intracellular malonyl-CoA decreased dramatically, but there was no change in glucose-stimulated insulin secretion [101]. When INS-1 cells were treated with an inhibitor of long-chain acyl-CoA synthetase (traesin C), total long-chain acyl-CoA was decreased about 50%, palmitate oxidation was attenuated, and there was decreased glucose or palmitate incorporation into lipids, but again no effect was observed on insulin exocytosis [101]. NADH and NADPH are
also produced by glucose metabolism. A rise in NAD(P)H was observed in rat islet \( \beta \)-cells before the rise in intracellular \( \text{Ca}^{2+} \) [96], implicating pyridine nucleotides in insulin exocytosis, although the exact mechanism of action has not been determined. Glutamate levels increased 4.8-fold within 30 min of incubation of rat INS-1 cells with a stimulating concentration of glucose [102]. The mitochondrial toxin FCCP (carbonyl cyanide \( p \)-trifluoromethoxyphenylhydrazone) inhibited the production of glutamate [102]. However, another study did not find increased intracellular glutamate levels following stimulation of \( \beta \)-cells with glucose, leucine, succinic acid methyl ester, or \( \alpha \)-oxoisocaproic acid, despite stimulation of insulin release by these compounds [103]. During glucose stimulation of the \( \beta \)-cell, glutamate dehydrogenase (GAD) favors glutamate formation over TCA cycle anaplerosis during glucose-stimulated insulin secretion, a finding that adds further support for glutamate as a messenger in GSIS [102]. In addition, mutations in GAD that result in hyperactivity of this enzyme are associated with hyperinsulinism [104].

**MODY2 and thiamine-responsive megaloblastic anemia syndrome**

Two rare causes of DM serve as further illustration of the importance of glycolytic and TCA cycle flux in GSIS. Maturity-onset diabetes of the young type 2 (MODY2) is caused by a decrease in functional glucokinase with subsequent decreased flux through glycolysis at a given glucose concentration and impaired insulin secretion [105]. Thiamine-responsive megaloblastic anemia syndrome (TRMA) is an autosomal recessive condition caused by mutations in \( SLC19A2 \), the plasma membrane thiamine transporter [105,106]. The primary clinical features of TRMA are anemia, deafness, and DM, but optic atrophy, stroke-like episodes and cardiomyopathy may occur. Pyruvate dehydrogenase is a thiamine-dependent enzyme that converts pyruvate to acetyl-CoA for entry into the TCA cycle. Patients with TRMA respond to high doses of thiamine [105].

**Hyperglycemia and the production of free radicals**

Hyperglycemia in IDDM and NIDDM potentiates cellular oxidative stress by increasing flux through the hexosamine and polyol pathways, advanced glycation end-product (AGE) formation, and activation of protein kinase C (PKC) [12,107–109]. All these pathways eventually result in the creation of mitochondrial superoxide, which can have further deleterious effects on mtDNA [107]. Ultimately, free radical generation leads to altered levels of transcription factors known to have significant roles in maintaining glucose homeostasis, such as transforming growth factor-\( \beta_1 \) (TGF-\( \beta_1 \)), plasminogen activator inhibitor-1 (PAI-1), pancreas duodenum homeobox-1 (PDX-1), NFkB, and Sp1 [12,108,110]. Mutations in various transcription factors underlie the etiology of 5 of 6 defined MODY subtypes, clearly illustrating the importance of transcription factors in abnormal insulin secretion [111]. For example, mutant HNF1\( \alpha \) (MODY3) produces changes that affect GSIS, including inhibition of expression of insulin, GLUT-2, l-pyruvate kinase, aldolase B, 3-hydroxy-3-methylglutaryl CoA reductase, and mitochondrial 2-oxoglutarate dehydrogenase E1 subunit, and upregulation of UCP-2 expression [112].

**Hexosamine pathway** activation by excess intracellular glucose results in superoxide formation and the diversion of fructose-6-phosphate from glycolysis to metabolism by glutamine:fructose-6-phosphate aminotransferase (GFAT) [108]. The glucosamine-6-phosphate thus formed is used for synthesis of UDP-N-acetylglucosamine (GlcNAc), which, in turn, leads to increased O-acetylgulosaminylation of the transcription factor Sp1, increased Sp1 transactivation, and Sp1-dependent gene expression [108,113]. Sp1 activates expression of genes linked to the pathogenesis of diabetic complications, including PAI-1 and TGF-\( \beta_1 \) [108]. Glucose flux through GFAT also leads to increased matrix production in porcine glomerular mesangial cells via increased production of TGF-\( \beta_1 \) [114]. Normal endothelial production of NOS is important in preventing vascular disease. The increased flux through the hexosamine pathway also increases \( N \)-linked \( N \)-acetylgulosamine modification of an Akt site of endothelial NOS, leading to NOS inhibition and altered vascular tone [115].

Increased glucose flux through the polyol pathway results in the formation of sorbitol through the action of aldose reductase, an NADPH-dependent enzyme [107]. Sorbitol is then converted to fructose by sorbitol dehydrogenase in a reaction that generates NADH. The resultant increased cytosolic NADH/NAD\(^+\) ratio inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH), leading to increased cellular triose phosphate concentration [107,116], which eventually serves to activate PKC and cause inhibition of the \( \text{Na}^+/\text{K}^+ \) ATPase (see below). NAD\(^+\) consumption by poly (ADP-ribose) polymerase (PARP) also results in an increased NADH/ NAD\(^+\) ratio. Activation of PARP is accomplished through increased ROS production [107]. It is also possible that decreased NADPH secondary to increased polyol pathway flux leads to depletion of cellular GSH stores and further susceptibility to oxidant stress [107].

**Advanced glycation endproducts** (AGEs) are formed via a non-enzymatic reaction of glyoxal, methyl glyoxal, and 3-deoxyglucosone with amino groups of intra- and extra-cellular proteins [107]. Acting through their receptors (RAGEs), AGEs have a number of toxic effects, including altered protein function, production of ROS,
blockage of the anti-proliferative effects of nitric oxide, and activation of NF-κB [107,109]. The production of ROS occurs through receptor–ligand interactions, whereby modified proteins bind to RAGEs on endothelial cells, mesangial cells, and macrophages and cause an increase of NF-κB and subsequent upregulation of genes implicated in hyperglycemic damage [107]. NF-κB levels are also increased in ob/ob mice. Inactivation of I-κB, a stimulator of NF-κB, in ob/ob mice leads to reversal of hyperglycemia, hyperinsulinemia, and dyslipidemia [117]. Non-enzymatic glycosylation of extracellular matrix proteins, including laminin and type I and IV collagen may lead to abnormal basement membrane function and altered extracellular matrix, which may contribute to the pathogenesis of diabetic vascular complications [107].

Protein kinase C (PKC) may be stimulated by the production of methyl glyoxal and diacylglycerol by increased flux through the polyol pathway (see above). Increased PKC activity leads to production of phospholipase A2 and increased arachidonate and prostaglandin E2 inhibit Na+/K+-ATPase and, therefore, interfere with GSIS [107,118]. Increased arachidonate and PGE2 inhibit Na+/K+-ATPase and, therefore, interfere with GSIS [107,118]. Increased ROS may also indirectly activate PKC [107]. Normal endothelial NOS production is important in preventing vascular complications of DM. In addition, PKC activation mediates endothelial blood flow, possibly by decreasing NO production and/or increasing endothelin-1 activity [119]. PKC activation also inhibits endothelial NOS mRNA in cultured endothelial cells [120]. Decreased NO production has been shown to induce expression of TGF-β1, fibronectin, and type IV collagen in cultured mesangial cells and glomeruli from diabetic rats [121].

Cancer

The hypothesis that damage to mtDNA and/or nDNA by ROS, which, in turn, cause mitochondrial ETC dysfunction and further DNA damage that could contribute to the onset of neoplasia, is not new [122]. It has been known for years that cancer cells have many abnormalities of their mitochondria, including ultrastructural changes, abnormal Ca2+ and K+ transport, decreased state III respiration, increased state IV respiration, and various ETC deficiencies [122]. However, a direct link between mitochondrial ETC dysfunction and cancer has only recently been established [123–129].

Mitochondrial ETC complex II (succinate dehydrogenase) and fumarate hydratase

Succinate dehydrogenase (SDH, EC 1.3.99.1) is a unique component of the mitochondrial ETC. It consists of four subunits (SDHA, SDHB, SDHC, and SDHD) that are coded for entirely by nDNA and catalyzes the conversion of succinate to fumarate. SDH is also a critical component of the TCA cycle and is embedded in the inner mitochondrial membrane. The SDHA (flavoprotein) and SDHB (Fe–S complex) subunits are necessary for catalytic activity, whereas the SDHC and SDHD subunits anchor the complex in the inner mitochondrial membrane.

Hereditary paragangliomas are usually benign, slow growing tumors that often occur in the carotid body. Phaeochromocytomas are catecholamine-secreting tumors that often arise from the adrenal medulla, but can develop in extra-adrenal parasympathetic ganglia as well. After identification of familial cases of paraganglioma, a gene (PGL1) was mapped to chromosome 11q23. Inheritance of PGL1 is autosomal dominant with incomplete penetrance when transmitted through the paternal lineage, but disease does not occur if there is maternal transmission [123]. Recently, heterozygous mutations in SDHD were identified in individuals with familial or isolated paragangliomas and phaeochromocytomas [122,123,127]. After initially finding mutations in SDHD, it became clear that linkage between paragangliomas and phaeochromocytomas and the SDHD locus was not always present. Subsequently, germline mutations in SDHB were identified in three of eight families with paraganglioma, phaeochromocytoma, or both [124] and SDHC germline mutations were found in autosomal dominant paraganglioma [126]. However, unlike SDHD mutations, mutations in SDHB and SDHC are not associated with maternal imprinting [129]. In a larger study of individuals with isolated phaeochromocytomas, 66 out of 271 had mutations in either VHL (30/66), RET (13/66), SDHB (12/66), or SDHD (11/66) [127]. On the other hand, homozygous germline mutations in SDHA are a cause of Leigh syndrome [129].

Fumarate hydratase (FH, EC 4.2.1.2) is a TCA cycle enzyme that catalyzes the next step after SDH, converting fumarate to malate. Familial autosomal dominant uterine leiomyomatia, skin leiomyomatia, and papillary renal cell cancer may be caused by germline heterozygous mutations in FH [128]. Homozygous FH mutations result in a neurodegenerative disorder [128].

Although the mechanisms are unknown, tumors associated with loss of SDH or fumarase activity demonstrate a loss of the remaining wild-type allele [129]. Deficient mitochondrial energy metabolism, ROS production, and aberrant apoptosis have been proposed to underlie the pathogenesis of cancer in SDH and fumarase deficiency [129]. In theory, mitochondrial ETC dysfunction could lead to generation of ROS, which then activate hypoxia inducible factors (HIFs), such as HIF-1. HIF-1 could then enter the nucleus and induce expression of anti-apoptotic and pro-proliferative genes (e.g., TGF-β1, platelet-derived growth-factor receptor β
PDGF], and epidermal growth-factor receptor (EGFR), resulting in cellular proliferation [129]. Mitochondrial membrane integrity could also play a role in cancer pathogenesis; disassembly or partial assembly of mutant SDH subunits may alter the composition of the inner mitochondrial membrane, making the mitochondrion more resistant to apoptosis (Fig. 2) [129]. At present time, these remain compelling, albeit unproven, hypotheses.

Potential therapies

Because generation of free radicals is a common theme that links mitochondrial dysfunction to the pathogenesis of neurodegenerative disorders, DM and cancer, interest in antioxidant therapy as a potential treatment has increased. N-acetylcysteine (NAC) is a precursor of glutathione synthesis and also stimulates cytosolic enzymes that regenerate glutathione. NAC can also interact directly with ROS through its thiol group to aid in detoxification of these compounds [130]. NAC has been shown to inhibit apoptosis in postmitotic and neuronal cells [131–133]. Treatment of rats with YM 737, a GSH analog, protects against cerebral ischemia by decreasing lipid peroxidation [134]. NAC treatment of wobbler mice, a model of ALS, resulted in a significant reduction of motor neuron loss, elevated levels of glutathione peroxidase in the cervical spinal cord, and increased axon caliber and muscle mass [135]. Therefore, NAC has been proposed to be a possible therapy for age-related neurodegenerative diseases, including PD, AD, HD, and FA [136]. However, it is unclear whether NAC has the ability to cross the blood-brain barrier sufficiently to be of any benefit and controlled clinical trials have not yet been undertaken. Similar concerns of efficacy have arisen following treatment with other GSH prodrugs. For example, treatment of 13 ALS patients with intravenous procysteine did not result in increased CSF GSH concentrations [137]. Nevertheless, the glutathione system will undoubtedly be an area of continued research for potential therapies for neurodegenerative diseases.

Similarly, it has been proposed that insufficient antioxidant defense by GSH plays a role in the development of DM complications [138–141]. Hepatic glutathione S-transferase expression may be decreased in DM [141]. In addition, islet β-cells have an intrinsically low level of antioxidant enzyme expression [12]. Significantly decreased GSH, GPx, and glutathione reductase levels were observed in red blood cells from individuals with IDDM after incubation with hydrogen peroxide when compared to controls [140]. Alpha lipoic acid, ascorbic acid-6-palmitate, and fish oil improve recycling of GSSG to GSH and suppress oxidative stress in diabetic rat erythrocytes [142]. In cultured bovine aortic endothelial cells, increased ROS production was prevented by a complex II inhibitor (thenoyltrifluoroacetone), an uncoupler of OXPHOS (carbonyl cyanide m-chlorophenylhydrazone), and overexpression of UCP-1 or MnSOD [143]. Treatment with each of these agents resulted in normal ROS levels and prevention of glucose-induced activation of PKC and NF-κB, sorbitol accumulation and formation of AGEs [143]. Treatment of insulin-producing RINm5F cells with overexpression of MnSOD, Cu/ZnSOD, and catalase offered protection against hydrogen peroxide and superoxide radicals. Optimal protection was observed with combined overexpression of both superoxide- and hydrogen peroxide-inactivating enzymes [144]. AGEs are known to induce ROS production and are linked to the production of diabetic vascular lesions. In a diabetic mouse model deficient in apoprotein E, treatment with the soluble extracellular domain of the AGE receptor (RAGE) completely suppressed atherosclerosis in a glycemia- and lipid-independent manner [145]. Therefore, RAGE may be a potential novel therapeutic target for diabetic macrovascular disease [145].

Anti-oxidant vitamins and coenzyme Q10 have also been used in animal models of DM and in human trials [6]. Vitamin C levels in plasma, liver, and kidney of streptozotocin-induced diabetic rats are significantly lower than controls [146]. Improved endothelial-dependent vasodilation in forearm resistance vessels in NIDDM patients improved after vitamin C administration [147], but low dose vitamin E failed to alter cardiovascular and retinal risks [148]. After treatment with α-tocopherol, reduction in PKC, ROS, and an increased nitrate to nitrite ratio was observed in corpus cavernosal vascular smooth muscle cells from rats exposed to high glucose [149]. Furthermore, long-term administration of ascorbate, α-tocopherol, NAC, β-carotene and selenium inhibited the development of
early stages of diabetic retinopathy in a diabetic rat model [150]. A number of groups have also studied the effects of coenzyme Q₁₀ supplementation in MIDD patients [151–155]. Coenzyme Q₁₀ prevented progressive hearing loss and improved post-exercise lactate levels in 28 MIDD patients with the A3243G mutation treated over 3 years, but did not affect insulin secretory capacity [154]. On the other hand, a single A3243G MELAS patient with DM treated with coenzyme Q₁₀ had improved insulin secretion as demonstrated by a significant increase in C-peptide levels following a glucose stimulation test and the ability to discontinue oral hypoglycemic agents [155]. No improvement of diabetic symptoms was observed after coenzyme Q₁₀ therapy in other patients carrying the A3243G mutation who have neuromuscular involvement [152,153].

Because mutations in SDH and FH genes have been clearly linked to cancer, hypotheses of disease pathogenesis are of the same sort invoked for neurodegenerative disorders and DM, i.e., free radical production, induction of angiogenesis factors, abnormal apoptosis etc. [129]. However, very little data exist to explain the exact nature of the causal link between dysfunction of the TCA cycle and OXPHOS and onset of neoplasia. Therapies that may have a theoretical benefit include elemental iron (to enhance Fe–S cluster function in SDH), malate supplements (for individuals with heterozygous SDHX mutations) or fumarate supplements (for individuals with heterozygous FH mutations) [129]. However, none of these therapeutic possibilities has been studied to date. Linus Pauling espoused the use of high doses of vitamin C for treating patients with cancer; a 4.2 times increased survival time was reported in a study of 100 terminal patients in Scotland [156]. However, the benefit of vitamin C therapy was not upheld in a double-blinded placebo-control study of 100 patients with advanced colorectal cancer at the Mayo Clinic [157]. The finding of decreased SDH and FH activities in some solid tumors may renew interest in antioxidants as potential therapy for some forms of cancer.

In conclusion, although mitochondria have been traditionally regarded as the major cellular energy producing organelles, generation of toxic reactive intermediates underlies the pathogenesis of many common disorders, including neurodegenerative diseases, DM, and cancer. Other areas of active investigation not touched upon, or only briefly mentioned, in this review include the contribution of mitochondrial dysfunction to retinopathy of prematurity, hypoxic-ischemic encephalopathy, bronchopulmonary dysplasia, acute respiratory distress syndrome, autoimmune disorders, stroke, idiopathic epilepsy, anti-retroviral drug side effects, and heart, liver, and kidney disease. Although further research is needed to define the role mitochondria play in the pathogenesis of common disorders with more precision, understanding this association has provided significant insight into seemingly unrelated common disorders and offers hope for potential new therapies.

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