

# Mitochondrial dysfunction and molecular pathways of disease

Steve R. Pieczenik, John Neustadt\*

Received 30 August 2006

Available online 18 January 2007

## Abstract

Since the first mitochondrial dysfunction was described in the 1960s, the medicine has advanced in its understanding the role mitochondria play in health, disease, and aging. A wide range of seemingly unrelated disorders, such as schizophrenia, bipolar disease, dementia, Alzheimer's disease, epilepsy, migraine headaches, strokes, neuropathic pain, Parkinson's disease, ataxia, transient ischemic attack, cardiomyopathy, coronary artery disease, chronic fatigue syndrome, fibromyalgia, retinitis pigmentosa, diabetes, hepatitis C, and primary biliary cirrhosis, have underlying pathophysiological mechanisms in common, namely reactive oxygen species (ROS) production, the accumulation of mitochondrial DNA (mtDNA) damage, resulting in mitochondrial dysfunction. Antioxidant therapies hold promise for improving mitochondrial performance. Physicians seeking systematic treatments for their patients might consider testing urinary organic acids to determine how best to treat them. If in the next 50 years advances in mitochondrial treatments match the immense increase in knowledge about mitochondrial function that has occurred in the last 50 years, mitochondrial diseases and dysfunction will largely be a medical triumph.

© 2006 Elsevier Inc. All rights reserved.

**Keywords:** Mitochondria; Antioxidant; L-carnitine; Coenzyme Q10; Lipoic acid; Thioctic acid; Schizophrenia; Bipolar disease; Dementia; Alzheimer's disease; Epilepsy; Migraine headache; Stroke; Neuropathic pain; Parkinson's disease; Ataxia; Transient ischemic attack; Cardiomyopathy; Coronary artery disease; Chronic fatigue syndrome; Fibromyalgia; Retinitis pigmentosa; Diabetes; Hepatitis C; Primary biliary cirrhosis; Electron; Transport chain; Krebs's cycle

Mitochondria are the powerhouses of our cells. They are responsible for generating energy as an adenosine triphosphate (ATP) and heat and are involved in the apoptosis-signaling pathway. Current theory holds that mitochondria are the descendants of aerobic bacteria that colonized an ancient prokaryote between 1 and 3 billion years ago (Spees et al., 2006; DiMauro and Schon, 2003; Wallace, 2005). This allowed for the evolution of the first eukaryotic cell capable of aerobic respiration, a necessary precursor to the evolution of multicellular organisms (Spees et al., 2006). Supporting this theory is the observation that mitochondria are the only other subcellular structure aside from the nucleus to contain DNA. However, unlike nuclear DNA, mitochondrial DNA (mtDNA) are not protected by histones (Croteau and Bohr, 1997). Nuclear DNA wraps around histones, which then physically shield the DNA from damaging free radicals (Milligan et al., 1993) and are also required to repair double-stranded DNA breaks (Celeste et al., 2003). Since mtDNA lacks the structural protection of histones

and their repair mechanisms, they are quite susceptible to damage.

The first mitochondrial disease was described by Luft and colleagues in 1962, when a euthyroid 35-year-old female presented with myopathy, excessive perspiration, heat intolerance, polydipsia with polyuria, and a basal metabolic rate 180% of normal (Luft et al., 1962). The patient suffered from an uncoupling of oxidative phosphorylation (ox-phos). Ox-phos is the major cellular energy-producing pathway. Energy, in the form of ATP, is produced in the mitochondria through a series of reactions in which electrons liberated from the reducing substrates nicotine adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) are delivered to O<sub>2</sub> via a chain of respiratory proton (H<sup>+</sup>) pumps (Brookes et al., 2004). The uncoupling of ox-phos leads to the generation of heat without generating ATP, which was the dysfunction underlying this patient's presentation. To compensate, her mitochondria enlarged and multiplied, which was evident in a histological examination of muscle biopsies.

Since this first documented case, mitochondrial dysfunction has been implicated in nearly all pathologic and toxicologic conditions (Aw and Jones, 1989). (These conditions are outlined

\* Corresponding author.

E-mail address: [drneustadt@gmail.com](mailto:drneustadt@gmail.com) (J. Neustadt).

in Tables 1–3.) The conditions include sarcopenia and nonalcoholic steatohepatitis; acquired diseases such as diabetes and atherosclerosis; neurodegenerative diseases such as Parkinson's and Alzheimer's diseases; and inherited diseases, collectively called mitochondrial cytopathies.

However, since symptoms vary from case to case, age of onset, and rate of progression, mitochondrial dysfunction can be difficult to diagnose when it first appears. According to Cohen, who wrote a July 2001 article in the *Cleveland Clinic Journal of Medicine*, "The early phase can be mild and may not resemble any known mitochondrial disease. In addition, symptoms such as fatigue, muscle pain, shortness of breath, and abdominal pain can easily be mistaken for collagen vascular disease, chronic fatigue syndrome, fibromyalgia, or psychosomatic illness" (Cohen and Gold, 2001).

### Mitochondria structure and function

Cellular energy requirements control how many mitochondria are in each cell. A single somatic cell can contain from 200 to 2000 mitochondria (Veltri et al., 1990; Gray, 1989), while human germ cells such as spermatozoa contain a fixed number of 16 mitochondria and oocytes have up to 100,000 (Szewczyk and Wojtczak, 2002). The largest number of mitochondria is found in the most metabolically active cells, such as skeletal and cardiac muscle and the liver and brain. Mitochondria are found in every human cell except mature erythrocytes (Cohen and Gold, 2001).

Mitochondria produce more than 90% of our cellular energy by ox-phos (Chance et al., 1979). Energy production is the result of two closely coordinated metabolic processes—the tricarboxylic acid (TCA) cycle, also known as the Krebs's or

Table 2

Acquired conditions in which mitochondrial dysfunction has been implicated

Diabetes (Wallace, 2005; Fosslie, 2001; West, 2000)
Huntington's disease (Stavrovskaya and Kristal, 2005)
Cancer (Wallace, 2005), including hepatitis-C virus-associated hepatocarcinogenesis (Koike, 2005)
Alzheimer's disease (Stavrovskaya and Kristal, 2005)
Parkinson's disease (Stavrovskaya and Kristal, 2005)
Bipolar disorder (Stork and Renshaw, 2005; Fattal et al., 2006)
Schizophrenia (Fattal et al., 2006)
Aging and senescence (Wallace, 2005; Savitha et al., 2005; Skulachev and Longo, 2005; Corral-Debrinski et al., 1992; Ames et al., 1993)
Anxiety disorders (Einat et al., 2005)
Nonalcoholic steatohepatitis (Lieber et al., 2004)
Cardiovascular disease (Fosslie, 2001), including atherosclerosis (Puddu et al., 2005)
Sarcopenia (Bua et al., 2002)
Exercise intolerance (Conley et al., 2000)
Fatigue, including chronic fatigue syndrome (Fulle et al., 2000; Buist, 1989), fibromyalgia (Park et al., 2000; Yunus et al., 1988), and myofascial pain (Yunus et al., 1988)

citric acid cycle, and the electron transport chain (ETC). The TCA cycle converts carbohydrates and fats into some ATP, but its major job is producing the coenzymes NADH and FADH so that they, too, are entered into the ETC.

The overall pathway for the TCA cycle is as follows: catabolism of glucose in the cytosol produces 2 molecules of pyruvate, which pass through the mitochondrion's double membrane to enter the TCA cycle. As the pyruvate molecules pass through the membranes, they encounter two enzymes, pyruvate carboxylase and pyruvate dehydrogenase (PDH). Although PDH is referred to as one enzyme, it is actually a complex of 3 separate enzymes—pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. The PDH complex requires a variety of coenzymes and substrates for its function—coenzyme A (CoA), which is derived from pantothenic acid (vitamin B5); NAD<sup>+</sup>, which contains niacin (vitamin B3); FAD<sup>+</sup>, which contains riboflavin (vitamin B2); lipoic acid; and thiamin pyrophosphate (TPP), which, as the name indicates, contains thiamin (vitamin B1).

Table 1

Signs, symptoms, and diseases associated with mitochondrial dysfunction (Cohen and Gold, 2001)

Organ system	Possible symptom or disease
Muscles	Hypotonia, weakness, cramping, muscle pain, ptosis, ophthalmoplegia
Brain	Developmental delay, mental retardation, autism, dementia, seizures, neuropsychiatric disturbances, atypical cerebral palsy, atypical migraines, stroke, and stroke-like events
Nerves	Neuropathic pain and weakness (which may be intermittent), acute and chronic inflammatory demyelinating polyneuropathy, absent deep tendon reflexes, neuropathic gastrointestinal problems (gastroesophageal reflux, constipation, bowel pseudoobstruction), fainting, absent or excessive sweating, aberrant temperature regulation
Kidneys	Proximal renal tubular dysfunction (Fanconi syndrome); possible loss of protein (amino acids), magnesium, phosphorus, calcium, and other electrolytes
Heart	Cardiac conduction defects (heart blocks), cardiomyopathy
Liver	Hypoglycemia, gluconeogenic defects, nonalcoholic liver failure
Eyes	Optic neuropathy and retinitis pigmentosa
Ears	Sensorineural hearing loss, aminoglycoside sensitivity
Pancreas	Diabetes and exocrine pancreatic failure
Systemic	Failure to gain weight, short stature, fatigue, and respiratory problems including intermittent air hunger

Table 3

Inherited conditions in which mitochondrial dysfunction has been implicated (Cohen and Gold, 2001)

Kearns–Sayre syndrome (KSS)—external ophthalmoplegia, cardiac conduction defects, and sensorineural hearing loss
Leber hereditary optic neuropathy (LHON)—visual loss in young adulthood
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS)—varying degrees of cognitive impairment and dementia, lactic acidosis, strokes, and transient ischemic attacks
Myoclonic epilepsy and ragged-red fibers (MERRF)—progressive myoclonic epilepsy, clumps of diseased mitochondria accumulate in the subsarcolemmal region of the muscle fiber
Leigh syndrome subacute sclerosing encephalopathy—seizures, altered states of consciousness, dementia, ventilatory failure
Neuropathy, ataxia, retinitis pigmentosa, and ptosis (NARP)—dementia, in addition to the symptoms described in the acronym
Myoneurogenic gastrointestinal encephalopathy (MNGIE)—gastrointestinal pseudoobstruction, neuropathy

When there is ample energy (relatively high concentrations of ATP), pyruvate carboxylase is activated and shuttles the pyruvates in the direction of gluconeogenesis. When energy demands are high (relatively low concentration of ATP), the two pyruvate molecules pass through the PDH complex to produce 2 molecules of acetyl-coenzyme A (acetyl CoA), which enter the TCA cycle. There are nine intermediates in the TCA cycle. To pass through this cycle completely, the enzymes catalyzing the biotransformation of the intermediates require the following cofactors: cysteine, iron, niacin, magnesium, manganese, thiamin, riboflavin, pantothenic acid, and lipoic acid (Bralley and Lord, 2001). Once the 2 molecules of acetyl CoA are produced, each acetyl CoA produces 3 molecules of NADH and 2 molecules of FADH, for a total of 6 NADH and 4 FADH per one molecule of pyruvate. Additionally, acetyl CoA can be produced by oxidation of fatty acids, which then requires the nutrient L-carnitine to shuttle the acetyl CoA into the mitochondria to enter the TCA cycle.

NADH and FADH carry electrons to the ETC, which is embedded in the inner mitochondrial membrane and consists of a series of five enzyme complexes, designated I–V. Electrons donated from NADH and FADH flow through the ETC complexes, passing down an electrochemical gradient to be delivered to diatomic oxygen ( $O_2$ ) via a chain of respiratory proton ( $H^+$ ) pumps (Brookes et al., 2004).

Complexes I–IV involve ubiquinone (Coenzyme Q10, abbreviated as CoQ10). Complex I is NADH dehydrogenase, or NADH:ubiquinone oxidoreductase; complex II is succinate dehydrogenase (SDH), or succinate:ubiquinone oxidoreductase; complex III is the bcl complex, or ubiquinone:cytochrome *c* oxidoreductase; complex IV is cytochrome *c* oxidase (COX), or reduced cytochrome *c*:oxygen oxidoreductase; and complex V is ATP synthase or proton-translocating ATP synthase (Wallace, 2005). Complexes I–IV contain flavins, which contain riboflavin, iron-sulfur clusters, copper centers, or iron-containing heme moieties.

Ubiquinone shuttles electrons from complexes I and II to complex III. Cytochrome *c*, an iron-containing heme protein with a binuclear center of a copper ion (Hunter et al., 2000), transfers electrons from complex III to IV. During this process, protons are pumped through the inner mitochondrial membrane to the intermembrane space to establish a proton motive force, which is used by complex V to phosphorylate adenosine diphosphate (ADP) by ATP synthase, thereby creating ATP. Proper functioning of the TCA cycle and ETC requires all the nutrients involved in the production of enzymes and all the cofactors needed to activate the enzymes.

### Mechanisms of mitochondria-induced injury

Damage to mitochondria is caused primarily by reactive oxygen species (ROS) generated by the mitochondria themselves (Wei et al., 1998; Duchon, 2004). It is currently believed that the majority of ROS are generated by complexes I and III (Harper et al., 2004), likely due to the release of electrons by NADH and FADH into the ETC. Mitochondria consume approximately 85% of the oxygen utilized by the cell during its

production of ATP (Shigenaga et al., 1994). During normal oxphos, 0.4–4.0% of all oxygen consumed is converted in mitochondria to the superoxide ( $O_2^-$ ) radical (Shigenaga et al., 1994; Evans et al., 2002; Carreras et al., 2004). Superoxide is transformed to hydrogen peroxide ( $H_2O_2$ ) by the detoxification enzymes manganese superoxide dismutase (MnSOD) or copper/zinc superoxide dismutase (Cu/Zn SOD), (Wallace, 2005) and then to water by glutathione peroxidase (GPX) or peroxidoredoxin III (PRX III) (Green et al., 2004). However, when these enzymes cannot convert ROS such as the superoxide radical to  $H_2O$  fast enough, oxidative damage occurs and accumulates in the mitochondria (James and Murphy, 2002; Sies, 1993). Glutathione in GPX is one of the body's major antioxidants. Glutathione is a tripeptide containing glutamine, glycine, and cysteine, and GPX requires selenium as a cofactor (Fig. 1).

Superoxide has been shown in vitro to damage the iron-sulfur cluster that resides in the active site of aconitase, an enzyme in the TCA cycle (Vasquez-Vivar et al., 2000). This exposes iron, which reacts with  $H_2O_2$  to produce hydroxyl radicals by way of a Fenton reaction (Vasquez-Vivar et al., 2000). Additionally, nitric oxide (NO) is produced within the mitochondria by mitochondrial nitric oxide synthase (mtNOS) (Carreras et al., 2004) and also freely diffuses into the mitochondria from the cytosol (Green et al., 2004). NO reacts with  $O_2^-$  to produce another radical, peroxynitrite ( $ONOO^-$ ) (Green et al., 2004). Together, these 2 radicals as well as others can do great damage to mitochondria and other cellular components.

Within the mitochondria, elements that are particularly vulnerable to free radicals include lipids, proteins, oxidative phosphorylation enzymes, and mtDNA (Shigenaga et al., 1994; Tanaka et al., 1996). Direct damage to mitochondrial proteins decreases their affinity for substrates or coenzymes and, thereby, decreases their function (Liu et al., 2002). Compounding the problem, once a mitochondrion is damaged, mitochondrial function can be further compromised by increasing the cellular requirements for energy repair processes (Aw and Jones, 1989). Mitochondrial dysfunction can result in a feed-forward process, whereby mitochondrial damage causes additional damage (Fig. 2).

Complex I is especially susceptible to nitric oxide (NO) damage, and animals administered natural and synthetic complex I antagonists have undergone death of neurons (Dauer and Przedborski, 2003; Betarbet et al., 2000; Qi et al., 2003). Complex I dysfunction has been associated with Leber hereditary optic neuropathy, Parkinson's disease, and other neurodegenerative conditions (Schon and Manfredi, 2003; Perier et al., 2005).

As a medical concern, hyperglycemia induces mitochondrial superoxide production by endothelial cells, which is an important mediator of diabetic complications such as cardiovascular disease (Green et al., 2004; Du et al., 2000). Endothelial superoxide production also contributes to atherosclerosis, hypertension, heart failure, aging, sepsis, ischemia-reperfusion injury, and hypercholesterolemia (Li and Shah, 2004).

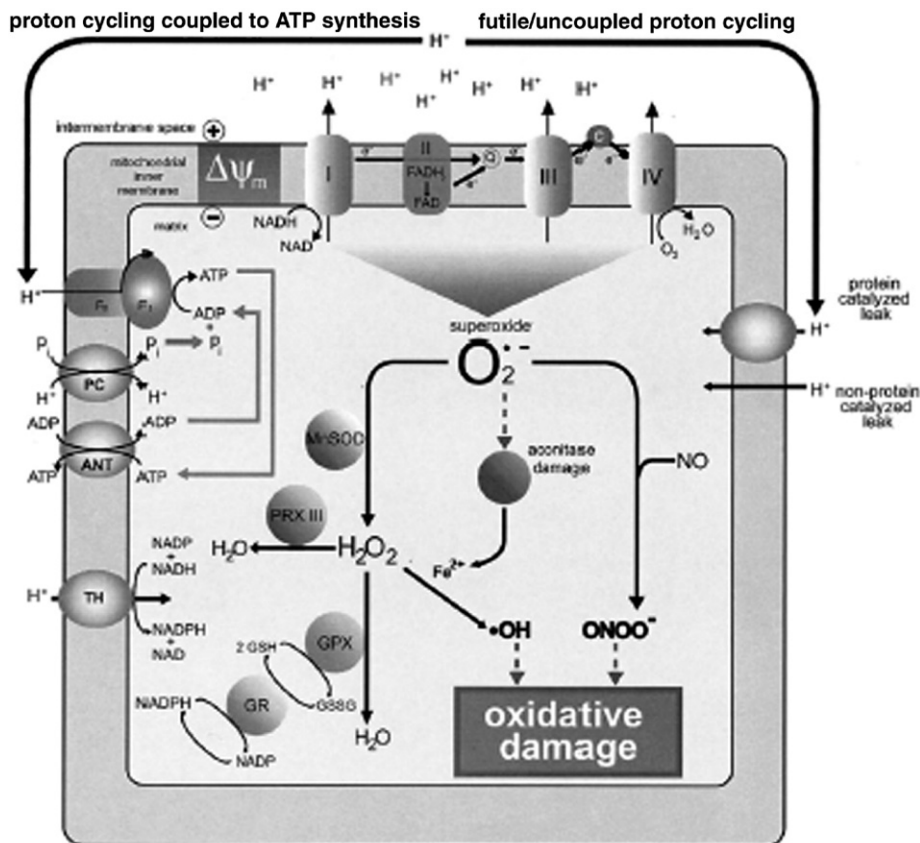


Fig. 1. Mitochondrial oxidative damage. The mitochondrial respiratory chain (top) passes electrons from the electron carriers NADH and FADH<sub>2</sub> through the respiratory chain to oxygen. This leads to the pumping of protons across the mitochondrial inner membrane to establish a proton electrochemical potential gradient ( $\Delta\mu_{H^+}$ ) negative inside: only the membrane potential ( $\Delta\psi_m$ ) component of  $\Delta\mu_{H^+}$  is shown. The  $\Delta\mu_{H^+}$  is used to drive ATP synthesis by the F<sub>0</sub>F<sub>1</sub> synthesis. The exchange of ATP and ADP across the inner membrane is catalyzed by the adenine nucleotide transporter (ANT) and the movement of inorganic phosphate (P<sub>i</sub>) is catalyzed by the phosphate carrier (PC) (top left). There are also proton leak pathways that dissipate  $\Delta\mu_{H^+}$  without formation of (ATP) (top right). The respiratory chain also produces superoxide (O<sub>2</sub><sup>•-</sup>), which can react with and damage iron sulfur such as aconitase, thereby ejecting ferrous iron. Superoxide also reacts with nitric oxide (HO) to form peroxynitrite (ONOO<sup>-</sup>). In the presence of ferrous iron hydrogen peroxide forms the very reactive hydroxyl radical (OH). Both peroxynitrite and hydroxyl radical can cause extensive oxidative damage (bottom right). The defense against oxidative damage (bottom left) includes MnSOD, and the hydrogen peroxide it produces is degraded by glutathione peroxidase (GPX) and peroxidoredoxin III (TRX III). Glutathione (GSH) is regenerated from glutathione disulfide (GSSG) by the action of glutathione reductase (GR), and the NADPH for this is in part supplied by a transhydrogenase (TH). Source: Green et al., 2004.

Inflammatory mediators such as tumor necrosis factor alpha (TNF- $\alpha$ ) have been associated in vitro with mitochondrial dysfunction and increased ROS generation (Moe et al., 2004). In a model for congestive heart failure (CHF), application of TNF- $\alpha$  to cultured cardiac myocytes increased ROS generation and myocyte hypertrophy (Nakamura et al., 1998). TNF- $\alpha$  results in mitochondrial dysfunction by reducing complex III activity in the ETC, increasing ROS production and causing damage to mtDNA (Suematsu et al., 2003).

Metabolic dysregulation can also cause mitochondrial dysfunction. Vitamins, minerals, and other metabolites act as necessary cofactors for the synthesis and function of mitochondrial enzymes and other compounds that support mitochondrial function (see Table 4), and diets deficient in micronutrients can accelerate mitochondrial decay and contribute to neurodegeneration (Ames, 2004). For example, enzymes in the pathway for heme synthesis require adequate amounts of pyridoxine, iron, copper, zinc, and riboflavin (Ames et al., 2005). Deficiencies of any component of the TCA cycle or ETC lead to increased production of free radicals and mtDNA damage (Table 5).

Medical research has found that iron-deficiency anemia is a major contributor to the global burden of disease, affecting an estimated 2 billion people, mostly women and children (Darnton-Hill et al., 2005; Ezzati et al., 2002). Low iron status decreases mitochondrial activity by causing a loss of complex IV and increasing oxidative stress (Atamna et al., 2001). The underlying mechanisms of how mitochondrial damage are well-defined.

Toxic metals, especially mercury, generate many of their deleterious effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation, depletion of protein sulfhydryls (eg, glutathione) and other effects (Valiko et al., 2005). These reactive radicals include a wide-range of chemical species, including oxygen-, carbon-, and sulfur-radicals originating from the superoxide radical, hydrogen peroxide, lipid peroxides, and also from chelates of amino acids, peptides, and proteins complexed with the toxic metals (Valiko et al., 2005).

One major mechanism for metals toxicity appears to be direct and indirect damage to mitochondria via depletion of glutathione, an endogenous thiol-containing (SH-) antioxidant,

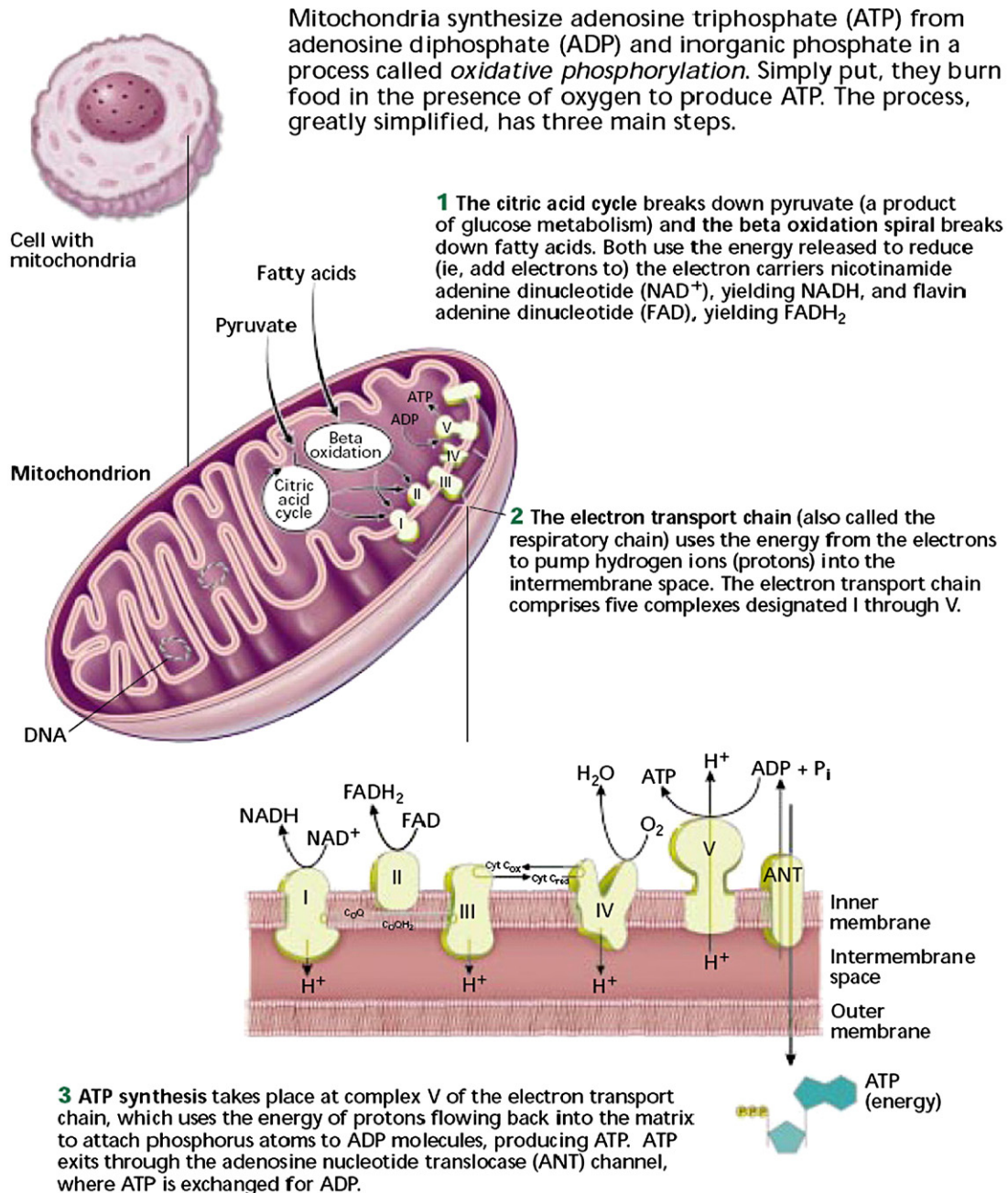


Fig. 2. Source: Cohen and Gold, 2001.

which results in excessive free radical generation and mitochondrial damage (Sanfeliu et al., 2001). Anecdotally, Dr. Neustadt, in his clinic, frequently observes an elevation of pyroglutamate, a urinary organic acid that is a specific marker for glutathione depletion (Bralley and Lord, 2001), in patients with confirmed mercury toxicity. Not surprisingly, these patients also complain of fatigue, a hallmark symptom of mitochondrial damage.

Mercury can accumulate in mitochondria and causes granular inclusions, which are visible with a scanning electron micrograph (Brown et al., 1985). Oxidative stress occurs in vitro and in vivo from both organic and inorganic mercury via their high affinity for binding thiols (sulfur-containing molecules), and the depletion of mitochondrial glutathione (Valko et al., 2005).

The central nervous system is particularly sensitive to damage by MeHg-induced glutathione depletion. In one study, ex vivo human neurons, astrocytes and neuroblastoma cells were exposed for 24 h to various levels of MeHg. The  $\text{LC}_{50}$  (concentration at which 50% of the cells died) was 6.5, 8.1, and 6.9  $\mu\text{M}$ , respectively (Sanfeliu et al., 2001). A second ex vivo study of mouse neurons and astrocytes confirmed the lower  $\text{LC}_{50}$  concentration for neurons (Kaur et al., 2006), and another ex vivo rat brain study demonstrated mitochondrial respiratory chain damage (Yee and Choi, 1996).

An in vitro, dose–response study of glutathione depletion and the  $\text{LC}_{50}$  of human neurons, astrocytes, and neuroblastoma cells exposed to MeHg showed an indirect relationship between GSH depletion and cell death and a direct relationship between

Table 4

Key nutrients required for proper mitochondrial function (Aw and Jones, 1989; Ames et al., 2005)

Required for the TCA cycle	<ul style="list-style-type: none"> <li>•Iron, sulfur, thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), cysteine, magnesium, manganese, and lipoic acid</li> <li>•Synthesis of heme for heme-dependent enzymes in the TCA cycle require several nutrients, including iron, copper, zinc, riboflavin, and pyridoxine (vitamin B6) (Ames et al., 2005)</li> <li>•Synthesis of L-carnitine requires ascorbic acid (vitamin C)</li> </ul>
Required for PDH complex	Riboflavin, niacin, thiamin, pantothenic acid, and lipoic acid
Required for ETC complexes	Ubiquinone (CoQ10), riboflavin, iron, sulfur, copper
Required for shuttling electrons between ETC complexes	Ubiquinone, copper, iron

length of MeHg exposure and cell damage (Sanfeliu et al., 2001). Cells were exposed to 6.5, 8.1, and 6.9  $\mu\text{M}$  MeHg for 7 days. After 24 h of exposure to MeHg, the  $\text{LC}_{50}$  for neurons, astrocytes and neuroblastoma cells was 6.5 (4.9–8.6), 8.1 (7.2–9.1), and 6.9 (6.4–7.5)  $\mu\text{M}$ , respectively, after 48 h it was 3.7 (3.2–4.3), 7.4 (6.9–7.9), and 5.5 (5.0–6.0)  $\mu\text{M}$ , respectively, after 72 h it was 2.9 (2.4–3.5), 7.0 (6.2–7.9), and 2.2 (0.5–8.9)  $\mu\text{M}$ , respectively, and after 7 days of exposure to MeHg it was 2.4 (1.5–3.6) and 4.4 (1.0–19.5)  $\mu\text{M}$ , respectively (toxicity of neuroblastoma cells after 7 days of exposure was not determined). Addition of buthionine sulfoximine (BSO), a glutathione-depleting agent, prior to MeHg exposure significantly increased the cytotoxicity of MeHg in all cell lines ( $P < 0.001$ ). In contrast, pre-incubation with 1 mM GSH for 24 h, followed by 10  $\mu\text{M}$  MeHg, resulted in protection of all cell lines from gross damage detected by phase contrast microscopy.

The pancreas has also been studied for its susceptibility to mercury poisoning. In one study, *ex vivo* mouse pancreatic beta-cells were exposed to 2 and 5  $\mu\text{M}$  MeHg (Chen et al., 2006). Compared to control cells, both levels of MeHg exposure significantly increased free radical generation ( $P < 0.05$ ), decreased mitochondrial membrane potential ( $P < 0.05$ ), and inhibited insulin secretion (percentage change not reported,  $P < 0.05$ ). This indicates a possible etiologic role for mercury toxicity in the pathogenesis of diabetes. Free radical production and the insulin response to MeHg, however, were reversed when the cells were treated with 0.5 mM *N*-acetyl-cysteine (NAC). Two *ex vivo* studies with NAC have shown an increase in intracellular glutathione and a decrease in reactive oxygen species formation in MeHg-treated neurons, astrocytes, and neuroblastoma cells (Sanfeliu et al., 2001; Kaur et al., 2006).

Inorganic mercury also impairs kidney function through depletion of glutathione, generation of free radicals, and mitochondrial damage. Rat kidney mitochondria treated with  $\text{Hg}^{2+}$  (as  $\text{HgCl}_2$ , 1.5 mg/kg *i.p.*) doubled hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) for 6 h afterwards, which occurred at the ubiquinone (coenzyme Q10)-cytochrome b region of the mitochondrial

respiratory chain *in vivo*. Concomitant with this increase in  $\text{H}_2\text{O}_2$  production, glutathione was depleted by more than 50%, and thiobarbiturate reactive substances (TBARS, an indicator of mitochondrial lipid peroxidation) increased 68% (Lund et al., 1993).

Other metals also damage cellular energy production pathways. Arsenic inhibits pyruvate dehydrogenase (PDH) activity (Valko et al., 2005). PDH catalyzes the transformation of pyruvate to acetyl-coenzyme A (acetyl-CoA), a post-glycolysis intermediate, to generate energy as adenosine triphosphate blocking (ATP) (Neustadt, 2006). Additionally, arsenic decreases mitochondrial energy production by directly blocks the Krebs cycle enzymes isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate dehydrogenase, NADH-dehydrogenase, and cytochrome C oxidase (Ramanathan et al., 2003).

### Testing and treatment options

How can physicians use the information to help patients? Since the major reason for mitochondrial dysfunction is ROS production and the accumulation of mitochondrial damage, antioxidant therapy is a viable strategy for attenuating the situation.

Along with providing the antioxidants, their precursors, and/or compounds that are known to increase their endogenous production, a systematic medical approach would also ensure that any cofactors required for detoxification enzyme function (e.g., selenium for GPX) are also present in clinically relevant amounts. But, while medical research supports this idea, the exact dosage and ratios of antioxidants and cofactors necessary to achieve clinically relevant responses in different diseases are still undefined. Additionally, prescribing all antioxidants known to quench free radicals and/or that are involved in proper mitochondrial function is prohibitively expensive for most patients and not covered by medical insurance.

Further complicating the issue, many of the mitochondrial dysfunction studies have only been conducted in animals, and most have provided only one or two antioxidants—instead of the total complement of antioxidants and minerals known to be involved in healthy mitochondrial function. For example, a randomized, placebo-controlled study performed at multiple centers in the United States administered 2000 IU of vitamin E, as alpha-tocopherol, to Alzheimer's disease patients. While some participants experienced a decrease in the rate of disease progression, there was much individual variation (Sano et al., 1997). In another study at the University of Bologna in Italy,

Table 5

Key enzymes and nutrients required for ox-phos (Wallace, 2005; Green et al., 2004)

Enzymes	Manganese superoxide dismutase (MnSOD), copper/zinc superoxide dismutase (Cu/Zn SOD), glutathione peroxidase (GPX), peroxidoredoxin III (PRX III)
Nutrients	Manganese, SOD, copper, zinc, glutathione (glutamine, glycine, and cysteine), selenium

CoQ10 administered at 150 mg/day for 6 months to 10 patients with mitochondrial cytopathies such as chronic progressive external ophthalmoplegia (CPEO) and Leber hereditary optic neuropathy (LHON) resulted in improved mitochondrial function, but a dose–response trial has not been conducted (Barbiroli et al., 1999).

To study whether or not declines in TCA enzyme activity associated with aging are reversible, L-carnitine and DL- $\alpha$ -lipoic acid—alone and in combination—were administered to male albino Wistar rats. L-carnitine was supplemented at dosages of 300 mg to kg of body weight per day (mg/kg bw/day) and DL- $\alpha$ -lipoic acid at 100 mg/kg bw/day (Savitha et al., 2005). At baseline, compared to young rats, enzyme levels in aged rats were 32% lower for succinate dehydrogenase, 33% lower for malate and isocitrate dehydrogenase, and 35% lower for  $\alpha$ -ketoglutarate dehydrogenase. After 30 days of combined supplementation with L-carnitine and  $\alpha$ -lipoic acid, enzyme levels in the aged rats increased 1.4-fold for succinate dehydrogenase, 1.5-fold for malate dehydrogenase, 1.4-fold for isocitrate dehydrogenase, and 1.5-fold for  $\alpha$ -ketoglutarate dehydrogenase. No significant change was detected in young rats that were supplemented with L-carnitine and  $\alpha$ -lipoic acid.

As previously discussed, TNF- $\alpha$  induces ROS production and mitochondrial decay. In one in vitro study, adding the antioxidants vitamin E and enzyme catalase to cultured rat cardiac myocytes negated the increase in ROS generation and cardiac myocyte hypertrophy (Nakamura et al., 1998). Lending further support are two animal studies where left ventricular hypertrophy (LVH) was attenuated using pharmacological anti-TNF- $\alpha$  therapy (etanercept in one study and the TNF- $\alpha$  blocking protein huTNFR:Fc) in a canine model of congestive heart failure (Moe et al., 2004; Bradham et al., 2002).

Since TNF- $\alpha$  expression is both controlled by and induces nuclear factor kappa-B (NF- $\kappa$ B) (Makarov, 2001), use of NF- $\kappa$ B inhibitors such as turmeric (*Curcumin longa*) (Jobin et al., 1999) may also prove an effective therapeutic strategy. TNF- $\alpha$  and NF- $\kappa$ B are important pro-inflammatory mediators, along with ROS, in many diseases, such as rheumatoid arthritis (Makarov, 2001), cardiovascular disease (Li and Shah, 2004), and renal fibrosis (Klahr and Morrissey, 2002).

Dietary supplements and drugs are not the only potential treatment strategy. Diets rich in antioxidants that are known to increase glutathione and other antioxidants also hold promise. Two diets were administered in a mice model of senescence to test which one was better at increasing glutathione (Rebrin et al., 2005). Diet I consisted of a base of 170 g/kg protein and 100 g/kg fat (type of fat was not specified) that was enhanced with 500 parts per million (ppm<sup>1</sup>) vitamins E, 80 ppm vitamin C, 300 ppm L-carnitine, and 125 ppm lipoic acid. Diet II contained the same base as Diet I, plus 500 ppm vitamin E, 80 ppm vitamin C, 5 g/kg glutamine, 0.15 g/kg lutein, and 11 additional food ingredients (e.g., 15 g/kg broccoli, 10 g/kg rice bran, 8.8 g/kg marine oil, 0.25 g/kg algae, 0.05 g/kg curcumin, 0.3 g/kg selenium-yeast); a combination high in flavonoids,

polyphenols, and carotenoids. Diet II, but not Diet I, increased reduced glutathione (GSH) in tissue homogenates. The greater efficacy of Diet II may be due to greater concentration and variety of antioxidants. Based on known molecular pathways, consumption of whey protein, which increases glutathione levels in vivo, might also be part of an effective therapeutic strategy (Grey et al., 2003; Middleton et al., 2004).

In our experience, the most important test for mitochondrial dysfunction is urinary organic acid testing. Organic acids are a class of compounds in biochemical pathways, which mostly have been used clinically to detect neonatal inborn errors of metabolism (Pitt et al., 2002). According to J. Alexander Bralley, PhD and Richard S. Lord, PhD, two of the world's leading organic acid researchers, the clinical relevance of organic acid testing is, among other benefits, its ability to determine dysfunction of mitochondrial energy production, the presence of functional nutrient deficiencies, and the presence of toxins that are adversely affecting detoxification pathways (Bralley and Lord, 2001). From this test come customized nutritional recommendations. Physicians will need to become comfortable in using these laboratory tools, which have not been an inherent part of our medical workup.

## Conclusion

Since the first mitochondrial dysfunction was described in the 1960s, the medicine has advanced in its understanding the role mitochondria play in health, disease, and aging. A wide range of seemingly unrelated disorders, such as schizophrenia, bipolar disease, dementia, Alzheimer's disease, epilepsy, migraine headaches, strokes, neuropathic pain, Parkinson's disease, ataxia, transient ischemic attack, cardiomyopathy, coronary artery disease, chronic fatigue syndrome, fibromyalgia, retinitis pigmentosa, diabetes, hepatitis C, and primary biliary cirrhosis have underlying pathophysiological mechanisms in common, namely ROS production, the accumulation of mtDNA damage, resulting in mitochondrial dysfunction. Antioxidant therapies hold promise for improving mitochondrial performance; however, medicine still has large gaps in its knowledge of which types and ratios of antioxidants, and in which forms, will result in the best outcomes. However, physicians seeking systematic treatments for their patients might consider testing urinary organic acids to determine how best to treat them. Clearly, additional clinical trials are needed, but if in the next 50 years advances in mitochondrial treatments match the immense increase in knowledge about mitochondrial function that has occurred in the last 50 years, mitochondrial diseases and dysfunction will largely be a medical triumph.

## References

- Ames, B.N., 2004. Delaying the mitochondrial decay of aging. *Ann. N.Y. Acad. Sci.* 1019 (1), 406–411.
- Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U. S. A.* 90 (17), 7915–7922 (Sep 1).
- Ames, B.N., Atamna, H., Killilea, D.W., 2005. Mineral and vitamin

<sup>1</sup> All ppm are approximate values.

- deficiencies can accelerate the mitochondrial decay of aging. *Mol. Aspects Med.* 26 (4–5), 363–378.
- Atamna, H., Liu, J., Ames, B.N., 2001. Heme deficiency selectively interrupts assembly of mitochondrial complex IV in human fibroblasts. Relevance to aging. *J. Biol. Chem.* 276 (51), 48410–48416.
- Aw, T.Y., Jones, D.P., 1989. Nutrient supply and mitochondrial function. *Annu. Rev. Nutr.* 9, 229–251.
- Barbiroli, B., Iotti, S., Lodi, R., 1999. Improved brain and muscle mitochondrial respiration with CoQ. An in vivo study by <sup>31</sup>P-MR spectroscopy in patients with mitochondrial cytopathies. *Biofactors* 9 (2–4), 253–260.
- Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3 (12), 1301–1306 (Dec).
- Bradham, W.S., Moe, G., Wendt, K.A., et al., 2002. TNF-alpha and myocardial matrix metalloproteinases in heart failure: relationship to LV remodeling. *Am. J. Physiol. Heart Circ. Physiol.* 282 (4), H1288–H1295.
- Bralley, J., Lord, R., 2001. Chapter 6: organic acids. *Laboratory Evaluations in Molecular Medicine: Nutrients, Toxicants, and Cell Regulators*. The Institute for Advances in Molecular Medicine, Norcross, GA, pp. 175–208.
- Brookes, P.S., Yoon, Y., Robotham, J.L., Anders, M.W., Sheu, S.-S., 2004. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell. Physiol.* 287 (4), C817–C833.
- Brown, A.C., Bullock, C.G., Gilmore, R.S., Wallace, W.F., Watt, M., 1985. Mitochondrial granules: are they reliable markers for heavy metal cations? *J. Anat.* 140 (Pt 4), 659–667 (Jun).
- Bua, E.A., McKiernan, S.H., Wanagat, J., McKenzie, D., Aiken, J.M., 2002. Mitochondrial abnormalities are more frequent in muscles undergoing sarcopenia. *J. Appl. Physiol.* 92 (6), 2617–2624.
- Buist, R., 1989. Elevated xenobiotics, lactate and pyruvate in C.F.S. patients. *Journal of Orthomolecular Medicine* 4 (3), 170–172.
- Carreras, M.C., Franco, M.C., Peralta, J.G., Poderoso, J.J., 2004. Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease. *Mol. Aspects Med.* 25 (1–2), 125–139 (Feb–Apr).
- Celeste, A., Difilippantonio, S., Difilippantonio, M.J., et al., 2003. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell.* 114 (3), 371–383 (Aug 8).
- Chance, B., Sies, H., Boveris, A., 1979. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59 (3), 527–605 (Jul).
- Chen, Y.W., Huang, C.F., Tsai, K.S., et al., 2006. Methylmercury induces pancreatic-cell apoptosis and dysfunction. *Chem. Res. Toxicol.* 19 (8), 1080–1085.
- Cohen, B.H., Gold, D.R., Mitochondrial cytopathy in adults: what we know so far. *Cleve Clin J Med.* Jul 2001;68(7):625–626, 629–642.
- Conley, K.E., Esselman, P.C., Jubrias, S.A., et al., 2000. Ageing, muscle properties and maximal O<sub>2</sub> uptake rate in humans. *J. Physiol.* 526 (Pt 1), 211–217 (Jul 1).
- Corral-Debrinski, M., Shoffner, J.M., Lott, M.T., Wallace, D.C., 1992. Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. *Mutat. Res.* 275 (3–6), 169–180 (Sep).
- Croteau, D.L., Bohr, V.A., 1997. Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. *J. Biol. Chem.* 272 (41), 25409–25412.
- Darnton-Hill, I., Webb, P., Harvey, P.W., et al., 2005. Micronutrient deficiencies and gender: social and economic costs. *Am. J. Clin. Nutr.* 81 (5), 1198S–1205S.
- Dauer, W., Przedborski, S., 2003. Parkinson's disease: mechanisms and models. *Neuron.* 39 (6), 889–909 (Sep 11).
- DiMauro, S., Schon, E.A., 2003. Mitochondrial respiratory-chain diseases. *N. Engl. J. Med.* 348 (26), 2656–2668 (June 26, 2003).
- Du, X.L., Edelstein, D., Rossetti, L., et al., 2000. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc. Natl. Acad. Sci. U. S. A.* 97 (22), 12222–12226.
- Duchen, M.R., 2004. Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol. Aspects Med.* 25 (4), 365–451 (Aug).
- Einat, H., Yuan, P., Manji, H.K., 2005. Increased anxiety-like behaviors and mitochondrial dysfunction in mice with targeted mutation of the Bcl-2 gene: further support for the involvement of mitochondrial function in anxiety disorders. *Behav. Brain. Res.* 165 (2), 172–180 (Dec 7).
- Evans, J.L., Goldfine, I.D., Maddux, B.A., Grodsky, G.M., 2002. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of Type 2 diabetes. *Endocr. Rev.* 23 (5), 599–622.
- Ezzati, M., Lopez, A.D., Rodgers, A., Vander Hoorn, S., Murray, C.J., 2002. Selected major risk factors and global and regional burden of disease. *Lancet* 360 (9343), 1347–1360.
- Fattal, O., Budur, K., Vaughan, A.J., Franco, K., 2006. Review of the literature on major mental disorders in adult patients with mitochondrial diseases. *Psychosomatics.* 47 (1), 1–7 (Jan–Feb).
- Fosslien, E., 2001. Mitochondrial medicine—Molecular pathology of defective oxidative phosphorylation. *Ann. Clin. Lab. Sci.* 31 (1), 25–67 (January 1, 2001).
- Fulle, S., Mecocci, P., Fano, G., et al., 2000. Specific oxidative alterations in vastus lateralis muscle of patients with the diagnosis of chronic fatigue syndrome. *Free Radic. Biol. Med.* 29 (12), 1252–1259 (Dec 15).
- Gray, M.W., 1989. Origin and evolution of mitochondrial DNA. *Annu. Rev. Cell. Biol.* 5, 25–50.
- Green, K., Brand, M.D., Murphy, M.P., 2004. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* 53 (Suppl 1), S110–S118.
- Grey, V., Mohammed, S.R., Smountas, A.A., Bahlool, R., Lands, L.C., 2003. Improved glutathione status in young adult patients with cystic fibrosis supplemented with whey protein. *J. Cyst. Fibros.* 2 (4), 195–198.
- Harper, M.E., Bevilacqua, L., Hagopian, K., Weindruch, R., Ramsey, J.J., 2004. Ageing, oxidative stress, and mitochondrial uncoupling. *Acta. Physiol. Scand.* 182 (4), 321–331 (Dec).
- Hunter, D.J., Oganessian, V.S., Salerno, J.C., Butler, C.S., Ingledew, W.J., Thomson, A.J., 2000. Angular dependences of perpendicular and parallel mode electron paramagnetic resonance of oxidized beef heart cytochrome *c* oxidase. *Biophys. J.* 78 (1), 439–450 (Jan).
- James, A.M., Murphy, M.P., 2002. How mitochondrial damage affects cell function. *J. Biomed. Sci.* 9 (6 Pt 1), 475–487.
- Jobin, C., Bradham, C.A., Russo, M.P., et al., 1999. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. *J. Immunol.* 163 (6), 3474–3483.
- Kaur, P., Aschner, M., Syversen, T., 2006. Glutathione modulation influences methyl mercury induced neurotoxicity in primary cell cultures of neurons and astrocytes. *NeuroToxicology* 27 (4), 492–500.
- Klahr, S., Morrissey, J., 2002. Obstructive nephropathy and renal fibrosis. *Am. J. Physiol. Renal. Physiol.* 283 (5), F861–F875 (November 1, 2002).
- Koike, K., 2005. Molecular basis of hepatitis C virus-associated hepatocarcinogenesis: lessons from animal model studies. *Clin. Gastroenterol. Hepatol.* 3 (10 Suppl 2), S132–S135 (Oct).
- Li, J.-M., Shah, A.M., 2004. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287 (5), R1014–R1030.
- Lieber, C.S., Leo, M.A., Mak, K.M., et al., 2004. Model of nonalcoholic steatohepatitis. *Am. J. Clin. Nutr.* 79 (3), 502–509 (March 1, 2004).
- Liu, J., Killilea, D.W., Ames, B.N., 2002. Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substrate-binding affinity and activity in brain by feeding old rats acetyl-L-carnitine and/or *R*-alpha-lipoic acid. *Proc. Natl. Acad. Sci. U. S. A.* 99 (4), 1876–1881.
- Luft, R., Ikkos, D., Palmieri, G., Ernster, L., Afzelius, B., 1962. A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study. *J. Clin. Invest.* 41, 1776–1804 (Sep).
- Lund, B.O., Miller, D.M., Woods, J.S., 1993. Studies on Hg(II)-induced H<sub>2</sub>O<sub>2</sub> formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. *Biochem. Pharmacol.* 45 (10), 2017–2024 (May 25).
- Makarov, S.S., 2001. NF-kappa B in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. *Arthritis Res.* 3 (4), 200–206.
- Middleton, N., Jelen, P., Bell, G., 2004. Whole blood and mononuclear cell glutathione response to dietary whey protein supplementation in sedentary and trained male human subjects. *Int. J. Food Sci. Nutr.* 55 (2), 131–141.



- Milligan, J.R., Aguilera, J.A., Ward, J.F., 1993. Variation of single-strand break yield with scavenger concentration for the SV40 minichromosome irradiated in aqueous solution. *Radiat. Res.* 133 (2), 158–162 (Feb).
- Moe, G.W., Marin-Garcia, J., Konig, A., Goldenthal, M., Lu, X., Feng, Q., 2004. In vivo TNF- $\alpha$  inhibition ameliorates cardiac mitochondrial dysfunction, oxidative stress, and apoptosis in experimental heart failure. *Am. J. Physiol., Heart Circ. Physiol.* 287 (4), H1813–H1820.
- Nakamura, K., Fushimi, K., Kouchi, H., et al., 1998. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor- $\alpha$  and angiotensin. *Circulation* 98 (8), 794–799.
- Neustadt, J., 2006. Mitochondrial dysfunction and disease. *Integr. Med.* 5 (3), 14–20.
- Park, J.H., Niermann, K.J., Olsen, N., 2000. Evidence for metabolic abnormalities in the muscles of patients with fibromyalgia. *Curr. Rheumatol. Rep.* 2 (2), 131–140 (Apr).
- Perier, C., Tieu, K., Guegan, C., et al., 2005. Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. *Proc. Natl. Acad. Sci. U. S. A.* 102 (52), 19126–19131.
- Pitt, J.J., Eggington, M., Kahler, S.G., 2002. Comprehensive screening of urine samples for inborn errors of metabolism by electrospray tandem mass spectrometry. *Clin. Chem.* 48 (11), 1970–1980.
- Puddu, P., Puddu, G.M., Galletti, L., Cravero, E., Muscari, A., 2005. Mitochondrial dysfunction as an initiating event in atherogenesis: a plausible hypothesis. *Cardiology* 103 (3), 137–141.
- Qi, X., Lewin, A.S., Hauswirth, W.W., Guy, J., 2003. Suppression of complex I gene expression induces optic neuropathy. *Ann. Neurol.* 53 (2), 198–205 (Feb).
- Ramanathan, K., Shila, S., Kumaran, S., Panneerselvam, C., 2003. Ascorbic acid and  $\alpha$ -tocopherol as potent modulators on arsenic induced toxicity in mitochondria. *J. Nutr. Biochem.* 14 (7), 416–420.
- Rebrin, I., Zicker, S., Wedekind, K.J., Paetau-Robinson, I., Packer, L., Sohal, R.S., 2005. Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse. *Free Radic. Biol. Med.* 39 (4), 549–557.
- Sanfeliu, C., Sebastia, J., Kim, S.U., 2001. Methylmercury neurotoxicity in cultures of human neurons, astrocytes, neuroblastoma cells. *Neurotoxicology* 22 (3), 317–327.
- Sano, M., Ernesto, C., Thomas, R.G., et al., 1997. A controlled trial of selegiline,  $\alpha$ -tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N. Engl. J. Med.* 336 (17), 1216–1222 (Apr 24).
- Savitha, S., Sivarajan, K., HariPriya, D., Kokilavani, V., Panneerselvam, C., 2005. Efficacy of levo carnitine and  $\alpha$  lipoic acid in ameliorating the decline in mitochondrial enzymes during aging. *Clin. Nutr.* 24 (5), 794–800.
- Schon, E.A., Manfredi, G., 2003. Neuronal degeneration and mitochondrial dysfunction. *J. Clin. Invest.* 111 (3), 303–312 (Feb).
- Shigenaga, M., Hagen, T., Ames, B., 1994. Oxidative damage and mitochondrial decay in aging. *PNAS* 91 (23), 10771–10778 (November 8, 1994).
- Sies, H., 1993. Strategies of antioxidant defense. *Eur. J. Biochem.* 215 (2), 213–219 (Jul 15).
- Skulachev, V.P., Longo, V.D., 2005. Aging as a mitochondria-mediated atavistic program: can aging be switched off? *Ann. N. Y. Acad. Sci.* 1057, 145–164 (Dec).
- Spees, J.L., Olson, S.D., Whitney, M.J., Prockop, D.J., 2006. Mitochondrial transfer between cells can rescue aerobic respiration. *PNAS* 103 (5), 1283–1288.
- Stavrovskaya, I.G., Kristal, B.S., 2005. The powerhouse takes control of the cell: is the mitochondrial permeability transition a viable therapeutic target against neuronal dysfunction and death? *Free Radic. Biol. Med.* 38 (6), 687–697.
- Stork, C., Renshaw, P.F., 2005. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol. Psychiatry* 10 (10), 900–919 (Oct).
- Suematsu, N., Tsutsui, H., Wen, J., et al., 2003. Oxidative stress mediates tumor necrosis factor- $\alpha$ -induced mitochondrial DNA damage and dysfunction in cardiac myocytes. *Circulation* 107 (10), 1418–1423.
- Szewczyk, A., Wojtczak, L., 2002. Mitochondria as a pharmacological target. *Pharmacol. Rev.* 54 (1), 101–127 (Mar).
- Tanaka, M., Kovalenko, S.A., Gong, J.S., et al., 1996. Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. *Ann. N. Y. Acad. Sci.* 786, 102–111 (Jun 15).
- Valko, M., Morris, H., Cronin, M.T., 2005. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* 12 (10), 1161–1208.
- Vasquez-Vivar, J., Kalyanaraman, B., Kennedy, M.C., 2000. Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation. *J. Biol. Chem.* 275 (19), 14064–14069.
- Veltri, K.L., Espiritu, M., Singh, G., 1990. Distinct genomic copy number in mitochondria of different mammalian organs. *J. Cell. Physiol.* 143 (1), 160–164 (Apr).
- Wallace, D.C., 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annual Review of Genetics* 39 (1), 359–407.
- Wei, Y.H., Lu, C.Y., Lee, H.C., Pang, C.Y., Ma, Y.S., 1998. Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. *Ann. N. Y. Acad. Sci.* 854, 155–170 Nov 20.
- West, I.C., 2000. Radicals and oxidative stress in diabetes. *Diabet. Med.* 17 (3), 171–180 (Mar).
- Yee, S., Choi, B.H., 1996. Oxidative stress in neurotoxic effects of methylmercury poisoning. *Neurotoxicology* 17 (1), 17–26 (Spring).
- Yunus, M.B., Kalyan-Raman, U.P., Kalyan-Raman, K., 1988. Primary fibromyalgia syndrome and myofascial pain syndrome: clinical features and muscle pathology. *Arch. Phys. Med. Rehabil.* 69 (6), 451–454 (Jun).

**Steve R. Pieczenik**, MD, PhD, trained at Cornell Medical College (MD), Harvard Medical College (Mass. Mental Health, Psychiatry), and MIT (PhD). Dr. Pieczenik is a Board Certified Psychiatrist and was a Board Examiner in Psychiatry and Neurology. He is the Chairman of Nutritional Biochemistry, Incorporated, which has conducted extensive research into the integration of medicine and mitochondrial dysfunction.

**John Neustadt**, ND, trained at Bastyr University (Naturopathic Medicine). Dr. Neustadt is the founder of Montana Integrative Medicine in Bozeman, Mont. Dr. Neustadt is also the President/CEO of Nutritional Biochemistry, Incorporated. He has written more than 100 published research reviews and is co-author with Jonathan Wright, MD, of the book, *Thriving through Dialysis*.