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REVIEW ARTICLE

MECHANISMS OF DISEASE

Monogenic Mitochondrial Disorders

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TO FUNCTION NORMALLY, HUMAN CELLS REQUIRE ENERGY IN THE FORM of ATP. In many cell types, ATP is primarily generated by mitochondria, which are also key players in other important cellular processes, such as adaptive thermogenesis,¹ ion homeostasis,² innate immune responses,³ production of reactive oxygen species,⁴ and programmed cell death (apoptosis).⁵ Mitochondria contain their own DNA (mtDNA), which encodes 13 mitochondrial proteins, 2 ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs). The replication, transcription, translation, and repair of mtDNA are controlled by proteins encoded by nuclear DNA (nDNA).^{6,7}

Mitochondrial dysfunction is not only observed in monogenic mitochondrial disorders but is also associated with more common pathologic conditions, such as Alzheimer's disease,⁸ Parkinson's disease,⁹ cancer,¹⁰ cardiac disease,¹¹ diabetes,¹² epilepsy,¹³ Huntington's disease,¹⁴ and obesity.¹⁵ In addition, a progressive decline in the expression of mitochondrial genes is a central feature of normal human aging.¹⁶ It is not entirely clear whether the changes in expression that occur with aging have positive or negative effects on life span. Given the aging of the population in developed societies and the increasing prevalence of conditions such as Alzheimer's disease and diabetes, the investigation of mitochondrial processes and diseases may make a timely contribution to our understanding of the human aging process and its relationship with the above conditions.

Other sources of interference in mitochondrial function include the off-target effects of environmental toxins (e.g., rotenone) and frequently used drugs (e.g., amiodarone, biguanides, haloperidol, statins, valproic acid, zidovudine), anesthetics (e.g., halothane), antibiotics (e.g., chloramphenicol), chemotherapeutic agents (e.g., doxorubicin), and even aspirin (acetylsalicylic acid).^{17,18} Certain drugs may lead more frequently to adverse reactions and side effects in patients with mitochondrial disorders than in healthy persons.^{17,19} The term "mitochondrial medicine"²⁰ refers to approaches that have been developed to manage mitochondrial dysfunction and, directly or indirectly, its consequences.²¹

In the past decade, the analysis of monogenic mitochondrial diseases has considerably advanced our understanding of the cellular pathophysiology of mitochondrial dysfunction. Here we summarize these insights and explain how they can contribute to the rational design of intervention strategies for mitochondrial dysfunction; we present our ongoing research on human mitochondrial complex I deficiency as an example of mitochondrial medicine. This information is preceded by a brief primer on the basics of mitochondrial structure and ATP generation.

 INTERNAL AND EXTERNAL
 STRUCTURE AND ATP GENERATION

STRUCTURE

Mitochondrial internal and external structure varies with cell type and metabolic state and often becomes altered during mitochondrial dysfunction (Fig. 1A and 1B).²² Net mitochondrial morphology depends on the balance between mitochondrial motility, fission, and fusion. This equilibrium is regulated by dedicated proteins and plays a role in metabolic energy dissipation, turnover of damaged mitochondria, distribution of mitochondria throughout the cell, induction of apoptosis, mitochondrial inheritance during cell division, and defense against aging.²³ Mitochondria consist of outer and inner membranes that envelop an aqueous compartment, the mitochondrial matrix (Fig. 1C and 1D). The space between the inner and outer membranes is referred to as the intermembrane space. The outer membrane is relatively smooth, whereas the inner membrane contains many matrix-protruding folds (or cristae) that increase the surface area of the inner membrane and have a dynamic structure.²² These structural dynamics may serve to regulate mitochondrial metabolism.²⁴

ATP GENERATION

The best-known mitochondrial function is the production of ATP. For this purpose, glucose is converted to pyruvate through the glycolysis pathway in the cytosol (Fig. 1E). Pyruvate can be converted into lactate¹⁰ or taken up by mitochondria, where it is converted to acetyl coenzyme A (CoA). Acetyl CoA enters the tricarboxylic acid cycle that generates the reduced forms of NADH and flavin adenine dinucleotide (FADH₂). Alternatively, fatty acids²⁵ and glutamine¹⁰ can be used as substrates. NADH and FADH₂ fuel the oxidative-phosphorylation system, which consists of five functionally coupled mitochondrial protein complexes.²⁶ NADH and FADH₂ are oxidized at mitochondrial complexes I and II, respectively (Fig. 1F). The released electrons are transported to complex III by coenzyme Q₁₀ (CoQ₁₀) and then to complex IV by cytochrome *c*, where they are donated to molecular oxygen to form water. The energy released by electron transport is used at complexes I, III, and IV to expel protons (H⁺) from the mitochondrial matrix, which establishes a proton gradient across the mitochondrial inner membrane. The latter is associated with an inside-negative inner-

membrane potential ($\Delta\psi$) and a matrix-directed proton-motive force. The proton-motive force is used at complex V to generate ATP, which is transported to the cytosol by the adenine nucleotide translocator. The proton-motive force also sustains many other mitochondrial functions (e.g., ion transport, metabolite exchange, protein import, and mitochondrial fusion).²⁷ As a consequence, defects in oxidative phosphorylation often induce multiple cellular aberrations.

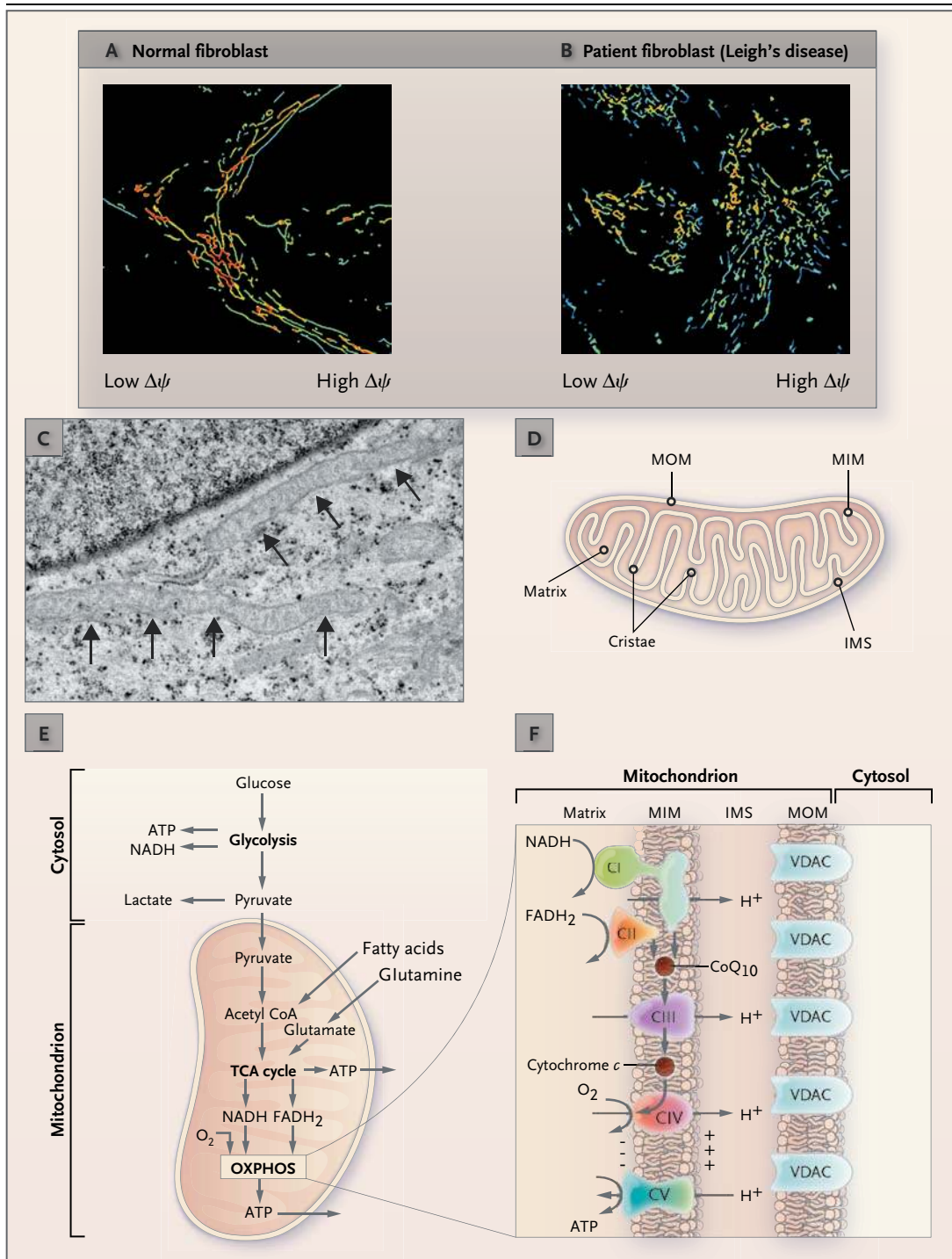
 MONOGENIC CELL MODELS OF
 MITOCHONDRIAL DYSFUNCTION

PRIMARY AND SECONDARY DISORDERS

With the use of mitochondrial proteome analysis,²⁸ approximately 1000 genes encoding mitochondrial proteins have been identified in humans (MitoCarta human inventory, Broad Institute). Mitochondrial dysfunction can arise from a mutation in one of these genes (causing a primary mitochondrial disorder) or from an outside influence on mitochondria (causing a secondary mitochondrial disorder). Causes of secondary disorders include viral infections²⁹ and off-target drug effects.^{17,18} To date, mutations in 228 protein-encoding nDNA genes and 13 mtDNA genes have been linked to a human disorder (Fig. 2). (For a list of these genes and their associated disease phenotypes, see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Given the preponderance of nDNA-encoded mutations, this review focuses mainly on these genes. A detailed discussion of mtDNA mutations and their associated pathology is provided elsewhere.³⁰⁻³⁵

MUTATIONS AND PHENOTYPE

Although it is evident that mutations in mitochondrial proteins induce mitochondrial dysfunction, it is less clear how specific genetic defects are linked to dysfunction at the level of cells, organs, and the whole organism. One major difficulty in determining the effects of specific defects is that the consequences of mitochondrial dysfunction are affected by the action of compensatory stress-response pathways. At the cellular level, these responses include mitochondrial biogenesis, increased expression of oxidative phosphorylation proteins, a switch to a more glycolytic mode of ATP production, and the removal of dysfunctional mitochondria by quality-control systems.^{9,36-39} To complicate matters even further, the substrate sup-



ply, the mode of ATP generation, the level of demand for ATP, mitochondrial dynamics, and the rate of oxidative phosphorylation differ among cell types and tissue types.

These factors increase the likelihood that the various types of tissue are not equally sensitive to mitochondrial dysfunction.^{27,40} At the level of the

organism, genetic background — both mitochondrial and nuclear — also modifies the phenotype of human mitochondrial diseases (e.g., as reported by Bénit et al.⁴¹). In this sense, mutations in mitochondrial proteins can evoke pathologic phenotypes specific to a cell, tissue, organ, or patient that are manifested only when a certain threshold

Figure 1 (facing page). Mitochondrial Structure and ATP Generation.

Panel A shows elongated mitochondria (filaments) in a living skin fibroblast from a healthy person (staining with the mitochondria-specific cation tetramethylrhodamine methyl ester [TMRM] and visualized with fluorescence microscopy). The more intense (red) the TMRM signal, the more negative the mitochondrial membrane potential ($\Delta\psi$). Panel B shows the fragmented mitochondrial structure in living skin fibroblasts from a patient with a mitochondrial disorder (Leigh's disease) caused by a mutation in *NDUFS2*, which encodes a subunit of complex I. TMRM staining is less intense (i.e., membrane potential is less negative) than in healthy fibroblasts. In the electron micrograph shown in Panel C, individual mitochondrial filaments (arrows) can be seen in the cytosol of a fixed primary-skin fibroblast from a healthy person. The dark line is the membrane of the cell nucleus. Panel D shows mitochondrial ultrastructure and compartmentalization, highlighting the mitochondrial outer membrane (MOM), mitochondrial inner membrane (MIM), and intermembrane space (IMS). Panel E summarizes the main ATP production pathway in human cells. In the cytosol, glucose is converted into pyruvate, which is subsequently processed in the mitochondrion to acetyl coenzyme A (CoA) and the reduced forms of NADH and flavin adenine dinucleotide (FADH₂). In addition to using pyruvate as a substrate, the mitochondrion can also use fatty acids and glutamine. Mitochondrial ATP production is carried out by the oxidative phosphorylation (OXPHOS) system, shown at the bottom of Panel E. This system is embedded in the MIM and consists of five multiprotein complexes (CI through CV), as shown in Panel F. NADH and FADH₂ are oxidized at CI and CII, and the electrons released are transported to CIII by coenzyme Q₁₀ (CoQ₁₀). The electrons are then transferred to cytochrome *c* and delivered to CIV, where they react with molecular oxygen (O₂). TCA denotes tricarboxylic acid. The energy released during electron transport is used to expel protons (H⁺) from the mitochondrial matrix across the MIM into the IMS. This creates a proton gradient across the MIM, which is associated with an inside-negative potential. At CV, the energy stored in the proton gradient is used to drive ATP production by allowing the reentry of H⁺ into the matrix. Because of the presence of voltage-dependent anion channels (VDACs), the MOM is ion-permeable.

of mitochondrial dysfunction or cellular demand on mitochondrial metabolism is exceeded. This phenomenon is exemplified by the broad variety of clinical presentations associated with mutations in nDNA-encoded mitochondrial genes (see Table 1 in the Supplementary Appendix).

DEVELOPING A RATIONAL INTERVENTION STRATEGY

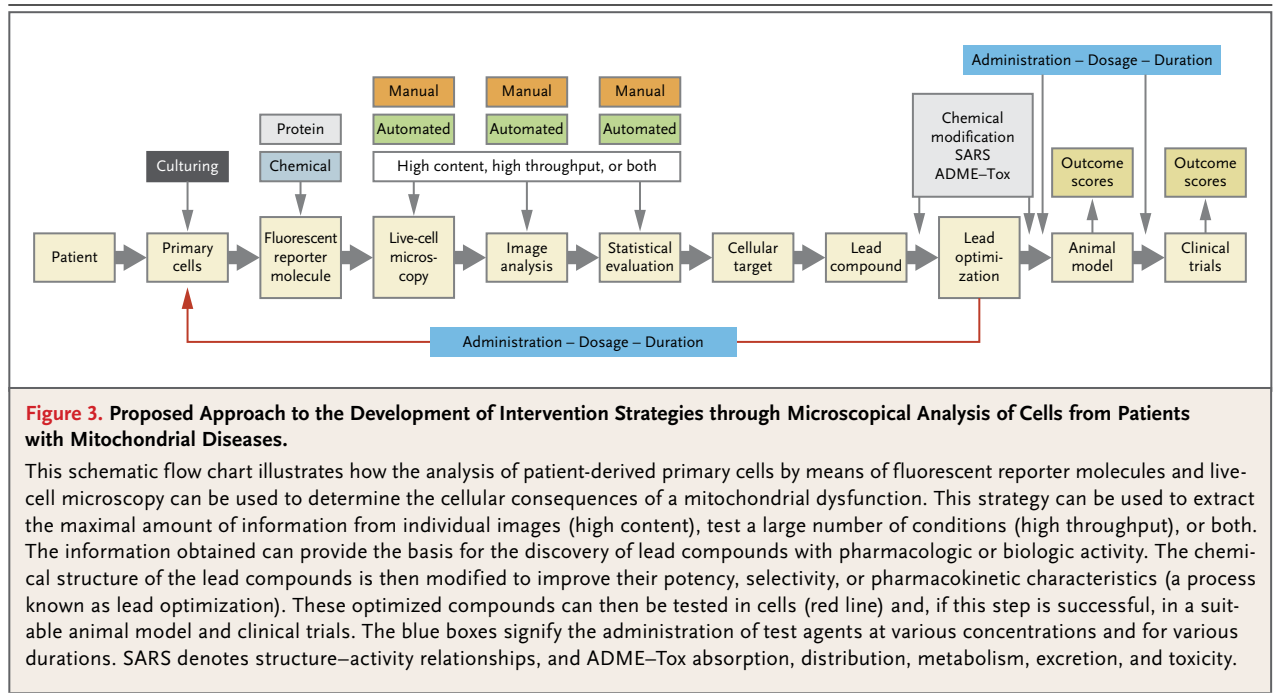
Mitochondrial function is often studied in mitochondrial suspensions that have been isolated from patient cells or tissues with the use of detergent

treatment.⁴² However, mitochondrial metabolism in situ is closely linked to that of the cell and is generally associated with submaximal rates of mitochondrial enzyme activity. In addition, the cellular milieu provides for communication among mitochondria, cytosol, and other organelles, and mitochondrial structure is greatly altered during isolation. As a consequence, the functional properties of isolated mitochondria differ considerably from those within the cell.⁴³⁻⁴⁵

Mitochondrial function can also be investigated in intact (patient) cells.^{22,24,25,27,42,46-48} Here we summarize our ongoing research on complex I deficiency to illustrate how live-cell analysis can be integrated into an approach to the development of treatment strategies (Fig. 3).

Using biopsy specimens from pediatric patients carrying nDNA-encoded complex I mutations, we have cultured primary fibroblast cell lines, which are maintained under standardized conditions.⁴⁷ Cellular and mitochondrial physiology is assessed with the use of fluorescent reporter molecules that are introduced into the cells and analyzed by means of live-cell microscopy (Fig. 4). The results are then compared with those in cells from healthy children.^{49,52-57} Statistical evaluation of 26 physiological variables in 14 control and 24 patient cell lines has revealed that complex I mutations lead to a reduction in the expression of complex I, depolarization of mitochondrial membrane potential, the accumulation of NADH, increased levels of reactive oxygen species, aberrations in mitochondrial structure, and aberrations in the homeostasis of cytosolic and mitochondrial Ca²⁺ and ATP.⁵⁸ The extent of these aberrations has been correlated with the age of the patient at disease onset and with death from the disease.⁵⁸

These results are currently being used in disease modeling and drug-target prediction to design an intervention strategy focused on the above aberrations. Once a lead compound has been identified, the dependence of its effects on time and concentration can be evaluated at the cellular level to assess structure–activity relationships and its properties of absorption, distribution, metabolism, and excretion (or toxicity).⁵⁹ In the case of complex I deficiency, lead compounds will then be evaluated in an animal model.^{57,60} Animal models are also available for several other primary and secondary mitochondrial disorders.^{31,32,56,61,62} Once the outcome scores in the animal model are satisfactory, the effects of any compounds that are developed can be evaluated in clinical trials.



stimulate mitochondrial biogenesis, or reduce levels of reactive oxygen species.⁶⁶ Targeting reactive oxygen species may not be entirely beneficial, however, since they can act as signaling molecules,^{4,27,48,53} in which case their elimination might also have detrimental effects.

MITOCHONDRIA-TARGETED SMALL MOLECULES

In contrast with membrane-permeable small molecules, which exert their effect throughout the cell, novel therapeutic strategies involve the specific targeting of mitochondria by small molecules (referred to as “cargo”).^{65,68-74} Targeting can be achieved by coupling the cargo to a delocalized lipophilic cation, leading to its accumulation in mitochondria. Several antioxidants have been successfully targeted using the cation approach (e.g., plastoquinones and CoQ variants). Protein-based cargo can be coupled to a mitochondrial targeting sequence recognized by the mitochondrial protein import machinery. Another strategy involves the use of mitochondria-penetrating peptides, engineered cell-penetrating peptides that target mitochondria on the basis of their membrane potential and lipophilicity (e.g., the antioxidant Szeto–Schiller peptide, SS-31). There are also vesicle-based transporters that target mitochondria through macropinocytosis, endosomal escape, and membrane fusion (e.g., DQAsomes and

MITO-porters). Recently, in a mouse model of mitochondrial lipoamide dehydrogenase (LAD) deficiency, mitochondrial enzyme-replacement therapy mediated by TAT (a transactivating transcriptional activator of human immunodeficiency virus 1) was used to introduce a TAT-LAD fusion protein.⁷⁵

TREATMENT OF MITOCHONDRIAL DISORDERS

In 2006, a large-scale review of published clinical trials of various treatments for primary mitochondrial disorders revealed no evidence supporting the use of any intervention.⁷⁶ More recently, the results of several trials in which a variety of treatments for mitochondrial disease were studied, including dichloroacetate, vitamins, and a cocktail of specific food components (nutraceuticals), were reviewed.⁷⁷ Although some of these trials showed a positive effect on one or more clinical or biochemical primary end points, none led to the filing of a New Drug Application with the Food and Drug Administration.

The drug idebenone (a CoQ₁₀ variant) has been approved for the treatment of Friedreich’s ataxia.⁴¹ A large multicenter, randomized, placebo-controlled trial of idebenone in patients with Leber’s hereditary optic neuropathy revealed that patients with discordant visual acuities were the most likely to benefit from this treatment.⁷⁸ In

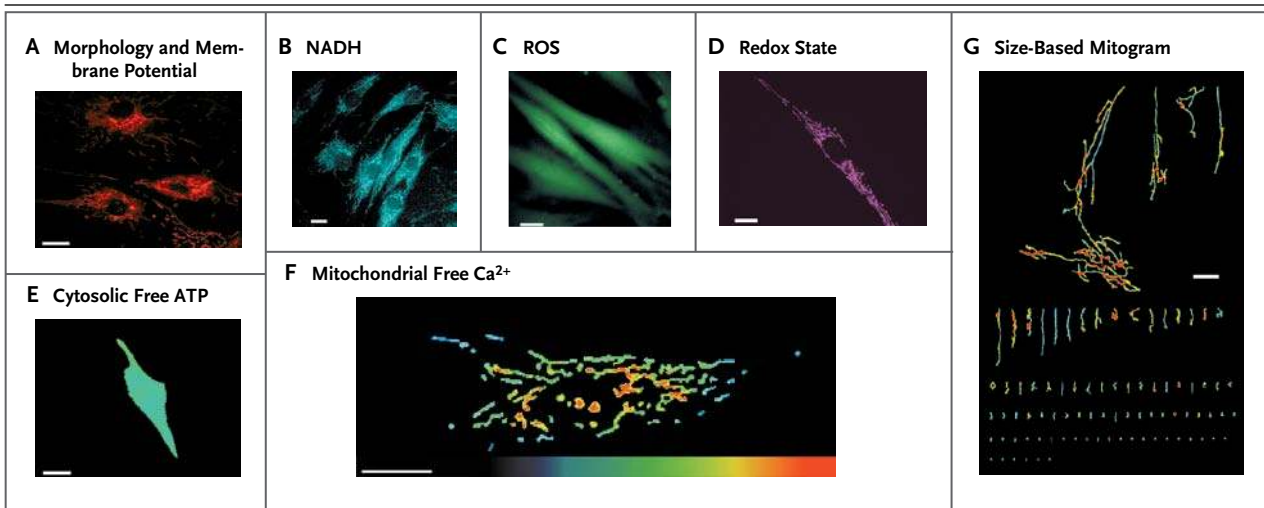


Figure 4. Typical Cell Readouts in Primary Fibroblasts from Human Skin.

Mitochondrial morphology and membrane potential can be visualized with the use of TMRM staining (Panel A),⁴⁶ NADH levels with autofluorescence (Panel B),⁴⁹ levels of reactive oxygen species with the chemical reporter CM-H₂DCFDA (Panel C),⁴⁸ mitochondrial redox state with the mitochondria-targeted protein-based sensor Mit-roGFP1⁴⁹ (400-nm image shown) (Panel D), the cytosolic free ATP concentration with the protein-based sensor ATeam (Panel E),⁵⁰ and levels of mitochondrial free Ca²⁺ in a cell stimulated with the hormone bradykinin and the calcium-sensitive cation Rhod-2 (Panel F).⁵¹ A mitogram shows all mitochondrial structures in the cell, sorted on the basis of size (Panel G).⁵²

one study, investigators found that a ketogenic diet was effective in preventing epileptic seizures in children with electron-transport-chain defects.⁷⁹ The findings in another study indicated that nutritional modulation affects mitochondrial function, suggesting that it may be worthwhile to pursue nutritional treatment strategies.⁶⁴

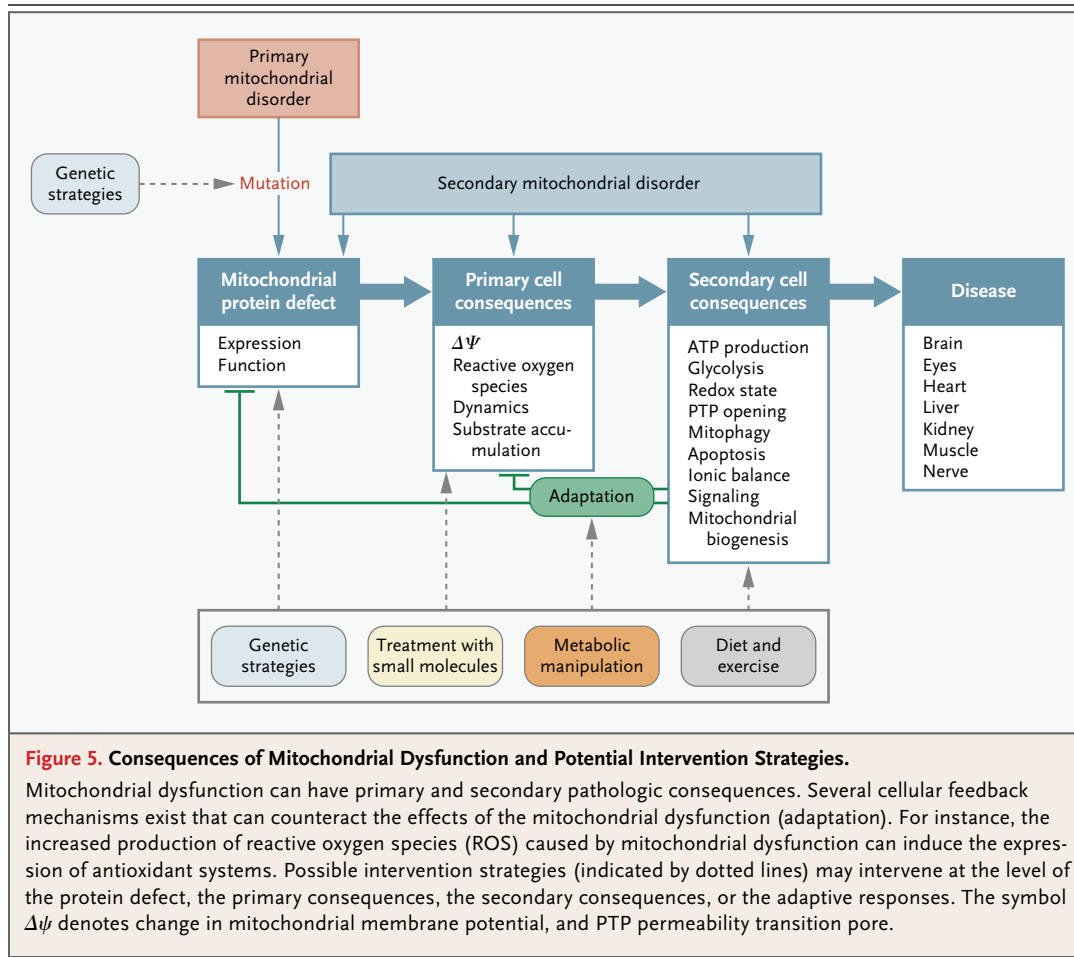
A successful treatment strategy has been developed for patients with a secondary mitochondrial disorder involving Ullrich's congenital muscular dystrophy and Bethlem's myopathy.⁸⁰⁻⁸² These myopathies, caused by mutations in the extracellular-matrix protein collagen VI, are associated with mitochondrial dysfunction and muscle-cell apoptosis. The pathogenic mechanism involves inappropriate opening of the mitochondrial permeability transition pore. This action was prevented in patients treated with a permeability-transition-pore desensitizer, cyclosporin A. Another study showed that cyclosporin A reduced the size of myocardial infarcts during cardiac reperfusion.⁸³ Other published intervention strategies are summarized in Table 2 in the Supplementary Appendix.

An overview of current clinical trials can be found online ([www.clinicaltrials.gov/ct2/results?](http://www.clinicaltrials.gov/ct2/results?term=mitochondrial+disease)

[term=mitochondrial+disease](http://www.clinicaltrials.gov/ct2/results?term=mitochondrial+disease)); these include studies of CoQ₁₀ for the treatment of muscle weakness and mitochondrial diseases, dietary supplements for MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), EPI-743 for mitochondrial diseases, human growth hormone for obesity, nutritional therapy for diabetes, pioglitazone for diabetes, idebenone for MELAS, and vitamin E for mitochondrial trifunctional protein deficiency.

FUTURE PERSPECTIVES

A field that began more than 50 years ago, when a physician detected a mitochondrial disorder in a single patient with hypermetabolism,⁸⁴ has now evolved into the discipline of mitochondrial medicine. Lessons learned from studies of rare diseases have implications for a broad range of medical disciplines. Within the next few years, the application of new technologies such as whole-exome sequencing can be expected to result in a huge expansion of the number of known causative nuclear gene defects in patients with mitochondrial disease (which is currently diagnosed by means of biochemical methods, mtDNA and nDNA se-



quencing, and candidate-gene studies). In the near future, the challenge will be to increase our understanding of the consequences of mitochondrial dysfunction at all levels of complexity in order to drive the development of rational treatment strategies. Although gene therapy has been proved beneficial, its use is still limited by the sheer number of potential mitochondrial gene defects. Direct enzyme-replacement therapy may be feasible in addressing single-protein enzymes, such as those in the tricarboxylic acid cycle, and perhaps mitochondrial multiprotein enzyme complexes. Another promising approach may be indirect enzyme-replacement therapy, which bypasses electron-transport defects with the use of alternative dehydrogenases or oxidases (see Table 2 in the Supplementary Appendix). Although more experimental data are required, exogenous stimulation of endogenous cellular adaptation programs

may also be useful to at least partially counterbalance the consequences of mitochondrial dysfunction.

Given the metabolic individuality in humans,⁸⁵ we do not expect monotherapeutic metabolic manipulation strategies to be a magic bullet but predict that the next step in treatment development will be the use of combinations of manipulation strategies applied in an individualized way. In the meantime, efforts must be made on a global scale to genetically categorize patient cohorts, monitor them in a standardized way by means of prognostic scoring systems,⁸⁶ and develop new biomarkers to allow for proper monitoring of the effect of intervention strategies.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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