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MITOCHONDRIAL MEDICINE

Metabolic manipulators: a well founded strategy to combat mitochondrial dysfunction

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Abstract Whilst the pathophysiology and genetics of mitochondrial disease are slowly being unraveled, currently no effective remedy for mitochondrial disorders is available. One particular strategy in mitochondrial medicine presently under study is metabolic manipulation. This approach is aimed at counteracting the deranged cell biological homeostasis caused by mitochondrial dysfunction, using dietary modifications or small molecule therapy. Cell biological alterations caused by mitochondrial dysfunction include increased reactive oxygen species production, enhanced lipid peroxidation and altered cellular calcium homeostasis. This review covers the five principles of metabolic manipulation: (1) prevention of oxidative damage by reactive oxygen species, (2) amelioration of lipid peroxidation, (3) correction of altered membrane potential, (4) restoration of calcium homeostasis, and (5) transcription regulation interference. We hypothesize that a combination of compounds targeting different metabolic pathways will abolish cellular disturbance arising as a consequences of mitochondrial dysfunction, and thereby improve or stabilize clinical features. However, only a handful of compounds have reached efficacy testing in mammals, and it remains unknown to what extent metabolic manipulation will affect the whole organism. Until a potent remedy is found, patients will remain dependent on supportive, not curative, interventions.

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References to electronic databases: e.g., OMIM disorder/gene accession number(s)
OMIM
#606426 Co-enzyme Q biosynthesis defects;
#535000 Leber Hereditary Optic Neuropathy;
#229300 Friedreich Ataxia;
#255110 carnitine palmitoyltransferase II deficiency (adult onset)

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Abbreviations

Ca^{2+}	Calcium
CoQ10	Co-enzyme Q10
CPEO	Chronic progressive external ophthalmoplegia
CPT2	Carnitine palmitoyltransferase II
COX	Cytochrome c oxidase
ER	Endoplasmic reticulum
FA	Friedreich ataxia
IMM	Inner mitochondrial membrane
LHON	Leber hereditary optic neuropathy
MitoQ	Mitoquinone
MMP	Mitochondrial membrane potential
MnSOD2	Manganese–super oxide dismutase 2
MPP	Mitochondria penetrating peptide
mtDNA	Mitochondrial DNA
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide reduced form
NARP	neuropathy, ataxia and retinitis pigmentosa
OXPHOS	Oxidative phosphorylation
PBN	Alpha-phenyl N-tertiary-butyl nitrone
PDHC	Pyruvate dehydrogenase complex
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator-1 alpha
PPARγ	Peroxisome proliferator-activated receptor gamma
rAAV-SOD2	Recombinant adeno-associated virus (rAAV) containing the SOD2 gene
ROS	Reactive oxygen species

Sir 2	Silent information regulator two
SIRT	Sirtuin
Sirtuins	Silent information regulator two (Sir2) proteins
SOD	Superoxide dismutase
SS	Szeto-Schiller
TIM	Translocase of the inner membrane
TOM	Translocase of the outer membrane
TPP	Triphenylphosphonium

Introduction

Despite progress in our current understanding of the pathophysiology and genetics of mitochondrial disease, no effective cure for mitochondrial disorders has been found (Smeitink et al. 2006). Supportive therapy is the only treatment approach we can offer our patients to date (Chinnery et al. 2006). Due to the increased knowledge of metabolism and pathophysiology, new therapeutic approaches are being discovered. Current treatment strategies applied in mitochondrial treatment development include (1) gene therapy (replacement or repair), (2) controlled regulation of specific transcriptional regulators, (3) metabolic manipulation, and (4) altering the balance between wild-type and mutated mtDNA (e.g., by exercise training) in the case of oxidative phosphorylation (OXPHOS) defects with a mitochondrial DNA (mtDNA) origin (Koene and Smeitink 2009). The effect of some of these interventions has already been explored in humans; however, most research in this field is still at the level of single cell research (Koene and Smeitink 2009).

Many in vitro experiments have been done using the metabolic manipulation strategy (Koene and Smeitink 2009). In the context of mitochondrial disease, this is defined as 'reversing the consequences of mitochondrial dysfunction using dietary modification or small molecule therapy to compensate for a deranged biological process'. Strategies used to correct the deranged cell biological processes in mitochondrial dysfunction include, for example, the prevention of reactive oxygen species damage using scavenging enzymes and compounds restoring disturbed mitochondrial calcium metabolism. Compounds altering these disturbed processes can, for example, be nutraceuticals, a contraction of "nutrition" and "pharmaceutical" used for a group of food components (such as vitamins, polyhenols, benzoquinones, etc.) claimed to have a beneficial effect on health or medical conditions.

Here, we review the current status of research in mitochondrial medicine regarding the application of metabolic manipulators in oxidative phosphorylation dysfunction.

Metabolic manipulators: compounds to repair mitochondrial dysfunction

Mitochondrial dysfunction leads not only to a reduced ATP production but also influences a variety of up- and downstream processes, including an altered cellular redox state (Distelmaier et al. 2009a), increased production of superoxide (Balaban et al. 2005), changes in membrane potential (Distelmaier et al. 2009a) and the mitochondrial morphology (Koopman et al. 2005b; Smeitink et al. 2006) (Fig. 1). We hypothesize that the metabolic and cellular alterations seen as a consequence of mitochondrial dysfunction work together to hamper cellular function resulting in the variety of clinical symptoms present in patients. Therefore, we propose that repairing the problems arising as a consequence of disturbed mitochondrial function is a well-founded way of developing further treatment for mitochondrial disease.

In the next paragraphs, we will summarize the consequences of mitochondrial dysfunction and subsequently discuss how metabolic manipulation might counteract these cell biological alterations (see also Table 1).

Preventing oxidative damage: introduction

The superoxide anion, hydrogen peroxide and the hydroxyl radical are collectively referred to as reactive oxygen species (ROS). ROS plays an important role in the expression of many proteins like transcription factors, kinases, ion channels and phosphatases (Koopman et al. 2009). However, ROS can lead to harmful effects such as DNA damage and lipid peroxidation, resulting in subsequent membrane damage and to changes in the mitochondrial network. Cells are equipped with their own antioxidant mechanisms to counteract these potentially harmful effects of ROS (Balaban et al. 2005; Murphy 2009). If the production of reactive oxygen species (ROS) becomes too great to be counterbalanced by its antioxidant system, damage to proteins, lipids and DNA might occur (Balaban et al. 2005; Smeitink et al. 2006), which is thought to have harmful effects on cellular and mitochondrial (ultra)structure, activity and matrix protein diffusion (Koopman et al. 2007, 2008a).

To what extent ROS are indeed involved in disease pathophysiology is largely dependent on model systems and experimental conditions used. For example, their role in mitochondrial disease is still under debate (Balaban et al. 2005; Murphy 2009). Chemical inhibition of complex I by rotenone, a frequently used model to study complex I deficiency, shows all of the above stated consequences (Fato et al. 2009). However, no mtDNA abnormalities or increased lipid peroxidation has been observed in genetically defective complex I human cell lines, although these cell lines do

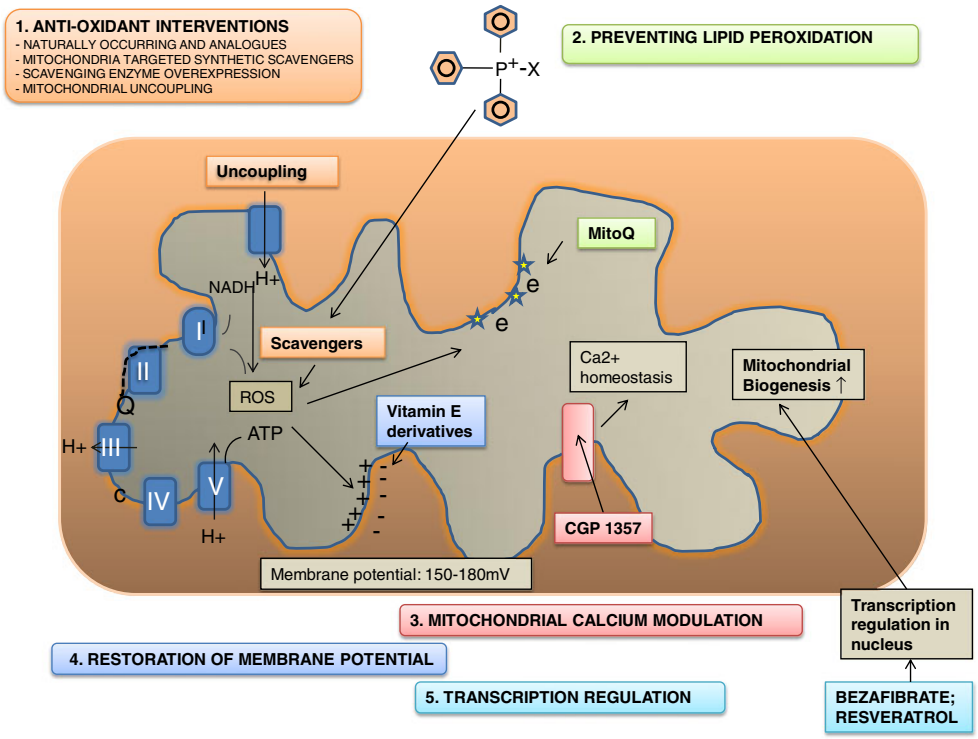

Fig. 1 Metabolic manipulation strategies. The mitochondrial oxidative phosphorylation system consists of five complexes (*I–V*; *blue*). Electrons are transported (*broken line*) through complex I and II to complex III via Co-enzyme Q10 (*Q*) and to complex IV via cytochrome oxidase (*c*), creating a proton gradient (for schematic purposes only proton transport at complex III is depicted). This gradient is the driving force behind the production of ATP by complex V. When gene mutations or secondary dysfunction causes failure in the electron transport chain, increased oxidative stress is thought to be one of the consequences. We describe five approaches which may correct the proposed cell biological consequences. Prevention of oxidative damage (1; *orange*) can be achieved by either stimulating or over expressing naturally occurring antioxidants, or by scavenger supplementation. To facilitate membrane transport several triphenylphosphonium-based compounds such as TTP-vitE have been generated. Uncoupling of the respiratory chain leads to reduced oxidative damage, but also to a reduced membrane potential. Since oxidative damage is thought to cause lipid peroxidation substances preventing lipid peroxidation were designed (2; *green*), e.g., *MitoQ*. Restoring the disturbed calcium homeostasis (3; *pink*) has been achieved on a cellular level by *CGP 1357*, a benzothiazepine drug inhibiting the mitochondrial sodium/calcium (Na^+/Ca^{2+}) exchanger. The mitochondrial membrane potential (4; *blue*) a key indicator of mitochondrial health, can be restored by the vitamin E derivates. Finally, transcription up-regulation (5; *turquoise*) of genes involved in cellular energy metabolism and subsequent mitochondrial biogenesis is achieved by over expressing the transcription factor PGC1A. *NADH* Nicotinamide adenine dinucleotide (reduced form); *Q/COQ* co-enzyme Q10; *c* cytochrome oxidase; *SOD* superoxide dismutase; *vit E* vitamin E; *CGP 1357* a benzothiazepine drug inhibiting the mitochondrial sodium/calcium (Na^+/Ca^{2+}) exchanger; *PGC-1A* peroxisome proliferator-activated receptor γ (PPAR-Γ) coactivator 1α; *MitoQ* TTP with co-enzyme Q10 attached; *ATP* adenosine triphospate; *Ca2+* calcium; *e* electron

show increased ROS production (Distelmaier et al. 2009a; Koopman et al. 2009). Despite the ongoing debate regarding the role of ROS in mitochondrial disease, much effort is devoted to studying the effects of an increased scavenger potential in mitochondrial dysfunction. Experiments to reduce ROS have explored the following strategies: (1) supplementing naturally occurring antioxidants and analogues (Chen et al. 1997; Quinzii et al. 2007), (2) treatment with synthetic scavenging compounds (Koopman et al. 2005a; Murphy 2008; Murphy and Smith 2007; Szeto 2006b), and (3) increased uncoupling of the mitochondrial respiratory chain (Sluse et al. 2006).

Table 1 A summary of therapeutic strategies in metabolic manipulation

1. Preventing oxygen damage
   i) Supplementation of naturally occurring antioxidants
   ii) Mitochondria-targeted scavenging compounds
   iii) Uncoupling of the mitochondrial respiratory chain
2. Preventing lipid peroxidation
3. Restoring the mitochondrial membrane potential
4. Modulation of mitochondrial calcium homeostasis
5. Transcription regulation

The following experiments have to be interpreted with great caution, since it is still uncertain whether ROS has a major role in the pathophysiology of mitochondrial disease. Furthermore, ROS is not only harmful to cells but also has a regulatory function in protein expression of, for example, transcription factors (Semenza 2004). Therefore, influencing its concentration might disturb cell viability.

Supplementation of naturally occurring antioxidants and analogues

The most well-studied anti-oxidant, Co-enzyme Q10 (CoQ10) (ubiquinone), acts as an electron transporter in the mitochondrial respiratory chain where it transports electrons from complex I and II to complex III (Lenaz and Genova 2009). In the rare case of CoQ10 biosynthesis defects (OMIM #606426), high doses of CoQ10 have proven to lead to clinical improvement (Quinzii et al. 2007; Di Giovanni et al. 2001). CoQ10 is frequently prescribed in patients suffering from other mitochondrial diseases with many case reports of beneficial effects (Barak et al. 1995; Wang et al. 2008). We reviewed two trials that studied the effects of CoQ10 in patients with mitochondrial encephalopathy and chronic progressive external ophthalmoplegia (CPEO) (Chen et al. 1997; Chinnery et al. 2006). One reports subjective improvement and globally a significant increase in muscle strength in 8 patients with different types of mitochondrial encephalopathy (Chen et al. 1997), but the other trial in 17 patients with CPEO (mutations not known) did not show any benefit (Chinnery et al. 2006). So, to date, there is no evidence that CoS10 should be structurally prescribed to patients with mitochondrial diseases other than CoQ10 biosynthesis defects. However, if a co-enzyme Q deficiency has not been ruled out, a clinical trial ($n=1$) should be considered.

Idebenone (hydroxydecyl benzoquinone), a synthetic CoQ10 compound, is thought to have two modes of action: (1) it acts as an electron carrier, shuttling electrons from complex I and II to complex III (Esposti et al. 1996; James et al. 2005), and (2) it acts as an antioxidant, for which the benzoquinone undergoes reversible redox reactions (Meier and Buyse 2009).

It has been suggested that Idebenone might be beneficial in Leber hereditary optic neuropathy (LHON) (OMIM #535000) (Mashima et al. 1992, 2000), a mitochondrial genetic disease often associated with a mitochondrial complex I gene mutation (Man et al. 2002), that preferentially causes blindness in young adult males. Results from a retrospective study with idebenone, vitamin B2, and vitamin C suggest a faster recovery of vision in LHON patients with m.11778G>A, m.3460G>A and m.14484T>C mutations (Mashima et al. 2000). It is difficult to interpret these data, since the natural disease progression of LHON differs significantly (Man et al. 2002). Besides, no prospective studies have been described.

Idebenone has been effectively used in Friedreich ataxia (FA) since 1999 (Meier and Buyse 2009). Friedreich ataxia (OMIM #229300) is a progressive, multisystem, degenerative disorder caused by a reduction in frataxin, resulting in mitochondrial dysfunction and oxidative damage. Treatment with idebenone is generally well tolerated and associated with improvement in neurological function and activities of daily life in about 200 patients with FA (Di Prospero et al. 2007; Meier and Buyse 2009). Whilst there are some similarities between FA patients and mitochondrial disease patients, no conclusions can be drawn from the observations in FA patients.

Oral supplementation, as is the case with CoQ10 and its variants, is the easiest way to administer antioxidants. Not all oral supplementations, however, are properly absorbed. Glutathione, for example, a major endogenous antioxidant produced by all aerobic cells (Lash 2006; Witschi et al. 1992), will be, following oral ingestion, hydrolyzed by intestinal and hepatic gamma-glutamyltransferase (Witschi et al. 1992). Increasing circulating glutathione to a proposed clinically beneficial concentration by oral administration of single doses is not possible (Witschi et al. 1992).

Several naturally occurring groups of compounds are known for their ability to manipulate the intracellular environment. These derivatives, also known as nutraceuticals, include the quinones, vitamins and polyphenols, the latter including flavenoids. Presently, numerous in vitro studies are being performed with these complex natural molecules; for example, the assessment of the interaction of quercetin derivatives, other than their antioxidant ability, with mitochondria, (Biasutto et al. 2009). The vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a phenolic antioxidant with a chromane structure similar to vitamin E but without the hydrophobic polyisoprenoid tail of the latter (Tafazoli et al. 2005), has been shown to quench cellular reactive oxygen species in genetically defective OXPHOS fibroblasts (Koopman et al. 2008b). The vitamin E remaining after its dehydrogenation is a relatively unreactive free radical (Distelmaier et al. 2009b; Koopman et al. 2008b). An increased restoration of ROS to lower levels and restoration of both the amount and activity of fully assembled complex I was observed in complex I-deficient fibroblasts treated with Trolox (Koopman et al. 2008b). Trolox had its largest effect on complex I protein levels and activity in patient cells with a mild complex I deficiency (Koopman et al. 2008b). All of these experiments are still only on a cellular level.

Mitochondria-targeted synthetic scavenging compounds

For a more efficient delivery of scavengers to the source of the ROS production, special transporters have been designed to bring the compounds to the mitochondrial inner membrane (IMM) (Hoye et al. 2008; Szeto 2006b). To move molecules into the mitochondrial matrix, both the cell membrane and the two mitochondrial membranes have to be passed, of these the IMM is impermeable to uncharged and water-soluble molecules. Targeted mitochondrial delivery can be obtained by (1) endogenous

mitochondrial import, using the natural import system of the cell (Neupert 1997), (2) fusion of closed compartments with the mitochondria (Muratovska et al. 2001), or by (3) facilitated transport across the lipid bilayer (Macias et al. 2007). We will only discuss the latter, including constructed shuttles like the lipophilic cation shuttles Triphenylphosphonium (Murphy and Smith 2007), Szeto-Schiller tetrapeptides (Szeto 2006a), Gramicidin S analogs (Macias et al. 2007), mitochondria penetrating peptides (Horton et al. 2008) and the SkQ cations (Skulachev 2009).

Lipophilic cation shuttles have a large hydrophobic surface area which, together with the negatively charged electron gradient that facilitates cation passage, enables these molecules to pass through the two lipid bilayers of the mitochondria (Hoye et al. 2008; Murphy and Smith 2007). These components and their cargo accumulate within the mitochondria up to 1,000-fold, in comparison to those in untargeted analogues (Murphy and Smith 2007). Triphenylphosphonium (TPP), one of such lipophilic cation shuttles is designed to shuttle anti-oxidants to the mitochondrial matrix (Murphy and Smith 2007). TPP-conjugated derivates of ubiquinone (James et al. 2005, 2007; Kelso et al. 2001), tocopherol (Smith et al. 1999), lipoic acid (Brown et al. 2007), spin traps (Murphy et al. 2003) and the peroxidase mimetic Ebselen (Filipovska et al. 2005) can successfully import anti-oxidants into the mitochondria in vitro (Murphy 2008) and might be able to decrease ROS production (Murphy 2008). Although these experiments seem promising, these shuttles depend on the electrical polarization of the mitochondrial membrane for their uptake and may, therefore, not be applicable in all cases of mitochondrial dysfunction (Szeto 2006b). Furthermore, some of these agents may be toxic to the cell, as is the case with high concentrations of the spin trap alpha-phenyl N-tertiary-butyl nitrone (PBN) (Albano et al. 1986).

In addition, the mode of action of some of these compounds might be other than predicted. This is for example the case for MitoQ (Antipodean Company), a TTP shuttle with CoQ10 as cargo. In a complex I rotenone inhibition experiment, MitoQ did not scavenge the increased ROS as measured by HEt oxidation (Koopman et al. 2005a), but did decrease lipid peroxidation and normalized mitochondrial morphology (Koopman et al. 2007; Murphy 2009). High doses of MitoQ were administered orally to young healthy mice for up to 28 weeks to study the effects on whole-body physiology, metabolism, and gene expression (Rodriguez-Cuenca et al. 2010). There were no changes in the expression of mitochondrial or antioxidant genes as assessed by DNA microarray analysis. There was also no increase in oxidative damage to mitochondrial protein, DNA, or cardiolipin, and the activities of mitochondrial enzymes were unchanged. No adverse effects were reported in the mice. Whether MitoQ will be beneficial in patients with mitochondrial disorders has to be studied in randomized clinical trials.

The Szeto-Schiller (SS) tetrapeptide is a vehicle for mitochondrial scavenger targeting, using a independent potential delivery (Adlam et al. 2005; Magwere et al. 2006; Murphy and Smith 2007; Smith et al. 1999, 2008; Szeto 2006a, b). The water-soluble SS tetrapeptides can have alternating aromatic residues and basic amino acids (aromatic-cationic peptides), which are designed to reduce ROS in mitochondria and protect against lipid peroxidation and cell death in neuronal and cardiac cells (Zhao et al. 2004). Chemically induced complex I deficiency by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a mouse model for Parkinson, showed the neuroprotective effects of the administration of SS peptides (Yang et al. 2009). Surprisingly, a SS peptide which did not have a scavenging capacity also demonstrated significant neuroprotective effects on dopaminergic neurons of chemically induced Parkinson mice (Yang et al. 2009). SS peptides are also claimed to have an inhibitory effect on lipid peroxidation (Szeto 2006a); however, there is no evidence to confirm this statement. Studies with isolated mitochondria showed that both SS-31 and SS-20 prevented MPP+-induced inhibition of oxygen consumption, ATP production and mitochondrial swelling. It is not clear what the biochemical or pharmacological mechanism is underlying these observations. It might be either a ROS reducing effect of the SS peptide alone, or another, unknown pathophysiological mechanism underlying the neurodegeneration observed in this Parkinson model.

Mitochondria penetrating peptides (MPP), cationic, but also lipophilic synthetic peptides, were also reported to exhibit efficient cellular uptake with a targeted mitochondrial localization (Horton et al. 2008). For example, the Gramicidin S analogue 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) peptides were designed to transport the TEMPO cargo into mitochondria (Macias et al. 2007). Gramicidin was used because of its high affinity to bacterial membranes (Sholtz et al. 1975) and demonstrates an ability to manipulate mitochondrial membrane potential. TEMPO peptides can be used to deliver antioxidants and radical scavengers to mitochondria and were shown to prevent ROS-induced apoptosis in a rat model for ischemia-reperfusion injury (Macias et al. 2007).

Finally, a penetrating cation (Sk) with plastoquinone (Q), a quinone acting as a electron carrier in the electron transport chain has been extensively studied by Skulachev and co-workers (Antonenko et al. 2008; Skulachev 2009; Skulachev et al. 2009). SkQ, of which several variants have been synthesized, are rechargeable antioxidants with a high affinity for the inner leaflet of the mitochondrial inner membrane. The antioxidant properties of these compounds have been studied extensively both in vitro and in vivo, and

there is evidence for results reaching from recovering blindness in mammals to geroprotection (Skulachev et al. 2009). The claimed success of SkQ claims to prevent lipid peroxidation, senescence and ischemia reperfusion injury (Skulachev 1998). Independent studies are awaited to confirm the observed results.

Mitochondrial uncoupling regulation

Mitochondrial uncoupling proteins (UCPs) increase proton leakage in the IMM, thereby reducing oxygen stress by lowering the electrochemical gradient (Cannon and Nedergaard 2004; Sullivan et al. 2004). The net effect of uncoupling is (1) a reduction of ROS production, (2) dissipation of heat, (3) reduction of ATP synthesis, and (4) reduction of calcium influx to the mitochondrial matrix. Uncoupling can be stimulated by either high levels of cellular/mitochondrial ROS (Sluse et al. 2006) or by circulating free fatty acids (FFA) (Jarmuszkiewicz et al. 2004).

The ketogenic diet, a high-fat, low-protein, low-carbohydrate diet, provides an increased free fatty acid (FFA) concentration in plasma, which stimulates uncoupling by the mitochondrial UCPs in mice (Sullivan et al. 2004; Davis et al. 2008). Also, a decreased ROS production was found in the hippocampus of these mice, without decreasing mitochondrial ATP production (Sullivan et al. 2004).

Ketogenic diet is not only known for its uncoupling activity, but is also thought to provide an alternative source of acetyl Co-A by inducing fatty acid beta-oxidation as well as changing the heteroplasmy state in favor of the wild-type mtDNA copy number (Santra et al. 2004). In children with pyruvate dehydrogenase complex (PDHC) deficiency and a variety of isolated and combined OXPHOS system deficiencies, the ketogenic diet was reported to improve epilepsy (Kang et al. 2007; Wexler et al. 1997) and mental development in PDHC deficiency (Wexler et al. 1997). Although these results in PDHC and mitochondrial diseases complicated by epilepsy seem promising, ketogenic diets are often complicated by metabolic disturbances, such as hypoglycemias and hypercholesterolemia as well as by gastrointestinal symptoms and lethargy (Duchowny 2005; Toshima et al. 1982; Wexler et al. 1997; Wijburg et al. 1992). We think it is worthwhile investigating the effect of a ketogenic diet in patients with mitochondrial disease. However, this may also have major side effects because the defective mitochondria are not able to process the excess of lipids in the ketogenic diet.

Preventing lipid peroxidation and mitochondrial network

Mitochondria are highly dynamic organelles, forming an extensive network with constant fusion and fission (Detmer and Chan 2007). Lipid peroxidation as a consequence of an increased ROS production changes the structural architecture of the mitochondrial network (Bach et al. 2003; Hood 2001), and is generally accompanied by changes in mitochondrial content, ultrastructure, and enzyme levels (Rossignol et al. 2004).

Treating chemically inhibited Complex I-deficient cells with the antioxidant MitoQ abolished lipid peroxidation and normalized mitochondrial shape (Koopman et al. 2005a). Fibroblasts of Complex I-deficient patients, showing increased ROS formation, displayed no signs of increased lipid peroxidation (Verkaart et al. 2007) and seem to have adapted to increased levels of ROS. Unraveling these adaptive mechanisms may provide new targets for therapeutic intervention.

Modulation of mitochondrial calcium homeostasis

Mitochondria play a central role in ensuring the homeostasis of ionic calcium (Ca^{2+}), an all round intracellular signaling molecule that regulates a large variety of cellular processes (Berridge et al. 2003). During cell stimulation, Ca^{2+} is released from the endoplasmic reticulum to the cytosol and taken up by mitochondria, where it stimulates mitochondrial dehydrogenases giving rise to increased ATP production (Brini et al. 1999; Korzeniewski 2007; Pinton et al. 2008; Valsecchi et al. 2009).

Cells harboring mutations in nuclear or mitochondrial DNA often show disturbances in Ca^{2+} homeostasis (Brini et al. 1999; Distelmaier et al. 2009a). Complex I-deficient cells have an almost normal resting Ca^{2+} balance in mitochondria and cytosol, but a lower endoplasmic reticulum Ca^{2+} concentration (Visch et al. 2004). However, the peak in mitochondrial Ca^{2+} concentration seen after hormonal stimulation with bradykinin was lower than expected (Visch et al. 2004). Moreover, Ca^{2+} was removed from the cytosol at a slower rate (Visch et al. 2004). In a heart-specific knockout of the mitochondrial transcription factor A (*Tfam*) mouse model, a clear association of a disturbed Ca^{2+} homeostasis with cathecholamine-induced arrhythmias was found (Tavi et al. 2005).

This suggests that Ca^{2+} homeostasis is inevitable for cell functioning, and above all that disturbances in cellular Ca^{2+} homeostasis lead to clinical abnormalities (Brini et al. 1999; Tavi et al. 2005; Visch et al. 2004).

CGP37157 is a benzothiazepine drug which inhibits the mitochondrial sodium/calcium (Na^{+}/Ca^{2+}) exchanger. CGP37157 restores Ca^{2+} handling and ATP production in cells with a hampered energy metabolism. In Complex I-deficient cells harboring a NDUFS7 or NDUFS4 mutation, treatment with a CGP37157 resulted in an normalization of stimulated mitochondrial Ca^{2+} concentration and ATP production, as well as a normal cytosolic Ca^{2+}

removal (Visch et al. 2004, 2006). Treatment of cybrid cells expressing the m.8356T>C (MERFF) or the m.8993T>G (NARP) mutation with CGP37157 mostly restored both the Ca^{2+} uptake and the stimulation of ATP production (Brini et al. 1999). We are not aware of clinical studies aimed at correcting cellular calcium homeostasis.

Transcription regulation

Peroxisome proliferator-activated receptor γ (PPAR-γ) co-activator 1α (PGC-1α) is a transcriptional factor regulator that stimulates transcription of genes involved in cellular energy metabolism (Wenz 2009). The study of PGC-1α has received much attention for its possible roles in the treatment of the metabolic syndrome (Canto and Auwerx 2009), its potential neuroprotective capacity (St-Pierre et al. 2006), and its roles in various other pathologies (Baur and Sinclair 2006).

PGC-1α is not only known for its role in the transcription of proteins involved in energy metabolism but is also under interest because of its ROS-mediated cell death prevention (St-Pierre et al. 2006), and the stimulation of mitochondrial biogenesis (Wenz 2009). PPARS also affects mtDNA levels by modulating transcription of the mtDNA transcription factor A (Tfam) gene (Handschin and Spiegelman 2006). PGC-1α is physiologically regulated by for example exercise (Taivassalo and Haller 2005) and calorie intake (Corton and Brown-Borg 2005) but can also be manipulated by pharmacologic agents, for example, bezafibrate (Bastin et al. 2008) and resveratrol (Oliva et al. 2008) (Fig. 2). The latter is known as a stimulator of silent information regulator two (Sir2) proteins (Sirtuins), which catalyze NAD^+-dependent deactetylation within proteins such as PGC-1α (Hallows et al. 2009). NAD+, also known as a biochemical electron carrier shuttling electrons from the Krebs cycle to complex I, is therefore considered the direct link between cellular stress and an increased metabolism (Houtkooper et al. 2009) (Fig. 2).

The stimulation of the PPAR/PGC-1α pathway also has consequences for mitochondrial biogenesis (Wenz 2009). Increased mitochondrial biogenesis is a natural compensation mechanism commonly seen in skeletal muscle of patients with OXPHOS deficiencies (DiMauro and Schon 2008), and has been described as be a maladaptive effect in an attempt to increase ATP supply (Murdock et al. 1999). Since an increase in the biogenesis of healthy mitochondria shifts the balance (heteroplasmy) and thereby increases cell metabolism, this strategy has been studied to treat patients with mtDNA mutations. PGC-1α stimulation through exercise is known to increase mitochondrial mass and oxidative capacity (Taivassalo and Haller 2005). To verify the role of PGC-1α in mitochondrial biogenesis, a mouse

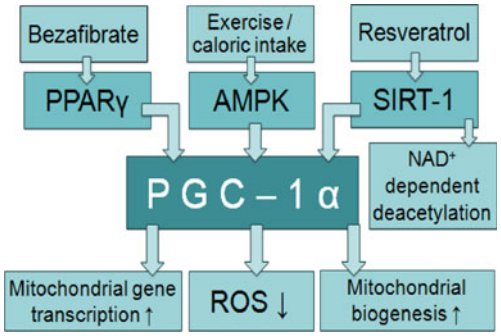

Fig. 2 Stimulation of transcription of mitochondrial genes. Peroxisome proliferator-activated receptor γ (*PPAR-γ*) co-activator 1α (*PGC-1α*) stimulates transcription of genes involved in mitochondrial energy metabolism, by increasing their nuclear transcription and expression. Stimulation of PGC-1α also causes a reduction in reactive oxygen species, an increase in mitochondrial biogenesis, and a beneficial shift in heteroplasmy. All this leads to an increased cellular energy production; however, the long-term effects of an increased mitochondrial biogenesis are unknown. PGC-1α is stimulated via adenosine monophosphate (AMP), activated protein kinase (*AMPK*), by physiological processes such as exercise, as well as by pharmacological agents for example bezafibrate and resveratrol. Bezafibrate directly stimulates PPARγ, the transcription factor working together with PGC-1α. Resveratrol stimulates silent information regulator two proteins (Sirtuins) which catalyze NAD^+-dependent deactetylation within PGC-1α. Since NAD^+ reflects the cells energy metabolism, this is described as the direct link between external physiological stimuli and the regulation of mitochondrial biogenesis. *PGC-1α* peroxisome proliferator-activated receptor γ (*PPAR-γ*) coactivator 1α; *PPAR-γ* peroxisome proliferator-activated receptor γ; *ROS* reactive oxygen species; *AMPK* adenosine monophosphate (AMP), activated protein kinase; *SIRT-1* silent information regulator 1 protein; *NAD* nicotinamide adenine dinucleotide

model for cytochrome *c* oxidase (COX) deficiency with transgenic expression of PGC-1α was used (Wenz et al. 2008). These PGC-1α-expressing mice have a delayed onset of myopathy, increased mitochondrial biogenesis, increased ATP levels and increased health and lifespan compared to COX deficient littermates (Wenz et al. 2008). PGC-1α stimulation thus positively affects mitochondrial function in COX-deficient cells.

Resveratrol (3,5,4′-trihydroxystilbene), a polyphenolic phytoalexin, stimulates sirtuin activity and thereby transcription of the nuclear genes involved in energy metabolism (Almeida et al 2009). Also, a spectacular increase in the cells' own anti-oxidant machinery, such as mitochondrial superoxide dismutase MnSOD2 and glutathione, was observed (Das et al. 2008; Kode et al. 2008; Robb et al. 2008).

Healthy mice were fed with either a normal (SD) or a high calorie diet (HC), either with resveratrol (HCR) or without (HC). The HCR mice tend to have an increased survival rate and rotarod performance compared to HC mice and do not significantly differ from the SD mice (Baur et al. 2006). Also, their cardiovascular risk profile was improved compared to the mice having no resveratrol. The livers of the HCR mice had considerably more mitochondria than those of HC

controls and were not significantly different compared to those of the SD group (Baur et al. 2006). In another experiment with 1-year-old mice on the HC diet that had been treated with resveratrol for 6 weeks, the acetylation status of PGC-1α in the resveratrol-fed mice was threefold lower than the diet-matched controls. This enhanced enzyme activity can be either stimulated by resveratrol or SIRT1. Since the latter was not detectably increased, it can be concluded that resveratrol stimulates PCG-1α expression in mice on a HC diet (Baur et al. 2006; Milne and Denu 2008).

A double-blind, randomized, placebo-controlled study with resveratrol in healthy volunteers showed that frequent administration of resveratrol was well tolerated, but low plasma concentrations were achieved despite a 4-hourly oral administration regime (Almeida et al. 2009), possibly due to a rapid and extensive first pass metabolism (Walle et al. 2004). There are no data on the side effects of continual resveratrol administration.

Studies in healthy volunteers focusing on the effects of resveratrol on cardiovascular parameters show a stimulation of human platelet nitric oxide production, increased HDL cholesterol and inhibited platelet aggregation, and oxidation of low-density lipoproteins (Bhat et al. 2001; Gresele et al. 2008). From a mitochondrial disease point of view, resveratrol theoretically has a stimulating effect on mitochondria and has proven to increase mitochondrial biogenesis in the liver of mice on continual resveratrol treatment. In addition, administration seems well tolerated. However, we are not aware of any studies assessing mitochondrial enzyme complex activity in animals or humans treated with resveratrol, or resveratrol administration in animal models for mitochondrial disease or human mitochondrial disease patients.

Another pharmacologic PPAR pan-agonist called bezafibrate, was administered in COX-deficient mice shortly before disease onset. Bezafibrate was shown to lead to mitochondrial proliferation and an enhanced OXPHOS capacity per muscle mass as well as a reduction of the myopathy and prolongation of the lifespan (Wenz et al. 2008). The increase in ATP production per muscle mass was thought to play the key role in the clinical improvement of the myopathic mice.

Bezafibrate was also studied in a clinical trial in six adults with mild carnitine palmitoyltransferase II (CPT2) deficiency (Bonnefont et al. 2009). CPT2 (OMIM #255110) is a translocation protein shuttling long-chain fatty acyl-CoAs over the inner mitochondrial membrane (McGarry and Brown 1997). Patients with CPT2 deficiency may present either in infancy or as adults; the latter presenting with recurrent attacks of myalgia and muscle stiffness or weakness, occasionally associated with myoglobinuria. The frequency of these attacks is highly variable and, inbetween attacks, patients have no clinical symptoms (Bonnefont et al. 2004). Administration of bezafibrate in patients with CPT2 deficiency fully restored fatty acid oxidation capacity in muscle cells, probably caused by stimulating the expression of the mutated gene (Bonnefont et al. 2009; Djouadi et al. 2005). Also, patients had fewer periods of rhabdomyolysis, and quality of life parameters reached the control ranges on all domains (Bonnefont et al. 2009). No adverse effects were reported.

Although transcription regulation seems a very promising strategy in treating mtDNA defects, the long-term complications of mitochondrial proliferation should be studied, since both healthy and hampered mitochondria will proliferate (Wenz 2009). Excessive mitochondrial proliferation in PPAR stimulation may result in cardiomyopathy (Lehman et al. 2000).

In addition to that, a recent study showed that the activation of sirtuins by resveratrol and other synthetic SIRT1 agonists was not caused by a direct interaction between sirtuins and its agonists (Pacholec et al. 2010). NMR and calorimetry techniques demonstrated that the reported effect was caused by the interaction of the SIRT1 with the fluorophore (Pacholec et al. 2010). In vivo experiments did not confirm the beneficial effects of SRT1720 on plasma glucose and mitochondrial capacity in mice on a high fat diet (Pacholec et al. 2010).These results show that the effect of small molecules on sirtuin expression is most uncertain.

Conclusion

Metabolic manipulation is one way in which to develop a well-founded and effective therapy for mitochondrial disorders. Some examples of metabolic manipulation intervention seem very promising at the cellular level, though very few compounds have been tested in vivo. The real challenge in mitochondrial medicine is to design an effective clinical trial to definitively address the question of whether or not such therapeutic compounds are beneficial. This has proven to be very difficult in this heterogeneous population with an unpredictable natural history.

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