Potential compounds for the treatment of mitochondrial disease

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Abstract

Introduction: Mitochondrial diseases are a group of heterogeneous disorders for which no curative therapy is currently available. Several drugs are currently being pursued as candidates to correct the underlying biochemistry that causes mitochondrial dysfunction.

Sources of data: A systematic review of pharmacological therapeutics tested using in vitro, in vivo models and clinical trials. Results presented from database searches undertaken to ascertain compounds currently being pioneered to treat mitochondrial disease.

Areas of agreement: Previous clinical research has been hindered by poorly designed trials that have shown some evidence in enhancing mitochondrial function but without significant results.

Areas of controversy: Several compounds under investigation display poor pharmacokinetic profiles or numerous off target effects.

Growing points: Drug development teams should continue to screen existing and novel compound libraries for therapeutics that can enhance mitochondrial function. Therapies for mitochondrial disorders could hold potential cures for a myriad of other ailments associated with mitochondrial dysfunction such as neurodegenerative diseases.

Key words: mitochondrial disease, oxidative phosphorylation, clinical trials, therapeutic intervention, treatment
Introduction

Mitochondria are dynamic organelles that are responsible for multiple metabolic processes vital to cellular function. These roles are highly varied and include calcium handling, the formation of iron sulphur clusters and β oxidation of fatty acids. The focus of this review will be on the most well-known function of mitochondria, their production of the majority of cellular ATP by oxidative phosphorylation (OXPHOS).1 OXPHOS is the process whereby cellular respiration is coupled to the production of ATP. It is executed by five protein complexes within the inner mitochondrial membrane and two soluble electron carriers, Co-enzyme Q10 (CoQ10) and cytochrome c. Thirteen subunits of the protein complexes are encoded by mitochondrial DNA (mtDNA) with the remainder encoded by nuclear DNA (nDNA) and transported into mitochondria from the cytosol. Four of these complexes function to transfer electrons from reducing equivalents to molecular oxygen (the electron transfer or respiratory chain), with the resultant proton electrochemical gradient being harnessed by the fifth complex, F,F ATP synthase to generate ATP. mtDNA is a 16.5 kb circular, intronless genome present in multiple copies within the organelle which encodes 22 tRNAs and 2 rRNAs in addition to the 13 polypeptides.2 The multiple copy nature of mtDNA enables mutated and wild-type species to coexist within the same organelle, a situation termed heteroplasmy. Mutations are normally recessive, with a threshold heteroplasmy level needing to be reached before a phenotype is expressed, the level being dependent on the mutation present.

Mitochondrial diseases, caused by mutations either in mtDNA or nDNA encoded mitochondrial genes, affect one in 5000 individuals,3 however acquired dysfunction of the organelle has been implicated in the pathology of numerous other conditions such Alzheimer’s and Parkinson’s disease, along with the process of aging.4 Mitochondrial diseases most commonly present as neurological abnormalities or as myopathies affecting the skeletal or cardiac muscle, though any tissue in the body has the potential to be affected. The age of onset and disease severity also varies, with the same mutations potentially causing different clinical phenotypes. These factors combine to make mitochondrial diseases particularly challenging to diagnose and manage (Table 1).

The treatment of mitochondrial disease is currently limited to symptomatic therapies that do not address the biochemical defects that underpin the disorders. However, a number of putative mitochondrial therapeutics are currently used in the clinic after promising reports in patient case studies or in vitro investigations. Yet, to date there have been very few double-blind clinical trials of patients with mitochondrial disease, thus complicating the use of therapies within a clinical setting.5

Whilst it is obvious that the provision of treatments that alleviate mitochondrial dysfunction in patients with mitochondrial diseases has been lacking thus far, there are numerous reports of compounds that claim to improve mitochondrial function (Table 2). The chemical structure and composition of compounds under investigation are as diverse as the pathways they affect, ranging from isoﬂavones to protein based therapeutics. This review will attempt to highlight these compounds and discuss the progress towards a clinically relevant treatment for mitochondrial disease. Figure 1 summarizes the areas of mitochondrial function targeted by each compound.

Targeting the electron transport chain (ETC)

One method to treat mitochondrial disorders is to increase the rate of OXPHOS, increasing ATP production and overcoming energy deﬁcits that arise from mitochondrial dysfunction. Two strategies can be used to increase the rate of OXPHOS. The first attempts to bypass or supplement deﬁcient complexes in order to rescue the normal rate of OXPHOS, the second provides more substrate for OXPHOS enzymes.

Bypassing respiratory complexes

Co-enzyme Q10

CoQ10 is a vital component of the respiratory chain, shuttling electrons from Complexes I and II to Complex
<table>
<thead>
<tr>
<th>Disease</th>
<th>Molecular aetiology</th>
<th>Clinical features</th>
<th>Major organs affected</th>
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</thead>
<tbody>
<tr>
<td>Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS)</td>
<td>m.3243A&gt;G MT-TL1</td>
<td>Epilepsy, encephalopathy, myopathy, severe constipation, failure to thrive</td>
<td>Skeletal muscle, brain, heart</td>
</tr>
<tr>
<td>Maternally Inherited Diabetes and Deafness (MIDD)</td>
<td>m.3243A&gt;G MT-TL1</td>
<td>Sensorineural deafness, diabetes, myopathy, constipation</td>
<td>Ear, endocrine pancreas</td>
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<td>Myoclonus Epilepsy with Ragged-Red fibres (MERRF)</td>
<td>m.8344A&gt;G MT-TK</td>
<td>Generalized epilepsy, ataxia and myopathy.</td>
<td>Skeletal muscle, brain, heart</td>
</tr>
<tr>
<td>Neuropathy, Ataxia and Retinitis Pigmentosa (NARP)</td>
<td>Mutations in MT-ATP6 gene affecting protein structure</td>
<td>Blindness, cerebellar ataxia, seizures, cognitive impairment and peripheral neuropathy</td>
<td>Brain, eye</td>
</tr>
<tr>
<td>Leber’s Hereditary Optic Neuropathy (LHON)</td>
<td>Three mutations in complex I genes m.3460G&gt;A, m.11778G&gt;A, m.14484T&gt;C</td>
<td>Bilateral optic atrophy</td>
<td>Eye, heart</td>
</tr>
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<td>Leigh syndrome</td>
<td>Point Mutations in mtDNA and/or nDNA genes such as SURF1</td>
<td>Lactic acidosis, failure to thrive, myopathy, bilateral symmetrical lesions in subcortical brain.</td>
<td>Brain, skeletal muscle, eye, peripheral nervous system</td>
</tr>
<tr>
<td>Kearns–Sayre syndrome (KSS)</td>
<td>Single, large-scale mtDNA deletion</td>
<td>Short stature, diabetes mellitus, cardiomyopathy, ataxia</td>
<td>Skeletal muscle, brain, heart, eye</td>
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<tr>
<td>Pearson’s syndrome (PS)</td>
<td>Single, large-scale mtDNA deletion</td>
<td>Lactic acidosis, sideroblastic anaemia, vomiting, diarrhoea and failure to thrive.</td>
<td>Bone-marrow, exocrine pancreas, liver, kidney, brain</td>
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<tr>
<td>Chronic Progressive External Ophthalmoplegia (CPEO)</td>
<td>Single, large-scale mtDNA deletion</td>
<td>Ptosis, muscle weakness,</td>
<td>Skeletal muscle, heart</td>
</tr>
<tr>
<td>Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE)</td>
<td>Mutation in nuclear thymidine phosphorylase gene causes mtDNA deletions and depletion</td>
<td>Muscle weakness, ptosis, ophthalmoplegia, peripheral neuropathy, gastrointestinal dysmotility, cachexia, leukoencephalopathy</td>
<td>Gut, skeletal muscle, brain, peripheral nervous system</td>
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<tr>
<td>Drug</td>
<td>Phase</td>
<td>Disease association</td>
<td>Registration</td>
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<td>Bezaifibrate</td>
<td>2</td>
<td>Mitochondrial myopathies</td>
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<td>2</td>
<td>Mitochondrial myopathy</td>
<td>2015</td>
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<td>EPI-743</td>
<td>2</td>
<td>Japanese MELAS syndrome</td>
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<td>2</td>
<td>Mitochondrial respiratory chain diseases</td>
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<td>Enhance vitality and vigour in elders</td>
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<td>Bendavia</td>
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<td>Mitochondrial myopathy</td>
<td>2015</td>
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<td>2</td>
<td>Skeletal muscle function in elderly</td>
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<td>RP103</td>
<td>2/3</td>
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<td>RTA 408</td>
<td>2</td>
<td>Mitochondrial myopathy</td>
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III via the quinone pool. Patients suffering from specific deficiencies in CoQ10 synthesis have been treated effectively with the compound. It was therefore suggested that supplementing CoQ10 in patients with respiratory chain deficiencies would improve the efficiency of electron transfer through the ETC. However in 2010, a randomized double-blind trial where patients were given 1200 mg daily found only a slight increase in post-exercise aerobic capacity and no difference in muscle strength or serum lactate levels. One reason for the failure of CoQ10 to have a more significant impact may have been its poor bioavailability. A Phase III multi-centre trial in 24 patients was recently completed, for which results are being awaited (www.clinicaltrials.gov, NCT00432744). An alternative molecule developed with better delivery of CoQ10 to the mitochondrion is MitoQ. This lipophilic cation conjugate is able to pass through the double membrane and exert potent antioxidant effects in the mitochondrial matrix. The compound in its quinol state has been shown to prevent lipid peroxidation. However the compound is not able to function as an electron carrier, preventing its use in bypassing compromised respiratory complexes.

**Idebenone**

Idebenone is based structurally upon CoQ10. Idebenone functions as an electron carrier and antioxidant, balancing reactive oxygen species (ROS) levels in cells and preventing lipid peroxidation in brain homogenate. The derivative was found to have higher efficacy than its coenzyme counterpart while functioning in a similar manner.

The antioxidant has been tested extensively in isolated Complex I deficiencies such as Leber’s Hereditary Optic Neuropathy. In 2011 it was reported that the RHODOS trial found no significant changes in visual acuity upon administration of Idebenone for 24 weeks. However a follow up report stated that any positive changes in outcome measures persisted in patients after treatment was discontinued.
also reported that administration of Idebenone was able to reduce the frequency of loss of visual acuity in patients suffering from LHON, suggesting the compound may halt disease progression with long-term administration. Furthermore, a Phase IIa pharmacodynamics study is currently ongoing in 21 patients diagnosed with MELAS (www.clinicaltrials.gov, NCT00887562). The European Medicines Agency has also recently recommended granting a marketing authorisation for Idebenone for the treatment of visual impairment in adolescent and adult patients with LHON.

Dimethylglycine
Dimethylglycine (DMG) is an amino acid derivative thought to be oxidized in mitochondria where it donates electrons to the quinone pool, therefore bypassing compromised upstream Complexes I and II. Its effect on mitochondrial function was assessed in a crossover, randomized double-blind trial in five children harbouring the Saguenay-Lac-Saint-Jean cytochrome-c oxidase deficiency at a dose of 51–5000 mg/kg every day. This small trial showed improvements in oxygen consumption (VO₂), though these values did not reach significance. DMG was well tolerated in patients with no observed side effects.

Supplementing substrate supply
Carnitine
Fatty acids can be utilized by mitochondria to produce ATP. Fatty acids are degraded and enter into the tricarboxylic acid (TCA) cycle after being metabolized via β oxidation. The carnitine palmitoyltransferase pathway catalyses the transport of long chain fatty acids in the form of fatty acyl carnitine into the organelle. Addition of carnitine has therefore been suggested to elevate the level of β oxidation in mitochondria. L-carnitine is an FDA approved compound and is used to treat a range of metabolic diseases. 10–1000 mg of the compound are typically administered either orally or intravenously. Few studies have reported any success with the compound as a mitochondrial therapeutic; this is due to the low bioavailability of only 10–20% when administered orally. L-carnitine undergoes rapid tissue absorption and a high rate of renal clearance. Although available on prescription, several side effects have been documented such as gastro intestinal irritation and in some cases, cardiac arrhythmias.

Dichloroacetate (DCA)
DCA has been investigated in the alleviation of lactic acidosis, caused by biochemical changes due to mitochondrial dysfunction. DCA functions by inhibiting pyruvate dehydrogenase kinase, an enzyme which catalyses the inactivation of the pyruvate dehydrogenase complex (PDHC). By preventing the inactivation of the PDHC, DCA ensures pyruvate is imported into the organelle, increasing its availability for catabolism and thus increasing the level of reduced cofactors (NADH, FADH₂) available for OXPHOS. To date, two studies have been conducted, both of which were double-blind, placebo-controlled crossover trials in patients of varying ages and disease backgrounds. The compound was found to reduce serum lactate levels but caused peripheral nerve toxicity in adults suffering from MELAS after long-term use.

Maintaining organelle homeostasis
A number of compounds have been investigated that exert their effect by maintaining normal homeostasis within the mitochondria. This includes compounds that prevent aberrant signalling, maintaining normal membrane composition and structure, reducing ROS levels or by regulating mitochondrial proteostasis. ROS are highly reactive molecules derived from oxygen. These species can oxidize macromolecules such as DNA, RNA, proteins or lipids in their immediate vicinity, damaging organelle integrity. Glutathione is a potent reducing agent that detoxifies ROS and other electrophiles present in the organelle.

Regeneration of glutathione
Epi-743
One line of defence against mitochondrial disease progression would be to prevent the oxidation of mitochondrial components by free radical species. Vitamin E is one such chemical that displays potent
antioxidant effects via the regeneration of glutathione. Unfortunately the low bioavailability of the compound and the risk of cardiac complications at high doses has prevented researchers pursuing its use in patients. Epi-743 is a synthetic analogue of vitamin E. The compound has been tested in both in vitro and in vivo studies, and previous open-label trials reported some improvement in the quality of life and clinical features of patients with Leigh syndrome. EPI-743 is currently in a Phase IIb randomized trial in which 30 children with Leigh syndrome are receiving between 5 and 15 mg/kg of compound a day (www.clinicaltrials.gov, NCT01721733). Two additional trials are also underway, one to treat 87 severely ill patients with inherited mitochondrial respiratory chain deficiency with high (50–100 mg) doses several times a day (www.clinicaltrials.gov, NCT01370447). Another to monitor the effect of EPI-743 on disease severity in paediatric patients with mitochondrial disorders. (www.clinicaltrials.gov, NCT01642056).

RP103
RP103 cysteamine bipartite is a whey-based product that has better pharmacokinetics than many other glutathione regenerators. Previously, a double-blind crossover trial showed no significant changes in lactate levels or other clinical markers, yet levels of oxidative stress were greatly reduced. Thirty-two paediatric participants are currently being recruited for a Phase II/III trial to treat Leigh Syndrome using a dose escalating study design to measure changes in the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) and pharmacodynamics markers (www.clinicaltrials.gov, NCT02023866).

ROS scavenging
Flupirtine
Direct ROS scavenging has also been investigated in the treatment of mitochondrial disease. Flupirtine is a cationic triaminopyridine derivative and is used as a non-opioid analgesic. It has also been found to display prominent antioxidant effects. In vitro addition of 20 μM of Flupirtine was found to increase the survival of PC12 cells after insult with hydrogen peroxide. Additionally, mitochondria isolated from rats were treated with varying levels of the compound (10–100 μM). At lower levels the drug was found to prevent lipid peroxidation by scavenging ROS near the mitochondrial membrane.

Ganoderma lucidum
Ganoderma lucidum or Reishi is a natural fungal component found in South Asia; it has been used in traditional medicine and was recently reported to enhance mitochondrial function via a number of mechanisms. The plant extract demonstrated antioxidant activity in rodent models, decreasing lipid peroxidation, increasing the activity of TCA cycle dehydrogenase enzymes and elevating levels of Respiratory Complexes I and II in the brains of rats administered the compound. The extract is a cocktail of terpenes, polyphenols, vitamin C, E and β carotenes. A recent study has found that Reishi may increase levels of mitochondrial biogenesis via activation of peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) in cellular models of Huntington’s disease.

Regulation of lipid dynamics
Bendavia
One mechanism for overcoming mitochondrial dysfunction is the restoration of normal organelle structure. Mutations that produce misfolded components of the respiratory chain can cause aberrant ROS production which can in turn perpetuate cardiolipin peroxidation. Cardiolipin is a key component of the inner mitochondrial membrane and serves to regulate cristae formation, allowing the invagination of the membrane which brings respiratory complexes into closer proximity to one another, enabling formation of supercomplexes and thus aiding electron transfer. Bendavia (MTP-131) a Szeto-Schiller tetra peptide, has been shown to prevent peroxidation of cardiolipin by binding to the molecule and preventing its interaction which cytochrome C, a reaction that prevents the latter functioning as a peroxidase rather than an electron carrier. Bendavia, is currently being evaluated in two studies, a Phase II trial to determine the effect of the compound on skeletal muscle function in elderly individuals (www.clinicaltrials.gov, NCT02245620).
and a study in patients with mitochondrial myopathy (www.clinicaltrials.gov, NCT02367014).

**Modifying mitochondrial biogenesis**

Mitochondrial biogenesis is the process by which the cell increases the number of mitochondria present. This is driven by the activation of PGC-1α, a transcriptional co-activator known as the master regulator of mitochondrial biogenesis. Activation of PGC-1α is caused by factors such as declining ATP production and increased NAD+. Activation causes transcription of nuclear encoded mitochondrial genes and therefore mitochondrial biogenesis, increasing the amount of ATP produced. Activation of the PGC-1α is attractive as it will alleviate the ATP deficient phenotype in patients with mitochondrial disease. An overview of mechanisms that activate PGC-1α are depicted in Figure 2.

**Upstream effectors**

**Bezafibrate**

Bezafibrate is commonly used to treat dyslipidemia and is a known peroxisome proliferator-activated receptor (PPAR) agonist. PPAR pathway activation leads to downstream stimulation of PGC-1α. Bezafibrate when administered to a cytochrome c oxidase (COX) 10 deficient mouse model was found to increase fatty acid oxidation, mtDNA and protein production and improve COX activity. In contrast, no difference in mitochondrial markers in Deleter mice was found, but several side effects including weight loss and hepatomegaly were observed. Similarly no change in COX activity was observed in Surf1 knockout mutants either. Bezafibrate however, was involved in an open-label trial to treat carnitine palmitoyltransferase II deficiency in which beneficial changes were observed. There is currently one ongoing trail for Bezafibrate and one study recruiting patients. The pharmacodynamics of Bezafibrate are being monitored in a randomized placebo controlled, double-blind study on mitochondrial myopathy (www.clinicaltrialsregister.eu, eudract number: 2012-002692-34). Additionally, a Phase II interventional study is also planned in 10 patients with MELAS (www.clinicaltrials.gov, NCT02398201).

**SRT2104**

Another upstream effector currently under scrutiny for its role in mitochondrial biogenesis, cellular responses to insulin and other health related changes is the NAD+-dependent deacetylase SIRT1. SIRT1 functions by propagating the deactylation (and activation) of PGC-1α, thus initiating mitochondrial biogenesis. The molecule SRT2104 has been identified as an activator of SIRT1 that exhibits high efficacy and low toxicity in preliminary experiments. The compound increased mitochondrial function when administration to elderly patients, causing increased ADP and creatine recovery after exercise, alluding to elevated mitochondrial function.

**Rapamycin**

The mammalian target of Rapamycin complex 1 (mTORC1) is a kinase responsible for activating anabolic pathways and promoting cellular growth. mTORC1 also influences mitochondrial metabolic processes in response to changes in nutrient availability. It has been demonstrated that in the presence of high levels of nutrients, mTORC1 phosphorylates the Yin Yang 1 factor (YY1) which interacts with PGC-1α, causing it to associate more closely with mitochondrial genes and initiate transcription. In contrast the inhibitor of mTORC1, Rapamycin alleviated symptoms of mitochondrial disease in a murine model of Leigh Syndrome. The Complex I knockout, Ndufs4−/− model was found to have an increased lifespan, reduced phenotypic weight loss and a reduction in neurological lesion formation after being administered 8 mg/kg of the compound every other day. These changes were attributed to abnormal changes in the metabolic status in the cells and causing aberrant phosphorylation of mTORC1 activity which was rectified by the addition of Rapamycin. Rapamycin also increases the presence of free amino acids in the cytosol which are utilized to enhance energy production. Further work is required to determine precisely how mTORC1 influences mitochondrial biogenesis and whether this can be harnessed in a clinical setting.
setting. The compound has been known to cause several side effects including immunosuppression, hyperlipidaemia and decreased wound healing.47

**AICAR**

AMP activated protein kinase (AMPK) is a potent activator of mitochondrial biogenesis in the presence of stress-induced elevated AMP: ATP ratio. AMPK functions by directly phosphorylating PGC-1α or by influencing SIRT1 signalling.48 Several compounds such as 1,2-dithiole-3-thione compound (Oltipraz) and 5-Aminoimidazole-4-carboxamide ribotide (AICAR) have demonstrated increased AMPK activity.

AICAR is an FDA approved compound administered intravenously to patients with hyperinsulineamia.1 The effect of AICAR was investigated in patient-derived fibroblasts containing a range of nuclear encoded Complex I subunit mutations.49

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**Fig. 2** Mitochondrial biogenesis is controlled by the co activator PGC-1α which can be targeted in a number of ways. mTORC1 has been shown to regulate the YY1 which associates with PGC-1α. Conversely, the upstream effector Rapamycin inhibits mTORC1 signalling, allowing AMPK to phosphorylate PGC-1α. AMPK is stimulated by elevated levels of AMP and can be targeted by AICAR. SIRT1 is also regulated by AMPK and is sensitive to an imbalance in NAD+: NADH. SRT2104 causes the activation of the deacetylase SIR1. L-arginine causes SIRT1 activation via the eNOS/cGMP pathway. Bezaflibrate binds PPARs to cause direct changes in mitochondrial biogenesis and to activate PGC-1α. The downstream effector Genistein is thought to stimulate changes in mitochondrial biogenesis via ERR signalling while RTA 408 increases Nrf 2 signalling.
The group found that the presence of AICAR increased mitochondrial biogenesis and ATP production while reducing the level of oxidative stress in COX deficient fibres. Additionally, the treatment of several mouse models with mitochondrial disease with AICAR has been recently reviewed.41

Downstream effectors

Polyphenols

Mitochondrial biogenesis is also intimately related to changes in the extracellular environment, as signalled to the cell by changes in hormone levels. The phytoestrogen Genistein is derived from leguminous plants and has been attributed to have many health benefits.50 Although the mechanisms of oestrogen signalling are not yet fully understood, it has been demonstrated that Genistein related increase in oestrogen-related receptor alpha (ERRα) signalling causes increased ATP concentrations, restoring COX activity and maintaining membrane integrity.51 One proposal for its mechanism of action is that the compound is able to increase TCA cycle turnover and therefore restore redox balance in the mitochondria.52

The polyphenol Resveratrol is thought to increase mitochondrial biogenesis by acting upon SIRT1.1 Mouse models of neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease have demonstrated the neuroprotective effects of the compound which may be attributed to its effect on biogenesis.53 Recent work has also suggested that Resveratrol may cause the translocation of human tyrosyl tRNA synthetase to the nucleus where it interacts with PARP1 and influences NAD+ signalling.54 A Phase Ia randomized, endpoint, double-blind study is currently recruiting in which a combination of polyphenols will be given to elderly patients to determine their effect on a number of parameters including mitochondrial respiration, citrate synthase activity and mtDNA copy number (clinicaltrials.gov, NCT02123121).

RTA 408

RTA 408 is a synthetic isoprenoid found to increase the activation of nuclear respiratory factor (Nrf) 2 in rat models of UV-induced dermal oxidative damage. Nrf2 is a downstream effector of PGC-1α and activator of mitochondrial biogenesis. The compound elevated levels of glutathione and increased mitochondrial biogenesis in mouse models of ALS.55 The compound is currently being trialled in the MOTOR study to treat mitochondrial myopathy. The study is currently recruiting patients who will be administered doses ranging from 2.5 to 10 mg for 12 weeks. Changes in peak work load and the distance walked in 6 min will be assessed as primary outcome measures (https://clinicaltrials.gov, NCT02255422).

Mitochondrial biogenesis and the unfolded protein response

Acipimox

The NAD+ precursor, Acipimox has also been found to increase mitochondrial function. The compound was originally developed to reduce hyperlipidaemia. In a Phase II randomized, crossover, blinded, multicentre trial, 31 patients with type 2 diabetes were administered the drug at a dose of 250 mg, three times a day for 2 weeks. The study found that levels of mitochondrial respiration and the expression of nuclear encoded mitochondrial proteins increased.56 The augmentation of NAD+ levels has been proposed to induce the mitonuclear unfolded protein response (UPRmt). This stress signalling pathway detects the imbalance in the ratio of nuclear to mitochondrial encoded proteins present in the organelle. Changes in mitochondrial proteostasis cause induction of the UPRmt, activating mitochondrial biogenesis via the deacetylases sirtuin (SIRT) 3 and FOXO1.57,58 A randomized, double-blind study to monitor the effect of Acipimox administration in cardiac and skeletal muscle has recently been completed (www.clinicaltrials.gov, NCT00943059). In addition, an ongoing trial to determine the effect of the compound in type 1 diabetes is currently underway (www.clinicaltrials.gov, NCT01816165).

Nicotinamide riboside

Nicotinamide riboside (vitamin B3) is also a precursor of the vital cofactor NAD+. The effect of the compound was tested on mtDNA Deletor mice for 4 months where increases in the rate of OXPHOS and higher levels of ATP synthase were observed.59
Several mitochondrial changes were also reported including an increase in mitochondrial mass, mtDNA copy number and cristae complexity. Elevated levels of NAD+ were found to contribute to the deacetylation of FOXO1 and activation of SIRT1 via the induction of the UPR\textsuperscript{mt}. In addition, a study using Sco2 knockout/knock-in mice, characterized by impaired COX biogenesis was conducted. The mice were administered 400 mg/kg for 4 weeks. A significant increase in motor function and the mRNA levels of key β oxidation enzymes were reported. Conversely, no changes in mtDNA content as reported previously were observed.\textsuperscript{60} A previous study also recapitulated the beneficial effect of the compound in mice fed a high fat diet; showing that elevated NAD+ increased levels of SIRT1 and SIRT3.\textsuperscript{61} Furthermore, a 6 month open-label trial was conducted on seven MELAS patients and was shown to reduce serum lactate levels while improving some clinical outcome measures.\textsuperscript{62}

**Mitochondrial biogenesis and vasoregulation**

**L-Arginine**

Compounds that target vasoregulation have also been utilized in the treatment of some symptoms of mitochondrial diseases and play a role in regulating biogenesis. Citrulline and L-Arginine have been prescribed by some clinicians to prevent stroke-like episodes in patients suffering from MELAS.\textsuperscript{63} Patients with MELAS have been shown to suffer from nitric oxide (NO) deficiency.\textsuperscript{64} L-arginine is metabolized and serves as a precursor of NO, a key molecule in signalling for vasodilation and hence is proposed to prevent metabolic strokes.\textsuperscript{65} The production of NO from such compounds also causes activation of SIRT1 via cGMP signalling, in turn activating PGC-1α and increasing mitochondrial biogenesis. L-arginine is administered intravenously or orally at 500 mg/day for 1–3 days. Citrulline also acts as an NO precursor and was reported to reduce stroke-like episodes in a Japanese Cohort.\textsuperscript{66} A Phase II open label study in which siblings suffering from MELAS who were administered L-arginine for 6 weeks displayed favourable increases in aerobic capacity and muscle metabolism along with significant decreases in serum lactate levels.\textsuperscript{67} A drawback to the use of L-arginine and Citrulline are the possible side effects which can manifest as hypotension, hyponatremia, headache, nausea and diarrhoea.\textsuperscript{1}

\textsuperscript{(-)}-Epicatechin

\textsuperscript{(-)}-Epicatechin, an isoflavone derived from cocoa and has been trialled alone and in conjunction with exercise in mouse models for cardiovascular disease.\textsuperscript{68} \textsuperscript{(-)}-Epicatechin functions by stimulating endothelial NO synthase (eNOS) and may increase mitochondrial mass and ATP production.\textsuperscript{69} Moreno-Ulloa \textit{et al.}, combined exercise with a 1 mg/kg oral dose of Epicatechin for 15 weeks in 1-year-old mice and found an increase in muscle performance, reduced fatigue and increased mitochondrial mass in cardiomyocytes.\textsuperscript{68} A clinical study has recently been completed, in which 60 individuals suffering from heart failure and diabetes were compared with healthy individuals upon \textsuperscript{(-)}-Epicatechin administration alongside an exercise regime (www.clinicaltrials.gov, NCT01671514).

\alpha-Lipoic acid

The antioxidant alpha Lipoic acid (LA) has been explored in several experimental models.\textsuperscript{70} Interest in LA was sparked by its alleged benefits in obesity and diabetes. LA is often administered as part of an antioxidant cocktail to patients with mitochondrial disease where the beneficial effect of LA has been attributed to the induction of PGC-1\textalpha via the eNOS pathway.\textsuperscript{13} Although clinical trials for the use of LA to treat mitochondrial disease specifically are still being awaited, studies using NARP hybrid cell lines were found to have beneficial effects on mitochondrial biogenesis.\textsuperscript{71}

**Discussion**

Several mechanisms for potential therapeutic intervention have been explored in this review. It is known that mitochondrial disease manifests once a critical threshold of dysfunction is reached, below which cellular function is able to continue normally. For this reason many compounds under investigation aim to increase mitochondrial function in order to alleviate the burden of mutation on organs and prevent symptoms from occurring.
Although the quality of clinical data reported has greatly improved over the last decade, moving from single case reports of compounds increasing mitochondrial function, to studies with larger patient cohorts, often spread over multiple centres and utilizing more robust trial strategies, many ongoing trials are still open label, preventing results from being supported by sound evidence that could be used as a therapeutic strategy by clinicians.

Several compounds introduced here suffer from drawbacks including poor efficacy, low bioavailability and high toxicity due to their pleiotropic function. However these compounds could be targeted by pharmaceutical companies in structure activity relationship studies to develop and screen libraries of compounds structurally similar to the parent compound but with better pharmacokinetics and safety profiles.

This paper highlights several areas of mitochondrial physiology targeted by compounds, many of which have been investigated in clinical trials that may be used to prevent mitochondrial disease progression, or in turn influence a new generation of derivative compounds that are able to do so.

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**Conflict of interest statement**

The authors have no potential conflicts of interest.

**References**


