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REVIEW

Turn up the power – pharmacological activation of mitochondrial biogenesis in mouse models

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The oxidative phosphorylation (OXPHOS) system in mitochondria is responsible for the generation of the majority of cellular energy in the form of ATP. Patients with genetic OXPHOS disorders form the largest group of inborn errors of metabolism. Unfortunately, there is still a lack of efficient therapies for these disorders other than management of symptoms. Developing therapies has been complicated because, although the total group of OXPHOS patients is relatively large, there is enormous clinical and genetic heterogeneity within this patient population. Thus there has been a lot of interest in generating relevant mouse models for the different kinds of OXPHOS disorders. The most common treatment strategies tested in these mouse models have aimed to up-regulate mitochondrial biogenesis, in order to increase the residual OXPHOS activity present in affected animals and thereby to ameliorate the energy deficiency. Drugs such as bezafibrate, resveratrol and AICAR target the master regulator of mitochondrial biogenesis PGC-1α either directly or indirectly to manipulate mitochondrial metabolism. This review will summarize the outcome of preclinical treatment trials with these drugs in mouse models of OXPHOS disorders and discuss similar treatments in a number of mouse models of common diseases in which pathology is closely linked to mitochondrial dysfunction. In the majority of these studies the pharmacological activation of the PGC-1α axis shows true potential as therapy; however, other effects besides mitochondrial biogenesis may be contributing to this as well.

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Abbreviations
AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated kinase; BAT, brown adipose tissue; CI–V, complex I–V; DMD, Duchenne muscular dystrophy; FAO, fatty acid oxidation; HD, Huntington's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PD, Parkinson's disease; PGC-1, PPAR-γ coactivator 1; POLG, polymerase γ; SIRT1, silent information regulator two (Sir2) protein 1, sirtuin 1

Mitochondria as the powerhouse and mitochondrial disease

Mitochondria are involved in numerous processes but they are best known for their role as the powerhouse of the cell. The oxidative phosphorylation (OXPHOS) system located in the mitochondrial inner membrane integrates the production of cellular energy in the form of ATP, with all the major catabolic pathways including fatty acid oxidation (FAO), the tricarboxylic acid cycle and amino acid oxidation. The OXPHOS system consists of five multimeric protein complexes, complex I–V (CI–V). Although critical OXPHOS subunits are encoded by the circular DNA molecules residing inside mitochondria (mtDNA), the majority of the subunits, together with all the proteins required for mtDNA transcription and translation, are encoded by the nuclear DNA (nDNA). This forms an extra layer of regulatory complexity in order to get the right balance in both the nuclear and mitochondrial subunits together for a functional OXPHOS system.

Historically, the term 'respiratory chain' refers only to CI–IV and has its starting point at the oxidation of NADH by CI (NADH-ubiquinone oxidoreductase), or succinate by CII (succinate-ubiquinone oxidoreductase). These metabolites originate from the degradation of sugars, protein and lipids, and their oxidation initiates a series of redox reactions up until the actual respiration at CIV, where oxygen is the final electron acceptor. The chain of redox reactions coincides with proton pumping at CI, CIII and CIV from the matrix into the intermembrane space, giving rise to an electrochemical gradient known as the mitochondrial membrane potential which is used by CV to drive ATP synthesis.

Inherited OXPHOS disorders, also known as mitochondrial diseases, are the most common group of inborn errors of metabolism, affecting approximately 1:5000 people (Skladal *et al.*, 2003). These diseases may affect any organ at any age of onset and most often affect tissues that have a high-energy demand such as heart, brain, skeletal muscle and liver. Because of the complex nature of the genetics of the OXPHOS system, OXPHOS disorders can occur via almost any mode of inheritance. Moreover, a clear genotype–phenotype relationship is only sometimes apparent. Mutations have been found in nuclear and mtDNA genes encoding OXPHOS subunits, mtDNA, tRNA and rRNA genes, and nuclear genes encoding proteins required for the assembly of the OXPHOS complexes or for mtDNA replication, transcription and translation (Ylikallio and Suomalainen, 2012).

In recent years enormous progress has been made in the molecular diagnosis of patients with clinically diagnosed OXPHOS disorders by combining technological advances in the area of bioinformatics and molecular biology (Calvo *et al.*, 2012; Falk *et al.*, 2012; Wong, 2013). This is very important for parents as this shortens their search for diagnosis and may provide them with reproductive options in case of planning for subsequent pregnancies. On the other hand, there is unfortunately still no effective treatment for patients with OXPHOS disorders (Pfeffer *et al.*, 2012). Current practice consists of management of symptoms and may include dietary intervention based on anecdotal success (Wallace *et al.*, 2010).

One of the reasons for the lack of therapeutic options has been a lack of relevant animal models, especially in mammals, for preclinical trials. The generation of viable mouse models with useful phenotypes has been difficult because of the crucial role of the OXPHOS system. Moreover, genetic manipulation of mtDNA has been a real technical challenge. Nevertheless, recently, there have been various mouse models for OXPHOS disorders generated with mutations in nDNA or mtDNA. It is no surprise that one model does not suffice for all OXPHOS disorders when considering the entire range of phenotypes in patients. For an overview of mouse models of OXPHOS disorders. please see the following reviews: Dogan and Trifunovic (2011); Komen and Thorburn (2012); and Torraco *et al.* (2009).

Mitochondrial biogenesis induction as treatment for mitochondrial disease

A popular treatment strategy for mouse models of OXPHOS disorders has been the stimulation of mitochondrial biogenesis. Boosting mitochondrial numbers would hypothetically increase the residual OXPHOS activity present in all patients. This would enhance the cellular ATP synthesis capacity and hopefully ameliorate the cellular energy deficit. This review will focus on the outcome of studies in mouse models of OXPHOS disorders in which pharmacological induction of mitochondrial biogenesis has been trialled as therapy. In addition, similar treatment studies with mouse models of generic diseases in which mitochondrial dysfunction has been linked to pathogenesis will be discussed with the emphasis on mitochondrial biology. These mouse models include models for Duchenne muscular dystrophy (DMD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD). Effects of the various treatments on these models outside the context of mitochondrial function will mainly be beyond the scope of this review.

Furthermore, mitochondrial dysfunction has been implicated as an integral part in the pathology of non-genetic metabolic disorders, such as obesity and metabolic syndrome (Joseph *et al.*, 2012; Szendroedi *et al.*, 2012). Whole-body energy balance is a key concept in these disorders, with a long-standing interest in the role of brown fat, which is rich in mitochondria and can dissipate energy efficiently through non-shivering thermogenesis due to high levels of the uncoupling protein 1 (Wu *et al.*, 2013). More recently, attention has also focused on the role of so-called beige fat, which can be generated from white adipose tissue by induction of a thermogenic pathway in which mitochondrial biogenesis plays a key role (Schulz *et al.*, 2013). Hence, stimulation of mitochondrial biogenesis has been a major target in the treatment of obesity and metabolic syndrome, as reviewed elsewhere (Joseph *et al.*, 2012; Wu *et al.*, 2013). Pharmacological stimulation of mitochondrial biogenesis simulates the positive effect of caloric restriction and exercise on these generic metabolic disorders. Inevitably, many of these drugs that aim to provide exercise in a pill have now been tested in mouse models of genetic OXPHOS disorders as well and will be reviewed mainly in the context of inherited mitochondrial disease here. On the other hand, outcome of these studies using mouse models in which pathogenesis is caused directly by a genetic mitochondrial defect may be very useful for understanding the outcome of studies in models of more complex diseases in which there is an indirect mitochondrial defect.

Not surprisingly, activation of mitochondrial biogenesis is not the only treatment strategy for OXPHOS disorders and mitochondrial dysfunction in general. For an overview of other treatment strategies, please see the following reviews: Schon *et al.* (2010); Wallace *et al.* (2010); Koene and Smeitink (2011); Suomalainen (2011); Yu-Wai-Man *et al.* (2011); and Andreux *et al.* (2013).

Activation of mitochondrial biogenesis and the central role of PPAR-γ coactivator 1 (PGC)-1α

It is well known that certain stressors such as caloric restriction, exercise and cold adaptation can induce proliferation of

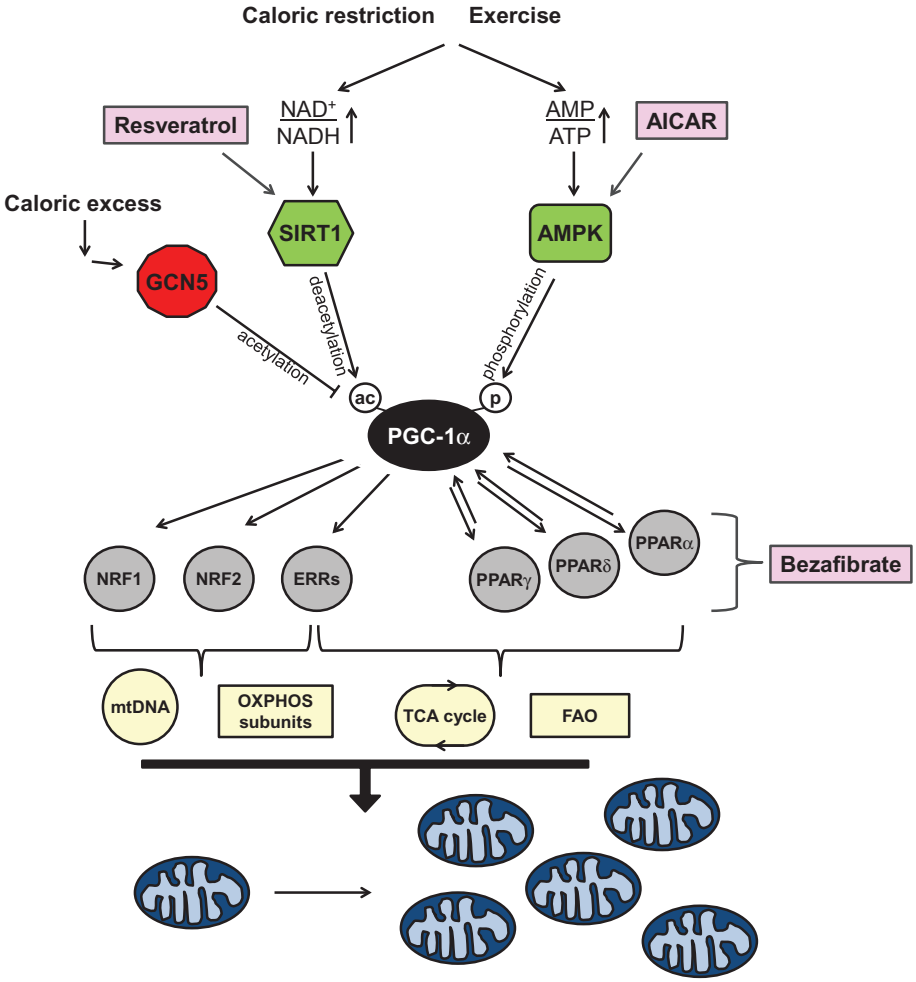

Figure 1

Overview of the induction of PGC1-α-mediated mitochondrial biogenesis by resveratrol, bezafibrate and AICAR. During caloric excess PGC-1α, the master regulator of mitochondrial biogenesis is in an inactive acetylated (ac) state. In times of caloric restriction, or after exercise, energy becomes scarce resulting in increases in the AMP/ATP ratio and NAD^+/NADH ratio. The former has a direct impact on the activation of AMPK as binding of AMP (or ADP) facilitates phosphorylation (p) of AMPK by upstream kinases. Activated AMPK phosphorylates PGC-1α directly, which may either activate this coactivator or prime PGC-1α for activation via deacetylation by SIRT1. SIRT1 requires increased NAD^+ levels for its activity as this nucleotide is a cosubstrate of the enzyme. Once in its deacetylated activated form PGC-1α is able to coactivate transcription of nuclear mitochondrial genes via transcription factors including NRF1/2, oestrogen-related receptor (ERR)α,β,γ (ERRs) and PPARα,β,γ. Consequently, this activates the mtDNA transcription, translation and replication machinery (represented by mtDNA); production of OXPHOS subunits; tricarboxylic acid (TCA) cycle enzymes and FAO enzymes. This ultimately leads to mitochondrial proliferation and increased mitochondrial mass. Resveratrol is an activator of SIRT1. The exact mechanism of activation is still unresolved but ultimately results in activation of the PGC-1α pathway via increased deacetylation of the coactivator. AICAR is phosphorylated into its ZMP analogue, an AMP mimetic, and can directly activate AMPK by promoting the phosphorylation of AMPK by upstream kinases. Activation of AMPK causes phosphorylation of PGC-1α, and SIRT1 activation via AMPK-induced increases in the NAD^+/NADH ratio. Bezafibrate is a PPAR pan-agonist and therefore able to activate PPAR-regulated gene expression, which includes the expression of *PGC-1α* via the PPAR-responsive element in its promoter region. The increase in PGC-1α levels is believed to be sufficient for activation of the mitochondrial biogenesis pathway. For more details see the text or reviews (Fernandez-Marcos and Auwerx, 2011; Scarpulla *et al.*, 2012; Wenz, 2013).

mitochondria, which may be accompanied by changes in size and shape (Scarpulla *et al.*, 2012). Members of the PGC-1 family play a central role in this process of mitochondrial biogenesis. The PGC-1 family consists of three members: PGC-1α, PGC-1β and PGC-1-related coactivator. Although all members have been shown to play a part in the regulatory network surrounding mitochondrial biogenesis, the major research focus has been on PGC-1α, which is considered to be the master regulator of this process (Figure 1; Fernandez-Marcos and Auwerx, 2011; Scarpulla, 2011; Wenz, 2013). This has made PGC-1α of special interest as a target of treatment intervention for various diseases in which mitochondrial dysfunction has been implicated, including the primary OXPHOS disorders. As a coactivator, PGC-1α requires other transcription factors for the trans-activation of mitochondrial biogenesis genes. Among these are the nuclear respiratory

factors 1 and 2 (NRF1/2), and the PPAR- and oestrogen-related receptor nuclear receptor families (see Scarpulla, 2011; Scarpulla *et al.*, 2012).

Activation of PGC-1α can be induced via gene expression and/or by post-translational modification. Transcriptional activation of PGC-1α expression is under the regulation of various transcription factors including cAMP response element-binding protein, thyroid hormones and PPARs (Wenz, 2013). The latter comprise three family members – PPARα, PPARβ/δ (in this review δ) and PPARγ – each responsible for tissue-specific activation of *PGC-1α* gene expression, albeit with some overlap. Although their endogenous ligands *in vivo* are still not well defined, each of the PPARs can be pharmacologically activated by specific agonists (Poulsen *et al.*, 2012; Monsalve *et al.*, 2013). PPARs form heterodimers with retinoid X receptors in the nucleus. These heterodimers bind to DNA together with corepressors. Ligand binding releases the corepressor and allows activation of coactivator, and results in changes in gene expression. Pharmacological activation of any of the PPARs is able to activate *PGC-1α* gene expression via the peroxisome proliferator response element (PPRE) located in the promoter region of the *PGC-1α* gene (Figure 1; Hondares *et al.*, 2006; 2007; 2011).

In addition to the induction of gene expression, PGC-1α activity is also tightly regulated via various post-translational modifications. These modifications make PGC-1α an inportant integration site for intra- and extracellular metabolic signals. For example, increases in cellular AMP levels, the indicator for cellular energy status, result in the activation of AMP-activated kinase (AMPK). Phosphorylation of PGC-1α by AMPK activates the coactivator and consequently induces *PGC-1α* gene expression via a feedback loop (Jager *et al.*, 2007). More recent studies have shown that phosphorylation of PGC-1α by AMPK may not directly activate PGC-1α but only prime PGC-1α for activation via deacetylation (Canto *et al.*, 2009).

Silent information regulator two (Sir2) protein 1 (sirtuin 1, SIRT1) is the enzyme responsible for deacetylation of PGC-1α in the nucleus (Rodgers *et al.*, 2005). SIRT1 is part of the sirtuin family of metabolic regulators that in mammals consists of seven proteins (SIRT1–7) and uses cellular NAD^+ as cofactor for the deacetylation of proteins. The activation of SIRT1 is closely linked to the availability of its substrate NAD^+ and therefore linked to the cellular redox state [the $NAD(P)^+$/NAD(P)H balance] (Fulco *et al.*, 2008; Canto *et al.*, 2009).

This coordinated response of AMPK and SIRT1 is very important as a physiological response to fasting and exercise in skeletal muscle, but the mechanism is not without controversy as recently shown by the study of Higashida *et al.* (Canto *et al.*, 2010; Higashida *et al.*, 2013). Downstream of PGC-1α activation the major mitochondrial sirtuin, SIRT3, plays an important role in this response although the exact mechanism is still somewhat unclear (Hirschey *et al.*, 2010; 2011; Qiu *et al.*, 2010; Shimazu *et al.*, 2010; Someya *et al.*, 2010; Hallows *et al.*, 2011; Jing *et al.*, 2011; 2013; Fernandez-Marcos *et al.*, 2012). SIRT3 modulates activities of mitochondrial enzymes via NAD^+-dependent deacetylation and has been shown to have a broad substrate specificity acting on mitochondrial proteins present in all major mitochondrial pathways including FAO, OXPHOS and reactive oxygen species (ROS) metabolism (Rardin *et al.*, 2013; see also Verdin *et al.*, 2010; Giralt and Villarroya, 2012; Pirinen *et al.*, 2012). Overexpression of SIRT3 induced mitochondrial biogenesis in HIB1B brown adipocytes and was anti-proliferative in cancer cells by decreasing ROS and thereby destabilizing HIF-1α (Finley *et al.*, 2011; Bell *et al.*, 2011). Furthermore, this SIRT3-mediated stimulation of the antioxidant response was also able to protect transgenic, SIRT3-overexpressing, mice against cardiac hypertrophy (Sundaresan *et al.*, 2009). No data were provided on activation of mitochondrial biogenesis as a result of the SIRT3 overexpression in the latter studies, so it is unknown whether this played any part in the observed effects.

In times of caloric excess, deactivation of PGC-1α occurs by general control of amino acid synthesis 5 through acetylation (Lerin *et al.*, 2006). Additionally, inhibition of PGC-1α also occurs by phosphorylation of a specific site by Akt/PKB in response to insulin signalling (Li *et al.*, 2007).

PGC-1α knockout and transgenic mouse models

Considering the fact that PGC-1α is the master regulator of mitochondrial biogenesis, the knockout of the *PGC-1α* gene in mice gives rise to a relatively mild phenotype (Lin *et al.*, 2004; Leone *et al.*, 2005). These mice are hyperactive, have reduced muscle function and exercise capacity, and an abnormal thermogenic response. The most logical explanation for the mild phenotype is the redundant function of the three PGC-1 family members. Accordingly, the PGC-1α/PGC-1β double knockout has a very severe phenotype, and the majority of pups die of cardiac failure within 24 h after birth (Lai *et al.*, 2008).

Besides these studies with knockout mice, studies with transgenic overexpressing PGC-1α mice have been very insightful for determining the function of PGC-1α in various tissues (Table 1). Transgenic overexpression of PGC-1α in mouse heart has been shown to induce proliferation of enlarged mitochondria (Lehman *et al.*, 2000). However, this coincides with the development of cardiomyopathy and oedema, which can be reversible depending on the period of PGC-1α overexpression (Lehman *et al.*, 2000; Russell *et al.*, 2004).

The overexpression of PGC-1α in skeletal muscle is phenotypically more beneficial and results in fibre-type switching towards the more oxidative type I (slow-twitch) fibres, which rely on mitochondrial OXPHOS for the generation of ATP rather than glycolysis (Lin *et al.*, 2002). Furthermore, it protects the mice against age-induced muscle wasting and metabolic disease, and has been shown to increase lifespan (Wenz *et al.*, 2009b). Interestingly, PGC-1β overexpression results in different fibre-type switching into type IIx fibres (Arany *et al.*, 2007), indicating slight differences in the regulatory network of these two closely related coactivators.

These initial studies led to the use of the PGC-1α transgenic mice in proof of principle experiments to determine the effect of induction of PGC-1α on the pathology in mouse models of various diseases as discussed below and summarized in Table 1.

Table 1

Rescue of mitochondrial dysfunction in mouse models by PGC-1α overexpression

Mouse model (mode of overexpression)	Effect on phenotype	Evidence for mitochondrial biogenesis	Reference
mdx model of DMD (MCK-PGC-1α)	Amelioration of muscle damage and motor performance	ND	Handschin *et al.* (2007)
mdx model of DMD (recombinant adeno-associated virus mediated in skeletal muscle)	Increased resistance against contraction-induced muscle damage	+	Selsby *et al.* (2012)
cox10 model of mitochondrial myopathy (MCK-PGC-1α)	Increase in lifespan and reduced progression of myopathy	+	Wenz *et al.* (2008)
Surf1$^{-/-}$ model of COX deficiency (MCK-PGC-1α)	No distinct phenotype	+	Viscomi *et al.* (2011)
Polg Mut mouse model of mitochondrial disease/premature ageing (MCK-PGC-1α)	Improvement of motor performance and cardiac function	+	Dillon *et al.* (2012b)
R6/2 model of HD (lentiviral vector via injection in striatum)	Prevention of atrophy of striatal neurons	ND	Cui *et al.* (2006)
SOD1 (G93A) DL model of ALS (Liang *et al.*, 2011, low-level whole-body human PGC-1α)	Slower disease progression and improved motor performance, prevention of neuron loss	ND	Liang *et al.* (2011)
SOD1 (G93A) Gurl model of ALS (hPGC-1α with rat neuron-specific enolase promoter)	Improvement of survival and motor performance, prevention of neuron loss	+	Zhao *et al.* (2011)
SOD1 (G37R) model of ALS (MCK-PGC-1α)	Improved motor function but no effect on motor neuron loss and survival	ND	Da Cruz *et al.* (2012)
MTPT-induced mouse model of PD (PGC-1α behind a neuronal thy-1 promotor)	Neuroprotective effect, amelioration of decreased dopamine levels	+	Mudo *et al.* (2012)

ND, not determined.

Rescue of mitochondrial dysfunction by transgenic PGC-1α expression

The phenotype in a mouse model of DMD, *mdx* mice, benefitted significantly from transgenic PGC-1α expression in skeletal muscle (Lin *et al.*, 2002; Handschin *et al.*, 2007). *Mdx* mice lack the dystrophin protein and develop a mild muscular dystrophy most pronounced in sedentary mice around 4–6 weeks of age (Handschin *et al.*, 2007). Transgenic PGC-1α overexpression decreased skeletal muscle damage in sedentary young mice and provided protection against exercise-induced muscle damage in older *mdx* mice. Unfortunately, no details were reported on changes in mitochondrial function due to increased expression of PGC-1α in the muscle of *mdx* mice. Recently, however, it was shown that PGC-1α overexpression by virally mediated gene transfer in skeletal muscle of *mdx* mice could result in increased expression of mitochondrial OXPHOS components suggestive of induction of mitochondrial biogenesis (Selsby *et al.*, 2012).

The same approach as used for the *mdx* mice was deployed using mice with mitochondrial CIV [cytochrome c oxidase (COX)] deficiency in skeletal muscle due to a conditional knockout of the CIV assembly factor gene *Cox10* (Diaz *et al.*, 2005; Wenz *et al.*, 2008). It was observed that muscle-specific PGC-1α overexpression improved the survival of *ΔCox10* mice in both males and females by slowing the progression of myopathy. Analysis of the muscle showed that the *ΔCox10* transgenic mice had more COX activity per muscle volume, whereas COX activity per mitochondrion was not increased and remained reduced compared with wild type (20–25%). The activity of citrate synthase, a marker enzyme for mitochondrial quantity, was also increased four- to five-fold in muscle of transgenic *ΔCox10* mice in agreement with a large induction of mitochondrial proliferation. On the other hand, non-transgenic *ΔCox10* mice already had a two- to threefold increase in citrate synthase activity compared with wild-type mice as a natural compensation mechanism for the OXPHOS defect. A similar induction has often been observed in humans with mitochondrial myopathy, as well as in the first mouse model of mitochondrial myopathy/cardiomyopathy (*Ant1*$^{-/-}$ mice) (Graham *et al.*, 1997).

In a different COX-defective mouse model with a mild decrease in COX activity due to ablation of the *Surf1* COX assembly factor gene, transgenic overexpression of PGC-1α in muscle induced an increase in mitochondrial mass as shown by increases in mtDNA and citrate synthase activity and increases in specific activities of most OXPHOS enzymes including the defective COX (Viscomi *et al.*, 2011). Because *Surf1*$^{-/-}$ mice have no overt phenotype it is not possible to look at correction of pathology in this model; however, no negative effects of the expression of the transgene were reported either (Dell'agnello *et al.*, 2007; Viscomi *et al.*, 2011).

Recently the same strategy has been used in another mouse model with mitochondrial dysfunction, the mutator (Mut) mouse (Dillon *et al.*, 2012b). Mut mice have a proof-reading mutation in the gene coding for polymerase γ (POLG). POLG is the polymerase responsible for mtDNA replication, and the mutation causes the accumulation of mutations in mtDNA causing progressive mitochondrial dysfunction and symptoms of premature ageing (Trifunovic *et al.*, 2004; Kujoth *et al.*, 2005). Transgenic expression of PGC-1α in muscle of Mut mice was analysed at 10 months of age and shown to result in increases in OXPHOS subunits, respiratory chain enzyme activity, citrate synthase activity and mtDNA per muscle volume. Surprisingly, cardiac function in transgenic PGC-1α Mut mice was restored to wild-type levels at 10 months of age despite the fact that there was no significant increase in PGC-1α expression and mitochondrial biogenesis in this tissue. There was an increased accumulation of low-abundance mtDNA mutations in muscle, which are assumed to be mainly acquired during the lifespan of mice rather than inherited. This was suggested to be caused by the increase in mtDNA replication rate that coincides with mitochondrial proliferation.

In addition to muscle, PGC-1α also plays an important role in the brain as demonstrated by the neurological component in the phenotype of the PGC-1α$^{-/-}$ mice (Lin *et al.*, 2004; Leone *et al.*, 2005). Moreover, it has been shown that part of the role of PGC-1α is as a regulator of ROS metabolism (St-Pierre *et al.*, 2006). This role is very relevant to OXPHOS disorders, many of which manifest with a form of neurodegeneration or brain abnormalities. Furthermore, the OXPHOS system is a major site of ROS generation, which may contribute to pathology when increased in the case of a malfunctioning system. In this respect the lentiviral-mediated delivery of PGC-1α in a mouse model of the fatal neurological disease HD is of note (Cui *et al.*, 2006). PGC-1α is down-regulated in the striata of HD patients and in a mouse model for HD (R6/2), which is suggested to play a role in the energy imbalance in HD striata (Cui *et al.*, 2006; Weydt *et al.*, 2006). Administration of a PGC-1α expression vector via lentivirus to the striata of HD mice prevented neural atrophy. Conversely, breeding a different and milder HD mouse model onto a PGC-1α$^{-/-}$ background aggravated the phenotype emphasizing the importance of PGC-1α in disease progression (Cui *et al.*, 2006).

Recently, studies have tested whether constitutive transgenic overexpression of PGC-1α in neuronal tissues would ameliorate the pathology in mouse models of the neurological diseases ALS (Liang *et al.*, 2011; Zhao *et al.*, 2011) and PD (Mudo *et al.*, 2012) (Table 1). In the ALS mouse models, transgenic overexpression of mutant SOD1 (G93A), a mutation found in familial ALS, causes progressive neurodegeneration. PGC-1α expression improved motor performance, attenuated neurodegeneration and, in the most severely affected model, extended survival (Liang *et al.*, 2011; Zhao *et al.*, 2011). Mitochondrial function was only assessed by *in situ* histological experiments, which showed a restoration towards wild-type levels for CI and CIV activity in the spinal cords of PGC-1α ALS mice while no change in CII activity observed (Zhao *et al.*, 2011).

A subsequent study using the ALS G37R SOD1 mouse model took a different approach and showed that overexpression of PGC-1α in the skeletal muscle specifically could not slow the disease progression and survival (Da Cruz *et al.*, 2012). Still, the improvement in muscle function in the mice did show that targeting peripheral activation of PGC-1α may improve quality of life in ALS patients (Da Cruz *et al.*, 2012).

In order to study the effect of PGC-1α in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model, a new mouse line with constitutive transgenic overexpression of PGC-1α behind a neuronal *thy-1* promotor was generated (Mudo *et al.*, 2012). In the substantia nigra of these PD mice, PGC-1α expression resulted in induction of the mitochondrial antioxidant enzymes SOD2 and TRX2 as well as the OXPHOS protein COX IV (Mudo *et al.*, 2012). The increased amount of mitochondrial proteins is in line with minimal experimental evidence showing a higher respiratory control ratio in isolated brain mitochondria from transgenic animals. These biochemical changes were suggested to contribute to the neuroprotective effect of PGC-1α expression against cell degeneration and decreases in dopamine levels by the PD inducing neurotoxin MPTP. The active component of this toxin, 1-methyl-4-phenylpyridinium (MPP+), is a very potent inhibitor of CI in substantia nigra mitochondria, which is believed to be causing the Parkinson's-like symptoms in this model. Hence, this model is not only a PD model but also a model with a direct OXPHOS defect. Therefore, this study highlights PGC-1α up-regulation as a potential therapeutic method for OXPHOS disorders in the brain.

The studies with PGC-1α transgenic mice clearly reveal the potential of pharmacological activation of this coactivator as part of treatment of various diseases affecting muscle, heart and brain. In the next sections we will discuss the current status on pharmacological treatments in mouse models, which aim to do just this.

Induction of mitochondrial biogenesis via PPAR activation: bezafibrate

Pharmacological activation of mitochondrial biogenesis can be achieved with a large range of compounds (see Andreux *et al.*, 2013). Here the main focus is on the treatments used in mouse models of OXPHOS disorders (Figure 1 and Tables 2–4).

The pan-PPAR (α, β/δ, γ) agonist bezafibrate is a commonly used drug for the treatment of dyslipidaemia (Tenenbaum *et al.*, 2005). Although historically fibrates are strongly linked to FAO and peroxisome proliferation, studies in recent years have shown that PPARs via PGC-1α are able to regulate other aspects of mitochondrial metabolism as well (Hondares *et al.*, 2006; 2007; 2011). Bezafibrate stimulates heart and skeletal muscle PGC-1α expression in mice in a PPARδ-dependent manner (Hondares *et al.*, 2007). Moreover, *in vitro* experiments have shown that bezafibrate treatment or overexpression of PGC-1α is able to up-regulate respiratory capacity and OXPHOS function in fibroblasts and myoblasts from OXPHOS patients (Bastin *et al.*, 2008; Srivastava *et al.*, 2009; Casarin *et al.*, 2012).

Bezafibrate was the drug of choice for Wenz *et al.* (2008) to study whether they could pharmacologically replicate the beneficial effect of transgenic overexpression of PGC-1α in

Table 2

Mouse models studies trialling PPAR pan-agonist bezafibrate for the activation of PGC-1α

Mouse model	Effect on phenotype	Evidence for mitochondrial biogenesis	Reference
Cox10 model of mitochondrial myopathy	Prolonged lifespan and delayed onset of myopathy	+	Wenz *et al.* (2008)
Surf1$^{-/-}$ model of COX deficiency	Loss of body weight, hepatomegaly	–	Viscomi *et al.* (2011)
ACTA-Cox15$^{-/-}$ model of mitochondrial myopathy	Lethality within 48 h	–	Viscomi *et al.* (2011)
Peo1 Deletor mouse model of late-onset mitochondrial myopathy	Loss of body weight, hepatomegaly, reduction in body temperature, reduction in COX-negative fibres and mtDNA deletion load	–	Yatsuga and Suomalainen (2012)
Polg Mut mouse model of mitochondrial disease/premature ageing	Delay in hair loss, reduction in skin damage, reduction in spleen size, reduction of body weight, hepatomegaly	–	Dillon *et al.* (2012a)
Forebrain-specific *ΔCox10* mice, a model of mitochondrial encephalopathy	Overall amelioration of the phenotype including attenuation of the loss of motor function, protection of brain damage, reduction of loss of bodyweight	+	Noe *et al.* (2013)
R6/2 model of HD	Increase in motor performance, increase in lifespan. Amelioration of phenotype in brain, skeletal muscle and BAT. Slight increase in liver weight and lipid vacuolization	+	Johri *et al.* (2012)
P301S transgenic mouse model of AD	Attenuation of locomotor and anxiety abnormalities, histological improvement in the brain pathology, improvement in lipid vacuoles in BAT, reduced body weight	+ (BAT)	Dumont *et al.* (2012)

ΔCox10 mice. Dietary bezafibrate administration at a concentration of 0.5% (w/w) in the diet, as used in all other studies described in this review, resulted in an induction of mitochondrial biogenesis in skeletal muscle of *ΔCox10* mice. Bezafibrate caused increased expression of *Ppar* and *Pgc-1* genes and induction of mtDNA and mitochondrial proteins in muscle homogenates. Importantly, treatment delayed the onset of myopathy and prolonged survival of *ΔCox10* mice.

After this first study demonstrated a beneficial effect of bezafibrate treatment for mitochondrial myopathy, more groups reported on the use of the drug in other mouse models of mitochondrial disease with varying success (Table 2). In the *Surf1*$^{-/-}$ model of mild COX deficiency, treatment with bezafibrate was not able to replicate the effect of PGC-1α overexpression (Viscomi *et al.*, 2011). Instead, treatment caused an induction in both PPARα and δ and resulted in the up-regulation of FAO in skeletal muscle. Phenotypically, bezafibrate caused a loss in body weight and severe hepatomegaly, clearly indicating toxicity of the drug. Viscomi *et al.* also evaluated the use of bezafibrate as treatment for a new mouse model with severe muscle-specific COX deficiency (*ACTA-Cox15*$^{-/-}$ mice) and early onset mitochondrial myopathy. In these mice bezafibrate was lethal within 48 h after the start of the treatment at the same dose used for *ΔCox10* and *Surf1*$^{-/-}$ mice previously (Wenz *et al.*, 2008; Viscomi *et al.*, 2011).

The studies of Viscomi *et al.* and Wenz *et al.* showed that the success rate with bezafibrate may be very dependent on the particular model and the severity of disease in the model. Therefore, it was not a surprise that bezafibrate was tolerated in Deletor mice, a mouse model for adult-onset mitochondrial myopathy (Yatsuga and Suomalainen, 2012). However, in this model significant induction of hepatic liver FAO, hepatomegaly and decreases in adipose tissue were also reported as a result of the treatment. The Deletor mice accumulate mtDNA deletions because of the transgenic expression of a mutated Twinkle helicase protein. The mutated Twinkle contains a mutation in *Peo1* similar to a mutation observed in the *C10orf2* gene in patients with progressive external ophthalmoplegia (Tyynismaa *et al.*, 2005; Yatsuga and Suomalainen, 2012). The effect of bezafibrate in Deletor mice was limited as it delayed the accumulation of COX negative fibres and reduced mtDNA deletion load without a change in phenotype and without induction of muscle mitochondrial biogenesis. The treatment strategy in this study was different from the previous studies in that treatment was started at the onset of symptoms at 1 year of age instead of at 5 weeks of age in the previous studies (Wenz *et al.*, 2008; Viscomi *et al.*, 2011).

Intermediate beneficial effects of bezafibrate were also observed in another mouse model with defects in mtDNA maintenance, Mut mice, which had previously been shown to be substantially rescued by PGC-1α expression, as described above (Dillon *et al.*, 2012a; 2012b). Again no evidence of mitochondrial biogenesis was observed in mice treated with bezafibrate. However, there was a delay of hair loss, reduction in damaged dorsal skin structure and amelioration of the abnormal spleen size and weight observed in Mut mice receiving bezafibrate. Biochemically there was evidence of increased FAO in skeletal muscle and liver of treated

Table 3

Treatment studies using resveratrol for the activation of PGC-1α in mouse models

Mouse model	Effect on phenotype	Evidence for mitochondrial biogenesis	Reference
The YG8R transgenic mice containing *FXN* with GAA repeat expansion in intron 1 as model for Friedreich's ataxia	ND, only increased *FXN* expression	ND	Li *et al.* (2013)
mdx model of DMD (skeletal muscle)	Amelioration of muscle damage and motor performance, increased mortality rate when dosage at 400 $mg \cdot kg^{-1} \cdot day^{-1}$	ND	Hori *et al.* (2011); Selsby *et al.* (2012); Gordon *et al.* (2013)
mdx model of DMD (cardiac muscle)	Prevention of cardiomyopathy and cardiac function	ND	Kuno *et al.* (2013)
Tg19959 mouse model of AD	Diminished plaque formation in specific brain areas	ND	Karuppagounder *et al.* (2009)
APP/PS1 mouse model of AD	Diminished plaque formation in specific brain areas	ND	Vingtdeux *et al.* (2010)
N171-82Q HD transgenic mouse model of HD	No effect on motor performance and striatal atrophy	+ (BAT)	Ho *et al.* (2010)
MTPT-induced mouse model of PD	Neuroprotective effect, amelioration of dopamine levels	ND	Blanchet *et al.* (2008); Mudo *et al.* (2012); Lofrumento *et al.* (2013)
SOD1 (G93A) Gurl model of ALS	No effect when administrated via diet (0.06%). Delay of disease onset and prolonged survival when administered via i.p. injection	ND	Markert *et al.* (2010); Han *et al.* (2012)

ND, not determined.

Table 4

Treatment studies using AICAR for the activation of mitochondrial biogenesis in mouse models

Mouse model	Effect on phenotype	Evidence for mitochondrial biogenesis	Reference
Surf1$^{-/-}$ model of COX deficiency	Improved biochemical phenotype, no motor impairment to improve	+	Viscomi *et al.* (2011)
ACTA-Cox15$^{-/-}$ model of mitochondrial myopathy	Improved biochemical phenotype without improved motor impairment	+	Viscomi *et al.* (2011)
Sco2KO/KI model of COX deficiency	Improved biochemical phenotype and improved motor impairment	+	Viscomi *et al.* (2011)
mdx model of DMD (skeletal muscle)	Amelioration of muscle pathology. May have positive effect on muscle function	+	Ljubicic *et al.* (2011); Jahnke *et al.* (2012)
mdx model of DMD (diaphragm)	Amelioration of muscle pathology. Evidence of increased mitophagy	–	Pauly *et al.* (2012)

mice, as previously observed by Viscomi *et al.* (2011) and Yatsuga and Suomalainen (2012) in their studies.

The results described above show considerable variability in the outcome of treatment with bezafibrate in mouse models with muscle disease due to mitochondrial dysfunction. Notwithstanding, bezafibrate has recently been reported to be beneficial in mouse models with neurological disorders, apparently mainly by improvement of mitochondrial function. Forebrain-specific *ΔCox10* mice develop progressive neurodegeneration and shrinkage of the cortex, which can be ameliorated by treatment with bezafibrate (Fukui *et al.*, 2007; Noe *et al.*, 2013). Mitochondrial mass was increased in the brain after treatment, including specific activity of COX and citrate synthase. Additionally, *Tfam* and *PGC-1α* gene expression were shown to be increased, which may have contributed to mitochondrial proliferation. Antioxidant enzymes SOD2 and

catalase were induced in brain of treated mice, and may have protected against oxidative damage that is believed to contribute to the pathology in forebrain-specific *ΔCox10* mice. The treatment resulted in an overall amelioration of the phenotype including attenuation of the loss of motor function.

In models with neurological disease not caused by primary mitochondrial dysfunction, bezafibrate was able to ameliorate pathology as well. Johri *et al*. (2012) demonstrated that the beneficial effect of lentiviral PGC-1α transgene expression in brain of the R6/2 HD mouse model by Cui *et al*. (2006) could be mimicked via bezafibrate treatment. This included restoring the expression levels of genes involved in mitochondrial biogenesis to the levels in non-treated wild-type mice in brain, skeletal muscle and brown adipose tissue (BAT) (Johri *et al*., 2012). Phenotypically, motor performance was improved by treatment and survival was increased by 20% in HD mice.

The same group also showed the amelioration of disease progression in a mouse model of AD (P301S transgenic mice) by bezafibrate diminishing locomotor and anxiety-related abnormalities (Dumont *et al*., 2012). The overall protective mechanism of bezafibrate in this model remains inconclusive despite some biochemical changes including activation of FAO in various tissues. Evidence of mitochondrial proliferation as a result of treatment was minimal in brain with slight increases in mtDNA quantity and *Sirt1* and *Tfam* gene expression, but more apparent in BAT with increased expression of regulators *Pgc-1α*, *Nrf1*, *Tfam* and *Sirt1*.

In summary, bezafibrate has been able to stimulate mitochondrial biogenesis in some mouse models with mitochondrial dysfunction (Wenz *et al*., 2008; Johri *et al*., 2012; Noe *et al*., 2013) (Table 2). In the majority of the other studies, increases in FAO have been found which may contribute to bezafibrate-induced changes in disease progression and normal development. Toxicity issues were identified in a number of studies, including impairment of growth. Bezafibrate causes a strong anabolic effect in mice including stimulation of FAO, most likely due to activation of PPARα by bezafibrate. Activation of PPARα is the major contributor responsible for the hepatomegaly resulting from bezafibrate treatment in mice and can result in hepatocarcinogenesis (Hays *et al*., 2005). Small differences between the mouse and human PPARα orthologues prevent proliferation of hepatic tissue in humans. This is demonstrated in the studies of Yang *et al*. (2008), which showed that the PPARα activator fenofibrate was unable to induce hepatomegaly in the PPARα-humanized mice whereas other metabolic effects of the drug were similar to wild type. Therefore, bezafibrate is relatively safe in humans although rhabdomyolysis and kidney failure have been reported as side effects (Charach *et al*., 2005; Wu *et al*., 2009). On a positive note, long-term bezafibrate treatment of patients with the mitochondrial FAO disorder CPT2 deficiency has shown a significant clinical improvement (Bonnefont *et al*., 2009; 2010).

Induction of mitochondrial biogenesis via SIRT1 activation: resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a natural component present in low concentrations in the skin of red grapes and has been extensively studied for its ability to activate sirtuins, including SIRT1 in mammals (Howitz *et al*., 2003). Resveratrol was originally shown to extend lifespan in various species and provide protection against the damaging effects of metabolic stress in mice (Howitz *et al*., 2003; Wood *et al*., 2004; Baur *et al*., 2006; Lagouge *et al*., 2006). Although the direct link between activation of SIRT1 and lifespan extension remains inconclusive (Bass *et al*., 2007; Burnett *et al*., 2011), the importance of SIRT1 as a metabolic sensor/regulator is well accepted but not without controversy. Resveratrol activates SIRT1 either directly or indirectly, which may be dose-dependent (Borra *et al*., 2005; Kaeberlein *et al*., 2005; Pacholec *et al*., 2010; Price *et al*., 2012; Hubbard *et al*., 2013). On the other hand, it is well documented that the activation of SIRT1 by resveratrol involves the activation of AMPK (Baur *et al*., 2006; Zang *et al*., 2006; Dasgupta and Milbrandt, 2007; Um *et al*., 2010). Consequently, AMPK activation results in increases in cellular NAD^+ levels, thereby activating SIRT1 through increasing its substrate (Fulco *et al*., 2008; Canto *et al*., 2009). The subsequent concerted action of both activated AMPK and SIRT1 on PGC-1α activates this coactivator, resulting in the induction of mitochondrial biogenesis (Canto *et al*., 2009). Recently, a direct inhibition of cAMP PDEs by resveratrol was demonstrated, which may be an important aspect in its mechanism of action (Park *et al*., 2012). Then again, the large number of proteins that are able to interact with resveratrol makes it very difficult to interpret the contribution of the effect of direct inhibition of PDEs by this compound, in the overall scheme of interactions (Pacholec *et al*., 2010).

In healthy mice, resveratrol increased lifespan and was protective under conditions of diet-induced obesity, although it did not extend their lifespan under normal conditions (Baur *et al*., 2006; Lagouge *et al*., 2006; Pearson *et al*., 2008). Induction of mitochondrial biogenesis via PGC-1α activation is a major contributor of the beneficial effects of the drug. Hence it is not surprising that resveratrol has been trialled for the treatment in mouse models of genetic disorders associated with mitochondrial dysfunction. No treatment studies with resveratrol in mouse models of primary OXPHOS disorders have been published so far. In this respect it is interesting to mention the use of resveratrol in a study with a mouse model of Friedreich's ataxia (Al-Mahdawi *et al*., 2006; Li *et al*., 2013). This neurological condition is caused by impaired expression of the *FXN* gene due to a pathological expansion of GAA repeats in intron 1 of this gene encoding mitochondrial frataxin (Li *et al*., 2013). Frataxin appears to be involved in biosynthesis of iron-sulfur clusters, which form integral parts of OXPHOS CI, CII and CIII. Accordingly, the OXPHOS system has been found to be dysfunctional in Friedreich's ataxia. Subcutaneous resveratrol injections (200 $mg \cdot kg^{-1}$ for 3 days) increased *FXN* mRNA and frataxin protein levels in the mice indicative of the potential of resveratrol for the treatment of Friedreich's ataxia (Li *et al*., 2013).

Some of the other mouse models previously described in the PGC-1α overexpression studies (Table 1) or bezafibrate trials (Table 2) have been treated with resveratrol as well (Table 3). In the *mdx* model of DMD, long-term resveratrol treatment mimicked the effect observed by PGC-1α overexpression including reduction of muscle tissue loss, changes in fibre-type composition and reduction of oxidative damage in

skeletal muscle of *mdx* mice (Handschin *et al.*, 2007; Hori *et al.*, 2011). Additional studies confirmed the positive results of resveratrol treatment on skeletal muscle in *mdx* mice (Selsby *et al.*, 2012; Gordon *et al.*, 2013). However, 8 weeks of 400 mg·kg^{-1}·day^{-1} was reported to result in a high mortality rate (Selsby *et al.*, 2012).

In a subsequent study, the effect of resveratrol on the development of cardiomyopathy in *mdx* mice was studied (Kuno *et al.*, 2013). Treatment with resveratrol prevented cardiomyopathy and restored cardiac function in *mdx* mice. This effect was suggested to be caused via attenuation of expression of the p300 coactivator, which is a key contributor to cardiac hypertrophy and fibrosis in the heart. In the context of this review it is unfortunate that the effect of resveratrol on mitochondrial status was not reported in any of the studies using *mdx* mice despite the important function of mitochondria in muscle and heart during health and disease.

Resveratrol has become a popular compound to test as treatment for a range of neurological disorders (Herskovits and Guarente, 2013; Table 3). In the majority of these disorders, SIRT1 activation by resveratrol is not primarily aiming to ameliorate mitochondrial metabolism but to improve other SIRT1-regulated biological processes (Herskovits and Guarente, 2013). Resveratrol has been used in mouse models of AD, HD, PD and ALS with some success but with minimal evidence of the mode of action.

Importantly, some of these studies have confirmed the stability of resveratrol in mouse food when stored at room temperature, which has been under debate (Karuppagounder *et al.*, 2009; Vingtdeux *et al.*, 2010). Furthermore, Vingtdeux *et al.* (2010) demonstrated the presence of resveratrol in the brain after oral treatment, indicating resveratrol can have a direct mode of action in neurological disorders. These two studies followed an initial treatment trial with p25 transgenic mice with features of AD in which resveratrol was injected directly into the brain via i.c.v. injections. In these mice neurodegeneration and cognitive decline was decreased (Kim *et al.*, 2007). Both subsequent studies, using two different models of AD (Table 3) showed that dietary administration of this compound [0.2 and 0.35% (w/w)] resulted in amelioration of plaque pathology in specific areas of the brain.

As mentioned previously, lentiviral-mediated overexpression of PGC-1α was beneficial in the R6/2 mouse model of HD (Cui *et al.*, 2006). However, administration of resveratrol (oral gavage, 25 mg/mouse/day) could not replicate this effect in N171-82Q HD transgenic mice where it was found to have an effect on *PGC1α* gene expression in BAT and not in the striatum, which is likely to explain the lack of improvement of phenotype (Ho *et al.*, 2010).

In terms of PD, several studies have shown the neuroprotective effect of resveratrol in the substantia nigra after MPTP-induced neurotoxicity in mice (Blanchet *et al.*, 2008; Mudo *et al.*, 2012; Lofrumento *et al.*, 2013). The outcomes of these studies were suggested to result from the induction of an antioxidant response and/or an anti-inflammatory effect induced by resveratrol (Mudo *et al.*, 2012; Lofrumento *et al.*, 2013).

In the SOD1G93A ALS mouse model, treatment with dietary resveratrol (0.06%, w/w) was ineffective (Markert *et al.*, 2010) but resveratrol injected i.p. (20 mg·kg^{-1} twice weekly) delayed disease onset and prolonged survival of mice (Han *et al.*, 2012), suggesting the dose and mode of administration can be critical when using resveratrol. SIRT1 activation by resveratrol resulted in deacetylation of heat shock factor 1 and a subsequent unfolded protein response. The latter effect is likely to be a significant contributor to the mechanism of action of resveratrol in this model in which the pathology is caused by misfolding of the mutated SOD1G93A protein (Westerheide *et al.*, 2009; Han *et al.*, 2012).

The studies described above illustrate the enormous potential for resveratrol in the treatment of a wide variety of diseases. The positive treatment outcomes of resveratrol in these studies have been attributed to induction of an antioxidant response, anti-inflammatory effect or an unfolded protein response. None of these studies have looked at the effect of resveratrol on mitochondrial status, which may well play an additional role in the amelioration of pathology in these disease models.

Induction of mitochondrial biogenesis via AMPK activation: AICAR

The activation of AMPK has been implicated as a key player for the mechanism of action of resveratrol on SIRT1 and consequent mitochondrial biogenesis (Baur *et al.*, 2006; Zang *et al.*, 2006; Dasgupta and Milbrandt, 2007; Um *et al.*, 2010). AMPK activation occurs through phosphorylation of Thr^{172} by AMPK kinases, including Ca^{2+}/calmodulin-dependent kinase kinase and liver kinase B1 (see Hardie *et al.*, 2011; Oakhill *et al.*, 2012). AMPK phosphorylation is directly promoted by AMP and ADP binding, and therefore linked to the cellular energy status that is reflected by the AMP/ATP and the ADP/ATP ratios. Furthermore, AMP is also able to stimulate the activity of phosphorylated AMPK via allosteric activation of the enzyme, although the significance of this effect *in vivo* is not known (Oakhill *et al.*, 2012). All these aspects make AMPK an ideal metabolic sensor that monitors and reacts to changes in the cellular energy status.

Moreover, it makes AMPK also an ideal drug target. AMPK can be directly activated via treatment with the drug 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). AICAR requires phosphorylation inside the cell and it is this phosphorylated derivative known as ZMP, that acts as an AMP mimetic (Sullivan *et al.*, 1994; Corton *et al.*, 1995; Henin *et al.*, 1995; 1996; Merrill *et al.*, 1997). Unlike other AMPK stimuli, AICAR promotes phosphorylation and activation of AMPK while not disturbing the energy status of the cell, that is, the AMP/ATP ratio remains the same.

AICAR is orally effective in mice and shown to increase endurance performance in sedentary mice by increasing expression of oxidative metabolism genes (Narkar *et al.*, 2008). This is believed to be via the direct phosphorylation/activation of PGC-1α by activated AMPK and subsequent induction of mitochondrial biogenesis (Winder *et al.*, 2000; Jager *et al.*, 2007). Unfortunately AICAR has a very poor ability to penetrate the blood–brain barrier, which limits its use to peripheral tissues (Marangos *et al.*, 1990). Nevertheless peripheral stimulation of AMPK and mitochondrial biogenesis may still have beneficial effects on brain function as demonstrated in healthy mice (Kobilo *et al.*, 2011).

In the case of OXPHOS disorders, in which there are typically defects in ATP generation, partial activation of AMPK may be already occurring in certain tissues. Additional stimulation of this pathway to maximum with AICAR may bring this rescue mechanism to full potential. Accordingly, in a drug screen using skin fibroblasts from a cohort of CI patients AICAR was shown to be the most promising candidate in ameliorating mitochondrial defects (Golubitzky *et al.*, 2011).

In mice, Viscomi *et al.* (2011) have demonstrated the potential of AICAR for OXPHOS disorders by the successful treatment of their muscle-specific COX-deficient mouse models of mitochondrial myopathy. The drug was injected s.c. from 8 weeks of age for 1 month (0.25 $mg \cdot day^{-1} \cdot g^{-1}$). AICAR treatment resulted in AMPK phosphorylation and induction of FAO and OXPHOS gene expression in muscle in *Surf1*$^{-/-}$, *Sco2KO/KI* and the *ACTA-Cox15*$^{-/-}$ mice (Table 4; Dell'agnello *et al.*, 2007; Yang *et al.*, 2010; Viscomi *et al.*, 2011). Moreover, COX enzyme activity in muscle was increased in all models as a result of increased amounts of COX subunits. As well as increasing the deficient COX activity, AICAR also increased other OXPHOS enzyme activities, indicating a general increase in mitochondrial biogenesis. Motor performance impairment present in *Sco2KO/KI* mice could be rescued by AICAR treatment. No changes were observed in the more severely affected *ACTA-Cox15*$^{-/-}$ mice, even when doubling the AICAR dose. This may be due to the severity of the muscle damage present in these mice at the start of treatment.

AICAR is also able to improve skeletal muscle pathology in the *mdx* model of DMD (Ljubicic *et al.*, 2011; Jahnke *et al.*, 2012). This coincided with the induction of mitochondrial biogenesis as shown by increases in COX activity in fast-twitch muscle (extensor digitorum longus) and a switch to more oxidative fibre types. In addition, AICAR evoked the expression of utrophin and PGC-1α plus increased expression of the OXPHOS components cytochrome c and COX IV. However, in the more oxidative diaphragm muscle, AICAR treatment did not result in changes in fibre type or induction of mitochondrial biogenesis (Pauly *et al.*, 2012). Contrariwise, data from this study suggested that AICAR caused increased mitophagy in the diaphragm of *mdx* mice (Pauly *et al.*, 2012). Mitophagy, the process responsible for degradation of dysfunctional and damaged mitochondria, has been shown to be impaired in *mdx* muscle (De Palma *et al.*, 2012). AICAR had a positive effect on diaphragm structure and contractile capacity, suggesting improvement of mitophagy may be an alternative pathway to target for the treatment of muscle diseases including DMD and mitochondrial myopathies (Pauly *et al.*, 2012).

Induction of mitochondrial biogenesis: miscellaneous compounds

This review has focussed on the treatment of mitochondrial dysfunction by bezafibrate, resveratrol and AICAR, with the rationale that these compounds have all been used in OXPHOS mouse models to induce mitochondrial biogenesis. Various other drugs have been used in preclinical trials with the other mouse models discussed in this review. Some of these may have potential for the induction of mitochondrial biogenesis. Among these is the drug metformin, which is widely used for the treatment of type 2 diabetes (Viollet *et al.*, 2012). The mode of action of metformin is still not completely resolved, but AMPK is believed to contribute to the effect of the drug. AMPK is not directly activated by metformin but through changes in the cellular energy state as a consequence of the mild inhibitory effect of metformin on CI of the OXPHOS system. Of course this makes metformin unlikely to be a treatment option for OXPHOS disorders where a pathogenic OXPHOS defect is already present.

NO is a gaseous signalling molecule that has been shown to be involved in mitochondrial biogenesis (see Nisoli and Carruba, 2006; Tengan *et al.*, 2012). The role of NO in mitochondrial biogenesis is not exactly clear but the downstream induction of PGC-1α expression is essential for this effect (Nisoli *et al.*, 2003). NO is able to activate PGC-1α through a cyclic GMP-dependent pathway, via AMPK, or in a p53-dependent manner, with a possibility of multiple pathways overlapping as well (Nisoli *et al.*, 2003; Lira *et al.*, 2010; McConell *et al.*, 2010; Aquilano *et al.*, 2013). Not only is NO involved in mitochondrial biogenesis but it is also implicated in myogenic differentiation in which the inhibition of the mitochondrial fission protein Drp1 by NO through activation of guanylate cyclases plays an important role (De Palma *et al.*, 2010). Because of the physiological effects that are under control of NO, its metabolism has been an attractive therapeutic target especially in case of muscle wasting conditions such as DMD and mitochondrial myopathy. NO levels can be influenced by stimulating the endogenous synthesis with arginine, the substrate for the NOS, or via drugs with NO-donating properties. These strategies have been trialled in the *mdx* mouse model of DMD and beneficial effects have been observed (see De Luca, 2012; De Palma and Clementi, 2012).

Moreover, positive results have been reported from attempts to treat the potential NO deficiency present in patients with the mitochondrial disease MELAS (mitochondrial encephalopathy, lactic acidosis, stroke-like episodes) with arginine or citrulline (Koga *et al.*, 2005; see also El-Hattab *et al.*, 2012; Tengan *et al.*, 2012). However, the rationale in these studies is focused on restoring the NO deficiency observed in some forms of mitochondrial disease and not increasing mitochondrial biogenesis. Contrariwise, mitochondrial proliferation resulting from a compensation mechanism in these mitochondrial disease patients has been hypothesized to be one of the underlying causes of impaired NO synthesis in the first place (El-Hattab *et al.*, 2012).

Apart from its role in mitochondrial biogenesis, NO also has a direct inhibitory effect on the protein complexes of the OXPHOS system. NO competitively inhibits oxygen binding at COX and, via reaction with haem iron, iron sulfur clusters or reduced protein thiols, NO also inhibits NADH-dehydrogenase activity at CI and the transfer of electrons at CIII and CI. All these direct effects on the respiratory chain make manipulation of NO levels in patients with OXPHOS defects a complex strategy (Tengan *et al.*, 2012). On the other hand, this effect of NO is cardioprotective during ischaemia-reperfusion injury. Recently, using a mitochondria-selective S-nitrosating agent, it was shown that reversible mitochon-

drial S-nitrosation of CI forms a major part of the mechanism underlying the protective effect of NO during ischaemic reperfusion, thereby forming a therapeutic target for the prevention of injury after infarct or stroke (Chouchani *et al.*, 2013).

The PPARδ agonist GW501516 induced the generation of oxidative myofibers and increased endurance, especially in combination with exercise or AMPK activation (Narkar *et al.*, 2008). This was followed by studies in *mdx* models in which GW501516 improved the DMD phenotype (Miura *et al.*, 2009; Bueno Junior *et al.*, 2012; Jahnke *et al.*, 2012).

In humans, GW501516 has beneficial effects on parameters linked to the metabolic syndrome (Olson *et al.*, 2012). Unfortunately long-term preclinical treatment trials have led to safety concerns, and clinical development for humans has been discontinued. Nevertheless, stimulation of PPARδ still has enormous potential for treatment of multiple conditions once a safer alternative for GW501516 can be identified.

PPARγ activation by drugs of the thiazolidinedione (TZD) class is widely used as treatment for diabetes. Although PPARγ interacts with PGC-1α in many different tissues, TZDs have been extensively tested in models of neurodegeneration because of their anti-inflammatory properties (Carta, 2013; Choi and Bothwell, 2012). The first studies were performed with the TZD pioglitazone in the G93A SOD1 mouse model of ALS (Kiaei *et al.*, 2005; Schutz *et al.*, 2005). Both studies found increases in lifespan, which were suggested to be due to the anti-inflammatory effect of pioglitazone in both models. This was subsequently translated into a clinical trial with ALS patients but, disappointingly, there was no additional effect of pioglitazone on the standard treatment with the drug riluzole (Dupuis *et al.*, 2012).

TZDs have also been tested successfully on multiple models of HD recently (Chiang *et al.*, 2010; Napolitano *et al.*, 2011; Jin *et al.*, 2013). Data from these studies suggest amelioration of both PPARγ and PGC-1α levels contributes to the improved phenotype in treated mice.

Furthermore, PPARγ activation has been used extensively for the treatment of various mouse models of PD (see Carta, 2013; Ridder and Schwaninger, 2012) and AD (see Mandrekar *et al.*, 2011). In all these models PPARγ activation was used to suppress the inflammatory response and not the induction of mitochondrial biogenesis. However, TZDs may still be beneficial for the treatment of OXPHOS disorders, especially when an inflammatory response in the brain is part of the symptoms.

The last treatment option discussed here does not require any pharmacological compounds but consists of endurance exercise, which is a strong endogenous inducer of PGC-1α, especially in skeletal muscle. Indeed, the endurance exercise regime that Wenz *et al.* (2008; 2009a) used on the *ΔCox10* mice was sufficient for prolonged survival and delayed onset of disease, mimicking transgenic PGC-1α overexpression or treatment with bezafibrate. The study showed clear evidence of mitochondrial proliferation and improved OXPHOS parameters in skeletal muscle. Similarly, a substantial effect of endurance exercise was also observed in the Mut mice (Safdar *et al.*, 2011). Again this mimicked the effect of overexpression of PGC-1α, but without a decrease in muscle weight indicating that exercise may act on additional metabolic pathways as well (Dillon *et al.*, 2012b). Both these studies in mice provide additional evidence for the current use of exercise therapy in the treatment of mitochondrial myopathies (Pfeffer and Chinnery, 2013).

Concluding remarks

The hypothesis that induction of mitochondrial biogenesis could be used as a treatment for OXPHOS disorders has been explored in various studies using bezafibrate, RSV and AICAR, as summarized here. *In vitro* studies in cell models have initially shown great potential for these compounds, and the recent development of appropriate mouse models for various OXPHOS disorders has resulted in the translation of this *in vitro* work into preclinical treatment trials.

The studies described in this review show that the translation from the *in vitro* results to the *in vivo* pharmacological outcomes is often not straightforward. There has been substantial variation in the success of treatment for each tested compound, even in terms of whether mitochondrial biogenesis is induced. In this review this has been summarized in detail for bezafibrate, but recently one study reported that dietary resveratrol treatment had no effect on mitochondrial biogenesis in skeletal muscle of wild-type mice and rats when using the same dosage shown previously by others to induce mitochondrial proliferation (Lagouge *et al.*, 2006; Higashida *et al.*, 2013). Differences of treatment effect may be partly due to the physical condition, age, genetic background and which tissues are affected in each mouse model. Bioavailability of the drugs could also be influenced by differences in drug quantities and administration routes. This has been demonstrated for both bezafibrate and resveratrol, for which the dose can contribute to differences in the specific mode of action (Nakajima *et al.*, 2009; Price *et al.*, 2012). This is even more important in the context of toxic side effects that have been reported for both compounds in studies with mouse models (Tables 2 and 3). However, it remains unclear why even wild-type mice treated with apparently the same quantity and administration route of bezafibrate show clear up-regulation of skeletal muscle mitochondrial biogenesis in some studies (Wenz *et al.*, 2008) but not others (Viscomi *et al.*, 2011; Yatsuga and Suomalainen, 2012).

Both resveratrol and bezafibrate are relatively safe compounds and widely used by humans. The damaging effects of bezafibrate on the liver in mice are not observed in humans, due to differences in PPARα signalling between species and the much lower doses typically used in humans (Hays *et al.*, 2005; Tenenbaum *et al.*, 2005; Yang *et al.*, 2008; Djouadi and Bastin, 2011). Kidney and muscle wasting have been reported as side effects of the drug and should be taken into account when trialling bezafibrate for disorders in which these tissues are already affected (Charach *et al.*, 2005; Wu *et al.*, 2009). Nevertheless, a clinical trial with bezafibrate in patients with CPT2 deficiency, who have muscle involvement as part of the pathology, has generated a positive outcome (Bonnefont *et al.*, 2009, 2010; Djouadi and Bastin, 2011).

Resveratrol is readily available as part of food and vitamin supplements but, unfortunately,it also has a very poor bioavailability and therefore has been used in trials at high doses in humans. At these high doses, resveratrol can have deleterious side effects, of which gastrointestinal symptoms are the most common (Patel *et al.*, 2011). Despite this, recent trials

with the drug in humans have shown the potential of resveratrol for the treatment of metabolic diseases (Brasnyo *et al.*, 2011; Timmers *et al.*, 2011; Bhatt *et al.*, 2012; Yoshino *et al.*, 2012; Poulsen *et al.*, 2013). The low bioavailability has resulted in the development of more potent small activators of sirtuins, also known as sirtuin-activating compounds, which may cause fewer off-target effects (Milne *et al.*, 2007; Feige *et al.*, 2008).

Bezafibrate, resveratrol and AICAR have been used in trials for the treatment of other mouse models with more common neurological and muscular diseases. Mitochondrial dysfunction has been linked to the pathology of all of these more common diseases described in this review (Kuznetsov *et al.*, 1998; Lin and Beal, 2006). Surprisingly, the majority of these treatment studies have had little focus on the effect of the treatment on mitochondrial parameters, despite an expectation that they may well have contributed to the outcomes (see Tables 2–4).

Finally the concept that inducing mitochondrial biogenesis may lead to increased numbers of dysfunctional mitochondria should be discussed. On the one hand, all patients with OXPHOS disorders have residual enzyme activity because a complete loss of activity is not compatible with life. Hence, up-regulation of the amount of less active mitochondria may increase ATP synthesis capacity above a certain cellular threshold. Of course, pharmacological induction may not help every cell in every tissue in a multicellular organism but could be helpful in a limited set of tissues. This is particularly relevant to AICAR, which does not cross the blood–brain barrier. Many studies have shown that, especially, skeletal muscle cells with their long multinucleated structure seem good candidates for the induction of mitochondria, resulting in fibre type switching towards a more oxidative type.

On the other hand, the mitochondrial dysfunction may not only lead to an ATP generation defect but also to an increased generation of ROS. ROS may be cellular signalling molecules under normal conditions but also have the capacity to damage protein, DNA and lipids. First of all, mitochondrial ROS production is not increased in all OXPHOS disorders. In those OXPHOS conditions where ROS are increased, it may be better to consider treatment with antioxidants instead of targeting biogenesis and potentially increasing the number of ROS generators in the cell. Second, activation of the PGC-1α pathway leads not only to mitochondrial biogenesis but also to a SIRT3-mediated antioxidant response, which may counteract any increases in ROS production (Bell and Guarente, 2011; Scarpulla, 2012; Scarpulla *et al.*, 2012). Third, recently it has been found that AMPK activation directly activates the autophagy/mitophagy pathway via phosphorylation of mitophagy regulator ULK1 (Egan *et al.*, 2011; Kim *et al.*, 2011). Mitophagy regulates the degradation/recycling of dysfunctional and damaged mitochondria. Simultaneous stimulation of mitophagy and mitochondrial biogenesis is likely to provide a protection mechanism against the proliferation of dysfunctional mitochondria.

Furthermore, the study of Dillon *et al.* (2012b) showed that induction of the PGC-1α pathway in Mut mice mainly resulted in an increase in newly acquired mtDNA mutations in skeletal muscle compared with control and not in an increase in the heteroplasmic mutant load of inherited mtDNA mutations. The increased introduction of new mtDNA mutations probably occurs during the proliferation of mtDNA and is due to the DNA proofreading defect in POLG in the Mut mice. Stability of the inherited mutations is very important when considering patients with mitochondrial DNA mutations as candidates for treatment with mitochondrial biogenesis inducers.

This review describes a number of mouse models of conditions that appear to benefit from the induction of mitochondria. These studies have identified challenges related to variation with genetic background or bioavailability, side effects, the blood–brain barrier, differing response between tissues and potential risks of increasing mtDNA mutation load or ROS generation. These issues are clearly relevant to human trials. Because of the enormous clinical variability in patients with OXPHOS disorders, it would be unrealistic to expect to develop a one-size-fits-all treatment. Current and future clinical trials in humans will reveal how well these mouse studies translate into actual therapeutic use.

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Conflict of interest

The authors have no financial links with manufacturers of any of the materials or devices described in the manuscript.

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