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Regulation of mitochondrial biogenesis

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Abstract

Although it is well established that physical activity increases mitochondrial content in muscle, the molecular mechanisms underlying this process have only recently been elucidated. Mitochondrial dysfunction is an important component of different diseases associated with aging, such as Type 2 diabetes and Alzheimer's disease. PGC-1α (peroxisome-proliferator-activated receptor γ co-activator-1α) is a co-transcriptional regulation factor that induces mitochondrial biogenesis by activating different transcription factors, including nuclear respiratory factor 1 and nuclear respiratory factor 2, which activate mitochondrial transcription factor A. The latter drives transcription and replication of mitochondrial DNA. PGC-1α itself is regulated by several different key factors involved in mitochondrial biogenesis, which will be reviewed in this chapter. Of those, AMPK (AMP-activated protein kinase) is of major importance. AMPK acts as an energy sensor of the cell and works as a key regulator of mitochondrial biogenesis. AMPK activity has been shown to decrease with age, which may contribute to decreased mitochondrial biogenesis and function with aging. Given the potentially important role of mitochondrial dysfunction in the pathogenesis of numerous diseases and in the process of aging, understanding the molecular mechanisms regulating mitochondrial biogenesis and function may provide potentially important novel therapeutic targets.

Introduction

Mitochondrial biogenesis can be defined as the growth and division of pre-existing mitochondria. Mitochondria are direct descendants of an α-protobacteria endosymbiont that became established in a host cell. Owing to their bacterial origin, mitochondria have their own genome and can autoreplicate. Mitochondrial proteins are encoded by the nuclear and the mitochondrial genomes. The mtDNA (mitochondrial DNA) is a double-stranded circular molecule of approx. 16.5 kb that contains 37 genes encoding 13 subunits of the electron transport chain complexes I, III, IV and V. The only other mitochondrial gene products are the 22 tRNAs (transfer RNAs) and two rRNAs (ribosomal RNAs) necessary for translation of the respiratory subunit mRNAs within the mitochondrial matrix. Correct mitochondrial biogenesis requires the co-ordinated synthesis and import of approx. 1000–1500 proteins encoded by the nuclear genome and synthesized on cytosolic ribosomes [1]. mtDNA replication, as well as mitochondrial fusion and fission, which are discussed elsewhere in this volume, must also be co-ordinated [2]. Mitochondrial biogenesis is influenced by

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environmental stress such as exercise, caloric restriction, low temperature, oxidative stress, cell division and renewal and differentiation. Mitochondrial biogenesis is accompanied not only by variations in number, but also in size and mass.

As the majority of proteins are encoded in the nucleus, a mechanism for targeting, import and correct assembly exists to ensure correct mitochondrial function and shape. mRNAs are translated in the cytosol after activation of the nuclear genome to precursor proteins. These precursor proteins are synthesized with an N-terminal positively charged presequence capable of forming a basic, amphipathic α-helix. They traverse inner and outer mitochondrial membranes in an unfolded conformation by crossing through protein translocases. The mitochondrial membrane potential and the action of matrix Hsp70 (heat-shock protein 70) are used to drive translocation. The presequence is then cleaved by a matrix protease and, often with the aid of molecular chaperones, the imported protein is folded. However, a majority of mitochondrial protein precursors use different protein-import pathways to the other mitochondrial compartments. Precursor proteins that follow these routes do not typically contain the N-terminal targeting signals, but harbour targeting information within their mature sequence. Currently, four major membrane protein translocase complexes are known. The TOM (translocase of the outer membrane) is the universal entry gate for all proteins that are imported into mitochondria. The different protein pathways then diverge at this point. TIM (translocase of the inner membrane) sorts matrix-targeted precursors. The PAM (presequence translocase-associated motor) regulates matrix Hsp70 action to drive precursors into the matrix. Finally, the outer membrane SAM (sorting and assembly machinery) inserts β-barrel proteins into the outer membrane. All of these processes are an integral part of mitochondrial biogenesis.

Mitochondrial biogenesis and endurance exercise

It is well established that physical activity increases mitochondrial content. The first observations to establish this association were done by comparing the mitochondrial content in different muscle groups in animals. For example, it has been found that the breast muscle of chickens, which fly infrequently, contain less mitochondria than the breast muscle of pigeons, which are able to fly for long periods [3]. Other studies demonstrated that continuously working muscles, such as the heart, had more mitochondrial activity and content than sporadically functioning muscles, such as back muscles [3]. These early studies in animals suggested that muscles involved in regular and sustained physical activity can increase their mitochondrial activity and content to achieve their goals. In one of those, 6-week-old rats subjected to exercise 5 days per week for 3 months showed an increase in skeletal muscle cytochrome *c* concentration, as well as increased activities of key mitochondrial enzymes and OXPHOS (oxidative phosphorylation) [4]. Further studies established the same observations in humans [5, 6]. Another study compared exercise-trained individuals, elite distance runners and sedentary individuals and found a much greater percentage of oxidative, slow-twitch skeletal muscle fibres and more succinate dehydrogenase activity in runners than in sedentary controls [7]. However, despite the well-recognized link between mitochondrial biogenesis and stimuli such as endurance exercise, the factors regulating mitochondrial biogenesis have remained elusive, until recently.

Factors regulating mitochondrial biogenesis

Transcription cascade of mitochondrial biogenesis

PGC-1α [PPAR (peroxisome proliferator-activated receptor)-γ coactivator-1α]—PGC-1α is a major regulator of mitochondrial biogenesis [8] that had been discovered after the NFRs (nuclear respiratory factors) 1 and 2, and Tfam (mitochondrial transcription factor A; also known as mtTFA). In the experiment leading to the discovery of PGC-1α,

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mice were exposed to the cold (4°C), which induced an increase in PGC-1α mRNA expression in thermogenic tissues, namely brown fat and skeletal muscle. This cold-induced expression of PGC-1α also increased the expression of key mitochondrial enzymes in the brown adipose tissue, such as ATP synthetase (β-subunit) and COX (cytochrome *c* oxidase) subunits (COX II and COX IV). Further experiments in rats provided *in vivo* evidence of a link between PGC-1α and mitochondrial biogenesis, as electrical stimulation induced an increase in COX activity [9].

PGC-1α is a co-transcriptional regulation factor that induces mitochondrial biogenesis by activating different transcription factors, including NRF-1 and NRF-2, which promote the expression of Tfam. NRF-1 and NRF-2 are important contributors to the sequence of events leading to the increase in transcription of key mitochondrial enzymes, and they have been shown to interact with Tfam, which drives transcription and replication of mtDNA [10]. A strong connection between PGC-1α and mitochondrial biogenesis was further established by examining mtDNA content in mouse C2C12 myotubes expressing PGC-1α. Indeed, it was demonstrated that PGC-1α stimulates mitochondrial biogenesis and respiration in muscle cells through an induction of UCP (uncoupling protein) 2 and through induction of NRF-1 and NRF-2 gene expression. Moreover, PGC-1α binds to and co-activates the transcriptional function of NRF-1 on the promoter for Tfam [11]. Another study confirmed the link between exercise and mitochondrial biogenesis by an acute swimming bout, which increased PGC-1α protein expression and NRF-1 binding to the δ-ALAS (δ-aminolevulinate synthase) promoter and NRF-2 binding to the COX IV promoter [12]. In addition to NRFs, PGC-1α also interacts with and co-activates other transcription factors such as PPARs, thyroid hormone, glucocorticoid, oestrogen and ERRs (oestrogen-related receptors) α and γ [13]. ERRs are orphan nuclear receptors that target vast gene networks involved in all aspects of energy homoeostasis, including fat and glucose metabolism, as well as mitochondrial biogenesis and function [14].

Taken together, these data indicate that PGC-1α plays a crucial role in linking stimuli such as cold or exercise to an internal metabolic response like mitochondrial biogenesis via, among others, the NRF transcription factors (Figure 1).

PGC-1β—PGC-1β shares similar molecular structure and function with PGC-1α, including nuclear-receptor binding and transcriptional activation, and also regulates mitochondrial biogenesis. Ectopic expression of PGC-1β, such as in L6 myoblasts, induces mitochondrial biogenesis and increases basal oxygen consumption. However, unlike PGC-1α, PGC-1β is not up-regulated in brown adipose tissue in response to cold and is also not up-regulated in muscle in response to exercise [15]. This suggests that PGC-1α and PGC-1β are stimulated independently, although both clearly regulate mitochondrial biogenesis through NRF-1 to enable mitochondria to meet the energetic requirements of the cell.

Signalling events leading to activation of the transcription cascade

AMPK (AMP-activated protein kinase)—AMPK is a major regulator of mitochondrial biogenesis which regulates intracellular energy metabolism in response to acute energy crises [16]. β-GPA (β-guanadinopropionic acid) is a creatine analogue acting as a chronic pharmacological activator of AMPK. Indeed, β-GPA mimics exercise training and leads to reductions in the intramuscular ATP/AMP ratio and phosphocreatine concentrations, which in turn activates skeletal muscle AMPK. Rats fed for 8 weeks with β-GPA had a chronic skeletal muscle AMPK activation, which resulted in increases in NRF-1-binding activity, δ-ALAS mRNA expression, cytochrome *c* protein expression and mitochondrial content, thus demonstrating that AMPK activation promotes mitochondrial biogenesis through PGC-1α and the NRFs [17]. To further examine the role of AMPK in mitochondrial biogenesis,

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transgenic mice overexpressing a DN (dominant-negative) mutant of AMPK in muscle (DN-AMPK) were treated with β-GPA. This treatment had no effect on AMPK activity or mitochondrial content in DN-AMPK mice, but induced an activation of muscle AMPK and mitochondrial biogenesis in wild-type mice. Furthermore, this AMPK inactivation abrogated the β-GPA-induced expression of PGC-1α and CaMKIV (calcium/calmodulin-dependent protein kinase IV) [18]. Pharmacological studies using other AMPK agonists, such as AICAR (5-amino-4-imidazolecarboxamide 1-β-D-ribofuranoside), also established the link between chronic AMPK activation and the up-regulation of key mitochondrial enzymes [19]. Recently Reznick et al. [20] examined AMPK activity in young and old rats and found that the acute stimulation of AMPK-α2 activity by AICAR and exercise was blunted in skeletal muscle of old rats. Furthermore, mitochondrial biogenesis was diminished in these old rats after the chronic activation of AMPK with β-GPA [20]. These findings suggest that AMPK activity is reduced with aging and that it may be an important contributing factor in mitochondrial dysfunction and dysregulated intracellular lipid metabolism. It has been shown recently that the AMPK agonist AICAR can increase endurance in sedentary mice by genetically reprogramming muscle metabolism [21]. Moreover, PPARδ activation also partially mimics exercise. This led to the hypothesis that activation of PPARδ, with an agonist such as GW1516, could induce mitochondrial biogenesis. However, mitochondrial biogenesis, measured as mtDNA expression, was not increased by drug alone, but was increased by approx. 50% with the combination of exercise and treatment. Furthermore, co-transfection experiments suggested that AMPK may be present in a transcriptional complex with PPARδ, leading to the hypothesis that the AMPK–PPARδ interaction could be the target of drugs to increase endurance, even without exercise [21].

Taken together, these data demonstrate that by sensing the energy status of the muscle cell, AMPK is a key regulator of mitochondrial biogenesis.

CaMKIV—CaMKIV is another major regulator of mitochondrial biogenesis. It was first noticed that CaMKIV influences gene expression in oxidative fibres of myocytes [22]. In order to assess the role of CaMKIV in mitochondrial biogenesis, a transgenic mouse model muscle-specifically expressing an active form of CaMKIV was created. It was shown that the skeletal muscle of these mice contained more copies of mtDNA and possessed more mitochondria as a percentage of the volume of myocytes [23]. In addition, mRNA expression of cytochrome *b* and carnitine palmitoyltransferase-1 was increased in the transgenic mice [23]. However, further studies using CaMKIV-null mice found similar protein expression levels of PGC-1α and COX IV compared with wild-type mice. Moreover, running increased PGC-1α and COX IV protein expression to the same amount in both wild-type and CaMKIV-null mice, indicating that CaMKIV may not be required for mitochondrial biogenesis [24]. It is also important to note that CaMKIV protein expression could not be detected in murine skeletal muscle in this study [24]. It is also important to note that CaMKIV does not seem to be expressed in human skeletal muscle, to the opposite of CaMKII, which plays an important role during exercise [25]. Thus the role of CaMKIV in the regulation of muscle mitochondrial biogenesis in response to exercise remains unclear.

NO (nitric oxide)—More recently, NO has been shown to be involved in mitochondrial biogenesis. HeLA cells expressing eNOS (endothelial NO synthase) display an increase in mtDNA content, cytochrome *c* and COX IV protein expression levels, as well as PGC-1α, NRF-1 and Tfam mRNA expression [26]. Moreover, the NO produced by eNOS activates guanylate cyclase to increase the amount of cGMP present, which transmits a signal to the nucleus through an unknown mechanism, leading to the induction of PGC-1α gene transcription and mitochondrial biogenesis as a consequence.

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SIRT1 [sirtuin (silent mating type information regulation 2 homologue) 1]—The silent information regulator protein (Sir2) homologue SIRT1 is another activator of PGC-1α. SIRT1 protein is induced in liver during fasting through a nutrient-signalling response mediated by pyruvate kinase. SIRT1 then activates PGC-1α by deacetylation [27]. Resveratrol is a phytoalexin produced by certain plants that activates SIRT1, and has been shown to enhance mitochondrial biogenesis in skeletal muscle and improve exercise tolerance [28]. However, recent studies have found that resveratrol may not be a direct activator of SIRT1 [28a].

TORC [transducer of regulated CREB (cAMP-response-element-binding protein)-binding protein] 1—PGC-1α gene transcription is also strongly activated by TORC1, a co-activator of CREB. The other two members of the TORC family, TORC2 and TORC3, also strongly activate PGC-1α transcription. Indeed, it has been shown that forced expression of TORCs in primary muscle cells induced the endogenous mRNA of PGC-1α and its downstream target genes in the mitochondrial respiratory chain and TCA (tricarboxylic acid) cycle (Krebs cycle), providing further evidence for the key role of PGC-1α in mitochondrial biogenesis [29].

Calcineurin—Calcineurin is a calcium/calmodulin-dependent protein phosphatase known to be the master regulator of fast-to-slow twitch muscle-fibre-type changes [30]. Transgenic mice overexpressing calcineurin display a substantial increase in the number of slow-twitch muscle fibres, as well as an increase in skeletal muscle PGC-1α expression, which in turn leads to improved insulin action [31]. Conversely, inhibition of calcineurin by cyclosporin promotes slow-to-fast fibre-type transformation [30]. Calcineurin also up-regulates a large number of genes involved in mitochondrial energy metabolism in cultured cardiomyocytes [32]. However, cyclosporin, which is a calcineurin inhibitor, did not prevent the up-regulation of mitochondrial markers when administered to training rats [33]. Taken together, these results indicate that despite playing an important role in mitochondrial biogenesis, calcineurin cannot fully account for exercise-training-induced adaptations in skeletal muscle.

p38 MAPK (mitogen-activated protein kinase)—p38 MAPK has been suggested to play an important role in myogenic cell differentiation [34]. p38 MAPK shows increased activation following muscle contraction or running exercise protocols in rodents [35] and humans [36]. Transgenic mice specifically overexpressing p38 MAPK in skeletal muscle show enhanced PGC-1α expression and increased mitochondrial proteins [37]. Finally, an acute bout of exercise in mice or rats increases p38 MAPK, leading to PGC-1α activation [37, 38]. Taken together, these studies indicate that p38 MAPK may have an important role in exercise-induced mitochondrial biogenesis in skeletal muscle.

RIP140 (receptor-interacting protein 140)—Several transcriptional co-repressors, of which RIP140 is one, have been implicated in the regulation of mitochondrial biogenesis [39]. RIP140 is ubiquitously present in many tissues and its expression is regulated by various hormones. It is abundantly expressed in skeletal muscle such as extensor digitorum longus and gastrocnemius, which are rich in glycolytic (or fast-twitch, type II) and mixed muscle fibres respectively. In contrast, RIP140 mRNA is lower in oxidative (or slow-twitch, type I) muscles such as soleus and diaphragm [40]. Studies in RIP140-null mice indicate that RIP140 deficiency results in increased mitochondrial gene expression and oxidative capacity. These mice are lean and protected against diet-induced obesity due to increased energy expenditure [41]. These metabolic changes are accompanied by increased oxidative muscle fibres in the extensor digitorum longus. Transgenic expression of RIP140 in skeletal muscle results in more glycolytic muscle fibres and decreased mitochondrial gene

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expression [39]. These findings indicate that RIP140 and PGC-1α have opposite effects on the metabolic properties of skeletal muscle fibres. RIP140 regulates mitochondrial oxidative metabolism in a similar way in adipocytes. RIP140-null adipocytes have increased mitochondrial biogenesis and fatty acid oxidation, as well as increased UCP1 gene expression [42].

Sin3A—Sin3A is a core component of a multiprotein co-repressor complex and it has been shown that its deficiency increases the expression of genes involved in oxidative metabolism [43]. It is, however, not clear how the Sin3A transcriptional complex controls mitochondrial gene expression, but it may modulate nuclear receptor function through its interaction with the nuclear receptor co-repressor and silencing mediator of retinoid and thyroid hormone receptor co-repressor complexes [44]. Taken together, these data suggest that these co-activators and co-repressors play an important function in maintaining cellular oxidative metabolism through modulation of mitochondrial gene expression.

Tissue-specific abnormalities in mitochondrial biogenesis and their clinical relevance

Reduction in mitochondrial function has been found to be associated with many pathologies associated with aging, such as Type 2 diabetes and Alzheimer's disease [45].

Hepatic and intramyocellular lipid content can be measured non-invasively *in vivo* with ^{1}H-MRS (magnetic resonance spectroscopy). The rate of ATP synthesis can be assessed by direct observation of ^{31}P-magnetization transfer between P_i and ATP using phosphorus ^{31}P-MRS. Finally, ^{13}C spectroscopy can be used to trace the incorporation of [2-^{13}C]-labelled acetate into glutamate in skeletal muscle during a constant infusion of [2–^{13}C]acetate. The rate of incorporation reflects TCA cycle (Krebs cycle) activity providing an index of mitochondrial function [46, 47].

Using these methods, Petersen et al. [46] found reductions in mitochondrial oxidative and phosphorylation activity, as well as increased intramyocellular and intrahepatocellular lipid content and skeletal muscle insulin resistance in aging [46]. More recent studies using similar ^{13}C-MRS methods have found similar reductions in neuronal mitochondrial TCA metabolism associated with healthy aging [48]. It is not clear why mitochondrial dysfunction occurs with aging, but age-associated accumulation of mutations in mtDNA may play a role.

In further studies, Petersen and co-workers [47, 49] measured basal rates of mitochondrial function in human skeletal muscle of young, lean and sedentary insulin-resistant offspring of parents with Type 2 diabetes. They found an ~30% reduction in rates of ATP synthesis in muscle using ^{31}P-MRS [39], and a similar reduction in rates of muscle mitochondrial TCA flux using ^{13}C-MRS [47]. These young, lean and sedentary insulin-resistant subjects also exhibited increased intramyocellular lipid content, which has previously been associated with insulin resistance [50]. Muscle biopsy studies were then conducted to assess mitochondrial content in a similar group of young, lean and sedentary insulin-resistant offspring of Type 2 diabetic parents to assess whether reduced mitochondrial activity could be attributed to reduced mitochondrial content and/or mitochondrial dysfunction [51]. Mitochondrial density was assessed by electron microscopy and was reduced by 38% in the insulin-resistant offspring compared with the control subjects. These findings suggest that reduced mitochondrial activity may be due to reduced mitochondrial content, and provide further evidence to support the concept that hereditary mitochondrial dysfunction contributes to the development of insulin resistance and Type 2 diabetes. These data further suggest that alterations in nuclear-encoded genes regulating mitochondrial biogenesis may form a genetic basis for inheritance of at least some forms of Type 2 diabetes. In this regard two studies found reduced NRF-1-dependent gene expression and reduced PGC-1α and PGC-1β gene expression in skeletal muscle of Type 2 diabetic subjects and overweight first-

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degree relatives of Type 2 diabetic subjects [52, 53], but these results could not be replicated in a group of young lean insulin-resistant offspring of parents with Type 2 diabetes [51], suggesting that other factors are responsible for the reduced mitochondrial content in these individuals.

More recently Petersen et al. [54] have also found profound reductions in insulin-stimulated rates of muscle mitochondrial ATP production in insulin-resistant offspring, demonstrating that these individuals have alterations in both basal and insulin-stimulated mitochondrial metabolism.

It is noteworthy to mention that physical inactivity by itself can be a confounding factor regarding mitochondrial biogenesis, especially in Type 2 diabetes. Indeed, the above-mentioned studies describe skeletal muscle mitochondrial biogenesis in physical-activity-matched subjects, which were sedentary.

Taken together, these data demonstrate decreased mitochondrial activity, which most likely can be attributed to reduced mitochondrial content, in healthy young lean individuals prone to develop Type 2 diabetes. A key question which remains to be answered is whether reduced mitochondrial function causes the increase in intramyocellular lipid content and insulin resistance, or whether the reduction in mitochondrial function is secondary to the insulin resistance or some other factor. Although this is a critical question either way, given the key role that increased intracellular lipid has on the pathogenesis of insulin resistance this reduction in mitochondrial activity would be expected to exacerbate their insulin resistance.

Diabetes drugs and their potential role in mitochondrial biogenesis

TZDs (thiazolidinediones) are activators of PPARγ. TZDs include pioglitazone and rosiglitazone, which are frequently used in the treatment of Type 2 diabetes. Pioglitazone has been shown to induce mitochondrial biogenesis by the activation of the PGC-1α pathway in human subcutaneous adipose tissue [55], but also in HUVECs (human umbilical vein endothelial cells), along with a reduction of mitochondrial ROS (reactive oxygen species) [56]. Rosiglitazone treatment for 8 weeks also increased expression of PGC-1α and activity of oxidative enzymes in skeletal muscle of patients with Type 2 diabetes [57]. These effects of TZDs on skeletal muscle mitochondria are secondary to PPARγ activation in adipocytes, leading to a subsequent increase in adiponectin and mtDNA copy number in human muscles [58]. A potential direct effect of TZDs on muscle cannot be excluded. Indeed, a study showed that rosiglitazone increased AMPK activity in muscle in Type 2 diabetes and obesity [59]. Metformin, another frequently used drug in the treatment of Type 2 diabetes, has also been shown to increase the skeletal muscle content of PGC-1α in rats, suggesting increased mitochondrial biogenesis. These effects seem to be mediated, at least in part, by an increase in AMPK phosphorylation [60].

Caloric restriction and mitochondrial biogenesis

Caloric restriction without malnutrition is a well-known dietary intervention that consistently increases median and maximal life expectancy by delaying the aging process in a wide variety of species, including flies and mice [61]. Oxidative stress theory suggests that the aging process involves the accumulation of oxidative damage to mitochondria and other cellular components. Oxidative damage is induced by ROS, which are a by-product of mitochondrial OXPHOS, a process that is responsible for approx. 85–90% of cellular oxygen consumption [62] (Figure 2). However, this theory remains controversial, as some studies involving mouse models with respiratory chain deficiency induced by tissue-specific mtDNA depletion or by a massive increase of point mutations in mtDNA have very minor or

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no increase of oxidative stress [63]. Therefore future studies are needed to address the relative importance of mitochondrial dysfunction and ROS in aging.

Several studies have demonstrated that caloric restriction modulates cellular ROS production and damage to cellular macromolecules in various tissues. Studies in mammals have shown that caloric restriction reduces the generation of free radicals by mitochondria, as well as whole-body energy expenditure, and paradoxically induces mitochondrial proliferation [64]. In humans, a study examined the effect of caloric restriction on skeletal muscle mitochondrial function in non-obese humans and found that the expression of genes involved in the regulation of mitochondrial biogenesis, including PGC-1α, Tfam, eNOS and SIRT1, was increased in the caloric restriction group. In parallel, mitochondrial content was significantly increased in the caloric restriction group compared with the control group [65]. SIRT1-null mice are hypermetabolic, utilize ingested food inefficiently, have inefficient liver mitochondria and have elevated rates of lipid oxidation [66]. Their absence of lifespan extension has been interpreted as evidence that SIRT1 is required for the caloric restriction response [67]. AMPK may also play a role linking caloric restriction and mitochondrial biogenesis. Indeed, AMPK also regulates food intake by responding to hormonal and nutrient signalling in the hypothalamus [68]. Moreover, fasting by itself induces AMPK in skeletal muscle [69], although caloric restriction seems less efficient. Long-term caloric restriction in mice does not change AMPK activity in skeletal muscle or heart, but increases it in liver [70]. However, others have reported a decreased AMPK activity in rat liver [71]. Short-term caloric restriction has finally been shown to increase AMPK activity in the myocardium of young and old rats [72]. Thus caloric restriction may increase AMPK in rat heart, but not in skeletal muscle or liver.

These data suggest that caloric restriction increases whole-body energy efficiency by inducing the biogenesis of mitochondria that utilize less oxygen and produce less ROS. However, the exact mechanisms linking caloric restriction and up-regulation of mitochondrial mass and function remain poorly understood.

Conclusion

Although it has been known for a long time that physical activity is associated with increased mitochondrial content, critical factors involved in the regulation of mitochondrial biogenesis have only been recently identified. PGC-1α plays a central role and is a major regulator of this process. It is itself modulated by numerous factors, such as AMPK and p38 MAPK. These may or may not be induced by exercise. Given the potentially important role of mitochondrial dysfunction in the pathogenesis of numerous diseases and in the process of aging, future research aiming at understanding the molecular mechanisms regulating mitochondrial biogenesis and function may provide potentially important novel therapeutic targets to treat and prevent these diseases.

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Summary

- Mitochondrial biogenesis is regulated at the transcriptional and post-transcriptional levels of gene expression.
- PGC-1α is a co-transcriptional regulation factor that plays a central role in the regulation of mitochondrial biogenesis.
- Mitochondrial biogenesis is increased, among other factors, by endurance exercise and caloric restriction.
- Understanding the molecular mechanisms regulating mitochondrial biogenesis may provide important therapeutic targets to prevent and treat numerous diseases, such as Type 2 diabetes.

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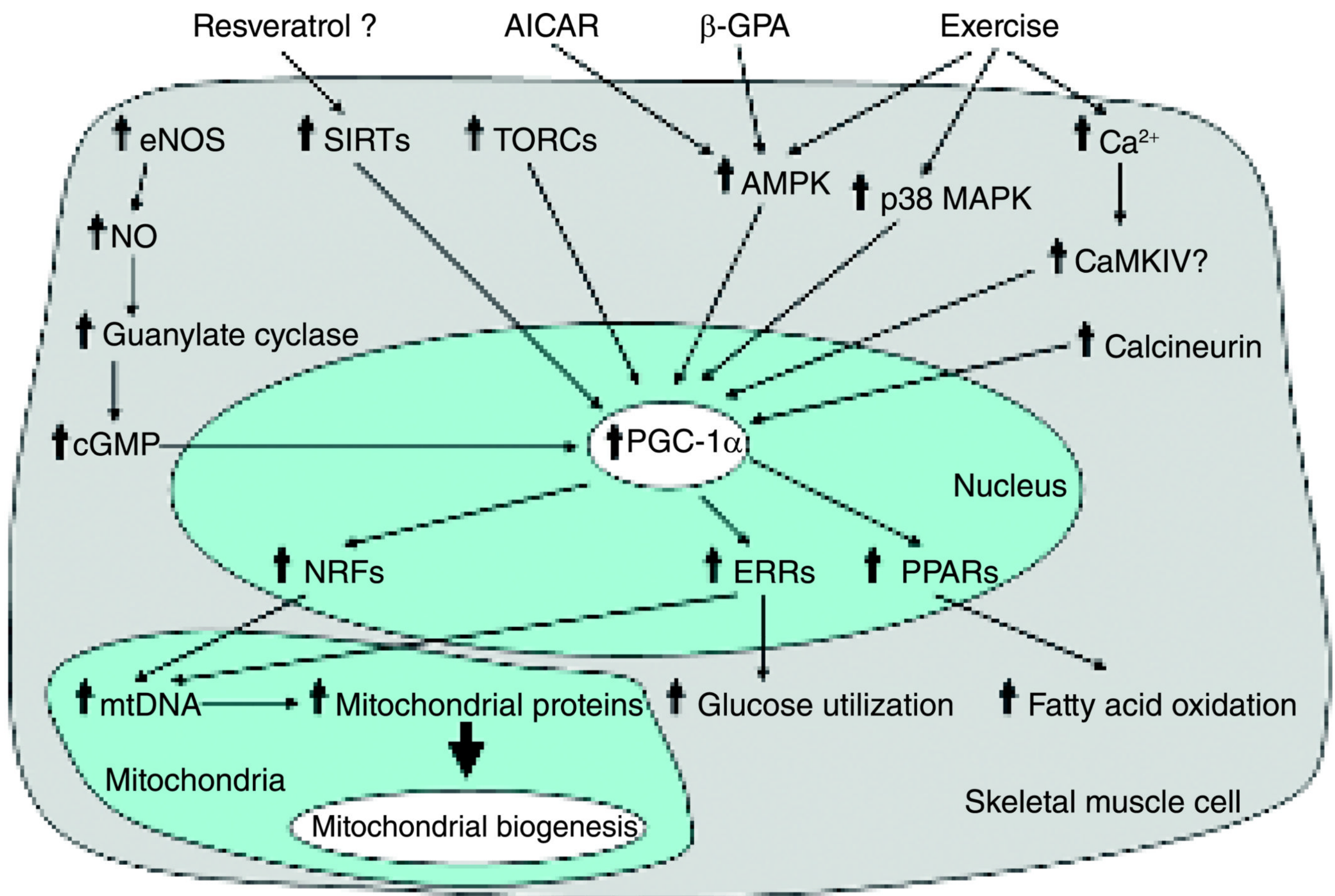

Figure 1. Pathways involved in mitochondrial biogenesis

eNOS, SIRTs, TORCs, AMPK and possibly CaMKIV cause an increase in PGC-1α gene transcription, which results in an increase in NRFs, leading to mtDNA expression and mitochondrial proteins, thus promoting mitochondrial biogenesis. An increase in PGC-1α also results in an activation of ERRs and PPARs.

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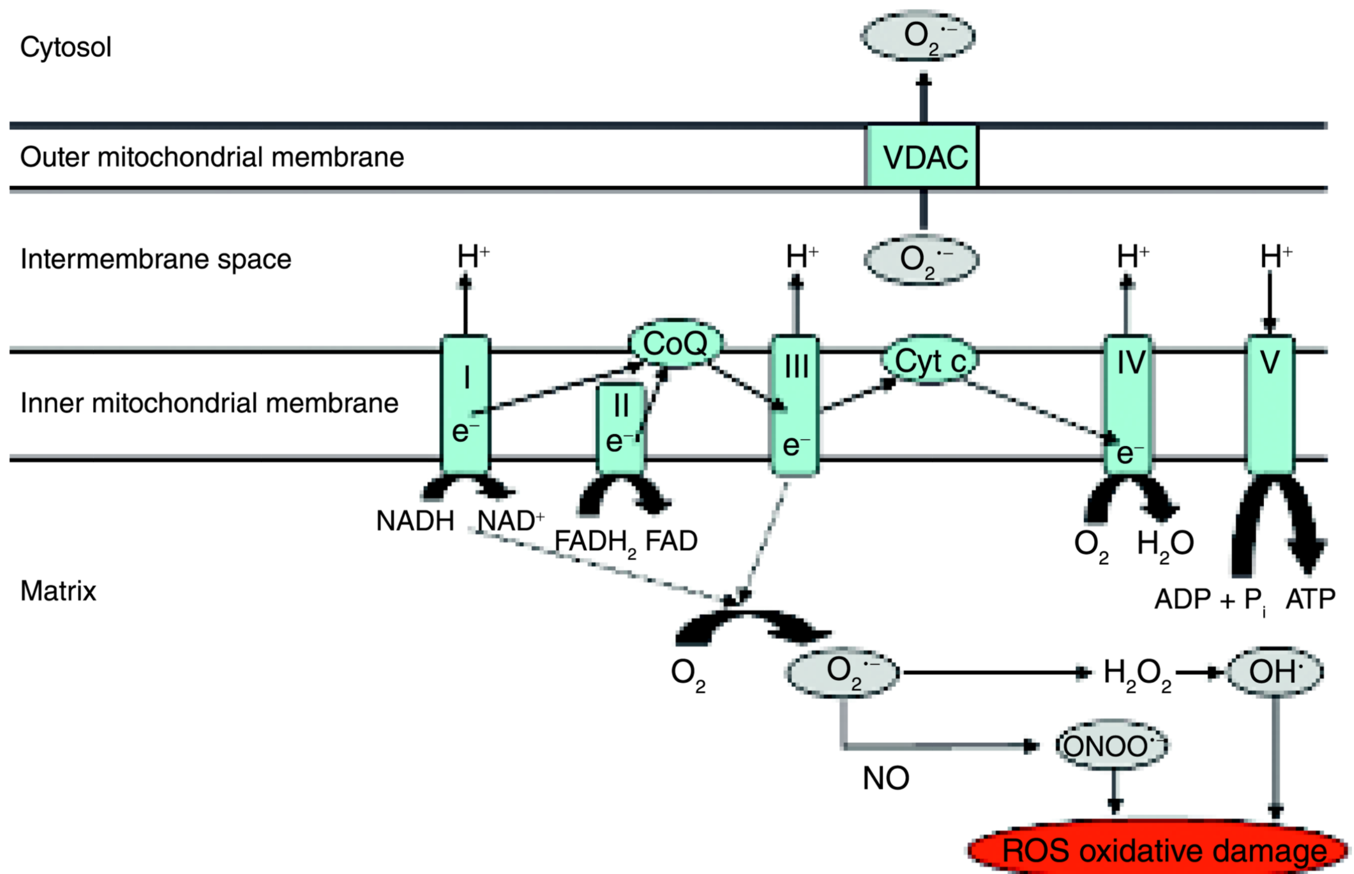

Figure 2. Superoxide production by the mitochondrial electron transport chain

Superoxide ($O_2^{\bullet-}$) anions are produced at complexes I and III of the electron transport chain. The superoxide released in the intermembrane space can enter the cytosol via the voltage-dependent anion channel (VDAC) and cause oxidative damage if not scavenged by superoxide dismutase. Hydrogen peroxide (H_2O_2) is converted into hydroxyl radical ($OH^\bullet$). Reactive nitrogen species can be formed with the interaction of superoxide with NO to produce peroxynitrite ($ONOO^{\bullet-}$). Superoxide, the hydroxyl radical and peroxynitrite are ROS that cause oxidative stress to organelles. CoQ, coenzyme Q; Cyt c, cytochrome *c*.