Mechanisms activating PGC-1α and consequential transcriptional mechanisms following exercise: A mini review

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Abstract

Skeletal muscle has the ability to adapt in response to exercise to increase the oxidative potential of the tissue. The adaptive response contributes to a favourable phenotype, increasing the ability of the tissue to augment superior fat oxidation and glucose uptake. In this respect, exercise, particularly endurance modes of exercise, forms a strong preventative and treatment strategy for metabolic diseases. The acute disturbance of homeostasis in response to muscle contraction during exercise stimulates a variety of intra-cellular mechanisms that signal to a putative stimulator of mitochondrial biogenesis, PGC-1α. The activation of these mechanisms is explored in this mini-review, drawing upon *in vivo, ex vivo and in vitro* data. Additionally, the effect of PGC-1α activation and the consequential transcriptional regulatory network is discussed in relation to stimulating mitochondrial biogenesis. Our understanding of these mechanisms has been hindered by the complex nature of the coordination of both nuclear and mitochondrial genomes. Further, the nature of *in vivo* experimentation with respect to exercise modalities and nutritional manipulation has often presented conflicting findings. Delineating the mechanisms further with highly controlled *in vivo* experiments, along with targeted *in vitro* experiments will define targets for potential genetic and pharmacological therapies in the future.

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Introduction

Skeletal muscle has the intrinsic ability to adapt in response to physical activity (exercise). At the onset of contraction, disturbances in muscle cellular homeostasis induce a broad spectrum of biochemical and molecular events. These events not only provide immediate energy supply for sustained contraction but also induce the synthesis of gene transcripts and proteins required for the adaptive response to change the phenotypic characteristics necessary to meet subsequent functional demands.

Increased glucose uptake and an alteration in substrate utilisation towards a greater reliance on fat metabolism, represent some of the fundamental adaptations in skeletal muscle metabolism in response to endurance exercise (Baldwin et al., 1972; Holloszy, 1967; Mole et al., 1971; Oscai and Holloszy, 1971). As such, endurance exercise has the ability to play a key role in the fight against epidemic metabolic disorders including; obesity, diabetes and hypertension. Despite the potential for physical activity in targeting these diseases, the exact cellular and molecular mechanisms that govern the adaptations have only recently begun to be understood (Coffey et al., 2006). The pioneering work of John Holloszy demonstrated an increase in mitochondrial content and enzymes following exercise, providing the basic mechanism behind an increase in aerobic capacity with exercise training (Holloszy, 1967). However, the complex nature of the mechanisms along with many experimental limitations has hindered progress with regards to the co-ordinated nature of the genes and proteins involved in regulating this response. In the post genomic era it is now possible to investigate the role of every candidate gene with regards to its effect in skeletal muscle adaptation. In light of this, a surge in research investigating the molecular adaptations to exercise has provided us with a broad spectrum of potential mechanisms.

The aim of this review is to discuss the acute biochemical, intra-cellular signalling and transcriptional responses to exercise that have been proposed to play key roles in the adaptive response known as mitochondrial biogenesis. Broadly, this term describes the process whereby new mitochondria are formed in the cell. The extant literature presents a very confusing nomenclature with regards to mitochondrial biogenesis, an issue that has been discussed recently (Miller and Hamilton 2012). The extent to which mitochondrial biogenesis is controlled through transcriptional or post translational mechanisms, including processes regulating mitochondrial protein synthesis and breakdown are yet to be fully elucidated. Therefore, the aim of this review is to explore the primary and secondary mechanisms activating a putative fundamental gene and protein in regulating the transcriptional control of mitochondrial biogenesis with particular focus on the transcriptional mechanisms proposed to play role in increasing mitochondrial DNA (mtDNA). Finally the ‘hot topics’ that are areas for future research are briefly outlined.

Signalling mechanisms to PGC-1α: 'The master regulator of mitochondrial biogenesis'

The peroxisome proliferator-activated receptor co-activator-1α (PGC-1α) has been identified as a tissue specific transcriptional co-activator capable of regulating the response of metabolic pathways to external stimuli (nutritional, physical and environmental) (Puigserver et al., 1998). The activity of this protein has therefore been proposed to play a primary role in the adaptive response to exercise. Over-expression of PGC-1α results in increased exercise capacity and peak oxidative capacity, underpinned by increases in mtDNA, mitochondrial gene expression and mitochondrial enzyme activity (Calvo et al., 2008). Furthermore, transgenic mice over-expressing PGC-1α at physiological levels, preferentially express Type I muscle fibres and have increased mitochondrial protein content (Lin et al., 2002). In contrast, the selective deletion of PGC-1α in skeletal muscle attenuates the exercise induced increases in mitochondrial enzymes (Geng et al., 2010). The mechanisms activating this co-activator to stimulate these responses are varied and complex and therefore it is beyond the scope of this review to detail every proposed mechanism.

Ca^{2+} and p38 MAPK

A variety of *in vitro* and *in vivo* approaches have demonstrated a role for Ca^{2+} as a signalling molecule, which is not surprising considering the inherent fluctuation in transients associated with repeated contraction. The activated mechanisms are complex in nature, therefore only highlighted investigations and specific proteins are discussed. Following the depolarisation of the cell surface membrane, Ca^{2+} is released from the sarcoplasmic reticulum to initiate contraction; a process termed Excitation-Contraction Coupling (Dulhunty, 2006). Despite the well characterised role for Ca^{2+} in ECC, a further role for this ion in regulating contraction induced-gene expression and muscle fibre phenotypes has been proposed (Berchtold et al., 2000). This hypothesis stemmed from the seminal work of Buller et al., (1960) who demonstrated the change in phenotype of skeletal muscles upon the transplantation of an opposing motor nerve (i.e. fast to slow and *vice versa,* Buller et al., 1960). Force output and longevity is in part controlled and driven by motor nerve stimulation, which relates to the amplitude and duration of Ca^{2+} transients (Chin, 2005). The particular expression and level of expression of genes directly relates to the Ca^{2+} transient, a term that has been described as excitation-transcription coupling (Chin, 2005; Gundersen, 2011). A large number of genes have been suggested to be activated upon increases in Ca^{2+} transients including; Myosin Heavy Chain (MHC) isoforms (Allen et al., 2002), Glucose Transporter 4 (GLUT4, Ojuka et al., 2002b; Olson and Williams, 2000) and genes related to mitochondrial biogenesis (Ojuka et al., 2002a).

Investigations into the acute effects of Ca^{2+} transients upon skeletal muscle related to exercise have specifically taken the form of *in vitro* based models, using Ca^{2+} stimulants including Caffeine and Ca^{2+} ionophores. Raising intra-cellular Ca^{2+} in L6 rat skeletal muscle myotubes increases the expression of proteins involved in mitochondrial biogenesis, an effect that was blocked by dantrolene, which prevents the release of Ca^{2+} from the sarcoplasmic reticulum (SR) (Ojuka et al., 2003). Furthermore, ionophore A23187 increases the gene expression of cytochrome *c* in L6 myotubes, suggesting a role for Ca^{2+} in regulating this gene transcript associated with oxidative phosphorylation (Freyssenet et al., 1999).

Ex vivo skeletal muscle preparations have further provided a mechanism of action for increases in genes and proteins associated with mitochondrial biogenesis. Increasing cytosolic Ca^{2+} using 3.5mM caffeine in rat epitrochlearis, does not elicit contraction as the Ca^{2+} concentrations do not reach a required threshold for contraction (Wright, 2007). This does however lead to significant increases in gene and protein expression of PGC-1α, COX-I (cytochrome *c* oxidase I) and ALAS (Wright, 2007). This increase is coupled with an increase in p38 MAPK, a response which is negated upon incubation with KN93, a Calmodulin Kinase (CaMK) inhibitor (Wright, 2007). Together, these data suggest a clear signalling cascade involving the Ca^{2+} activation of CaMK leading to the activation of p38 MAPK. The increase in the mitochondrial enzymes was also completely diminished by the addition of a p38 MAPK inhibitor SB202190, suggesting a p38 MAPK-dependent mechanism of action (Wright, 2007). Recently, isoform specific data has revealed a significant role for p38γ MAPK in the adaptive response of mitochondrial biogenesis and vascular angiogenesis. Using a muscle specific gene deletion in mice, a dominant negative allele for p38γ MAPK but not p38α MAPK and p38β MAPK, blocked the phenotypic and underpinning transcriptional mechanisms (PGC-1α and vascular endothelial growth factor, VEGF) to motor nerve stimulated contraction (Pogozelski et al., 2009). The specific activation of PGC-1α following activation of p38 MAPK has been revealed with the overexpression of MAPK Kinase 3 and MAPK Kinase 6, which are upstream p38 MAPK activators (Akimoto et al., 2005).

In vitro experiments demonstrated that over-expression of MKK3 increases PGC-1α promoter activity and over-expression of muscle specific MKK6 *in vivo* increases PGC-1α protein content in type II skeletal muscle fibres (Akimoto, et al., 2005). These findings provide evidence for the mechanism in which the cell signal is transduced through p38 MAPK to increase both the gene expression and protein content of PGC-1α. Not only does the activation of PGC-1α stimulate the transcription and activation of a multitude of transcription factors (discussed later), it has been shown that it can work in an auto-regulatory loop stimulating its own promoter activity through the induction of myocyte enhancer factor-2C and –D (Handschin et al., 2003). The *in vitro, ex vivo* and *in vivo* data presented here provides substantiation for the role of Ca^{2+} in activating a signalling cascade through CaMK and p38γ MAPK to induce the activation of PGC-1α to increase mitochondrial biogenesis and metabolic adaptation.

AMPK

Muscle contraction is sustained by the production of ATP, generated from oxidation of stored and non-stored fuels sources alike; principally plasma glucose, stored muscle glycogen, plasma lipids and intramuscular triglycerides. The 5' AMP-activated protein kinase (AMPK) is activated in response to a reduction in high energy phosphates (Xiao et al., 2011) and hence acute energy deprivation. As such this protein has received attention as a putative energy sensor (Reznick and Shulman, 2006). The main function for AMPK is to increase the potential for ATP synthesis, whilst inhibiting energy consuming anabolic processes, along with acting as a signalling protein for gene transcription during and following rapid energy depletion (Jorgensen et al., 2006).

Given this function of AMPK, it is not surprising that it has received widespread attention with respect to responses to exercise, a potent energy depleting stimulus. Various forms of experimental design have been used to investigate the role for AMPK as a signalling protein to activate PGC-1α. Pharmacological agents have provided a tool to understand the influence of activation of AMPK in regulating increases in mitochondrial enzymes, increasing the potential for oxidative phosphorylation production of ATP. Bergeron et al., (2001) used *β*-guanadinopropionic acid (*β*-GPA) to chronically activate AMPK in rats for 8 weeks. Data demonstrated the increased

binding of nuclear respiratory factor-1 (NRF-1) to the promoter region of ALAS, along with an increase in cytochrome *c* protein expression and total mitochondrial content (Bergeron et al., 2001). Moreover, a dominant negative form of AMPK in mice ablates the *β*-GPA stimulated increases in mitochondrial content in WT littermates (Zong et al., 2002). These data provide key mechanistic evidence for the role of AMPK activation and subsequent signalling in inducing mitochondrial biogenesis through the activation of NRF-1 DNA binding activity (a downstream target of PGC-1α).

The use of 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR) to stimulate AMPK activation both in vitro and *in vivo*, without the need to exercise, has also demonstrated a clear association between AMPK activation of PGC-1α and consequential increases in mitochondrial proteins (Leick et al., 2010). Repeated injection of AICAR resulted in increases in cytochrome *c* oxidase I and cytochrome *c* protein expression (relative to a saline vehicle control) in white gastrocnemius of wild type (WT) animals, but not in whole body PGC-1α knock-out mice (Leick et al., 2010). Experiments using primary skeletal muscle cells and mice deficient in PGC-1α have demonstrated specific phosphorylation sites on the PGC-1α protein required for its activation by AMPK (Jager et al., 2007). Moreover, the threonine-177 and serine-538 phosphorylation sites have been shown to be necessary for the PGC-1α-dependent induction of the PGC-1α promoter (Jager, et al., 2007). These data together offer a clear pathway of AMPK-PGC-1α activation for increases in the mitochondrial proteins measured.

Evidence for the exercise/contraction specific activation of AMPK-PGC-1α has been established using *ex vivo* and *in vivo* models. Atherton et al., (2005) used low frequency stimulation (LFS) of isolated rat extensor digitorum longus (EDL) to mimic endurance exercise and high frequency stimulation (HFS) to mimic resistance exercise. AMPK was activated at 3 hrs post LFS, whilst HFS failed to increase AMPK activity (Atherton et al., 2005). *In vivo* data has provided contrasting findings with regards to the role of endurance and resistance exercise activation of AMPK, with particular emphasis on training status. Some indication for the training specific nature of AMPK activation was shown by Coffey et al., (2006), who demonstrated an increase in AMPK activation following endurance exercise in non-endurance trained weight lifters, whilst no AMPK activation was evident in endurance trained athletes (Coffey et al., 2006).

Conversely, AMPK was activated in endurance trained athletes that undertook strength training, whilst no activation was observed in strength trained athletes (Coffey et al., 2006). This data suggests the activation of AMPK is dependent on a novel stimulus to induce the metabolic milieu required. Contrary to this data, it has been shown that 10 weeks of training is not sufficient to suppress the activation of AMPK in either strength or endurance trained athletes, data that is coupled with increases in mitochondrial protein fractional synthetic rate (Wilkinson et al., 2008). Together these data demonstrate the necessity for AMPK activation during and following exercise for the resulting increases in mitochondrial protein, leading to enhanced oxidative potential for future periods of metabolic challenge.

Reactive oxygen and nitrogen species (ROS and RNS)

It has become clear that skeletal muscle contraction stimulates both ROS and RNS (Powers and Jackson, 2008). Despite early concerns surrounding the possible negative influence of ROS and RNS on skeletal muscle structure and function, it is now clear that these free radicals play an important role in the physiological adaptation of skeletal muscle to exercise (Powers et al., 2011). Indeed, the production of free radicals has been associated with disuse skeletal muscle atrophy (Min et al., 2011), whilst moderate increases in reactive species production allow for the cellular signalling pathways required for mitochondrial biogenesis, amongst others. It may therefore be suggested that acute increases in ROS and/or RNS may have positive roles in the adaptive response to exercise, whilst prolonged increases in ROS and RNS may result in the activation of signalling cascades associated with skeletal muscle tissue breakdown, such as the observations in the aged population.

PGC-1α has been termed 'redox sensitive', in that it has been shown on many occasions to be activated upon the increase in reactive species with muscle tissue or cells. In C_2C_{12} skeletal muscle cells, increasing the production of ROS by incubation with 300 μM hydrogen peroxide (H_2O_2), results in increased PGC-1α promoter activity and PGC-1α mRNA expression requiring the activation of AMPK (Irrcher et al., 2009). The use of this model in understanding the role of ROS in muscle adaptation provides evidence for the mechanisms activated; however the nature of synthesis of the ROS and their effect or longevity may be considerably different to *in vivo* skeletal muscle.

Nitric oxide (NO), a potent signalling molecule also stimulates the PGC-1α expression in L6 rat myotubes; a mechanism that is dependent upon NO-activated AMPK activity, demonstrated by a reduced effect with co-incubation of specific inhibitor of AMPK (Lira et al., 2010). These *in vitro* experiments provide mechanistic evidence for the role of both ROS and RNS in the activation and transcription of PGC-1α, mediated through the activation of AMPK. A body of *in vivo* evidence has developed in recent years with respect to the roles of ROS and RNS in mitochondrial biogenesis. Sprint exercise in rats increases xanthine oxidase and ROS, inducing significant increases in PGC-1α, NRF-1 and mitochondrial transcription factor A (Tfam) content (Kang et al., 2009) proteins required for mitochondrial biogenesis. Upon the administration of allopurinol (a specific xanthine oxidase inhibitor) this effect was abolished, thus providing evidence for a redox sensitive activation of PGC-1α (Kang et al., 2009). However, evidence for the roles of specific antioxidants in preventing the mechanisms involved in mitochondrial biogenesis are unclear. Gomez-Cabrera et al., (2008) demonstrated a reduction in key signalling mechanisms; PGC-1α protein expression, along with reductions in NRF-1 and Tfam mRNA expression compared to placebo control, following supplementation of vitamin C during a six week endurance protocol in rats.

Contradictory to these findings, Wadely and McConnell (2010) demonstrated that 7 days prior supplementation of vitamin C did not prevent the acute increases in PGC-1α, NRF-1, NRF-2 and Tfam mRNA (Wadley and McConell, 2010). Similarly, Higashida et al., (2011) supplemented rats with a combination of both vitamin C and E for 9 days, with no reduced effect of exercise on the final 3 days of supplementation on the expression of COX I and IV; components of the respiratory chain (Higashida et al., 2011). This disparity in findings may be a result of the shorter duration of supplementation of vitamin C and E resulting in a reduced total antioxidant effect. This notion is supported by the findings of Strobel et al., (2011), where supplementation of vitamin E and α-lipoic acid during a 14 week exercise period prevented increases in genes and proteins required for mitochondrial biogenesis (Strobel et al., 2011). Collectively, this data suggests a clear effect of duration of supplementation and may provide evidence for both acute transient and more chronic roles for ROS in activating mitochondrial biogenesis.

Notwithstanding the evidence suggesting a role for a reduced signalling response relating to mitochondrial biogenesis, the effect upon increasing oxidative capacity and $\dot{V}O_{2max}$, relating to gross physiological function is a point of contention (Gomez-Cabrera et al., 2008); Yfanti et al., 2010). The influence of RNS in the adaptation of skeletal muscle has not received as much attention, however evidence suggests a significant effect of this group of reactive species. Administration of N(G)-nitro-l-arginine methyl ester (l-NAME, a nitric oxide synthase (NOS) inhibitor) to rats for two days reduced basal Tfam mRNA in the soleus, COX I and COX 4 mRNA expression along with COX IV protein expression in the EDL (Wadley and McConell, 2007). Evidence for the isoform specific NOS regulation of mitochondrial biogenesis has demonstrated that mice deficient in neuronal NOS (nNOS), display greater levels of endothelial NOS (eNOS) and *vice versa* (Wadley et al., 2007), suggesting a compensatory increase in the opposing NOS isoform depending on the deficiency. nNOS-/- mice displayed significantly higher basal levels of Tfam, NRF-1 and NRF-2 mRNA in soleus and EDL, with increases in citrate synthase activity and PGC-1α higher in the EDL alone (Wadley et al., 2007). eNOS-/- mice displayed significantly higher citrate synthase activity in the soleus but not in the EDL, whilst exercise did not activate markers of mitochondrial biogenesis to a greater or lesser extent in either nNOS-/- mice or eNOS-/- mice compared to WT (Wadley et al., 2007). This suggests NOS isoforms have specific roles in regulating mitochondrial biogenesis in the rested state, with no identifiable influence on exercise induced mitochondrial biogenesis.

PGC-1α: Transcriptional mechanisms

Mitochondrial biogenesis occurs through the co-ordinated expression of both nuclear and mitochondrial genomes, with the transcriptional mechanisms relating to this response detailed here. Upon the activation of PGC-1α following exercise through the many mechanisms discussed here, it co-activates transcription factors required to induce mitochondrial biogenesis. PGC-1α activates many genes indirectly associated with increasing mitochondrial respiratory chain content, including NRF-1 and NRF-2 (Scarpulla, 2011). PGC-1α has been shown to activate the estrogen-related receptor-α (ERRα) and in conjunction with NRF-2α increases the expression of respiratory chain genes (Scarpulla, 2006; Zhang et al., 2006). The gene and protein content of cytochrome *c* has also been used in a multitude of experiments as a marker of mitochondrial density, as it forms a key element of the electron transport across the mitochondrial membrane. Therefore, understanding the transcriptional regulation of this gene is key to determining what regulates mitochondrial density. Evans and Scarpulla (1989) identified key elements on the promoter region of the cytochrome *c* gene, one of which binds NRF-1 protein, along with a cAMP response element (CRE) site (Evans and Scarpulla, 1989). If the NRF-1 or CRE sites are mutated, any effect in cytochrome *c* gene transcription to electrical stimulation of skeletal muscle is attenuated, suggesting an NRF-1 and CRE dependent mechanism for the increased transcription of cytochome *c* (Xia et al., 1998). The co-ordinated expression of NRF-1 and NRF-2 also activates Tfam, which co-ordinates the transcription of mitochondrial DNA (mtDNA, Scarpulla, 2008). Tfam has also been shown to play a significant role in mtDNA replication, providing an RNA primer required for initial mtDNA synthesis (Hood, 2001).

The MEF-2 is a transcription factor required for the expression of many muscle specific genes, including the regulation of the glucose transporter-4 (GLUT-4) promoter (Ojuka et al., 2002b) and subunits of the respiratory chain COXVIaH and COXVIII (Lenka et al., 1996). The transcriptional activity of MEF-2 is controlled as a balance between suppression by histone deacetylases (HDAC's) and activation by PGC-1α and p38 MAPK (McGee and Hargreaves, 2004). Following cycling exercise HDAC-5 is exported from the nucleus, allowing for the association between PGC-1α and MEF-2 (McGee and Hargreaves, 2004). Moreover, at rest the majority of PGC-1α is found in cytosolic fractions, translocating to the nucleus 3 hours following an acute bout of high intensity exercise (Little et al., 2011), an association that is related with increased levels of mitochondrial protein content 24 hours following exercise (Safdar et al., 2011). These data together provide strong evidence for the role of PGC-1α in the activation of MEF-2 and nuclear encoding genes associated with inducing mitochondrial biogenesis.

PGC-1α independent mitochondrial biogenesis, gene splicing and future directions

There is evidence to suggest mitochondrial biogenesis can occur independently of PGC-1α, as demonstrated by PGC-1α gene knockout (KO) studies. Of note Leick et al., (2008) reported increases in cytochrome *c*, ALAS1 and COXI protein expression following 5 weeks of exercise training in the PGC-1α KO mice. However, it is important to note that in the rested state these animals have reduced protein and gene expression of cytochrome *c*, ALAS1 and COXI and the increases in protein expression observed in the exercise condition are 20% lower compared to WT controls (Leick et al., 2008). Interestingly cytochrome *c* mRNA was up-regulated in WT soleus muscle only (Leick, et al., 2008), suggesting the requirement of PGC-1α for the transcription of this gene in this particular muscle. These data provide evidence for alternative mechanisms of action for stimulating mitochondrial biogenesis, whilst clearly demonstrating the necessity for PGC-1α to obtain the greatest response. Exercise studies in humans have also demonstrated that the expression of PGC-1α mRNA does not occur and hence is not associated with the changes in phenotype observed following endurance training (Timmons et al., 2010). It has been suggested that increases in proteins that regulate phenotype are increased through multiple transient increases in gene expression. In light of this, increases in PGC-1α mRNA may occur at early time points during a training protocol contributing to the adaptation in phenotype, whilst further training may not provide any enhanced stimulus to induce the transcription of PGC-1α. Future experiments should investigate the transient increases in PGC-1α related to potential for adaptation, along with delineating the extent to which PGC-1α alone modulates mitochondrial adaptations.

Given the recent evidence demonstrating the localisation of PGC-1α in particular cellular compartments immediately and hours post exercise (Little et al., 2011; Safdar et al., 2011), the identification of a novel truncated form of PGC-1α (NT-PGC-1α) that appears to be missing key domains that regulate nuclear localisation and interaction with transcription factors (Zhang, et al., 2009) warrants further investigation. The characterisation of alternate splice forms of the PGC-1α gene are now being investigated, with isoform specific effects being demonstrated in pharmacological (Miura et al., 2008) and exercise studies (Norrbom et al., 2011). The control mechanisms regulating this alternative splicing, including preventing the nuclear localisation of PGC-1α, may be apparent in individuals who respond with reduced effect to endurance training. Further, such mechanisms may have a role in metabolic diseases characterised by mitochondrial dysfunction. Research utilising *in vitro* approaches to investigate the effect of each isoform may provide further evidence as to the effect of each isoform singularly and collectively.

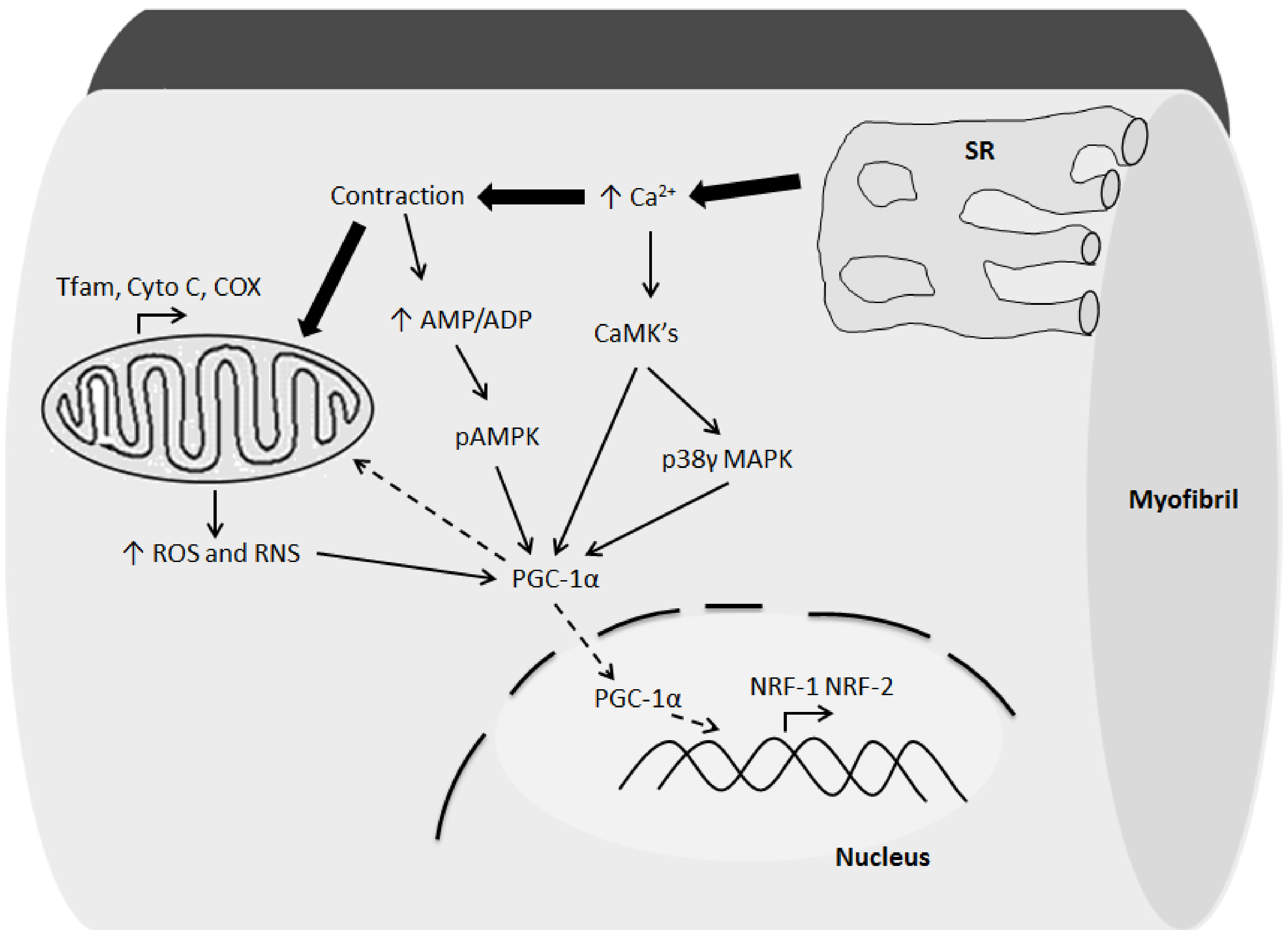

Figure 1. *Intra-cellular signalling mechanisms regulating PGC-1α activation and post-activation transcriptional mechanisms*. Abbreviations: SR (sarcoplasmic reticulum), Ca^{2+} (Calcium ion), CaMK's (calmodulin kinases), p38γ MAPK (p38 gamma mitogen activated protein kinase), PGC-1α (peroxisome proliferator-activated receptor co-activator-1 alpha), NRF-1 and NRF-2 (nuclear respiratory factors 1 and 2), AMP (adenosine mono-phosphate), ATP (adenosine tri-phosphate), pAMPK (phosphorylated 5' AMP-activated protein kinase), Tfam (mitochondrial transcription factor A, mTFA), Cyto C (cytochrome *c*), COX (cytochrome *c* oxidase subunits), ROS (reactive oxygen species), RNS (reactive nitrogen species).

Summary and conclusion

Exercise is a potent stimulator of mitochondrial biogenesis, a compensatory mechanism to increase the oxidative potential of skeletal muscle. In doing so, skeletal muscle has the greater potential to oxidise fat and as such, exercise forms a key preventative and treatment modality for metabolic diseases.

The molecular mechanisms that govern this response have begun to be elucidated; however the co-ordination of *in vivo* and targeted *in vitro* based experiments will provide further evidence into this complex cellular adaptation.

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