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Symposium 3: Mechanisms involved in exercise-induced mitochondrial biogenesis in skeletal muscle

Involvement of PPARγ co-activator-1, nuclear respiratory factors 1 and 2, and PPARα in the adaptive response to endurance exercise

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Endurance exercise training induces an increase in the respiratory capacity of muscle, resulting in an increased capacity to generate ATP as well as improved efficiency of muscle contraction. Such adaptations are largely the result of a coordinated genetic response that increases mitochondrial proteins, fatty acid oxidation enzymes and the exercise- and insulin-stimulated glucose transporter GLUT4, and shifts the contractile and regulatory proteins to their more efficient isoforms. In recent years a number of the transcriptional regulators involved in this genetic response have been identified and these factors can be classified into two different groups. The first group comprises transcription factors such as nuclear respiratory factors (NRF) 1 and 2 and PPARα that bind DNA in a sequence-specific manner. The second group, referred to as transcriptional co-activators, alter transcription without directly binding to DNA. The PPARγ co-activator (PGC) family of proteins have been identified as the central family of transcriptional co-activators for induction of mitochondrial biogenesis. PGC-1α is activated by exercise, and is sufficient to produce the endurance phenotype through direct interactions with NRF-1 and PPARα, and potentially NRF-2. Furthering the understanding of the activation of PGC proteins following exercise has implications beyond improving athletic performance, including the possibility of providing targets for the treatment of frailty in the elderly, obesity and diseases such as mitochondrial myopathies and diabetes.

Mitochondrial biogenesis: Skeletal muscle: Transcriptional co-activators

Mitochondria are the site of oxidative energy production in eukaryotic cells. Within the mitochondrial matrix, enzymes oxidize fatty acids and carbohydrates producing the reducing equivalents, NADH and $FADH_2$. These reducing equivalents are then used to produce a proton gradient across the inner mitochondrial membrane. Dissipation of this gradient through the F_0F_1-ATPase results in the resynthesis of the ATP that drives every energy-dependent process in the cell.

Changes in metabolic demand can directly alter the concentration of mitochondria within the cell. Proliferation of mitochondria occurs in muscle in response to endurance exercise training (Holloszy, 1967), chronic electrical stimulation (Reichmann *et al*. 1985) and thyroid hormone (Irrcher *et al*. 2003), while loss of mitochondria is associated with inactivity (Wibom *et al*. 1992) and aging (Rooyackers *et al*. 1996).

The present review will describe the transcriptional control of mitochondrial biogenesis with particular emphasis on the adaptations to endurance exercise through nuclear respiratory factors (NRF) 1 and 2, PPARα and the PPARγ co-activator (PGC) family. A more global perspective of mitochondrial biogenesis can be found in a number of very good recent reviews (Hood, 2001; Scarpulla, 2002; Puigserver & Spiegelman, 2003).

Exercise and mitochondrial biogenesis

All mitochondrial enzymes and the insulin- and contraction-induced glucose transporter GLUT4 are increased by

Abbreviations: NRF, nuclear respiratory factor; PGC, PPARγ co-activator.

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50–100% following endurance exercise training (Holloszy *et al*. 1998). This coordinated increase in mitochondrial proteins is impressive because, unlike any other process in the human body, mitochondrial biogenesis requires the coordination of two distinct genomes.

Mitochondria are semiautonomous organelles that contain their own genome. Their circular genetic material encodes thirteen electron transport chain proteins, and the tRNA and rRNA required for their translation. The nucleus encodes the remaining proteins of electron transport, as well as enzymes for the synthesis of haem, oxidation of pyruvate, ketones and fatty acids, and the expression and replication of mitochondrial DNA. While elucidation of how this process is controlled has been ongoing for >30 years, improved molecular techniques have enabled substantial advances in the last 5 years.

The current paradigm for the control of mitochondrial biogenesis involves two key classes of nuclear regulatory proteins. The first class is made up of transcription factors that bind to sites within the promoter region of mitochondrial genes. The binding of these factors is sequence-specific and functions to identify the genes to be transcribed. The second class of regulatory proteins is made up of a family of transcriptional co-activators. Unlike transcription factors, co-activators do not bind DNA. Instead, co-activators bind to transcription factors and promote the formation of a protein complex. This protein complex includes molecules that contain histone acetyltransferase activity. Histone acetyltransferase proteins function to change the local structure of the chromosome, allowing the DNA to unwind and thus promoting the binding of RNA polymerase II and the initiation of transcription. The transcription factors and co-activators required for mitochondrial biogenesis and the effects of exercise on their activities will be described in detail.

Transcription factors

The first insight into how the expression of mitochondrial proteins are controlled came from the identification of NRF-1 and -2 by Scarpulla and his colleagues (Scarpulla, 2002). Binding sites for these proteins are present in many of the respiratory genes, as well as genes for haem biosynthesis, mitochondrial protein import and mitochondrial DNA transcription, replication and splicing (Table 1), implicating these proteins in the coordination of mitochondrial biogenesis. In order to determine the function of NRF-1 in mitochondrial biogenesis a transgenic mouse that overexpresses NRF-1 in skeletal muscle has recently been generated (Baar *et al*. 2003). These mice have increased levels of cytochrome c, 5-aminolevulinate synthase and ubiquinol–cytochrome c reductase. However, neither the levels of cyclooxygenase-IV, citrate synthase or succinate:ubiquinol oxidoreductase, nor the rate of O_2 consumption of these muscles were increased. This finding suggests that NRF-1 alone is not sufficient to induce complete mitochondrial biogenesis and that other factors and/or cofactors are required for this process.

The PPAR are a family of proteins involved in the proliferation of peroxisomes, organelles that function to eliminate toxic substances from the body. Peroxisomes are similar to mitochondria in that they have a crystalline internal structure, contain oxidative enzymes and self-replicate. Gulick *et al*. (1994) and other researchers (Vega *et al*. 2000) have demonstrated that PPARα also controls the expression of key mitochondrial fatty acid and β-oxidation genes.

Table 1. A representative list of genes containing consensus binding sites for nuclear respiratory factors (NRF) 1 or 2, or both transcription factors (adapted from Scarpulla, 2002)

Category and target gene	NRF-1	NRF-2
Oxidative phosphorylation		
Rat cytochrome c	+	
Complex I		
Human NADH dehydrogenase subunit 8 (TYKY)	+	
Complex II		
Human succinate dehydrogenase subunit B	+	+
Complex III		
Human ubiquinone-binding protein	+	
Complex IV		
Rat cytochrome oxidase subunit IV		+
Rat cytochrome oxidase subunit Vb	+	+
Complex V		
Bovine ATP synthase g subunit	+	
mtDNA transcription and replication		
Human TFAM	+	+
Mouse MRP RNA	+	
Human MRP RNA	+	
Haem biosynthesis		
Rat 5-aminolevulinate synthase	+	
Mitochondrial protein import machinery		
Human Tom 20	+	+
Protein synthesis		
eIF 2α	+	

TFAM, mitochondrial transcription factor A; MRP, mitochondrial RNA-processing enzyme; Tom, translocase of the outer mitochondrial membrane; eIF 2α, eukaryotic initiation factor 2α; +, binding site present.

Transcriptional co-activators

PGC-1α is a transcriptional co-activator that was first discovered by Spiegelman's group (Puigserver *et al*. 1998) as a cold-inducible protein that binds to PPARγ and increases the expression of uncoupling protein 1 in the brown fat of mice. While PGC-1α mRNA was not detected in resting muscle in this study, on exposure to cold muscle PGC-1α became detectable. In contrast, Boss *et al*. (1999) detected three PGC-1α mRNA in control skeletal muscle that did not increase with cold. Since the initial characterization of PGC-1α, two homologous proteins have been described: PGC-1β (Lin *et al*. 2002*a*) and the PGC-1-related cofactor (Andersson & Scarpulla, 2001). These proteins have different tissue distributions and are activated by different stimuli, suggesting that each member may have a distinct tissue-specific role.

PGC-1α overexpression in C_2C_{12} cells induces a 2- to 3-fold increase in the expression of respiratory genes, NRF-1, NRF-2 and mitochondrial transcription factor A. Beyond the control of mitochondrial proteins, PGC-1α may also increase the expression of GLUT4 (Michael *et al*. 2001), although this suggestion has recently been

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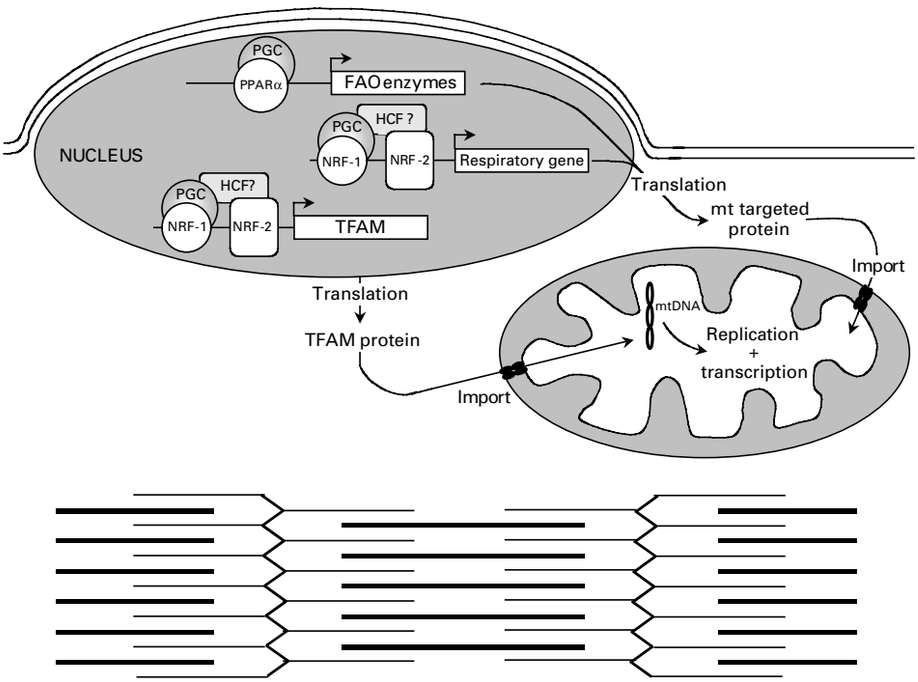

Fig. 1. A model of the transcriptional control of mitochondrial biogenesis in muscle. The schematic depicts the central role of PPARγ co-activator (PGC) family members in the regulation of mitochondrial transcription via transcription factor co-activation. The co-activation of PPARα plays an important role in regulating the transcription of enzymes of the citric acid cycle and fatty acid oxidation. Nuclear respiratory factors (NRF) 1 and 2 are important in the regulation of mitochondrial (mt) DNA transcription and replication, as well as in the expression of proteins of the electron transport chain, glucose transport and haem biosynthesis. The schematic includes the interaction between host cell factor (HCF) and PGC and NRF-2, even though this interaction has yet to be demonstrated experimentally. FAO, fatty acid oxidation; TFAM, mitochondrial transcription factor A.

challenged in transgenic animals (Miura *et al*. 2003). Nonetheless, a PGC-1α-induced increase in GLUT4 would explain why mitochondrial biogenesis following exercise is associated with an increase in this glucose transporter. Another interesting effect of PGC-1α overexpression is its ability to induce an approximate 10% shift in muscle fibre type from fast to slow (Lin *et al*. 2002*b*). However, it is unclear whether this shift is a direct effect of PGC-1α or an indirect effect associated with a shift in metabolism within the cell. The alterations in muscle phenotype are the result of a direct interaction between PGC-1α and a number of important transcription factors, including NRF-1, PPARα and myocyte-enhancing factor-2, and similar to the effects of endurance exercise, suggesting that PGC-1α plays a prominent role in exercise-induced mitochondrial biogenesis.

While PGC-1α is known to bind and co-activate NRF-1, PPARα and myocyte-enhancing factor-2, it does not directly interact with NRF-2. One possible mechanism for the activation of NRF-2 involves the recent discovery that PGC-1α and -β both interact with another transcriptional cofactor termed the host cell factor (Lin *et al*. 2002*a*). Host cell factor has been shown to directly interact with the β-subunit of GA-binding protein, the mouse homologue of human NRF-2, and stimulate transcription (Vogel & Kristie, 2000). Thus, it appears that mitochondrial biogenesis may be the result of a series of protein–protein interactions coordinated by PGC-1 (Fig. 1). If this scenario is true, then increases in mitochondria will correlate with the level and/or activation of PGC family members and the presence of the appropriate transcription factor binding partner.

Exercise and transcription factor activity

Endurance exercise training of sufficient frequency, intensity and duration can increase NRF-1 and PPARα protein (Horowitz *et al*. 2000; Irrcher *et al*. 2003). While training for periods of weeks or days are required to increase levels of transcription factor protein, a more immediate effect is seen in the DNA binding of these factors. NRF-1 and -2 DNA-binding activities increase in parallel 12–18 h following an acute bout of swimming (Baar *et al*. 2002), suggesting a rapid and coordinated activation of these proteins after acute exercise.

Exercise and PPARγ co-activator 1

PGC-1 mRNA increases between 50% and 7- to 10-fold following a single bout of exercise (Baar *et al*. 2002; Terada *et al*. 2002; Pilegaard *et al*. 2003). This difference

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may be the result of using different primers for the quantitative PCR reactions. It has been shown by Northern blot that the full-length PGC-1α mRNA is increased by about 2-fold 6 h after an acute bout of swimming. However, there is a much greater increase in a shorter variant of the PGC-1α mRNA that lacks exon 8 (Baar *et al*. 2002). Beyond the importance of verifying the increase in message by Northern blot, this finding is especially interesting as the 5′ region of exon 8 is believed to serve an inhibitory function, decreasing the capacity for transcription factor co-activation (Vega *et al*. 2000). If this inhibitory domain is eliminated from the mRNA following exercise, the resulting protein could be as much as 40-fold more active according to the *in vitro* studies. Recent work by Irrcher *et al*. (2003) has shown that both *in vitro* and *in vivo* chronic electrical stimulation increase PGC-1 protein. After 4 d of *in vitro* stimulation for 3 h/d PGC-1α protein increases by 80%, while 5–10 d of chronic electrical stimulation of the rat tibialis anterior muscle for 3 h/d increases PGC-1α by 30–50%.

Conclusions and future directions

In the last few years researchers have brought us much closer to understanding how endurance exercise of sufficient frequency, intensity and duration leads to an increase in mitochondrial mass. Nevertheless, a number of important questions still remain. Such questions include: which PGC family members are activated in skeletal muscle by exercise; what is the mechanism involved in their activation; how do they coordinate the activity of the targetted transcription factors?

With the discovery of two new members of the PGC family, it will be important to experimentally determine whether one of the members is preferentially activated by exercise. Similarly, it will be of interest to determine whether there is in fact an 'exercise responsive' PGC transcript, as preliminary evidence suggests (Baar *et al*. 2002). Once this evidence has been confirmed, the next step will be to determine the mechanism of PGC activation. A number of molecules are known to increase the expression of PGC, including AMP kinase, cyclic GMP (Nisoli *et al*. 2003) and Ca^{2+}/calmodulin-dependent protein kinase. The question is do all these factors have a direct effect on PGC expression, do they signal through independent pathways, or do they all work via the same upstream activator? If a common activator exists, then can this master regulator be pharmacologically targeted to augment mitochondrial mass independent of exercise?

While overexpression or chronic activation of upstream signalling molecules can increase the expression of PGC, it is unclear whether this process is the primary mechanism used by exercise. Many mitochondrial and metabolic proteins appear to increase slowly after the onset of exercise training, while others are rapidly increased following exercise. Since it is likely that all these proteins are coordinately controlled, this disparity may be the result of differences in the half-life of the proteins being measured. In fact, it is proteins with a short half-life, such as 5-aminolevulinate synthase and GLUT4, that increase most rapidly after exercise (Ren *et al*. 1994), suggesting that the increase in expression of mitochondrial and metabolic genes is rapid. This increase coupled with the fact that PGC-1α may be increased immediately after a bout of exercise (S Terada and I Tabata, personal communication) suggests that the initial effects of exercise on PGC may be post-translational. If this interpretation is correct, the stabilization of PGC protein as a result of phosphorylation by p38 mitogen-activated kinase kinase presents an attractive model to explain these data (Puigserver *et al*. 2001). However, further research is required to confirm such conjecture.

Mitochondrial biogenesis is a primary adaptation to endurance exercise, while a decrease in mitochondrial function is synonymous with aging. Understanding the deficit that occurs with age may in the future allow us to return normal muscle function to individuals who, because of disease or advanced age, can no longer exercise at a sufficient intensity to induce mitochondrial adaptations. To this end, a recent discovery that the expression of PGC-1α is controlled by histone deacetylation is encouraging (Czubryt *et al*. 2003). Aging can result in changes in chromatin structure as a result of alterations in histone deacetylation and DNA methylation (Burzynski, 2003). Deacetylation and then methylation of DNA can result in gene silencing, decreasing the expression of the associated genes. Since PGC-1α expression is sensitive to deacetylation and there is a decrease in mitochondrial protein with age, it would be very interesting to determine whether the PGC-1α promoter is hypermethylated in 'old' muscle.

It seems likely that many but not all the proteins involved in exercise-induced mitochondrial biogenesis have been identified. It is now important to piece together the individual parts and find ways of genetically or pharmacologically manipulating mitochondrial mass. This process will probably not elevate performance in athletes who have reached elite status, but it could have important implications for the treatment of frailty, and metabolic diseases such as diabetes and obesity.

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