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MUSCLE PHYSIOLOGY

PGC-1α-mediated adaptations in skeletal muscle

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Abstract Lifestyle-related diseases are rapidly increasing at least in part due to less physical activity. The health beneficial effects of regular physical activity include metabolic adaptations in skeletal muscle, which are thought to be elicited by cumulative effects of transient gene responses to each single exercise, but how is this regulated? A potential candidate in this is the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, which has been identified as a master regulator of mitochondrial biogenesis, but also been shown to regulate proteins involved in angiogenesis and the anti-oxidant defence as well as to affect expression of inflammatory markers. Exercise increases PGC-1α transcription and potentially PGC-1α activity through post-translational modifications, and concomitant PGC-1α-mediated gene regulation is suggested to be an underlying mechanism for adaptations in skeletal muscle, when exercise is repeated. The current review presents some of the key findings in PGC-1α-mediated regulation of metabolically related, anti-oxidant and inflammatory proteins in skeletal muscle in the basal state and in response to exercise training, and describes functional significance of PGC-1α-mediated effects in skeletal muscle. In addition, regulation of PGC-1α expression and activity in skeletal muscle is described. The impact of changes in PGC-1α expression in mouse skeletal muscle and the ability of PGC-1α to regulate multiple pathways and functions underline the potential importance of PGC-1α in skeletal muscle adaptations in humans. The absence of exercise-induced PGC-1α-mediated gene regulation during a physical inactive lifestyle is suggested to lead to reduced oxidative capacity of skeletal muscle and concomitant impaired metabolism.

Keywords PGC-1α · Skeletal muscle · Adaptations · Exercise

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Exercise training-induced skeletal muscle adaptations

Lifestyle-related diseases are rapidly increasing in many parts of the world. Although several factors likely are involved, increasing physical inactivity seems to be one important contributor, but how does physical activity exert such beneficial effects?

Endurance exercise training leads to many kinds of adaptations in the body, and especially skeletal muscle is characterised by an extraordinary plasticity adjusting to the degree of use and disuse [6]. Repeated endurance exercise will typically increase the expression/activity of membrane transporters and mitochondrial metabolic enzymes as well as increase capillarisation in skeletal muscle [6] together enhancing the oxidative capacity of the muscle and the ability to oxidise both carbohydrates and fatty acids. In addition, exercise training can also increase the expression of enzymes in the anti-oxidant defence in skeletal muscle [14, 30], potentially ensuring a better protection against reactive oxygen species (ROS) in muscles.

The molecular mechanisms behind these exercise training-induced skeletal muscle adaptations likely involve regulation at several different steps in protein synthesis, but regulation at the gene level seems to be particularly important. Thus, a single exercise bout has been shown to induce transient increases in transcription and/or mRNA of metabolically related proteins in both rodent [40, 41] and

human [26, 27, 31, 43, 52] skeletal muscle, and it is suggested that cumulative effects of such transient increases are one important mechanism behind adaptations to repeated physical activity [43, 65].

The question is, however, what regulates these gene responses to acute exercise? Several factors may be involved, but peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α is of special interest not the least, because PGC-1α mediates regulation of multiple pathways and thereby possesses the potential to coordinate adaptations in skeletal muscle. Therefore, PGC-1α will be the focus of this review, where we will present some of the key findings showing the impact of PGC-1α in skeletal muscle both in the basal state and in response to exercise training as well as functional significance of PGC-1α-mediated regulation in skeletal muscle. Some of the factors regulating PGC-1α expression and post-translational modifications of PGC-1α in skeletal muscle will also be described, and the potential role of this regulation in response to exercise will be discussed.

Impact of PGC-1α in skeletal muscle

Discovery

Much attention has been given to the transcriptional coactivator PGC-1α since the discovery in 1998, where PGC-1α was identified as a coactivator of PPARγ in brown adipose tissue and a player in adaptive thermogenesis [47].

A coactivator

PGC-1α coactivates a broad range of transcription factors including PPARs, nuclear respiratory factors (NRF's), myocyte enhancing factors (MEFs), oestrogen-related receptor (ERR), forkhead box (FOX)O1 and yin-yang (YY)1 [24, 35, 49]. Moreover, transcriptional induction by PGC-1α involves the recruitment of coactivators with histone acetyl transferase activity as well as interaction with proteins involved in RNA processing and transcriptional initiation [60].

PGC-1α and mitochondrial biogenesis

Since the discovery of PGC-1α, a broad tissue distribution has been described with special attention to heart, liver, brain, adipose tissue and skeletal muscle, and several functions have been identified in these tissues including the regulation of proteins in gluconeogenesis, the antioxidant defence and especially mitochondrial biogenesis [35]. Thus, high PGC-1α expression levels in cell culture [39, 68] and mouse skeletal muscle [36, 63] convincingly demonstrated PGC-1α as a very potent stimulator of mitochondrial biogenesis. Muscle-specific overexpression (MCK) of PGC-1α in mice turned white, glycolytic skeletal muscle into red, oxidative muscles with increased mRNA content of cytochrome oxidase (COX)II, COX IV and the ATP synthase [36], elevated cytochrome c (Cyt c), myoglobin [36] and COXI protein content [64] and elevated citrate synthase (CS) activity [7]. Although this was shown to be associated with an increase in myosin heavy chain I [36], it may be noted that this effect was small relative to the massive metabolically related effects. Generating inducible muscle-specific PGC-1α mice revealed similar findings with regard to mRNA expression of oxidative enzymes and elevated CS activity [63] underlining the power of elevating PGC-1α in mouse skeletal muscle. In accordance with these results, both whole body PGC-1α knockout (KO) and muscle-specific knockout (MKO) of PGC-1α in mice have been shown to result in reduced mRNA and/or protein content of mitochondrial respiratory chain proteins and ATP synthase [3, 17, 32, 33, 37]. Although one study did not report any effect of PGC-1α KO on mitochondrial yield [3], other studies have demonstrated lower mitochondrial yield and mitochondrial respiration in PGC-1α KO muscles than in wild type (WT) [1, 33]. Taken together, there is strong evidence for a major impact of PGC-1α on regulation of mitochondrial metabolic proteins in skeletal muscle (Fig. 1).

PGC-1α and fat metabolism

A role of PGC-1α in regulating proteins in fat metabolism was first demonstrated by enhanced mRNA content of fat oxidation enzymes like carnitine palmitoyltransferase (CPT)I and medium-chain acyl-coenzyme A dehydrogenase (MCAD), when PGC-1α expression was increased in cell culture [59]. These findings were further supported by increased mRNA content of fatty acid translocase/CD36 (CD36), CPTI and MCAD in skeletal muscle of both chronic and inducible PGC-1α overexpression mice [7, 63] and reduced MCAD mRNA in PGC-1α KO mice relative to WT [16]. However, data showing the impact of PGC-1α KO and overexpression on protein expression of these proteins are still limited (Fig. 1).

PGC-1α and carbohydrate metabolism

Also, the non-mitochondrial protein glucose transporter (GLUT)4 has been shown to be regulated by PGC-1α in cell culture [39], to be reduced at the mRNA and protein level in PGC-1α KO mice [17, 63] and be elevated at the mRNA level in PGC-1α overexpression mice [63], indicating that PGC-1α can regulate GLUT4 expression in skeletal muscle. However, the cytosolic enzyme hexokinase (HK)II was unchanged at both the mRNA and protein level

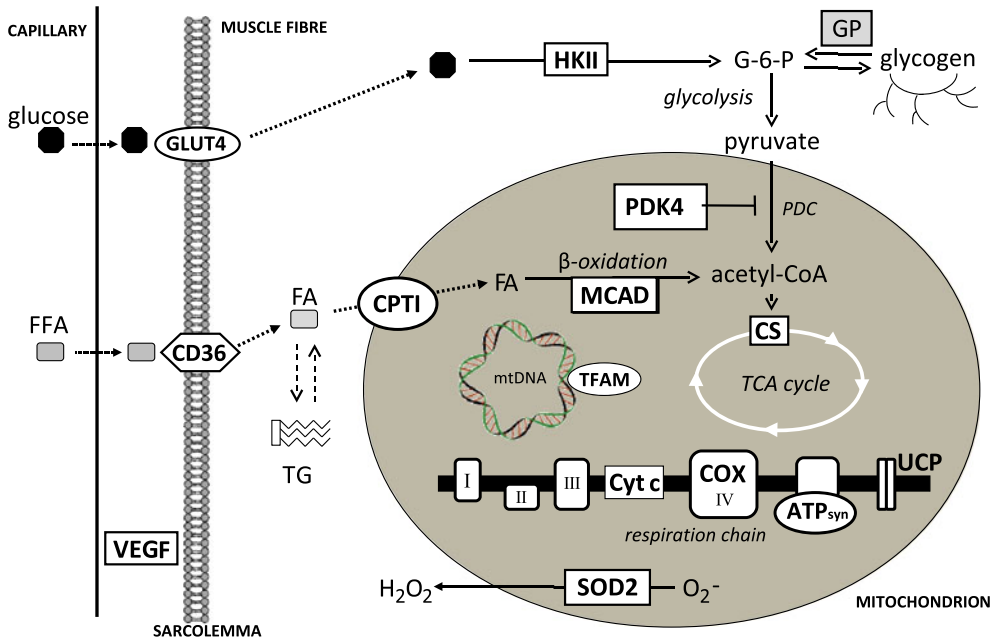

Fig. 1 Schematic overview of some of the metabolically, anti-oxidant- and angiogenesis-related proteins regulated by peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α in skeletal muscle. Proteins written with *bold* are induced by PGC-1α, whereas the protein in a *grey box* is reduced. *GLUT4* glucose transporter 4, *HKII* hexokinase II, *G-6-P* glucose-6-phosphate, *GP* glycogen phosphorylase, *PDC* pyruvate dehydrogenase complex, *PDK4* pyruvate dehydrogenase kinase 4, *CS* citrate synthase, *TCA* tricarboxylic acid, *FFA* free fatty acids, *CD36* fatty acid translocase/CD36, *FA* fatty acids, *TG* triglycerides, *CPTI* carnitine palmitoyltransferase I, *MCAD* medium-chain acyl-coenzyme A dehydrogenase, *mtDNA* mitochondrial DNA, *TFAM* transcription factor A mitochondria, *Cyt c* cytochrome c, *COX* cytochrome oxidase, *ATPsyn* ATP synthase, *UCP* uncoupling protein, *SOD2* superoxide dismutase 2, *VEGF* vascular endothelial growth factor

in skeletal muscle of PGC-1α KO mice [29, 32] and at the mRNA level in MCK PGC-1α overexpression mice [7]. However, HKII protein content has been demonstrated to be elevated in both inducible [63] and MCK PGC-1α overexpression mice (Olesen and Pilegaard, unpublished data). This shows that although high levels of PGC-1α are associated with increased HKII protein, PGC-1α is not required for basal HKII expression, suggesting that HKII is not a direct target of PGC-1α, but secondary effects of PGC-1α overexpression affects HKII protein expression (Fig. 1).

PGC-1α and substrate utilisation

PGC-1α does not only affect the oxidative capacity of skeletal muscle but may also exert more acute regulation of substrate choice. The pyruvate dehydrogenase complex (PDC) is central in such regulation, because it catalyses the irreversible transformation of pyruvate to acetyl-CoA and hence links glycolysis and the tricarboxylic acid cycle. The activity of PDC is regulated by the phosphorylation state of the pyruvate dehydrogenase (PDH)-E1α component of the complex, which is determined by the activity of PDH kinases (PDK) and PDH phosphatases (PDP), where PDK inhibits and PDP activates PDC [56]. A role of PGC-1α in PDC regulation is supported by findings in both cell culture and mice, which showed that PGC-1α regulated the expression of PDK4 [9, 62, 63]. In addition, the observation in rat skeletal muscle that a PPARδ agonist both down-regulated PDC activity and upregulated PDK4 [11] further supports that PGC-1α may regulate PDK4 expression and potentially substrate utilisation. Together, these observations suggest that a PGC-1α-mediated upregulation of PDK4 may contribute to inhibition of PDC and a concomitant increase in fat oxidation in skeletal muscle (Fig. 2).

PGC-1α-mediated regulation also affects skeletal muscle glycogen levels, because glycogen levels were shown to be reduced in PGC-1α KO mice [32] and be elevated in PGC-1α overexpression mice [7, 63]. PGC-1α induced effects on oxidative capacity and on the flux through PDC likely contribute to these findings. However, Wende et al. [63] have demonstrated that inducible PGC-1α mice do not—as WT mice—use muscle glycogen during running and revealed that this glycogen sparing was associated with both lower glycogen phosphorylase content and reduced phosphorylase kinase-mediated phosphorylation of glycogen phosphorylase. This shows that additional regulatory steps may play a role in the effect of PGC-1α on muscle glycogen levels and glycogen use. Our recent findings with

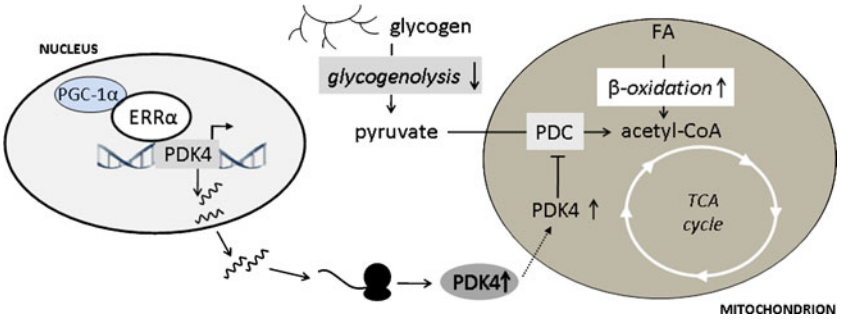

Fig. 2 Schematic illustration of the suggested peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α-mediated upregulation of pyruvate dehydrogenase kinase (PDK)4 leading to increased fat oxidation in skeletal muscle. *ERRα* oestrogen-related receptor α, *PDC* pyruvate dehydrogenase complex, *TCA* tricarboxylic acid

MCK PGC-1α overexpression mice do however show that these mice are able to use muscle glycogen during exercise [25], but this difference between the two PGC-1α overexpression mice strains remains to be elucidated (Fig. 2).

Coordinated regulation

The observations that manipulations of the PGC-1α content in cell culture and mouse skeletal muscle affect the mRNA and/or protein expression of both mitochondrial (like COXI and II) and nuclear-encoded (like COXIV and cyt c) mitochondrial proteins [32, 33, 36, 39, 63, 68] further point at PGC-1α as a coordinator ensuring parallel changes in the various components of the respiratory chain despite being encoded by the two different genomes. In accordance, PGC-1α was shown to regulate the expression of the mitochondrial transcription factor Tfam in cell culture [68], and lack of PGC-1α reduces the mRNA content of Tfam in mouse skeletal muscle [33]. A coordinating role of PGC-1α is further supported by the reduced expression of the mitochondrial enzyme aminolevulinate synthase (ALAS)1 (the rate limiting enzyme in heme production) in skeletal muscle of PGC-1α KO mice [17, 32], revealing PGC-1α also to be a regulatory factor in providing heme for the cytochromes in the respiratory chain. In addition, the increased protein levels of myoglobin in PGC-1α overexpression mice [36, 63] and reduction of myoglobin mRNA in PGC-1α KO mice [32] further underline the PGC-1α-mediated concerted regulation of several pathways important for the oxidative metabolism in skeletal muscle.

PGC-1α and angiogenesis

Regulation of the expression of proteins involved in oxidative metabolism in skeletal muscle contributes to determining the oxidative capacity of the muscle, but capillarisation is equally important. The first evidences for a role of PGC-1α in angiogenesis were the failure of PGC-1α KO mice to normally reconstitute blood flow to the limb after an ischemic insult, while MCK PGC-1α overexpression mice were protected, as well as the demonstration of an increased staining of the capillary marker CD31 in skeletal muscles of MCK PGC-1α mice [2]. In accordance, CD31 staining has been shown to be reduced in skeletal muscle of PGC-1α KO mice [29]. As the vascular endothelial growth factor (VEGF) is known as a critical factor in angiogenesis, the observations that expression of PGC-1α in cell culture increased VEGF mRNA [2] and that PGC-1α KO mice have markedly reduced VEGF protein content in skeletal muscle [29] indicate that VEGF exerts PGC-1α-mediated effects on angiogenesis (Fig. 1). Together, these findings provide strong evidence for PGC-1α-mediated regulation of skeletal muscle oxidative capacity through both effects on capillarisation and the expression of oxidative enzymes.

PGC-1α and anti-oxidant defence

An increased oxidative capacity renders the mitochondria with the potential to produce more ROS in the mitochondria, and a parallel regulation of respiratory chain proteins and mitochondrial anti-oxidant enzymes would be an advantage. Indeed PGC-1α does also regulate the expression of anti-oxidant proteins. Reduced superoxide dismutase (SOD)1, SOD2 and/or glutathione peroxidase (GPx)1 mRNA [17, 32], as well as SOD2 protein content [14, 30], were observed in skeletal muscle from PGC-1α KO mice relative to WT, while MCK PGC-1α overexpression mice had increased SOD2 protein content in skeletal muscle [64]. Moreover, the findings that PGC-1α KO fibroblasts exhibit a reduced H_2O_2 induced increase in SOD2, catalase and GPx1 mRNA content relative to WT fibroblasts [54] further indicate a role of PGC-1α in the upregulation of ROS removing enzymes in response to increases in ROS. In addition, PGC-1α has been shown to regulate the mRNA expression of uncoupling protein (UCP) 2 and 3 in cell culture [55], suggesting that PGC-1α may also increase the

uncoupling capacity and concomitantly reduce ROS production in the mitochondria.

Taken together, PGC-1α seems to improve the anti-oxidant defence of skeletal muscle by regulating the expression of both proteins, which reduce ROS production and proteins, which remove ROS (Fig. 1).

Other factors than PGC-1α

Of notice is that knockout of PGC-1α in skeletal muscle does in general lead to at most a 50% reduction in respiratory chain proteins [30, 32], anti-oxidant proteins [14, 30, 32] and VEGF protein [29], showing that although PGC-1α is required for normal basal expression levels, PGC-1α is not exclusively required for the expression of these proteins. Hence, other factors must also be involved. It might be speculated that the related PGC-1β could play a role and take over, but PGC-1β mRNA is unchanged in PGC-1α KO muscles [17, 37] and similarly, the transcriptional coactivator, PR domain containing (PRDM)16, shown to induce similar effects in brown adipose tissue as PGC-1α [51], is not changed at the mRNA level, when PGC-1α is lacking [32]. Although regulation of these coactivators at the protein level cannot be excluded, these findings suggest that another factor must be in play.

Functional role of PGC-1α-mediated metabolic modifications

Functional importance of the observed molecular effects of PGC-1α KO and overexpression in mice is evident by reduced running endurance of both whole body PGC-1α KO and PGC-1α MKO mice [16, 32, 33, 63] and increased running endurance and VO_2 max of MCK PGC-1α overexpression mice [7]. Of notice is, however, that inducible PGC-1α overexpression mice was shown to have reduced running capacity during high-intensity exhaustive exercise, which was suggested to be related to the inability to use muscle glycogen [63].

Additionally, the observed lower respiratory exchange ratio (RER) in MCK PGC-1α overexpression mice during both low-intensity and high-intensity running exercise [7], indicating that high skeletal muscle PGC-1α levels lead to an elevation in fat oxidation during exercise, further underlines the functional significance of the PGC-1α-mediated regulation of oxidative enzymes, transporters, PDK4 and angionesis. Interestingly, the MCK PGC-1α overexpression mice even maintain an RER value below 1 during maximal running exercise (while WT mice exhibit a normal level above 1) [7] likely reflecting the ability of these mice to maintain a high carbohydrate oxidation at maximal exercise and consequently avoiding lactate accumulation in muscle and blood. Together, these findings manifest the impact of the molecular changes due to PGC-1α KO and overexpression in skeletal muscle on physiological changes during exercise.

PGC-1α and inflammation

Interestingly, recent observations further indicate that PGC-1α may also exert anti-inflammatory effects [19]. PGC-1α MKO mice had higher basal mRNA expression of inflammatory markers such as tumour necrosis factor (TNF)α, interleukin (IL)-6, suppressor of cytokine signalling 1 and 3 in skeletal muscle, as well as higher serum IL-6 level than WT [16, 17]. Along these lines, MCK PGC-1α overexpression mice had lower expression of TNFα and IL-6 mRNA in skeletal muscle, and lifelong overexpression of PGC-1α reduced an age-associated increase in TNFα and IL-6 protein content in skeletal muscle and reduced serum TNFα and IL-6 levels in old mice compared with age-matched WT mice [64]. This further supports that PGC-1α lowers skeletal muscle expression and systemic levels of inflammatory cytokines. Moreover, a single exercise bout elicited a marked increase in skeletal muscle TNFα mRNA and serum TNFα content in PGC-1α MKO mice, but not in WT mice [16], strongly suggesting that skeletal muscle PGC-1α normally protects against exercise-induced increases in TNFα. A potential relevance of these observations in humans is supported by the negative correlations between PGC-1α mRNA and IL-6 mRNA expression as well as between PGC-1α mRNA and TNFα mRNA expression observed in skeletal muscle of type 2 diabetes patients [17].

The underlying mechanism behind the apparent PGC-1α-mediated anti-inflammatory effect is still not known. A possible explanation could be through PGC-1α-mediated regulation of the anti-oxidant defence, because increased oxidative stress due to an impaired balance between ROS production and removal can induce an inflammatory response through activation of the redox sensitive transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) [61]. However, whether this coupling is involved remains to be determined.

Regulation of PGC-1α

Expression

A single exercise bout regulates PGC-1α transcription and mRNA content in skeletal muscle. Thus, PGC-1α mRNA content was shown to be upregulated in rat skeletal muscle after a single swimming exercise bout [4, 58] and similarly,

PGC-1α transcription and mRNA content increased transiently in human skeletal muscle in response to a single prolonged exercise bout peaking within the first hours of recovery [45]. Several initiating stimuli and intracellular signalling pathways may contribute to eliciting the exercise-induced PGC-1α gene response.

Experiments in cell culture, with in vitro incubated rat muscles and with transgenic mice [20, 28, 42, 66, 67], provide strong evidence that cytosolic calcium concentrations play a role in inducing the PGC-1α gene in contracting skeletal muscle. Incubation of primary rat skeletal muscle cell culture with the calcium ionophore ionomycin or caffeine increased PGC-1α mRNA [28, 42], and calcineurin and Ca^{2+}/calmodulin-dependent protein kinase (CAMK) inhibitors prevented or reduced this effect [28] as was the case with electrical stimulation increases in PGC-1α mRNA in in vitro incubated rat extensor digitorum longus muscle [28]. In accordance, cotransfection experiments using C2C12 cells [18], as well as studies with transgenic mice selectively expressing a constitutively active form of calcineurin in skeletal muscle or CAMKIV transgenic mice [67], indicated that each of these calcium-signalling pathways can be involved in regulating the PGC-1α gene.

Increased ROS is also a potential mechanism during exercise. For example, the findings that anti-oxidant incubation prevented the electrical stimulation-induced increase in PGC-1α mRNA in primary rat skeletal muscle cell culture [53] indicate that increases in ROS may also contribute to exercise-induced increases in skeletal muscle PGC-1α mRNA content.

The energy sensitive AMP protein kinase (AMPK) has also been implicated in regulation of PGC-1α expression. Although exercise-induced PGC-1α mRNA expression does not require the presence of AMPKα2, the observation that the AMP analogue and AMPK activator, AICAR, induced PGC-1α mRNA in WT mice, but not AMPKα2 KO mice [23], provides evidence that AMPK does regulate the expression of PGC-1α in skeletal muscle, but the role of AMPK in PGC-1α gene regulation during exercise is still uncertain.

In addition, keeping muscle glycogen content low after exercise through diet manipulation maintains PGC-1α mRNA elevated in human skeletal muscle throughout 8 h of recovery [44], suggesting that substrate availability may also affect PGC-1α expression, although the mechanism behind this regulation still remains unresolved.

Finally, hormonal control of PGC-1α expression in skeletal muscle also seems to take place, because clenbuterol injections were shown to increase PGC-1α mRNA in mouse skeletal muscle [9], indicating a role of β-adrenergic signalling in PGC-1α gene regulation in skeletal muscle (Fig. 3).

Taken together, calcium signalling, AMPK signalling, ROS-mediated regulation, β-adrenergic signalling and substrate availability seem to regulate PGC-1α expression in skeletal muscle. These factors may all be changing during exercise and can therefore potentially contribute to the exercise-induced PGC-1α gene regulation (Fig. 3). In addition, PGC-1α has been shown to regulate the expression of itself, because a positive feedback loop was demonstrated between PGC-1α and MEF2, where MEF2 binds to and activates the PGC-1α promoter mainly when coactivated by PGC-1α [18]. Such a regulation may ensure a more powerful PGC-1α-mediated gene regulation.

Post-translational modifications

PGC-1α is not only regulated by changes in expression but also by several kinds of covalent modifications including phosphorylation [21, 46], acetylation [13, 34, 50] methylation and ubiquitination [49]. Thus in vitro experiments showed that p38 MAPK phosphorylates PGC-1α at three residues resulting in a more active and a more stable PGC-1α protein [46], and similarly that AMPK phosphorylates PGC-1α at two residues leading to a more active protein [21]. In addition, the acetyl transferase general control of amino-acid synthesis (GCN)5 acetylates PGC-1α at several lysine residues leading to relocalisation of PGC-1α to subnuclear foci with the transcriptional repressor receptor-interacting protein 140 [34], while the NAD^+-dependent sirtuin (Sirt)1 deacetylates PGC-1α, maintaining it in an active form bound to the chromatin [15]. Interestingly, experiments using C2C12 cells indicated that AMPK-mediated phosphorylation primes PGC-1α for deacetylation by Sirt1 concomitant with an AMPK-mediated increase in NAD^+, leading to activation of Sirt1 [8]. This suggestion places AMPK, Sirt1 and PGC-1α together as central players in metabolic regulation potentially with a special role during physical activity (Fig. 3).

PGC-1α and training-induced adaptations

The clear effects of knockout and overexpression of PGC-1α on the expression of VEGF, mitochondrial metabolic and anti-oxidant proteins, together with the induction of PGC-1α mRNA in response to exercise, indicate that PGC-1α could be a major player in endurance exercise training-induced adaptations in skeletal muscle. Moreover, the post-translational modifications of PGC-1α by AMPK [21], p38 MAPK [46] and Sirt1, together with exercise-induced activation of AMPK and p38 MAPK [48] and increased Sirt1 expression [57], suggest that exercise-induced regulation of PGC-1α activity also could be important in exercise-induced gene regulation. In accordance, both young and old

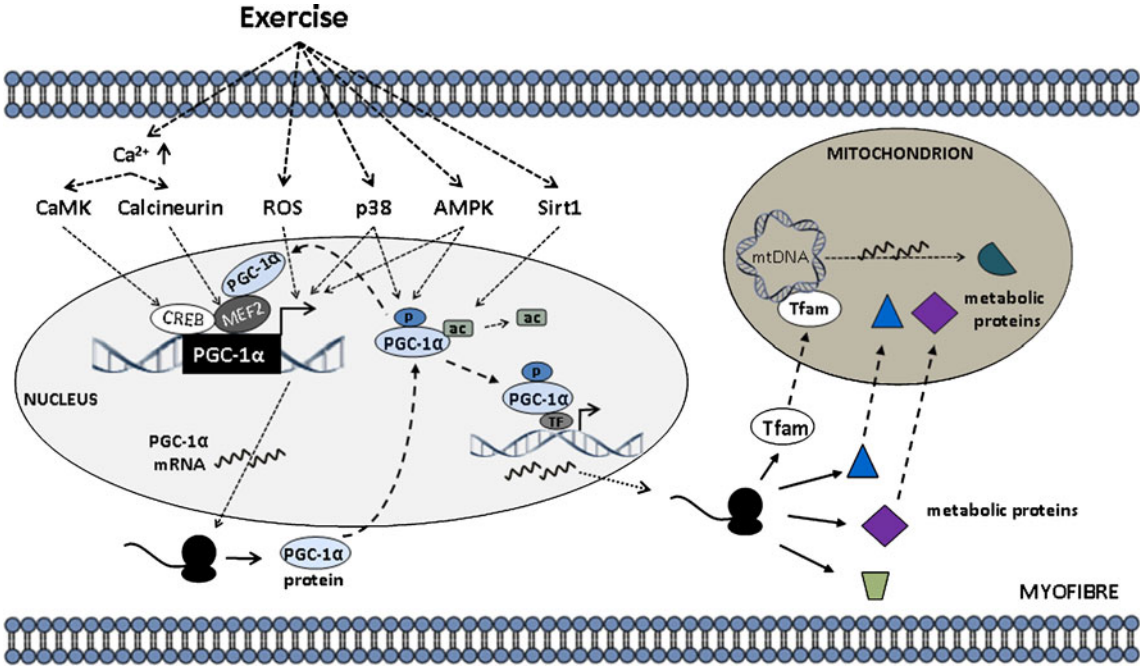

Fig. 3 Schematic presentation of the suggested coupling between exercise factors potentially involved in inducing and activating peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, PGC-1α gene expression and PGC-1α-mediated regulation of genes encoding metabolic proteins and transcription factor A mitochondria (TFAM), which translocates to the mitochondria to regulate the expression of mitochondrial-encoded metabolic proteins. *CAMK* Ca^{2+}/calmodulin-dependent protein kinase, *ROS* reactive oxygen species, *p38* p38 MAPK, *AMPK* AMP protein kinase, *Sirt1* sirtuin 1, *CREB* cAMP response element binding, *MEF2* myocyte enhancing factor 2, *P* phosphorylation, *ac* acetylation, *TF* transcription factor, *mtDNA* mitochondrial DNA

PGC-1α KO mice lacked the ability to increase VEGF protein expression in skeletal muscle with exercise training as WT mice were able to [29]. However, our findings that skeletal muscle of young whole body PGC-1α KO mice increased the protein expression of not only the cytosolic HKII but also the mitochondrial proteins cytochrome c, COXI and ALAS1 as well as CD31 in response to 5 weeks of combined treadmill and wheel running exercise training [32] are surprising. Thus, although PGC-1α clearly is required to maintain normal basal expression level of mitochondrial metabolic proteins and capillarisation, these observations [32] demonstrate that PGC-1α is not required for training-induced adaptations in these parameters in young mice and suggest that additional mechanisms exist. However, our recent findings show that in contrary to WT mice, whole body PGC-1α KO mice lacked the ability to rescue an age-associated reduction in CS activity in skeletal muscle through wheel running exercise training [30] and to induce an apparent increase in CD31 protein with training in 13-month-old mice [29]. This indicates that either is the capability of other factors to compensate lost with increasing age or exercise intensity, volume and/or mode is critical for such factors to play a role. In support of the latter suggestion are the findings that COXIV and cytochrome c protein content as well as CD31 positive endothelial cells increased less in skeletal muscle of young PGC-1α MKO mice than in WT in response to 4 weeks of wheel running exercise training [14], and that 2 weeks of exercise training failed to increase capillary density in PGC-1α MKO mice as in WT mice [9].

As for mitochondrial metabolic enzymes, PGC-1α was also found to be required for training-induced prevention of an age-associated decline in SOD2 protein in skeletal muscle of 13-month-old mice using whole body PGC-1α KO mice [30], while young PGC-1α MKO mice increased SOD2 protein during 4 weeks of training by wheel running [14]. These findings support that PGC-1α can play a role in exercise-induced SOD2 adaptations in skeletal muscle, but with additional mechanisms also being involved.

Of notice is that potential compensatory mechanisms are not only observed in PGC-1α KO mice [32] as similar findings have been obtained in AMPK KO mice in response to exercise training [22], supporting that more than one mechanism is in play. Taken together, it is likely that multiple regulatory pathways are involved in eliciting exercise-induced gene responses in skeletal muscle with PGC-1α-mediated regulation being one of these (Fig. 3).

Physical activity level and PGC-1α

Based on the above presented findings, a reduced skeletal muscle oxidative capacity associated with a physical

inactive lifestyle may be due to the lack of induction and/or activation of PGC-1α. Both aging [10] and type 2 diabetes [5] have been reported to be associated with reduced content/activity of oxidative enzymes in skeletal muscle, and skeletal muscle PGC-1α mRNA content has also been shown to be reduced with increasing age [38] and in type 2 diabetic patients [5]. Clearly, a part of these effects may be due to reduced physical activity and concomitant lack of exercise-induced gene responses. However, the exercise-induced PGC-1α mRNA increase was shown to be reduced in insulin-resistant subjects [12], indicating that the induction of the PGC-1α gene was compromised in these subjects and suggesting that type 2 diabetic subjects may need a larger exercise stimulus to obtain a given training adaptation. The recent reported PGC-1α hypermethylation in skeletal muscle of type 2 diabetic subjects could be one mechanism behind such changes, because PGC-1α promoter methylation was shown to cause a marked reduction in PGC-1α gene activity [5]. Based on the above described effects of PGC-1α on metabolic proteins, it is suggested that low oxidative capacity and associated low capacity for carbohydrate and fat oxidation in skeletal muscle when living a physical inactive lifestyle at least in part is due to the lack of exercise-induced PGC-1α expression and/or activation.

Conclusions and future directions

Studies in PGC-1α KO and overexpression mice have clearly demonstrated that PGC-1α plays an important role in maintaining the expression of mitochondrial metabolic and anti-oxidant enzymes in skeletal muscle and does influence training-induced adaptations of mitochondrial proteins. However, of notice is that PGC-1α is not exclusively required, and additional factors must be involved in the regulation of both basal expression and training-induced adaptations. The functional significance of PGC-1α-induced molecular adaptations in mouse skeletal muscle is evident on both performance and substrate utilisation during treadmill running emphasising the impact of PGC-1α-mediated regulation.

Exercise-induced PGC-1α expression and potentially also increased PGC-1α activity are likely mechanisms contributing to skeletal muscle mitochondrial adaptations and concomitant health beneficial effects of regular physical activity. Although several studies indicate potential roles of specific exercise-induced signalling factors in regulation of PGC-1α during exercise, a direct evidence for this coupling in vivo would be an interesting task for future studies. Also, investigations of potential exercise-induced post-translational modifications of PGC-1α in vivo and the impact of epigenetic changes for PGC-1α-mediated regulation would be of special interest for the understanding of the regulation of skeletal muscle adaptations to exercise in health and disease states.

A possible anti-inflammatory role of PGC-1α further gives PGC-1α the potential to protect against low-grade inflammation. Further exploration of such possible association between PGC-1α and inflammation is much warranted in both rodents and humans.

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