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The PGC-1 coactivators promote an anti-inflammatory environment in skeletal muscle in vivo

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Abstract

The peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) is abundantly expressed in trained muscles and regulates muscle adaptation to endurance exercise. Inversely, mice lacking a functional PGC-1 α allele in muscle exhibit reduced muscle functionality and increased inflammation. In isolated muscle cells, PGC-1 α and the related PGC-1 β counteract the induction of inflammation by reducing the activity of the nuclear factor κ B (NF κ B). We now tested the effects of these metabolic regulators on inflammatory reactions in muscle tissue of control and muscle-specific PGC-1 α /1 β transgenic mice *in vivo* in the basal state as well as after an acute inflammatory insult. Surprisingly, we observed a PGC-1-dependent alteration of the cytokine profile characterized by an increase in anti-inflammatory factors and a strong suppression of the pro-inflammatory interleukin 12 (IL-12). In conclusion, the anti-inflammatory environment in muscle that is promoted by the PGC-1s might contribute to the beneficial effects of these coactivators on muscle function and provides a molecular link underlying the tight mutual regulation of metabolism and inflammation.

Graphical Abstract

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Author contributions

P.S.E. designed and performed experiments, analyzed data and wrote the paper; R.F. wrote the paper; M.B. performed experiments; and C.H. supervised the study and wrote the paper.

Conflict of interests

The authors declare that they have no conflict of interests.

Keywords

inflammation; PGC-1; skeletal muscle; metabolism; mitochondria

Introduction

Metabolic and immune pathways intersect at multiple levels in the human body, both in the healthy but maybe even more importantly in the diseased state often with detrimental consequences. For example, a metabolic imbalance in obesity not only leads to metabolic dysregulation, but also a chronic systemic inflammation [1,2,3]. The latter event often originates from fat tissue with pronounced accumulation of pro-inflammatory macrophages (also referred to as classically activated or M1 macrophages) [4]. These macrophages release large quantities of pro-inflammatory cytokines, e.g. tumor necrosis factor α (TNF α), interleukin 6 (IL-6), and monocyte chemotactic protein 1 (MCP-1), that further foster inflammation [5,6]. In contrast, adipose tissue of lean animals and humans harbors mainly alternatively activated (or M2) macrophages that express a distinct set of anti-inflammatory cytokines, e.g. C-C motif chemokine 1 (CCL1), CCL22, IL-1 receptor antagonist (IL-1Ra), transforming growth factor β (TGF β), and IL-10 [5]. While M1 macrophages drive inflammation and are thus physiologically important during the innate immune response against pathogens, M2 macrophages constrain and modulate inflammatory reactions in the resolution of inflammation, tissue remodeling and repair.

The aberrant activation of M1 macrophages in obesity is not only seen in adipose tissue but also – in other peripheral organs such as liver or skeletal muscle. Obesity promotes the build-up of intramuscular adipose depots and, analogous to fat tissue, M1-type macrophage accumulation [4]. This chronic low grade inflammation contributes to the development of insulin resistance, a hallmark of diabetes. . Similar to diet-induced weight loss, regular exercise also counteracts type 2 diabetes and limits systemic inflammation [7,8]. An

important factor mediating many of the beneficial effects of exercise is the PPAR γ coactivator 1 α (PGC-1 α) [9,10]. PGC-1 α and the related PGC-1 β coactivate an array of nuclear receptors and transcription factors in skeletal muscle to induce the transcription of genes involved in mitochondrial biogenesis and oxidative phosphorylation and thereby enable a higher endurance capacity [11,12]. Mice with skeletal muscle-specific overexpression of PGC-1 α (MCK α mice) consequently score better in running tests and have a larger proportion of slow-twitch type I and IIa fibers than wild-type (WT) littermates [13,14]. Mice with transgenic overexpression of PGC-1 β (MCK β mice) also exhibit lower fatigability but unlike the MCK α mice, a switch towards IIx fibers [15]. Inversely, skeletal muscle-specific deletion of PGC-1 α [16] or of PGC-1 β [17] results in poorer running performance.

In contrast to the elevated levels of PGC-1 α in endurance athletes, the expression of PGC-1 α and PGC-1 β is reduced in skeletal muscle of human diabetic patients with a coordinate depression of mitochondrial oxidative phosphorylation, at least in certain populations [18,19]. Importantly, in these patients, PGC-1 α levels inversely correlate with the pro-inflammatory cytokines IL-6 and TNF α independent of body mass index (BMI) [20]. This suggests a mutual negative relationship between PGC-1 coactivators and inflammation in skeletal muscle.

Further experimental evidence for such a link derives from mice with skeletal muscle-specific PGC-1 α deletion that exhibit elevated levels of muscle and systemic inflammation [20]. Accordingly, PGC-1 α and PGC-1 β overexpression constrains pro-inflammatory cytokine expression upon TNF α exposure by inhibiting the transcriptional activity of nuclear factor κ B (NF- κ B) in muscle cells *in vitro* [21]. Inversely, classical NF- κ B activation in muscle cells dampens the expression of proteins involved in oxidative phosphorylation, including PGC-1 α and PGC-1 β [22]. Thus, an inverse regulation between the PGC-1 coactivators and pro-inflammatory gene expression exists in muscle cells. In the present study, we expanded the *in vitro* findings and now explored this mutual negative relationship *in vivo*. Specifically, we defined the role of PGC-1 α and PGC-1 β overexpression in skeletal muscle on local and systemic inflammatory events triggered by injection of the inflammatory agents lipopolysaccharide (LPS) and TNF α . Thereby, the previously reported muscle cell autonomous effects of the PGC-1 coactivators in cell culture models can be separated from the consequences of muscle fiber-specific elevation of PGC-1 α and PGC-1 β on different cell types, including immune cells in muscle tissue *in vivo*. Intriguingly, we observed that elevation of the PGC-1 coactivators promotes an anti-inflammatory environment in muscle.

Material and Methods

Mice and treatments

C57BL/6 mice expressing PGC-1 α (MCK α) [14] and FVB/N mice expressing PGC-1 β (MCK β) [15] under the control of the muscle creatine kinase (MCK) promoter were bred with respective WT mice to obtain WT and transgenic littermates. Male mice were maintained on a standard rodent chow with 12h light/dark cycle and subjected to experiments at 8 – 12 weeks of age. Injections were performed under sevoflurane anesthesia.

Mice were randomly assigned to one experimental group and subsequently injected intramuscularly (*i.m.*) into the TA with either PBS (30 μ l/TA), or LPS (2 μ g/TA) or TNF α (50ng/TA) in both legs. 4h post injection, mice were euthanized and blood as well as TAs collected for further analysis. All animal experiments were approved by the institutional and federal authorities.

ELISA

To determine serum cytokine concentrations, sandwich immunoassays for TNF α and IL-6 were performed according to the manufacturer's instructions (Quantikine, R&D Systems).

Semiquantitative real-time PCR

Muscle tissue was homogenized using matrix particles (Qbiogene) in a FastPrep FP120 cell disrupter (Thermo Scientific) and RNA isolated with Trizol (Invitrogen) while residual DNA contamination was removed by DNase I (Invitrogen) digestion. 1 μ g of RNA was reverse transcribed with SuperScript II (Invitrogen) and the resulting cDNA used as template for RT-PCR. To detect relative expression levels, cDNA was amplified with the SYBR Green Master mix (Applied Biosystems) and analyzed on a StepOnePlus RT-PCR System (Applied Biosystems). The respective primer pairs are listed in Supplemental Table 1. All values are normalized to the expression of TATA-Box binding protein (TBP) and expressed as fold induction over untreated WT animals.

Histology

TA muscles were snap frozen in liquid nitrogen-chilled isopentane and cut into cross sections on a microtome/cryostat (Leica CM1950). Specimen were stained with Mayer's hematoxylin and eosin (H&E, Sigma) and light microscopically analyzed.

Statistical analysis

Data were analyzed with Student's *t* test using $P < 0.05$ as significance threshold.

Results

Muscle PGC-1 α and PGC-1 β do not suppress systemic pro-inflammatory factors after LPS/TNF α injection in vivo

To delineate the role of skeletal muscle PGC-1 in an acute inflammatory insult *in vivo*, we injected bacterial LPS, TNF α or PBS as vehicle control *i.m.* into the tibialis anterior (TA) muscle of wildtype (WT) control, PGC-1 α (MCK α) and PGC-1 β (MCK β) muscle-specific transgenic animals. Importantly, separate WT control cohorts were used to reflect the difference in the mouse strain background (C57BL/6 for MCK α , FVB/N for MCK β). After 4 hours, the animals were sacrificed, blood collected and the TA muscle isolated. First, plasma levels of the pro-inflammatory cytokines TNF α and IL-6 were measured. Both cytokines were not detectable in WT and MCK α mice after PBS injection but were strongly induced by LPS (Fig. 1A). TNF α *i.m.* injection moderately elevated systemic TNF α levels (Fig. 1A). PGC-1 α expression in skeletal muscle did not have any effect on these cytokine levels (Fig. 1A). Similarly, the induction of systemic TNF α and IL-6 was not affected by

transgenic overexpression of PGC-1 β even though PBS injected MCK β mice had significantly lower plasma TNF α levels than WT controls (Fig. 1B). Therefore, PGC-1 coactivators *in vivo* were not able to suppress LPS- or TNF α -mediated systemic induction of pro-inflammatory M1 cytokines.

Muscle PGC-1 α and PGC-1 β do not suppress acute inflammation, but reduce the expression of the M1 cytokine IL-12

Even though muscle-specific overexpression of PGC-1 α or PGC-1 β did not affect circulating pro-inflammatory cytokine levels, the PGC-1s may have a local effect on inflammation in muscle. To test this, gene expression changes were determined in the injected TA muscles. First, PGC-1 α expression in MCK α mice was not affected by any of the injections and accordingly remained about 6 fold above WT levels (Fig. 2A). PGC-1 β levels were not changed in PBS-injected muscles of the PGC-1 α muscle transgenics, but were reduced in LPS-treated mice (Fig. 2A). LPS injection also lowered PGC-1 β expression in the MCK β model, although PGC-1 β levels remained considerably higher compared to WT controls (about 9 fold, Fig. 2B). In all conditions, PGC-1 α levels in the MCK β animals were reduced to about a third of WT levels (Fig. 2B).

We next mapped the expression of pro-inflammatory cytokines in the TA under these conditions. The cytokines TNF α , IL-6, MIP-1 α and MCP-1 were strongly induced in WT muscles after injection of inflammatory agents (Figs. 2C, D). Skeletal muscle-specific overexpression of neither PGC-1 α nor PGC-1 β affected the elevation of these pro-inflammatory cytokines in stimulated muscle (Figs. 2C, D). Intriguingly, muscle-specific overexpression of PGC-1 α and PGC-1 β dramatically reduced the expression of the pro-inflammatory IL-12 regardless of the respective stimulation (Figs. 2C, D). These findings imply that muscle-specific overexpression of PGC-1 α or PGC-1 β is insufficient to prevent local and systemic inflammation upon a strong ectopic, acute stimulus.

The local inflammation induced by LPS and TNF α injections is also evident in TA cross sections stained with H&E. LPS and TNF α both evoked a substantial influx of immune cells compared to PBS-injected muscles (Figs. 2E, F). The difference in non-muscle mononuclear cells is already observed under basal conditions between transgenic and WT animals and persisted after injection of inflammatory agents, but neither PGC-1 α nor PGC-1 β had an inhibitory impact on that influx (Figs. 2E, F). Interestingly, the high number of regenerating fibers with centrally located nuclei in some areas of the TA in the MCK β model indicated a basal, low level of continuous fiber damage and regeneration in these animals that is not observed in MCK α mice. In summary, we could not confirm an influence of PGC-1 α or PGC-1 β on several pro-inflammatory cytokine expression levels and immune cell infiltration in muscle *in vivo*. However, expression of the pro-inflammatory cytokine IL-12 was potently suppressed in PGC-1 α and PGC-1 β overexpressing muscles.

Muscle PGC-1 α and PGC-1 β induce local anti-inflammatory M2 cytokines in the TA after LPS/TNF α injection

IL-12 expression is closely linked to M1 macrophages and absence of IL-12 promotes an anti-inflammatory environment favoring M2-type activation [23,24]. Therefore, the gene

expression of anti-inflammatory cytokines was determined to obtain a comprehensive picture of the inflammatory environment created by the PGC-1 coactivators in skeletal muscle. Since IL-12 levels were strongly suppressed in the basal condition as well as after injections of inflammatory agents, the inflammatory environment may already differ between genotypes in PBS injected muscles. Indeed, in control muscles of MCK α mice, TGF β expression was elevated compared to WT, whereas in MCK β mice, all tested M2 cytokines were higher compared to WT animals (Fig. 3).

In WT mice, CCL1, CCL22, IL-1Ra and TGF β were all significantly augmented by LPS and TNF α while IL-10 was only induced by LPS and not by TNF α (Fig. 3). PGC-1 α did not alter the levels of CCL22, IL-1Ra and IL-10, but it clearly enhanced the expression of CCL1 after LPS and TNF α injection compared to WT muscles (Fig. 3A). Notably, TGF β expression was elevated in MCK α mice across all experimental conditions (Fig. 3A). The higher levels of most of the tested M2 cytokines in PBS-injected MCK β animals were further elevated after LPS injection except for TGF β . TNF α did not elicit such clear differences between genotypes and only CCL1 expression was significantly increased in MCK β muscles compared to WT (Fig. 3B). These results indicate that the PGC-1s alter the cytokine environment in control muscle as well as after inflammatory stimulation by differentially boosting the expression of anti-inflammatory markers.

Discussion

Metabolism and inflammation are two biological processes that are tightly linked. We now report that PGC-1 α and PGC-1 β constitute a molecular link between metabolism and inflammation in skeletal muscle. Strikingly, these two PGC-1 coactivators alter the inflammatory environment with a corresponding muscle cytokine expression profile. In particular, anti-inflammatory factors such as CCL1, CCL22, IL-1Ra, TGF β or IL-10 were elevated in one or both muscle-specific overexpression models depending on the experimental context, thereby creating an anti-inflammatory environment. Inversely, the pro-inflammatory cytokine IL-12 was strongly suppressed in both models. Studies of mice with an IL-12 deletion revealed deficits in M1-type cytokines and more importantly, a bias in macrophage activation towards M2 [23,24], suggesting that defective IL-12 production causes an altered macrophage polarization.

Systemically, we did not find a difference between the inflammation in PGC-1 transgenic and WT animals as plasma levels of TNF α and IL-6 were comparable after the acute insults. The PGC-1s in skeletal muscle are therefore not able to suppress an acute inflammatory reaction of the immune system on whole-body level. A previous study using the same MCK α animals that were injected *i.p.* with LPS yielded similar results: plasma IL-6 and TNF α levels were not different between genotypes after LPS injection even though there was a reduction in basal TNF α levels [25]. This is reminiscent of the situation in our MCK β model. However, it remains to be determined if PGC-1 α and PGC-1 β in skeletal muscle are beneficial in conditions of chronic systemic inflammation, e.g. obesity-related morbidities, where cytokine levels are only slightly but permanently elevated as opposed to the acute, strong but transient inflammatory response in the study by Olesen and colleagues as well as ours.

Importantly, in contrast to the inhibited induction of IL-6, TNF α and MIP-1 α by PGC-1 α and PGC-1 β in C2C12 myotubes, we did not detect a strong repression of pro-inflammatory cytokines after the inflammatory challenge *in vivo* [21]. This seeming discrepancy might reflect the absence of immune cells in cultures of isolated myotubes that allows the assessment of muscle cell-autonomous effects. *In vivo*, immune and other non-muscle cell types that reside within the TA most likely contribute much more to the overall amounts of cytokines since these cells express significantly higher levels of cytokines compared to muscle fibers. We therefore speculate that even though the muscle cells of transgenic animals might release lower quantities of pro-inflammatory cytokines (as observed *in vitro*), this effect is outweighed by the phenotype of resident immune cells in the muscle.

Curiously, signs of fiber damage and inflammation were detected even in sedentary MCK β mice. It is thus conceivable that the supraphysiological expression of the PGC-1 β transgene in these animals confers some detrimental effects on muscle fibers, similar to the pathological changes described in higher-expressing muscle-specific PGC-1 α transgenic lines [14,26]. In contrast, no signs of fiber damage and inflammation were detected in the MCK α mice. Importantly, despite these potentially confounding effects of increased inflammation intrinsic to the animal models, PGC-1 α and PGC-1 β are still able to promote an anti-inflammatory cytokine environment in skeletal muscle.

In the respective animal models, PGC-1 α and PGC-1 β improve the pathology of Duchenne muscular dystrophy and other muscle wasting diseases [27,28]. Injured and dystrophic muscles also depend on macrophage activity to undergo repair. Following a first phase in which necrotic muscle fibers are removed by M1 cells, M2 macrophages control the second phase of regeneration [29,30]. Given the beneficial effect of physiological PGC-1 α and PGC-1 β overexpression in Duchenne muscular dystrophy and their ability to induce an anti-inflammatory environment after an inflammatory insult, it is tempting to speculate that PGC-1 α and PGC-1 β are instrumental in muscle regenerative processes by modulating immune responses that are vital for proper recovery.

In conclusion, an anti-inflammatory role of the metabolic regulators PGC-1 α and PGC-1 β was confirmed based on an altered cytokine environment in skeletal muscle tissue of transgenic animals *in vivo*. Due to crosstalk between muscle and immune cells, the greater anti-inflammatory and immunomodulatory context compared to WT control animals may be explained by skewed balance of macrophages after an inflammatory insult from M1 to M2 cells. Potential mediators of this effect include the secreted phosphoprotein 1 (SPP1), a protein that is regulated by PGC-1 α in muscle to coordinate immune and endothelial cell activation for angiogenesis [31]. Likewise, meteorin-like (Metrl) is a PGC-1 α -controlled myokine with immunomodulatory effects [32]. However, SPP1 promotes a M1-type polarization with increased expression of MCP-1 [31] while Metrl is predominantly regulated by the PGC-1 α 4 isoform and subsequently promotes alternative activation of macrophages in adipose tissue in an indirect manner involving eosinophile-secreted IL-4 [32]. Therefore, the identity of a PGC-1 α - and PGC-1 β -controlled myokine that promotes this anti-inflammatory environment and possibly a M2-type macrophage polarization in skeletal muscle in a paracrine manner remains elusive. Nevertheless, pharmacological targeting of the immunomodulatory effect of the PGC-1s in muscle and thereby promotion

of an anti-inflammatory environment could be a novel strategy for metabolic and muscle wasting diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IL	interleukin
LPS	lipopolysaccharide
MCKα/β	muscle-specific PGC-1 α/β transgenic mice
NFκB	nuclear factor κ B
PGC-1	peroxisome proliferator-activated receptor γ coactivator 1
TA	tibialis anterior
TNFα	tumor necrosis factor α
WT	wildtype

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Highlights

- Muscle PGC-1s are insufficient to prevent acute systemic inflammation
- The muscle PGC-1s however promote a local anti-inflammatory environment
- This anti-inflammatory environment could contribute to the therapeutic effect of the PGC-1s

Figure 1. Muscle PGC-1 α and PGC-1 β do not suppress systemic pro-inflammatory factors after LPS/TNF α injection in vivo

A, B. PBS, LPS or TNF α were injected *i.m.* into the TA of MCK α (**A**) and MCK β (**B**) mice and their respective WT controls. Serum levels of TNF α and IL-6 were determined 4h post injection. Values represent the mean of at least 7 animals +SEM. ** $P < 0.01$, * $P < 0.05$, WT versus MCK α or MCK β , respectively.

Figure 2. Muscle PGC-1 α and PGC-1 β do not suppress acute inflammation, but reduce the expression of the M1 cytokine IL-12

A-F. PBS, LPS or TNF α were injected *i.m.* into the TA of MCK α (**A, C, E**) and MCK β (**B, D, F**) mice and their respective WT controls. **C, D.** mRNA expression levels of PGC-1 isoforms and different M1 cytokines in TA were measured by RT-PCR. Values represent the mean of at least 7 animals $\pm$ SEM. ** $P < 0.01$, * $P < 0.05$, WT versus MCK α or MCK β , respectively. **E, F.** TA cross sections were stained with hematoxylin and eosin (H&E) 4h post injection for histological analysis. Representative images are shown.

Figure 3. Muscle PGC-1 α and PGC-1 β induce local anti-inflammatory M2 cytokines in the TA after LPS/TNF α injection

A, B. PBS, LPS or TNF α were injected *i.m.* into the TA of MCK α (**A**) and MCK β (**B**) mice and their respective WT controls. mRNA expression levels of different M2 cytokines in TA were measured by RT-PCR. Values represent the mean of at least 7 animals +SEM. ** $P < 0.01$, * $P < 0.05$, WT versus MCK α or MCK β , respectively.