Remodeling of calcium handling in skeletal muscle through PGC-1α: impact on force, fatigability, and fiber type

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MORE THAN 600 DIFFERENT MUSCLES are present in the human body, and every skeletal muscle is composed of a distinct set of heterogeneous muscle fibers with various contractile properties (10, 43). On the basis of their twitch characteristic, muscle fibers are broadly classified into two major groups: fast-twitch fibers, which are capable of strong, explosive contractions, and slow-twitch fibers, which are suitable for prolonged physical activities (54).

A cardinal difference between different fiber types is their respective peak amplitude and rate of calcium transients (5). Compared with slow-twitch fibers, fast-twitch fibers express higher amounts of proteins involved in calcium release. The voltage sensor DHPR (1,4-dihydropyridine receptor) and the calcium channel ryanodine receptor 1 (RyR1) are both abundant in fast-twitch muscles (17). Thus, more calcium can be released in response to motor neuron activation (5). Once released from the sarcoplasmic reticulum (SR), calcium binds to troponin, thereby pulling away tropomyosin, exposing the myosin-binding sites and allowing contraction. A direct quantitative relationship exists between calcium concentration and force generation (35, 36). Moreover, fast-twitch fibers are endowed with a high amount of sarcoplasmic/endoplasmic reticulum calcium-ATPase 1 (SERCA1), a protein that pumps calcium back into the SR, and with parvalbumin, a protein that sequesters calcium and enhances calcium reuptake (17, 30). The interplay of these proteins allows rapid muscle relaxation. With respect to their amplitude and dynamics of calcium handling, the respective muscle fibers thus clearly differ, with fast-twitch fibers displaying much higher peak amplitudes and faster rates of calcium turnover compared with slow-twitch fibers.

The fiber type composition of muscle displays considerable plasticity even in the adult, differentiated state. In response to certain environmental demands (e.g., exercise), skeletal muscle remodels accordingly to adapt its functional capacity (4, 16). A prominent hallmark of adaptation to chronic endurance exercise is the fiber type switching towards an increased proportion of slow-twitch fibers. The underlying molecular networks are complex and only partially elucidated. An interplay of various, independent signaling pathways mediates exercise adaptation, many of which seem to converge ultimately on common key molecules where these signals are integrated (4, 15). The peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) represents such a systemic hub in muscle plasticity (4, 21, 22, 33).

The central importance of PGC-1α in exercise adaptation is impressively exemplified by studies using PGC-1α transgenic mice. Muscle-specific overexpression of PGC-1α, even in the absence of physical activity, is sufficient to drive changes that are typical of endurance training. PGC-1α increases the oxidative capacity by promoting mitochondrial biogenesis (32), improves oxygen supply to muscle by promoting angiogenesis (1), and increases peak oxygen consumption (12), lipid oxidation, and energy refueling (44, 47). Most importantly, PGC-1α has been shown to drive fiber type switching from fast, glycolytic towards slow, oxidative fibers as defined by changes in myosin heavy chain (MHC) composition and metabolic parameters (33). Intriguingly, muscle-specific PGC-1α transgenic mice (MPGC-1α TG) exhibit an increased endurance capacity in treadmill exercise tests (12), which is associated with an
elevated content of slow-type fibers. However, a modulation of calcium handling in MPGC-1α/TG mice to meet the specific demands of slow-type, high-endurance fiber contractions has not yet been investigated. Thus, it is unknown whether PGC-1α changes calcium handling cell-autonomously in skeletal muscle, although this would be a prerequisite to permit slow-type contractions. Thus, we investigated whether PGC-1α modulates calcium levels, whether it quantitatively and/or qualitatively affects force generation in skeletal muscle, and which mechanisms might underlie such changes. To address these issues, we used a mouse model with physiological overexpression of PGC-1α that is limited to skeletal muscle (33).

MATERIALS AND METHODS

Ethical approval. All studies were performed according to criteria outlined for the care and use of laboratory animals and with approval of the Swiss authorities. MPGC-1α TG mice (33) and control littersmates were maintained according to institutional guidelines in a conventional facility with a fixed 12:12-h light-dark cycle on a commercial pellet chow diet and free access to tap water. All experiments were performed in 8-wk-old male mice.

In vivo and ex vivo muscle strength assessment. Maximal force was tested in vivo using a grip strength meter (Chatillon, DFE Series Digital Force Gauge). In brief, mice were held by their tail and gently lowered towards the apparatus. They were allowed to grip the grid with their front or hindlimbs and were then slowly pulled backwards in a horizontal plane. Each measurement was repeated three times per mouse, and the average was taken as maximal strength. To test force ex vivo, extensor digitorum longus (EDL) muscles were dissected and mounted into a muscle testing setup (Heidelberg Scientific Instruments). Muscle force was digitized at 4 kHz by using an AD Instruments converter. EDL tetanus was recorded in response to 350-ms pulses at 100 Hz as previously described (13). Specific force was normalized to the muscle cross-sectional area (CSA = wet weight / wet weight).

![Table 1. Primer list](https://www.ajpcell.org)

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Fig. 1. Reduced maximal force in muscle-specific peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) transgenic (MPGC-1α TG) animals. A and B: maximal force of front legs (A) and hind legs (B) in MPGC-1α TG and control mice. C and D: total body weight (C) and fat-free dry mass (FFDM) (D) in MPGC-1α TG and control mice. WT, wild type. All values are expressed as means ± SE (n = 7 per group); *P < 0.05; **P < 0.01.
(mg)/length (mm) × 1.06 (density mg/mm³)] (13). To test resistance to fatigue, repeated tetani were recorded in response to 350-ms pulses at 100 Hz with intervals of 3.65 s (18).

Electron microscopy and morphometry. Electron microscopy and morphometry to determine muscle composition was performed as described previously (25). In brief, EDL muscles of control and transgenic mice were fixed in 6.5% (vol/vol) glutaraldehyde diluted in 0.1 M sodium cacodylate buffer, pH 7.4, at 4–8°C for several days and then subdivided into ~20 tissue blocks (1 mm³ in size). These blocks were used to prepare ultrathin sections of 50–70 nm thickness which were stained with lead citrate and uranyl acetate before viewing in a Philips EM-400 electron microscope. Transversely or slightly obliquely oriented sections from randomly selected blocks of each EDL muscle were subjected to morphometry. To this end, 20 micrographs obtained by systematic sampling were recorded at a magnification of ×6,000. These microphotographs were overlaid with a quadratic grid of 12 × 12 lines in distance of 0.916 μm. In accordance with standard stereological rules, the interceptions were counted to determine the volume density of individual muscle components (53).

RNA extraction and RT-PCR. Frozen tissues were homogenized under liquid nitrogen, and total RNA was isolated using TRIzol reagent (Invitrogen). RNA concentrations were adjusted and reverse transcription was carried out using random hexamer primers (Promega). Real-time PCR analysis (Power SYBR Green Master Mix, Applied Biosystems) was performed using the ABI Prism 7000 Sequence Detector. Relative expression levels for each gene of interest were calculated with the ΔΔCt method (where Ct is the threshold cycle number) and normalized to the expression of the TATA-box binding protein (TBP), whose expression was equal between wildtype and transgenic animals. The amplification efficiency of all investigated genes was similar to TBP. Primer sequences are listed in Table 1.

Calcium transients. Flexor digitorum brevis (FDB) muscles were enzymatically dissociated at 37°C for 60 min in an incubator for cell culture in Tyrode’s solution containing 0.20% collagenase (Sigma C0130-16). The muscles were rinsed in DMEM-10% FCS, transferred to DMEM and mechanically dissociated with fire-polished Pasteur pipettes. The dissociated fibers were placed on glass coverslips previously coated with laminin (Invitrogen catalog no. 23017-015). FDB fibers were loaded for 20 min at 20°C in Tyrode’s solution (137 mM NaCl, 5.4 mM KCl, 0.5 mM MgCl₂, 1.8 mM CaCl₂, 11.8 mM HEPES-NaOH, pH 7.4, 0.1% glucose) containing 10 μM Mag-Fluo-4-AM and 50 μM BTS [4 methyl-N-(phenylmethyl)benzenesulfonamide], and calcium transients were triggered by field stimulation with a 40-V pulse of 0.5 ms duration (11, 24). Fluorescent signals were recorded with a Nikon EclipseTE2000-U fluorescence microscope equipped with a P101 photomultiplier and digitized at 10 kHz. Calcium transients were calculated as (Fmax/Frest)/Frest.

**Fig. 2. Reduced tetanic force, but unaltered kinetics of force generation in extensor digitorum longus (EDL) muscle of MPGC-1α TG animals. A and B: absolute (A) and relative (B) tetanic force in EDL muscle of MPGC-1α TG and control mice in response to 100 Hz stimulation ex vivo. C and D: muscle weight (C) and length (D) of EDL muscle of MPGC-1α TG and control mice. E and F: half-contraction time (E) and half-relaxation time (F) of EDL muscle of MPGC-1α TG and control mice following tetanic stimulation at 100 Hz. All values are expressed as means ± SE (n = 8 per group); **P < 0.01; ***P < 0.001.**
Calcium transients in myotubes, grown on glass coverslips, were performed 24 h after adenoviral infection. In brief, myotubes were loaded with 5 μM fura-red at 37°C for 30 min, after which the coverslips were mounted on a 37°C thermostatically controlled chamber which was continuously perfused with Krebs-Ringer medium. Myotubes were first visualized by epifluorescence. Calcium transients were elicited by addition of 600 μM 4-chloro-m-cresol in 100 μM La³⁺, and measurements were made on a Nikon Eclipse TE2000- E inverted microscope. Fura-red was excited with a 405 nm laser, and the changes in fura-red fluorescence were followed at 625 nm using a brightline HC 625/26 cut-off filter (AHF Analysentechnik). Images were acquired every 100 ms through an oil immersion CFI Plan Apochromat 60 TIRF objective (1.49 numerical aperture) as previously described (49). Changes in fluorescence were analyzed with Metamorph imaging software.

SERCA activity. SERCA activity was determined as described by Simonides and van Hardeveld (41). In brief, fresh muscle homogenates were incubated in 1 mM EGTA, 10 mM phosphoenolpyruvate, 18 U/ml each of pyruvate kinase and lactate dehydrogenase, 0.2 mM NADH, 20 mM HEPES, pH 7.5, supplemented with 200 mM KCl, 15 mM MgCl₂, 200 mM NaN₃, and Triton X-100 (0.005%). The assay was started by addition of 4 mM MgATP followed by calcium at 12%, 18 U/ml each of pyruvate kinase and lactate dehydrogenase, 0.2 mM NADH, 20 mM HEPES, pH 7.5, supplemented with 200 mM KCl, 15 mM MgCl₂, 200 mM NaN₃, and Triton X-100 (0.005%). The assay was started by addition of 4 mM MgATP followed by calcium at various concentrations.

Calcineurin activity. Calcineurin activity was assayed using a commercially available kit from Enzo Life Sciences. In brief, muscles were lysed in lysis buffer containing protease inhibitors and passed through 18-gauge needles to loosely break up the tissue. After centrifugation at 100 g at 4°C for 45 min, the supernatant was desalted by gel filtration. Calcineurin activity was determined colorimetrically according to the manufacturer’s instructions.

Western blot analysis. Protein extraction was performed as described previously (45, 46). In brief, frozen tissues were crushed under liquid nitrogen, homogenized in lysis buffer [20 mM Tris·HCl, 138 mM NaCl, 2.7 mM KCl, 5% (vol/vol) glycerol, 1% (vol/vol) NP-40, and various hydrolase inhibitors] and incubated for 60 min. After centrifugation at 15,294 g for 15 min, protein concentration in the supernatant was quantified and equal amounts of protein extracts were separated by SDS-PAGE. The gels were then blotted on PVDF membranes and analyzed with the following antibodies: calsequestrin 1 [molecular mass (MM): 60 kDa] and 2 (MM: 55 kDa) (Sigma), SERCA 1 (MM: 100 kDa), 2 (MM: 114 kDa) and tubulin (MM: 55 kDa) (Cell Signaling), thyroid hormone receptor-α (MM: 47 kDa) (Abcam), sarcolipin (MM: 4 kDa), phospholamban and phosphorylated phospholamban (MM: 6 kDa) (Santa Cruz Biotechnology). Tubulin was used as loading control and for normalization. Bands within the linear range were quantified densitometrically.

Data analysis and statistics. All data are presented as means ± SE. The data were analyzed by two-tailed, unpaired Student’s t-test or Mann-Whitney test when the difference between the two SDs was significantly different. Statistical significance was set at \( P < 0.05 \).

RESULTS

Reduced muscle strength, but unchanged muscle mass in MPGC-1α TG mice. PGC-1α drives the formation of slow-twitch, oxidative fibers (33). However, it is unresolved whether characteristic features of fast-twitch fibers are lost upon PGC-1α-induced fiber type transition. We thus first tested whether PGC-1α-induced fiber type switching affects muscle strength in vivo. MPGC-1α TG mice generated less force [forelimbs, \(-12\%, \ P < 0.05\) (Fig. 1A); hindlimbs, \(-15\%, \ P < 0.01\) (Fig. 1B)] than control animals in the absence of alterations in body weight (Fig. 1C) or fat-free dry mass (Fig. 1D).

![AJP-Cell Physiol • doi:10.1152/ajpcell.00190.2011 • www.ajpcell.org](http://ajpcell.physiology.org/)

Fig. 3. Elevated subsarcolemmal and intermyofibrillar mitochondria, but diminished myofibrillar structures, in EDL muscle of MPGC-1α TG animals. A: quantification of morphometrical analysis of EDL muscle of MPGC-1α TG and control mice. B: detailed quantification of different mitochondrial subpopulations. C: representative micrographs from electron microscopy of EDL muscle of MPGC-1α TG and control mice. Arrows indicate intermyofibrillar mitochondria and arrowheads indicate subsarcolemmal mitochondria. All values are expressed as means ± SE (\( n = 8 \) per group): ** \( P < 0.01 \); *** \( P < 0.001 \).
To scrutinize further the nature of the reduced muscle force generation, the experiments were extended to intact muscle preparations ex vivo. Isolated EDL muscle of MPGC-1α TG mice showed reduced absolute (−25%, *P < 0.001) (Fig. 2A) and specific (−25%, *P < 0.01) (Fig. 2B) tetanic force compared with their control littermates. EDL weight (Fig. 2C) and length (Fig. 2D) were not different between control and transgenic animals, indicating that the reduced force was not due to loss in muscle mass. Despite the lower force in MPGC-1α TG mice, kinetics of muscle force contraction (Fig. 2E) and relaxation (Fig. 2F) in response to tetanic stimulation remained similar in both groups.

Altered muscle morphology in EDL of MPGC-1α TG mice. Since muscle mass was equal between transgenic and control mice, we speculated that the reduced force generation could conceivably result from altered muscle morphology. To test whether structural changes account for the reduced force, the composition of EDL muscle was determined by electron microscopy. Because of the overexpression of PGC-1α, transgenic mice showed elevated mitochondrial mass (+187%, *P < 0.001), at the expense of total myofibrillar structures, which were reduced by 20% (*P < 0.001) (Fig. 3A).

Detailed quantitative analyses revealed that both mitochondrial subpopulations were elevated to a similar extent, the centrally located intramyofibrillar mitochondria, which are in close contact to the SR, by +198% (*P < 0.001) and the subsarcolemmal mitochondria by +175% (*P < 0.01) (Fig. 3B).

Capacity for calcium release is diminished in EDL of MPGC-1α TG mice. Calcium release from the sarcoplasmic reticulum is an important determinant of muscle force generation. The skeletal muscle L-type Ca2+ channel (DHPR) serves

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**Fig. 4.** Decreased capacity for calcium release and fiber type switching in muscle of MPGC-1α TG animals. A: relative expression of genes involved in calcium release from the sarcoplasmic reticulum in muscle of MPGC-1α TG and control mice. RyR, ryanodine receptor 1; DHPR, 1,4-dihydropyridine receptor; Tdn, triadin; Jnc, junctin. B and C: relative mRNA expression of fast-twitch specific calsequestrin 1 (B) and slow-twitch specific calsequestrin 2 (C). D and E: protein levels of calsequestrin 1 (CQS 1; D) and calsequestrin 2 (CQS 2; E). F: comparison of calsequestrin 2 protein levels across different muscle tissues. All values are expressed as means ± SE (*n = 6–8 per group); *P < 0.05; **P < 0.01; ***P < 0.001.
as the voltage sensor for excitation-contraction (EC) coupling and activates Ca\(^{2+}\) release from the sarcoplasmic reticulum via RyR1 (40). RyR1 entertain a complex network with regulatory proteins, such as calsequestrin, triadin, and junctin (6).

While the mRNA expression of DHPR was similar between wild-type and transgenic animals, the mRNA expression levels of RyR1, triadin, and junctin were reduced in EDL muscles of transgenic animals (Fig. 4A). In addition, the mRNA expression of calsequestrin 1 was reduced, while calsequestrin 2 was elevated (Fig. 4B and C, respectively). The same pattern was observed at the protein level (Fig. 4D and E, respectively). This is in line with the fiber type switching towards oxidative fibers in the MPGC-1\(\alpha\) TG animals, as calsequestrin 1 and 2 are specific for fast-twitch and slow-twitch fibers, respectively (6). A comparison of calsequestrin 2 across different muscle tissues was then performed to determine the extent of fiber type transition. Calsequestrin 2 levels in EDL muscles of transgenic animals were lower than in hearts but similar to levels in soleus of wild-type animals as assessed by ANOVA and Tukey’s post hoc test (Fig. 4F).

To test functionally whether reduced expression of the calcium release machinery impairs calcium release from the SR, single fibers were isolated from FDB muscle and stimulated electrically. The kinetics of the calcium rise was unaltered in the transgenic animals (Fig. 5A). However, quantitatively less calcium was released into the myoplasm in transgenic animals compared with controls (Figs. 5B and 6A).

To demonstrate that the reduced calcium release was not primarily due to altered neuronal activity during development or changes at the neuromuscular junction, calcium release was determined in cultured myotubes following adenoviral infection with green fluorescent protein (GFP) or bicistronic PGC-1\(\alpha\)-GFP. To this end, the ryanodine receptor was directly stimulated by 4-chloro-\(m\)-cresol. Calcium release was reduced following adenoviral overexpression of PGC-1\(\alpha\) (Fig. 6B). Quantification of the traces showed reduced calcium release following overexpression of PGC-1\(\alpha\) (GFP: 0.23 ± 0.013 vs. PGC-1\(\alpha\)-GFP: 0.14 ± 0.003; \(n = 12–15\); \(P < 0.001\)).

Improved resistance to fatigue in EDL of MPGC-1\(\alpha\) TG mice. Interestingly, when muscles of MPGC-1\(\alpha\) TG mice and control littermates were repeatedly stimulated, they showed different temporal development of force generation. The first tetanic stimulation generated less force in transgenic animals than in their control littermates. However, following repeated tetanic stimulation, transgenic animals displayed higher force generation than wild type animals over time (Fig. 6C) and were therefore more resistant to fatigue, similar to previously published results.
lished data that demonstrated an increased time of stimulation in muscles of MPGC-1α TG animals until force generation dropped to 30% (33).

**Calcium reuptake capacity is diminished in EDL of MPGC-1α TG mice.** A possible explanation for the relative higher tetanic force in transgenic animals in response to repeated tetani could reside within a delayed decay of the calcium transient. Reduced calcium reuptake into the SR has been demonstrated in fatigue-resistant endurance-trained athletes (31). SERCAs are largely responsible for postcontraction calcium clearance. In adult skeletal muscle, two major isoforms of SERCA exist: SERCA1, which is very abundant in glycolytic muscle, and SERCA2, which has generally a low expression in all muscles, but which is the predominant isoform in slow oxidative fibers (6).

EDL muscle overexpressing PGC-1α showed reduced mRNA levels of SERCA1 (Fig. 7A), but unaltered levels of SERCA2 (Fig. 7B). These results were confirmed at the protein level (Fig. 7, C and D). Total SERCA activity was significantly reduced in transgenic animals (Fig. 7E). In line with this finding, the half-relaxation time of the calcium transient was increased in transgenic animals (Fig. 7F).

Besides calcium reuptake by the sarcoplasmic reticulum, calcium binding to the buffering molecule parvalbumin (30), mitochondrial calcium uptake regulated by mitochondrial calcium uptake 1 (MICU1) (39), and calcium export by sodium-calcium exchangers (NCX) (7) might contribute to muscle relaxation. Parvalbumin expression was significantly reduced in PGC-1 transgenic animals (Fig. 7G), while MICU1 was unaltered (Fig. 7G). Moreover, NCX levels were significantly reduced in MPGC-1α TG mice (Fig. 7G).

**Potential inhibitory mechanisms of SERCA in MPGC-1α TG mice.** The expression of SERCA is regulated by thyroid hormones (42). We thus tested whether thyroid hormone receptor expression is altered in skeletal muscle of PGC-1 transgenic mice. We observed a significant reduction in thyroid hormone receptor mRNA expression in transgenic mice (Fig. 8A). Moreover, protein levels of the thyroid hormone receptor were reduced (Fig. 8B). Finally, we tested the expression of inhibitors of SERCA activity. Relative mRNA and protein levels of

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**Fig. 7.** Diminished calcium removal in muscle of MPGC-1α TG animals. A and B: relative mRNA expression of sarcoplasmic/endoplasmic reticulum calcium-ATPase 1 (SERCA1; A) and SERCA2 (B) in muscle of MPGC-1α TG and control mice. C and D: protein levels of SERCA1 (C) and SERCA2 (D) in muscle of MPGC-1α TG and control mice. E: total SERCA activity in skeletal muscle of MPGC-1α TG and control mice. F: half-decay time of calcium following single-pulse electrical stimulation ex vivo. G: relative expression of genes involved in calcium removal in muscle of MPGC-1α TG and control mice. PVA, parvalbumin; MICU1, mitochondrial calcium uptake 1; NCX, sodium-calcium exchanger. All values are expressed as means ± SE (n = 6–8 per group); **P < 0.01; ***P < 0.001.
sarcolipin were elevated in MPGC-1α TG mice (Fig. 8C and D, respectively). Furthermore, we found elevated mRNA and protein levels of phospholamban in MPGC-1α TG mice (Fig. 8E and F, respectively). However, the inhibition of SERCA through phospholamban can be relieved by phosphorylation of phospholamban. MPGC-1α TG mice had a tendency to display higher levels of phosphorylated phospholamban (P = 0.066) (Fig. 8G), yet the ratio of phosphorylated phospholamban to total phospholamban was lower in the transgenic mice (Fig. 8H).

**Elevated calcineurin activity in MPGC-1α TG mice.** Sustained elevated calcium levels can activate calcineurin, which is involved in the establishment and maintenance of a slow fiber type-specific gene program (38, 55). We thus tested whether the prolonged calcium transients in MPGC-1α TG mice would affect calcium-dependent calcineurin activity. In the transgenic animals, altered calcium handling was associated with increased activity of calcineurin in skeletal muscle (Fig. 9).

**DISCUSSION**

Muscle fibers are distinguished on the basis of their physiological features, such as appearance (red vs. white), predominant MHC isoform (MHC I and IIA vs. MHC IIB and X), metabolic parameters (oxidative vs. glycolytic), or contractile properties (slow- vs. fast-twitching) (3, 4, 9, 23, 43). The latter concept is inextricably linked to calcium handling. The speed of calcium release, clearance, and its peak amplitude determine the characteristic of the twitch (5, 50).

Interconversion of different fiber types can occur because of the high plasticity of skeletal muscle. PGC-1α confers a reddish appearance to skeletal muscle, increases slow MHC isoform expression, and metabolically promotes a more oxidative phenotype (33). We have now shown that the transcriptional coactivator PGC-1α remodels calcium release by reducing the mRNA expression of several components of the sarcoplasmic reticulum calcium channel complex (namely, RyR1, triadin, and junctin). The ensuing lower myoplasmic concentration of calcium in combination with a decrease in contractile
elements (myofibrils), possibly as a result from increased mitochondrial biogenesis, culminates in a diminished maximal force generation. Furthermore, PGC-1α prolongs myoplasmic calcium transients by impairing SERCA mRNA and protein expression, as well as activity, thereby inhibiting calcium reuptake into the SR, by decreasing the mRNA levels of the cytosolic calcium buffer parvalbumin, and by reducing NCX mRNA levels and hence the potential to export calcium from the muscle fiber.

In contrast, the mRNA level of MICU1, a molecule that regulates mitochondrial calcium import, remains unaltered. Given the increase in mitochondrial mass, relatively lower levels of MICU1 per mitochondria occur in MPGC-1α TG mice.

Taken together, our findings demonstrate that PGC-1α slows down calcium handling in skeletal muscle. Altered calcium transients secondarily influence force generation, fatigability, and fiber type switching (Fig. 10).

Importantly, while PGC-1α regulates the expression of postsynaptic genes in skeletal muscle (19), the modulation of calcium signaling by PGC-1α is at least in part exerted in a cell autonomous manner. Thus, we observed altered calcium transients following electrical stimulation ex vivo in the absence of a motor neuron. Moreover, our experiments on muscle cells in vitro demonstrate that overexpression of PGC-1α is sufficient to alter calcium transients (Fig. 5B). This is further underlined by the finding that PGC-1α diminishes the mRNA levels of both the ryanodine receptor (calcium release) and SERCA1 (calcium reuptake) in myotubes following adenoviral overexpression of PGC-1α (Fig. 11). Thus, muscle PGC-1α per se is able to change calcium handling in skeletal muscle.

PGC-1α strongly promotes mitochondrial biogenesis in skeletal muscle and other tissues (33). We now demonstrate that subsarcolemmal and intermyofibrillar mitochondria are both elevated to a similar extent. Intermyofibrillar mitochondria are often positioned adjacent to calcium release units (CRUs) and are even tethered to them (8, 52). This proximity facilitates the communication between mitochondria and sarcoplasmic reticulum (14). Emerging evidence suggests that mitochondria exert an inhibitory action on local SR calcium release presumably by controlling the local redox environment of CRUs (26, 27, 34). Support for this hypothesis derives from studies on isolated slow- and fast-twitch fibers. Mitochondria-rich slow-twitch fibers display diminished local SR calcium

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**Fig. 9.** Elevated calcineurin activity in muscle of MPGC-1α TG animals. Calcineurin activity in skeletal muscle of MPGC-1α TG and control mice. All values are expressed as means ± SE (n = 6 per group); *P < 0.05.

**Fig. 10.** PGC-1α slows down calcium handling in skeletal muscle. This model integrates the findings of the present study. PGC-1α promotes mitochondrial biogenesis and downregulates the calcium releasing unit (CRU), which consists of RyR1, Tdn, Jnc, and calsequestrin. Subsequently, lower levels of calcium are released from the sarcoplasmic reticulum and force generation is altered. Moreover, NCX, PVA, and SERCA1, which are responsible for postcontraction calcium removal, are reduced in MPGC-1α TG mice. Concomitantly, the levels of SERCA inhibitors (SLN and PLB) are elevated. Thus calcium transients in the cytoplasm are slowed down and can influence muscle fatigability and fiber type switching.
release compared with mitochondria-poor fast-twitch fibers (26, 27).

SERCA accounts for the majority of calcium removal in skeletal muscle, and its expression is regulated by thyroid hormones (42). We now show that PGC-1α diminishes the levels of thyroid hormone receptor in skeletal muscle. Diminished levels of thyroid hormone receptor are associated with reduced SERCA transcription (28). Moreover, it has been demonstrated that calcium represses thyroid hormone-dependent transcription of SERCA1 (48). The coordinated effect of reduced thyroid hormone receptor and sustained elevated myoplasmic calcium levels through PGC-1α thus explains the transcriptional reduction in SERCA1. In addition, we observed elevated mRNA and protein levels of sarcolipin in MPGC-1α TG mice. Sarcolipin inhibits SERCA activity, and mice that overexpress sarcolipin in muscle are resistant to fatigue but have weaker muscles compared with their control littermates (51), a phenotype that is remarkably similar to the PGC-1α muscle-specific transgenic animals in that regard.

Similarly, we found elevated mRNA and protein levels of the SERCA inhibitor phospholamban. Phospholamban is mainly expressed in slow-twitch muscle, where it interacts with SERCA2 and thereby inhibits calcium reuptake into the sarcoplasmic reticulum. Interestingly, the inhibitory effect of phospholamban can be amplified by sarcolipin (2). Although the quantitative contribution of the different inhibitors remains unclear, their interplay clearly results in inhibition of total SERCA activity and delayed calcium removal from the myoplasm.

Taken together, PGC-1α interferes with multiple mechanisms that can lower myoplasmic calcium levels postcontraction, but it mainly affects SERCA expression and activity. Overall, PGC-1α slows down calcium removal and thus induces features of calcium handling in fast-twitch fibers that are reminiscent of fatigue-resistant slow-twitch fibers. How PGC-1α reduces the expression of these genes mechanistically remains unclear.

Beyond its key role in excitation-contraction coupling, calcium could be implicated in fiber type switching. Inhibition of the calcium release channel is crucial for fiber type transformation since RyR activity in fast muscle fibers contributes to the repression of slow muscle-specific genes (29). Furthermore, calcium is involved in transcriptional regulation through calcium-dependent enzymes such as calcineurin and calmodulin-dependent kinases. In adult mouse skeletal muscle fibers, the concerted action of the two pathways is required to accomplish a fast-to-slow fiber type transformation (37). Our results suggest that modified calcium handling in PGC-1α muscle-specific transgenic animals could relieve the repression on slow muscle-specific genes and support sustained activation of calcium-dependent enzymes that drive fiber type switching. Interestingly, PGC-1α thus appears to be downstream and upstream of calcium signaling in muscle: the induction of PGC-1α gene expression in endurance exercise is mediated to a large extent by calcium signaling (20), and, in turn, PGC-1α promotes a slow fiber type-specific calcium handling in muscle. Thereby, PGC-1α promotes all three prerequisites for high-endurance contractions: availability of calcium, slow-type myofibrillar proteins, and adequate supply of energy.

In conclusion, we have provided the first evidence that PGC-1α drives changes in muscular calcium handling by reducing calcium release and clearance. Decreased calcium release accounts for reduced maximal force and relieves the inhibition of slow muscle-specific genes, while diminished calcium clearance promotes resistance to fatigue and fiber type switching. Our data suggest that elevated PGC-1α levels, in combination with muscle contraction, promote fiber type switching. PGC-1α alters calcium handling capacity, and elevated myoplasmic calcium levels, which support fiber type switching, are achieved in response to contraction. Our findings provide new physiological insights into the role of PGC-1α in skeletal muscle adaptation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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