The transcriptional coactivator PGC-1α is dispensable for chronic overload-induced skeletal muscle hypertrophy and metabolic remodeling

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Skeletal muscle mass loss and dysfunction have been linked to many diseases. Conversely, resistance exercise, mainly by activating mammalian target of rapamycin complex 1 (mTORC1), promotes skeletal muscle hypertrophy and exerts several therapeutic effects. Moreover, mTORC1, along with peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), regulates skeletal muscle metabolism. However, it is unclear whether PGC-1α is required for skeletal muscle adaptations after overload. Here we show that although chronic overload of skeletal muscle via synergist ablation (SA) strongly induces hypertrophy and a switch toward a slow-contractile phenotype, these effects were independent of PGC-1α. In fact, SA down-regulated PGC-1α expression and led to a repression of energy metabolism. Interestingly, however, PGC-1α deletion preserved peak force after SA. Taken together, our data suggest that PGC-1α is not involved in skeletal muscle remodeling induced by SA.

muscle overload | transcriptional regulation | resistance training

Skeletal muscle size exhibits drastic changes throughout life, mainly dependent on mechanical load and nutritional supply (1). For example, loss of muscle mass is observed during immobilization and starvation, but also under pathological conditions like heart failure and cancer (2). To date, resistance exercise is considered as one of the most efficient ways to induce skeletal muscle hypertrophy and to revert the adverse effects of muscle wasting (2, 3). However, because pharmacological interventions are scarce, identification of the molecular regulation of muscle remodeling via resistance exercise is of great therapeutic interest.

Activation of mammalian target of rapamycin complex 1 (mTORC1) is the main regulatory step by which resistance exercise enhances protein synthesis and skeletal muscle size (4). In fact, inhibition of mTORC1 drastically abrogates plantaris hypertrophy via chronic overload (5), even though muscle size is not affected uniformly by muscle knockout of the mTORC1 inhibitor tuberous sclerosis complex 1 (6). Interestingly, the activity of the mTORC1 protein kinase complex positively correlates with oxidative capacity (7, 8). Moreover, mTORC1 is recruited to the promoter of a wide range of metabolism-related genes to regulate their expression (9–11). In skeletal muscle, mTORC1 regulates oxidative metabolism by facilitating the activation of Yin Yang 1 by the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (10, 12). The physiological context in which the mTORC1–PGC-1α axis regulates energy metabolism, however, is unknown.

Protein degradation is an important limiting factor of skeletal muscle hypertrophy. This process is fine-tuned by the transcription factor forkhead box O3 (FOXO3), which regulates the expression of the E3 ubiquitin ligases muscle ring finger protein 1 (MuRF1) and F-box protein 32 (Fbxo32/atrogin-1) (13). Interestingly, PGC-1α represses FOXO3 activity and consequently ameliorates skeletal muscle mass loss during denervation and aging (14–16). Finally, a recently discovered transcript variant of PGC-1α called PGC-1α4 also protects against skeletal muscle wasting and even promotes skeletal muscle hypertrophy (17). Inversely however, the functional requirement for PGC-1α in muscle hypertrophy and its associated adaptations has not been studied so far. Here, we therefore examined the role of PGC-1α in the molecular and functional adaptations to chronic overload of skeletal muscle.

Results

PGC-1α Is Not Required for Chronic Overload-Induced Plantaris Hypertrophy. To induce skeletal muscle hypertrophy via mechanical overload, we have taken advantage of the well-established method of synergist ablation (SA) (18, 19). The role of PGC-1α in skeletal muscle was studied using WT, PGC-1α muscle-specific transgenic (mTg), and PGC-1α muscle-specific knockout (mKO) mice (Fig. S1*A* and *B*), thereby avoiding confounding factors of whole-body KO and Tg models (20). Under basal conditions, neither body weight nor wet weight of gastrocnemius, plantaris, tibialis anterior, and quadriceps showed significant differences between genotypes (Table S1). However, soleus was slightly heavier in mTg mice, whereas extensor digitorum longus was slightly lighter in both mTg and mKO animals (Table S1).

After SA, we observed a ∼87% increase in the relative mass of WT and mTg overloaded plantaris (OVL) (Fig. 1*A*). Interestingly, this response was slightly attenuated in mKO mice (Fig.

Significance

Skeletal muscle hypertrophy is mainly induced by growth hormones and mechanical overload and exerts health beneficial effects. The mammalian target of rapamycin complex 1 (mTORC1) and the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) are key regulators of skeletal muscle mass and energy metabolism, respectively. Thus, acting in concert, mTORC1 and PGC-1α interplay is thought to regulate skeletal muscle function. Our results indicate that PGC-1α is not required for skeletal muscle hypertrophy and a slow-contractile phenotype induced by chronic overload of the plantaris muscle. In fact, PGC-1α gene expression and global energy metabolism were repressed in this experimental context of muscle hypertrophy. Hence, these results exclude PGC-1α as the main regulator of skeletal muscle remodeling after chronic overload.

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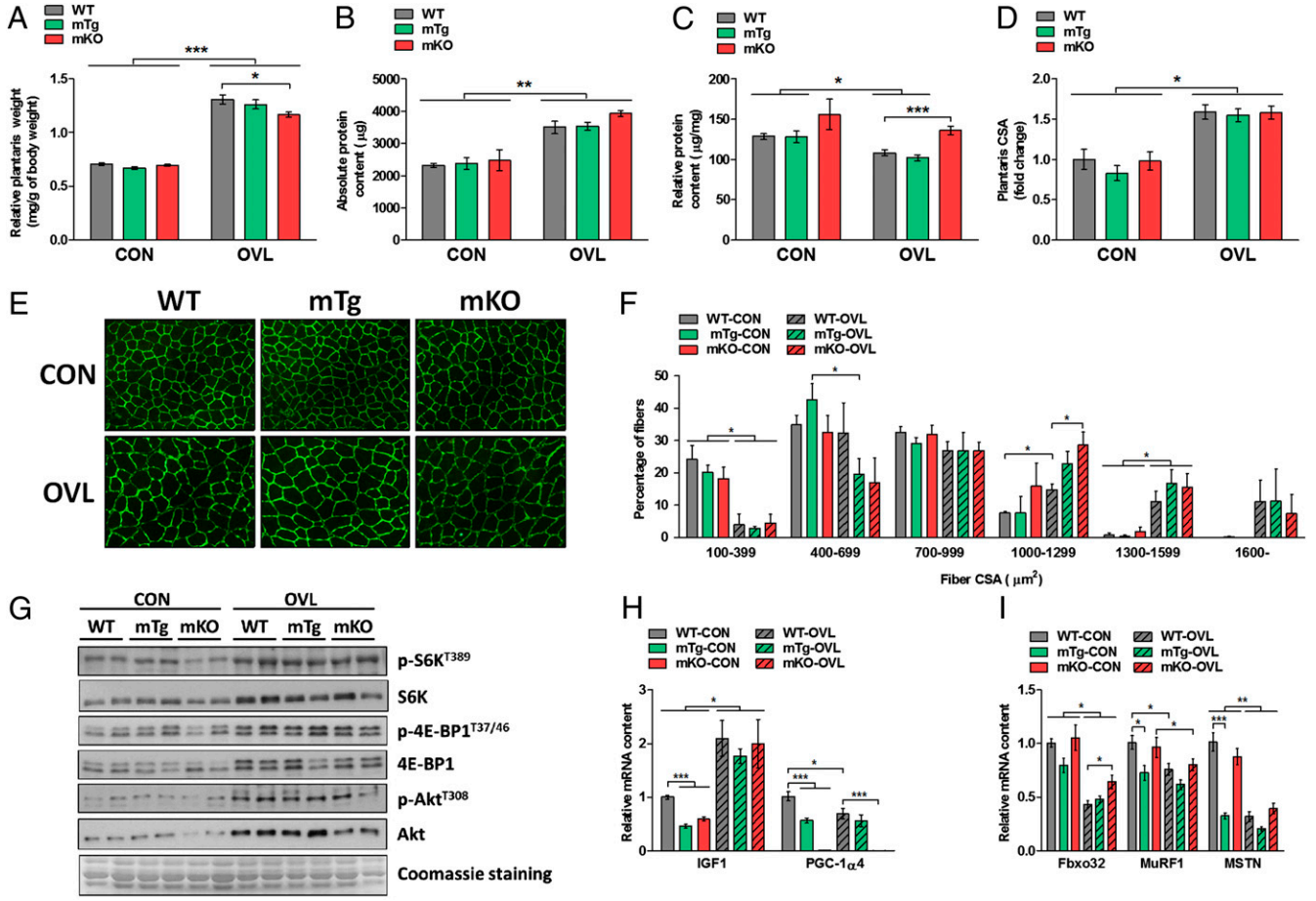

Fig. 1. Effects of SA on plantaris hypertrophy. (*A*) Relative plantaris weight (n = 15–18 per group). (*B* and *C*) Absolute and relative plantaris protein levels (n = 6 per group). (*D*) Whole plantaris CSA (n = 3 per group). (*E* and *F*) Representative pictures of laminin staining and quantification of fiber CSA (n = 3 per group). (*G*) Western blot analysis of mTORC1 and PI3K target proteins (n = 6 per group). (*H* and *I*) Quantitative PCR (qPCR) analysis of prohypertrophic and proatrophic genes (n = 6 per group). Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

1*A*). Moreover, absolute protein and RNA content were elevated by ∼53% and ∼63%, respectively, in OVL of all genotypes (Fig. 1*B* and Fig. S1*C*). Whereas relative RNA content remained higher after SA (Fig. S1*D*), relative protein content was decreased in WT and mTg OVL (Fig. 1*C*). All genotypes showed an increase in whole plantaris cross-sectional area (CSA) in response to SA (Fig. 1*D*). The analysis of single muscle fiber CSA also revealed an overall shift toward a higher proportion of large fibers in WT, mTg, and mKO OVL (Fig. 1 *E* and *F*).

Next, plantaris hypertrophy was further studied at the molecular level. Interestingly, relative phosphorylation levels of the mTORC1 target protein ribosomal protein S6 kinase (S6K) were increased and decreased in mTg and mKO control plantaris (CON), respectively (Fig. 1*G* and Fig. S1*E*). After SA, relative p-S6K^{T389} levels remained elevated in mTg OVL, whereas they significantly increased in WT and mKO mice compared with CON (Fig. 1*G* and Fig. S1*E*). Surprisingly, this positive effect on S6K phosphorylation was particularly pronounced in mKO mice (Fig. 1*G* and Fig. S1*E*). In contrast, relative phosphorylation levels of the mTORC1 and PI3K target proteins eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and Akt, respectively, were not increased by SA (Fig. 1*G* and Fig. S1*E*). The total protein content of these proteins was elevated by SA in all genotypes (Fig. 1*G* and Fig. S1*F*).

Next, transcriptional analysis revealed lower levels of insulin-like growth factor 1 (IGF1) mRNA in mTg and mKO CON, whereas IGF1 expression was increased by SA in all genotypes (Fig. 1*H*). Importantly, PGC-1α4 mRNA levels were significantly decreased in both mTg and mKO CON (Fig. 1*H*). Unexpectedly, we found that SA decreased PGC-1α4 mRNA levels by 32% in WT mice (Fig. 1*H*). The analysis of genes involved in muscle atrophy showed lower mRNA content of MuRF1 and myostatin (MSTN) in mTg CON (Fig. 1*I*). SA down-regulated Fbxo32 and MSTN in all genotypes, but the effect on Fbxo32 was slightly reduced in mKO mice (Fig. 1*I*). MuRF1 mRNA was also lower in WT and mKO OVL, whereas expression remained low in mTg OVL (Fig. 1*I*). Thus, these data suggest that PGC-1α does not modulate the overall effects of SA on plantaris hypertrophy.

Metabolic Remodeling Induced by Chronic Overload. Considering the proposed metabolic role of the mTORC1–PGC-1α axis, we also explored the metabolic adaptations to SA. Most of the genes involved in the electron transport chain (ETC), tricarboxylic acid cycle, β-oxidation, and fatty acid transport showed higher mRNA levels in mTg CON, whereas the opposite effects were observed in mKO CON (Fig. 2*A* and Fig. S2*A*). Genes involved in glucose and lactate metabolism revealed a different pattern, mainly reflecting an enhanced lactate catabolism in mTg CON (Fig. 2*A* and Fig. S2*A*). Surprisingly, SA down-regulated most of these genes, regardless of the metabolic function (Fig. 2*A* and Fig. S2*A*). Protein content of different components of the ETC also showed a similar expression pattern, both under basal conditions and after SA (Fig. 2*B* and Fig. S2*B*). To further explore the

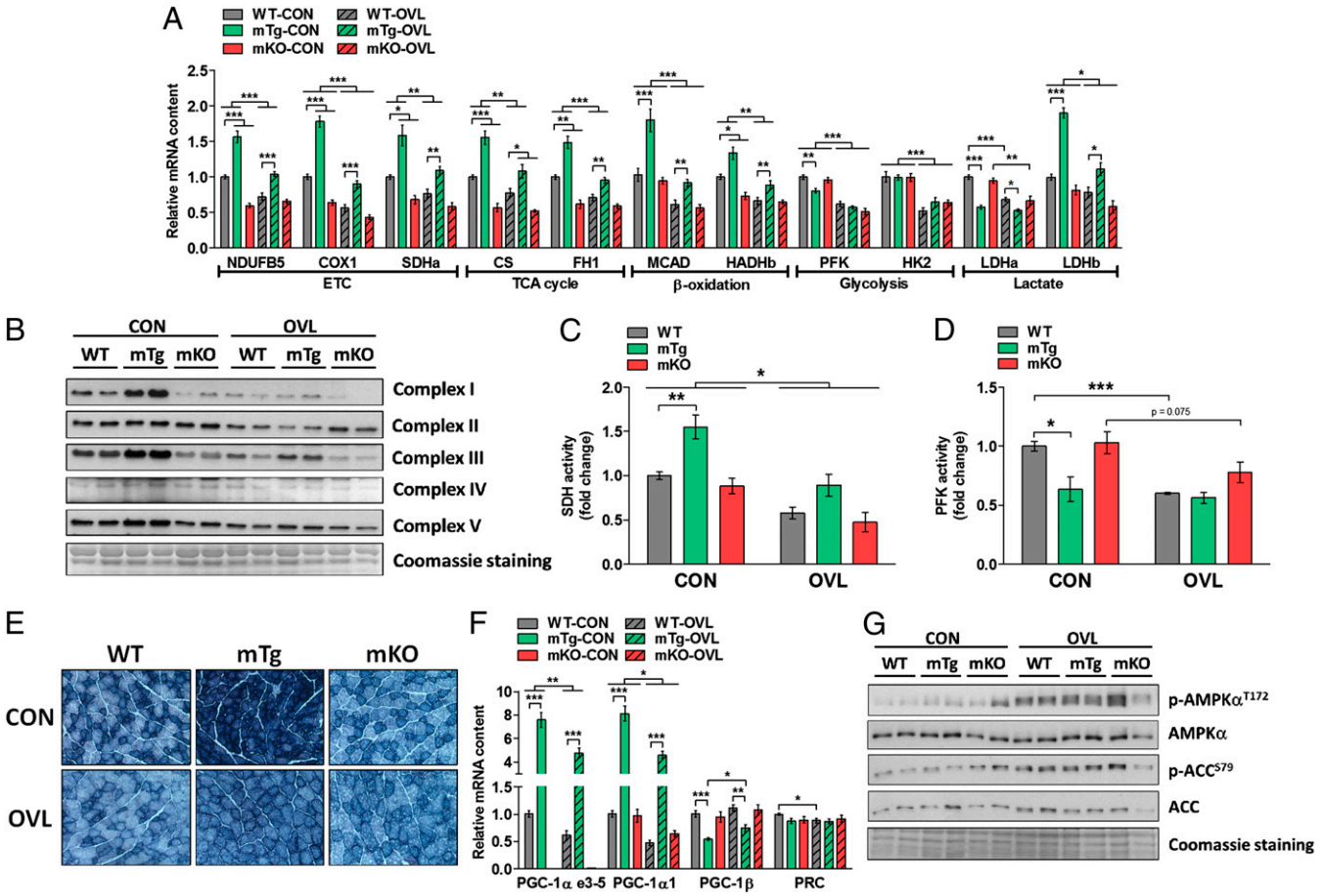

Fig. 2. Plantaris metabolism is repressed by SA. (*A*) qPCR analysis of metabolic-related genes (n = 6 per group). (*B*) Western blot analysis of different components of the ETC (n = 6 per group). (*C* and *D*) SDH and PFK activity assessment (n = 6 per group). (*E*) Representative pictures of NADH staining (n = 3 per group). (*F*) qPCR analysis of PGC-1 family of coactivators (n = 6 per group). (*G*) Western blot analysis of AMPK activation (n = 6 per group). Values are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

functional effect of SA on oxidative and glycolytic metabolism, we assessed the enzymatic activities of succinate dehydrogenase (SDH) and phosphofructokinase (PFK), respectively. As expected, SDH and PFK activities were higher and lower in mTg CON, respectively (Fig. 2*C* and *D*). After SA, SDH activity was significantly decreased in all genotypes (Fig. 2*C*), whereas PFK activity decreased in WT OVL and showed a trend (P = 0.075) toward a reduction in mKO OVL (Fig. 2*D*). Consistently, NADH staining implied an increase and slight decrease in the proportion of oxidative fibers in mTg and mKO CON, respectively, which was reduced in WT and mTg upon SA (Fig. 2*E*). Additionally, on the basis of NADH staining, either positive fibers (oxidative) and negative fibers (glycolytic) separately or all muscle fibers collectively showed a similar increase in CSA after SA (Fig. S2*C*). We also found that SA significantly decreased PGC-1α1 mRNA content by 53% and 44% in WT and mTg OVL, respectively (Fig. 2*F*), similar to the reduction in PGC-1α4 content (Fig. 1*H*). Importantly, it should be noted that the genetic ablation of PGC-1α in mKO mice was assessed with primers targeting the floxed region of the gene (exon 3–5; PGC-1α e3-5) that is shared in all different PGC-1α isoforms (17). In contrast, because specific detection of PGC-1α1, -2, -3, and -4 relies on amplification of transcript regions outside this deleted area, mRNA is detected in mKO mice with primers that are specific for PGC-1α1 (Fig. 2*F*). Nevertheless, however, because of the knockout strategy aimed at introducing a frame shift in the gene, no protein will be made from these nonfunctional transcripts in PGC-1α knockout models (21, 22). In contrast to PGC-1α, no major changes in PGC-1β- and PGC-1-related coactivator were induced by SA (Fig. 2*F*). The AMP-activated protein kinase (AMPK) is another key regulator of cellular energy metabolism in various tissues (23). Interestingly, relative phosphorylation levels of AMPK and its target protein acetyl-CoA carboxylase (ACC) were highly increased by SA, whereas absolute levels of ACC were only increased in WT OVL (Fig. 2*G* and Fig. S2 *D* and *E*). Our results imply a negative effect of SA on energy metabolism, which seems to be related to the down-regulation of PGC-1α and was consequently attenuated by PGC-1α overexpression.

Functional Adaptations to Chronic Overload. Finally, we investigated the effects of SA on plantaris contractility and fatigue resistance. Peak force in response to a single twitch and tetanic stimulation was lower in mTg CON (Fig. 3 *A*–*C*). In WT, SA significantly decreased force generation independently of the stimulation pattern, whereas in mTg mice only twitch force was further decreased (Fig. 3 *A*–*C*). Surprisingly, force generation did not change in mKO mice after SA (Fig. 3 *A*–*C*). SA also delayed the contractile kinetics observed in response to tetanic stimulation (Table S2). Furthermore, fatigue resistance was higher in mTg CON, and SA significantly improved this parameter in all genotypes (Fig. 3 *D* and *E*). Thus, the area under the curve of the fatigue resistance protocol was increased by SA in all genotypes (Fig. 3*F*).

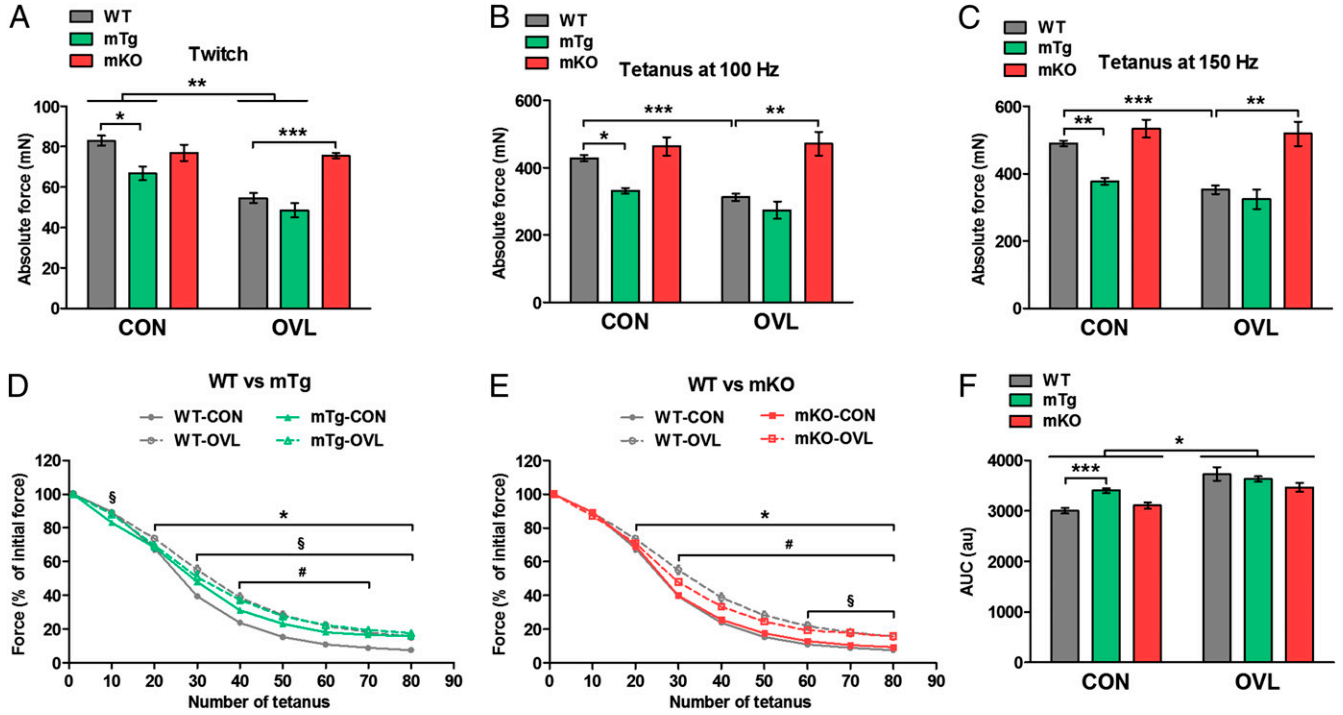

Fig. 3. Effects of SA on plantaris contractility. (*A–C*) Peak force in response to a single twitch or tetanic stimulation ($n = 5–6$ per group). (*D–F*) Assessment of fatigue resistance and the corresponding area under the curve ($n = 5–6$ per group). Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. In *D* and *E*: *$P < 0.05$ CON vs. OVL WT, $^{\#}P < 0.05$ CON vs. OVL mTg or mKO, $^{\S}P < 0.05$ CON WT vs. mTg or mKO.

The contractile properties of muscle cells are in part determined by the specific expression of genes involved in calcium handling and excitation–contraction coupling (24, 25). Thus, mTg CON showed a shift toward a slow phenotype, mainly reflected by a lower mRNA level of calsequestrin 1 (CSQ1) and myosin heavy chain 2 B (MyHC-2B), in contrast to higher levels of the respective slow isoform of these genes, CSQ2 and MyHC-1 (Fig. 4 *A* and *B*). On the other hand, mKO CON showed a higher mRNA level of CSQ1, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 1 (SERCA1), and actin α1 (ACTC1) (Fig. 4 *A* and *C*). In line with the functional analysis, SA induced CSQ2, SERCA2, MyHC-2A, ACTC1, and troponin C1 (TNNC1) gene expression in WT OVL (Fig. 4 *A–C*). Moreover, mRNA level of SERCA1, MyHC-2B, ACTN3, and TNNC2 were lower in WT OVL (Fig. 4 *A–C*). These effects of SA were not substantially altered in mTg or mKO mice (Fig. 4 *A–C*). Importantly, the genes up- and down-regulated by SA are characteristic of slow- and fast-twitch skeletal muscle, respectively (24, 25). At the

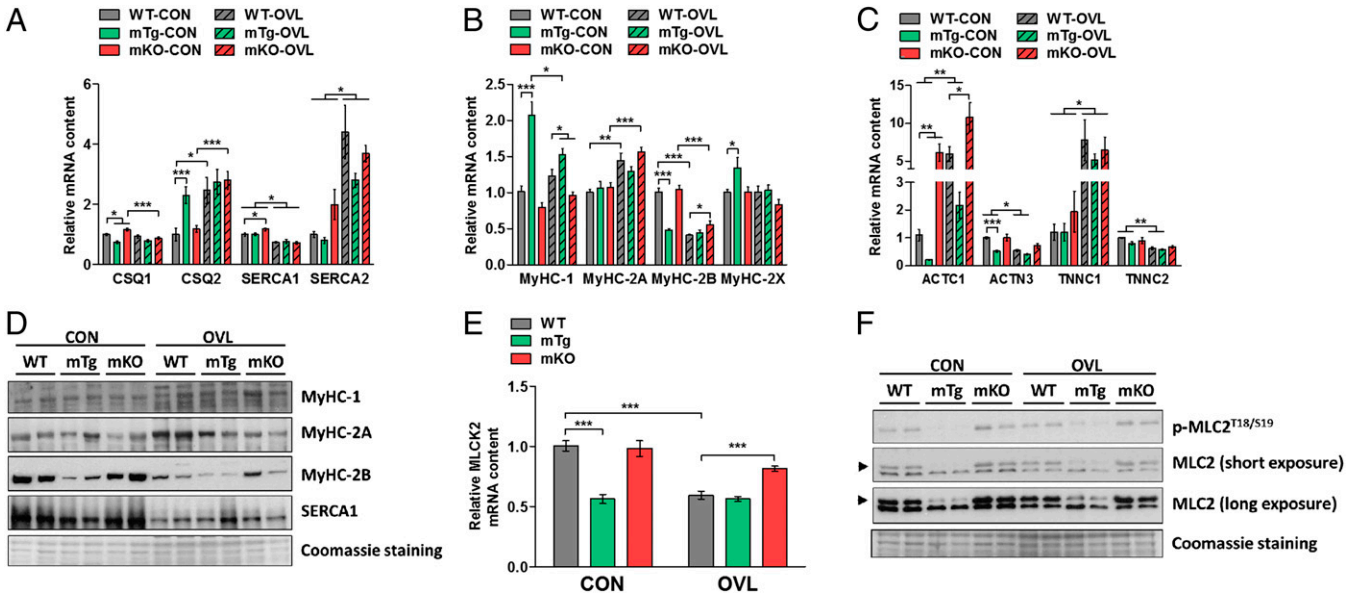

Fig. 4. SA modulates the expression of excitation–contraction coupling-related genes. (*A–F*) qPCR and Western blot analysis of key regulators of excitation–contraction coupling in plantaris (arrowhead indicates MLC2; $n = 6$ per group). Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

protein level, we observed a reduction of MyHC-2B and SERCA1 in mTg CON, whereas SA strongly decreased MyHC-2B in all genotypes and SERCA1 in WT and mKO OVL, further supporting the functional analysis (Fig. 4*D* and Fig. S3*A*). We finally analyzed myosin regulatory light chain (MLC) phosphorylation by MLC kinase 2 (MLCK2), a key process for the potentiation of force generation (26). MLCK2 mRNA content decreased by ~42% in mTg CON and WT OVL (Fig. 4*E*). In contrast, the effect of SA on MLCK2 mRNA was attenuated in mKO OVL (Fig. 4*E*), strongly supporting the ex vivo assessment of peak force. Interestingly, although relative levels of p-MLC2$^{T18/S19}$ were lower in mTg mice, the opposite effect was found in mKO CON and OVL (Fig. 4*F* and Fig. S3*B*). In addition, we observed that MLC2 protein content was strongly reduced in mTg CON, and it was increased by SA in mTg and mKO OVL (Fig. 4*F* and Fig. S3*C*). Thus, taken together, the functional and molecular analysis strongly suggests that SA induces a switch toward a slow-contractile phenotype.

Discussion

Different exercise-based and pharmacological strategies have been proposed to prevent skeletal muscle atrophy under pathological conditions, with mTORC1 activation playing a key role in this process (2, 3). In fact, mTORC1 is a well-known mediator of protein synthesis and cell growth and has recently emerged also as a metabolic regulator (27). The link between mTORC1 and skeletal muscle metabolism is thought to be PGC-1α (10, 12). PGC-1α and PGC-1α4 positively modulate skeletal muscle mass under catabolic conditions (14, 16, 17). However, we have now found that regardless of mTORC1 activity, PGC-1α is not required for skeletal muscle remodeling via chronic overload.

The role of PGC-1α in anabolic pathways is poorly understood. In aged mice, PGC-1α preserves skeletal muscle mass along with Akt and mTOR phosphorylation (15). We have found that mTg CON showed higher p-S6K^{T389} relative levels, whereas the opposite effect was observed in mKO CON. Interestingly, enhanced oxidative metabolism is associated with increased mTORC1 activity (7, 8), implying that PGC-1α might modulate mTORC1 through its effects on mitochondrial function. Moreover, mTg CON exhibited lower levels of MuRF1 and MSTN mRNA, but neither mTg nor mKO CON showed signs of skeletal muscle hypertrophy, similar to previous reports (16, 28–31). Consistently, we have previously shown that a cluster of genes related to ribosomes and anabolic pathways is down-regulated in mTg skeletal muscle (32). Finally, in vitro, PGC-1α does not regulate the rate of protein synthesis in C_2C_{12} myotubes (14). As a consequence, the assessment of a wide range of parameters related to skeletal muscle hypertrophy revealed that the overall effect of SA was not different in mTg or mKO mice. In fact, endogenous PGC-1α1 and PGC-1α4 were down-regulated by SA in WT mice. Importantly, the fact that both of these coactivators were fully deleted in mKO mice strongly indicates that they are not required for chronic overload-induced skeletal muscle hypertrophy. In line with these data, PGC-1α overexpression does not overcome skeletal muscle atrophy induced by mTORC1 inhibition via raptor knockout (28). It is important to note that PGC-1α4 was proposed to promote muscle growth via IGF1 and MSTN expression (17), which seem to play a minor role in the context of SA. In fact, even though IGF1 was up-regulated by SA, p-Akt^{T308} relative levels were not increased. Therefore, these data support the concept that mechanical overload-induced skeletal muscle hypertrophy is PI3K-independent (4) and thus might not involve PGC-1α4. Our results do not preclude PGC-1α4 to be required for muscle hypertrophy in other contexts. Accordingly, some studies show no changes in PGC-1α expression in human skeletal muscle after resistance exercise (33–35), whereas others have reported opposite results (17, 36).

PGC-1α is a central mediator of mitochondrial biogenesis (37). Consistently, concomitantly with a reduction in PGC-1α mRNA, SA strongly repressed the expression and activity of several metabolic-related proteins in both WT and mTg mice. Microarray analysis of human skeletal muscle has also revealed that resistance exercise does not increase the expression of genes involved in oxidative metabolism (38, 39). In fact, resistance-trained athletes exhibit the same (38) or lower relative peak oxygen consumption compared with untrained healthy people (40). These data disagree with the metabolic role of mTORC1, even though it should be noted that this aspect of mTORC1 function has been mainly studied using genetic and pharmacological approaches. In stark contrast, mechanical overload of skeletal muscle modulates a wide spectrum of signal pathways (1), suggesting that the regulation of additional molecules could blunt the metabolic effects of mTORC1, at least in part via PGC-1α down-regulation.

Interestingly, SA has previously been demonstrated to lower peak force (41, 42), which might be interpreted as a negative effect. However, our data suggest that it actually reflects an extreme switch to a slow-contractile phenotype. In fact, SA increased the expression of the slow-twitch specific isoform of several genes regulating excitation–contraction coupling in a PGC-1α–independent way, whereas the fast-twitch specific isoforms of these genes were concomitantly down-regulated. Importantly, our findings imply that the reduction in peak force induced by SA is mainly related to MyHC-2B, SERCA1, MLCK2, and MLC2 expression. These proteins are highly expressed in fast-twitch muscles, and they play a pivotal role in muscle force potentiation (25, 26). In accordance, we found that peak force, MyHC-2B mRNA/protein and MLCK2 mRNA levels were preserved in mKO OVL, whereas MLC2 protein levels were even increased. Moreover, p-MLC2$^{T18/S19}$ relative levels were higher in mKO mice, and the opposite results were observed in mTg CON, supporting thus the effects of PGC-1α on skeletal muscle contractility (29). Interestingly, SA improved fatigue resistance primarily in a PGC-1α–independent way. The metabolic remodeling induced by SA seems to disagree with the functional adaptations. However, our data as well as previous reports (5, 43) show that SA strongly increases AMPK activity, and considering its metabolic role (23), it seems likely that AMPK activation compensates for the negative effects of SA on energy metabolism.

In summary, we have now shown that besides inducing skeletal muscle hypertrophy, SA strongly promotes a switch toward a slow-contractile phenotype that seems to be dissociated from the metabolic phenotype. Moreover, we have identified MyHC-2B, MLCK2, and MCL2 as PGC-1α targets involved in skeletal muscle contractility. However, our data demonstrate that PGC-1α and PGC-1α4 are not involved in skeletal muscle remodeling induced by chronic overload. In fact, SA seems to disrupt the mTORC1–PGC-1α axis by down-regulation of this coactivator. It should be noted that SA does not fully resemble the effects of resistance exercise in human skeletal muscle in several aspects, and therefore the relevance of PGC-1α as a therapeutic target aiming at promoting skeletal muscle growth remains to be further explored under different conditions.

Materials and Methods

Animals. Mice were housed in a conventional facility with a 12-h night and day cycle, with free access to food and water. All experiments were performed on adult male mice with approval of the Swiss authorities. Description of mouse generation and genotyping is provided in *SI Materials and Methods*.

Synergist Ablation. Mechanical overload of plantaris was performed as previously described (43). Briefly, unilateral SA was performed under anesthesia (2.5% isoflurane) and sterile conditions, then soleus and gastrocnemius were surgically removed, and animals were allowed to recover for a period of 2 wk. The contralateral nonoperated leg was used as control.

Ex Vivo Assessment of Muscle Function, Histology and Immunohistochemistry, RNA Isolation and Quantitative PCR, Protein Isolation and Western blot, and SDH and PFK Activity Assays. These analyses were performed by standard procedures as described in *SI Materials and Methods*. See Table S3 for details on primer sequences.

Statistical Analysis. Values are expressed as mean ± SEM. Statistical significance was determined with unpaired two-tailed *t* tests or one-way ANOVA with Dunnett post hoc test. Significance was considered with a P value of <0.05.

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