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ERRγ promotes angiogenesis, mitochondrial biogenesis and oxidative remodeling in PGC1α/β-deficient muscle

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Summary

PGC1α is a pleiotropic co-factor that affects angiogenesis, mitochondrial biogenesis and oxidative muscle remodeling via its association with multiple transcription factors including the master oxidative nuclear receptor ERRγ. To decipher their epistatic relationship, we explored ERRγ gain-of-function in muscle-specific PGC1α/β double knockout mice (PKO). ERRγ-driven transcriptional reprogramming largely rescues muscle damage and improves muscle function in PKO mice, inducing mitochondrial biogenesis, antioxidant defense, angiogenesis, and a glycolytic-to-oxidative fiber-type transformation independent of PGC1α/β. Furthermore, in combination with voluntary exercise, ERRγ gain-of-function largely restores mitochondrial energetic deficits in PKO muscle, resulting in a 5-fold increase in running performance. Thus, while PGCs can interact with multiple transcription factors, these findings implicate ERRs as the major molecular target through which PGC1α/β regulates both innate and adaptive energy metabolism.

Introduction

Originally identified as a co-activator for PPARγ in brown fat (Puigserver et al., 1998), PPAR Gamma Co-activator 1α (PGC1α) is a master regulator of mitochondrial energy metabolism (Mouchiroud et al., 2014). In skeletal muscle, PGC1α and its closely related homologue PGC1β are required for maintaining basal mitochondrial energy metabolism and muscle functions (Rowe et al., 2013; Zechner et al., 2010). In addition, numerous studies

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Author Contributions

W.F., M.D., and R.M.E. designed the study. W.F., N. He, C.S.L., Z.W., N. Hah, W.W., and M.H. conducted all experiments. C.L. and R.T.Y. analyzed genomic data. W.F., A.R.A., M.D., and R.M.E. drafted and revised the manuscript.

Accession Numbers

RNA-Seq and ChIP-Seq data reported in this paper have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database, Accession # SRP110311.

have implicated muscle PGC1α in exercise-induced oxidative muscle remodeling, including glycolytic-to-oxidative fiber type transformations, increased vasculature development (angiogenesis), elevated mitochondrial biogenesis and OXPHOS activity, and a shift from glucose to fatty acid as the energy source (Egan and Zierath, 2013; Geng et al., 2010; Handschin et al., 2007; Holloszy and Booth, 1976; Lin et al., 2005; Lin et al., 2002). It is now clear that exercise training activates and induces muscle PGC1α, likely through both AMPK and SIRT1 signaling pathways, which subsequently induces downstream genes involved in oxidative muscle remodeling through its interaction with a number of transcription factors (TFs) (Canto and Auwerx, 2009).

As PGC1α interacts with over 20 TFs, the role and importance of specific downstream effectors of exercise-induced oxidative muscle remodeling is not clear (Villena, 2015). With regard to mitochondrial oxidative metabolism, the estrogen-related receptors ERRα, β, and γ have been suggested as key partners (Fan et al., 2013) (Mouchiroud et al., 2014). In particular, expression of ERRγ is highly specific to oxidative muscle fibers, and is enhanced during exercise-induced oxidative remodeling (Narkar et al., 2011) (Rangwala et al., 2010). Furthermore, due to its intrinsic transcriptional activity ectopic expression of ERRγ in glycolytic muscle is sufficient to drive oxidative muscle remodeling and increase endurance performance in the absence of PGC1α induction (Narkar et al., 2011) (Rangwala et al., 2010) (Gan et al., 2013). However, unlike exercise, ERRγ overexpression does not induce the expression or activation of PGC1α/β, suggesting PGC1-independent transcriptional regulation may naturally occur during ERRγ-driven muscle remodeling. However, while PGC1α/β are not induced, it is possible that basal levels of PGC1α/β are sufficient to drive ERRγ activity. Moreover, it remains to be demonstrated what features of PGC1α induction are dependent on ERR and what aspects of PGC1α/β deficient muscle can be rescued by ERRγ overexpression.

In addition to PGC1α, transcriptional co-repressors such as NCoR and RIP140 also participate in oxidative muscle remodeling induced by exercise, whereby reductions in their expression and the resulting de-repression of downstream TFs activates oxidative gene expression (Seth et al., 2007) (Yamamoto et al., 2011) (Fan et al., 2013). Furthermore, exercise is known to directly induce the expression of TFs such as ERRγ and PPARδ, which can activate their target genes without changes in expression or activity of co-factors (Wang et al., 2004) (Narkar et al., 2011) (Rangwala et al., 2010). Therefore, exercise training could beneficially affect muscle remodeling independent of PGC1α/β. Indeed, it has been shown that adult muscle PGC1α and β are dispensable for endurance exercise-induced oxidative muscle remodeling. However, the short-term (5 day) induction of a mature muscle cell *cre* driver in this model would allow for the incorporation of wild-type satellite cells during exercise-induced muscle regeneration, confounding the conclusion (Ballmann et al., 2016).

Here we show that ERRγ overexpression largely rescues autonomous muscle damage in a germ-line muscle-specific PGC1α/β double knockout mouse model (PKO). Despite the complete loss of muscle PGC1α/β, ERRγ overexpression induces multiple aspects of oxidative muscle remodeling, including increased mitochondrial biogenesis and fatty acid oxidation, a glycolytic-to-oxidative fiber-type transformation, and angiogenesis. Consistent with this, genomic analyses identify gene networks in these pathways that are directly bound

and activated by ERRγ independent of PGC1α/β. Furthermore, when combined with exercise training, ERRγ overexpression almost completely restores the mitochondrial energetic deficiency of PKO muscle. These findings identify ERRγ as a pro-oxidative transcription factor that directly regulates oxidative remodeling in a PGC1-independent fashion, and implicate the ERR subfamily as the dominant mediators of PGC1α in mitochondrial biogenesis and adaptive energy metabolism.

Results

Defects in PGC1-deficient muscle are improved by ERRγ

To determine the regulatory hierarchy in oxidative remodeling and mitochondrial function in muscle, we generated muscle-specific PGC1α/β double knockout (PKO) mice with comprehensive depletion of both PGC1α and β in skeletal muscle (>90% depletion, Fig. S1A–B **and RNA-seq data in SRP110311**). Loss of PGC1α/β caused a pronounced change in the color of the skeletal muscle, suggestive of an oxidative-to-glycolytic transformation (Fig. 1A). Consistent with this color change, the expression of myoglobin (*Mb*), a major determinant of muscle color, was reduced ~35% in PKO muscle (Fig. 1B). Notably, PKO mice showed evidence of severe muscle damage under sedentary conditions, as seen by the increased number of centrally localized nuclei (Fig. 1C–D), the marked increases in expression of developmental myosin genes *Myh3* and *Myh8* (Fig. S1C–D), and the elevated serum levels of muscle protein creatine kinase (CK, Fig. 1E). Furthermore, the endurance capacity of PKO mice was severely compromised. When subjected to a treadmill endurance test, the running time of PKO mice was reduced to ~5% of WT mice (Fig. 1F). The dramatic reduction in running performance was likely caused by a sharp elevation in lactate production (Fig. 1G) (Robergs et al., 2004), as circulating glucose – an independent determinant of endurance capacity – was not exhausted in PKO mice (Fan et al., 2017) (Fig. 1H).

To determine the capacity of ERRγ to rescue muscle defects, we crossed PKO mice with muscle-specific ERRγ transgenic (HE) mice and examined muscle function and running performance between WT, HE, PKO, and HEPKO mice. While overexpression of ERRγ intensified the reddish color of transgenic muscle as previously described (HE compared to WT muscle) (Narkar et al., 2011), it also largely restored WT-like color to PKO muscle (HEPKO vs PKO in Fig. 1A). Consistent changes in the expression of *Mb* were seen, with the overexpression of ERRγ increasing its level in HEPKO muscle to those similar to WT mice (Fig. 1B). Of note, the muscle damage seen in sedentary PKO mice was almost completely rescued in HEPKO mice, with a marked reduction in centrally localized nuclei and normalized expression of *Myh3* and *Myh8*, as well as circulating CK levels (Fig. 1C–E and Fig. S1C–D). Furthermore, the overexpression of ERRγ nearly tripled the running time of PKO mice (HEPKO, 24 minutes compared to PKO, 8.8 minutes, Fig. 1F), although the persistently elevated lactate levels (not fully rescued by ERRγ overexpression) eventually resulted in pre-exhaustion running failure (Fig. 1F–H).

ERRγ improves mitochondrial energetic defects in PGC1-deficient muscle

Severe defects in mitochondrial energetics were apparent in muscle from PKO mice, consistent with previous findings (Zechner et al., 2010). In particular, the activities of the electron transport chain (ETC) complexes were dramatically reduced (Fig. 2A–B and S2A–B), as was the TCA cycle enzyme citrate synthase (CS, Fig. S2C). Consistent with this, the expression of core genes involved in OXPHOS and the TCA cycle including *Cox6a2*, *mt-Nd4*, *Idh2*, *Atp5a*, and *Cs*, as well as the mtDNA copy number, were all reduced in PKO muscle, with equivalent reductions seen in mitochondrial protein levels (Fig. 2C–D and S2D–F). As a consequence, mitochondrial oxygen consumption rates determined using succinate and palmitate as substrates were reduced 45% and 65%, respectively (Fig. 2E and S2G). Notably, each of these mitochondrial energetic defects was significantly restored by the overexpression of ERRγ. Interestingly, the magnitude of the changes induced by ERRγ in the PKO mice was comparable, and in some cases greater than those induced in WT mice (HEPKO vs PKO compared to HE vs WT, Fig. 2A–E and S2A–G). This suggests that ERRγ-driven improvements in mitochondrial energy metabolism are not contingent on the function of PGC1α/β.

Compromised mitochondrial function is commonly associated with elevated production of reactive oxygen species (ROS) (Fan et al., 2012), which is known to cause oxidative muscle damage (Powers et al., 2011). Indeed, mitochondria isolated from PKO muscle showed >40% increase in ROS production compared to those from WT muscle (Fig. 2F), while their membrane potential remained unaffected (Fig. S2H). In addition to the mitochondrial energetic defects described above, the increase in ROS is also attributed to a marked reduction in the expression of the key mitochondrial anti-oxidant gene *Sod2* (Fig. 2G). In contrast, the levels of ROS in mitochondria isolated from HEPKO muscle approached those seen in WT muscle (Fig. 2F), consistent with the doubling of *Sod2* expression (Fig. 2G) and increases in additional anti-oxidant genes such as *Gpx3* upon ERRγ overexpression (Fig. S2I).

The dramatic increase in circulating lactate levels in PKO mice upon exercise (Fig. 1G) suggests that lactate metabolism may be dysregulated in PKO mice. The inter-conversion of lactate and pyruvate is controlled by the relative expression of the two subunits of lactate dehydrogenase (LDH), with LDHA driving lactate formation and LDHB promoting pyruvate production (Summermatter et al., 2013). Indeed, *Ldha* was significantly increased in PKO muscle (Fig. 2H). Notably, ERRγ overexpression coordinately suppressed *Ldha* and activated *Ldhb* expression in both WT and PKO muscle (Fig. 2H–I), consistent with the reduction in serum lactate levels in HEPKO mice (Fig. 1G).

ERRγ directly controls PGC1 target genes in mitochondrial energy metabolism

To understand the privileged interplay between PGC1α/β and ERRγ in regulating the expression of genes involved in mitochondrial energy metabolism, we compared the transcriptomic changes induced in muscle in the above described loss- and gain-of-function mouse models. Consistent with a central role of PGC1α/β in mitochondrial function, the down-regulated gene set in the oxidative soleus (SOL) muscle from PKO mice was enriched in gene ontology (GO) categories related to mitochondrial energetic functions, including

OXPHOS, TCA cycle, and fatty acid oxidation (FAO) (Fig. S3A). These same GO categories were enriched in the 1321 genes upregulated by ERRγ overexpression in the glycolytic white quadriceps (WQ) (Fig. S3A), providing mechanistic insight into the functional rescue of the mitochondrial energetic defects in PKO muscle (Fig. 2 and S2), and confirming the overlapping roles of both PGC1α/β and ERRγ in regulating mitochondrial energetic functions. Of note, two GO categories (muscle protein and vasculature development) were uniquely enriched in the ERRγ-upregulated gene set (Fig. S3A), suggesting PGC1α/β play a minimal role in these pathways.

To further delineate the roles of PGC1α/β and ERRγ in muscle, we identified the genome-wide binding sites (cistrome) of ERRγ by performing chromatin immunoprecipitation coupled with high-throughput deep sequencing (ChIP-Seq) assays. While 9387 ERRγ binding sites were identified in HE skeletal muscle, only three peaks were found in WT glycolytic muscle where ERRγ is minimally expressed. Motif analysis identified a highly enriched consensus ERR-response element (ERRE) present in over 40% of the total peaks ($P=10^{-1594}$) (Fig. S3B and Table S1). The second most enriched motif is the myocyte enhancer factor 2c (MEF2C, $P=10^{-422}$) (Fig. S3B and Table S1), suggesting a potential interaction between this critical muscle transcription factor and ERRγ (Estrella et al., 2015). The majority of the ERRγ binding sites are located in introns and intergenic regions with about 15% in promoters (Fig. S3B), suggesting much of its transcriptional regulation is mediated through distal enhancers. Interestingly, ~15% of ERRγ binding sites coincide with the H3K27Ac active enhancer mark, however, no correlation with ERRγ-induced gene expression changes is evident, suggesting H3K27Ac-independent transcriptional mechanisms (Fig. S3C). Overlaying the ERRγ cistromic and transcriptomic data revealed that 48% (629 out of 1321) of the genes up-regulated by ERRγ overexpression contain ERRγ binding sites, based on the proximity to the closest transcriptional start site. Notably, this directly regulated ERRγ target gene set is enriched for the same GO categories as found in the total ERRγ-induced gene set, including OXPHOS, TCA cycle, FAO, vasculature, and muscle proteins (Fig. S3D). In addition, the lactate metabolism genes *Ldha* and *Ldhb* that are repressed and activated by ERRγ overexpression, respectively, both contain strong ERRγ binding sites and are also direct ERRγ target genes (Fig. S3E). Together the gene expression and the ERRγ DNA binding data sets demonstrate that ERRγ directly binds to and regulates not only PGC1-targeted genes in mitochondrial energetic metabolism but also PGC1-independent genes in vasculature development and fiber-type determination.

ERRγ-specified oxidative remodeling is independent of PGC1α/β

Interestingly, while PGC1α/β are required for the basal expression of mitochondrial energetic genes in OXPHOS, TCA cycle, and FAO (Fig. 3A and S3A), ERRγ overexpression was able to significantly induce the expression of these genes in PGC1α/β-deficient muscle (HEPKO/PKO, Fig. 3A). Furthermore, ERRγ directly activated genes involved in vasculature development including *Vegfa* and *Fgf1,* independent of PGC1α/β expression (Fig. 3A–C and S3D). Loss of PGC1α/β minimally affected angiogenic gene expression (Fig. 3A–C and S3A), and the vasculature density (CD31 staining) in PKO muscle was not significantly lower than that in WT (Fig. 3D and S3F), consistent with the lack of vasculature gene enrichment in the downregulated gene set in PKO muscle (Fig.

S3A). However, overexpression of ERRγ in PKO muscle induced the expression of angiogenic genes to levels comparable to those in HE muscle (Fig. 3A–C), and the extent of vascularization in HEPKO muscle was similar to that seen in HE muscle (Fig. 3D and S3F), suggesting PGC1α/β are dispensable for both innate and adaptive angiogenesis in skeletal muscle.

ERRγ-induced oxidative muscle remodeling also involves muscle fiber-type changes through its direct regulation of muscle genes, including the different myosin heavy chain (MHC) genes such as *Myh1* (intermediate type IIx), *Myh2* (oxidative IIa), and *Myh4* (glycolytic IIb) (Fig. 3A, 3E, and S3D). Overall ERRγ drives a glycolytic-to-oxidative fiber type switch in different ways dependent on specific muscle types: in the oxidative SOL, ERRγ mainly switches intermediate type IIx fibers to oxidative type IIa (reduced *Myh1* and increased *Myh2* expression) (Fig. 3D and S3G); while in the more glycolytic WQ and PL (plantaris) it induces a switch from glycolytic IIb to intermediate IIx and oxidative IIa fibers (reduced *Myh4* and increased *Myh1/2* expression) (Fig. 3D–E and S3H–I). Surprisingly, the slow-twitch oxidative type I fiber (*Myh7*) is not affected by ERRγ overexpression in all muscles examined (SOL, WA, and PL) (Fig. 3D–E and S3G–I). These effects are consistent with direct and extensive ERRγ binding throughout the genomic locus containing the *Myh* gene cluster comprised of *Myh1*, *2*, *3*, *4*, and *8* (Fig. 3F) but not to the loci containing the slow-twitch *Myh7* (Fig. S3J).

It has been previously reported that the knockout of muscle PGC1α/β increases type I fibers (*Myh7*) (Zechner et al., 2010), although the use of a whole-body PGC1α knockout model in that study raised concerns as to whether loss of PGC1α in other tissues contributed to the phenotype (Handschin and Spiegelman, 2011) (Zechner et al., 2011). In our muscle-specific PGC1α/β knockout mice, both SOL and PL consistently show increased type I fibers and *Myh7* expression (Fig. 3D–E and S3F–G), clearly demonstrating that loss of PGC1α/β in muscle drives an increase in type I fibers. Although the exact mechanism remains elusive, ERRγ is unlikely involved in this effect since its overexpression has minimal effect on *Myh7* expression in both WT and PKO mice (Fig. 3D–E and S3F–H).

Combined effects of exercise and ERRγ improve PKO muscle function

Endurance exercise induces oxidative muscle remodeling and improves performance (Holloszy, 1967). Given our finding that overexpression of ERRγ improved PKO muscle function by inducing genes involved in oxidative muscle remodeling, we explored whether exercise could also rescue muscle dysfunction in PKO mice and whether exercise could combine with the benefits of ERRγ overexpression in the absence of PGC1α/β.

Voluntary wheel running was used as an exercise training regimen for WT, HE, PKO, and HEPKO mice. Consistent with our treadmill studies, the initial wheel-running activities of PKO mice were dramatically reduced compared to WT mice (green and red lines, respectively, Fig. 4A–B). However, after 8 weeks of exercise, significant improvements in voluntary running activity and treadmill sustained running performance were seen in PKO mice (Fig. 4A–C). Mechanistically, while both PGC1α and β as well as ERRα are not affected by exercise in PKO muscle, we found that ERRγ itself is induced and its co-repressor NCOR significantly reduced by exercise (Fig. S4A), potentially contributing to the

improvements. Another exercise-related transcriptional co-factor, RIP140, however is not changed (Fig. S4A), which could be due to the relatively low exercise activity of PKO mice. By comparison, these exercise-induced improvements were further potentiated by the overexpression of ERRγ (Fig. 4A–B), such that exercised HEPKO mice ran ~5 times longer than PKO mice on the treadmill running test (55 vs 9.5 minutes, respectively) (Fig. 4C). This improvement is in agreement with the lower serum lactate levels measured after forced running, where the reduction attributed to ERRγ overexpression (sedentary HEPKO compared to PKO mice) was further attenuated by exercise training (Fig. S4B). We found that exercise training and ERRγ overexpression coordinately affected the expression of *Ldha* and *Ldhb* in PKO muscle (down- and up-regulated, respectively, Fig. S4C–D), to suppress glycolysis and promote oxidative metabolism to improve muscle function. Furthermore, in PKO muscle, additive effects of exercise and ERRγ overexpression were seen on the levels of mitochondrial proteins (Fig. 4D), mitochondrial complex I activity (Fig. 4E), and the expression of OXPHOS and FAO genes such as *Ndufa4* and *Acadl* (Fig. S4E–F), suggesting the presence of additional transcriptional regulators that are independent of ERRγ and PGC1α/β. It is noteworthy that exercise training fails to further induce oxidative remodeling in the already highly oxidative HE muscle (Fig. 4C–E and S4B–F).

Discussion

The intrinsic pleiotropic nature of co-regulatory factors such as PGC1α/β creates a mechanistic challenge in deconstructing the role of individual TF targets. As PGC1 broadly impacts muscle oxidative metabolism and performance it becomes key to decipher principal targets that mediate these effects. The muscle-specific PGC1α/β KO mouse provides a means to identify defining factors by virtue of their ability to rescue defects resulting from PGC1α/β deficiency. Thus, via gain of function studies, we genetically establish a pivotal role for ERRγ in mitochondrial energy metabolism as well as its epistatic relationship with PGC1α/β in driving a broad oxidative platform. For example, while PKO mice show reduced gene expression in major mitochondrial energetic pathways (including OXPHOS, TCA cycle, and FAO metabolism), ERRγ overexpression significantly boosts expression of these genes in PKO muscle, as well as restoring a multitude of the above-mentioned mitochondrial energetic dysfunctions (Fig. S4F). ERRγ overexpression also significantly, though not completely, improves exercise performance in PKO mice (by about 3-fold). Unexpectedly, PGC1 deficiency in muscle shows little change in vasculature or oxidative myofibers such that ERRγ overexpression enhances both into the realm of highly trained animals. This indicates that baseline vasculature and oxidative fiber determination are PGC1-independent pathways (Fig. S4F). Interestingly, voluntary exercise in PKO mice still confers many benefits (Fig. S4F), suggesting that major adaptive functions of exercise, such as angiogenesis, mitochondrial biogenesis and oxidative remodeling can be elicited in absence of PGC1α/β as long as ERRγ-dependent signaling is intact. This suggests that ERRγ synthetic agonists could have substantial and predictable benefits in treatment of muscle disease when exercise (and/or PGC1α/β induction) is not possible or practical.

Differences in phenotype severity in previous PGC1α/β double-knockout models appear to correlate with the efficiencies of the muscle depletion, suggesting that the absolute levels of PGC1α/β are important (Zechner et al., 2010) (Rowe et al., 2013). Indeed, the severely

compromised muscle phenotype described here is similar to that shown in Zechner et al., where both models efficiently deplete both PGC1α and β in muscle (Zechner et al., 2010). While injury is considered a product of exercise, PKO mice show evidence of severe muscle damage even under sedentary conditions, indicating a basal role for PGC1α/β in this process. Although the exact mechanism causing muscle damage in PKO mice is not clear, mitochondrial energy deficit and increased ROS production are likely involved (Powers et al., 2011). Notably, such damage is almost completely rescued by ERRγ overexpression, with mitochondrial ROS in HEPKO muscle fully restored to WT levels. This is associated with ERRγ-induced upregulation of antioxidant genes such as *Sod2* and *Gpx3* and suppression of developmental myosin heavy chain genes *Myh3* and *Myh8*.

Previously, we have shown that the overexpression of ERRγ induces exercise-like oxidative muscle remodeling without engaging changes in the expression level or activity of PGC1α (Narkar et al., 2011). In this study, overexpression of ERRγ in PKO muscle reveals that ERRγ-activated target genes can achieve almost all aspects of oxidative muscle remodeling in the absence of PGC1α/β, including oxidative fiber-type transformation, angiogenesis, and increased mitochondrial energy metabolism. These data highly suggest that PGC1α/β are dispensable for ERRγ-induced oxidative muscle remodeling. Interestingly, both oxidative fiber-type determination and angiogenesis are minimally affected in PKO muscle, with no significant reduction in genes involved in these pathways, suggesting PGC1α/β are not required for their basal functions.

Our RNA-Seq and ChIP-Seq studies identify a network of 629 ERRγ directly controlled genes that are induced by its overexpression. These genes are highly enriched in all of the above-mentioned oxidative muscle remodeling processes, confirming the direct role of ERRγ in transcriptionally regulating oxidative muscle remodeling. PGC1α/β are transcriptional co-activators and require DNA-binding TFs to activate downstream genes. PGC1α is known to drive oxidative muscle remodeling by activating the same set of genes that are ERRγ direct targets (Lin et al., 2002) (Arany, 2008), indicating ERRα/γ as its primary partner TFs during PGC1α-induced oxidative muscle remodeling, although further studies are required to confirm this.

We have also demonstrated that exercise training alone is sufficient to significantly restore mitochondrial energetic dysfunctions and improve running performance in PKO mice (Fig. S4F). Surprisingly, these improvements are comparable to those achieved by ERRγ overexpression. This confirms that exercise has concurrent benefits on oxidative muscle remodeling that are PGC1α/β-independent. Notably, training combined with ERRγ overexpression further boosts mitochondrial energetic functions, resulting in 5-fold increase in running time indicating that ERRγ is itself not simply an exercise surrogate. In summary, these results suggest that while exercise activates PGC1α/β many of its benefits can be achieved independently with notable cross talk with ERRγ and its target genes (Fig. 4F).

METHODS

Mouse models

PKO ($Cre^{+}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$) mice were generated by crossing $Pgc1a^{fl/fl}$ (JAX 009666) with $Pgc1b^{fl/fl}$ ((Sonoda et al., 2007) (Wei et al., 2010)) and ACTA1-Cre (JAX 006139) mice. HE mice (ACTA1-Esrrg (Narkar et al., 2011)) were also crossed to $Pgc1a^{fl/fl}$ and $Pgc1b^{fl/fl}$ mice to generate $HE^{+}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$ mice. $Cre^{+}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$ mice were then crossed to $HE^{+}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$ mice to generate WT ($Cre^{-}HE^{-}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$), HE ($Cre^{-}HE^{+}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$), PKO ($Cre^{+}HE^{-}Pgc1a^{fl/fl}Pgc1b^{fl/fl}$), and HEPKO ($Cre^{+}HE^{+}Pgc1a^{fl/fl}$ $Pgc1b^{fl/fl}$) mice.

For exercise training, 2-month-old mice were transferred to cages with low-profile wireless running wheels (ENV-044, Med Associates Inc.) and housed for 2 months. Daily running activity was monitored and recorded with the wheel manager software.

Prior to exhaustion running, mice were pre-adapted to the treadmill for 5 minutes per day for 3 days at 5 meter/min. After 1 day recovery mice were subjected to the run-to-exhaustion test, which included running for 1 minute each at speeds of 5, 6, 7, 8 and 9 meter/min, 30 minutes at 10 meter/min, 2 minutes each at speeds of 11, 12, 13, 14 and 15 meter/min, and run at 15 meter/min till exhaustion. Blood lactate was measured in all mice using a Lactate Scout analyzer (EKF Diagnostic) with tail bleed upon exhaustion of the first mouse. Blood glucose was measured using a glucometer (Nova max) in individual mice upon exhaustion.

All animal protocols were reviewed and approved by the Institute of Animal Care and Use Committee (IACUC) of the Salk Institute, and studies were conducted in compliance with institutional and national guidelines.

Quantification and statistical analysis

ANOVA and post-hoc analysis were used to evaluate statistical significance in all studies.

Detailed experimental procedures are described in supplemental material.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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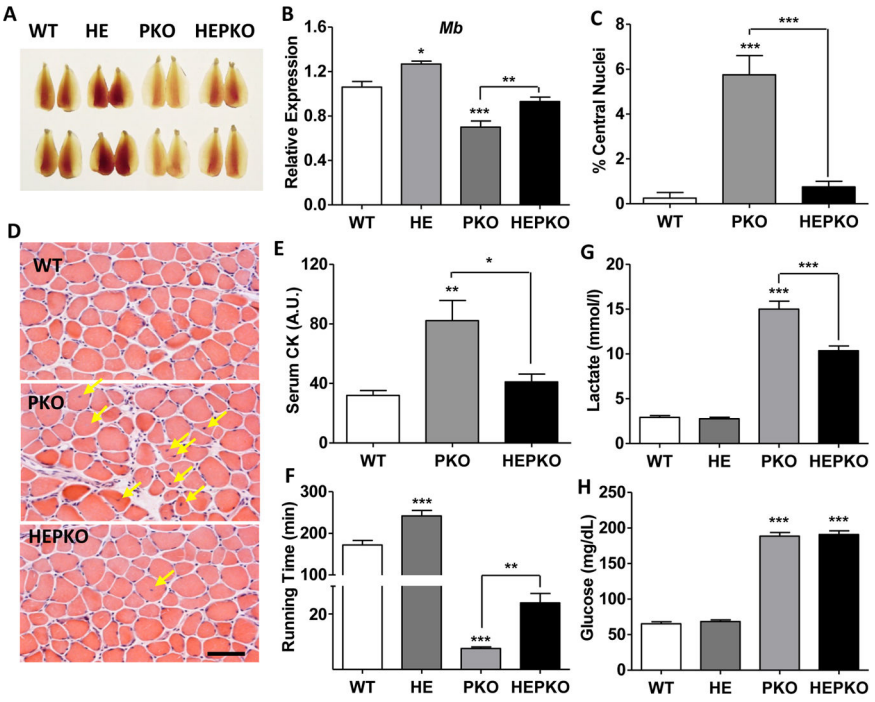

Figure 1. ERRγ improves running defect and muscle damage in PGC1-null mice

(A) Images of Tibialis anterior (TA) muscle from wild-type (WT), ERRγ transgenic (HE), muscle-specific PGC1α/β knockout (PKO), and HEPKO mice; (B) Relative expression of Myoglobin (*Mb*) in plantaris muscle; (C–D) Percentage centralized-nuclei in muscle and representative H&E staining (E) serum creatine kinase (CK) levels in sedentary mice; (F) running time during low-speed endurance test; (G) blood lactate levels after 6 minutes of endurance running (upon failure of the first mouse); and (H) blood glucose level at failure in endurance test. n=5. Data represent mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar, 50μm. See also Figure S1.

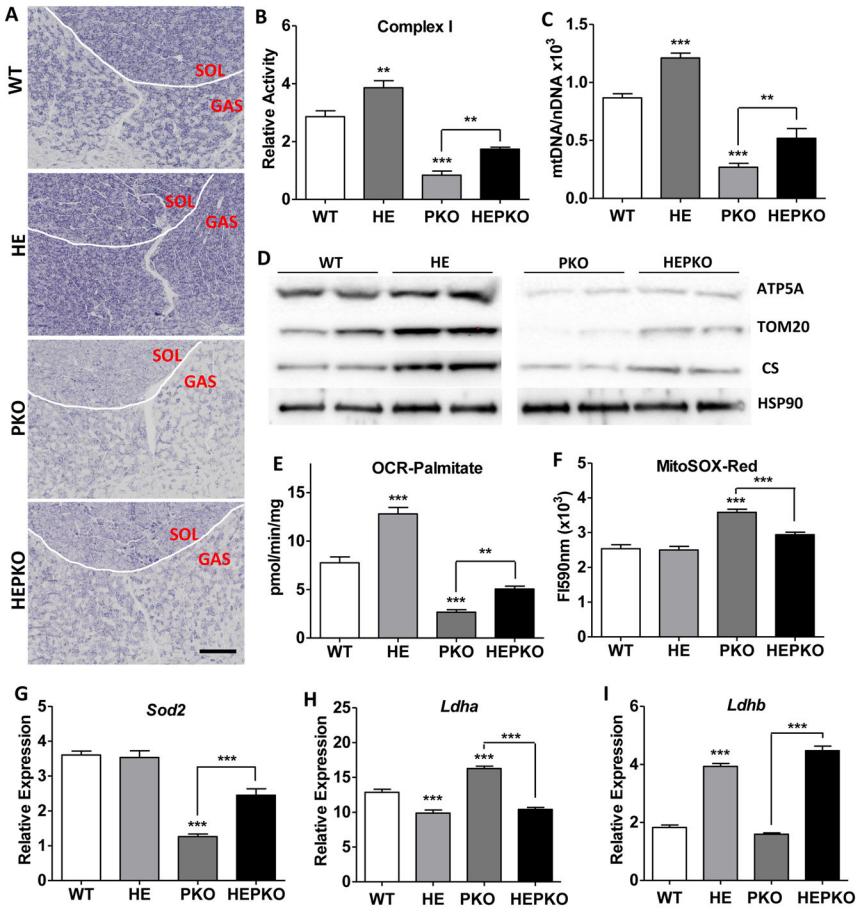

Figure 2. ERRγ improves mitochondrial energetic defects in PGC1-null muscle

(A) Immunohistochemical staining for mitochondrial complex I activity in cryosections from soleus (SOL) and gastrocnemius (GAS); (B) mitochondrial complex I activity measured in isolated mitochondrial from quadriceps muscle; (C) relative mtDNA copy numbers in plantaris; (D) western blots showing the levels of mitochondrial proteins ATP5A, TOM20, and CS, as well as a cytoplasmic control HSP90 in plantaris; (E) oxygen consumption rates measured in freshly isolated quadriceps mitochondria using palmitoylcarnitine as substrate; (F) ROS measured as MitoSOX fluorescence intensity in freshly isolated quadriceps mitochondria; and (G–I) Relative expression levels of *Sod2* (G), *Ldha* (H), and *Ldhb* (I) in plantaris. n=5. Data represent mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar, 500μm. See also Figure S2.

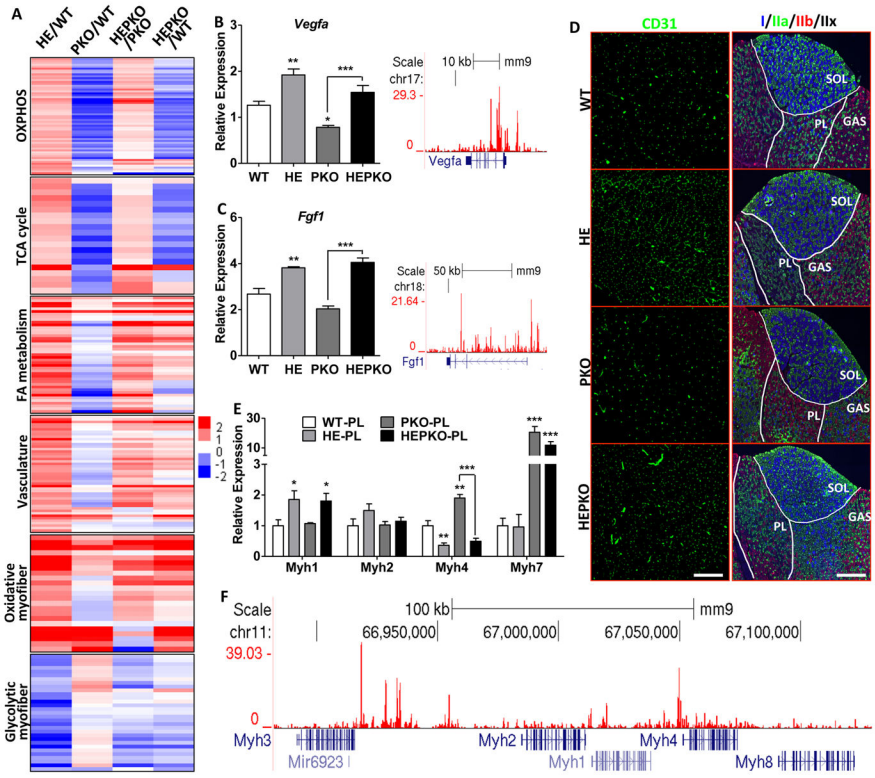

Figure 3. ERRγ induces oxidative muscle remodeling independent of PGC1

(A) Heat maps showing relative changes in expression of selected genes involved in OXPHOS, TCA cycle, FAO, vasculature, oxidative-specific myofiber, and glycolytic-specific myofiber in plantaris; (B–C) Relative expression of *Vegfa* and *Fgf1* in plantaris (left) and genome browser tracks showing ERRγ binding (right); (D) Immunostaining of CD31 for vasculature (left) and Myh I/IIa/IIb for fiber-typing (right); (E) Relative expression of *Myh1/2/4/7* in plantaris; and (F) genome browser track showing ERRγ binding at the *Myh* cluster locus. n=5. Data represent mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bars, 300μm (CD31) and 750μm (Myh). See also Figure S3.

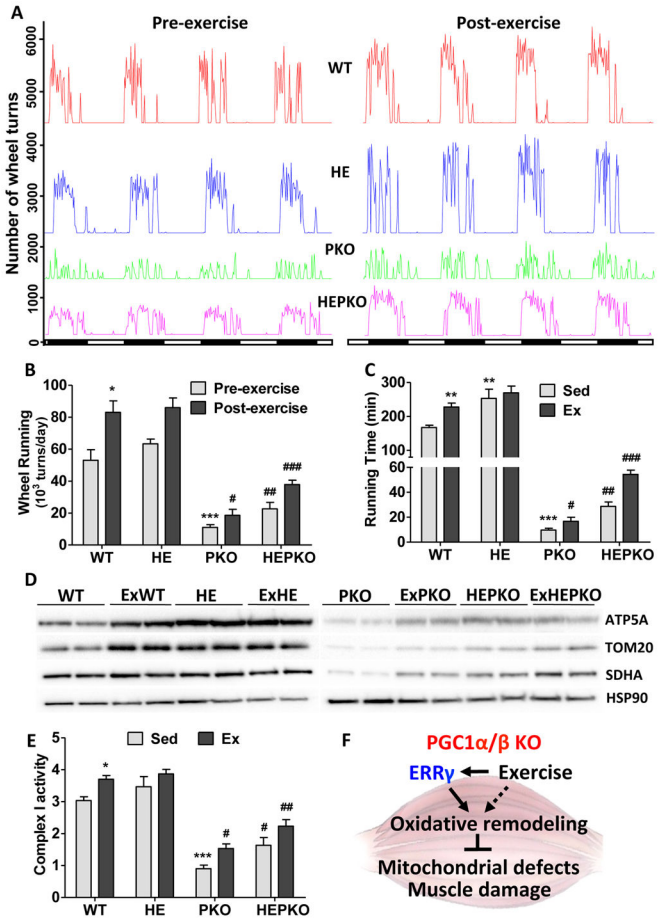

Figure 4. Exercise and ERRγ synergistically improve oxidative functions in PKO muscle

(A) Running wheel activity of WT (red), HE (blue), PKO (green), and HEPKO (purple) mice before (left) and after (right) 6 weeks of voluntary wheel running; (B) Cumulative daily wheel running from (A); (C) Total running time of sedentary and exercised (8 weeks of voluntary wheel running) mice in endurance test; (D) Western blots showing the levels of mitochondrial proteins ATP5A, TOM20, and SDHA, as well as a cytoplasmic control HSP90 in plantaris; (E) Mitochondrial complex I activity in isolated mitochondrial from quadriceps; and (F) Diagram showing ERRγ- and exercise-induced oxidative muscle remodeling in PKO mice that improves muscle damage and mitochondrial defects. Data represent mean ± SEM. * and # show statistical difference from WT and PKO, respectively. n=5. Data represent mean ± SEM, * and # $p < 0.05$, ** and ## $p < 0.01$, *** and ### $p < 0.001$. See also Figure S4.