Estrogen-related receptor γ (ERRγ) regulates the perinatal switch to oxidative metabolism in the myocardium. We wanted to understand the significance of induction of ERR expression in skeletal muscle by exercise. Muscle-specific VP16ERRγ transgenic mice demonstrated an increase in exercise capacity, mitochondrial enzyme activity, and enlarged mitochondria despite lower muscle weights. Furthermore, peak oxidative capacity was higher in the transgensics as compared with control litters. In contrast, mice lacking one copy of ERRγ exhibited decreased exercise capacity and muscle mitochondrial function. Interestingly, we observed that increased ERRγ in muscle generates a gene expression profile that closely overlays that of red oxidative fiber-type muscle. We further demonstrated that a small molecule agonist of ERRβ/γ can increase mitochondrial function in mouse myotubes. Our data indicate that ERRγ plays an important role in causing a shift toward slow twitch muscle type and, concomitantly, a greater capacity for endurance exercise. Thus, the activation of this nuclear receptor provides a potential node for therapeutic intervention for diseases such as obesity, which is associated with reduced oxidative metabolism and a lower type I fiber content in skeletal muscle.

The estrogen-related receptors (ERRs) are orphan nuclear receptors that were identified through their homology to the estrogen family of receptors (1). ERRs are expressed in tissues with high metabolic demand, with ERRα being more ubiquitously expressed, whereas ERRβ and ERRγ show a more restricted pattern of expression. The levels of ERR activity are dramatically enhanced by the addition of peroxisome proliferator-activated receptor γ-coactivator 1α (PGC-1α) (2), such that the PGC-1 proteins are sometimes referred to as protein ligands for this class of nuclear receptors.

ERRα is an important mediator of adaptive mitochondrial biogenesis under situations of increased physiological stress as evidenced by the inability of ERRα knock-out mice in regulating body temperature upon cold challenge (3). The ERRα null mice are also more susceptible to cardiac occlusion by banding.
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dinated network of fuel uptake and oxidation and mitochondrial and muscle structural genes.

EXPERIMENTAL PROCEDURES

Animal Studies—Mice were maintained on 12-h light/dark cycle and cared for in accordance with the Animal Care and Use Committee protocol in the Novartis Institutes for BioMedical Research animal facility. Body composition was determined using EchoMRI (Echo Medical Systems, Houston, TX). ERRγ heterozygous null mice were obtained from Deltagen (San Mateo, CA) and backcrossed to C57Bl/6 for four generations (7). ERRγ transgenic mice were generated on a C57Bl/6 background using standard procedures. The transgenic construct consists of the 4.8-kb mouse muscle creatine kinase promoter driving a hemagglutinin-tagged VP16-ERRγ fusion or a hemagglutinin-tagged ERRγ gene.

Primary Mouse Myotube Culture, Adenoviral Transduction, and Compound Treatment—Mouse myoblasts were isolated as described previously (20). Human ERRγ-RES green fluorescent protein adenovirus was generated by Welgen (Worcester, MA). Following a 48-h differentiation period, myotubes were transduced with green fluorescent protein or ERRγ adenovirus for 48 h. The titers to achieve low and high levels of ERRγ expression were 1 × 10⁸ and 5 × 10⁸ virus particles/ml, respectively. GSK4716 was synthesized as described previously (9) and dissolved in DMSO for cell culture experiments. Cells treated with a corresponding volume of DMSO were used as controls.

Analysis of mRNA Expression and mtDNA Content—For both tissues as well as cell lysates, total RNA was isolated using TRIzol reagent (Invitrogen). For muscle tissue, tissues were pulverized, and the RNAeasy® Fibrous Tissue Minikit (Qiagen, Valencia, CA) was utilized for isolation of RNA. Quantitative real time PCR was performed using Assay-on-Demand® primer probes (Applied Biosystems, Foster City, CA) (supplemental Table S1). Mitochondrial DNA levels were assessed as described previously (17).

Protein Analysis—Tissues or cell lysates were prepared in RIPA buffer and separated using standard techniques. Normalization for loading was done using Ponceau staining. The polyclonal anti-human ERRγ antibody was generated using a 229-amino acid peptide corresponding to the ligand-binding domain (Covance Research Products Inc, Denver, PA). A donkey anti-rabbit IgG horseradish peroxidase-linked secondary antibody (GE Healthcare) and SuperSignal West Dura extended duration ECL substrate (Pierce) were used for detection. Citrate synthase activity was determined using 2 μg of total protein as prepared above, using the citrate synthase assay kit (Sigma).

Microarray Analysis—RNA isolated from myotubes transduced with adenovirus or intact gastrocnemius muscle were used for microarray analysis. RNA was hybridized to mouse 430_2 Affymetrix chip (Affymetrix, Santa Clara, CA) in triplicate.

Histology, Immunohistochemical Staining, and Morphometrics—Muscle samples were rapidly frozen by submerging for 20 s in a chilled isopentane bath wrapped in pre-cooled plastic wrap and placed in a −80 °C freezer for storage until cryo-sectioning. Samples were sectioned at 6 μm in a cryomicrotome and mounted on glass slides immediately prior to staining. Succinate dehydrogenase activity was measured histochromically according to published protocols (21), and muscle fiber size was measured by immunohistochemical staining using a polyclonal rabbit anti-laminin antibody (Sigma) at room temperature for 1 h at a 1:800 dilution. Detection was performed using a rabbit-on-rodent APL polymer (Biocare, Concord, CA) and Vulcan Fast Red chromogen (Biocare).

Stained slides were digitized using an Aperio ScanScope XT slide scanner and analyzed using algorithms from Aperio (color deconvolution for succinate dehydrogenase staining intensity and positive pixel count for total tissue area) as well as a custom watershed algorithm (ASTORIA) developed to segment the muscle fibers from the laminin-stained sections. Area of positive staining was reported as the percent tissue area that stained positive for succinate dehydrogenase, and staining intensity was reported as the total optical density of all positive pixels divided by the total tissue area.

Transmission Electron Microscopy—Transmission electron microscopy was performed using standard techniques. Gastrocnemius muscles were dissected unilaterally and placed in a modified Karnovsky’s fixative. Samples were routinely processed and were embedded in EMBed (Epon) 812 such to maintain a longitudinal orientation of the resulting sections. Thick sections were stained with toluidine blue to identify the best longitudinal block for each animal. Ultrathin sections cut for transmission electron microscopy survey were double-stained with uranyl acetate and lead citrate and examined using a FEI Tecnai G2 BioTwin electron microscope. Photomicrographs were captured using an Olympus-SIS Morada digital camera. Exercise Protocols—C57Bl/6 mice (age 15 weeks) were trained for a period of 8 days by running on a treadmill (10 m/min, zero degree incline) for 2 h each day. Tissues were isolated immediately after the last bout of exercise training. To determine exercise capacity, 4-month old male mice were placed on a 6-lane treadmill (Columbus Instruments, Columbus, OH) and run with a fixed upward slope of 10°. The speed during the 1st h was 10 m/min and increased 2 m/min every 15 min thereafter. Work, peak VO₂, and respiratory exchange ratios were assessed as described previously (17).

Data Analysis—Statistical analysis was performed using a two-tailed Student’s t test or analysis of variance as appropriate and was considered statistically significant if p < 0.05. For analysis of microarray data, the Probe Logarithmic Intensity Error (22) method was used to normalize the raw data and generate the summarized probe-set level gene expression values. A moderated t test (23) was applied to identify the significantly altered genes using the “limma” package from Bioconductor. p values were adjusted using the BH (24) method to control for false discovery rate in multiple testing. Functional enrichment analyses were performed at DAVID (Data base for Annotation, Visualization and Integrated Discovery) (25).

RESULTS

Expression of ERRγ in Oxidative Skeletal Muscle—Exercise is a physiological intervention that results in the dynamic induction of mitochondrial biogenesis in skeletal muscle. We showed...
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ERRγ Transgenic Mice Exhibit Skeletal Muscle Fiber-type Conversion and Enhance Exercise Capacity—The muscle creatine kinase promoter was used to generate muscle-specific ERRγ transgenic mice (Fig. 2A, top panel). We analyzed two independent founder lines, one where the ERRγ was expressed as a fusion with the VP16 viral transactivator and the other expressing the “native” version of the protein. Expression of transcriptional factors as VP16 fusions is a strategy often utilized in vivo to ascertain effects elicited by increasing intrinsic activity of the factor beyond that achieved by enhanced protein levels alone (29, 30). Fig. 2A, lower left panel, demonstrates expression of the VP16ERRγ transgenic protein in founder line 431. The transgene was expressed at equal levels in gastrocnemius, soleus, and tibialis anterior muscle but was not expressed in the heart (supplemental Fig. 2A). We observed that VP1ERRγ transgenic muscles were noticeably redder and all hind limb muscles exhibited the same color as the soleus muscle (Fig. 2A, lower right panel). The animals had a similar body weight and body composition as compared with the control littermates (supplemental Fig. 2, B and C). The tibialis anterior (glycolytic type II fiber type muscle) and gastrocnemius (mixed fiber type muscle) muscles were smaller in the transgenics (Fig. 2B), although the mass of an oxidative type I fiber muscle such as soleus was not decreased. The transgenic line 339, expressing increased native ERRγ protein in muscle (supplemental Fig. 3A), exhibited a similar decrease in muscle weight of glycolytic and mixed fiber muscles but no change in soleus muscle, in the absence of any body weight changes (supplemental Fig. 3, B–E).

Individual fiber size was determined in the entire muscle cross-section by laminin staining. The fiber size distribution for the VP16ERRγ transgenic muscle was left-shifted, indicating a smaller muscle fiber size as evidenced by the histogram in Fig. 2C. Analysis of mitochondrial DNA levels in gastrocnemius muscle demonstrated a trend toward an increased content in the transgenic mice (Fig. 2D, p = 0.069). Electron microscopy of gastrocnemius muscle demonstrated an increase in numbers of large mitochondria, characterized by an enlargement of mitochondrial surface area in the transgenics (Fig. 2E). These large mitochondria were more pronounced in the subsarcolemmal region as opposed to the intramyofibrillar space (Fig. 2E, High, lower panels).

The VP16ERRγ transgenics did not show any alterations in energy expenditure, activity, or respiratory exchange ratios in the basal state (data not shown). When challenged with an endurance treadmill test, the VP16ERRγ transgenic mice demonstrated a significant increase in the work performed and distance traveled compared with the wild type controls (Fig. 2, F and G). In a peak VO2, the VP16ERRγ transgenic mice surpassed their control littermates (Fig. 2H). Interestingly, the respiratory exchange ratio values in the transgenic mice were lower during this exercise challenge, indicating a greater preference for fat oxidation (supplemental Fig. 2D). The respiratory exchange ratio values for the transgenics do not increase beyond a value of 1, implying that the transgenic mice are protected from the lactic acid-induced hyperventilation seen under maximal exercise. The non-VP16-tagged ERRγ transgenic mice also demonstrated an increase in distance run dur-
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increasing an exercise capacity challenge and decreased peak oxidative capacity (Fig. 2, I–K).

Assessment of Mitochondrial Enzymatic Function and Gene Expression—Analysis of succinate dehydrogenase enzyme activity in the muscle cross-sections demonstrated a robust increase in the succinate dehydrogenase positive area as well as intensity in the transgenic muscle (Fig. 3, A and B). Succinate dehydrogenase is situated in the mitochondrial inner membrane and is a component of the electron transport chain as well as the tricarboxylic acid cycle. Aconitase, like citrate...
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ERRγ improves mitochondrial function in vivo.

Gene Expression Profile in ERRγ Transgenic Mice Resembles That Observed in Type I Muscle—We analyzed our microarray data in context of the gene signatures observed in two distinct muscle fiber types (Fig. 4A, left panel) that were generated based on a published data set described for tibialis anterior muscle, a representative of type II fast twitch glycolytic muscle, and soleus muscle, a representative of type II fast twitch oxidative muscle.

Effects of ERRβ/γ Agonist on Mitochondrial Gene Expression and Function in Primary Mouse Myotubes—Treatment of primary mouse myotubes with GSK4716, an ERRβ/γ agonist (9), resulted in a concerted increase in the expression levels of *Ppargc1a*, *Ppargc1b*, and *Esrrb* genes (Fig. 6A). Furthermore, *Cpt1b*, *Atp5b*, and *Idh3* genes in key mitochondrial pathways, were also induced by GSK4716 (Fig. 6A). The concentration of the compound used in our study is similar to that observed for the activation of both ERRβ and ERRγ in the Gal4 transactivation assay (9). Additionally, GSK4716 increased citrate synthase transgenic mice; conversely, the gene pathways decreased in soleus but increased in the tibialis are decreased in the ERRγ transgenic muscle (Fig. 4A, right panel).

Gastrocnemius muscle isolated from ERRγ transgenic mice exhibited decreased expression of MHC IIb and a concomitant increase in expression of MHC IId and MHC IIa and a trend toward an increase in slow MHC I, which did not reach statistical significance (Fig. 3E). This is indicative of a muscle fiber-type conversion from fast type II toward slow type I muscle fibers. Furthermore, we observed increased expression of myoglobin, a gene essential for the facilitated oxygen transport required by oxidative skeletal muscle (Fig. 3E). Candidate gene analysis shows decreases in *Ppargc1a* and *Esrra* but an increase in ERRβ, fatty acid oxidation genes (*Lpl* and *Fabp3*), and *Epas1* (Fig. 3F). The down-regulation of *Ppargc1a* and *Esrra* suggests that there exist feedback regulatory loops for the control of mitochondrial function. Global gene expression was assessed in an unbiased fashion using microarrays, followed by gene set enrichment analysis. The top regulated pathways by their GO categories in the transgenic mice are described in Tables 1 and 2. Genes representing pathways of lipid metabolism, angiogenesis, and muscle calcium handling and contractility were robustly up-regulated in the transgenic muscle, whereas genes of the electron transport chain showed a smaller magnitude of up-regulation. Among the gene pathways that were down-regulated in transgenic muscle were genes representing fast twitch myosins and calcineurin Aa (Fig. 3G).

Gene expression analyses using microarray in gastrocnemius muscle demonstrated similar patterns of changes in this muscle as in the VP16ERRγ muscle (supplemental Fig. 3F).

Gene expression analyses using microarray in gastrocnemius muscle from wild type (WT) and VP16ERRγ transgenic muscle as compared with the wild type. Statistical significance (Fig. 4B). Thus, we can closely overlay the molecular genetic signature of ERRγ onto that of a pure oxidative muscle.

**ERRγ Heterozygotes Exhibit Impairments in Muscle Function and Oxidative Capacity**—Tissues from ERRγ heterozygous mice displayed a 50% reduction in ERRγ mRNA (Fig. 5A) and protein (Fig. 5B). No significant differences in muscle, heart, and total body weights were observed in these mice (data not shown). These mice did not show any alterations in energy expenditure, activity, or respiratory exchange ratios in the basal state (data not shown). The ERRγ heterozygotes ran a shorter distance and performed less work during an exercise capacity test (Fig. 5, C and D). These mice attained a reduced maximum speed (16.71 ± 0.24 versus 21.22 ± 0.29 m/min) compared with their wild type controls. Furthermore, ERRγ heterozygotes demonstrated a lower peak VO2 measurement (Fig. 5E). Citrate synthase levels trended to be lower, although the difference did not reach statistical significance (Fig. 5F). Gastrocnemius muscle demonstrated no changes in levels of *Ppargc1a* and *Esrra* to compensate for the partial loss of ERRγ in the heterozygous mice, although *Esrrb* levels were reduced. Additionally, fatty acid uptake and oxidation genes such as *Lpl* and *Cpt1b* were decreased in the muscle of these mice, indicating that these animals may have impairment in the utilization of fatty acids as fuel.
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A. SDH staining

B. SDH staining intensity and area

C. CS activity (µmol/min/mg)

D. Aconitase activity (mU/mg)

E. Relative expression of ERRα, ERRβ, PGC-1α, EPAS-1, LpI, FABP3

F. Relative expression of ERRα, ERRβ, PGC-1α, EPAS-1

G. Gene expression analysis:
   - Electron transport
   - Lipid metabolism
   - Myofibrillar genes
   - Angiogenesis
activity (Fig. 6B) and cytochrome c protein levels (Fig. 6C). These results indicate that the activation of ERRβ/γ transcriptional activity with a small molecule agonist can produce a coordinate increase in mitochondrial gene expression and function.

**DISCUSSION**

We demonstrate that ERRγ expression in skeletal muscle is sufficient and necessary to increase exercise capacity and activate mitochondrial function. In addition to its role in the regulation of mitochondrial and metabolic gene levels, ERRγ controls the expression of key genes controlling angiogenic, myofibrillar, and calcium handling pathways in skeletal muscle.

**TABLE 1**

Over-represented GO terms for genes significantly up-regulated in VP16-ERRγ mice

| GO category          | Term                        | Fold enrichment | p value  |
|----------------------|-----------------------------|-----------------|----------|
| Biological process   | Angiogenesis                | 3.47            | 3.98E-13 |
|                      | Fatty acid metabolic process| 3.26            | 1.19E-12 |
|                      | Electron transport          | 2.04            | 1.28E-09 |
|                      | Cell migration              | 2.3             | 3.56E-09 |
| Cellular compartments| Actin cytoskeleton          | 2.73            | 4.31E-11 |
|                      | Adherens junction           | 4.02            | 7.11E-09 |
|                      | Basolateral plasma membrane | 3.71            | 2.01E-08 |
|                      | Contractile fiber           | 3.61            | 3.48E-08 |
|                      | Myofibril                   | 3.6             | 7.31E-08 |
|                      | Mitochondrial lumen         | 3.86            | 1.70E-07 |
| Molecular function    | Oxidoreductase activity     | 1.98            | 7.52E-18 |
|                      | Actin binding               | 2.71            | 5.31E-13 |
|                      | FAD binding                 | 3.86            | 8.03E-8  |
|                      | Electron carrier activity    | 2.32            | 3.78E-07 |

**TABLE 2**

Over-represented GO terms for genes significantly down-regulated in VP16-ERRγ mice

| GO category          | Term                        | Fold enrichment | p value  |
|----------------------|-----------------------------|-----------------|----------|
| Biological process   | Post-translational protein modification | 2.05 | 8.85E-27 |
|                      | Ubiquitin cycle             | 2.81            | 7.78E-23 |
|                      | Protein amino acid          | 1.67            | 3.73E-06 |
|                      | Phosphorylation             |                 |          |
| Molecular functions  | Chromatin modification      | 2.44            | 3.99E-06 |
|                      | Transcription factor binding| 2.51            | 1.53E-08 |
|                      | Magnesium ion binding       | 1.93            | 4.71E-06 |
|                      | Transforming growth factor β| 8.39            | 6.20E-05 |
|                      | Cytoskeletal protein binding| 1.77            | 6.47E-05 |
|                      | Ubiquitin thiol esterase activity | 2.95 | 4.16E-04 |
| Cellular compartments| Contractile fiber part      | 3.56            | 3.52E-06 |
|                      | Myofibril                   | 3.33            | 9.76E-06 |
|                      | Nucleoplasm part            | 1.63            | 5.92E-05 |
|                      | Endoplasmic reticulum       | 1.52            | 1.05E-04 |
|                      | Histone deacetylase complex | 4.82            | 1.24E-04 |

**FIGURE 4. Gene expression profile of ERRγ transgenic muscle is similar to that of type I oxidative muscle.** A, heat map representing the coordinate gene expression pathways in tibialis anterior (TA) and soleus (Sol) (left panel) and wild type (WT) and VP16ERRγ transgenic (right panel) gastrocnemius muscle. Each individual column within the heat map represents a single mouse subject (n = 3 each for the soleus and tibialis anterior, n = 6 for WT and 7 for TG mice). The expression values are standardized within the data sets. Red color means up-regulation; green is down-regulation, and black is no change. The color scale is represented in Fig. 5A. The significantly over-represented GO terms by each group of genes is marked on the right. The p values used to determine significance for the individual genes are as follows: p < 0.01 for TG versus WT and p < 0.05 for soleus versus tibialis anterior. B, table represents the numbers of genes significantly changed in both datasets. The p value of Fisher’s exact test is 1.47e-170 for the null hypothesis that the genes regulated by VP16ERRγ are unrelated to those differentially expressed between soleus and tibialis anterior (TA) muscle.

**FIGURE 3. Transgenic VP16ERRγ mice have increased muscle mitochondrial function.** A, representative images of succinate dehydrogenase (SDH) staining in tibialis muscle in littermate control (WT) (left panel) and VP16ERRγ transgenic (TG) (right panel) mice; B, staining intensity (left panel) and total staining area (right panel) of the muscle cross-sections (n = 5–6 mice per group). Enzymatic activities for citrate synthase (CS) (C) and aconitase (D) were determined in gastrocnemius muscle (n = 6–8 mice per group). Gene expression for MHCs (E) and metabolic and mitochondrial genes (F) (n = 6–8 mice per group) are shown. Data are presented as mean ± S.E. Statistical significance is indicated as follows: *p < 0.05; **p < 0.01; ***p < 0.005. G, heat maps denoting individual genes and pathways that were altered in gastrocnemius muscle of TG mice relative to littermate control mice. The color scheme representing the fold change is shown.
that are required for longer term adaptation to exercise. Thus, ERRγ emerges as a key regulator of the “slow, oxidative” muscle phenotype, serving as an integrator to ensure the concerted modulation of ultrastructural and metabolic transcriptional pathways that determine the identity of this muscle subtype.

Studies have shown impaired mitochondrial function via diminished ATP production in skeletal muscle of individuals with a family history of diabetes and in older insulin-resistant individuals (14). Additional studies have shown a decrease in mitochondrial number and function as a correlation of age (16), focusing the attention on improving mito-
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FIGURE 6. Primary mouse myotubes were treated with ERRβ/γ agonist, GSK4716 (10 μM), for 48 h. A, gene expression was determined using real-time PCR. The levels of the test genes were expressed relative to the housekeeping gene, B2M, and for each sample normalized to the average signal for vehicle-treated cells. Citrate synthase activity (B) and cytochrome c (Cyt c) (C) protein levels were determined in cells treated with GSK4716. Data are expressed as mean ± S.E. (n = 3). Statistical significance is indicated as follows: *, p < 0.05; **, p < 0.01; ***, p < 0.005; ****, p < 0.001.

PGC-1α regulates the expression of mitofusin-2 (Mfn2) in an ERRα-dependent manner (43), and the overexpression of PGC-1α in skeletal muscle causes an increase in the number of mitochondria (44). The levels of Mfn2 in skeletal muscle are decreased in obese individuals as compared with lean individuals (45). These data have led to the hypothesis that Mfn2 links mitochondrial dysfunction to the etiology of disease (43). Interestingly, through our unbiased gene expression profiling efforts, we discovered that the ERRγ transgenic muscle does not demonstrate an increase in Mfn2 and other genes of mitochondrial fusion/fission. This was further evidenced by the lack of an increase in the number of mitochondria observed by electron microscopy. Our data imply that the absence of increased mitochondrial fusion/fission does not negatively impact the energy production/functionality of the mitochondria at the organismal level. Furthermore, increased subsarcolemmal mitochondria as seen in the transgenics imply an increase in ATP supply for membrane activities, including substrate and ion transport and fatty acid oxidation (46).

Increased ERRγ expression in skeletal muscle results in a decrease in size of fast twitch muscle and a distinct shift toward slow fiber type and greater endurance. Unbiased gene expression profiling uncovered striking increases in pathways of slow type myosin heavy chain, tropomyosins, and cytoskeletal com-
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ponent proteins, which are activated by MEF2/histone deacetylase, key regulators of fiber type specification. Similar to our muscle creatine kinase-VP16ERRγ transgenic model, transgenic expression of activated MEF2 in muscle increases endurance capacity and increases expression of the slow contractile proteins (29). Nuclear factor of activated T-cells is a crucial partner of MEF2 in skeletal muscle, and its activity is controlled by calcineurin. Calcineurin signaling via nuclear factor of activated T-cells has been demonstrated to be essential for fiber-type switching from a fast/glycolytic phenotype to a slow/oxidative phenotype in skeletal muscle (47). Furthermore, the genetic ablation of the calcineurin inhibitor, calscarin-2, in skeletal muscle results in a decrease in fast glycolytic muscle mass and an increase in endurance exercise in mice (48).

Given the role of ERRγ in the myocardial switch to oxidative metabolism, the effects on exercise capacity observed with ERRγ haploinsufficiency may be due to diminished cardiac function. We did not observe a decrease in the heart or muscle size in adult ERRγ heterozygous mice in contrast to what was observed in E18.5 embryos, indicating a postnatal compensation in these animals (5). Unlike the heart, we did not see a compensatory increase in ERRα or PGC-1α in skeletal muscle in the heterozygous mice. The study of tissue type-specific ERRγ null mouse models will contribute significantly toward the understanding of the role of ERRγ in cardiac versus skeletal muscle.

Because the phenotype of the transgenic ERRγ mice is strongly reminiscent of mouse models for peroxisome proliferator-activated receptor δ (30) or the MEF2/histone deacetylase/calcineurin pathway (29, 47), an important question that arises is whether these transcriptional factors have redundant roles in muscle. There are salient differences among these genetic models that point to a specific role for each protein in the control of muscle structure, function, and metabolism. Unlike the peroxisome proliferator-activated receptor δ transgenic models, we did not observe a resistance of our animals to a high fat diet, nor did they demonstrate an improvement in glucose tolerance.3 The MEF2 pathway controls expression of the slow muscle fiber genes but does not impact the mitochondrial or metabolic program. Our gene expression studies indicate that ERRγ plays a broader role in the control of biological processes such as angiogenesis and calcium handling in skeletal muscle. Thus, future studies will aim at comparing the gene expression patterns in these models directly to be able to tease apart the specific roles of these pathways in skeletal muscle. The schematic in Fig. 7 illustrates the complex transcriptional network regulating aspects of muscle gene expression.

Investigations into the molecular mechanism of ERRγ activation have shown that unlike other nuclear receptors ERRγ does not undergo rearrangement of its AF-2 helix upon agonist binding (49). Instead, it was observed that agonist binding increases thermal stability of the receptor, leading the authors to speculate that activation occurs via an increase in the cellular half-life of the protein. Thus, our studies with overexpression of the receptor provide a qualitative indication of the biological changes that would be elicited with an ERRγ agonist. It is noteworthy in this context that our results show that mice expressing the “activated” form of the receptor (VP16ERRγ) have a very similar phenotype compared with the native ERRγ-overexpressing strain. There may be a yet unknown endogenous agonist or antagonist ligand for ERRγ, in addition to common environmental contaminants that bind to the protein. Antagonistic agents would alter basal receptor activity and dampen downstream biological effects that may result in muscle or metabolic pathologies. From a therapeutic standpoint, we provide evidence that it is feasible to further activate ERRγ with a low molecular weight compound, despite its constitutive activity, and subsequently increase mitochondrial function in myocytes. Because of the lack of exposure in vivo, the use of GSK4716 for such investigations was not possible. The discovery of more potent and selective agonists with improved in vivo exposure will enable proof of concept studies linking ERRγ agonism to alleviation of disease symptoms. This will pave the path to the establishment of a novel mechanism in the arsenal against obesity and metabolic and muscle disease.

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