Road to exercise mimetics: targeting nuclear receptors in skeletal muscle

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Abstract

Skeletal muscle is the largest organ in the human body and is the major site for energy expenditure. It exhibits remarkable plasticity in response to physiological stimuli such as exercise. Physical exercise remodels skeletal muscle and enhances its capability to burn calories, which has been shown to be beneficial for many clinical conditions including the metabolic syndrome and cancer. Nuclear receptors (NRs) comprise a class of transcription factors found only in metazoans that regulate major biological processes such as reproduction, development, and metabolism. Recent studies have demonstrated crucial roles for NRs and their co-regulators in the regulation of skeletal muscle energy metabolism and exercise-induced muscle remodeling. While nothing can fully replace exercise, development of exercise mimetics that enhance or even substitute for the beneficial effects of physical exercise would be of great benefit. The unique property of NRs that allows modulation by endogenous or synthetic ligands makes them bona fide therapeutic targets. In this review, we present an overview of the current understanding of the role of NRs and their co-regulators in skeletal muscle oxidative metabolism and summarize recent progress in the development of exercise mimetics that target NRs and their co-regulators.

Introduction

Exercise has been known for its health benefits since ancient times. It is now widely accepted that physical activity positively affects a variety of clinical conditions including obesity, type 2 diabetes, metabolic syndrome, neurodegenerative diseases, cardiovascular diseases, and cancer (Perseghin et al. 1996, Grazina & Massano 2013, Lemanne et al. 2013, Mellett & Bousquet 2013). On the other hand, physical inactivity has major negative influences on these disease conditions (Hu et al. 2004).

How exactly exercise exerts its beneficial effects is not fully understood; however, skeletal muscle is believed to play a vital role (Hamilton & Booth 2000). As the largest organ in the human body, skeletal muscle comprises ~40% of total body mass and accounts for ~30% of whole-body energy metabolism during rest (Zurlo et al. 1990). Upon insulin stimulation, skeletal muscle can be responsible for ~85% of total glucose disposal (Defronzo et al. 1981). During peak activity, whole-body energy metabolism can be increased by up to 20-fold, ~90% of which is contributed by skeletal muscle (Zurlo et al. 1990). Hence, muscle is the major site of calorie burning of energy substrates such as glucose and free fatty acids. Exercise training remodels skeletal muscle to more efficiently clear these substrates, the excess levels of which negatively affect many tissues.

In mammals, skeletal muscle is a mosaic of heterogeneous myofibers with diverse structural and functional properties (Schiaffino & Reggiani 2011). Based on the expression patterns of different myosin heavy chain (MYH) isoforms, which coincide with various biochemical
characteristics, myofibers can be classified into four major groups: slow-twitch type I and fast-twitch types IIA, IIX/d, and IIB. Type I and IIA fibers are red in appearance due to their high myoglobin content. They are rich in mitochondria and predominantly powered by complete oxidation of glucose and fatty acids. These oxidative fibers are also dense with vasculature and resistant to fatigue. By contrast, the glycolytic type IIX/d and IIB fibers are generally white in color, have less myoglobin content and mitochondria, mainly rely on glycolysis for energy production, have less vasculature, and fatigue rapidly (Schiaffino & Reggiani 2011). In humans, fiber-type composition is strongly associated with metabolic health, with more glycolytic fibers being seen in obese and type 2 diabetic patients (Hickey et al. 1995).

It has been well documented that skeletal muscle undergoes a series of physiological and biochemical adaptations upon exercise training (Hamilton & Booth 2000), of which the most intriguing is fiber-type transformation. Many human and animal studies have clearly demonstrated that prolonged exercise induces the glycolytic type IIX/d and IIB fibers to transform to the more oxidative type IIA fibers (Gollnick et al. 1973, Foster et al. 1978, Wu et al. 2001). Although some professional athletes have an increased proportion of type I fibers (Gollnick et al. 1972), it remains unclear whether exercise training can switch type II fibers completely to type I. While exercise has a positive effect on the glycolytic-to-oxidative fiber-type transformation, physical inactivity and obesity usually have the opposite effect and lead to the reverse transformation (Bergouignan et al. 2011). During fiber-type transformation, not only is the expression of MYH isoforms switched, but other fiber-type-specific properties, such as mitochondrial density, oxidative phosphorylation (OXPHOS) activity, vasculature, and fatigue resistance, are also changed accordingly (Yan et al. 2011).

Skeletal muscle adaptation during exercise involves numerous transcriptional and epigenetic changes, which are regulated by multiple signaling pathways (Bassel-Duby & Olson 2006, Barrès et al. 2012). In addition to the widely known calcineurin/NFAT and HDAC/MEF pathways, it has recently been shown that nuclear receptors (NRs) and their co-regulatory factors also play important roles in skeletal muscle adaptation.

NRs are ligand-modulated transcription factors that respond to a variety of hydrophobic molecules including hormones, lipids, steroids, retinoids, and xenobiotics. All NRs share similar modular domains, including a highly conserved DNA-binding domain (DBD), a ligand-binding domain (LBD), variable N- and C-terminal domains, and a hinge domain between the DBD and LBD (Mangelsdorf et al. 1995). The DBD is characterized by a zinc finger motif that recognizes the hormone response element on target chromatin and the LBD by a hydrophobic ligand-binding pocket. Upon ligand binding, NRs undergo conformational changes, which alter their interactions with other proteins and trigger epigenetic chromatin changes and downstream transcriptional regulation (Wurz et al. 1996).

A major goal of exercise science is to find substitutes for physical exercise that achieve its beneficial effects in people unable to exercise. The ability of NRs to sense and respond to small-molecule ligands makes them ideal pharmacological targets. This review focuses on the roles of NRs and their co-regulatory factors in the regulation of skeletal muscle functions, including fiber-type determination, mitochondrial biogenesis, vasculature development, and fatigue resistance, with the goal of shedding some light on the development of the ‘exercise in a pill’.

The peroxisome proliferator-activated receptor subfamily

The peroxisome proliferator-activated receptor (PPAR) subfamily of NRs is composed of three members: PPARα, PPARδ (also referred to as PPARβ), and PPARγ. PPARα was the first PPAR identified during a screen for the molecular target of fibrate, a class of cholesterol-lowering compounds that increase hepatic fatty acid oxidation and peroxisome proliferation (hence the name) (Issemann & Green 1990). Based on sequence homology, PPARδ and PPARγ, which do not induce peroxisome proliferation, were later cloned from mouse tissue (Zhu et al. 1993, Klierer et al. 1994).

PPARs are predominantly localized in the nucleus. They form heterodimers with retinoid X receptors (RXRs) and can be activated by both PPAR ligands and RXR ligands. In the absence of a ligand, the PPAR/RXR heterodimers bind to PPAR response elements (PPREs) in association with transcriptional co-repressors such as nuclear receptor co-repressor (NCoR) and SMRT. Ligand binding leads to a conformational change and recruitment of co-activators such as PPARγ co-activator 1α and PPARγ co-activator 1β (PGC1α and PGC1β) to replace the co-repressors, resulting in the activation of downstream target gene expression. PPARs play essential roles in the regulation of lipid metabolism. They sense and respond to free fatty acids and their derivatives to regulate genes involved at almost all levels of lipid metabolism, including lipid import/export, synthesis, storage, breakdown, and oxidation (Evans et al. 2004). Although the PPAR
subfamily shares certain common target genes, PPARα and PPARδ are typically involved in the regulation of lipid catabolism and oxidation, while PPARγ is responsible for adipogenesis and lipid synthesis. All the three PPARs are expressed in skeletal muscle (Muioio et al. 2002, Amin et al. 2010), and over the last decade, both gain-of-function and loss-of-function studies have contributed significantly to our understanding of their roles in muscle.

PPARδ is the most abundant PPAR in skeletal muscle (Muioio et al. 2002, Amin et al. 2010) and plays important roles in the regulation of fiber-type determination, mitochondrial function, lipid metabolism, and fatigue resistance (Fig. 1). It is expressed relatively highly in oxidative fibers compared to glycolytic fibers. Exercise, in both acute and prolonged forms (Luquet et al. 2003, Watt et al. 2004), induces the expression of Pparδ (Ppard) in skeletal muscle. Similar to exercise, fasting also triggers a fuel-source switch in skeletal muscle from glucose to fatty acid utilization. Consistently, 6–48 h of fasting dramatically increases the expression of Pparδ in skeletal muscle (de Lange et al. 2006).

Two independent studies have shown that the overexpression of Pparδ in skeletal muscle induces a glycolytic-to-oxidative fiber-type transformation (Luquet et al. 2003, Wang et al. 2004). Mice overexpressing WT Pparδ have more oxidative fibers, higher OXPHOS enzyme activities, and more uncoupling proteins (UCPs). These transgenic mice also have reduced fat content with smaller adipocyte size, similar to what is observed in exercised animals (Luquet et al. 2003). Mice expressing a constitutively active form of Pparδ were nicknamed ‘marathon mice’ as they can run for up to twice the distance of their WT littermates. They have more type I and less type II fibers, have increased mitochondrial biogenesis and uncoupling, are resistant to diet-induced obesity, and have improved glucose tolerance (Wang et al. 2004). Conversely, conditional knockout of Pparδ in skeletal muscle leads to an oxidative-to-glycolytic fiber-type switch. The knockout muscle has lower expression of genes involved in fatty acid catabolism and oxidation, as well as reduced OXPHOS activities (Schuler et al. 2006). Upon a high-fat diet challenge, the mutant mice gain more weight mainly due to increased fat content and are more susceptible to developing insulin resistance and glucose intolerance (Schuler et al. 2006). Therefore, Pparδ appears to be necessary for the maintenance of oxidative metabolism and energy homeostasis.

Figure 1
NR regulation of energy metabolism and remodeling in skeletal muscle. The NR ring of physiology is shown on the left (Bookout et al. 2006). It clusters 49 mouse NRs into six groups based on their tissue distribution patterns. The NRs that have been found to play crucial roles in skeletal muscle function (highlighted in red/bold) are clustered mainly in two groups: group IC, the members of which are broadly expressed and are linked to lipid metabolism and energy homeostasis, including PPARα, PPARδ, PPARγ, and ERRα. These NRs work in concert with exercise and co-regulators to regulate many aspects of skeletal muscle physiology. Synthetic ligands targeting NRs and their co-regulators can enhance or replace the physiological benefits induced by exercise, which is of great value to public health.

REV-ERBα, and groups IIB and IIC, the members of which are typically expressed in highly metabolic tissues and are involved in CNS, circadian, and basal metabolic functions, including NOR1, NUR77, NURR1, ERRα, ERRγ, REV-ERBα, and REV-ERBβ, and groups IIB and IIC, the members of which are broadly expressed and are linked to lipid metabolism and energy homeostasis, including PPARα, PPARδ, PPARγ, and ERRα. These NRs work in concert with exercise and co-regulators to regulate many aspects of skeletal muscle physiology. Synthetic ligands targeting NRs and their co-regulators can enhance or replace the physiological benefits induced by exercise, which is of great value to public health.
fibers and their oxidative functions in skeletal muscle. However, it remains to be demonstrated whether Pparγ is required for exercise-induced muscle remodeling.

Pparα (Ppara) is abundantly expressed in tissues with high fatty acid catabolism, such as liver and heart (Fig. 1; Braissant et al. 1996), where it is activated by free fatty acids and promotes fatty acid oxidation (Kersten et al. 1999). Ppara is also expressed at significant levels in skeletal muscle. Both Ppara and Pparγ regulate fatty acid catabolism and share common target genes. Similar to Pparγ, the overexpression of Ppara in skeletal muscle also induces the expression of genes involved in fatty acid catabolism, tricarboxylic acid (TCA) cycle, and mitochondrial OXPHOS. As a result, fatty acid oxidation is increased in the transgenic muscle and the mice are resistant to diet-induced obesity (Finck et al. 2005).

However, the transgenic mice are more prone to developing insulin resistance and glucose intolerance due to the reduced expression of genes involved in glucose uptake and glycolysis (Finck et al. 2005). Thus, although Ppara has a positive role in the regulation of fatty acid oxidation in skeletal muscle, its activity needs to be finely regulated to balance glucose and fatty acid metabolism.

In addition to their different roles in metabolic regulation, Ppara also functions distinctly from Pparγ in fiber-type determination. In contrast to Pparγ, the overexpression of Ppara in skeletal muscle does not increase endurance but rather reduces it by more than 50% (Gan et al. 2011). Consistently, an oxidative-to-glycolytic fiber-type switch is found in these mice, as shown by the expression of MYH genes, metachromatic ATPase staining, and MYH immunohistochemistry staining (Gan et al. 2013). The opposing functions of PPARα and PPARγ in the induction of glycolytic and oxidative fiber-type transformations respectively seem to be mediated by a miRNA network involving two specific miRNAs, mir-208b and mir-499 (Gan et al. 2013), which play important roles in fiber-type determination by activating the oxidative and repressing the glycolytic myofiber gene program (van Rooij et al. 2009). In contrast to the overexpression model, knockout of Ppara in skeletal muscle induces a glycolytic-to-oxidative fiber-type switch (Gan et al. 2013). Therefore, endogenous PPARα counteracts PPARγ to maintain a proper fiber-type composition in skeletal muscle.

Pparγ (Pparγ) is expressed most highly in adipose tissues, where it plays an essential role in adipogenesis and whole-body lipid homeostasis (Fig. 1). Its ablation in adipose tissues leads to severe lipodystrophy and elevated levels of blood triglycerides and free fatty acids. The knockout mice are more susceptible to diet-induced insulin resistance. However, treatment with thiazolidinediones (TZDs), a class of PPARγ-specific ligands, can still improve insulin sensitivity in these knockout mice, suggesting that PPARγ in non-adipose tissues also contributes to its regulation of lipid homeostasis and insulin sensitivity (He et al. 2003). The strongest evidence showing a positive role for muscle PPARγ in metabolic regulation comes from the generation of a mouse model with Pparγ specifically deleted in skeletal muscle (Hevener et al. 2003). These knockout mice develop glucose intolerance and insulin resistance. Moreover, they are less responsive to TZD-induced skeletal muscle insulin sensitization, while the effects of TZDs in the liver and adipose tissues remain unaffected (Hevener et al. 2003). A similar study seems to have drawn a different conclusion, showing that the knockout mice only have mild insulin resistance and respond normally to TZD treatment (Norris et al. 2003). However, the two studies were carried out in mice with different genetic backgrounds, one being a pure C57BL/6j background (Hevener et al. 2003) and the other a mixed 129/sv, C57BL/6, and FVB background, which might account for the different phenotypes observed. In addition to the knockout models, the overexpression of Pparγ in skeletal muscle also demonstrates its importance in metabolic regulation (Amin et al. 2010). These transgenic mice are protected from diet-induced insulin resistance and glucose intolerance. Interestingly, these mice produce significant amounts of adiponectin in skeletal muscle, despite their reduced intramuscular adiposity. Furthermore, the activation of AMP-activated protein kinase (AMPK), a known adiponectin target, in the transgenic muscle suggests that the increased adiponectin functions locally. Similar to Pparα, the overexpression of Pparγ induces a glycolytic-to-oxidative fiber-type switch and an increase in mitochondrial gene expression, which may be a secondary effect from the activated AMPK pathway (Amin et al. 2010). Therefore, PPARγ is required in skeletal muscle for glucose and lipid homeostasis. In addition, its role in the generation of muscle adiponectin provides another layer of metabolism regulation.

The estrogen-related receptor subfamily

The estrogen-related receptor (ERR) subfamily includes three members: ERRα, ERRβ, and ERRγ. ERRα was the first to be identified based on its high sequence homology with the estrogen receptor α (ERα; Giguère et al. 1988). ERRβ was cloned in the same study using Erro (Esra) cDNA as a probe (Giguère et al. 1988). Last but not least, ERRγ was discovered in three independent studies using different
strategies (Eudy et al. 1998, Hong et al. 1999, Heard et al. 2000). Although all the three ERRs share high structural similarities with ERs at both the DNA and protein levels, they are distinct from ERs in both their functions and their regulation of target gene transcription (Eichner & Giguere 2011).

All the three ERRs are believed to be constitutively active and, to date, no natural ligand(s) has been identified (Eichner & Giguere 2011). Instead, the transcriptional activities of ERRs are regulated by a number of co-regulatory factors, the most studied of which include the steroid receptor co-activators (SRC1, SRC2, and SRC3; Hong et al. 1999, Xie et al. 1999, Zhang & Teng 2000), the PGC1α and PGCβ (Huss et al. 2002, Kamei et al. 2003), and the NR co-repressors RIP140 (receptor-interacting protein 140) and NCoR1 (Sanyal et al. 2004, Pérez-Schindler et al. 2012).

Extensive studies in the past decade have clearly established a central role of ERRs in the regulation of energy metabolism (Eichner & Giguere 2011), which is further supported by their tissue expression patterns. ERRα is the most abundant of the three. It is ubiquitously expressed but peaks in tissues with high energy needs including brain, heart, muscle, and kidney (Fig. 1; Giguere et al. 1988, Bookout et al. 2006). ERRβ (Esrb) and ERRγ (Esrg) have similar tissue distribution patterns. Both are selectively expressed in metabolically active tissues such as retina, spinal cord, heart, muscle, and kidney, with ERRγ generally being expressed at a higher level (Fig. 1; Bookout et al. 2006). All the three ERRs are highly expressed in skeletal muscle, and their roles in the regulation of muscle energy metabolism have been explored in both gain-of-function and loss-of-function studies (Luo et al. 1997, 2003, Huss et al. 2004, Wende et al. 2005, Alaynick et al. 2007, Chinsomboon et al. 2009, Rangwala et al. 2010, Narkar et al. 2011, Gan et al. 2013, Matsakas et al. 2013).

Studies of ERRα in skeletal muscle have mainly focused on its synergistic interaction with PGC1α in target gene regulation. No phenotypic change in skeletal muscle is observed after whole-body Errα ablation, possibly due to a compensatory induction of Pgc1α (Ppargc1a; Luo et al. 2003, Huss et al. 2004). ERRα seems to play a role in the regulation of fatty acid metabolism and fuel selection in skeletal muscle as its overexpression induces the expression of Ppargc1a, a key regulator of fatty acid metabolism, and Pdk4, the mitochondrial gatekeeper for pyruvate oxidation. The overexpression of its co-activator PGC1α can further enhance the expression of these genes (Huss et al. 2004, Wende et al. 2005). Such regulation is mediated by the direct binding of ERRα to the ERR response element (ERRE) on the promoters of Ppargc1a and Pdk4 (Huss et al. 2004, Wende et al. 2005). In addition, ERRα also regulates myocyte differentiation. The overexpression of Errα in C2C12 myoblasts accelerates myotube formation, while Errα-null primary myocytes show delayed myogenesis and mitochondrial dysfunction (Murray & Huss 2011). Although ERRα positively regulates lipid metabolism and mitochondrial OXPHOS in cooperation with PGC1α in heart and brown adipose tissue (Dufour et al. 2007, Villena et al. 2007), its physiological function in skeletal muscle remains to be elucidated.

Similar to ERRα, ERRγ also plays an important role in the regulation of energy metabolism. Errγ-null mice die within the first week of life, possibly from heart failure due to disrupted mitochondrial energy production (Alaynick et al. 2007). The importance of ERRγ in energy metabolism is also indicated by its distribution in skeletal muscle, where it is exclusively expressed in oxidative muscles such as soleus and red gastrocnemius but not in glycolytic muscles such as white gastrocnemius or quadriceps (Narkar et al. 2011). Transgenic mice with muscle-specific overexpression of Errγ have a remarkable conversion of glycolytic to oxidative fibers, with all white muscles appearing red (Narkar et al. 2011). The transgenic mice are fatigue resistant and can run about twice the distance of the controls. They also have a higher energy expenditure rate and a lower respiratory exchange ratio (RER), indicating a fuel preference for fatty acids. Both mitochondrial biogenesis and vascularization are induced. Gene expression analysis has further revealed a gene signature change from glycolytic to oxidative muscles, including the induction of genes involved in lipid metabolism, TCA cycle, angiogenesis, and mitochondrial OXPHOS (Rangwala et al. 2010, Narkar et al. 2011). In addition, the overexpression of ERRγ also alleviates the symptoms of Duchenne muscular dystrophy and promotes muscle recovery from ischemic damage (Matsakas et al. 2012, 2013). Therefore, genetic activation of ERRγ can induce an exercise-like phenotype in skeletal muscle with positive impacts on muscle diseases. However, its endogenous roles in the regulation of skeletal muscle function and exercise-induced muscle remodeling remain to be demonstrated.

Unlike ERRα and ERRγ, little is known about whether and how ERRβ regulates energy metabolism. Loss-of-function studies have demonstrated the crucial roles of ERRβ in placental development (Luo et al. 1997), germ cell development (Mitsunaga et al. 2004), inner ear development (Chen & Nathans 2007), and retinal photoreceptor survival (Onishi et al. 2010). In skeletal muscle, it has been briefly shown that both ERRβ and ERRγ are required to...
maintain type I muscle fibers in the oxidative/glycolytic mixed muscle gastrocnemius but not in the mostly oxidative muscle soleus (Gan et al. 2013). However, the extent of functional redundancy between ERRβ and ERRγ in skeletal muscle is unclear, and more work is needed to fully understand the role of ERRβ in the regulation of energy metabolism and skeletal muscle function.

**The NR4A subfamily**

The NR4A subfamily of NRs consists of three closely related members: NR4A1 (NUR77), NR4A2 (NURR1), and NR4A3 (NOR1). Similar to ERRs, the NR4As are also orphan receptors that do not bind to any natural agonist (Pearen & Muscat 2010). They are constitutively active and their transcriptional activities appear to be primarily regulated by their abundance and post-translational modifications (Chao et al. 2012).

Based on their tissue expression patterns, the NR4As are clustered in the same group as ERRβ and ERRγ; they are preferentially expressed in tissues with high energy needs such as brain, muscle, and brown adipose tissue (Fig. 1; Bookout et al. 2006). While little is known about the function of NURR1 in skeletal muscle, both NUR77 and NOR1 have been clearly shown to play important roles in the regulation of skeletal muscle metabolism (Maxwell et al. 2005, Chao et al. 2007, 2012, Pearen et al. 2008, 2012).

In skeletal muscle, Nur77 is selectively expressed in glycolytic vs oxidative muscles, suggesting a positive role in the regulation of glucose metabolism (Chao et al. 2007). The expression of Nur77 can be significantly induced by β-adrenergic signaling from the sympathetic nervous system to regulate muscle energy metabolism (Maxwell et al. 2005). Contrarily, skeletal muscle denervation reduces the expression of Nur77, as well as a subset of glucose metabolism genes, which is restored by the ectopic expression of Nur77 in denervated muscle (Chao et al. 2007). The importance of Nur77 in the regulation of glucose metabolism can be further demonstrated by the overexpression of Nur77 in C2C12 cells, which not only induces glucose metabolism genes but also enhances cellular glucose transport (Chao et al. 2007). Despite its role in the regulation of glucose metabolism, muscle-specific overexpression of Nur77 induces an oxidative fiber-type switch, similar to Pparo and Errγ (Chao et al. 2012). The transgenic muscle has typical characteristics of oxidative fibers such as increased fatty acid oxidation, higher mitochondrial OXPHOS activity, and fatigue resistance. However, the level of glycogen, which is usually high in glycolytic fibers and low in oxidative fibers, is increased in the Nur77 transgenic muscle, suggesting a different working model for its fiber-type determination compared with PPARo and ERRγ. More detailed analysis of fiber-type composition, endurance performance, and gene expression profiling will be required to understand the mechanism of muscle remodeling induced by Nur77. In addition, the endogenous role of Nur77 in the β-adrenergic signaling cascade remains to be elucidated.

Similar to Nur77, Nor1 is also induced by β-adrenergic signaling in skeletal muscle (Pearen et al. 2008). However, NOR1 seems to participate more in the regulation of fatty acid metabolism rather than in that of glucose. Knockdown of NOR1 in C2C12 cells reduces fatty acid oxidation and mitochondrial OXPHOS, but induces glycolysis (Pearen et al. 2008). The overexpression of an active form of Nor1 in skeletal muscle leads to a fiber-type switch from glycolytic to oxidative fibers (Pearen et al. 2012). The transgenic mice have increased running endurance, improved insulin sensitivity and glucose tolerance, and higher energy expenditure. Both myoglobin expression and mitochondrial activity are induced in the transgenic muscle. The fiber-type switch phenotype seems to be dependent on muscle groups, with overall more type IIa and IIx fibers but less type I and IIb fibers. This intermediate oxidative fiber-type switch might be due to the enhanced HDAC5 activity, which has been shown to promote oxidative fiber formation (Potthoff et al. 2007). However, the direct targets of NOR1 remain to be identified. It is also not clear how NOR1 activates HDAC5 and whether or not other pathways are involved in the fiber-type conversion induced by NOR1.

**The REV-ERB subfamily**

There are two members in the REV-ERB subfamily of NRs: REV-ERBα and REV-ERBβ. REV-ERBs were originally discovered as orphan receptors (Miyajima et al. 1989), but were later ‘adopted’ by the identification of heme as their physiological ligand (Raghuram et al. 2007). Upon heme binding, REV-ERBs recruit co-repressors such as NCoR1 and repress target gene expression (Raghuram et al. 2007, Yin et al. 2007). REV-ERBs are active components of the circadian clock (Preitner et al. 2002, Bass 2012), and recent studies have also linked their functions to metabolic regulation in adipose tissues, liver, and muscle (Yang et al. 2006, Kumar et al. 2010, Cho et al. 2012, Woldt et al. 2013). Anatomical profiling of NRs clusters REV-ERBs in the same group as ERRβ, ERRγ, Nur77, and NOR1, all of...
which are preferentially expressed in metabolically active tissues (Fig. 1; Bookout et al. 2006). This further indicates an active role of REV-ERBs in the regulation of energy metabolism.

While little is known about the function of REV-ERBβ in skeletal muscle, REV-ERBα has recently been shown to positively regulate energy metabolism and mitochondrial OXPHOS function in muscle (Woldt et al. 2013). Rev-erbα (Nr1d1) is expressed at higher levels in oxidative muscles than in glycolytic muscles and exercise can further induce its expression (Woldt et al. 2013). The importance of REV-ERBα in skeletal muscle has been demonstrated in Rev-erbα-null mice. These mice have reduced voluntary wheel-running activity, diminished endurance exercise performance, and lower energy expenditure during exercise. The knockout muscle has decreased mitochondrial density, reduced OXPHOS activity, and downregulated fatty acid metabolism genes (Woldt et al. 2013). On the other hand, the overexpression of Rev-erbα in C2C12 cells increases mitochondrial biogenesis and OXPHOS activity, accompanied by the induction of fatty acid metabolism genes. The in vivo overexpression of Rev-erbα in muscle via adeno-associated viral (AAV) infection also induces mitochondrial OXPHOS activity. These physiological changes seem to be mediated by the AMPK–Sirt1–PGC1α signaling pathway, which is downregulated in the knockout muscle but upregulated in Rev-erbα-overexpressing muscle cells. In addition to its roles in the regulation of mitochondrial biogenesis and OXPHOS activity, muscle REV-ERBα is also involved in the modulation of mitochondrial autophagy (mitophagy, Woldt et al. 2013). Mitophagy is induced in Rev-erbα-knockout muscle but suppressed in overexpressing C2C12 cells. REV-ERBα seems to directly bind to and repress genes in multiple steps of mitophagy, including the mitophagy regulator Park2, the autophagosome initiation factor Ulk1, the autophagosome elongation factors Atg5 and Bnip3, and the lysosomal enzymes Ctsl and Atpase6v1b2. Therefore, REV-ERBα increases mitochondria number by both inducing mitochondrial biogenesis through the AMPK–Sirt1 pathway and reducing mitochondrial turnover by inhibiting mitophagy. However, it is not clear how AMPK is activated by the overexpression of Rev-erbα since the level of ATP is much lower in Rev-erbα-knockout muscle (Woldt et al. 2013), which is usually associated with AMPK activation. Also, the inhibition of mitophagy might be deleterious in the long term due to the diminished clearance of dysfunctional mitochondria (Narendra et al. 2008, Jin & Youle 2012).

**NR co-regulatory factors**

The functions of NRs are finely modulated by associated co-activators and co-repressors. The abundance of these co-regulators and their post-translational modifications are regulated in response to a variety of physiological stimuli such as exercise and fasting, which then induce conformational changes in the NR–chromatin complexes and regulate their transcriptional activities. Recent studies have demonstrated important roles for NR co-regulators in energy metabolism and fiber-type determination in skeletal muscle.

**PPARγ co-activator 1**

The PGC1α and PGC1β are probably the best-known and most studied NR co-regulators implicated in energy metabolism. Both are highly expressed in metabolically active tissues such as brain, heart, muscle, and brown adipose tissue, where they serve as co-activators for a number of transcription factors involved in the regulation of energy metabolism, including the PPAR and ERR NRs, and the nuclear respiratory factors 1 and 2 (NRF1 and NRF2/GABPA).

PGC1α was first identified as a cold-inducible thermogenic factor in brown adipose tissue (Puigserver et al. 1998). In skeletal muscle, Pgc1α is predominantly expressed in oxidative muscles such as soleus (Wu et al. 1999). The expression of Pgc1α can be induced by exercise or cold exposure in skeletal muscle (Puigserver et al. 1998, Baar et al. 2002, Russell et al. 2003). In addition to expression level, its co-transcriptional activity can also be modulated by a variety of post-translational modifications such as phosphorylation (Puigserver et al. 2001, Jäger et al. 2007), acetylation (Rodgers et al. 2005), and methylation (Teysier et al. 2005). When overexpressed in C2C12 muscle cells, Pgc1α stimulates mitochondrial biogenesis by upregulating the mitochondrial transcription factor A (Tfam) as well as the mitochondrial regulators Nrf1 and Nrf2 (Nfe2L2). It can further function as a co-activator for Nrf1 and Nrf2 in the upregulation of the expression of mitochondrial genes. In addition to mitochondrial biogenesis, Pgc1α also stimulates mitochondrial uncoupling by upregulating the mitochondrial Uncp2, to further enhance mitochondrial energy expenditure (Wu et al. 1999). In vivo ectopic expression of Pgc1α in skeletal muscle not only induces mitochondrial biogenesis and OXPHOS activity but also switches type Iib and Iix/d glycolytic fibers to type I and Ia oxidative fibers (Lin et al. 2002b). As a result, the transgenic mice have improved endurance running performance.
PGC1β was identified by its high homology with PGC1α (Kressler et al. 2002, Lin et al. 2002a). It is also highly involved in the regulation of mitochondrial function and energy metabolism (Kamei et al. 2003). In vitro overexpression of Pgc1β (Ppargc1b) in muscle cells has effects similar to that of Pgc1α in terms of promoting mitochondrial biogenesis and oxidative fiber-type transformation (Mortensen et al. 2006). Similarly, the overexpression of Pgc1β in skeletal muscle stimulates mitochondrial OXPHOS and fatty acid oxidation, along with oxidative fiber-type transformation (Arany et al. 2007). However, instead of a switch toward the most oxidative type I and IIa fibers as seen in the PGC1α model, PGC1β induces a more intermediate switch toward type IIx/d fibers (Arany et al. 2007), suggesting a different working mechanism. Whole-body or muscle-specific knockout of Pgc1β causes reduced mitochondrial OXPHOS function in skeletal muscle but does not change fiber-type composition (Lelliott et al. 2006, Sonoda et al. 2007, Zechner et al. 2010). It would be expected that PGC1α and PGC1β compensate for each other when one is absent. This is true for their contributions to the regulation of mitochondrial function. Double-knockout mice lacking Pgc1α and Pgc1β in skeletal muscle have significantly lower mitochondrial OXPHOS activity compared with the single-knockout mice. However, the fiber-type composition of the double-knockout mice is not different from that of the WT controls (Zechner et al. 2010). Therefore, PGC1α and PGC1β are necessary for mitochondrial OXPHOS function in skeletal muscle, but appear dispensable for oxidative fiber-type determination.

Receptor-interacting protein 140

In addition to the co-activators of NRs, their co-repressors also contribute to the regulation of energy metabolism in skeletal muscle, one of which is the RIP140. It was originally identified as a co-regulatory factor for the ERs (Cavalliès et al. 1995). RIP140 (NRIP) is highly expressed in metabolic tissues such as fat and muscle (Leonardsson et al. 2004). In skeletal muscle, it is selectively expressed in glycolytic vs oxidative muscles (Seth et al. 2007), indicating a repressive role in the regulation of oxidative metabolism. Rip140-null mice exhibit ~70% reduction in total fat content, mainly due to increased fatty acid oxidation and mitochondrial energy consumption in muscle and white adipose tissue (Leonardsson et al. 2004). The knockout mice exhibit ~25% increase in whole-body energy expenditure and a lower RER, suggesting a shift toward fat utilization as energy source. In primarily glycolytic muscles where Rip140 is endogenously expressed, loss of Rip140 induces an oxidative fiber-type switch toward type Ila and IIx/d fibers, as well as increases in myoglobin content and mitochondrial biogenesis. Gene expression profiling further reveals significant induction of genes involved in fatty acid oxidation and mitochondrial OXPHOS in the knockout muscle (Seth et al. 2007). On the contrary, ectopic expression of Rip140 in oxidative muscles causes a reduction of oxidative fibers and myoglobin content. However, the exercise-induced fiber-type conversion is still retained in these transgenic mice (Seth et al. 2007). A subset of oxidative genes repressed by RIP140 are known targets of PPARs and ERRs and can be co-activated by PGC1α, including Mcad (Cdhl15), Cidea, Cpt1b, and Fabp3 (Christian et al. 2006, Hallberg et al. 2008). Additionally, RIP140 is recruited to either known or predicted PPREs and ERREs at the promoters of these genes (Seth et al. 2007). Hence, RIP140 and PGC1 could work in a yin–yang fashion in the regulation of the transcriptional activity of NRs such as PPARs and ERRs.

Nuclear receptor co-repressor 1

The NCoR1 was first identified as a ligand-independent transcriptional co-repressor for thyroid hormone receptor and retinoic acid receptor (Hörlein et al. 1995). It is ubiquitously expressed and is required for normal embryonic development (Jepsen et al. 2000). In skeletal muscle, NCoR1 is expressed at similar levels in oxidative and glycolytic muscles (Schuler et al. 1999). However, in conditions when fatty acid metabolism is stimulated, such as during fasting, high-fat diet challenge, and exercise, its expression in skeletal muscle is significantly reduced (Yamamoto et al. 2011, Pérez-Schindler et al. 2012), indicating that NCoR1 is involved in the repression of fatty acid metabolism. Muscle-specific deletion of NCoR1 increases muscle mass and exercise endurance (Yamamoto et al. 2011). The Ncor1-null mice have higher locomotor
activity and whole-body energy expenditure. Similar to the overexpression of Pgc1α or deletion of Rip140, the knockout of Ncor1 induces an oxidative fiber-type switch, associated with increased mitochondrial biogenesis and enhanced oxidative metabolism. In addition, there is a high overlap between the genes induced by the overexpression of Pgc1α and knockout of Ncor1 or Rip140 in skeletal muscle. Similar to RIP140, NCoR1 functions through PPARs and ERRs in opposition to PGC1α. It is recruited to PREs or ERREs at their target gene promoters to repress their transcriptional activity, which can be antagonized by PGC1α (Christian et al. 2006, Pérez-Schindler et al. 2012). Thus, the three co-regulatory factors work cooperatively with PPARs and ERRs in the regulation of skeletal muscle adaptation and energy metabolism. However, the abundance of NCoR1 and PGC1α, but not of RIP140, fluctuates in response to exercise, suggesting that they both play an important role in the exertion of exercise-induced muscle remodeling (Frier et al. 2011).

AMP-activated protein kinase

The AMPK is a central mediator of metabolism that functions by sensing and regulating cellular energy supplies. It is activated when energy levels are low to restore energy balance by promoting catabolism and inhibiting anabolism (Hardie 2007). In skeletal muscle, the activity of AMPK is significantly higher in oxidative vs glycolytic muscles, indicating its contribution to the maintenance of the basal oxidative metabolism (Narkar et al. 2011). This is further confirmed by the in vivo overexpression of an inactive form of AMPK in skeletal muscle, which dramatically reduces endurance exercise capacity and induces insulin resistance and glucose intolerance (Fujii et al. 2007, 2008). In addition to the basal oxidative metabolism, the activation of AMPK is also required for exercise-induced mitochondrial biogenesis via PGC1α (Zong et al. 2002, Jäger et al. 2007), in which AMPK is activated by exercise and directly phosphorylates PGC1α and upregulates its co-transcriptional activity (Jäger et al. 2007, Narkar et al. 2008). In some NR genetic models where oxidative fiber-type conversion is induced, such as the muscle-specific overexpression of Pparα, Pparγ, Errγ, or Rev-erba, AMPK activity is also significantly increased (Narkar et al. 2008, 2011, Amin et al. 2010, Woldt et al. 2013). Furthermore, direct interaction between AMPK and PPARα has been observed to synergistically activate target genes involved in oxidative metabolism (Narkar et al. 2008, Gan et al. 2011). Thus, although AMPK is not a canonical NR co-regulator, it interacts with NRs and is highly involved in their regulation of energy metabolism (Fan et al. 2011).

Road to exercise mimetics

A common feature of NRs and AMPK is that their activities can be modulated by small-molecule ligands, which makes them ideal pharmacological targets. Toward this end, a number of synthetic ligands have been developed for NRs including the ones described above. Some of these ligands have already been shown to promote skeletal muscle oxidative metabolism, including the PPARα agonist GW501516 (Narkar et al. 2008), ERRβ/γ agonist GSK4716 (Rangwala et al. 2010), and REV-ERBα/β agonists SR9009 and SR9011 (Woldt et al. 2013).

GW501516 was originally developed as a potent and selective PPARα agonist (Oliver et al. 2001). Its activation of PPARα in cultured C2C12 muscle cells induces the expression of genes involved in fatty acid catabolism, mitochondrial OXPHOS, and cholesterol efflux (Dressel et al. 2003). GW501516 also works in vivo to enhance oxidative metabolism in skeletal muscle. Oral doses of 5 mg/kg per day for 4 weeks have been shown to significantly upregulate oxidative genes such as Ucp3, Pdk4, and Cpt1α, similar to that seen with the muscle-specific overexpression of Pparα (Luquet et al. 2003, Wang et al. 2004, Narkar et al. 2008). The ligand activation of PPARα alone does not stimulate any oxidative fiber-type switch or mitochondrial biogenesis in skeletal muscle, which is different from the muscle overexpression model. However, when co-administered with exercise training, GW501516 treatment increases the proportion of type I oxidative fibers by ~38% and mitochondrial biogenesis by ~50%, while training alone has little effect. In addition, the pairing of GW501516 treatment with exercise training has been shown to dramatically increase endurance running performance compared with GW501516 treatment or training alone. Gene expression profiling has revealed a unique oxidative gene signature, which is also found in the Pparα transgenic muscle but not during either GW501516 treatment or training alone (Wang et al. 2004, Narkar et al. 2008). Thus, in vivo activation of PPARα by oral administration of GW501516 enhances the effect of exercise training.

GSK4716 was identified as a specific agonist for ERRβ and ERRγ, without any crossover activity with the ERs (Zuercher et al. 2005). It seems to have good potential for promoting oxidative metabolism in skeletal muscle. In primary mouse myotubes, treatment with GSK4716 leads
to the upregulation of all the three Err genes and their co-activators Pgc1α and Pgc1β. Additionally, it induces the expression of genes involved in fatty acid oxidation, TCA cycle, and mitochondrial OXPHOS, such as Cpt1b, Idh3, and Atp5b. It also stimulates mitochondrial biogenesis as both the mitochondrial citrate synthase activity and the amount of cytochrome c are increased (Rangwala et al. 2010). However, no in vivo trial has been reported and more functional studies will be needed to fully assess its effect in skeletal muscle.

The synthetic REV-ERB agonists SR9009 and SR9011 have been developed recently (Solt et al. 2012). Treatment with SR9009 or SR9011 increases the transcriptional repression of REV-ERBs on their target genes. In vivo, a single injection of SR9009 or SR9011 has been shown to result in the induction of genes involved in glycolysis, fatty acid catabolism, and mitochondrial OXPHOS, including Hk1, Pkm2, Pgc1α, Cpt1b, Fatp1, and Ucp3. Mice treated with SR9011 for 12 days have increased energy expenditure with no change in RER, indicating that the oxidation of both fatty acids and glucose is induced. Additionally, 30 days of treatment with SR9009 has been found to significantly increase mouse running endurance. In C2C12 myotubes, treatment with SR9009 or SR9011 has been reported to increase mitochondria number (Woldt et al. 2013). While the effects of these agonists on skeletal muscle seem promising, questions regarding the requirement for skeletal muscle REV-ERBs and how REV-ERBs activate energy metabolism genes remain to be answered.

In addition to the NR ligands, the AMPK activator AICAR also works as an exercise mimetic (Narkar et al. 2008). AICAR treatment for 4 weeks increases mouse energy expenditure and enhances running endurance by ~40%. It induces the expression of a number of genes linked to oxidative metabolism, including Scd1, Pdk4, Fasn, Lip, and Dgat, most of which are also induced by the overexpression of Pparo in skeletal muscle (Wang et al. 2004). The stimulation of oxidative genes by AICAR seems to be dependent on PPARα as AICAR fails to induce these genes in Pparα-null muscle cells. In addition, when administered together, AICAR and GW501516 synergistically activate PPARα target genes such as Ucp3, Pdk4, and Lpl (Narkar et al. 2008). Therefore, the activation of AMPK by its activator AICAR induces an oxidative gene signature change mediated by PPARα, which causes skeletal muscle remodeling and enhances endurance. However, the mechanism as to how AMPK synergistically activates PPARα target genes remains to be elucidated.

Conclusions

Studies over the past decade have made it clear that NRs and their co-regulators are key regulatory components of energy metabolism and exercise-induced remodeling in skeletal muscle. Synthetic ligands targeting NRs and their co-regulators, including GW501516, AICAR, GSK4716, and SR9009/9011, have been developed and proven to be effective in enhancing or mimicking exercise effects. To date, many issues remain with the current generation of exercise mimetics, such as toxicity, side effects, and high dosage, which prevent their immediate clinical applications. However, with advances in our understanding of the molecular mechanism by which NRs regulate skeletal muscle physiology, we are optimistic that the next generation of exercise mimetics is not far away.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

This work was supported by US National Institutes of Health grant numbers (DK057978, DK090962, HL088093, HL105278, CA014195, and ES010337), the Glenn Foundation for Medical Research, the Leona M and Harry B Helmsley Charitable Trust, Ipsen/Biomeasure, and the Ellison Medical Foundation.

Acknowledgements

The authors thank L Ong and C Brondos for administrative assistance. RM Evans is an Investigator of the Howard Hughes Medical Institute at the Salk Institute and March of Dimes Chair in Molecular and Developmental Biology.

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