Regulation of Muscle Fiber Type and Running Endurance by PPARδ

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Endurance exercise training can promote an adaptive muscle fiber transformation and an increase of mitochondrial biogenesis by triggering scripted changes in gene expression. However, no transcription factor has yet been identified that can direct this process. We describe the engineering of a mouse capable of continuous running of up to twice the distance of a wild-type littermate. This was achieved by targeted expression of an activated form of peroxisome proliferator-activated receptor δ (PPARδ) in skeletal muscle, which induces a switch to form increased numbers of type I muscle fibers. Treatment of wild-type mice with PPARδ agonist elicits a similar type I fiber gene expression profile in muscle. Moreover, these genetically generated fibers confer resistance to obesity with improved metabolic profiles, even in the absence of exercise. These results demonstrate that complex physiologic properties such as fatigue, endurance, and running capacity can be molecularly analyzed and manipulated.

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Introduction

Skeletal muscle fibers are generally classified as type I (oxidative/slow) or type II (glycolytic/fast) fibers. They display marked differences in respect to contraction, metabolism, and susceptibility to fatigue. Type I fibers are mitochondria-rich and mainly use oxidative metabolism for energy production, which provides a stable and long-lasting supply of ATP, and thus are fatigue-resistant. Type II fibers comprise three subtypes, IIa, IIx, and IIb. Type IIb fibers have the lowest levels of mitochondrial content and oxidative enzymes, rely on glycolytic metabolism as a major energy source, and are susceptible to fatigue, while the oxidative and contraction functions of type IIa and IIx lie between type I and IIb (Booth and Thomason 1991; Berchtold et al. 2000; Olson and Williams 2000). Adult skeletal muscle shows plasticity and can undergo conversion between different fiber types in response to exercise training or modulation of motoneuron activity (Booth and Thomason 1991, Jarvis et al. 1996; Pette 1998; Olson and Williams 2000; Hood 2001). This conversion of muscle fiber from type IIb to type IIa and type I is likely to be mediated by a calcium signaling pathway that involves calcineurin, calmodulin-dependent kinase, and the transcriptional cofactor Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) (Naya et al. 2000; Olson and Williams 2000; Lin et al. 2002; Wu et al. 2002). However, the targeted transcriptional factors directly responsible for reprogramming the fiber-specific contractile and metabolic genes remain to be identified.

Muscle fiber specification appears to be associated with obesity and diabetes. For instance, rodents that gain the most weight on high-fat diets possess fewer type I fibers (Abou et al. 1992). In obese patients, skeletal muscle has been observed to have reduced oxidative capacity, increased glycolytic capacity, and a decreased percentage of type I fibers (Hickey et al. 1995; Tanner et al. 2002). Similar observations have been made in type 2 diabetic patients (Lillioja et al. 1987; Hickey et al. 1995). Recently, it has been shown that increasing oxidative fibers can lead to improved insulin action and reduced adipocyte size (Luquet et al. 2003; Ryder et al. 2003).

We have previously established that peroxisome proliferator-activated receptor (PPAR) δ is a major transcriptional regulator of fat burning in adipose tissue through activation of enzymes associated with long-chain fatty-acid β-oxidation (Wang et al. 2003). Although PPARδ is the predominant PPAR isoform present in skeletal muscle, its in vivo function has not been determined. Our current study uncovers PPARδ as the first transcription factor able to drive the formation of functional type I muscle fibers, whose activation entrains complex pathways both enhancing physical performance and creating a state of obesity resistance.

Results

Activation of PPARδ Leads to Muscle Fiber Transformation

A role of PPARδ in muscle fiber was suggested by its enhanced expression—at levels 10-fold and 50-fold greater than PPARα and γ isoforms, respectively (unpublished data). An examination of PPARδ in different muscle fibers reveals a significantly higher level in type I muscle (soleus) relative to type II–rich muscle (extensor digitorum longus) or type I and type II mixed muscle (gastrocnemius) (Figure 1A); this

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Abbreviations: COX, cytochrome c oxidase; mCPT1, muscle carnitine palmitoyltransferase-1; PGC-1α, Peroxisome proliferator-activated receptor-gamma coactivator 1α; PPAR, peroxisome proliferator-activated receptor; UCP, uncoupling protein

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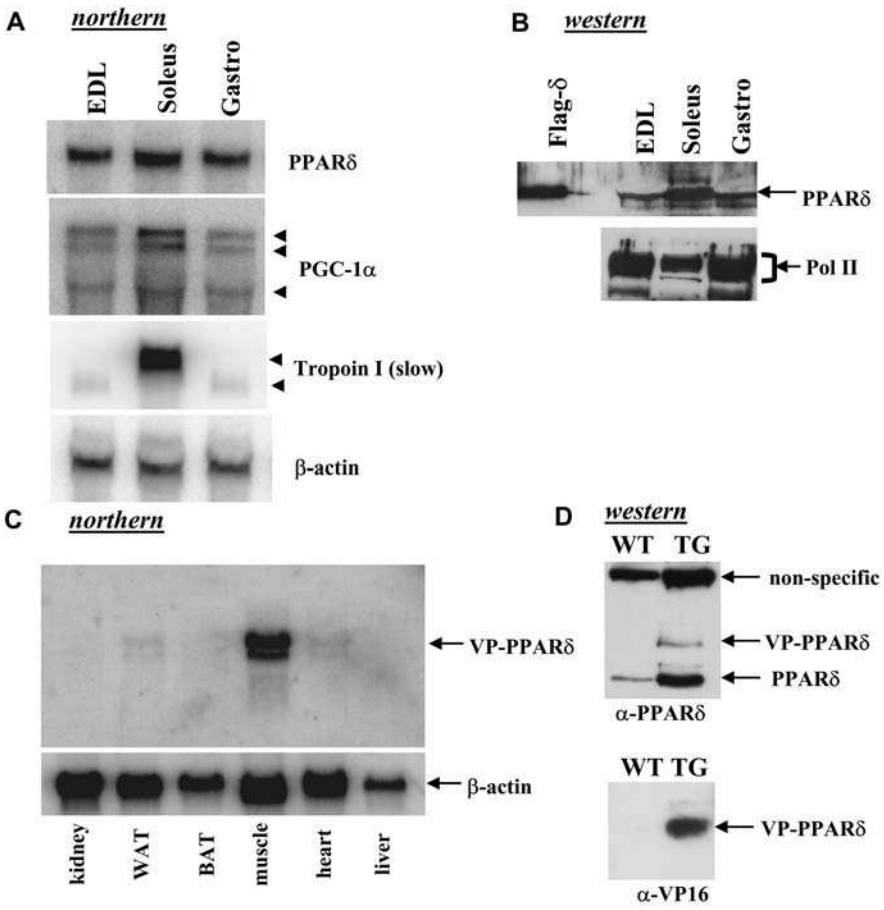

Figure 1. Expression of Endogenous PPARδ and VP16-PPARδ Transgene in Muscle

(A) Pooled RNA isolated from various muscles of five wild-type male C57B6 mice was hybridized with indicated probes. EDL, extensor digitorum longus; Gastro, gastrocnemius.

(B) Pooled nuclear proteins (15 μg/lane) isolated from muscles of five wild-type male C57B6 were probed with anti-PPARδ antibody. RNA polymerase II (Pol II) is shown as a loading control.

(C) Expression of the VP16-PPARδ transgene in various tissues. 10 μg of total RNA from each tissue was hybridized with a VP16 cDNA probe. Gastrocnemius muscle was used here.

(D) Nuclear proteins (15 μg/lane) isolated from gastrocnemius muscle of the transgenic mice (TG) and the wild-type littermates (WT) were probed with indicated antibodies. The upper, nonspecific band that cross-reacted with the anti-PPARδ antibody serves a loading control.

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expression pattern closely resembles that of PGC-1α (Lin et al. 2002). A similar pattern but with more pronounced differences was found at the protein level (Figure 1B).

To directly assess the role of activation of PPARδ in control of muscle fiber plasticity and mitochondrial biogenesis, we generated mice expressing a transgene in which the 78-amino-acid VP16 activation domain was fused to the N-terminus of full-length PPARδ, under control of the 2.2-kb human α-skeletal actin promoter. In agreement with the previous characterization of this promoter (Brennan and Hardeman 1993; Clapham et al. 2000), the VP16-PPARδ transgene was selectively expressed in skeletal muscle, with 10-fold less in the heart (Figure 1C). Among different types of muscle fibers, the levels of VP16-PPARδ expression appeared to be similar (unpublished data). As shown in Figure 1D for gastrocnemius muscle, VP16-PPARδ fusion protein was produced at a level similar to that of endogenous PPARδ in wild-type littermates. Interestingly, the level of endogenous muscle PPARδ protein in the transgenic mice was much higher than in the control littermates. The substantial increase of endogenous PPARδ may have been caused by a switch to type I fiber (see below), which intrinsically expresses higher levels of PPARδ (Figure 1A and 1B).

Type I muscle can be readily distinguished from type II or mixed muscle by its red color, because of its high concentration of myoglobin, a protein typically expressed in oxidative muscle fibers. We found that muscles in the transgenic mice appeared redder (Figure 2A), which is particularly evident in the mixed type I/II fibers of the hindlimb (Figure 2B). Indeed, metachromatic staining revealed a substantial muscle fiber transformation (Figure 2C). In gastrocnemius muscle, we estimated that there was a 2-fold increase of type I fibers. A diagnostic component of oxidative fibers is their high myoglobin and mitochondrial content, which is supported by the mRNA analysis shown in Figure 3A. In addition to myoglobin, mitochondrial components for electron transfer (cytochrome c and cytochrome c oxidase [COX] II and IV) and fatty-acid β-oxidation enzymes were elevated (Figure 3A; unpublished data). These effects appear to be direct consequences of PPARδ activation, as levels of PGC-1α, a coactivator involved in muscle fiber switch and mitochondrial biogenesis (Wu et al. 1999; Lehman et al. 2000; Lin et al. 2002), remained unchanged. Southern blot analysis detected a substantially higher copy number of the mitochondrial genome–encoded COXII DNA in the transgenic mice (Figure 3B). Mitochondrial DNA was increased 2.3-fold in gastrocnemius muscle of the transgenic mice (Figure 3C). These results reveal a marked stimulation of mitochondrial biogenesis and further support the idea that there is a muscle fiber switch. This conclusion was also confirmed by Western blot analysis. As shown in Figure 3D, the characteristic type I fiber proteins, such as myoglobin and cytochrome c and b, were significantly increased. More importantly, the specialized contraction protein troponin I (slow) of type I fiber was robustly induced; this was accompanied by a marked reduction of the specialized contraction protein troponin I