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ARTICLE

The coactivator PGC-1 α regulates skeletal muscle oxidative metabolism independently of the nuclear receptor PPAR β/δ in sedentary mice fed a regular chow diet

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Abstract

Aims/hypothesis Physical activity improves oxidative capacity and exerts therapeutic beneficial effects, particularly in the context of metabolic diseases. The peroxisome proliferator-activated receptor (PPAR) γ coactivator-1 α (PGC-1 α) and the nuclear receptor PPAR β/δ have both been independently discovered to play a pivotal role in the regulation of oxidative metabolism in skeletal muscle, though their interdependence remains unclear. Hence, our aim was to determine the functional interaction between these two factors in mouse skeletal muscle *in vivo*.

Methods Adult male control mice, PGC-1 α muscle-specific transgenic (mTg) mice, PPAR β/δ muscle-specific knockout (mKO) mice and the combination PPAR β/δ mKO + PGC-1 α mTg mice were studied under basal conditions and following PPAR β/δ agonist administration and acute exercise. Whole-

body metabolism was assessed by indirect calorimetry and blood analysis, while magnetic resonance was used to measure body composition. Quantitative PCR and western blot were used to determine gene expression and intracellular signalling. The proportion of oxidative muscle fibre was determined by NADH staining.

Results Agonist-induced PPAR β/δ activation was only disrupted by PPAR β/δ knockout. We also found that the disruption of the PGC-1 α –PPAR β/δ axis did not affect whole-body metabolism under basal conditions. As expected, PGC-1 α mTg mice exhibited higher exercise performance, peak oxygen consumption and lower blood lactate levels following exercise, though PPAR β/δ mKO + PGC-1 α mTg mice showed a similar phenotype. Similarly, we found that PPAR β/δ was dispensable for PGC-1 α -mediated enhancement of an oxidative phenotype in skeletal muscle.

Conclusions/interpretation Collectively, these results indicate that PPAR β/δ is not an essential partner of PGC-1 α in the control of skeletal muscle energy metabolism.

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Keywords Coregulators · Exercise · Nuclear receptors ·
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Abbreviations

AMPK	AMP-activated protein kinase
CON	Control mice
GTT	Glucose tolerance test
IMTG	Intramyocellular triacylglycerol
ITT	Insulin tolerance test
mKO	Muscle-specific knockout
mTg	Muscle-specific transgenic
PGC	PPAR γ coactivator
PPAR	Peroxisome proliferator-activated receptor
qPCR	Quantitative PCR
RER	Respiratory exchange ratio

TBP	TATA binding protein
UCP3	Uncoupling protein 3
$\dot{V}O_{2\text{peak}}$	Peak oxygen consumption

Introduction

The regulation of energy metabolism in skeletal muscle is highly controlled by the peroxisome proliferator-activated receptor (PPAR) γ coactivator-1 α (PGC-1 α) [1]. PGC-1 α drives the expression of genes involved in catabolic processes leading to aerobic ATP synthesis [1] while concomitantly promoting anabolic processes, including de novo lipogenesis [2]. Once activated, PGC-1 α boosts the activity of different transcription factors to control gene programmes resembling an endurance-trained phenotype in skeletal muscle [1, 3]. These adaptations are associated with an enhanced oxidative capacity, which contributes to an increased skeletal muscle fatigue resistance *ex vivo* and exercise performance *in vivo* [4–6]. Importantly, exercise is in fact one of the most efficient stimuli to induce PGC-1 α in skeletal muscle [3].

Among the transcription factors regulated by PGC-1 α , the nuclear receptor PPAR β/δ has been proposed to be a key partner of PGC-1 α in the regulation of skeletal muscle metabolism and function, though mainly based on cell culture and pharmacological studies [7]. PGC-1 α acts as a coactivator of PPAR β/δ [8–10], while PPAR β/δ can directly regulate PGC-1 α expression [11, 12], indicating that this nuclear receptor acts both upstream and downstream of PGC-1 α . Furthermore, transgenic mouse models for PPAR β/δ exhibit a similar phenotype to their counterparts for PGC-1 α [4, 5, 13, 14]. Nevertheless, although the PGC-1 α –PPAR β/δ axis appears to play a key role in the regulation of energy metabolism, the epistatic interaction between these proteins is currently unclear. We therefore aimed at directly assessing the functional interplay between PGC-1 α and PPAR β/δ in the regulation of skeletal muscle oxidative metabolism *in vivo*.

Methods

Animals Mice were housed in a conventional facility with a 12 h night/day cycle and had free access to food/water. Experiments were performed on adult male mice with approval of the Swiss authorities. PGC-1 α muscle-specific transgenic (mTg) mice have been described previously [5]. PPAR β/δ muscle-specific knockout (mKO) mice were generated by crossing PPAR $\beta/\delta^{\text{loxP/loxP}}$ mice with HSA-Cre transgenic mice [11]. Finally, PGC-1 α mTg and HSA-Cre positive PPAR $\beta/\delta^{\text{loxP/loxP}}$ mKO mice were crossed to generate PPAR β/δ mKO + PGC-1 α mTg mice. PPAR $\beta/\delta^{\text{loxP/loxP}}$ mice without *Cre* and *Pgc-1 α* (also known as *Ppargc1a*) transgene expression were

used as control (CON) mice. All mice had mixed sv129 and C57BL/6 background. Genotypes were confirmed through PCR procedures (data not shown) and quantitative PCR analysis in kidney and skeletal muscle (Fig. 1a, b).

PPAR β/δ agonist administration CON mice were subjected to an intraperitoneal injection of either 0.9% NaCl (control) or 1 mg/kg of body weight of the PPAR β/δ agonist GW0742 (Tocris No. 2229; Tocris, Bristol, UK), as previously described [15]. Muscles were collected 8 h following drug administration.

Body composition analysis Lean and fat mass were measured via magnetic resonance imaging (EchoMRI, Houston, TX, USA).

Blood and plasma analysis Blood samples were collected under basal conditions or immediately after maximal exercise from fed and/or overnight-fasted mice, as previously described [9].

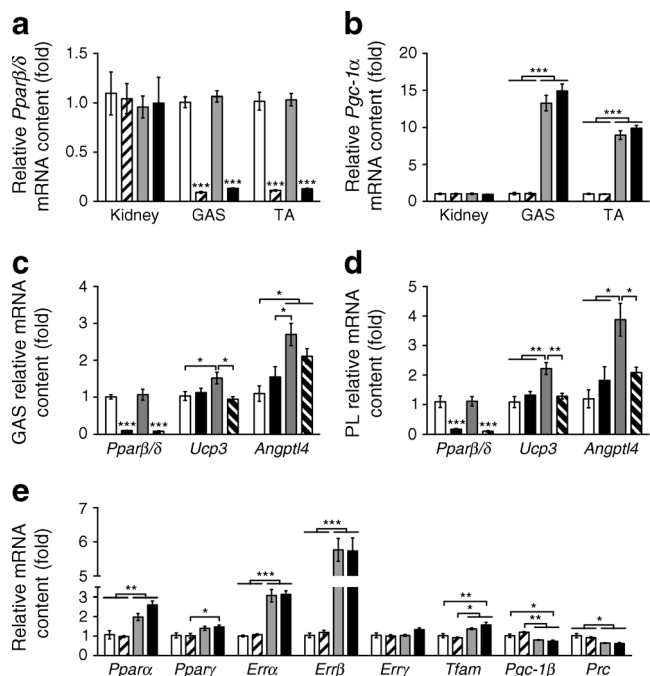


Fig. 1 PGC-1 α and PPAR β/δ mouse models. (a, b) PPAR β/δ and Pgc-1 α mRNA levels in kidney, gastrocnemius (GAS) and tibialis anterior (TA) ($n=6$ per group). (c, d) PPAR β/δ , Ucp3 and Angptl4 mRNA levels in GAS and plantaris (PL) 8 h after the injection of 0.9% NaCl (as control) or 1 mg/kg of body weight of the PPAR β/δ agonist GW0742 ($n=6$ per group). (e) mRNA level of different transcriptional regulators in GAS ($n=6$ per group). In (a), (b) and (e): white bars, CON; hatched bars, PPAR β/δ mKO; grey bars, PGC-1 α mTg; black bars, PPAR β/δ mKO + PGC-1 α mTg. In (c) and (d): white bars, CON + NaCl; black bars, PPAR β/δ mKO + NaCl; grey bars, CON + GW0742; hatched bars, PPAR β/δ mKO + GW0742. Values are mean \pm SEM. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ for the indicated comparisons. In (a), (c) and (d) *** $p<0.001$ vs CON and/or PGC-1 α mTg for same tissue/treatment. When shown, fold changes are reported vs CON group

Glucose and insulin tolerance test Intraperitoneal glucose tolerance tests (GTTs) were carried out by injecting 2 g/kg of body weight of glucose after mice had been fasted for 16 h. Insulin tolerance tests (ITTs) were performed by injecting 0.8 U/kg of body weight of insulin (Novo Nordisk, Bagsvaerd, Denmark) after mice had been fasted for 6 h.

Indirect calorimetry Mice were individually housed in a Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH, USA) for an acclimatisation period of 48 h during which they were allowed free access to food and water. Subsequently, indirect calorimetry was performed for 48 h and data was analysed with the Oxymax software (Columbus Instruments).

Maximal exercise test Exercise tests were performed as previously described [9]. Briefly, 2 days after acclimatisation, mice performed a maximal exercise test in a closed treadmill (Columbus Instruments), allowing the measurement of peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) and respiratory exchange ratio (RER; CO_2 produced divided by consumed O_2 [$\dot{V}\text{CO}_2 / \dot{V}\text{O}_2$]).

Histology NADH staining was performed on 10 μm cross sections from tibialis anterior by exposing the sections to 1 mg/ml NADH (Sigma, St Louis, MO, USA) in the presence of 1 mg/ml nitro blue tetrazolium (Sigma).

NEFA measurement Plasma NEFA were measured using a commercial kit (HR Series NEFA-HR(2); Wako Diagnostics, Richmond, VA, USA), according to the manufacturer's instructions. Blood samples were collected under basal conditions and following 1 h of treadmill running at 13 m/min with 5° slope.

Intramyocellular triacylglycerol extraction Quadriceps intramyocellular triacylglycerols (IMTGs) were extracted by standard procedures using a solid-phase extraction column (UPTI-CLEAN NH2-S 100 mg/1 mL SPE Columns; Interchim, Montluçon, France) and quantified with a commercial kit (Triglyceride enzymatique PAP 150; Biomérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions.

RNA isolation and quantitative PCR Total RNA isolation from fed (ad libitum) mice and quantitative PCR (qPCR) analysis was performed by standard procedures [9]. Sequences of qPCR primers are depicted in electronic supplementary material (ESM) Table 1. Analysis was performed by the $\Delta\Delta C_t$ method using TATA binding protein (TBP) as endogenous control. TBP transcript levels were not different between genotypes or between experimental conditions.

Protein isolation and western blot Protein isolation and western blot was conducted as previously described [9]. Proteins were detected with primary antibodies to Akt (Cell Signaling

No.9272; Cell Signaling, Danvers, MA, USA), p-Akt^{T308} (Cell Signaling No. 4056), AMP-activated protein kinase (AMPK) α (Cell Signaling No. 2603), p-AMPK α ^{T172} (Cell Signaling No. 2535), total OXPHOS (ab110413; Abcam, USA) and eEF2 (Cell Signaling No. 2332).

Statistical analysis Values are expressed as mean \pm SEM. Statistical significance was determined with unpaired two-tailed *t* tests or one-way ANOVA with Tukey's post hoc test. Significance was considered with a $p < 0.05$.

Results

PGC-1 α overexpression and PPAR β/δ deletion in mouse skeletal muscle To elucidate the functional requirement for PPAR β/δ in the metabolic adaptations induced by PGC-1 α , we crossed PPAR β/δ mKO mice with PGC-1 α mTg mice, referred to as PPAR β/δ mKO + PGC-1 α mTg mice. As expected, both PPAR β/δ mKO and PPAR β/δ mKO + PGC-1 α mTg mice showed a reduction in *Ppar β/δ* (*Ppard*) mRNA specifically in skeletal muscle, while *Pgc-1 α* mRNA was upregulated by ~12-fold in skeletal muscle of PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice compared with control (CON) mice (Fig. 1a, b). To validate the functional consequence of *Ppar β/δ* deletion in skeletal muscle, we assessed the effects of the PPAR β/δ agonist GW0742 on the expression levels of PPAR β/δ target genes [7, 16]. Acute treatment with GW0742 did not affect *Ppar β/δ* mRNA in gastrocnemius and plantaris muscles whereas *Ucp3* mRNA levels were induced in CON, but not in PPAR β/δ mKO mice (Fig. 1c, d). Moreover, as previously reported [16], *Angptl4* was upregulated by GW0742 in a way that was partially dependent on PPAR β/δ (Fig. 1c, d). Importantly, *Ppar β/δ* deletion did not affect the transcript levels of *Ppar α* (*Ppara*) and *Ppar γ* (*Pparg*) (Fig. 1e). We subsequently measured the expression levels of other transcription factors and coactivators regulating metabolism, including the oestrogen-related receptors, mitochondrial transcription factor A, Pgc-1 β and PGC-1-related coactivator. The expression levels of genes encoding these factors/coactivators (*Err α* [*Esrra*], *Err β* [*Esrrb*], *Tfam*, *Pgc-1 β* [*Ppargc1b*], *Pre* [*Pprc1*]) were altered in skeletal muscle of PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice, thus independent of PPAR β/δ ablation (Fig. 1e).

Effects of skeletal muscle disruption of the PGC-1 α –PPAR β/δ axis on whole-body metabolism Body composition assessment revealed equal body weight, fat mass and lean mass in PPAR β/δ mKO, PGC-1 α mTg, PPAR β/δ mKO + PGC-1 α mTg and CON mice (Fig. 2a). Analysis of plasma triacylglycerol, cholesterol, LDL-cholesterol and HDL-cholesterol

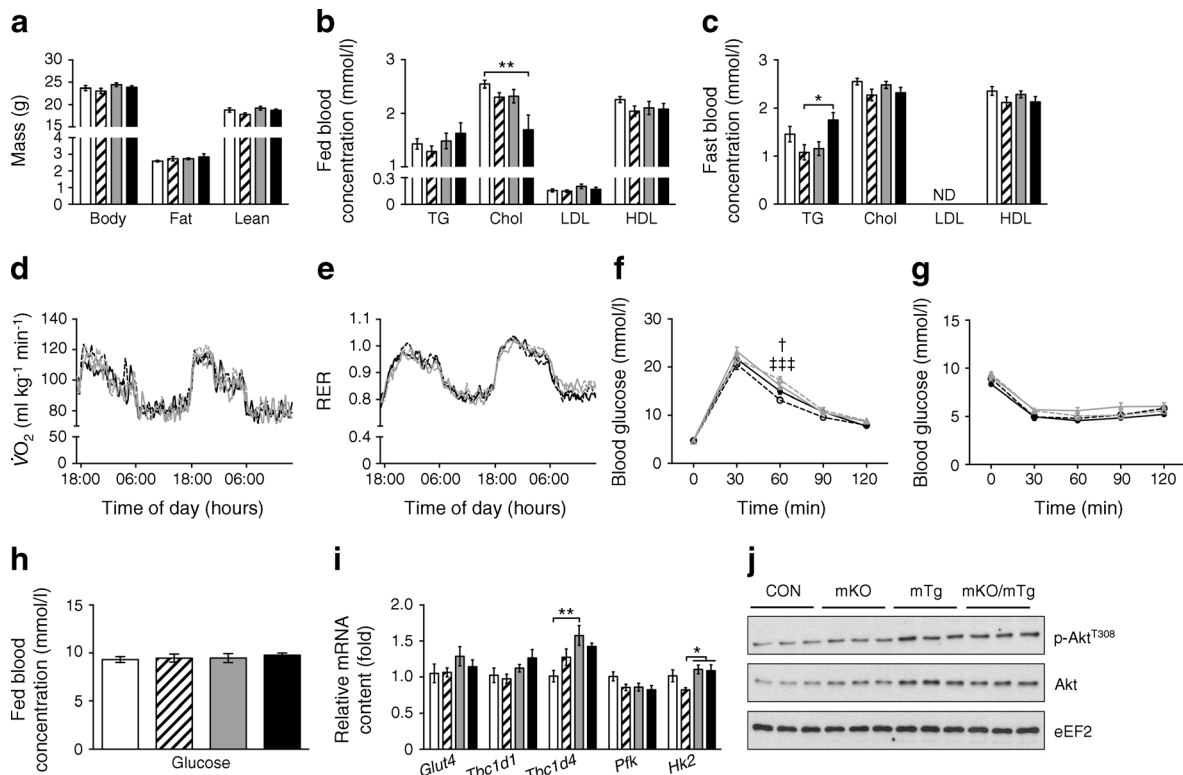


Fig. 2 Body composition, systemic variables and glucose handling. **(a)** Assessment of body composition ($n=10$ – 12 mice per group). **(b, c)** Plasma concentration of TG, cholesterol (Chol), LDL-cholesterol and HDL-cholesterol under fed and fasted conditions ($n=10$ – 12 mice per group). **(d)** $\dot{V}O_2$ **(e)** RER over a period of 48 h ($n=10$ – 14 mice per group). **(f, g)** Blood glucose levels during GTTs **(f)** and ITTs **(g)**. **(h)** Blood glucose levels in fed mice ($n=10$ – 12 per group). **(i)** Gastrocnemius mRNA levels of genes involved in glucose metabolism ($n=6$ mice per group). **(j)** Western blot assessment of Akt

phosphorylation status in gastrocnemius muscle ($n=6$ mice per group). In **(a–c)**, **(h)** and **(i)**: white bars, CON; hatched bars, PPAR β/δ mKO; grey bars, PGC-1 α mTg; black bars, PPAR β/δ mKO + PGC-1 α mTg. * $p<0.05$ and ** $p<0.01$ for the indicated comparisons. In **(d–g)**: solid black line, CON; dashed black line, PPAR β/δ mKO; solid grey line, PGC-1 α mTg; dashed grey line, PPAR β/δ mKO + PGC-1 α mTg. † $p<0.05$, PPAR β/δ mKO vs PGC-1 α mTg; ††† $p<0.001$ PPAR β/δ mKO vs PPAR β/δ mKO + PGC-1 α mTg. In graphs, values are mean \pm SEM. When shown, fold changes are reported vs CON group

during the fed and fasted state exhibited no differences except for a significant decrease in fed cholesterol in the PPAR β/δ mKO + PGC-1 α mTg mice (Fig. 2b, c). Moreover, indirect calorimetry during 48 h revealed no differences in $\dot{V}O_2$ or RER between any of the genotypes (Fig. 2d, e and ESM Fig. 1a, b).

Pharmacological activation of PPAR β/δ attenuates the detrimental effects of obesity and type 2 diabetes on systemic glucose homeostasis [13, 17, 18]. Compared with CON mice, neither GTTs nor ITTs were affected by PGC-1 α overexpression and/or Ppar β/δ deletion in skeletal muscle in mice fed a regular chow diet (Fig. 2f, g and ESM Fig. 1c, d). Moreover, we did not find any differences in blood glucose levels in fed mice between the four different genotypes (Fig. 2h). These findings were corroborated by unchanged expression of genes involved in glucose transport and catabolism, such as *Glut4* (*Slc2a4*), *Tbc1d1*, *Pfk* and *Hk2* (encoding glucose transporter 4, TBC domain family member 1, phosphofructokinase and hexokinase 2, respectively), in skeletal muscle of PPAR β/δ mKO, PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice (Fig. 2i). In contrast, *Tbc1d4* (*As160*), and which encodes

Akt substrate of 160 kDa) was significantly upregulated in PGC-1 α mTg mice (Fig. 2i). Finally, we observed an increase in total Akt protein levels following PGC-1 α overexpression, with no substantial effect of Ppar β/δ deletion (Fig. 2j and ESM Fig. 1e). Consistently, PGC-1 α overexpression slightly decreased relative Akt^{T308} phosphorylation levels, although this effect was not statistically significant (ESM Fig. 1f). These data hence suggest that the PGC-1 α –PPAR β/δ axis is not essential for the modulation of whole-body metabolism and glucose homeostasis under basal conditions in chow-fed mice.

Modulation of skeletal muscle metabolism by the PGC-1 α –PPAR β/δ axis Skeletal muscle PGC-1 α and PPAR β/δ have been proposed to be key regulators of exercise performance and lactate metabolism [19, 20]. Consequently, we next assessed exercise performance in treadmill-based tests, which revealed a higher exercise performance in PGC-1 α mTg mice as expected (Fig. 3a–c). Interestingly, Ppar β/δ muscle knockout did not reduce this difference when PPAR β/δ mKO + PGC-1 α mTg mice were compared with CON mice (Fig. 3a–c). Moreover, $\dot{V}O_2$ was significantly enhanced in PGC-1 α mTg

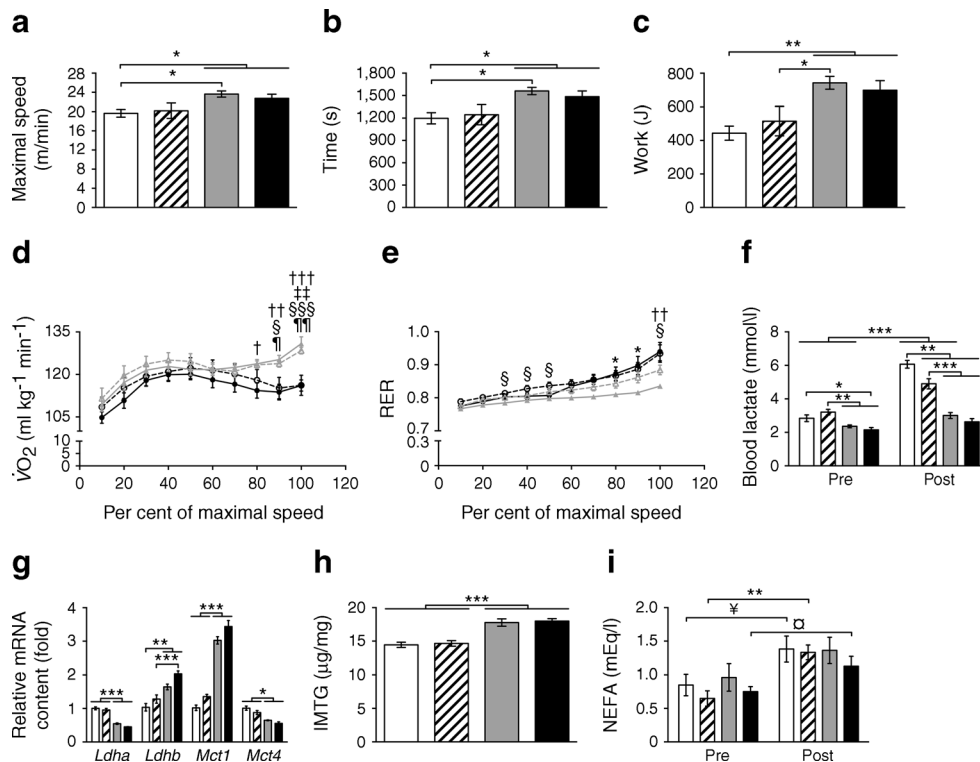


Fig. 3 Skeletal muscle PGC-1 α modulates whole-body metabolism in mice during maximal exercise. (**a–c**) Maximal speed, time and work achieved during exercise tests to exhaustion ($n=10$ – 12 mice per group). (**d, e**) Measurement of $\dot{V}O_2$ and RER during the maximal exercise test ($n=10$ – 12 mice per group). (**f**) Blood lactate levels before (Pre) and after (Post) maximal exercise ($n=10$ – 12 mice per group). (**g**) mRNA levels of key genes of lactate metabolism in gastrocnemius ($n=6$ per group). (**h**) Quadriceps IMTG content ($n=5$ per group). (**i**) Plasma levels of NEFA before (Pre) and after (Post) exercise ($n=4$ – 6 mice per group). Values are mean \pm SEM. In (**a–c, f–i**): white bars, CON; hatched bars, PPAR β/δ

mKO; grey bars, PGC-1 α mTg; black bars, PPAR β/δ mKO + PGC-1 α mTg. In (**d, e**): black continuous line, CON; black discontinuous line, PPAR β/δ mKO; grey continuous line, PGC-1 α mTg; grey discontinuous line, PPAR β/δ mKO + PGC-1 α mTg. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ for the indicated comparisons. In (**d, e**): † $p<0.05$, †† $p<0.01$ and ††† $p<0.001$, CON vs PGC-1 α mTg; ‡ $p<0.01$, PPAR β/δ mKO vs PPAR β/δ mKO + PGC-1 α mTg; § $p<0.05$ and §§§ $p<0.001$, PPAR β/δ mKO vs PGC-1 α mTg; ¶ $p<0.05$ and ¶¶ $p<0.01$, CON vs PPAR β/δ mKO + PGC-1 α mTg. In (**i**) ¶ $p=0.067$; ¶¶ $p=0.065$. When shown, fold changes are reported vs CON group

and PPAR β/δ mKO + PGC-1 α mTg mice during maximal exercise (Fig. 3d), thus altered by PGC-1 α independent of PPAR β/δ . In contrast, the decrease in the RER in PGC-1 α mTg mice was attenuated by concomitant *Ppar β/δ* deletion (Fig. 3e). Blood lactate concentration increased following maximal exercise in CON mice (Fig. 3f). This effect was attenuated in PPAR β/δ mKO mice and virtually abolished in both PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice (Fig. 3f). Similarly, pre-exercise blood lactate levels were reduced only in the mouse models with elevated skeletal muscle PGC-1 α (Fig. 3f). Consistently, mRNA levels of genes encoding lactate dehydrogenase A (*Ldha*) and monocarboxylic acid transporter 4 (*Mct4* [*Slc16a3*]) were reduced only by PGC-1 α overexpression in skeletal muscle, while in the same mice, *Ldhb* and *Mct1* (*Slc16a1*) genes were upregulated (Fig. 3g), reflecting an attenuated lactate production as well as higher catabolism. To assess substrate availability, we measured IMTG content and, consistent with the function of PGC-1 α in de novo lipogenesis [2], both PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice showed elevated IMTG levels, though *Ppar β/δ* knockout had no effect (Fig. 3h).

Finally, we measured plasma levels of NEFA before and after exercise. Exercise significantly increased plasma NEFA levels in PPAR β/δ mKO mice, while CON and PPAR β/δ mKO + PGC-1 α mTg mice showed a trend toward an increase (Fig. 3i). These data show that, in response to maximal exercise, skeletal muscle PGC-1 α is a pivotal regulator of whole-body metabolism, mainly in a PPAR β/δ -independent manner.

Next, we investigated the relevance of PGC-1 α and PPAR β/δ interaction in the regulation of skeletal muscle metabolism. We therefore determined the mRNA levels of genes regulating skeletal muscle oxidative metabolism, several of which have been suggested to be both PGC-1 α and PPAR β/δ targets. Interestingly, we observed that *Ppar β/δ* deletion in skeletal muscle did not change the transcript abundance of genes involved in the tricarboxylic acid cycle, β -oxidation and electron transport chain (Fig. 4a, b). In contrast, most of these genes were strongly upregulated by PGC-1 α overexpression in a PPAR β/δ -independent manner (Fig. 4a, b). Assessment of the protein content of different components of mitochondrial complexes supported the mRNA data, although the overall effects were milder

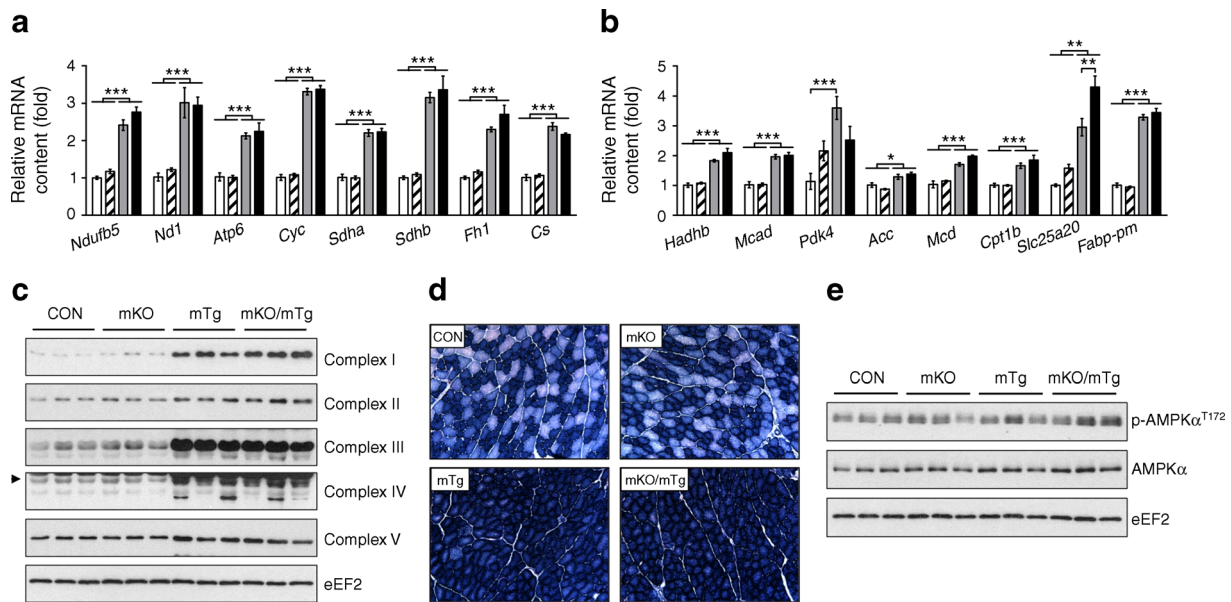


Fig. 4 Oxidative metabolism of gastrocnemius is enhanced by PGC-1 α even in the absence of PPAR β/δ . **(a, b)** mRNA levels of genes regulating oxidative and fatty acid metabolism ($n=6$ per group). **(c)** Western blot analysis of key proteins regulating the electron transport chain ($n=6$ per group). **(d)** Assessment of oxidative muscle fibres (dark blue) via NADH staining ($n=3$ per group). **(e)** Western blot analysis of AMPK

phosphorylation status ($n=6$ per group). In **(a, b)**: white bars, CON; hatched bars, PPAR β/δ mKO; grey bars, PGC-1 α mTg; black bars, PPAR β/δ mKO + PGC-1 α mTg. Values are mean \pm SEM. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ for the indicated comparisons. When shown, fold changes are reported vs CON group

(Fig. 4c and ESM Fig. 2a). We then assessed the metabolic muscle phenotype by determining the proportion of oxidative fibre using NADH staining. This revealed a higher oxidative activity and proportion of oxidative fibres in PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice independent of a functional *Ppar β/δ* gene (Fig. 4d). The total protein content and phosphorylation levels of the key metabolic regulator AMPK did not differ between PPAR β/δ mKO, PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice, suggesting that there was no alteration in energy status in any of these models (Fig. 4e and ESM Fig. 2b, c). We finally explored the relevance of the PGC-1 α –PPAR β/δ axis in the context of the PPAR β/δ agonist GW0742-induced gene expression. As shown in ESM Fig. 2d, GW0742 enhanced the expression of the PPAR β/δ target genes *Angptl4* and *Ucp3*, whereas it did not affect the mRNA levels of the key regulators of oxidative metabolism. Moreover, the effects of PGC-1 α overexpression on gene expression were not affected by GW0742 (ESM Fig. 2d). Finally, as expected, *Ppar β/δ* gene ablation likewise abrogated any effect of the synthetic ligand (Fig. 1c, d and ESM Fig. 2d).

Discussion

The oxidative phenotype of skeletal muscle is strongly linked to physical activity levels and it has been associated with

beneficial health effects in metabolic diseases and other pathologies. Even though the molecular mechanisms controlling exercise-induced adaptation in skeletal muscle have not been fully elucidated, PGC-1 α is thought to promote mitochondrial function, myofibrillar gene expression, vascularisation and other gene programmes that are characteristic of oxidative muscle fibres [1]. Interestingly, PPAR β/δ is able to recapitulate several of these effects [7], although the functional interaction between PGC-1 α and PPAR β/δ has not been elucidated in this tissue so far. We now provide strong evidence indicating the almost complete PPAR β/δ independence of PGC-1 α overexpression in its effects on the metabolic phenotype of skeletal muscle.

Importantly, supporting our hypothesis, contrary to the effects observed in PGC-1 α muscle-specific transgenic mice, the enhancement of skeletal muscle oxidative metabolism is weaker in a bona fide muscle-specific PPAR β/δ gain-of-function mouse model [14]. Moreover, ligand-based activation of PPAR β/δ only increases exercise performance in trained mice and not in sedentary animals [10]. Interestingly, oxidative metabolism and exercise performance can be boosted by fusing the PPAR β/δ protein to the heterologous VP16 activation domain, which strongly increases its transcriptional activity in the absence of ligand or coactivator recruitment [13]. These data demonstrate that the reported functions of PPAR β/δ upstream and downstream of PGC-1 α thereby are dispensable for PGC-1 α function in an overexpression context. These observations are consistent

with cell culture-based experiments showing that PGC-1 α strongly increases oxidative metabolism in the absence of PPAR β/δ in skeletal muscle cells [21]. It thus appears that PGC-1 α regulates skeletal muscle oxidative metabolism by increasing the transcriptional activity of alternative transcription factors, some of which might even compensate for the loss of PPAR β/δ . In fact, *Ppar α* , *Err α* and *Err γ* were significantly upregulated in the skeletal muscle of both PGC-1 α mTg and PPAR β/δ mKO + PGC-1 α mTg mice, suggesting that these transcription factors might have a more relevant function in this context. Importantly, our results indicate that *Ppar β/δ* deletion by itself does not result in a compensatory activation of such related transcription factors. In fact, PPAR β/δ mKO mice do not exhibit an upregulation of PPARs, oestrogen-related receptors or mitochondrial transcription factor A in skeletal muscle. In addition, several target genes of these transcription factors were unaltered in PPAR β/δ mKO mice.

The contribution of PPAR β/δ to the regulation of skeletal muscle metabolism seems to be more relevant in the context of ligand-induced activation. Accordingly, PPAR β/δ activation with synthetic ligands is an efficient treatment for metabolic disorders [13, 17, 18, 22], though it remains unclear whether this effect is mediated by skeletal muscle PPAR β/δ . Conversely, overexpression of PGC-1 α in skeletal muscle is insufficient to evoke similar therapeutic benefits in young mice and even accelerates the development of insulin resistance when such mice are fed a high-fat diet [23], unless the mice are concomitantly exercised [24]. In elderly animals, however, overexpression of PGC-1 α in muscle prevents age-induced insulin resistance [25]. These findings indicate that in some pathological settings, PPAR β/δ activation might be more relevant than PGC-1 α , particularly in the absence of physical activity.

Surprisingly, in our study, PPAR β/δ mKO mice had a similar phenotype to CON mice, with minimal or no changes in body composition, blood variables and gene expression. In contrast, Schuler et al have reported higher body weight and fat, in addition to increased serum levels of glucose, insulin and TG, in the same mouse model [11]. Intriguingly, similar discrepancies have been reported in global PPAR β/δ KO mouse models in regard to whole-body metabolism assessed under basal conditions [18, 26–29]. These differences in the phenotype of PPAR β/δ KO mouse models in the chow-fed sedentary condition might stem from different environmental factors (e.g. diet and temperature), which could lead to a partial PPAR β/δ activation in CON mice and thus lead to more pronounced phenotypic differences in metabolic variables when compared with knockout mice. Importantly, most of the effects of skeletal muscle *Ppar β/δ* deletion reported by Schuler et al on energy metabolism are observed following high-fat diet feeding and/or in elderly mice [11]. Moreover, in the same study, the phenotype of adult PPAR β/δ mKO mice fed chow diet is rather mild and not substantially different from our results, reflected by the magnitude and variability of the data [11].

During exercise, skeletal muscle exerts a greater impact on whole-body metabolism. Accordingly, PGC-1 α mTg mice exhibit a higher $\dot{V}O_2$ and lower RER during treadmill running, reflecting an enhanced oxidative capacity and increased fatty acid oxidation [4]. Interestingly, while the PGC-1 α -mediated improvement in $\dot{V}O_2$ during exercise was maintained in the absence of a functional PPAR β/δ gene, knockout of *Ppar β/δ* attenuated the decrease in the RER in PPAR β/δ mKO + PGC-1 α mTg mice. In line with our observations, it has been shown that PPAR β/δ overexpression in skeletal muscle does not affect $\dot{V}O_2$ and RER during treadmill running [20]. Moreover, PPAR β/δ has been proposed to specifically regulate fatty acid metabolism and, only to a smaller extent, other oxidative metabolic genes in cultured muscle cells [21]. Surprisingly, the effect of PPAR β/δ knockout on RER during maximal exercise appears to be unrelated to mRNA level of genes controlling fatty acid transport and oxidation. Interestingly, *Ppar β/δ* deletion attenuated the upregulation of *Pdk4* induced by PGC-1 α overexpression. Importantly, skeletal muscle pyruvate dehydrogenase kinase 4 has been extensively shown to be a key regulator of fatty acid oxidation during exercise [30], suggesting a possible mechanism by which PPAR β/δ modulates RER and thus energy substrate use during maximal exercise. It should be noted that *Ppar β/δ* knockout induced the upregulation of *Pdk4*, an effect that supports the idea that this nuclear receptor can actively repress target genes in the absence of ligand [16, 31]. Together, these data suggest that the effects of skeletal muscle PGC-1 α on $\dot{V}O_2$ are not dependent upon PPAR β/δ , even though this nuclear receptor appears to be partially involved in the PGC-1 α -mediated increase in β -oxidation during exercise. In addition, our findings support previous data suggesting that PGC-1 α -controlled lactate metabolism is predominantly regulated by oestrogen-related receptor α and not by PPAR β/δ [19].

In summary, our results reveal important insights into the regulatory networks that control skeletal muscle plasticity. Here, we show that in normal/physiological conditions, PPAR β/δ is dispensable for the effect of PGC-1 α on skeletal muscle remodelling. Importantly, the different therapeutic effects of PPAR β/δ and PGC-1 α in the context of metabolic diseases during sedentary vs exercise/ageing state, strongly suggest that the relative importance of these molecules in controlling the metabolic phenotype of skeletal muscle varies significantly depending on the physiological and pathological context. Therefore, we hope that these findings will allow a more targeted dissection and modulation of skeletal muscle plasticity in health and disease in the future.

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