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The coactivator PGC-1 α regulates mouse 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 γ (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 remain 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 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 signaling. Proportion of oxidative muscle fiber 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 does 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

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Contribution statement JPS, WW and CH contributed to the study conception and design, being responsible for the integrity of the work as a whole. All the authors contributed to acquisition of data or analysis and interpretation of data, in addition to drafting the article and the approval of its final version.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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.

Keywords

skeletal muscle metabolism; nuclear receptors; coregulators; exercise

Introduction

The regulation of energy metabolism in skeletal muscle is highly controlled by the peroxisome proliferator-activated receptor γ (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 programs 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 as well as downstream of PGC-1 α . Furthermore, transgenic mouse models for PPAR β/δ exhibit a similar phenotype compared 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, with 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 $\beta/\delta^{loxP/loxP}$ mice with HSA-Cre transgenic mice [11]. Finally, PGC-1 α mTg and HSA-Cre positive PPAR $\beta/\delta^{loxP/loxP}$ mKO mice were crossed to generate PPAR β/δ mKO+PGC-1 α mTg mice. PPAR $\beta/\delta^{loxP/loxP}$ animals without *Cre* and PGC-1 α 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 qPCR analysis in kidney and skeletal muscle (Fig. 1a, b).

PPAR β/δ agonist

CON mice were subjected to an intra-peritoneal injection of either 0.9% NaCl (control) or 1 mg/kg of body weight of the PPAR β/δ agonist GW0742 (Tocris #2229, 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 (EchoMRITM, 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].

Glucose and insulin tolerance test

Intra-peritoneal glucose tolerance tests (GTT) were carried out by injecting 2 g/kg of body weight of glucose after 16 h of fasting. Insulin tolerance tests (ITT) were performed by injecting 0.8 U/kg of body weight of insulin (Novo Nordisk, Bagsvaerd, Denmark) after 6 h of fasting.

Indirect calorimetry

Animals were individually housed in a Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH, USA) during an acclimatization period of 48 h with free access to food and water. Subsequently, indirect calorimetry was performed during 48 h and data analyzed with the Oxymax software (Columbus Instruments).

Maximal exercise test

Exercise tests were performed as previously described [9]. Briefly, two days after acclimatization animals performed a maximal exercise test in a closed treadmill (Columbus Instruments), allowing the measurement of peak oxygen consumption (VO_{2peak}) and respiratory exchange ratio (RER).

Histology

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

Non-esterified fatty acids (NEFA) measurement

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

Intramyocellular triacylglycerol (IMTG) extraction

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

RNA isolation and quantitative PCR (qPCR)

Total RNA isolation from fed (*ad libitum*) animals and qPCR analysis was performed by standard procedures [9]. Sequences of qPCR primers are depicted in Table S1. Analysis was performed by the C_T method using TATA binding protein (TBP) as endogenous control. TBP transcript levels were not different between genotypes or 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, Danvers, MA, USA; #9272), p-Akt^{T308} (Cell Signaling #4056), AMPK α (Cell Signaling #2603), p-AMPK α ^{T172} (Cell Signaling #2535, USA), total OXPHOS (Abcam #ab110413, USA) and eEF2 (Cell Signaling: #2332).

Statistical analysis

Values are expressed as mean \pm standard error of the mean (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 muscle-specific knockout (mKO) mice with PGC-1 α muscle-specific transgenic (mTg) mice, referred as PPAR β/δ mKO+PGC-1 α mTg animals. As expected, both PPAR β/δ mKO and PPAR β/δ mKO+PGC-1 α mTg mice showed a reduction of *Ppar* β/δ mRNA specifically in skeletal muscle, while *Pgc-1 α* mRNA was up-regulated by ~12 fold in skeletal muscle of PGC-1 α mTg and PPAR β/δ mKO+PGC-1 α mTg mice compared to control (CON) animals (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 uncoupling protein 3 (*Ucp3*) mRNA levels were induced in CON, but not in PPAR β/δ mKO mice (Fig. 1c, d). Moreover, as previously reported [16], angiopoietin-like 4 (*Angptl4*) was up-regulated 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 *Ppara* and *Ppar* γ (Fig. 1e). We subsequently measured the expression levels of other transcription factors and coactivators regulating metabolism, including the estrogen related receptors (*Err*), mitochondrial transcription factor A (*Tfam*), *Pgc-1 β* and PGC-1-related coactivator

(*Prc*). The expression of these genes was 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 (TG), cholesterol (CHOL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) during fed and fasted state exhibited no differences except for a significant decrease in fed CHOL in the PPAR β/δ mKO+PGC-1 α mTg mice (Fig. 2b, c). Moreover, indirect calorimetry during 48 h revealed no differences in VO₂ or RER between any of the genotypes (Fig. 2d, e, electronic supplementary material (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 to CON mice, neither glucose nor insulin tolerance tests 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, ESM Fig. 1c, d). Moreover, we did not find any differences in blood glucose levels in fed mice of the four different genotypes (Fig. 2h). These findings were corroborated by unchanged expression of genes involved in glucose transport and catabolism such as the glucose transporter 4 (*Glut4*), TBC1 domain family member 1 (*Tbc1d1*), phosphofruktokinase (*Pfk*) and hexokinase 2 (*Hk2*) 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* (also known as Akt substrate of 160 kDa, *As160*) was significantly up-regulated in PGC-1 α mTg animals (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, ESM Fig. 1e). Consistently, PGC-1 α overexpression slightly decreased relative Akt^{T308} phosphorylation levels, though 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 animals.

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, b, c). Interestingly, PPAR β/δ muscle knockout did not reduce this difference when PPAR β/δ mKO+PGC-1 α mTg mice were compared to CON animals (Fig. 3a, b, c). Moreover, VO₂ was significantly enhanced in PGC-1 α mTg 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 animals was attenuated by concomitant PPAR β/δ deletion (Fig. 3e). Blood lactate concentration increased following maximal exercise in CON animals (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, lactate dehydrogenase A (*Ldha*) and monocarboxylic acid transporters 4 (*Mct4*) mRNA levels were reduced only by PGC-1 α overexpression in skeletal muscle, while in the same mice, *Ldhb* and *Mct1* were up-regulated (Fig. 3g) reflecting an attenuated lactate production as well as higher catabolism. In order 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 pre- and post-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 determined 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 TCA cycle, β -oxidation and electron transport chain (ETC) (Fig. 4a, b). In contrast, most of these genes were strongly up-regulated by PGC-1 α overexpression in a PPAR β/δ independent manner (Fig. 4a, b). The assessment of protein content of different components of mitochondrial complexes supports mRNA data, though the overall effects were milder (Fig. 4c, ESM Fig. 2a). We then assessed the metabolic muscle phenotype by determining the proportion of oxidative fiber using NADH staining, which revealed a higher oxidative activity and proportion of oxidative fibers 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). Total protein content and phosphorylation levels of the key metabolic regulator AMP-activated protein kinase (AMPK) were not different in PPAR β/δ mKO, PGC-1 α mTg or PPAR β/δ mKO+PGC-1 α mTg mice, suggesting no altered energy status in any of these models (Fig. 4e, 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 the ESM Fig. 2d, GW0742 enhanced the expression of the PPAR β/δ targets *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 (ESM Fig. 2d, Fig. 1c and 1d).

Discussion

The oxidative phenotype of skeletal muscle is strongly linked to physical activity levels and it has been associated with health beneficial 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, vascularization and other gene programs that are characteristic of oxidative muscle fibers [1]. Interestingly, PPAR β/δ is

able to recapitulate several of these effects [7], though 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 almost complete PPAR β/δ independence of PGC-1 α overexpression 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, but 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 that 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 increased oxidative metabolism in the absence of PPAR β/δ in skeletal muscle cells [21]. It appears hence 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, *Ppara*, *Erra* and *Erry* were significantly up-regulated in both PGC-1 α mTg and PPAR β/δ mKO+ PGC-1 α mTg skeletal muscle, suggesting that these transcription factors might play 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 animals do not exhibit an up-regulation of PPARs, ERRs and TFAM 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 animals are fed a high-fat diet [23], unless the mice are concomitantly exercised [24]. In old 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 , in particular in the absence of physical activity.

Surprisingly, in our study, PPAR β/δ mKO animals had a similar phenotype compared to CON mice, with minimal or no changes in body composition, blood parameters and gene expression. In contrast, Shuler 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 chow fed sedentary condition

might stem from different environmental factors (e.g. diet and temperature) that could lead to a partial PPAR β/δ activation in CON animals and thus lead to more pronounced phenotypic differences in metabolic parameters when compared to KO 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 old 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 to our results, which is reflected by the magnitude and variability of the data [11].

During exercise, skeletal muscle exerts a bigger impact on whole body metabolism. Accordingly, PGC-1 α mTg mice exhibit a higher VO₂ 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 VO₂ during exercise is 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 VO₂ 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 up-regulation of *Pdk4* induced by PGC-1 α overexpression. Importantly, skeletal muscle PDK4 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 utilization during maximal exercise. It should be noted that PPAR β/δ knockout induced the up-regulation 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 VO₂ are not dependent of 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 ERR α and not 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 remodeling. Importantly, the different therapeutic effects of PPAR β/δ and PGC-1 α in the context of metabolic diseases during sedentary vs. exercise/aging state, strongly suggest that the relative importance of these molecules in controlling the metabolic phenotype of skeletal muscle varies significantly depending of 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

| | |
|--------------------------------------|---|
| AMPK | AMP-activated protein kinase |
| ANGPTL4 | angiopoietin-like 4 |
| CHOL | Cholesterol |
| CON | control mice |
| ERR | estrogen related receptors |
| ETC | electron transport chain |
| GLUT4 | glucose transporter 4 |
| GTT | glucose tolerance tests |
| HK2 | hexokinase 2 |
| ITT | Insulin tolerance tests |
| LDHA | lactate dehydrogenase A |
| LDHB | lactate dehydrogenase B |
| MCT1 | monocarboxylic acid transporters 1 |
| MCT4 | monocarboxylic acid transporters 4 |
| mKO | PPAR β/δ muscle-specific knockout |
| mTg | PGC-1 α muscle-specific transgenic |
| PFK | phosphofructokinase |
| PGC-1α | PPAR γ coactivator-1 α |
| PGC-1β | PPAR γ coactivator-1 β |
| PPARα | peroxisome proliferator-activated receptor α |
| PPARβ/δ | peroxisome proliferator-activated receptor β/δ |
| PPARγ | peroxisome proliferator-activated receptor γ |
| PRC | PGC-1-related coactivator |
| RER | respiratory exchange ratio |
| TBC1D1 | TBC1 domain family member 1 |
| TBC1D4 | TBC1 domain family member 4 |

| | |
|---------------------------|--------------------------------------|
| TBP | TATA binding protein |
| TFAM | mitochondrial transcription factor A |
| TG | triglycerides |
| UCP3 | uncoupling protein 3 |
| VO_{2peak} | peak oxygen consumption |

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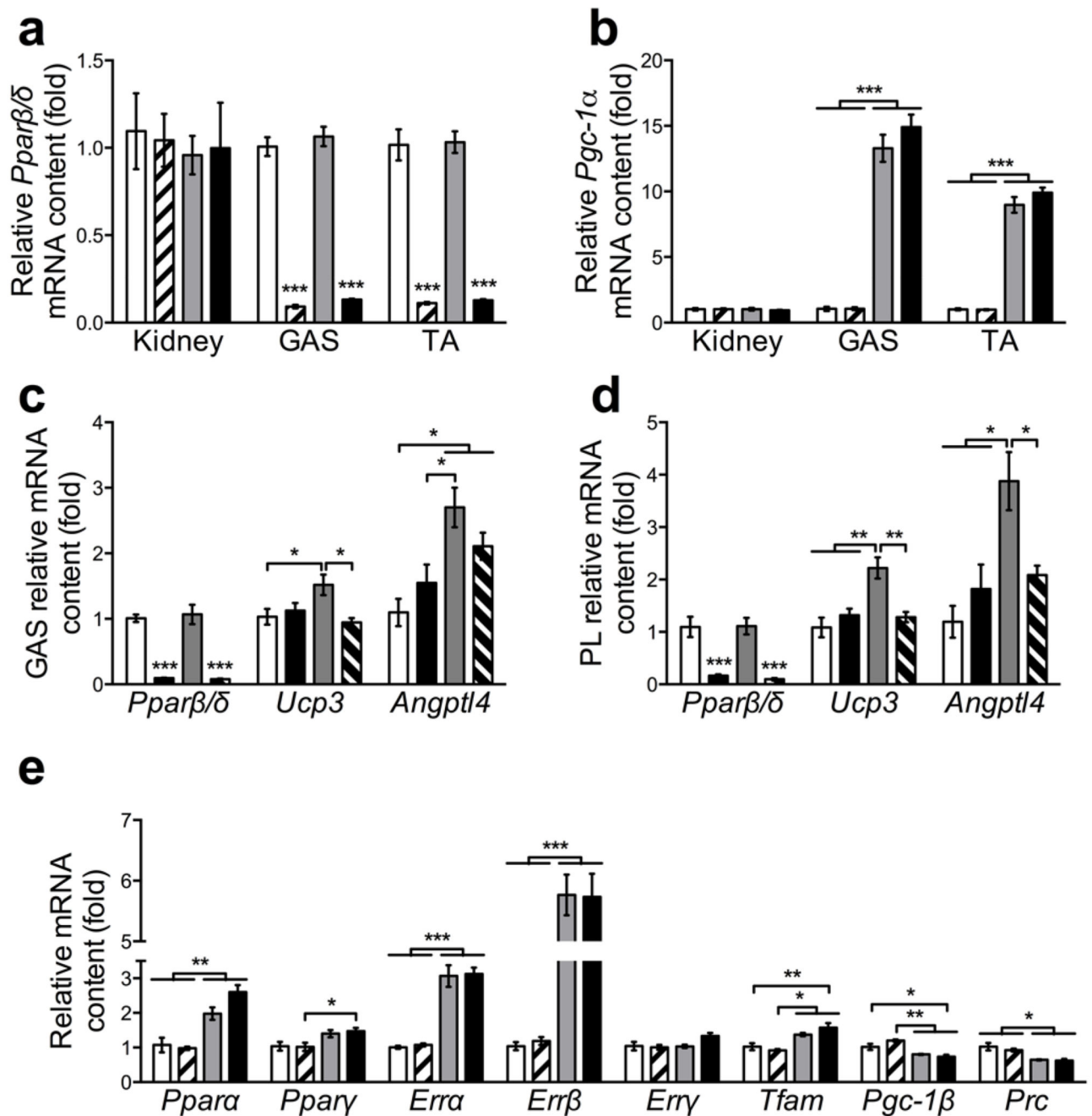


Fig. 1. PGC-1 α and PPAR β/δ mouse models. (a and b) *Pgc-1 α* and *Ppar β/δ* mRNA levels in kidney, gastrocnemius (GAS) and tibialis anterior (TA) (n = 6 per group). (c and d) *Ppar β/δ* , *Ucp3* and *Angptl4* mRNA levels in GAS and plantaris (PL) 8 h after the injection of 0.9% NaCl (as control) or 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 CON: white, PPAR β/δ mKO: white with stripes, PGC-1 α mTg: grey, PPAR β/δ mKO+ PGC-1 α mTg: black. In c and d CON+NaCl: white, PPAR β/δ mKO+NaCl: black,

CON+ GW0742: grey, PPAR β/δ mKO+ GW0742: black with white stripes. Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. In **a**, **c** and **d** *** $p < 0.001$ vs. CON and/or PGC-1 α mTg for same tissue/treatment.

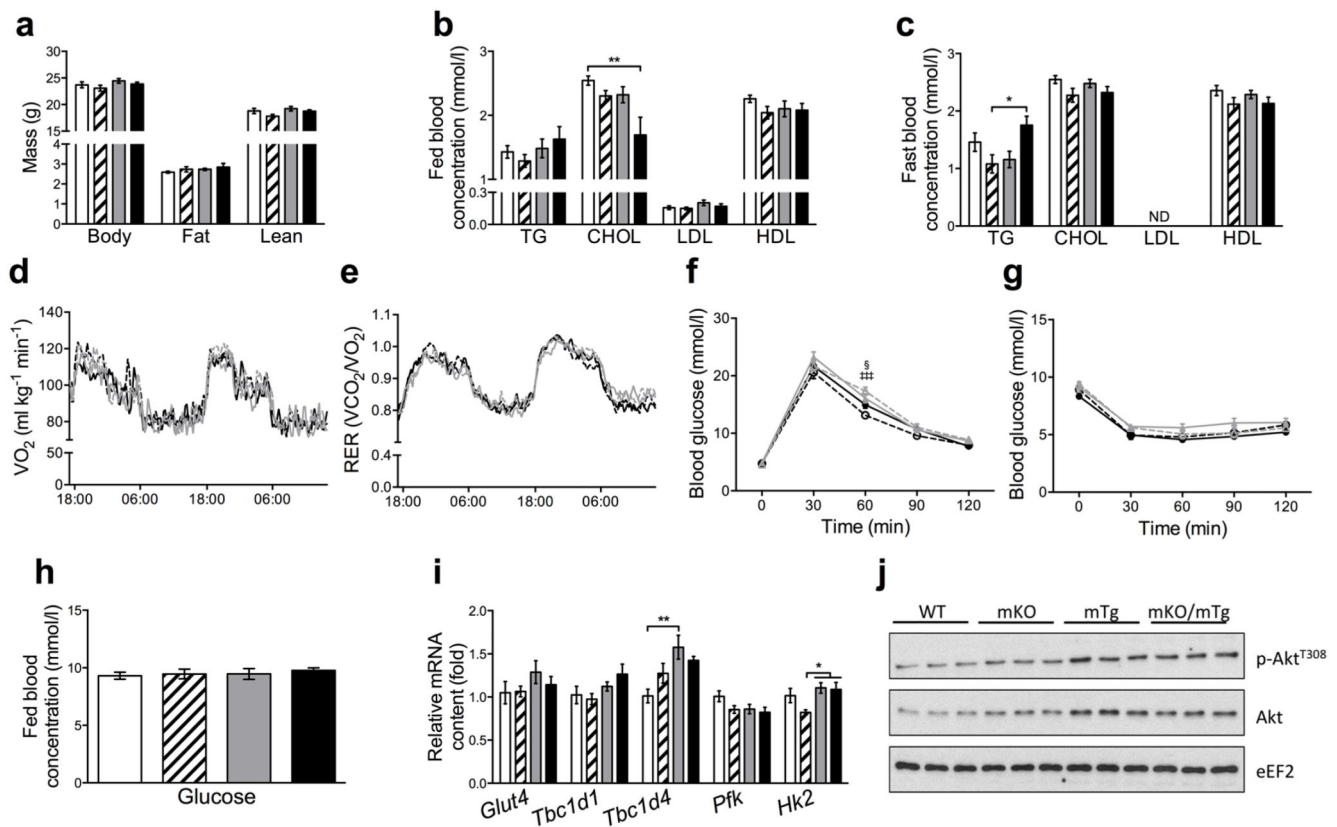
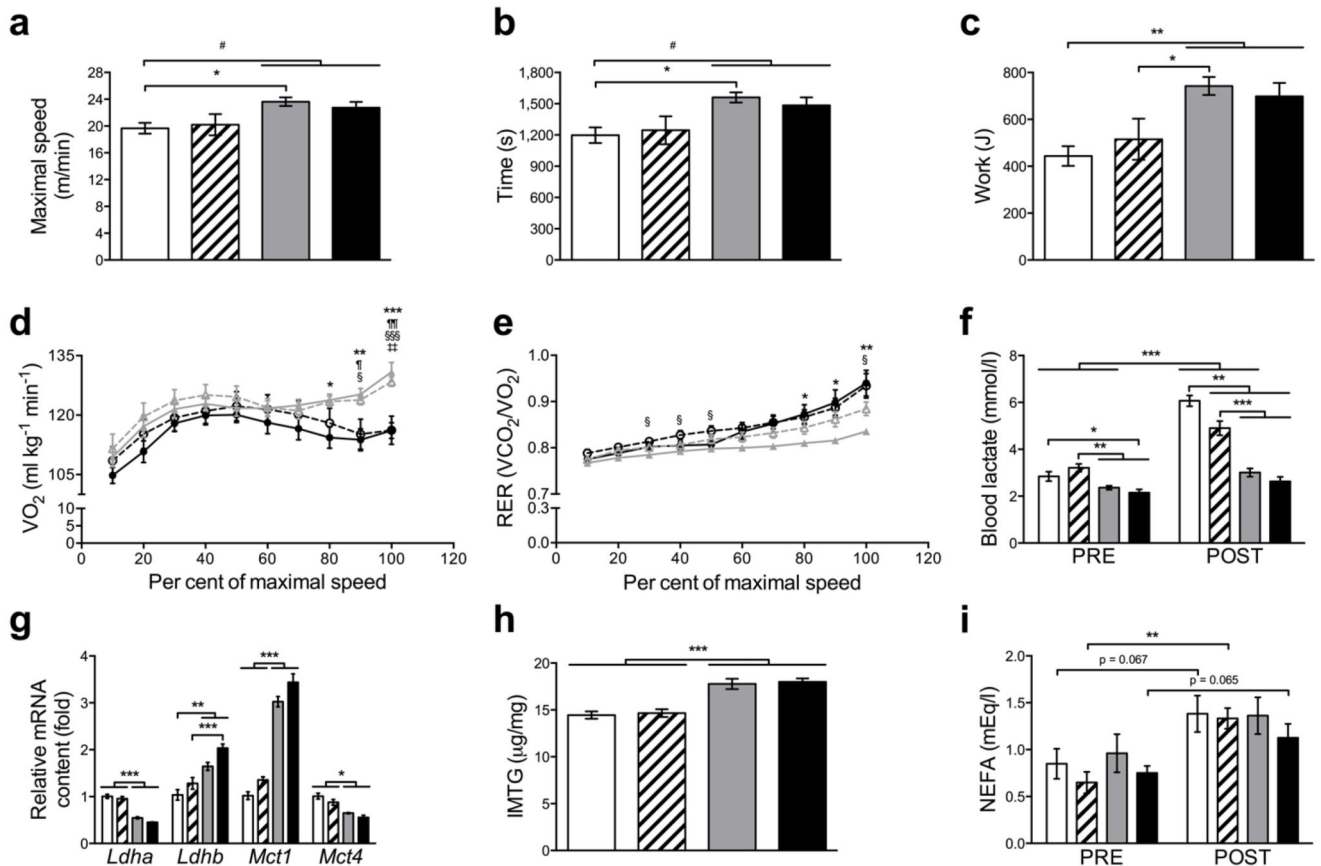


Fig. 2. Body composition, systemic parameters and glucose handling. **(a)** Assessment of body composition ($n = 10-12$ per group). **(b and c)** Plasma concentration of TAG, CHOL, LDL and HDL under fed and fasted conditions ($n = 10-12$ per group). **(d and e)** Measurement of VO_2 **(d)** and RER **(e)** over a period of 48 h ($n = 10-14$ per group). **(f-h)** Blood glucose levels during glucose **(f)** and insulin tolerance tests **(g)**. **(h)** Blood glucose levels in fed animals ($n = 10-12$ per group). **(i)** Gastrocnemius mRNA levels of genes involved in glucose metabolism ($n = 6$ per group). **(j)** Western blot assessment of Akt phosphorylation status in gastrocnemius ($n = 6$ per group). In **a, b, c, h** and **i** CON: white, PPAR β/δ mKO: white with stripes, PGC-1 α mTg: grey, PPAR β/δ mKO + PGC-1 α mTg: black. In **d - g** CON: black continuous line, PPAR β/δ mKO: black discontinuous line, PGC-1 α mTg: grey continuous line, PPAR β/δ mKO + PGC-1 α mTg: grey discontinuous line. Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$. In **f** § $p < 0.05$ 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.

**Fig. 3.**

Skeletal muscle PGC-1 α modulates whole body metabolism during maximal exercise. (**a**, **b**, **c**) Maximal speed, time and work achieved during exercise tests to exhaustion (n = 10-12 per group). (**d** and **e**) Measurement of VO_2 and RER during the maximal exercise test (n = 10-12 per group). (**f**) Blood lactate levels before (PRE) and after (POST) maximal exercise (n = 10-12 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 per group). In **a**, **b**, **c**, **f**, **g**, **h** and **i** CON: white, PPAR β/δ mKO: white with stripes, PGC-1 α mTg: grey, PPAR β/δ mKO + PGC-1 α mTg: black. In **d** and **e** CON: black continuous line, PPAR β/δ mKO: black discontinuous line, PGC-1 α mTg: grey continuous line, PPAR β/δ mKO + PGC-1 α mTg: grey discontinuous line. Values are mean \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001. In **d** and **e** *p < 0.05, **p < 0.01, ***p < 0.001 CON vs. PGC-1 α mTg; ¶p < 0.05, ¶¶p < 0.01 CON vs. PPAR β/δ mKO + PGC-1 α mTg; §p < 0.05, §§§p < 0.001 PPAR β/δ mKO vs. PGC-1 α mTg; ‡‡p < 0.01 PPAR β/δ mKO vs. PPAR β/δ mKO + PGC-1 α mTg.

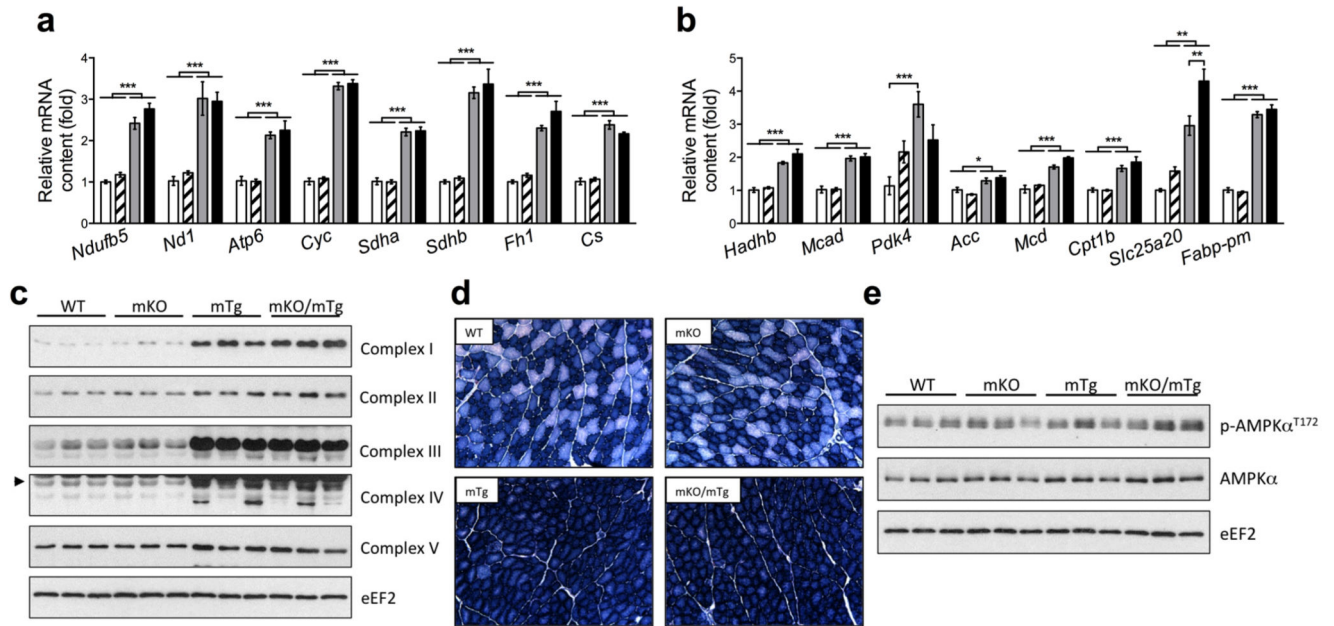


Fig. 4. Oxidative metabolism of gastrocnemius is enhanced by PGC-1 α even in the absence of PPAR β/δ . (**a** and **b**) mRNA levels of genes regulating oxidative and fatty acid metabolism ($n = 6$ per group). (**c**) Western blot analysis of key proteins regulating the ETC ($n = 6$ per group). (**d**) Assessment of oxidative muscle fibers (dark blue) via NADH staining ($n = 3$ per group). (**e**) Western blot analysis of AMPK phosphorylation status ($n = 6$ per group). In **a** and **b** CON: white, PPAR β/δ mKO: white with stripes, PGC-1 α mTg: grey, PPAR β/δ mKO + PGC-1 α mTg: black. Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.