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Skeletal muscle PGC-1α controls whole body lactate homeostasis through ERRα-dependent activation of LDH B and repression of LDH A

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Abstract

The peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) controls metabolic adaptations. We now show that PGC-1α in skeletal muscle drives the expression of lactate dehydrogenase (LDH) B in an estrogen-related receptor α (ERRα)-dependent manner. Concomitantly, PGC-1α reduces the expression of LDH A and one of its regulators, the transcription factor Myc. PGC-1α thereby coordinately alters the composition of the LDH complex and prevents the rise in blood lactate during exercise. Our results show how PGC-1α actively coordinates lactate homeostasis and provide a novel molecular explanation for PGC-1α-mediated muscle adaptations to training that ultimately enhance exercise performance and improve metabolic health.

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Introduction

Skeletal muscle adaptations to endurance exercise are largely mediated by PGC-1α (reviewed in (1, 2)). Elevated expression of PGC-1α in skeletal muscle is consequently sufficient to mimic an endurance-trained phenotype (3), which is partially achieved by a pronounced fiber-type switching from fast, glycolytic towards slow, oxidative fibers, including slow fiber type-specific calcium handling (4, 5). Additionally, PGC-1α improves oxygen supply to muscle by promoting angiogenesis (6) and remodels the neuromuscular junction (7). Most importantly, however, PGC-1α alters skeletal muscle metabolism by inducing mitochondrial biogenesis (5, 8) and promoting lipid oxidation (9). However, PGC-1α also drives anabolic processes like lipid (10) and glucose refueling in skeletal muscle (11). Concomitantly, substrate flux through glycolysis is inhibited by elevated levels of PGC-1α (11) while pentose phosphate pathway activity is increased (10).

Exercise performance and lactate synthesis are strongly linked (12). For example, strenuous exercise leads to the production of lactate in working skeletal muscles and to a steady increase in blood lactate levels. Inversely, regular training counters the excessive rise in blood lactate levels as indicated by the reduced blood lactate levels in trained athletes during acute exercise bouts (13). Similarly, reduced basal and post-exercise blood lactate levels have previously been reported in mice with elevated levels of PGC-1α in skeletal muscle (11). However, it is unclear whether this observation is caused by diminished lactate generation, enhanced lactate clearance or both. Moreover, the molecular mechanisms that underlie the putative PGC-1α-mediated reduction in blood lactate levels are unknown. We hypothesized that PGC-1α

actively drives a transcriptional program to enhance lactate metabolism and have now

unraveled the direct molecular mechanism by which PGC-1α remodels lactate homeostasis.

Results

PGC-1α in skeletal muscle controls whole body lactate levels

Since exhaustive exercise boosts blood lactate levels, we first investigated the impact of PGC-1α in skeletal muscle on whole body lactate homeostasis. During a maximal endurance performance test, blood lactate levels continuously increased in wild-type mice and returned to basal levels within 40mins following fatigue-induced exercise cessation (**Fig. 1A, B**). In stark contrast, blood lactate levels did not substantially rise in muscle-specific PGC-1α transgenic (MPGC-1α TG) animals during exercise at any point (**Fig. 1A, B**). Since skeletal muscle is the main site for lactate production, we next assessed the mRNA expression of lactate dehydrogenase A (LDH A), which encodes for the LDH muscle subunit (M) that metabolizes pyruvate to lactate (14). LDH A mRNA expression in tibialis anterior was decreased by 53.5% ($p < 0.001$) in MPGC-1α TG animals compared to control littermates (**Fig. 1C**). Consistently, the enzymatic activity of LDH-mediated pyruvate to lactate conversion was diminished by 40.1% ($p < 0.001$) in skeletal muscle of transgenic animals (**Fig. 1D**). These differences in LDH A mRNA expression and activity between MPGC-1α TG and control animals persisted in response to an acute bout of exercise (**Fig. S1A**, **B**).

To test whether lactate removal is also altered by PGC-1α, lactate tolerance tests were performed. The excursion (**Fig. 1E**) and total area under the curves (**Fig. 1F**) clearly show that MPGC-1α TG animals more efficiently cleared lactate from the circulation compared to control animals. To further characterize the enhanced capacity of muscle for lactate clearance, we determined the mRNA expression of LDH B which encodes for the LDH heart subunit (H) that drives the conversion of lactate to pyruvate (14). LDH B mRNA expression in tibialis anterior was significantly elevated in MPGC-1α TG animals by 110.2% ($p < 0.001$) (**Fig. 1G**).

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Moreover, the enzymatic activity of LDH to convert lactate to pyruvate was enhanced by 60.7% (p<0.01) (**Fig. 1H**). In response to exercise, LDH B mRNA levels did not change, but LDH B activity further increased in MPGC-1α TG animals (**Fig. S1C**, **D**).

PGC-1α drives LDH isoenzyme-type switching in skeletal muscle

The tetrameric lactate dehydrogenase complex consists of LDH M and H subunits (14, 15). According to its subunit composition, the complex is referred to as LDH 1 (H_4), LDH 2 (H_3M), LDH 3 (H_2M_2), LDH 4 (HM_3) or LDH 5 (M_4) (14, 16). To gain insights into the individual isoenzyme composition of wild-type and MPGC-1α TG animals, we performed native gel electrophoresis of muscle homogenates (**Fig. 1I**). MPGC-1α TG animals displayed a pronounced shift towards an isoenzyme composition enriched in LDH H subunits, namely LDH 1, 2 and 3 (**Fig. 1I, J**). In contrast, protein levels of LDH 5, which exclusively contains LDH M subunits, were reduced in MPGC-1α TG animals (**Fig. 1I, J**).

Elevated capacity for lactate uptake in MPGC-1α TG animals

Given the enhanced potential of skeletal muscle of MPGC-1α TG animals to convert lactate to pyruvate, we also investigated the mRNA expression of key genes implicated in lactate import and export in this organ. Relative mRNA levels of MCT1, which mediates lactate import into muscle (17) and mitochondria (18) was significantly increased in MPGC-1α TG animals (**Fig. 1K**). In contrast, the mRNA levels of MCT4, which exports lactate (17), and CD147, an ancillary molecule of MCT1 and 4 (19), were both unaltered (**Fig. 1K**). The elevated muscle MCT1 content in MPGC-1α TG animals is consistent with the enhanced

whole-body lactate removal during lactate tolerance tests and with the previous demonstration that PGC-1α increases lactate uptake into skeletal muscle (20).

PGC-1α promotes LDH B transcription by co-activating ERRα on the LDH B promoter

Biocomputational predictions were then applied to unravel the potential molecular mechanism by which PGC-1α promotes LDH B and MCT1 transcription. To this end, microarray data from differentiated C2C12 myotubes adenovirally infected with either GFP or bicistronic GFP-PGC-1α (21) were analyzed. We first used Motif Activity Response Analysis (MARA) to identify motifs that are most active following overexpression of PGC-1α (**Fig. 2A**). Then, we screened the lactate dehydrogenase B and the MCT1 promoters for putative binding sites for transcription factors. No transcription factors were found in the MCT1 promoter that showed a high activity in MARA. In contrast, there were two transcription factors that displayed very high activities in MARA and concomitantly were predicted to bind to the LDH B promoter, namely the estrogen-related receptor α (ERRα) and retinoid X receptors (RXRs) (**Fig. 2A, Fig. S2**). Recruitment of ERRα to the LDH B promoter has previously been reported in global ChIP-on-ChIP assays in mouse liver cells; however, the functional consequences of this observation have not been investigated (22).

To experimentally verify our biocomputational predictions, pharmacological inhibitors and siRNA silencing technology for ERRα and RXR were used. In differentiated C2C12 myotubes, PGC-1α led to a very robust induction of LDH B, which was completely prevented by the ERRα inverse agonist XCT-790 (**Fig. 2B**). Inhibition of RXRs by HX-531 likewise resulted in a significant reduction in PGC-1α-mediated induction of LDH B, however to a smaller extent compared to ERRα inhibition (**Fig. 2B**). Similarly, RNA silencing of ERRα in

myoblasts led to a significant reduction in LDH B expression, while silencing of RXRα or β reduced LDH B expression to a smaller extent (**Fig. S3**).

Interestingly, analysis of the LDH B promoter (http://www.swissregulon.unibas.ch) (23) identified overlapping binding sites for ERRα and RXR in the LDH B promoter **(Fig. S4A)**. Using chromatin immunoprecipitated DNA from muscle samples, we confirmed that PGC-1α is recruited to the ERRα/RXR binding site on the proximal LDH B promoter (**Fig. 2C**). Moreover, activation of a 684bp fragment of the LDH B promoter by PGC-1α in reporter gene assays was dependent on functional integrity of the ERRα response element (**Fig. S4B and C**). In contrast, association of PGC-1α with the recently identified distal MEF2 binding site in the LDH promoter region (24) was not elevated in MPGC-1α TG mice compared to wild-type controls (**Fig. 2C**). In line with this finding, pharmacological inhibition of the MEF2 upstream activator PPARβ/δ did not prevent PGC-1α-mediated LDH B transcription (**Fig. S5**).

Decreased levels of LDH A and Myc in MPGC-1α TG animals

LDH A transcription is regulated by HIF-1α (hypoxia-inducible factor 1 α) and Myc (myelocytomatosis oncogene) (25). While HIF-1α mRNA levels were similar in wild-type and transgenic animals, Myc transcript levels were reduced in skeletal muscle of MPGC-1α TG mice compared to controls (**Fig. 2D**). Consistently, protein levels of Myc were also significantly decreased in MPGC-1α TG mice compared to their control littermates (**Fig. 2E, F**). Interestingly, the administration of AM6-36, a drug that binds to RXR responsive elements and mimics the effects of RXRs, has recently been reported to abrogate Myc expression (26, 27). Indeed, also in our experimental context, pharmacological inhibition of

RXRs by HX-531 was sufficient to restore Myc (**Fig. 2G**) and consequently LDH A expression (**Fig. 2H**).

Increased levels of MCT1 and decreased levels of LDH A are not caused by elevated LDH B

To investigate whether the increased MCT1 and the decreased LDH A levels constitute a secondary effect of LDH B induction, we next inhibited LDH B transcription in differentiated C2C12 myotubes. While LDH B transcription was successfully reduced in this experiment (**Fig. S6A**), the effect of PGC-1α on MCT1 and LDH A persisted (**Fig. S6B, C**).

Selective regulation of muscle lactate homeostasis by PGC-1β

Since PGC-1α and β exert similar effects, we also examined the potential impact of PGC-1β on LDH B, LDH A and MCT1 mRNA levels. Overexpression of PGC-1β in differentiated C2C12 myotubes resulted in elevated levels of LDH B (**Fig. S6D**), but the effect was less pronounced than with PGC-1α (**Fig. 2B**). LDH A levels were reduced, but MCT1 levels unaltered following overexpression of PGC-1β (**Fig. 6E** and **F**). Importantly however, PGC-1β is not induced in skeletal muscle by exercise (28) and might thus be less relevant in mediating altered lactate handling in the adaptation to exercise.

PGC-1α is important for the regulation of LDH B transcription and proper lactate handling

To corroborate the importance of PGC-1α in regulating lactate homeostasis, and especially LDH B expression, we then studied mice with a muscle-specific knock-out of PGC-1α. MPGC-1α KO animals fatigued rapidly and accumulated blood lactate to a higher extent than their control littermates in endurance exercise trials (**Fig. 3A, B**). LDH A mRNA showed a non-significant trend towards higher expression in MPGC-1α KO animals (**Fig. 3C**). Myc mRNA did not differ beween MPGC-1α KO and control animals (**Fig. S7**). In terms of lactate tolerance, MPGC-1α KO animals were less efficient in lactate clearance upon injection of lactate (**Fig. 3D, E**). Consistently, MPGC-1α KO animals displayed a significant reduction of LDH B expression (**Fig. 3F**). Moreover, the analysis of LDH isoenzyme composition revealed lower levels of isoenzymes typically enriched in H subunits (**Fig. 3G, H, I**). Together, these data clearly demonstrate that muscle PGC-1α is important for the transcription of LDH B and for maintaining whole body lactate homeostasis.

Acute bouts of exercise had no effect on LDH A mRNA expression, but led to a significant increase in LDH A activity in control animals (**Fig. S8A, B**). In MPGC-1α KO mice- LDH A activity was already elevated at the basal state and was not further inducible by exercise (**Fig. S8B**). LDH B mRNA levels and activity were lower in MPGC-1α KO animals and these differences persisted after exercise (**Fig. S8C, D**).

The low blood lactate levels in MPGC-1α TG are not due to altered metabolism in heart or liver

Besides skeletal muscle, liver and heart play important roles in buffering blood lactate levels (29, 30). In the "Cori Cycle", the liver takes up lactate and converts it into pyruvate, which then serves as a substrate for gluconeogenesis. Glucose can subsequently be transported back

to different organs including skeletal muscle, where it is again metabolized to lactate. In contrast, the heart directly uses lactate as energy source (29). Lactate is completely oxidized to water and carbon dioxide in the heart and is even preferred as energy source compared to glucose (29). We thus analyzed the mRNA levels of genes involved in lactate production, removal and transport in these two tissues. In the heart, no differences in mRNA expression between wild-type and transgenic animals were observed (**Fig. 4A**). In the liver, there was a significant increase in CD147 mRNA levels, while the other genes remained unchanged (**Fig. 4B**). Analogous to our studies in skeletal muscle, we subsequently assessed the enzymatic activities of LDH in heart and liver. The conversion of pyruvate to lactate was significantly reduced in the heart (**Fig. 4C**), while the conversion of lactate to pyruvate was unaltered (**Fig. 4D**). In the liver, no changes in LDH activity were detectable (**Fig. 4E, F**). Iso-enzyme compositions between MPGC-1α TG and wild-type animals were undistinguishable for both the heart (**Fig. 4G, H**) and the liver (**Fig. 4I, J**). An acute bout of exercise affected LDH in both tissues. In the heart, exercise increased LDH A activity in wild-type, but not in MPGC-1α TG mice (**Fig. S9**). Cardiac LDH B mRNA levels and activity were both induced by exercise to a similar extent in both genotypes (**Fig. S9C, D**). The liver was less responsive to exercise and only an elevation in LDH B mRNA levels was detected (**Fig. S10**).

Discussion

Disarranged metabolism in skeletal muscle is a common, early event in the etiology of obesity and type 2 diabetes. One trait of these diseases is a decreased oxidative capacity along with elevated lactate production (31, 32). Remodeling of the muscular metabolic profile might thus constitute a potential approach to treat or prevent such disorders (33). Exercise is one of the most important stimuli for improving the metabolic phenotype of skeletal muscle. Glucose and lipid metabolism are extensively altered in response to chronic exercise, largely mediated by PGC-1α (reviewed in (1, 2)). We have now shown that PGC-1α also is a key regulator of tissue and systemic lactate homeostasis.

Intriguingly, a shift towards a muscle LDH isoenzyme composition enriched in LDH H subunits has been observed in mice and humans in response to chronic electrical stimulation and regular endurance exercise, respectively (34, 35). The molecular mechanism that underlay the remodeling of the LDH complex remained, however, unresolved. Our study now demonstrates that this adaptation to exercise is directly mediated by the interaction of PGC-1α and ERRα and, moreover, that PGC-1α is required for the elevated LDH B transcription and proper lactate homeostasis during exercise. PGC-1α thus remodels LDH isoenzyme composition distinctly and independently of coactivation of PPARβ/δ, which recently has been described to drive LDH B transcription via MEF2 activation in a distal enhancer region of LDH B (24). In addition, PGC-1α reduces LDH A gene expression, which could potentially counteract the enzymatic activity of LDH B of converting lactate into pyruvate. How PGC-1α, a transcriptional co-activator, can exert repressive effects remains elusive. A previous study revealed that stimulation of mitochondrial activity diminishes Myc expression (36). Conceivably, the PGC-1α-mediated boost in mitochondrial activity might repress Myc expression analogously. In addition, our results demonstrating restoration of Myc following

RXR inhibition strongly suggest a role of RXRs in regulating Myc expression. This is further underlined by our previous data showing induction of RXRs gene expression in skeletal muscle by PGC-1α (10). Furthermore, in the present study, we found RXR binding motifs to be predicted to be associated with PGC-1α-dependent gene expression in muscle cells by MARA. However, a detailed analysis of the mechanistic aspects of the repression of LDH A gene transcription by the transcriptional co-activator PGC-1α through Myc is hampered by the very low expression of Myc in skeletal muscle (37). Importantly, our findings that PGC-1α elevates LDH H and diminishes LDH M subunit expression are further corroborated by mouse and human studies on muscle beds with different fiber type compositions. In fact, fast-twitch muscles, which typically express low levels of PGC-1α display high amounts of LDH M subunits, while the LDH complexes in slow-twitch muscles with higher expression of PGC-1α are enriched in LDH H subunits (38, 39).

Lactate production is important in working muscle to maintain glycolytic fluxes for ATP production (15, 40). Presumably, the conversion of pyruvate to lactate rapidly regenerates NAD^+ from NADH. This requirement is mainly due to the limited pool of cytosolic NAD^+ in skeletal muscle (25). The prevention of NAD^+ regeneration by PGC-1α and the lower levels of the transcription factor Myc, a well-known activator of glycolysis (41) now suggest a molecular explanation for the reduced glycolytic rates occurring at high PGC-1α levels in muscle (11). Moreover, the potentially enhanced production of NADH by lactate oxidation triggered by PGC-1α ensures adequate levels of this cofactor for the electron transport chain and thus for ATP generation during muscle contractions. This concept of lactate as energy source during acute exercise is corroborated by previous studies showing that lactate oxidation was higher in exercising compared to resting muscle (42-44).

Importantly, our study also sheds new light onto the role of lactate in skeletal muscle fatigue. For many decades lactate was considered as a side product of contracting muscle and viewed as a metabolite that causes muscle fatigue. MPGC-1α TG animals display reduced lactate levels and are more resistant to fatigue, which would further support the concept of lactate as fatiguing metabolite. Importantly however, MPGC-1α TG animals reach exhaustion at a time point when blood lactate levels are still in a normal range, indicating that factors other than lactate contribute to muscle fatigue.

Recently, the perception of lactate as a harmful metabolite has drastically waned (12, 45). During exercise, lactate accumulation and mild lactic acidosis cause vasodilatation and dissociation of oxygen from hemoglobin and thus oxygen transport to muscle. In this context, the reduction in blood lactate levels by PGC-1α might be viewed as a performance-limiting factor during strenuous exercise. However, PGC-1α likely overcomes this effect by increasing myoglobin expression (4) and enhancing angiogenesis (6).

In conclusion, we have demonstrated that PGC-1α and ERRα orchestrate the transcription of LDH B, that PGC-1α reduces the levels of the oncogenic LDH A activator Myc and that the subsequent shift in LDH composition promotes lactate oxidation in skeletal muscle. Lactate produced during exercise by predominantly glycolytic muscles is thus used as fuel in oxidative muscle fibers that express elevated levels of PGC-1α. Importantly, our results suggest a direct regulatory role for PGC-1α in the metabolic remodeling of lactate metabolism. Thus, by driving lactate uptake and oxidation, PGC-1α promotes alternative metabolic pathways for energy generation during muscle contraction. Finally, ectopic PGC-1α antagonizes the disarranged lactate metabolism observed under pathological conditions such as type 2 diabetes and obesity, in addition to restoring mitochondrial functions. Our results

therefore provide new insights into the molecular mechanisms by which PGC-1α metabolically enhances exercise performance and improves muscle function.

Materials and Methods

Animals

Male muscle-specific PGC-1α transgenic mice (MPGC-1α TG) (4), muscle-specific PGC-1α knock-out mice (MPGC-1α KO) (46) and control littermates were maintained in a conventional facility with a fixed 12-h light/dark cycle on a commercial pellet chow diet and free access to tap water. Studies were performed with 8 weeks old animals according to criteria outlined for the care and use of laboratory animals and with approval of the Swiss authorities.

Treadmill running and lactate tolerance tests

Detailed description in the supplementary material.

RNA extraction and RT-PCR

Frozen tissues were homogenized under liquid nitrogen and total RNA was isolated using Trizol reagent (Invitrogen). RNA concentrations were adjusted and reverse transcription was carried out using random hexamer primers (Promega). Real-time PCR analysis (Power SYBR Green Master Mix, Applied Biosystems) was performed using the StepONE Detector. Relative expression levels for each gene of interest were calculated with the $\Delta\Delta C_t$ method and normalized to the expression of the Tata box binding protein (TBP).

Lactate dehydrogenase activity

Specific lactate dehydrogenase activities (pyruvate to lactate and lactate to pyruvate conversions) were determined according to Howell *et al* (47). The enzymatic reactions were carried out either in the presence of 1mmol sodium pyruvate and 150µmol NADH or of

7mmol lactic acid and 5.5mmol NAD$^+$. Changes in absorbance were assessed by a spectrophotometer at 340nm and 30°C.

Isoenzyme shift

LDH isoenzyme patterns were determined colorimetrically. Protein isolation from tibialis anterior was performed as described previously (48). Twenty, 50 and/or 100 μg of protein were loaded onto a 6% native polyacrylamide gel. Following electrophoresis, the gel was stained in 10 mL of staining solution containing 0.1 M sodium lactate, 1.5 mM NAD$^+$, 0.1 M Tris-HCl (pH 8.6), 10 mM NaCl, 5 mM MgCl2, 0.03 mg/mL phenazinmethosulphate (PMS), and 0.25 mg/mL nitrobluetetrazolium (NBT).

Cell culture experiments

C$_2$C$_{12}$ myoblasts were fused into myotubes and infected with adenovirus expressing GFP or bicistronic PGC-1α-GFP. The transfection efficiency was similar between GFP and PGC-1α-GFP and PGC-1α-GFP efficiently increased PGC-1α protein levels (**Fig. S11**). In addition, cells were treated for 48 h with 0.1% DMSO (as control), 10 μM XCT-790 (Sigma-Aldrich) to inhibit ERRα or 2 μM HX-531 (Tocris Bioscience) to inhibit RXRs, together with the corresponding adenovirus. Silencing of LDH B, ERRα, RXRα and RXRβ was performed using Dharmacon smartpool siRNAs according to the manufacturer's instructions.

ChIP assays

Detailed description in the supplementary material.

LDH B promoter cloning and reporter gene assays

Detailed description in the supplementary material.

Bioinformatics, data analysis and statistics

Detailed description in the supplementary material.

SUPPORTING INFORMATION MATERIALS AND METHODS

Treadmill running

Treadmill running was performed on a motorized treadmill (Columbus Instruments) equipped with an electric shock grid. After acclimatization, the mice were forced to run till exhaustion. The detailed protocol was as follows: 10m/min for 5min and increase by 2m/min every 5 minutes until 26m/min with 5° inclination. The speed of 26m/min was then kept until exhaustion. The experiments were conducted according to established guidelines (49). Tissues were harvested 3 hrs post-exercise.

Lactate tolerance test

Lactate tolerance test was performed following i.p. injection of 2g/kg body weight of lactate. Blood was obtained at intervals of 10 minutes from the tail vein, and lactate levels were determined using a standard lactometer (Nova Biomedical).

ChIP assays

Chromatin immunoprecipitation from muscle tissue was performed as previously described (10). In brief, muscle tissues were fixed in 1.5% formaldehyde at room temperature for 15mins. The reaction was stopped by adding glycine and tissues were homogenized on ice using a Dounce homogenizer (20strokes). Following cell lysis, chromatin was enzymatically sheared and subjected to immunoprecipitation using anti-PGC-1α antibodies (PGC-1 (H-300), Santa Cruz Biotechnology) or unrelated IgG bound to protein G-coupled magnetic beads. Precipitated DNA was recovered and amplified by RT-PCR.

Bioinformatics

Previously published microarray data from differentiated C2C12 myotubes adenovirally infected with GFP or bicistronic PGC-1α-GFP under the cytomegalo virus (CMV) promoter (GEO Data set: GSE4330, ref. (21)) were analyzed using Motif Activity Response Analysis (http://www.mara.unibas.ch/cgi/mara). Prediction of potential transcription factors binding to the LDH B promoter were performed with SwissRegulon (http://www.swissregulon.unibas.ch) (23).

LDH B promoter cloning and reporter gene assays

A 681-bp fragment of the LDH B promoter was amplified using the primers LDHB prom forward (CGACGCGTGG AGGAACTGTG AAAGGCAGAA ATC) and LDHB prom reverse (GAAGATCTGC AAAGTCAGCA GGCTGCTGCT CTGC), which added an MluI and BglII restriction sites to the 5' and 3' ends of the PCR product, respectively. This promoter fragment was subcloned into the pGL3 basic luciferase reporter gene vector (Promega) and subsequently validated by sequencing. Site-directed mutagenesis of the ERRα response element in the LDH B promoter was performed by using the following, overlapping mutagenesis primers: ERRa mut forward CAGCGGAAGG **GGTACC**CTAA CTTTAGAGAG C and ERRa mut reverse CTAAAGTTAG **GGTACC**CCTT CCGCTGAGGC AC. The nucleotides depicted in bold represent the mutagenesis of the core ERRα response element in the LDH B promoter (TGACCT) into a KpnI restriction site. The plasmid with the mutated promoter was verified by sequencing.

Reporter gene assays were performed on 12 well plate using C2C12 myoblasts and COS-7 cells. Cells were transfected using LipofectamineTM 2000 (Invitrogen) with 0.1 μg pRL-SV40 (Promega: E2231) and 0.7 μg pLDHB-luc or pLDHB-mut-luc. Total amount of plasmid DNA was kept constant at 1.6 μg per well by using the control plasmid pShuttle-CMV

(ATCC). Twenty four hours after transfection cells were infected with GFP or PGC-1α adenovirus for another 24 h. Following a total period of 48 h, cells were lysed with 250 μL of 1X Passive Lysis 5X Buffer (Promega) and luciferase activity was measured in 75 μL of lysate in a 96 well plate using the Dual-Glo® Luciferase Assay System (Promega). Renilla (pRL-SV40) luciferase activity was used for normalization. Two independent experiments were performed in triplicate each.

Data analysis and statistics

All data are presented as means ± SE. The data were analyzed by Student's two-tailed unpaired t test or the Mann-Whitney test when the difference between the two S.D. values was significantly different. For the analysis of pharmacological inhibition, multifactorial ANOVA was performed followed by Tukey's *post hoc* test or t-test where indicated.

Acknowledgements

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Figure legends

Figure 1. *Muscle PGC-1α controls blood lactate levels by shifting LDH composition*

(**A, B**) Blood lactate excursion curves of wild-type (black dotted line) and MPGC-1α TG (black continuous line) animals during maximal endurance test (**A**) and corresponding area under the curve (**B**). Arrows indicate the time-point of exhaustion.

(**C, D**) LDH A mRNA levels (**C**) and activity (**D**) in tibialis anterior muscle.

(**E, F**) Lactate tolerance test (LTT) excursion curves of wild-type (dotted line) and MPGC-1α TG (continuous line) animals (**E**) and corresponding area under the curve (**F**)

(**G, H**) LDH B mRNA levels (**G**) and activity (**H**) in tibialis anterior muscle.

(**I**) LDH isoenzyme composition in tibialis anterior of MPGC-1α TG and control littermates.

(**J**) Quantification of the LDH isoenzyme composition.

(**K**) Relative mRNA levels of MCT1, MCT4 and CD147 in MPGC-1α TG and control littermates

All values are expressed as means ±SE (n =8 per group). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Figure 2. *PGC-1α interacts with ERRα on the LDH B promoter*

(**A**) Venn Diagram with red circle denoting the number of transcription factors from MARA of microarray data from C2C12 myotubes following adenoviral overexpression of GFP or bicistronic PGC-1α-GFP. Only transcription factors with Z-scores set above a cut-off of 2 were considered. The 18 transcription factors predicted to bind to LDH B are shown in the blue circle. Two predicted transcription factors were simultaneously found by MARA.

(**B**) Relative mRNA levels of LDH B in C2C12 myotubes following adenoviral overexpression of GFP or bicistronic PGC-1α-GFP and in the absence or presence of XCT-790 or HX-531. All values are expressed as means ±SE (n =6 per group); Effect of PGC-1α (GFP *vs*. PGC-1α-GFP): @; Effect of treatment (DMSO *vs*. XCT-790 or HX-531): #; interaction: x. triple symbols: $p < 0.001$. Symbols at the left refer to the comparison of XCT-790 treated C2C12 myotubes to controls. Symbols at the right refer to the comparison of HX-531 treated C2C12 myotubes to controls,

* denotes results from *post hoc* analysis. ***: $p < 0.001$ compared to GFP-PGC-α untreated.

(**C**) Chromatin Immunoprecipitation assay on mouse skeletal muscle. Recruiting of PGC-lα to the ERRα and RXR or to the MEF2 binding site in the LDH B promoter of MPGC-1α TG mice and control animals.

(**D**) Relative mRNA expression levels of Myc and Hif-1α.

(**E**, **F**) Representative western blot of Myc (**E**) and corresponding quantification (**F**).

(**G, H**) Relative mRNA levels of Myc (**G**) and LDH A (**H**) in muscle cells following adenoviral overexpression of GFP or bicistronic PGC-1α-GFP and in the absence or presence of HX-531. All values are expressed as means ±SE (n =6 per group); Effect of PGC-1α (GFP *vs*. PGC-1α-GFP): @; Effect of treatment (DMSO *vs*. HX-531): #; interaction: x. single symbol: p<0.05; double symbols: p<0.01; triple symbols: p<0.001.

* denotes the result from *post hoc* analysis. **: p<0.01 compared to GFP-PGC-α untreated.

Figure 3. *PGC-1α is important for the regulation of LDH B transcription*

(**A, B**) Blood lactate excursion curves of wild-type (dotted line) and MPGC-1α KO (continuous gray line) animals during maximal endurance test (**A**) and corresponding area under the curve (**B**). Arrows indicate the time-point of exhaustion.

(**C**) LDH A mRNA levels in tibialis anterior muscle of wild-type and knock-out mice.

(**D, E**) Lactate tolerance test (LTT) excursion curves of wild-type (dotted line) and MPGC-1α KO (continuous gray line) animals (**D**) and corresponding area under the curve (**E**)

(**F**) LDH B mRNA levels in tibialis anterior muscle of wild-type and knock-out mice.

(**G**) LDH isoenzyme composition in tibialis anterior from MPGC-1α KO and control littermates with 50 µg (left side) and 100 µg of protein extract.

(**H, I**) Quantification of the LDH isoenzyme composition.

All values are expressed as means ±SE (n =6 per group). *: p<0.05; **: p<0.01; ***: p<0.001.

Figure 4. *Blood lactate levels are not controlled by heart or liver lactate metabolism following elevated PGC-1α expression*

(**A**) Relative mRNA expression of LDH A, LDH B, MCT1, MCT4 and CD147 in the heart of MPGC-1α TG and control littermates.

(**B**) Relative mRNA expression of LDH A, LDH B, MCT1, MCT4 and CD147 in the liver of MPGC-1α TG and control littermates.

(**C, D**) Conversion of pyruvate to lactate (**C**) and reverse reaction (**D**) in the heart of MPGC-1α TG and control littermates.

(**E, F**) Conversion of pyruvate to lactate (**E**) and reverse reaction (**F**) in the liver of MPGC-1α TG and control littermates.

(**G, H**) LDH isoenzyme composition (**G**) and quantification (**H**) in the heart of MPGC-1α TG and control littermates.

(**I, J**) LDH isoenzyme composition of 20 µg (left) and 100 µg of protein extract (**I**) and quantification (**J**) in the liver of MPGC-1α TG and control littermates.

All values are expressed as means ±SE (n =8 per group). *: p<0.05; **: p<0.01; ***: p<0.001.

Figure 5. *PGC-1α promotes rapid energy provision by lactate oxidation*

(**A**) Scheme illustrating the action of PGC-1α on the LDH B promoter. PGC-1α promotes the transcription of ERRα, which then binds to an ERRα-responsive element (ERRE) in the LDH B promoter. The subsequent activation of ERRα is enhanced by PGC-1α.

(**B**) PGC-1α promotes the expression of RXRs, which then by unknown mechanisms diminish Myc and thereby LDH A expression.

(**C**) Scheme integrating the coordinate actions of PGC-1α on genes regulating lactate homeostasis. The enhanced transcription of MCT1 drives lactate import into skeletal muscle. Lactate is then converted to pyruvate through the action of LDH B. This process is further facilitated by the concomitant reduction in LDH A. NADH is then generated and serves as substrate for the electron transport chain. PGC-1α thereby promotes a lactate oxidizing phenotype, which is associated with improved endurance capacity and metabolic health.

Fig. S1. *Muscle LDH mRNA expression and activity in MPGC-1α TG mice after exercise*

(**A, B**) Relative mRNA expression (**A**) and activity (**B**) of LDH A in gastrocnemius muscle of MPGC-1α TG and control littermates in response to exercise.

(**C, D**) Relative mRNA expression (**C**) and activity (**D**) of LDH B in gastrocnemius muscle of MPGC-1α TG and control littermates in response to exercise.

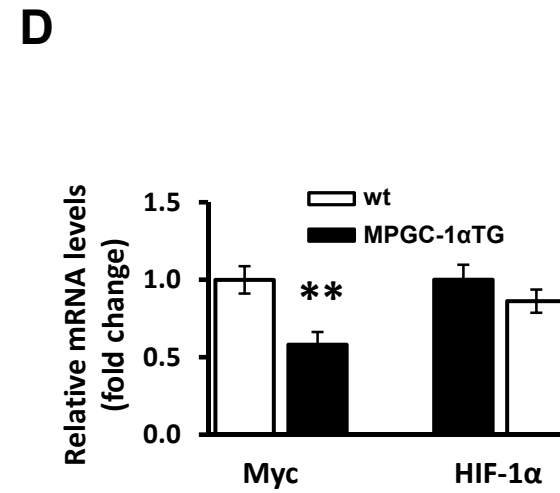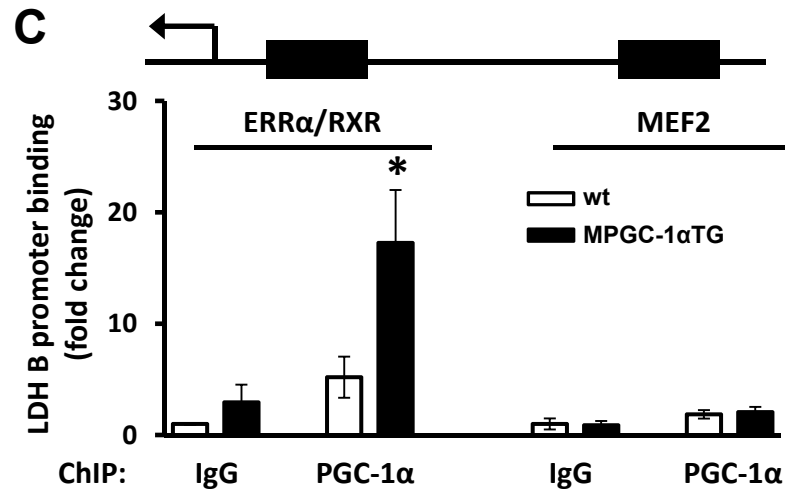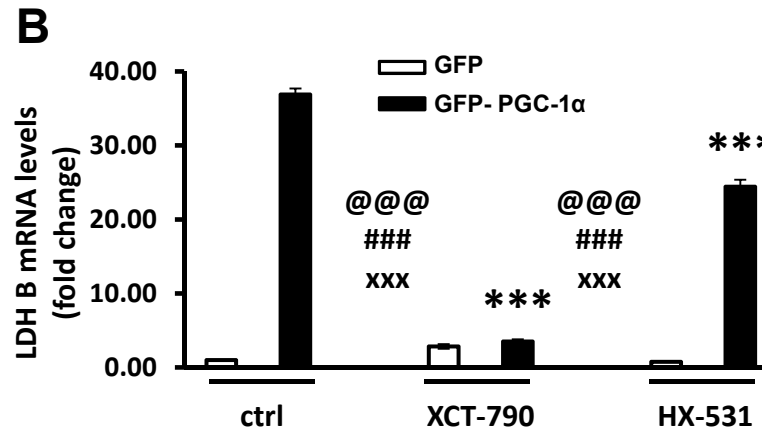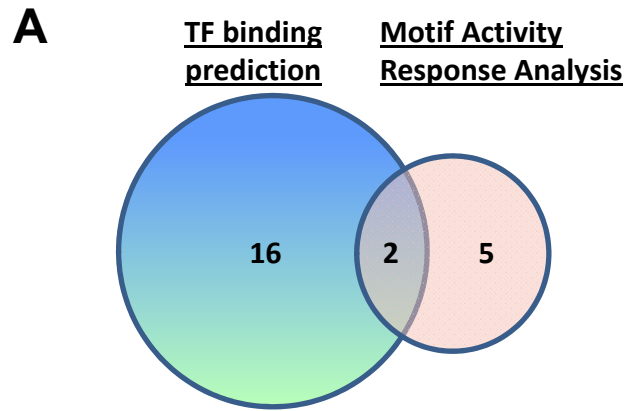All values are expressed as means ±SE (n =4-6 per group); Overall effect of genotype (wild-type *vs.* MPGC-1α TG): @; Effect of training (sedentary *vs.* exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *vs.* exercised)(*) and genotype (wild-type *vs.* MPGC-1α TG mice)(§) were assessed by t-test. Single symbol: $p < 0.05$; double symbols: $p < 0.01$; triple symbols: $p < 0.001$.

Fig. S2. *MARA analyses*

MARA analyses of microarray data from muscle cells infected with either GFP or bicistronic GFP-PGC-1α.

Fig. S3. *LDH B mRNA levels in response to ERRα, RXRα or RXRβ silencing*

(**A**) Relative mRNA levels of LDH B in confluent myoblasts transfected with siRNAs against ERRα, RXRα or RXRβ followed by adenoviral transfection with GFP or PGC-1α-GFP.

(**B-D**) Control experiments: Silencing efficiency of ERRα (**B**), RXRα (**C**) and RXRβ (**D**) by their corresponding siRNA.

Symbols at the left, middle and right refer to the comparison of siERRα, siRXRα and siRXRβ treated C2C12 myotubes to controls, respectively.

Effect of PGC-1α (GFP *vs.* PGC-1α-GFP): @; Effect of treatment (ctrl *vs.* siRNA): #; interaction: x. single symbol: $p < 0.05$; double symbols: $p < 0.01$; triple symbols: $p < 0.001$.

* denotes results from *post hoc* analysis.*: $p < 0.05$; ***: $p < 0.001$ compared to GFP-PGC-α untreated.

Fig. S4. *Reporter gene activation of the LDH B promoter by PGC-1α depends on the integrity of the ERRα responsive element.*

(**A**) Evolutionary conservation of the ERRα response element in the LDH B promoter. This element was identified with Swissregulon (ESRRA.p2 motif logo on the right) and confirmed using Matinspector scanning the TRANSFAC database. Nucleotides that are conserved in comparison to the mouse sequence are indicated in bold.

(**B and C**) Reporter gene assay using LDHB promoter containing wild type (pLDHB-luc) or mutated (pLDHB-mut-luc) ERRα response elements. (**B**) Autologous C2C12 and (**C**) heterologous COS7 cells were transfected with pLDHB-luc or pLDHB-mut-luc for 24 h, followed by additional 24 h in the presence of GFP (control, CON) or PGC-1α adenovirus (2 independent experiments were performed in triplicate each). Values represent mean ± SEM. ***$p < 0.001$ CON vs. PGC-1α.

Fig. S5. *LDH B mRNA levels in response to PPARβ/δ inhibition*

(**A**) Relative mRNA levels of LDH B in differentiated C2C12 myotubes following adenoviral overexpression of GFP or bicistronic PGC-1α-GFP and in the absence or presence of GSK0660.

(**B**) Control experiment: Relative mRNA levels of the PPARβ/δ target gene UCP3 in differentiated C2C12 myotubes following adenoviral overexpression of GFP or bicistronic PGC-1α-GFP and in the absence or presence of GSK0660.

All values are expressed as means ±SE (n =6 per group); Effect of PGC-1α (GFP *vs.* PGC-1α-GFP): @; Effect of treatment (DMSO *vs.* GSK0660): #; interaction: x. double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. S6. *Effect of LDH B silencing or PGC-1β overexpression on LDH B, LDH A and MCT1*

(**A-C**) Relative mRNA levels of LDH B (**A**), LDH A (**B**) and MCT1 (**C**) in confluent myoblasts transfected with pcDNA or PGC-1α expression plasmids in the absence or presence of siRNA against LDH B. All values are expressed as means ±SE (n =4-6 per group); Overall effect of PGC-1α (pcDNA *vs.* PGC-1α): @; Effect of treatment (ctrl *vs.* siRNA): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of treatment (ctrl *vs.* siRNA)(*) and PGC-1α (pcDNA *vs.* PGC-1α)(§) were assessed by t-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

(**D-F**) Relative mRNA levels of LDH B (**D**), LDH A (**E**) and MCT1 (**F**) in differentiated C2C12 myotubes following adenoviral overexpression of GFP or bicistronic PGC-1β-GFP. Values are expressed as means ±SE (n =6 per group).

Fig. S7. *Myc mRNA levels in skeletal muscle of MPGC-1α KO mice*

Relative mRNA levels of Myc in MPGC-1α KO mice and control animals. Values are expressed as means ±SE (n =8 per group).

Fig. S8. *Muscle LDH mRNA expression and activity in MPGC-1α KO mice after exercise*

(**A, B**) Relative mRNA expression (**A**) and activity (**B**) of LDH A in gastrocnemius muscle of MPGC-1α KO and control littermates in response to exercise.

(**C, D**) Relative mRNA expression (**C**) and activity (**D**) of LDH B in gastrocnemius muscle of MPGC-1α KO and control littermates in response to exercise.
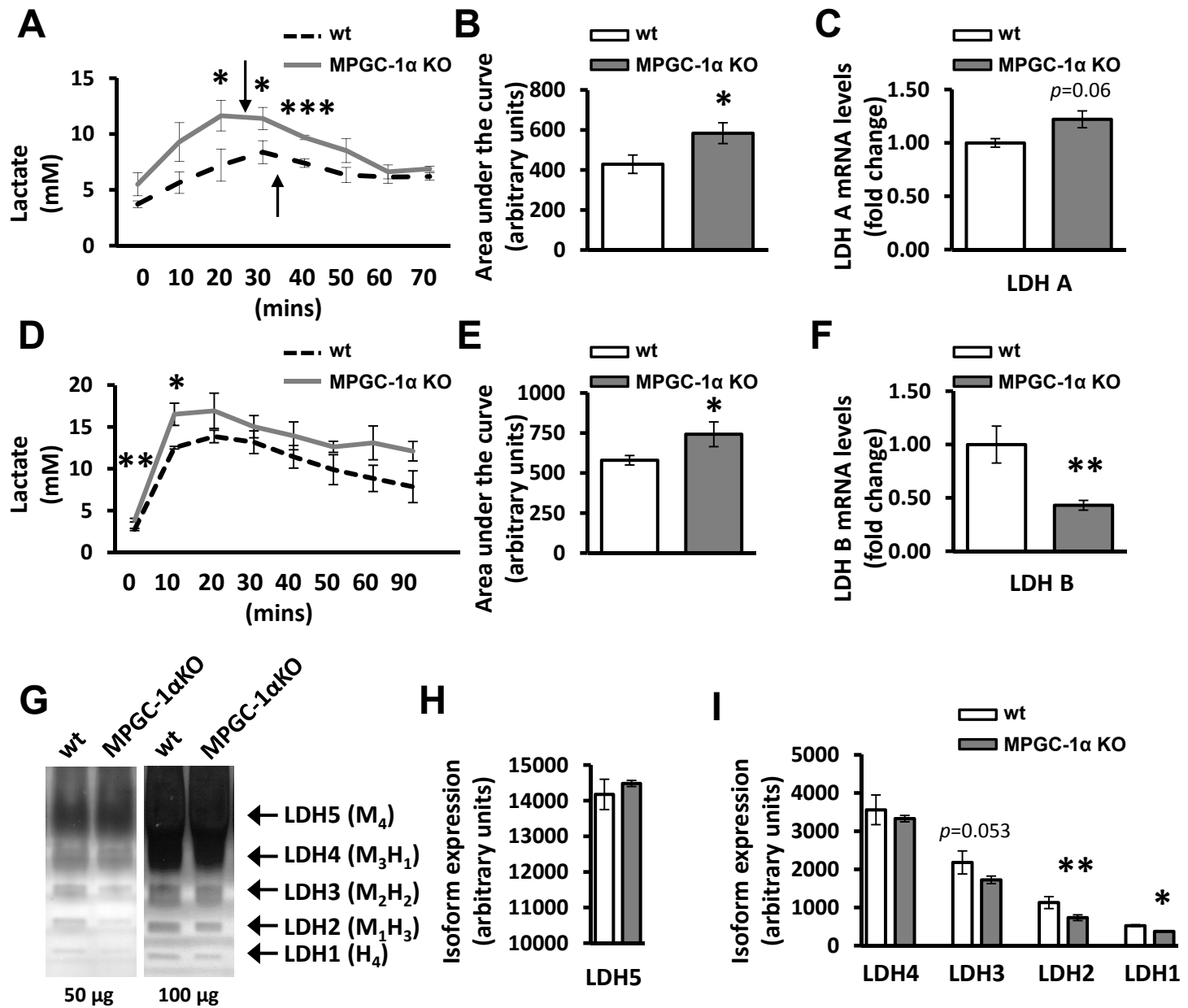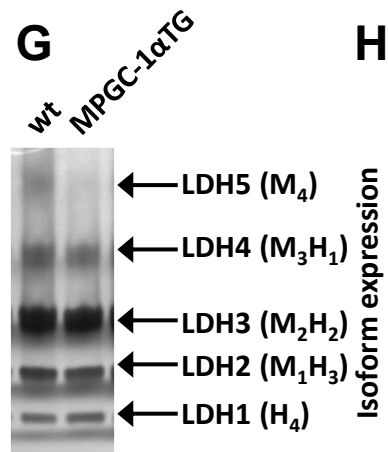
All values are expressed as means ±SE (n =4-6 per group); Overall effect of genotype (wild-type *vs.* MPGC-1α KO): @; Effect of training (sedentary *vs.* exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *vs.* exercised)(*) and genotype (wild-type *vs.* MPGC-1α KO mice)(§) were assessed by t-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. S9. *LDH mRNA expression and activity in the heart of MPGC-1α TG mice after exercise*

(**A, B**) Relative mRNA expression (**A**) and activity (**B**) of LDH A in the heart of MPGC-1α TG and control littermates in response to exercise.

(**C, D**) Relative mRNA expression (**C**) and activity (**D**) of LDH B in the heart of MPGC-1α TG and control littermates in response to exercise.

All values are expressed as means ±SE (n =4-6 per group); Overall effect of genotype (wild-type *vs.* MPGC-1α TG): @; Effect of training (sedentary *vs.* exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *vs.* exercised)(*) and genotype (wild-type *vs.* MPGC-1α TG mice)(§) were assessed by t-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. S10. *LDH mRNA expression and activity in the liver of MPGC-1α TG mice after exercise*

(**A, B**) Relative mRNA expression (**A**) and activity (**B**) of LDH A in the liver of MPGC-1α TG and control littermates in response to exercise.

(**C, D**) Relative mRNA expression (**C**) and activity (**D**) of LDH B in the liver of MPGC-1α TG and control littermates in response to exercise.

All values are expressed as means ±SE (n =4-6 per group); Overall effect of genotype (wild-type *vs.* MPGC-1α TG): @; Effect of training (sedentary *vs.* exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *vs.* exercised)(*) and genotype (wild-type *vs.* MPGC-1α TG mice)(§) were assessed by t-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. S11. *Transfection efficiency and PGC-1α protein expression in differentiated C2C12 myotubes*
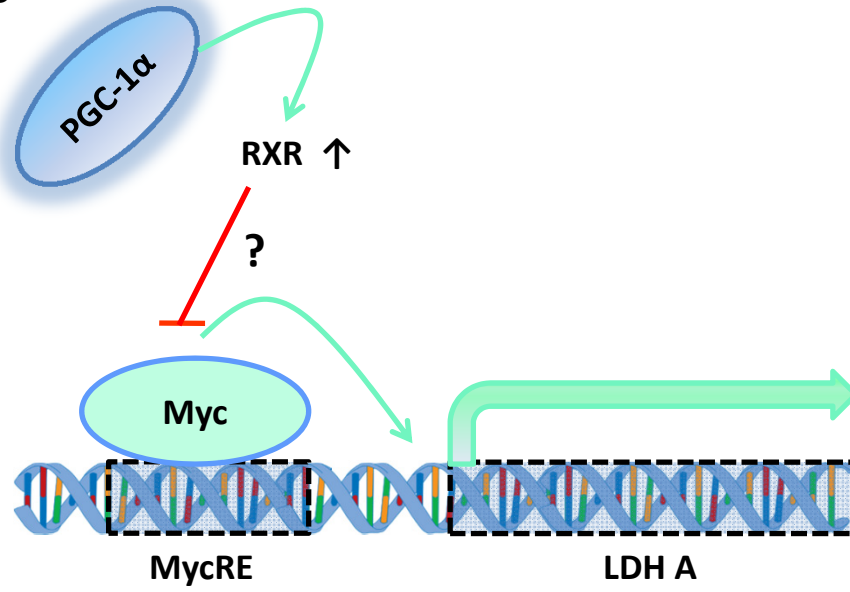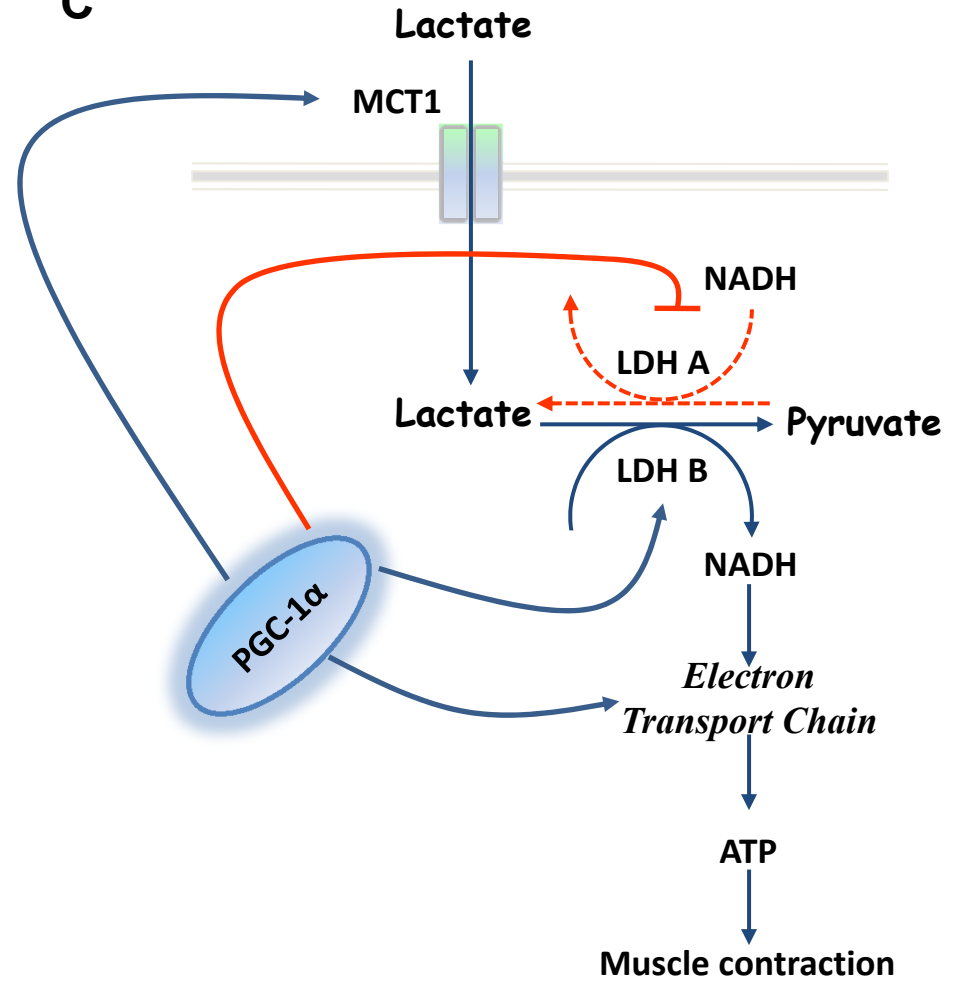
Green fluorescence in differentiated C2C12 myotubes adenovirally infected with GFP or PGC-1α-GFP and resulting overexpression of PGC-1α protein.
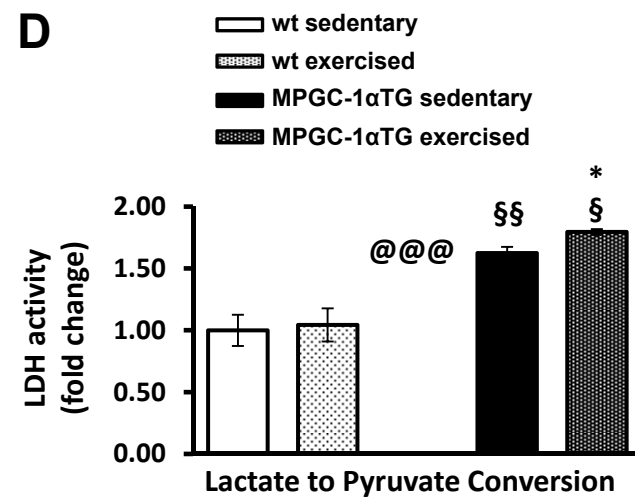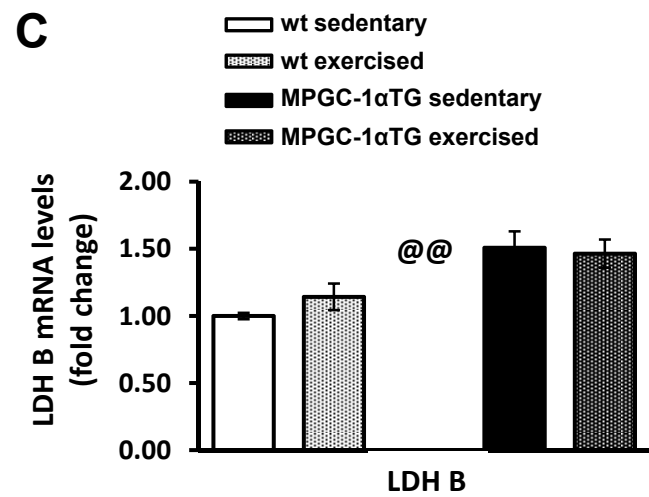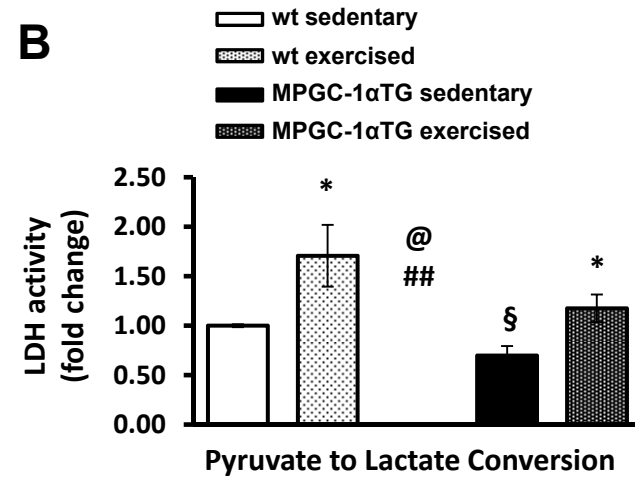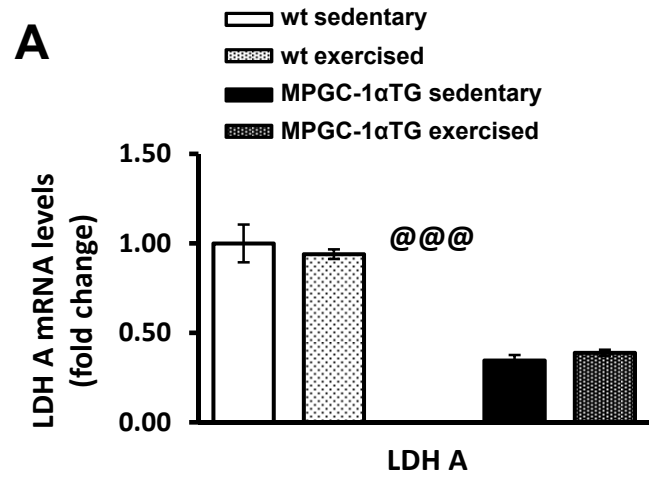
A

LDH A mRNA levels (fold change)

- □ wt sedentary
- ▦ wt exercised
- ■ MPGC-1αTG sedentary
- ▨ MPGC-1αTG exercised

@@@

LDH A

B

LDH activity (fold change)

- □ wt sedentary
- ▦ wt exercised
- ■ MPGC-1αTG sedentary
- ▨ MPGC-1αTG exercised

*

@
##

§

*

Pyruvate to Lactate Conversion

C

LDH B mRNA levels (fold change)

- □ wt sedentary
- ▦ wt exercised
- ■ MPGC-1αTG sedentary
- ▨ MPGC-1αTG exercised

@@

LDH B

D

LDH activity (fold change)

- □ wt sedentary
- ▦ wt exercised
- ■ MPGC-1αTG sedentary
- ▨ MPGC-1αTG exercised

@@@

§§

§
*

Lactate to Pyruvate Conversion

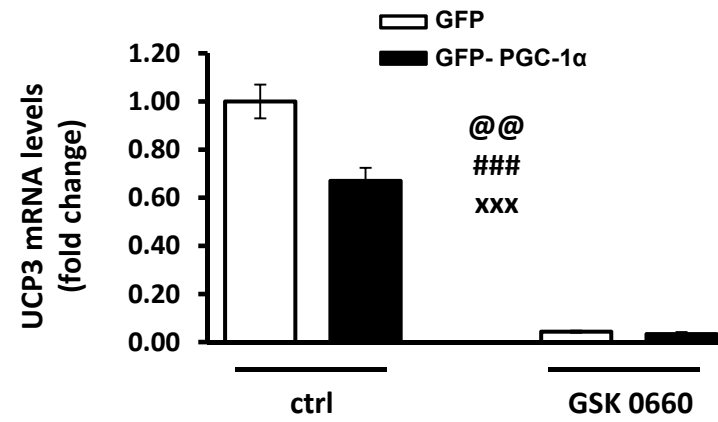| WM name	Z-value	Associated genes	Profile	Logo
ESRRA.p2	7.854	Esrra (ERRalpha, Estrra, Err1, Nr3b1)		
NR5A1,2.p2	6.836	Nr5a1 (Ad4BP, ELP, adrenal 4-binding protein, SF1, Ftz-F1, Ftzf1, SF-1, steroidogenic factor 1) Nr5a2 (D1Ertd308e, Ftf, LRH-1)		
RXR{A,B,G}.p2	3.004	Rxra (RXR alpha 1, RXRalpha1) Rxrb (Nr2b2, H-2RIIBP, RCoR-1, Rub) Rxrg (Nr2b3)		
ELK1,4_GABP{A,B1}.p3	2.858	Gabpa (GABPalpha) Gabpb1 (NRF2B2, E4TF1-47, BABPB2, E4TF1, NRF2B1, E4Tf1B, GABPB1-1, GABPB1-2, E4TF1-53) Elk4 (Sap1) Elk1 (Elk-1)		
NFY{A,B,C}.p2	2.853	Nfyc Nfyb (Cbf-A) Nfya (Sez10, Cbf-b)		
ZNF143.p2	2.402	Zfp143 (KRAB14, Zfp80-rs1, Zfp79, Staf, D7Ertd805e, pHZ-1)		
HNF4A_NR2F1,2.p2	2.313	Nr2f1 (COUP-TF1, Tcfcoup1, Erbal3, COUP-TFI) Nr2f2 (Tcfcoup2, COUP-TFII, ARP-1, EAR3, COUP-TF2, Aporp1) Hnf4a (Tcf4, Hnf4, Tcf14, Nuclear receptor 2A1, Nr2a1, HNF4 alpha, HNF-4, MODY1)		
GATA1..3.p2	1.979	Gata1 (Gata-1, Gf-1) Gata2 (Gata-2) Gata3 (Gata-3)		
TBX4,5.p2	1.887	Tbx5 Tbx4		
TCF4_dimer.p2	1.887	Tcf4 (E2.2, bHLHb19, SEF-2, TFE, ME2, ITF-2b, ASP-I2, MITF-2A, SEF2-1, E2-2, ITF-2, MITF-2B)		

A

wt sedentary
wt exercised
MPGC-1α KO sedentary
MPGC-1α KO exercised

LDH A mRNA levels (fold change)

1.50
1.00
0.50
0.00

LDH A

B

wt sedentary
wt exercised
MPGC-1α KO sedentary
MPGC-1α KO exercised

LDH activity (fold change)

4.00
3.00
2.00
1.00
0.00

*

§
p=0.053

Pyruvate to Lactate Conversion

C

wt sedentary
wt exercised
MPGC-1α KO sedentary
MPGC-1α KO exercised

LDH B mRNA levels (fold change)

1.50
1.00
0.50
0.00

*

@@@
xx

§§§   §§§

LDH B

D

wt sedentary
wt exercised
MPGC-1α KO sedentary
MPGC-1α KO exercised

LDH activity (fold change)

1.50
1.00
0.50
0.00

@@

§§

Lactate to Pyruvate Conversion

Heart

Liver

A

Legend: wt sedentary, wt exercised, MPGC-1αTG sedentary, MPGC-1αTG exercised

LDH A mRNA levels (fold change)

1.50, 1.00, 0.50, 0.00

LDH A

B

Legend: wt sedentary, wt exercised, MPGC-1αTG sedentary, MPGC-1αTG exercised

LDH activity (fold change)

1.50, 1.00, 0.50, 0.00

Pyruvate to Lactate Conversion

C

Legend: wt sedentary, wt exercised, MPGC-1αTG sedentary, MPGC-1αTG exercised

LDH B mRNA levels (fold change)

4.00, 3.00, 2.00, 1.00, 0.00

** ### **

LDH B

D

Legend: wt sedentary, wt exercised, MPGC-1αTG sedentary, MPGC-1αTG exercised

LDH activity (fold change)

1.50, 1.00, 0.50, 0.00

$p=0.066$

Lactate to Pyruvate Conversion

GFP

GFP-PGC-1α

PGC-1α →

GFP

GFP-PGC1α