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

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

transcriptional regulation | mitochondria | oxidative metabolism | metabolic reprogramming | muscle plasticity

Skeletal muscle adaptations to endurance exercise are largely mediated by peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α ; reviewed in refs. 1, 2). Elevated expression of PGC-1 α 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 fibers toward slow, oxidative fibers, including slow fiber type-specific calcium handling (4, 5). Additionally, PGC-1 α improves oxygen supply to muscle by promoting angiogenesis (6) and remodels the neuromuscular junction (7). Most importantly, however, PGC-1 α alters skeletal muscle metabolism by inducing mitochondrial biogenesis (5, 8) and promoting lipid oxidation (9). However, PGC-1 α 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-1 α (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 increase in blood lactate levels as indicated by the reduced blood lactate levels in trained athletes during acute exercise bouts (13). Similarly, reduced basal and postexercise blood lactate levels have previously been reported in mice with elevated levels of PGC-1 α 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-1 α -mediated reduction in blood lactate levels are unknown. We hypothesized that PGC-1 α actively drives a transcriptional program to enhance lactate metabolism and have now unraveled the direct molecular mechanism by which PGC-1 α remodels lactate homeostasis.

Results

PGC-1 α in Skeletal Muscle Controls Whole-Body Lactate Levels. As exhaustive exercise boosts blood lactate levels, we first investigated the impact of PGC-1 α in skeletal muscle on whole-body lactate homeostasis. During a maximal endurance performance test, blood lactate levels continuously increased in WT mice and returned to

basal levels within 40 min following fatigue-induced exercise cessation (Fig. 1*A* and *B*). In stark contrast, blood lactate levels did not substantially increase in muscle-specific PGC-1 α transgenic (MPGC-1 α TG) animals during exercise at any point (Fig. 1*A* and *B*). As skeletal muscle is the main site for lactate production, we next assessed the mRNA expression of *lactate dehydrogenase (LDH) A*, which encodes for the LDH muscle subunit (LDH M) that metabolizes pyruvate to lactate (14). LDH A mRNA expression in tibialis anterior was decreased by 53.5% ($P < 0.001$) in MPGC-1 α TG animals compared with control littermates (Fig. 1*C*). 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. 1*D*). These differences in LDH A mRNA expression and activity between MPGC-1 α TG and control animals persisted in response to an acute bout of exercise (Fig. S1*A* and *B*).

To test whether lactate removal is also altered by PGC-1 α , lactate tolerance tests were performed. The excursion (Fig. 1*E*) and total area under the curves (Fig. 1*F*) clearly show that MPGC-1 α TG animals more efficiently cleared lactate from the circulation compared with 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 (LDH H) that drives the conversion of lactate to pyruvate (14). LDH B mRNA expression in tibialis anterior was significantly elevated in MPGC-1 α TG animals by 110.2% ($P < 0.001$; Fig. 1*G*). Moreover, the enzymatic activity of LDH to convert lactate to pyruvate was enhanced by 60.7% ($P < 0.01$; Fig. 1*H*). In response to exercise, LDH B mRNA levels did not change, but LDH B activity further increased in MPGC-1 α TG animals (Fig. S1*C* and *D*).

PGC-1 α Drives LDH Isoenzyme-Type Switching in Skeletal Muscle. The tetrameric LDH 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 WT and MPGC-1 α TG animals, we performed native gel electrophoresis of muscle homogenates (Fig. 1*I*). MPGC-1 α TG animals displayed a pronounced shift toward an isoenzyme composition enriched in LDH H subunits, namely LDH 1, 2, and 3 (Fig. 1*I* and *J*). In contrast, protein levels of LDH 5, which exclusively contains LDH M subunits, were reduced in MPGC-1 α TG animals (Fig. 1*I* and *J*).

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Fig. 1. Muscle PGC-1 α controls blood lactate levels by shifting LDH composition. (A and B) Blood lactate excursion curves of WT (black dotted line) and MPGC-1 α 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 and D) LDH A mRNA levels (C) and activity (D) in tibialis anterior muscle. (E and F) Lactate tolerance test excursion curves of WT (dotted line) and MPGC-1 α TG (continuous line) animals and (E) corresponding area under the curve (F). (G and H) LDH B mRNA levels (G) and activity (H) in tibialis anterior muscle. (I) LDH isoenzyme composition in tibialis anterior of MPGC-1 α TG and control littermates. (J) Quantification of the LDH isoenzyme composition. (K) Relative mRNA levels of MCT1, MCT4, and CD147 in MPGC-1 α TG and control littermates. All values are expressed as means $\pm$ SE ($n = 8$ per group; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

Elevated Capacity for Lactate Uptake in MPGC-1 α TG Animals. Given the enhanced potential of skeletal muscle of MPGC-1 α 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 monocarboxylate transporter 1 (MCT1), which mediates lactate import into muscle (17) and mitochondria (18), was significantly increased in MPGC-1 α TG animals (Fig. 1K). In contrast, the mRNA levels of MCT4, which exports lactate (17), and CD147, an ancillary molecule of MCT1 and MCT4 (19), were unaltered (Fig. 1K). The elevated muscle MCT1 content in MPGC-1 α TG animals is consistent with the enhanced whole-body lactate removal during lactate tolerance tests and with the previous demonstration that PGC-1 α increases lactate uptake into skeletal muscle (20).

PGC-1 α Promotes LDH B Transcription by Coactivating Estrogen-Related Receptor- α on LDH B Promoter. Biocomputational predictions were then applied to unravel the potential molecular mechanism by which PGC-1 α promotes *LDH B* and *MCT1* transcription. To this end, microarray data from differentiated C2C12 myotubes adenovirally infected with GFP or bicistronic GFP-PGC-1 α (21) were analyzed. We first used Motif Activity Response Analysis (MARA) to identify motifs that are most active following overexpression of PGC-1 α (Fig. 2A). Then, we screened the *LDH 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 (ERR α) and retinoid X receptors (RXRs; Fig. 2A and Fig. S2). Recruitment of ERR α 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 ERR α and RXR were used. In differentiated C2C12 myotubes, PGC-1 α led to a very robust induction of LDH B, which was completely prevented by the ERR α inverse agonist XCT-790 (Fig. 2B). Inhibition of RXRs by HX-531 likewise resulted in a significant reduction in PGC-1 α -mediated induction of LDH B, but to a smaller extent compared with ERR α inhibition (Fig. 2B).

Similarly, RNA silencing of ERR α in myoblasts led to a significant reduction in LDH B expression, whereas silencing of RXR α or β reduced LDH B expression to a smaller extent (Fig. S3).

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

Decreased Levels of LDH A and Myelocytomatosis Oncogene in MPGC-1 α TG Animals. *LDH A* transcription is regulated by hypoxia-inducible factor-1 α (HIF-1 α) and myelocytomatosis oncogene (Myc) (25). Although HIF-1 α mRNA levels were similar in WT and transgenic animals, Myc transcript levels were reduced in skeletal muscle of MPGC-1 α TG mice compared with controls (Fig. 2D). Consistently, protein levels of Myc were also significantly decreased in MPGC-1 α TG mice compared with their control littermates (Fig. 2E and 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. Although LDH B transcription was successfully reduced in this experiment (Fig. S6A), the effect of PGC-1 α on MCT1 and LDH A persisted (Fig. S6B and C).

Selective Regulation of Muscle Lactate Homeostasis by PGC-1 β . Because PGC-1 α and β exert similar effects, we also examined the

Fig. 2. PGC-1 α interacts with ERR α 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-1 α -GFP. Only transcription factors with Z-scores set above a cutoff 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-1 α -GFP and in the absence or presence of XCT-790 or HX-531. All values are expressed as means $\pm$ SE ($n = 6$ per group). @, Effect of PGC-1 α (GFP vs. PGC-1 α -GFP); #, effect of treatment (DMSO vs. XCT-790 or HX-531); x, interaction. Triple symbols indicate $P < 0.001$. Symbols at left refer to the comparison of XCT-790-treated C2C12 myotubes vs. controls. Symbols at right refer to the comparison of HX-531-treated C2C12 myotubes vs. controls (*Results from post hoc analysis; *** $P < 0.001$ vs. GFP-PGC- α untreated). (C) ChIP assay on mouse skeletal muscle. Recruiting of PGC-1 α to the ERR α and RXR or to the MEF2 binding site in the LDH B promoter of MPGC-1 α TG mice and control animals. (D) Relative mRNA expression levels of Myc and Hif-1 α . (E and F) Representative Western blot of Myc (E) and corresponding quantification (F). (G and H) Relative mRNA levels of Myc (G) and LDH A (H) in muscle cells following adenoviral overexpression of GFP or bicistronic PGC-1 α -GFP and in the absence or presence of HX-531. All values are expressed as means $\pm$ SE ($n = 6$ per group). @, Effect of PGC-1 α (GFP vs. PGC-1 α -GFP); #, effect of treatment (DMSO vs. HX-531); x, interaction. Single symbols, $P < 0.05$; double symbols, $P < 0.01$; triple symbols, $P < 0.001$ (*Results from post hoc analysis; ** $P < 0.01$ vs. GFP-PGC- α untreated).

potential impact of PGC-1 β on LDH B, LDH A, and MCT1 mRNA levels. Overexpression of PGC-1 β in differentiated C2C12 myotubes resulted in elevated levels of LDH B (Fig. S6D), but the effect was less pronounced than with PGC-1 α (Fig. 2B). LDH A levels were reduced, but MCT1 levels were unaltered following overexpression of PGC-1 β (Fig. 6E and F). Importantly, however, PGC-1 β 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-1 α Is Important for Regulation of LDH B Transcription and Proper Lactate Handling. To corroborate the importance of PGC-1 α in regulating lactate homeostasis, and especially LDH B expression, we then studied mice with a muscle-specific KO of *PGC-1 α* (MPGC-1 α KO). MPGC-1 α KO animals fatigued rapidly and accumulated blood lactate to a higher extent than their control littermates in endurance exercise trials (Fig. 3A and B). LDH A mRNA showed a nonsignificant trend toward higher expression in MPGC-1 α KO animals (Fig. 3C). Myc mRNA did not differ between MPGC-1 α KO and control animals (Fig. S7). In terms of lactate tolerance, MPGC-1 α KO animals were less efficient in lactate clearance upon injection of lactate (Fig. 3D and E). Consistently, MPGC-1 α 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–I). Together, these data clearly demonstrate that muscle PGC-1 α 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 and B). In MPGC-1 α 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-1 α KO animals, and these differences persisted after exercise (Fig. S8C and D).

Low Blood Lactate Levels in MPGC-1 α TG Are Not Caused by 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 with 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 WT and transgenic animals were observed (Fig. 4A). In the liver, there was a significant increase in CD147 mRNA levels, whereas 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), whereas the conversion of lactate to pyruvate was unaltered (Fig. 4D). In the liver, no changes in LDH activity were detectable (Fig. 4E and F). Isoenzyme compositions between MPGC-1 α TG and WT animals were undistinguishable for the heart (Fig. 4G and H) and the liver (Fig. 4I and J). An acute bout of exercise affected LDH in both tissues. In the heart, exercise increased LDH A activity in WT mice, but not in MPGC-1 α 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 and 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-1 α (reviewed in refs.

Fig. 3. PGC-1 α is important for the regulation of LDH B transcription. (A and B) Blood lactate excursion curves of WT (dotted line) and MPGC-1 α 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 WT and KO mice. (D and E) Lactate tolerance test excursion curves of WT (dotted line) and MPGC-1 α KO (continuous gray line) animals (D) and corresponding area under the curve (E). (F) LDH B mRNA levels in tibialis anterior muscle of WT and KO mice. (G) LDH isoenzyme composition in tibialis anterior muscle of MPGC-1 α KO and control littermates with 50 μ g (Left) and 100 μ g (Right) of protein extract. (H and I) Quantification of LDH isoenzyme composition. All values are expressed as means $\pm$ SE (n = 6 per group; * P < 0.05, ** P < 0.01, and *** P < 0.001).

1, 2). We have now shown that PGC-1 α also is a key regulator of tissue and systemic lactate homeostasis (Fig. 5).

Intriguingly, a shift toward 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-1 α and ERR α and, moreover, that PGC-1 α is required for the elevated LDH B transcription and proper lactate homeostasis during exercise. PGC-1 α thus remodels LDH isoenzyme composition distinctly and independently of coactivation of peroxisome

proliferator-activated receptor- β - 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-1 α reduces *LDH A* gene expression, which could potentially counteract the enzymatic activity of LDH B of converting lactate into pyruvate. How PGC-1 α , a transcriptional coactivator, can exert repressive effects remains elusive. A previous study revealed that stimulation of mitochondrial activity diminishes Myc expression (36). Conceivably, the PGC-1 α -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

Fig. 4. Blood lactate levels are not controlled by heart or liver lactate metabolism following elevated PGC-1 α expression. (A) Relative mRNA expression of LDH A, LDH B, MCT1, MCT4, and CD147 in the heart of MPGC-1 α TG and control littermates. (B) Relative mRNA expression of LDH A, LDH B, MCT1, MCT4, and CD147 in the liver of MPGC-1 α TG and control littermates. (C and D) Conversion of pyruvate to lactate (C) and reverse reaction (D) in the heart of MPGC-1 α TG and control littermates. (E and F) Conversion of pyruvate to lactate (E) and reverse reaction (F) in the liver of MPGC-1 α TG and control littermates. (G and H) LDH isoenzyme composition (G) and quantification (H) in the heart of MPGC-1 α TG and control littermates. (I and J) LDH isoenzyme composition (I) and quantification (J) in the liver of MPGC-1 α TG and control littermates. All values are expressed as means $\pm$ SE (n = 8 per group; * P < 0.05, ** P < 0.01, and *** P < 0.001).

Fig. 5. PGC-1 α promotes rapid energy provision by lactate oxidation. (A) Scheme illustrating the action of PGC-1 α on the LDH B promoter. PGC-1 α promotes the transcription of ERR α , which then binds to an ERR α -responsive element (ERRE) in the LDH B promoter. The subsequent activation of ERR α is enhanced by PGC-1 α . (B) PGC-1 α 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-1 α 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-1 α thereby promotes a lactate oxidizing phenotype, which is associated with improved endurance capacity and metabolic health.

showing induction of RXRs gene expression in skeletal muscle by PGC-1 α (10). Furthermore, in the present study, we found RXR binding motifs to be predicted to be associated with PGC-1 α -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 coactivator PGC-1 α through Myc is hampered by the very low expression of Myc in skeletal muscle (37). Importantly, our findings that PGC-1 α 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-1 α , display high amounts of LDH M subunits, whereas the LDH complexes in slow-twitch muscles with higher expression of PGC-1 α 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 a result of the limited pool of cytosolic NAD⁺ in skeletal muscle (25). The prevention of NAD⁺ regeneration by PGC-1 α 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-1 α levels in muscle (11). Moreover, the potentially enhanced production of NADH by lactate oxidation triggered by PGC-1 α 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 with resting muscle (42–44).

Importantly, our study also sheds light on 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-1 α 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-1 α 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 vasodilation and dissociation of oxygen from hemoglobin and thus oxygen transport to muscle. In this context, the reduction in blood lactate levels by PGC-1 α might be viewed as a performance-limiting factor during strenuous exercise. However, PGC-1 α likely overcomes this effect by increasing myoglobin expression (4) and enhancing angiogenesis (6).

In conclusion, we have demonstrated that PGC-1 α and ERR α orchestrate the transcription of LDH B, that PGC-1 α 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-1 α . Importantly, our results suggest a direct regulatory role for PGC-1 α in the metabolic remodeling of lactate metabolism. Thus, by driving lactate uptake and oxidation, PGC-1 α promotes alternative metabolic pathways for energy generation during muscle contraction. Finally, overexpressed PGC-1 α 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 insights into the molecular mechanisms by which PGC-1 α metabolically enhances exercise performance and improves muscle function.

Materials and Methods

Animals. Male MPG-1 α TG (4), PGC-1 α 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-wk-old animals according to criteria outlined for the care and use of laboratory animals and with approval of the veterinary office of the canton Basel.

Treadmill Running and Lactate Tolerance Tests. Detailed descriptions of treadmill running and lactate tolerance tests are provided in *SI Materials and Methods*.

RNA Extraction and RT-PCR. Frozen tissues were homogenized under liquid nitrogen, and total RNA was isolated by using TRIzol reagent (Invitrogen). RNA concentrations were adjusted, and reverse transcription was carried out by using random hexamer primers (Promega). Real-time PCR analysis (Power SYBR Green Master Mix; Applied Biosystems) was performed by 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.

LDH Activity. Specific LDH activities (pyruvate-to-lactate and lactate-to-pyruvate conversions) were determined according to Howell et al. (47). The enzymatic reactions were carried out in the presence of 1 mmol sodium pyruvate and 150 μ mol NADH or 7 mmol lactic acid and 5.5 mmol NAD⁺. Changes in absorbance were assessed by a spectrophotometer at 340 nm 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 μ g of protein were loaded onto a 6% (wt/vol) 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 phenazine methosulfate, and 0.25 mg/mL nitro blue tetrazolium.

Cell Culture Experiments. C2C12 myoblasts were fused into myotubes and infected with adenovirus expressing GFP or bicistronic PGC-1 α -GFP. The

transfection efficiency was similar between GFP and PGC-1 α -GFP, and PGC-1 α -GFP efficiently increased PGC-1 α protein levels (Fig. S11). In addition, cells were treated for 48 h with 0.1% DMSO (as control), 10 μ M XCT-790 (Sigma-Aldrich) to inhibit ERK α , or 2 μ M HX-531 (Tocris Bioscience) to inhibit RXR α , together with the corresponding adenovirus. Silencing of LDH B, ERK α , RXR α , and RXR β was performed by using Dharmacon SMARTpool siRNAs according to the manufacturer's instructions.

ChIP, LDH B Promoter Cloning, Reporter Gene Assays, Bioinformatics, Data Analysis, and Statistics. Detailed descriptions of ChIP, LDH B promoter cloning, reporter gene assays, bioinformatics, data analysis, and statistics are provided in *SI Materials and Methods*.

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- Summhammer S, Handschin C (2012) PGC-1 α and exercise in the control of body weight. *Int J Obes* 36(11):1428–1435.
- Handschin C (2010) Regulation of skeletal muscle cell plasticity by the peroxisome proliferator-activated receptor γ coactivator 1 α . *J Recept Signal Transduct Res* 30(6):376–384.
- Calvo JA, et al. (2008) Muscle-specific expression of PPAR γ coactivator-1 α improves exercise performance and increases peak oxygen uptake. *J Appl Physiol* 104(5):1304–1312.
- Lin J, et al. (2002) Transcriptional co-activator PGC-1 α drives the formation of slow-twitch muscle fibres. *Nature* 418(6899):797–801.
- Summhammer S, et al. (2012) Remodeling of calcium handling in skeletal muscle through PGC-1 α : Impact on force, fatigability, and fiber type. *Am J Physiol Cell Physiol* 302(1):C88–C99.
- Arany Z, et al. (2008) HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1 α . *Nature* 451(7181):1008–1012.
- Handschin C, et al. (2007) PGC-1 α regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy. *Genes Dev* 21(7):770–783.
- Lin J, Handschin C, Spiegelman BM (2005) Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab* 1(6):361–370.
- Summhammer S, Troxler H, Santos G, Handschin C (2011) Coordinated balancing of muscle oxidative metabolism through PGC-1 α increases metabolic flexibility and preserves insulin sensitivity. *Biochem Biophys Res Commun* 408(1):180–185.
- Summhammer S, Baum O, Santos G, Hoppeler H, Handschin C (2010) Peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) promotes skeletal muscle lipid refueling in vivo by activating de novo lipogenesis and the pentose phosphate pathway. *J Biol Chem* 285(43):32793–32800.
- Wende AR, et al. (2007) A role for the transcriptional coactivator PGC-1 α in muscle refueling. *J Biol Chem* 282(50):36642–36651.
- Cairns SP (2006) Lactic acid and exercise performance: Culprit or friend? *Sports Med* 36(4):279–291.
- Hagberg JM, et al. (1988) Metabolic responses to exercise in young and older athletes and sedentary men. *J Appl Physiol* 65(2):900–908.
- Dawson DM, Goodfriend TL, Kaplan NO (1964) Lactic dehydrogenases: Functions of the two types rates of synthesis of the two major forms can be correlated with metabolic differentiation. *Science* 143(3609):929–933.
- Draoui N, Feron O (2011) Lactate shuttles at a glance: from physiological paradigms to anti-cancer treatments. *Dis Model Mech* 4(6):727–732.
- Van Hall G (2000) Lactate as a fuel for mitochondrial respiration. *Acta Physiol Scand* 168(4):643–656.
- Juel C, Halestrap AP (1999) Lactate transport in skeletal muscle - role and regulation of the monocarboxylate transporter. *J Physiol* 517(pt 3):633–642.
- Hashimoto T, Hussien R, Brooks GA (2006) Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate oxidation complex. *Am J Physiol Endocrinol Metab* 290(6):E1237–E1244.
- Kirk P, et al. (2000) CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. *EMBO J* 19(15):3896–3904.
- Benton CR, et al. (2008) PGC-1 α increases skeletal muscle lactate uptake by increasing the expression of MCT1 but not MCT2 or MCT4. *Physiol Genomics* 35(1):45–54.
- Calvo S, et al. (2006) Systematic identification of human mitochondrial disease genes through integrative genomics. *Nat Genet* 38(5):576–582.
- Charest-Marcotte A, et al. (2010) The homeobox protein Prox1 is a negative modulator of ERK α /PGC-1 α bioenergetic functions. *Genes Dev* 24(6):537–542.
- Pachkov M, Erb I, Molina N, van Nimwegen E (2007) SwissRegulon: A database of genome-wide annotations of regulatory sites. *Nucleic Acids Res* 35(database issue):D127–D131.
- Gan Z, et al. (2011) The nuclear receptor PPAR β / δ programs muscle glucose metabolism in cooperation with AMPK and ME2F. *Genes Dev* 25(24):2619–2630.
- Fan J, et al. (2011) Tyrosine phosphorylation of lactate dehydrogenase A is important for NADH/NAD(+) redox homeostasis in cancer cells. *Mol Cell Biol* 31(24):4938–4950.
- Park EJ, Kiselev E, Conda-Sheridan M, Cushman M, Pezzuto JM (2012) Induction of apoptosis by 3-amino-6-(3-aminopropyl)-5,6-dihydro-5,11-dioxo-11H-indeno[1,2-c] isoquinoline via modulation of MAPKs (p38 and c-Jun N-terminal kinase) and c-Myc in HL-60 human leukemia cells. *J Nat Prod* 75(3):378–384.
- Park EJ, et al. (2011) Induction of retinoid X receptor activity and consequent upregulation of p21WAF1/CIP1 by indenoisoquinolines in MCF7 cells. *Cancer Prev Res (Phila)* 4(4):592–607.
- Mortensen OH, et al. (2007) PGC-1 β is downregulated by training in human skeletal muscle: No effect of training twice every second day vs. once daily on expression of the PGC-1 family. *J Appl Physiol* 103(5):1536–1542.
- Gertz EW, Wisneski JA, Stanley WC, Neese RA (1988) Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J Clin Invest* 82(6):2017–2025.
- Ahlborg G, Juhlin-Dannfelt A (1994) Effect of beta-receptor blockade on splanchnic and muscle metabolism during prolonged exercise in men. *J Appl Physiol* 76(3):1037–1042.
- Oberbach A, et al. (2006) Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. *Diabetes Care* 29(4):895–900.
- Py G, et al. (2001) Impaired sarcolemmal vesicle lactate uptake and skeletal muscle MCT1 and MCT4 expression in obese Zucker rats. *Am J Physiol Endocrinol Metab* 281(6):E1308–E1315.
- DeBerardinis RJ, Thompson CB (2012) Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* 148(6):1132–1144.
- Apple FS, Rogers MA (1986) Skeletal muscle lactate dehydrogenase isozyme alterations in men and women marathon runners. *J Appl Physiol* 61(2):477–481.
- Simoneau JA, Pette D (1989) Species-specific responses of muscle lactate dehydrogenase isozymes to increased contractile activity. *PLoS Arch* 413(6):679–681.
- Seyer P, et al. (2006) Mitochondrial activity regulates myoblast differentiation by control of c-Myc expression. *J Cell Physiol* 207(1):75–86.
- Veal EA, Jackson MJ (1998) C-myc is expressed in mouse skeletal muscle nuclei during post-natal maturation. *Int J Biochem Cell Biol* 30(7):811–821.
- Kemp JG, Blazev R, Stephenson DG, Stephenson GM (2009) Morphological and biochemical alterations of skeletal muscles from the genetically obese (ob/ob) mouse. *Int J Obes (Lond)* 33(8):831–841.
- Apple FS, Tesch PA (1989) CK and LD isozymes in human single muscle fibers in trained athletes. *J Appl Physiol* 66(6):2717–2720.
- Bayley JP, Devilee P (2012) The Warburg effect in 2012. *Curr Opin Oncol* 24(1):62–67.
- Yeung SJ, Pan J, Lee MH (2008) Roles of p53, MYC and HIF-1 in regulating glycolysis - the seventh hallmark of cancer. *Cell Mol Life Sci* 65(24):3981–3999.
- Richter EA, Kiens B, Saltin B, Christensen NJ, Savard G (1988) Skeletal muscle glucose uptake during dynamic exercise in humans: Role of muscle mass. *Am J Physiol* 254(5 pt 1):E555–E561.
- Jorfeldt L, Wahren J (1970) Human forearm muscle metabolism during exercise. V. Quantitative aspects of glucose uptake and lactate production during prolonged exercise. *Scand J Clin Lab Invest* 26(1):73–81.
- Omachi A, Lifson N (1956) Metabolism of isotopic lactate by the isolated perfused dog gastrocnemius. *Am J Physiol* 185(1):35–40.
- van Hall G (2010) Lactate kinetics in human tissues at rest and during exercise. *Acta Physiol (Oxf)* 199(4):499–508.
- Handschin C, et al. (2007) Abnormal glucose homeostasis in skeletal muscle-specific PGC-1 α knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J Clin Invest* 117(11):3463–3474.
- Howell BF, McCune S, Schaffer R (1979) Lactate-to-pyruvate or pyruvate-to-lactate assay for lactate dehydrogenase: a re-examination. *Clin Chem* 25(2):269–272.
- Summhammer S, et al. (2009) Adipose tissue plasticity during catch-up fat driven by thrifty metabolism: relevance for muscle-adipose glucose redistribution during catch-up growth. *Diabetes* 58(10):2228–2237.