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This research was originally published in FASEB J. 2016 30(5), 1976-86. PMID: 26849960. doi: 10.1096/fj.201500128

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Skeletal muscle PGC-1α modulates systemic ketone body homeostasis and ameliorates diabetic hyperketonemia in mice

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Running title: Muscle PGC-1α regulates ketone body metabolism

Non-standard abbreviations

AcAc, acetoacetate; βOHB, β-hydroxybutyrate; gKO, global PGC-1α knockout mouse; KB, ketone body; LCKD, low-carbohydrate ketogenic diet; mKO, muscle-specific PGC-1α knockout mouse; mTG, muscle-specific PGC-1α transgenic mouse; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; STZ, streptozotocin

Abstract

Ketone bodies are crucial energy substrates during states of low carbohydrate availability. However, an aberrant regulation of ketone body homeostasis can lead to complications such as diabetic ketoacidosis. Exercise and diabetes affect systemic ketone body homeostasis, but the regulation of ketone body metabolism is still enigmatic. Using mice with either a knockout or overexpression of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) in skeletal muscle, we show that PGC-1α regulates ketolytic gene transcription in muscle. Furthermore, ketone body homeostasis of these mice was investigated during fasting, exercise, ketogenic diet feeding and after streptozotocin injection. In response to these ketogenic stimuli, we show that modulation of PGC-1α levels in muscle affects systemic ketone body homeostasis. Moreover, our data demonstrate that skeletal muscle PGC-1α is necessary for the enhanced ketolytic capacity in response to exercise training and overexpression of PGC-1α in muscle enhances systemic ketolytic capacity and is sufficient to ameliorate diabetic hyperketonemia in mice. Using cultured myotubes, we also show that the transcription factor estrogen related receptor α (ERRα) is a partner of PGC-1α in the regulation of ketolytic gene transcription. Collectively, these results demonstrate a central role of skeletal muscle PGC-1α in the transcriptional regulation of systemic ketolytic capacity.

Keywords

Ketone body metabolism; Diabetes; Exercise; Ketoacidosis; Skeletal muscle; PGC-1α; transcriptional regulation

Introduction

During prolonged starvation, when carbohydrate availability is low, the ketone bodies (KB) β-hydroxybutyrate (βOHB) and acetoacetate (AcAc) are important metabolic fuels to help maintain energy homeostasis (1). KBs are produced in the liver and subsequently metabolized to acetyl-CoA in extra-hepatic organs. The majority of KB metabolism occurs in the mitochondria and is catalyzed by the enzymes 3-hydroxybutyrate dehydrogenase, type 1 (BDH1), succinyl-CoA:3-ketoacid-coenzyme A transferase 1 (OXCT1) and acetyl-CoA acetyltransferase 1 (ACAT1) (2). Mutations of genes encoding these enzymes are associated with exacerbated ketosis in humans (3). Moreover, knockout of the rate-limiting ketolytic enzyme OXCT1 leads to severe hyperketonemia and lethality in mice (4). Hyperketonemia is a common complication in diabetic patients, which can lead to severe and possibly lethal ketoacidosis (5), and has in part been attributed to impaired peripheral KB oxidation (6). However, relatively little is known about the transcriptional regulation of ketolytic enzymes (7). The peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) is an important transcriptional coactivator, and has a well-established role in the regulation of mitochondrial metabolic processes such as oxidative phosphorylation and TCA cycle (8). While these metabolic pathways are important for complete oxidation of KBs, it is not known whether PGC-1α can directly affect expression of ketolytic genes in skeletal muscle. Here, we demonstrate that PGC-1α is a transcriptional regulator of ketolytic enzymes and KB transporters in skeletal muscle. Moreover, we show that modulation of ketolytic gene transcription in skeletal muscle by PGC-1α affects systemic ketosis in response to various stimuli, such as fasting, low-carbohydrate diet feeding and exercise. In line with this, muscle-specific overexpression of PGC-1α can reduce hyperketonemia in both healthy and diabetic mice, and PGC-1α thus constitutes a novel therapeutic target to protect against hyperketonemia and diabetic ketoacidosis.

Materials and Methods

Mice and diets - Animals were housed in a facility with 12-h light/12-h dark cycle with free access to food and water. Experiments were performed in accordance with federal guidelines and were approved by the Kantonales Veterinäramt of Kanton Basel-Stadt. PGC-1α muscle-specific transgenic mice (mTG) and global PGC-1α-knockout mice (gKO) are described elsewhere (9, 10). The PGC-1α muscle-specific knockout mice (mKO) used in this study differ from previous publications (11), and were generated by crossing PGC-1α$^{loxP/loxP}$ mice (10) with HSA-Cre transgenic mice. Mice used were males, aged between 10 – 14 weeks, unless otherwise noted. Chow diet (AIN-93G; 7% fat, 58.5% carb. and 18% protein) and ketogenic diet (XL75:XP10; 74.4% fat, 3% carb. and 9.9% protein) were provided by Provimi Kliba AG, Kaiseraugst, Switzerland.

Fasting and cold exposure – Mice were fasted for 24 hours with free access to water. For cold-exposure, cages without bedding were pre-cooled at 4°C overnight. Mice were transferred to these cages at 4°C for 8 hours without access to food.

βOHB tolerance test - Mice were fasted for 5 hours in the morning, and then received a bolus intraperitoneal (I.P.) injection of 1.5 g/kg bodyweight Na-βOHB (Sigma). Blood βOHB levels were measured in tail vein blood at 0, 15, 30, 45, 60 and 90 minutes after injection.

Streptozotocin – Male mice, aged 20-24 weeks, were fasted for 10 hours and subsequently injected with either citrate buffer (CB) or Streptozotocin (STZ) (Sigma) in CB, I.P. at a dose of 150 mg/kg. 10% sucrose was administered to the drinking water of the mice during the first 24 hours after injection.

Acute and chronic exercise - Exercise training was performed by giving mice free access to running wheels (Columbus instruments) in their home cages for 8 weeks, starting from the age of 10-12 weeks. Sedentary control groups were housed in cages without running wheels. Mice were removed from their running wheel cages 24 hours prior to either βOHB tolerance test or sacrifice. For post-exercise ketosis tests, animals were acclimatized to treadmill running 2 days before the start of the experiment, for 5 minutes at 8 meters/minute (m/m) followed by 5 minutes at 10 m/m, at an incline of 5°. For the actual experiment, treadmill (Columbus instruments) was kept at an incline of 5°. The program started at 5 m/m for 5 minutes, followed by 8 m/m for 10 minutes. The speed of the treadmill was then increased by 2 m/m every 15 minutes. Basal blood βOHB was assessed in tail-vein blood pre-exercise. Mice were removed from the treadmill after 80 minutes of running, before any of the mice

reached terminal exhaustion. Blood βOHB levels were measured in tail vein blood at 0, 30 and 180 minutes post-exercise.

Blood analysis - Blood glucose and βOHB were measured in a tail vein blood using a handheld glucose meter (Accu-Chek, Roche) or βOHB-meter (Precision Xtra, Abbott). For plasma analysis, whole tail-vein blood was collected in Microvette tubes (Sarstedt). Plasma analysis was performed using colorimetric tests according to the manufacturers' instructions; non-esterified fatty acids (HR Series NEFA-HR(2); Wako Diagnostics), acetoacetate (Acetoacetate Assay Kit; Abcam) and insulin (Ultra-sensitive mouse insulin ELISA kit, Crystal Chem Inc).

RNA extraction and qRT-PCR – All tissue samples for RNA extraction were collected between Zeitgeber (ZT) 2-5. For basal measurements, mice were fasted for 2 hours before tissue samples were collected. For fasting experiments, mice were fasted for 24 hours before tissue samples were collected. Frozen tissue or cells were homogenized and total RNA was extracted using TRIzol reagent (Invitrogen). cDNA synthesis was performed using 1 μg of total RNA. Semi-quantitative Real-time PCR analysis was performed using Fast SYBR Green master mix (Applied Biosystems) on a StepOnePlus Real-Time PCR System (Applied Biosystems). Relative expression levels for each gene of interest were calculated with the ΔΔCt method, using either 18s, eEF2, Tbp or Rpl0 as normalization control. Primer sequences are listed in SI Table 1.

Immunoblotting - Tissues were homogenized in RIPA buffer, and equal amounts of proteins were separated on SDS-PAGE under reducing conditions, and transferred to a nitrocellulose membrane (Whatman). Proteins of interest were detected using the following antibodies: OXCT1 (ab105320; Abcam), ACAT1 (HPA004428; Sigma), eEF2 (2332; Cell signaling), Polyclonal Swine Anti-Rabbit Immunoglobulins/HRP (P0399, Dako). Densitometric analysis of immunoblots was performed on 6 individual samples using Image-J software, and a representative selection from this group is presented in each figure.

Cell culture - C2C12 myoblasts were grown to confluence in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum and 1% penicillin/streptomycin (P/S). Cells were differentiated for 4 days in DMEM containing 2% horse serum and 1% P/S. All experiments were performed on differentiated C2C12 myotubes. Three independent experiments were performed in triplicates. PGC-1α was overexpressed by transduction with either GFP-PGC-1α or GFP (control) adenovirus. ERRα was knocked down using shERRα or

shLacZ (control) adenovirus. Cells were harvested 48 hours after infection. Myotubes were treated with either 10μM XCT-790 (Sigma) or 0.2% DMSO (vehicle) for 48 hours, or 30μm DY131 (Sigma) or 0.2% DMSO (vehicle) for 24 hours.

Transcription factor binding sites prediction - Based on our previously published results from ChIP-Seq PGC-1α occupancy in muscle cells (12), a 10 kb long region located around the Oxct1 transcription start site (on chromosome 15 from 3'974'000 to 3'984'000 bp, mm9 genome assembly) was scanned for ERRα transcription factor binding sites (TFBSs). In order to reduce false positive predictions, this 10 kb long region was aligned using the software T-Coffee (13) to its orthologous regions from 6 different mammalian species (human, opossum, dog, rhesus macaque, horse and cow) and the resulting alignments were scanned with the motif search tool MotEvo (14) to predict TFBSs for the ERRα transcription factor (whose weight matrix was downloaded from the SwissRegulon database (15)).

Statistical analysis - Data are presented as means ± SEM. Unpaired student two-tailed t-test was used to determine differences between groups. Significance was considered with $p<0.05$.

Results

PGC-1α is a regulator of ketolytic gene transcription – Ketolytic capacity is high in oxidative organs such as brain, kidney, heart and skeletal muscle (2). To investigate the role of PGC-1α in ketolytic gene transcription in these organs, we used mice with a global deletion of PGC-1α (gKO) (10). Ablation of PGC-1α led to reduced transcript levels of ketolytic genes (e.g. *Bdh1*, *Oxct1* and *Acat1)* in brain, kidney, heart and skeletal muscle (Fig. 1A). Since, skeletal muscle is an important tissue for maintenance of systemic KB homeostasis (2), we chose to investigate the role of skeletal muscle PGC-1α in systemic KB homeostasis. To this end, we used mice with a skeletal muscle-specific deletion of PGC-1α (mKO) (16). Similar to gKO mice (Fig. 1A), PGC-1α mKO mice displayed reduced transcription of ketolytic genes in both soleus (Fig. 1B) and gastrocnemius (Fig. 1C) muscles. We also detected reduced protein levels of OXCT1 and ACAT1 in gastrocnemius muscle (Fig. 1D). However, ketolytic gene transcription was similar in heart and kidney of mKO and control mice (SI Fig. 1A and B). In line with previous findings (17, 18), loss of PGC-1α led to a reduced transcription of genes involved in skeletal muscle lactate and KB uptake (*Mct1*) and TCA cycle genes (*Cs, Aco2, Idh3a)* important for mitochondrial oxidation of KBs (Fig. 1E). No difference could be seen for transcription of genes involved in non-oxidative KB metabolism (*Aacs*, *Acat2*) (Fig.1 E). Thus, PGC-1α regulates a broad transcriptional program in muscle necessary for KB uptake and mitochondrial KB oxidation. To test whether ablation of PGC-1α affects ketolytic capacity, we injected mice with βOHB and measured its clearance from the blood. Supporting a physiological relevance of the reduced ketolytic gene transcription in muscle, PGC-1α mKO mice showed an impaired βOHB excursion rate (Fig. 1F). Thus, PGC-1α mKO mice display reduced ketolytic gene transcription in muscle and ketolytic insufficiency.

PGC-1α mKO mice exhibit hyperketonemia in response to fasting and ketogenic diet-feeding – We next investigated whether loss of PGC-1α in muscle impacts systemic adaptation to physiological ketogenic stimuli, e.g. fasting and ketogenic diet feeding. PGC-1α mKO displayed a significant hyperketonemia compared to control mice after 24 hours of food withdrawal, in regard to both βOHB (Fig. 2A) and AcAc (Fig. 2B). These findings support the hypothesis of a ketolytic insufficient phenotype in mice lacking a functional PGC-1α gene in muscle. Intriguingly, this hyperketonemic phenotype was also observed in PGC-1α mKO mice after three weeks of low-carbohydrate ketogenic diet (LCKD) feeding (Fig. 2C). We also examined the ketogenic response in liver during fasting and LCKD feeding. In contrast to ketolytic gene transcription in muscle, control and PGC-1α mKO mice showed a similar induction of β-oxidation- and ketogenic gene programs in liver in response to both fasting

(Fig. 2D) and LCKD feeding (Fig. 2E). These findings indicate that the hyperketonemic phenotype of PGC-1α mKO mice is not due to alterations in the hepatic ketogenic response to either fasting or LCKD feeding.

PGC-1α is necessary for the improved systemic ketolytic capacity with exercise training – Since PGC-1α mKO mice displayed hyperketonemia during fasting and LCKD feeding, we investigated whether this also occurs in response to other ketogenic stimuli, e.g. cold exposure and exercise. In line with our earlier findings (Fig. 2A-C), PGC-1α mKO mice developed hyperketonemia compared to control mice during cold exposure (Fig. 3A) and after an acute exercise bout (Fig. 3B). These data imply that muscle PGC-1α is important for systemic KB homeostasis, regardless of the ketogenic stimulus. Interestingly, exacerbated post-exercise ketosis is associated with an untrained phenotype in both rodents and humans, and can be ameliorated with exercise training (19, 20). Skeletal muscle PGC-1α could therefore be important for the adaptation of systemic ketolytic capacity with exercise training. To test this hypothesis, control and PGC-1α mKO mice had free access to running wheels for 8 weeks (trained group), while sedentary mice were kept in identical cages without running wheels. We first investigated the transcriptional profile in skeletal muscle from trained and sedentary mice. In line with previous reports (21), we observed a PGC-1α dependent induction of genes involved in oxidative phosphorylation (*Uqcrc2*, *Sdhb*, *Ndufb8*) and TCA-cycle (*Aco2*, *Idh3a*) in muscle from trained compared to sedentary mice (Fig. 3C). Interestingly, also ketolytic gene (*Bdh1*, *Oxct1* and *Acat1)* transcription was significantly induced in muscle from trained compared to sedentary control mice, and this induction was blunted in trained PGC-1α mKO mice compared to control mice (Fig. 3D). To test whether the altered transcriptional profiles in muscle from control and PGC-1α mKO mice would affect systemic ketolytic capacity we injected mice with βOHB and measured its clearance from the blood. Importantly, while ketolytic capacity was improved in trained control mice, as indicated by the enhanced βOHB excursion rate (Fig. 3E), PGC-1α mKO mice displayed no improvement in ketolytic capacity with exercise (Fig. 3F). Thus, skeletal muscle PGC-1α is important for transcriptional induction of ketolytic genes in muscle and for improvement of systemic ketolytic capacity in trained mice.

Elevation of PGC-1α levels in skeletal muscle improves systemic ketolytic capacity and ameliorates diabetic hyperketonemia – Since increased transcription of ketolytic genes in skeletal muscle correlated with an enhanced ketolytic capacity in trained mice, we were interested whether muscle-specific overexpression of PGC-1α in sedentary mice could

enhance the systemic ketolytic capacity. For this, we used mice with a skeletal muscle-specific overexpression of PGC-1α (mTG mice) (9). PGC-1α mTG mice displayed an enhanced transcription of ketolytic genes (*Bdh1*, *Oxct1* and *Acat1)* (Fig. 4A) and elevated protein levels of OXCT1 and ACAT1 in skeletal muscle (Fig. 4B). We also detected a minor overexpression of PGC-1α in heart of PGC-1α mTG mice (SI Fig. 1C). However, this was only associated with an induction of *Bdh1* transcription, and did not further affect transcript levels of *Acat1* or *Oxct1* (SI Fig. 1C). In kidney, no alterations in transcript levels of either PGC-1α or ketolytic genes were observed in PGC-1α mTG mice (SI Fig. 1D). In skeletal muscle, PGC-1α mTG mice displayed increased transcript levels of genes involved in lactate and KB uptake (*Mct1*) and TCA cycle genes (*Cs, Aco2, Idh3a)* important for mitochondrial oxidation of KBs (Fig. 4C). While *Aacs* mRNA levels were unaltered in muscle from PGC-1α mTG mice, *Acat2* transcription showed a small but significant induction (Fig. 4C). Thus, PGC-1α overexpression in muscle exerts only a minor transcriptional effect on genes involved in non-oxidative KB metabolism. Moreover, in line with the induction of a ketolytic gene program in muscle, PGC-1α mTG mice showed an enhanced βOHB excursion rate after βOHB injection (Fig. 4D), and thus an enhanced systemic ketolytic capacity. Importantly, in response to physiological ketogenic stimuli, such as fasting (Fig. 4E and F), LCKD feeding (Fig.4G) and exercise (Fig. 4H), mTG mice exhibited significantly reduced KB levels compared to control mice. Finally, in analogy with our findings in PGC-1α mKO mice (Fig. 2D and E), mTG mice displayed a similar induction of β-oxidation- and ketogenic gene programs in liver in response to LCKD feeding (SI Fig. 1E) compared to control mice. Thus, elevation of PGC-1α levels increases expression of ketolytic enzymes in skeletal muscle and is sufficient to enhance the systemic ketolytic capacity in mice without altering the hepatic ketogenic response.

Since elevation of PGC-1α levels in skeletal muscle led to reduced circulating KB levels, we hypothesized that activation of muscle PGC-1α could reduce diabetic hyperketonemia, which is a common and often fatal complication in type 1 diabetic patients (5). To assess whether elevated levels of PGC-1α in skeletal muscle reduced circulating KB levels during diabetes, we induced type 1 diabetes by injecting streptozotocin (STZ) (22) in both control and mTG mice. STZ-injected mice developed hallmark signs of type 1 diabetes, such as hyperglycemia (Fig. 5A), insulinopenia (Fig. 5B), elevated levels of free fatty acids in blood (Fig. 5C) and hyperketonemia (Fig. 5D). Importantly, while glucose, insulin and free fatty acid levels in blood were comparable between control and PGC-1α mTG mice, overexpression of PGC-1α in skeletal muscle efficiently reduced circulating βOHB levels in

diabetic mice (Fig. 5D). Furthermore, the reduced ketonemia in PGC-1α mTG mice could not be attributed to an impaired induction of β-oxidation- and ketogenic gene programs in liver of diabetic mice (Fig. 5E). Thus, without affecting the underlying insulin deficiency caused by the STZ injection, overexpression of PGC-1α in skeletal muscle can ameliorate hyperketonemia in type 1 diabetic mice.

PGC-1α regulates ketolytic gene transcription through ERRα – Next, we aimed to identify the transcriptional partner of PGC-1α involved in the regulation of ketolytic gene transcription. Through overexpression of PGC-1α in C2C12 myotubes, we could confirm that elevated PGC-1α levels results in induction of ketolytic gene transcription (*Bdh1*, *Oxct1* and *Acat1*) (Fig. 6A). Overexpression of PGC-1α also led to a concomitant increase in ERRα transcription in C2C12 myotubes (Fig. 6A). Since ERRα is a known transcriptional partner of PGC-1α in the regulation of mitochondrial and metabolic gene programs (23), we hypothesized that ERRα is also involved in the regulation of ketolytic gene transcription. To investigate the basal role of ERRα in ketolytic gene transcription, we performed a knockdown of ERRα in C2C12 myotubes using shERRα adenovirus. Knockdown of ERRα reduced transcript levels of both *Oxct1* and *Acat1*, while *Bdh1* transcription was unaffected (Fig. 6B). In contrast, activation of ERRβ and ERRγ with the dual ERRβ/γ agonist DY131 did not affect ketolytic gene transcription, while transcript levels of ERRγ and of the known ERR target gene pyruvate dehydrogenase kinase isozyme 4 (*Pdk4*) (24) were increased (Fig. 6C). Hence, activation of ketolytic gene transcription is specific for the ERRα-isoform. To substantiate the role of ERRα as a transcriptional partner of PGC-1α in the regulation of ketolytic gene transcription, we knocked down ERRα using shERRα adenovirus and concomitantly overexpressed PGC-1α (SI Fig. 1F), or in a second setup, overexpressed PGC-1α in C2C12 myotubes in the presence of the inverse ERRα-agonist XCT790 (25) (Fig. 6D). In both setups, knockdown or inhibition of ERRα abrogated the induction of several shared PGC-1α/ERRα target genes such as *ERRα* and *Idh3a* (SI Fig. 1F and Fig. 6D). Importantly, inhibition of ERRα blocked the induction of ketolytic genes (*Bdh1*, *Oxct1, Acat1*) elicited by PGC-1α overexpression (SI Fig. 1F and Fig. 6D), indicating that ERRα is important for the induction of ketolytic gene transcription by PGC-1α in muscle cells. Finally, to elucidate whether *Bdh1*, *Oxct1* and *Acat1* are directly regulated by interaction of PGC-1α and ERRα, we investigated data-sets on genome-wide occupancy of PGC-1α (12) and ERRα (unpublished data). Surprisingly, we could not detect any peaks for either PGC-1α or ERRα in the vicinity of the *Acat1* and *Bdh1* genes (data not shown), indicating that these genes are most likely indirectly regulated by PGC-1α and ERRα in skeletal muscle. On the other hand, we found overlapping

peaks for PGC-1α and ERRα within the *Oxct1* gene (Fig. 6E), indicating that these transcriptional regulators are bound to the DNA at the same site within the *Oxct1* gene. Moreover, by using MotEvo (14) to predict transcription factor binding sites, a putative response element for ERRα was identified within the genomic region occupied by PGC-1α and ERRα in the *Oxct1* gene (Fig. 6E). These findings indicate that PGC-1α and ERRα likely regulate *Oxct1* transcription by directly binding to this gene.

Discussion

KBs are vital metabolic substrates during states of reduced carbohydrate availability (1). Systemic KB levels are determined by the interplay of hepatic ketone body production and KB utilization in extra-hepatic organs. We now show that PGC-1α is a transcriptional regulator of ketolytic enzymes in several extra-hepatic organs, and that skeletal muscle PGC-1α determines systemic KB homeostasis in contexts of elevated KB levels, including fasting, cold exposure and exercise (Fig. 7). It is important to note that the overall effect of muscle PGC-1α on systemic KB homeostasis is likely due to two interrelated factors: 1) The role of PGC-1α in transcription of KB transporters and ketolytic enzymes in muscle, and 2) the established role of PGC-1α for the transcription of related mitochondrial metabolic pathways, such as TCA cycle and oxidative phosphorylation (8). PGC-1α thus regulates transcription of several interconnected metabolic pathways in muscle necessary for complete oxidation of KBs. Importantly, we could show that elevated PGC-1α levels in skeletal muscle reduces pathophysiological hyperketonemia in mice. Aberrant regulation of KB levels in type 1 diabetic patients can lead to diabetic ketoacidosis, which is a potentially fatal complication arising from exacerbated lipolysis and elevated ketogenesis associated with diabetic insulinopenia. Thus, patients suffering from acute diabetic ketoacidosis are primarily treated with insulin to reverse the insulin-deficient state (5). In analogy with the KB-lowering effect of exercise in diabetic rats (26), we now report that elevation of muscle PGC-1α levels reduces hyperketonemia in a mouse model of type 1 diabetes. These findings imply that chronic elevation of PGC-1α levels in muscle could help reduce the intensity of hyperketonemic bouts in type 1 diabetic patients. Here, it is important to point out that diabetic ketoacidosis is an acute and life-threatening condition, which requires immediate treatment. Thus, acute activation of PGC-1α in muscle would not be a feasible therapeutic strategy in this case. However, by chronically increasing the basal levels of PGC-1α in muscle through regular exercise training (27), and consequently increasing the ketolytic capacity of muscle, this would be useful as a preventive measure to reduce the incidence and intensity of hyperketonemic bouts in diabetic patients. In this case, it is also important to distinguish between the beneficial effects of continuous exercise training, compared to the acute metabolic alterations associated with exercise, which could precipitate or even exacerbate a diabetic hyperketonemic bout (28).

Exercise can modulate the systemic response to ketosis, which is evident by the enhanced KB tolerance (29) and resistance to post-exercise ketosis in trained humans (20) and rodents (19). Resistance to post-exercise ketosis with exercise has been attributed to a sparing

of liver glycogen content during exercise (19). However, reduction in post-exercise ketosis could also be mediated by adaptations of cardiac and skeletal muscle, two main consumers of KBs. In line with this, endurance training in rats enhances KB uptake into muscle and increases the levels of ketolytic enzymes in muscle (30, 31). We now demonstrate that muscle PGC-1α plays an important role in the adaptation of ketolytic capacity with long-term exercise training. These findings underline the importance of skeletal muscle in the regulation of KB homeostasis, and imply PGC-1α as a major regulator of this process. Additionally, we show that transcription of ketolytic genes is either directly or indirectly controlled by the interaction of PGC-1α with ERRα. It is well established that ERRα and PGC-1α stimulate the transcription of genes involved in several metabolic processes in skeletal muscle, such as oxidative phosphorylation, lactate metabolism and fatty acid β-oxidation (18, 25, 32). Thus, the functional interaction between PGC-1α and ERRα in the regulation of ketolysis most likely allows an integrated coordination of different metabolic pathways in skeletal muscle and other organs. It would thus be interesting in future studies to evaluate the role of PGC-1α and ERRα in the regulation of KB homeostasis in other ketolytic organs, such as the brain. This is of particular importance in the context of long-term fasting, where KB oxidation is essential for maintenance of energy homeostasis in neurons (1).

In summary, we have identified PGC-1α as a transcriptional regulator of KB oxidation in skeletal muscle (Fig. 7). Moreover, we show that muscle PGC-1α plays an important role in the regulation of systemic KB levels, both in physiological contexts of KB utilization, but also in pathological settings such as diabetic ketoacidosis. Hence, activation of PGC-1α in skeletal muscle could have an important therapeutic impact by reducing the extent of hyperketonemic bouts in disease states characterized by pathological elevation of KB levels, e.g. type 1 diabetes. However, until pharmacological means to therapeutically elevate PGC-1α in skeletal muscle become available, regular exercise training is the safest and most efficient way to increase the baseline ketolytic capacity in this organ and thus affect systemic KB homeostasis.

Acknowledgments

We thank Prof. Anastasia Kralli from the Scripps Research Institute, La Jolla, for the shERRα adenovirus used in this study.

Funding

This project was funded by the ERC Consolidator grant 616830-MUSCLE_NET, the Swiss National Science Foundation, SystemsX.ch, the Swiss Society for Research on Muscle Diseases (SSEM), the "Novartis Stiftung für medizinisch-biologische Forschung", the University of Basel and the Biozentrum. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Duality of interest

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

Contribution statement

K.S. and C.H. designed research and wrote the manuscript; K.S., V.A., B.C. and S.S. performed research and analyzed data.

References

1. Cahill, G. F., Jr. (2006) Fuel metabolism in starvation. *Annu. Rev. Nutr.* **26**, 1-22
2. Cotter, D. G., Schugar, R. C., and Crawford, P. A. (2013) Ketone body metabolism and cardiovascular disease. *American journal of physiology. Heart and circulatory physiology* **304**, H1060-1076
3. Shafqat, N., Kavanagh, K. L., Sass, J. O., Christensen, E., Fukao, T., Lee, W. H., Oppermann, U., and Yue, W. W. (2013) A structural mapping of mutations causing succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency. *J. Inherited Metab. Dis.* **36**, 983-987
4. Cotter, D. G., d'Avignon, D. A., Wentz, A. E., Weber, M. L., and Crawford, P. A. (2011) Obligate role for ketone body oxidation in neonatal metabolic homeostasis. *J. Biol. Chem.* **286**, 6902-6910
5. Westerberg, D. P. (2013) Diabetic ketoacidosis: evaluation and treatment. *Am. Fam. Physician* **87**, 337-346
6. Kamel, K. S., and Halperin, M. L. (2015) Acid-base problems in diabetic ketoacidosis. *The New England journal of medicine* **372**, 546-554
7. Newman, J. C., and Verdin, E. (2014) Ketone bodies as signaling metabolites. *Trends Endocrinol. Metab.* **25**, 42-52
8. Austin, S., and St-Pierre, J. (2012) PGC1alpha and mitochondrial metabolism--emerging concepts and relevance in ageing and neurodegenerative disorders. *J. Cell Sci.* **125**, 4963-4971
9. Lin, J., Wu, H., Tarr, P. T., Zhang, C. Y., Wu, Z., Boss, O., Michael, L. F., Puigserver, P., Isotani, E., Olson, E. N., Lowell, B. B., Bassel-Duby, R., and Spiegelman, B. M. (2002) Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* **418**, 797-801
10. Lin, J., Wu, P. H., Tarr, P. T., Lindenberg, K. S., St-Pierre, J., Zhang, C. Y., Mootha, V. K., Jager, S., Vianna, C. R., Reznick, R. M., Cui, L., Manieri, M., Donovan, M. X., Wu, Z., Cooper, M. P., Fan, M. C., Rohas, L. M., Zavacki, A. M., Cinti, S., Shulman, G. I., Lowell, B. B., Krainc, D., and Spiegelman, B. M. (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* **119**, 121-135
11. Handschin, C., Choi, C. S., Chin, S., Kim, S., Kawamori, D., Kurpad, A. J., Neubauer, N., Hu, J., Mootha, V. K., Kim, Y. B., Kulkarni, R. N., Shulman, G. I., and Spiegelman, B. M. (2007) Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J. Clin. Invest.* **117**, 3463-3474
12. Baresic, M., Salatino, S., Kupr, B., van Nimwegen, E., and Handschin, C. (2014) Transcriptional Network Analysis in Muscle Reveals AP-1 as a Partner of PGC-1alpha in the Regulation of the Hypoxic Gene Program. *Mol. Cell. Biol.* **34**, 2996-3012
13. Notredame, C., Higgins, D. G., and Heringa, J. (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **302**, 205-217
14. Arnold, P., Erb, I., Pachkov, M., Molina, N., and van Nimwegen, E. (2012) MotEvo: integrated Bayesian probabilistic methods for inferring regulatory sites and motifs on multiple alignments of DNA sequences. *Bioinformatics* **28**, 487-494
15. Pachkov, M., Balwierz, P. J., Arnold, P., Ozonov, E., and van Nimwegen, E. (2013) SwissRegulon, a database of genome-wide annotations of regulatory sites: recent updates. *Nucleic Acids Res.* **41**, D214-220
16. Perez-Schindler, J., Summermatter, S., Santos, G., Zorzato, F., and Handschin, C. (2013) The transcriptional coactivator PGC-1alpha is dispensable for chronic

overload-induced skeletal muscle hypertrophy and metabolic remodeling. *Proc Natl Acad Sci U S A* **110**, 20314-20319
17. Burgess, S. C., Leone, T. C., Wende, A. R., Croce, M. A., Chen, Z., Sherry, A. D., Malloy, C. R., and Finck, B. N. (2006) Diminished hepatic gluconeogenesis via defects in tricarboxylic acid cycle flux in peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha)-deficient mice. *J. Biol. Chem.* **281**, 19000-19008
18. Summermatter, S., Santos, G., Perez-Schindler, J., and Handschin, C. (2013) Skeletal muscle PGC-1alpha controls whole-body lactate homeostasis through estrogen-related receptor alpha-dependent activation of LDH B and repression of LDH A. *Proc Natl Acad Sci U S A* **110**, 8738-8743
19. Adams, J. H., and Koeslag, J. H. (1988) Carbohydrate homeostasis and post-exercise ketosis in trained and untrained rats. *The Journal of physiology* **407**, 453-461
20. Johnson, R. H., Walton, J. L., Krebs, H. A., and Williamson, D. H. (1969) Metabolic fuels during and after severe exercise in athletes and non-athletes. *Lancet* **2**, 452-455
21. Geng, T., Li, P., Okutsu, M., Yin, X., Kwek, J., Zhang, M., and Yan, Z. (2010) PGC-1alpha plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle. *Am. J. Physiol. Cell Physiol.* **298**, C572-579
22. McNeill, J. H. (1999) *Experimental models of diabetes*, CRC Press LLC, Boca Raton, Fla.
23. Villena, J. A., and Kralli, A. (2008) ERRalpha: a metabolic function for the oldest orphan. *Trends in endocrinology and metabolism: TEM* **19**, 269-276
24. Zhang, Y., Ma, K., Sadana, P., Chowdhury, F., Gaillard, S., Wang, F., McDonnell, D. P., Unterman, T. G., Elam, M. B., and Park, E. A. (2006) Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. *The Journal of biological chemistry* **281**, 39897-39906
25. Mootha, V. K., Handschin, C., Arlow, D., Xie, X., St Pierre, J., Sihag, S., Yang, W., Altshuler, D., Puigserver, P., Patterson, N., Willy, P. J., Schulman, I. G., Heyman, R. A., Lander, E. S., and Spiegelman, B. M. (2004) Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 6570-6575
26. El Midaoui, A., Chiasson, J. L., Tancrede, G., and Nadeau, A. (2005) Physical training reverses defect in 3-ketoacid CoA-transferase activity in skeletal muscle of diabetic rats. *Am J Physiol Endocrinol Metab* **288**, E748-752
27. Pilegaard, H., Saltin, B., and Neufer, P. D. (2003) Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. *The Journal of physiology* **546**, 851-858
28. Pugliese, G., Zanuso, S., Alessi, E., Simonelli, P., Fallucca, S., Fallucca, F., and Balducci, S. (2009) Self glucose monitoring and physical exercise in diabetes. *Diabetes/metabolism research and reviews* **25 Suppl 1**, S11-17
29. Johnson, R. H., and Walton, J. L. (1972) The effect of exercise upon acetoacetate metabolism in athletes and non-athletes. *Quarterly journal of experimental physiology and cognate medical sciences* **57**, 73-79
30. Ohmori, H., Kawai, K., and Yamashita, K. (1990) Enhanced ketone body uptake by perfused skeletal muscle in trained rats. *Endocrinologia japonica* **37**, 421-429
31. Winder, W. W., Baldwin, K. M., and Holloszy, J. O. (1974) Enzymes involved in ketone utilization in different types of muscle: adaptation to exercise. *Eur. J. Biochem.* **47**, 461-467

32. Huss, J. M., Torra, I. P., Staels, B., Giguere, V., and Kelly, D. P. (2004) Estrogen-related receptor alpha directs peroxisome proliferator-activated receptor alpha signaling in the transcriptional control of energy metabolism in cardiac and skeletal muscle. *Mol. Cell. Biol.* **24**, 9079-9091

Figure legends

Figure 1 - PGC-1α mKO mice display reduced expression of ketolytic enzymes in skeletal muscle

Experiments performed with control and (A) PGC-1α gKO mice or (B-F) PGC-1α mKO. (A) Gene expression in brain, kidney, heart and gastrocnemius normalized to *18s* (n=5/group). (B-C) Gene expression in (B) soleus and (C) gastrocnemius muscle normalized to *18s* (n=8/group). (D) Representative immunoblots of OXCT1, ACAT1 and eEF2 in gastrocnemius. Graph shows quantification of band intensities of OXCT1 and ACAT1 relative to eEF2 (n=6/group). (E) Gene expression in gastrocnemius normalized to *18s* (n=8/group). (F) Blood βOHB levels after an intraperitoneal βOHB-injection (n=6/group). Error bars represent mean ±SEM. Significant differences (p-value<0.05) between genotypes are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Figure 2 – Skeletal muscle PGC-1α modulates systemic ketone body homeostasis

Experiments performed with control and PGC-1α mKO mice. (A-B) Blood β-hydroxybutyrate (βOHB) (A) or plasma acetoacetate (AcAc) (B) levels in fed or 24-hour fasted mice (n=7-8/group). (C) βOHB levels in mice fed either chow or a LCKD for 3 weeks (n=7-13/group). (D) Liver gene expression from *ad libitum* fed or 24 hour fasted mice, normalized to *18s* (n=6-9/group). (E) Liver gene expression of mice fed a chow diet or LCKD diet for 3 weeks, normalized to *18s* (n=7-8/group). Error bars represent mean ±SEM. Significant differences (p-value<0.05) between genotypes are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Figure 3 - Skeletal muscle PGC-1α is necessary for improved systemic ketolytic capacity with exercise training

Experiments performed with control and PGC-1α mKO mice. (A) Blood βOHB levels of mice at room temperature (RT) or 4°C (Cold exp.) (n=5-8/group). (B) βOHB levels pre-exercise and at 0/30/180 minutes post-exercise (n=5/group). (C-D) Gastrocnemius gene expression normalized to *18s* (n=6-8/group). (E-F) Blood βOHB levels after an intraperitoneal βOHB-injection in sedentary or trained (E) control and (F) PGC-1α mKO mice (n=6/group). Error bars represent mean ±SEM. Significant differences (p-value<0.05) between genotypes are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Figure 4 - Muscle-specific overexpression of PGC-1α increases systemic ketolytic capacity and ameliorates diabetic hyperketonemia

Experiments performed with control and PGC-1α mTG mice. (A) Gene expression in gastrocnemius muscle normalized to *18s* (n=8/group). (B) Representative immunoblots of OXCT1, ACAT1 and eEF2 in gastrocnemius muscle. Bar graph shows quantification of band intensities of OXCT1 and ACAT1 relative to eEF2 (n=6/group). (C) Gene expression in gastrocnemius muscle normalized to *18s* (n=8/group). (D) Blood βOHB levels after an intraperitoneal βOHB-injection (n=10-11/group). (E-F) Blood βOHB (E) or plasma AcAc (F) levels in fed or 24-hour fasted mice (n=7-10/group). (G) βOHB levels in mice fed a chow or LCKD diet for 3 weeks (n=6-8/group). (H) βOHB levels pre-exercise and at 0/30/180 minutes post-exercise (n=6/group). Error bars represent mean ±SEM. Significant differences (p-value<0.05) between genotypes are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Figure 5 - Muscle-specific overexpression of PGC-1α ameliorates hyperketonemia in diabetic mice

Experiments performed with control and PGC-1α mTG mice. (A-D) Blood glucose (A), plasma insulin (B), plasma free fatty acids (C) and blood βOHB levels (D) in mice 3 days after injection with either citrate buffer (CB) or 150 mg/kg streptozotocin (STZ) (n=7-17/group). (E) Liver gene expression of mice injected with either CB or STZ, normalized to *Tbp* (n=6-9/group). Error bars represent mean ±SEM. Significant differences (p-value<0.05) between genotypes are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Figure 6 - PGC-1α regulates ketolytic gene transcription through co-activation of ERRα

(A) C2C12 myotubes transduced with GFP or GFP-PGC-1α adenovirus. Gene expression normalized to *18s*. (B) ERRα was knocked down in C2C12 myotubes using shERRα adenovirus or shLacZ (control). Gene expression normalized to *RPL0*. (C) C2C12 myotubes treated for 24 hours with 30μM DY131 or 0.2% DMSO (VEH). Gene expression normalized to *eEF2*. (D) C2C12 myotubes transduced with GFP or GFP-PGC-1α adenovirus, and co-treated with 0.2% DMSO (VEH) or 10μM XCT-790 (XCT). Gene expression normalized to *18s*. (E) ChIP-Seq peaks, depicted as read profiles for PGC-1α (dark blue) and ERRα (light blue). The most prominent peak is located ~7kb downstream of the *Oxct1* transcription start site (TSS) and within the first intron. The predicted binding site for ERRα is represented by a

yellow circle and its genomic position is referred to the *Oxct1* TSS. The bottom panel represents an enlargement of the above relevant regions. Error bars represent mean ±SEM. Significant differences (p-value<0.05) between treated and control condition are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Figure 7 - PGC-1α is an important regulator of skeletal muscle ketone body oxidation.

(a) PGC-1α/ERRα directly regulates transcription of *Oxct1,* while *Acat1* and *Bdh1* are indirectly regulated (b) Modulation of PGC-1α levels in skeletal muscle alters systemic ketone body levels in response to either fasting, ketogenic diet feeding or exercise (c) PGC-1α levels in skeletal muscle modulate expression of proteins important for mitochondrial KB uptake and oxidation, such as *Mct1*, *Bdh1*, *Oxct1*, *Acat1* and components of the TCA-cycle.

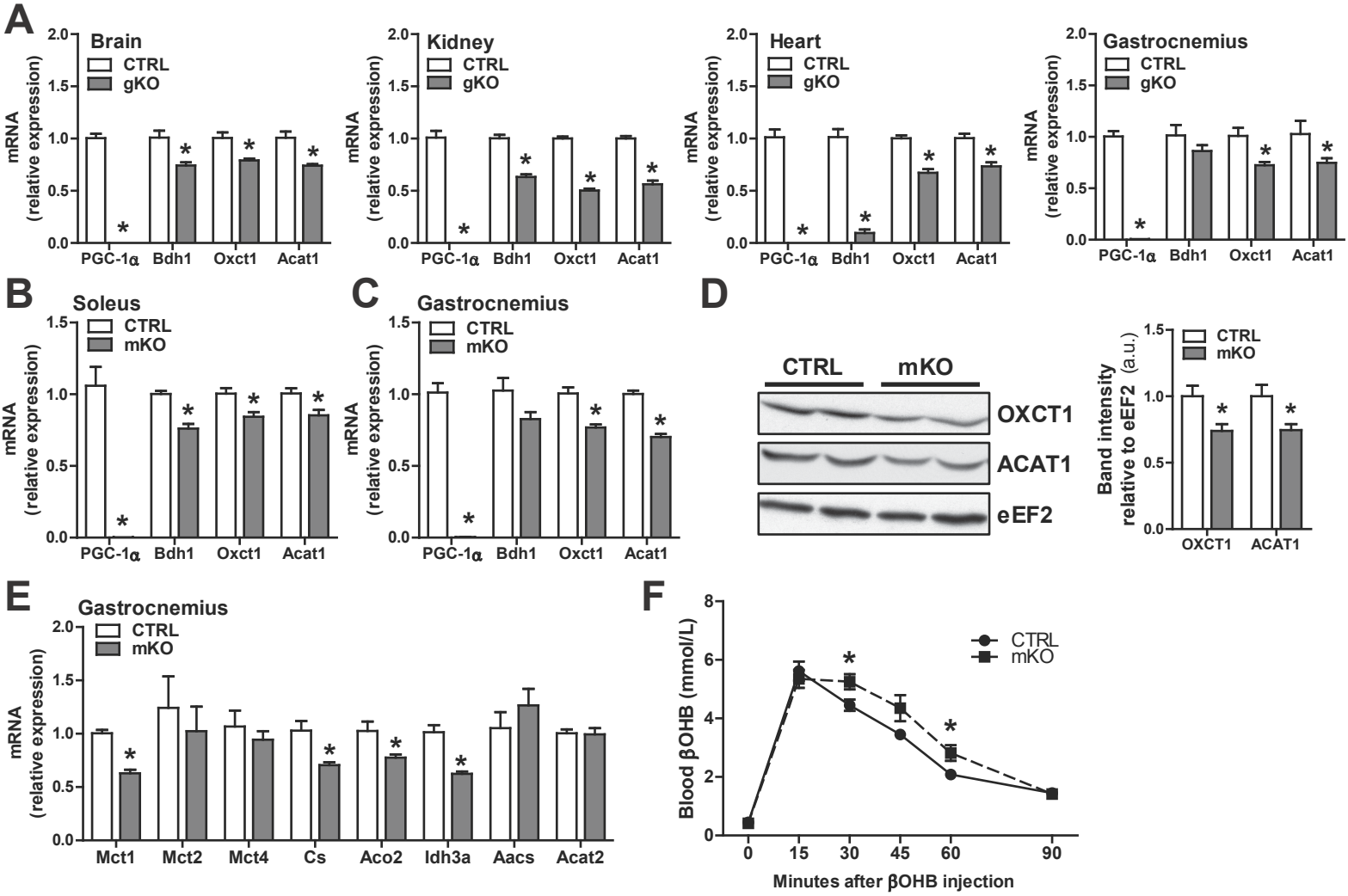
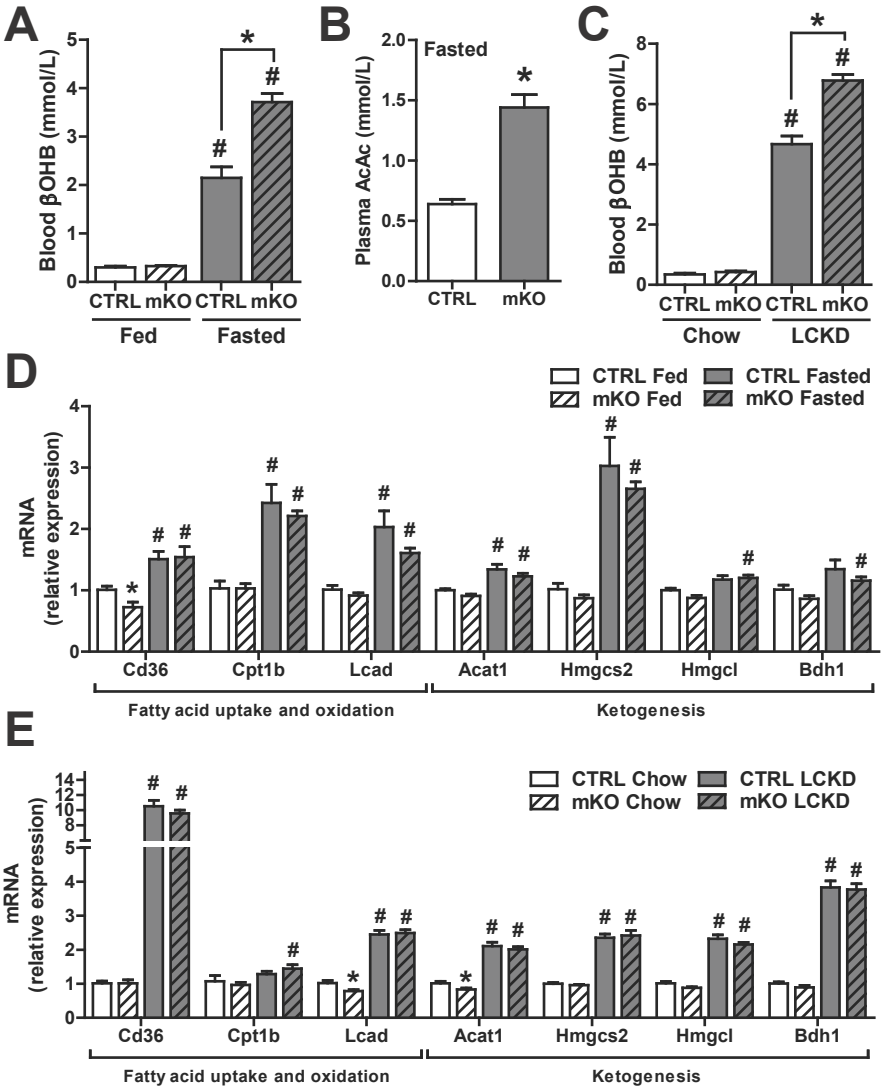

A
Brain
Kidney
Heart
Gastrocnemius
CTRL
gKO
mRNA (relative expression)
PGC-1α
Bdh1
Oxct1
Acat1
B
Soleus
mKO
C
Gastrocnemius
D
CTRL
mKO
OXCT1
ACAT1
eEF2
Band intensity relative to eEF2 (a.u.)
E
Gastrocnemius
Mct1
Mct2
Mct4
Cs
Aco2
Idh3a
Aacs
Acat2
F
Blood βOHB (mmol/L)
Minutes after βOHB injection

Figure 1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CTRL mKO CTRL mKO
Fed
Fasted
B
Fasted
Plasma AcAc (mmol/L)
CTRL
mKO
C
Blood βOHB (mmol/L)
CTRL mKO CTRL mKO
Chow
LCKD
D
CTRL Fed
mKO Fed
CTRL Fasted
mKO Fasted
mRNA (relative expression)
Cd36
Cpt1b
Lcad
Acat1
Hmgcs2
Hmgcl
Bdh1
Fatty acid uptake and oxidation
Ketogenesis
E
CTRL Chow
mKO Chow
CTRL LCKD
mKO LCKD
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Cd36
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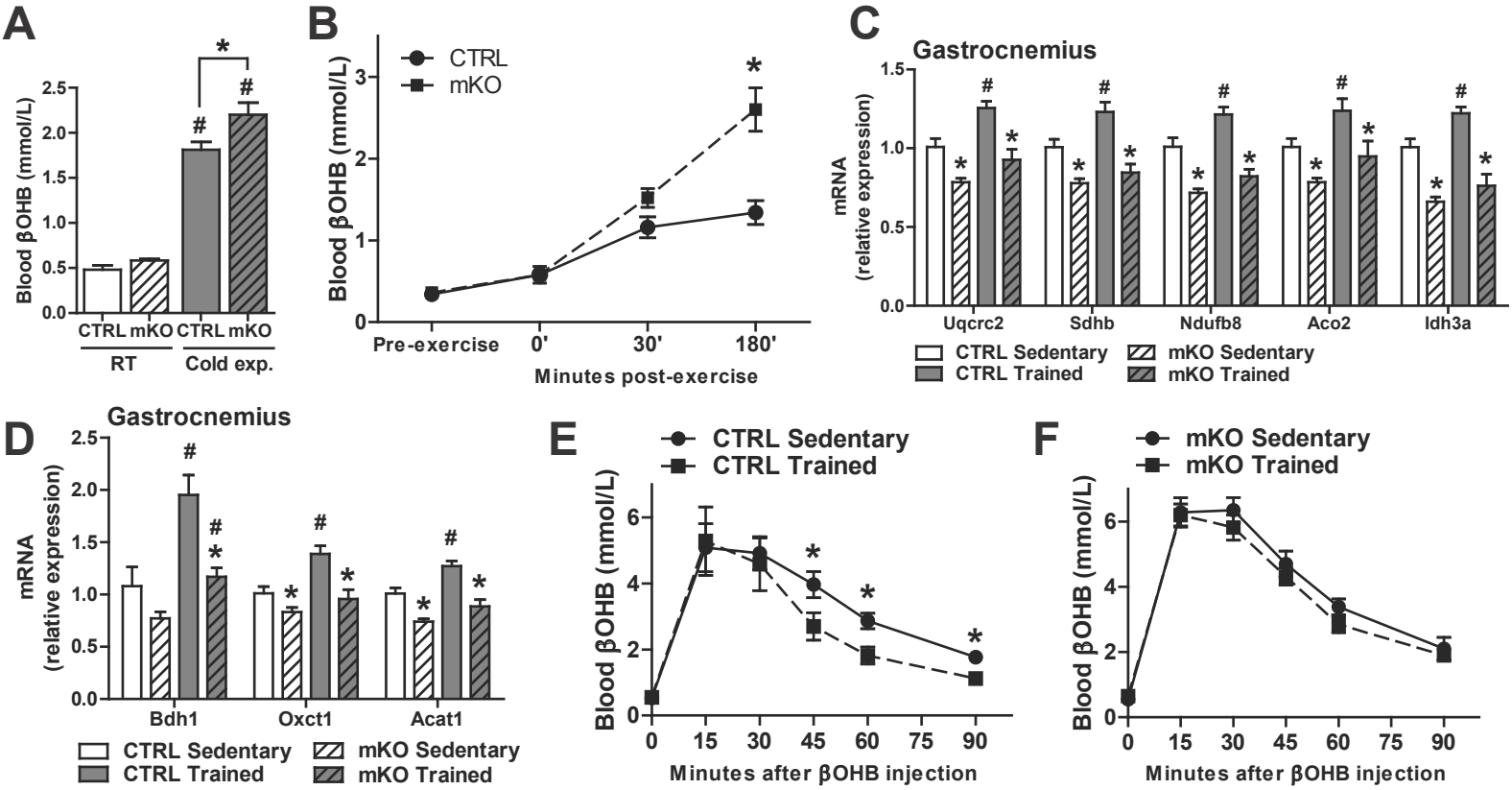
Bdh1
Fatty acid uptake and oxidation
Ketogenesis

Figure 2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Blood βOHB (mmol/L)
CTRL mKO CTRL mKO
RT
Cold exp.
B
CTRL
mKO
Blood βOHB (mmol/L)
Pre-exercise
0'
30'
180'
Minutes post-exercise
C
Gastrocnemius
mRNA (relative expression)
Uqcrc2
Sdhb
Ndufb8
Aco2
Idh3a
CTRL Sedentary
CTRL Trained
mKO Sedentary
mKO Trained
D
Gastrocnemius
mRNA (relative expression)
Bdh1
Oxct1
Acat1
CTRL Sedentary
CTRL Trained
mKO Sedentary
mKO Trained
E
CTRL Sedentary
CTRL Trained
Blood βOHB (mmol/L)
Minutes after βOHB injection
F
mKO Sedentary
mKO Trained
Blood βOHB (mmol/L)
Minutes after βOHB injection

Figure 3

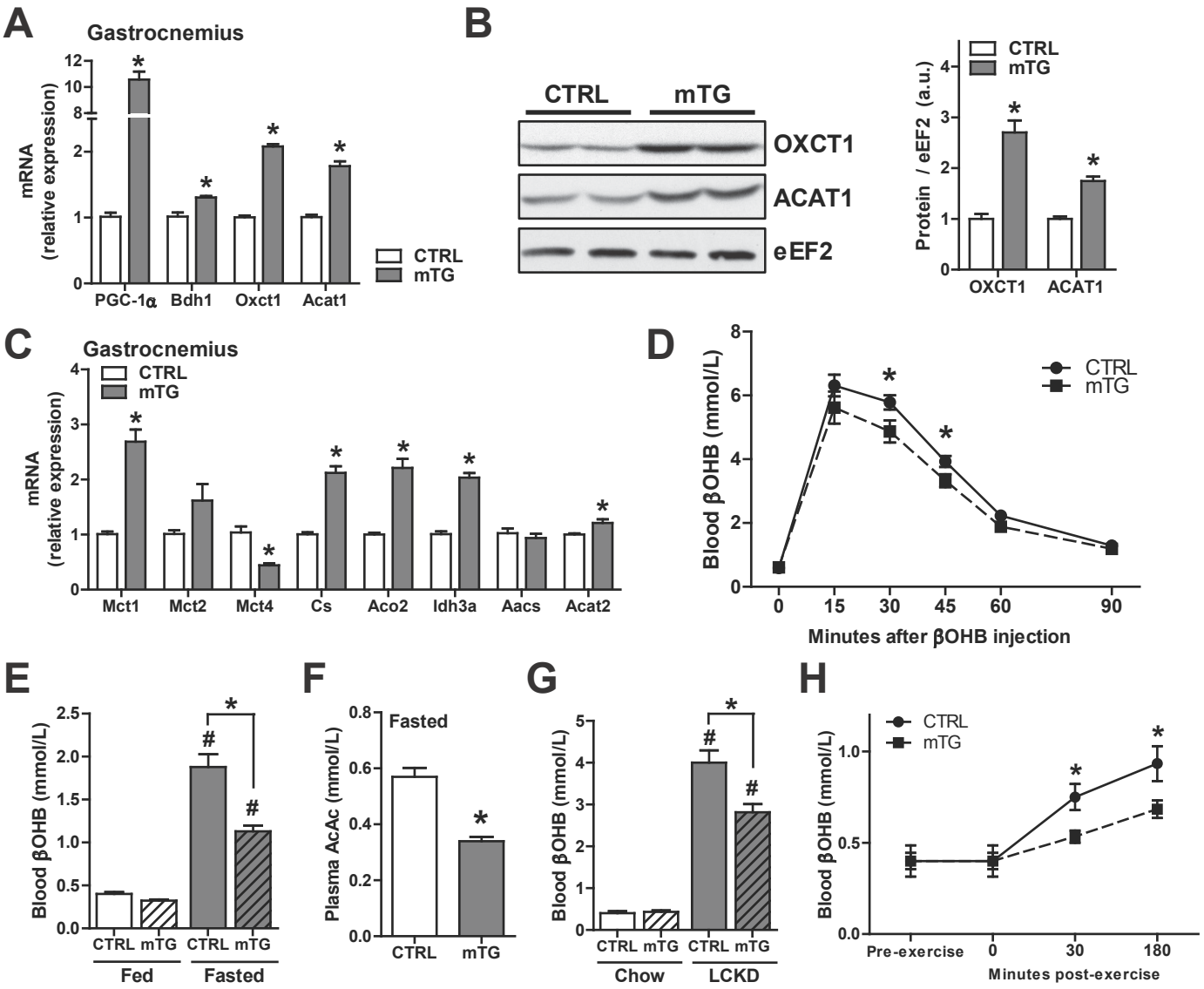
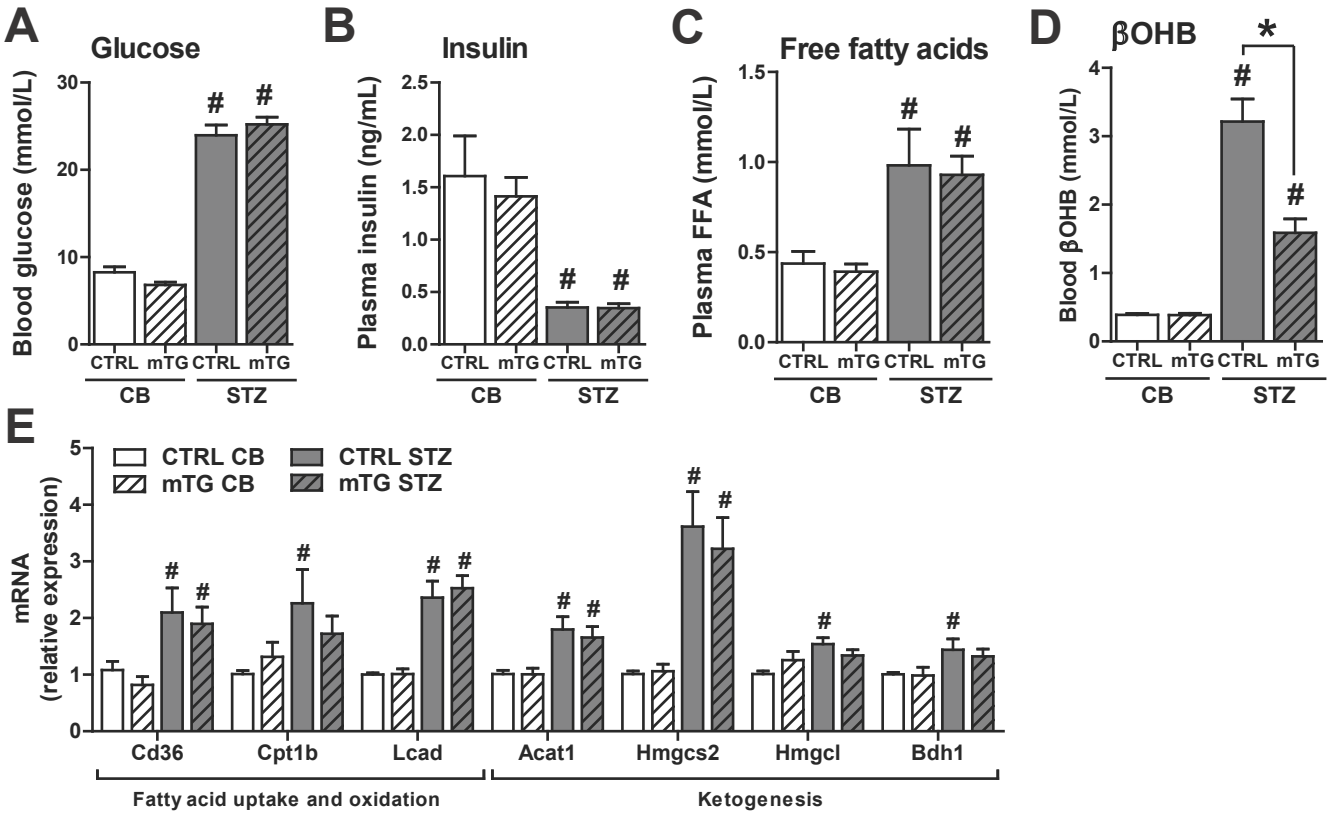

A
Gastrocnemius
mRNA (relative expression)
PGC-1α
Bdh1
Oxct1
Acat1
CTRL
mTG
B
CTRL
mTG
OXCT1
ACAT1
eEF2
Protein / eEF2 (a.u.)
C
Gastrocnemius
mRNA (relative expression)
Mct1
Mct2
Mct4
Cs
Aco2
Idh3a
Aacs
Acat2
D
Blood βOHB (mmol/L)
Minutes after βOHB injection
E
Blood βOHB (mmol/L)
Fed
Fasted
F
Fasted
Plasma AcAc (mmol/L)
G
Blood βOHB (mmol/L)
Chow
LCKD
H
Blood βOHB (mmol/L)
Pre-exercise
Minutes post-exercise

Figure 4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Glucose
Blood glucose (mmol/L)
30
20
10
0
#
#
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CB
STZ
B
Insulin
Plasma insulin (ng/mL)
2.5
2.0
1.5
1.0
0.5
0.0
#
#
CTRL mTG CTRL mTG
CB
STZ
C
Free fatty acids
Plasma FFA (mmol/L)
1.5
1.0
0.5
0.0
#
#
CTRL mTG CTRL mTG
CB
STZ
D
βOHB
*
Blood βOHB (mmol/L)
4
3
2
1
0
#
#
CTRL mTG CTRL mTG
CB
STZ
E
CTRL CB
mTG CB
CTRL STZ
mTG STZ
mRNA (relative expression)
5
4
3
2
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Acat1
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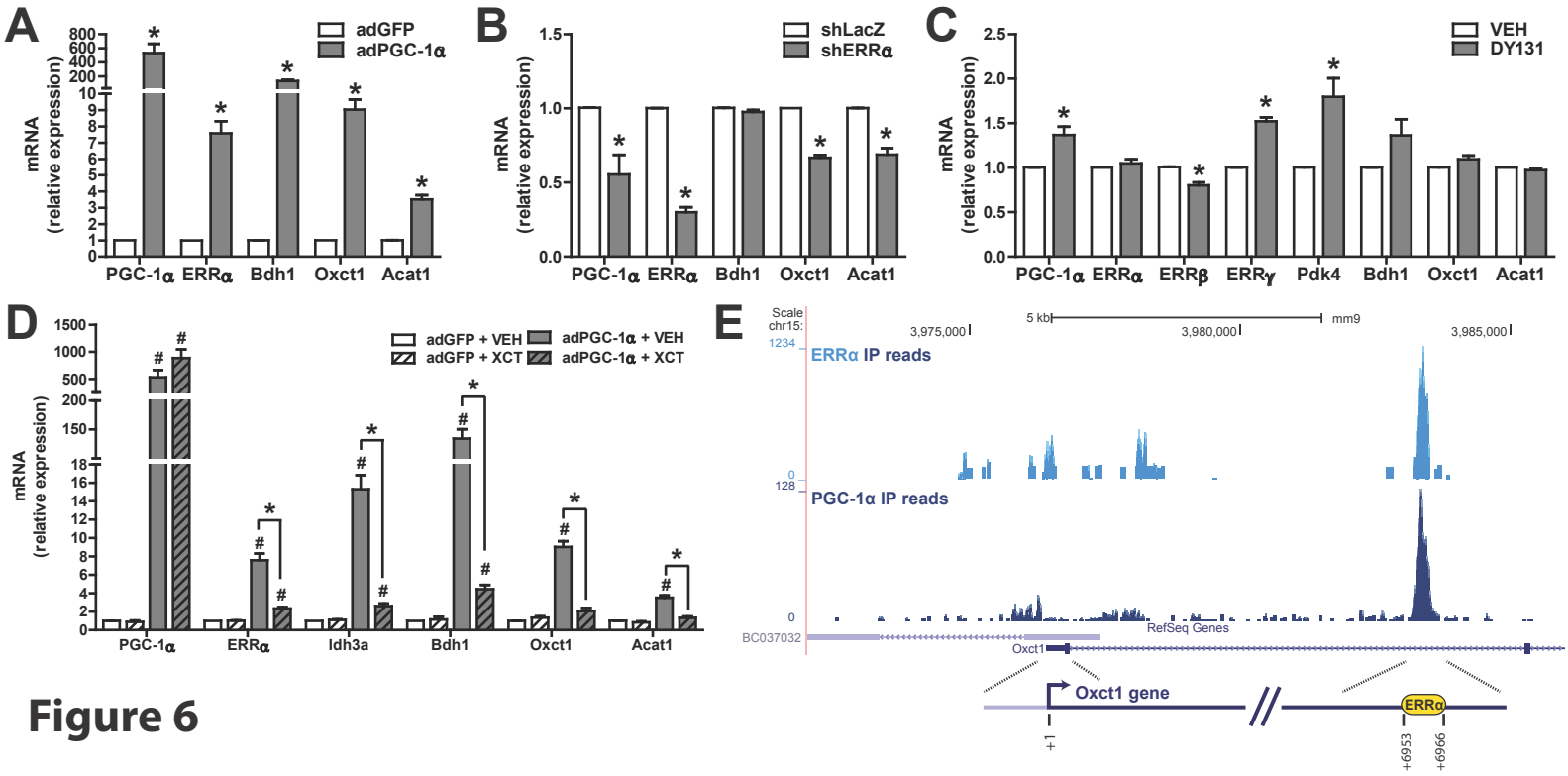
Hmgcl
Bdh1
Fatty acid uptake and oxidation
Ketogenesis

Figure 5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mRNA (relative expression)
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B
shLacZ
shERRα
mRNA (relative expression)
PGC-1α ERRα Bdh1 Oxct1 Acat1
C
VEH
DY131
mRNA (relative expression)
PGC-1α ERRα ERRβ ERRγ Pdk4 Bdh1 Oxct1 Acat1
D
adGFP + VEH
adGFP + XCT
adPGC-1α + VEH
adPGC-1α + XCT
mRNA (relative expression)
PGC-1α ERRα Idh3a Bdh1 Oxct1 Acat1
E
Scale
chr15:
3,975,000
5 kb
mm9
3,980,000
3,985,000
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Oxct1 gene
ERRα
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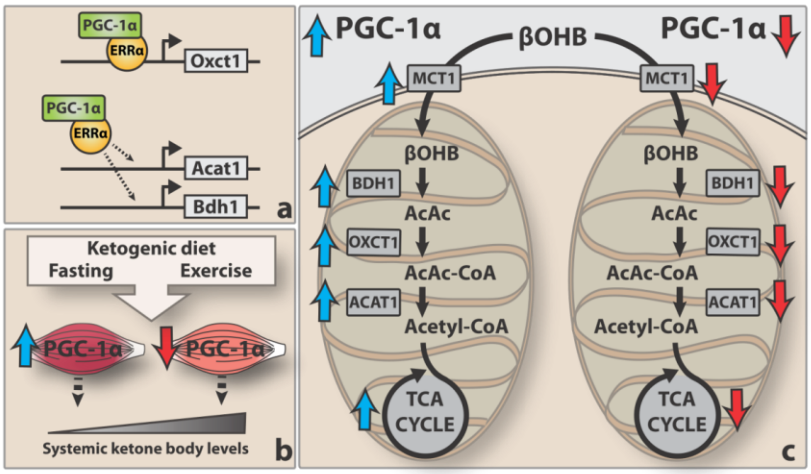
+6953
+6966

Figure 6


PGC-1α
ERRα
Oxct1
PGC-1α
ERRα
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Bdh1
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Ketogenic diet
Fasting
Exercise
PGC-1α
PGC-1α
Systemic ketone body levels
b
PGC-1α
βOHB
PGC-1α
MCT1
MCT1
βOHB
BDH1
AcAc
OXCT1
AcAc-CoA
ACAT1
Acetyl-CoA
TCA CYCLE
βOHB
BDH1
AcAc
OXCT1
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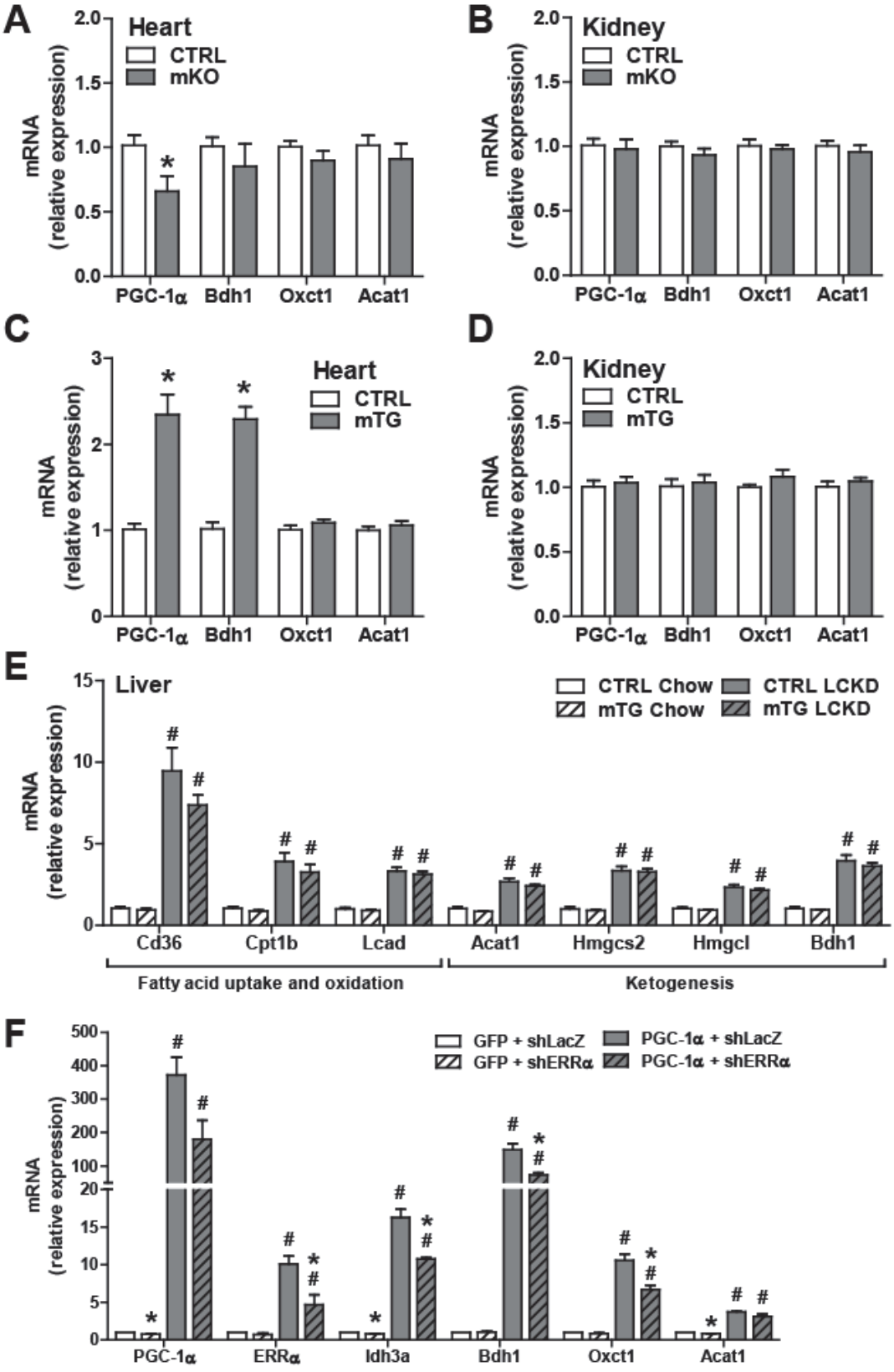
ACAT1
Acetyl-CoA
TCA CYCLE
c

Figure 7

Supplemental data



Supplemental data Figure 1

(A-B) Gene expression in heart (A) and kidney (B) from PGC-1α mKO mice, normalized to *18s* (n=6/group). (C-D) Gene expression in heart (C) and kidney (D) from PGC-1α mTG mice, normalized to *18s* (n=7/group). (E) Gene expression in liver from control and PGC-1α mTG mice, normalized to *18s* (n=6/group). (F) C2C12 myotubes transduced with adenovirus expressing either GFP or shERRα. GFP or shLacZ were used as controls. Gene expression normalized to *Tbp*. Error bars represent mean ±SEM. Significant differences (p-value<0.05) between genotypes are indicated by an asterisk (*) and between experimental conditions by a number-sign (#).

Table S1. qPCR Primer sequences

Target gene	Forward primer	Reverse primer
Aacs	AAGGACATGAAGCCAGTGGAAA	ACTCGTAACTCTGGGCTTTCAG
Acat1	GTGAAGGAAGTCTACATGGGCA	TGTGGTGCATGGAGTGGAAATA
Acat2	ATTCAAAACATGGGGATTCGGC	TCAATGGGAAACCGAGAGACAG
Aco2	ACATTGTCCGTAAACGGTTGAA	TATGTCTTTCCCCGCTCGATCT
Bdh1	TTTGCTGGCTGTTTGATGAAGG	TTGAGCTGGATGGTTCTCAGTC
Cd36	GGCAAAGAACAGCAGCAAAAT	TGGCTAGATAACGAACTCTGTATGTGT
Cpt1b	ATCATGTATCGCCGCAAACT	CCATCTGGTAGGAGCACATGG
Cs	CCCAGGATACGGTCATGCA	GCAAACTCTCGCTGACAGGAA
Errα	CGGTGTGGCATCCTGTGA	CTCCCCTGGATGGTCCTCTT
Errβ	CAGATCGGGAGCTTGTGTTC	TGGTCCCCAAGTGTCAGACT
Errγ	GGAAGAATTCGTCACCCTCA	TTCTGCACAGCTTCCACATC
Hmgcl	CCAGCTTTGTTTCTCCCAAGTG	GATGCCGGGAAACTTCTGAATG
Hmgcs2	CCACAAGGTGAACTTCTCTCCA	TGCATCTCATCCACTCGTTCAA
Idh3a	GCTGGTGGTGTTCAGACAGTAA	CACTGAATAGGTGCTTTGGCAG
Lcad	CCAGCTAATGCCTTACTTGGAGA	GCAATTAAGAGCCTTTCCTGTGG
Mct1	TGCAACGACCAGTGAAGTATCA	ACAACCACCAGCGATCATTACT
Mct2	AGGAAACCCCCAAAGCAAGAG	GGAAGGCTCTGATGGCATTTC
Mct4	AGAGCACTTAAAGTCGCCCCC	GGGCTGCTTTCACCTGTTACC
Ndufb8	CAAGAAGTATAACATGCGAGTGGAA	CCATACCCCATGCCATCATC
Oxct1	CCCATACCCACTGAAAGACGAA	CTGGAGAAGAAAGAGGCTCCTG
Pdk4	AAAATTTCCAGGCCAACCAA	CGAAGAGCATGTGGTGAAGGT
PGC-1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Sdhb	TGACGTCAGGAGCCAAAATGG	CCTCGACAGGCCTGAAACTG
Uqcrc2	CCCATCTTGCTTTGCTGTCTG	AATAAAATCTCGAGAAGGACCCG