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Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) promotes skeletal muscle lipid refueling *in vivo* by activating *de-novo* lipogenesis and the pentose phosphate pathway

Serge Summermatter[1,2], Oliver Baum[3], Gesa Santos[1], Hans Hoppeler[3], and Christoph Handschin[1,2]

[1]Biozentrum, Department of Pharmacology/Neurobiology, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland

[2]Institute of Physiology and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

[3]Institute of Anatomy, University of Bern, Baltzerstrasse 2, CH-3009 Bern, Switzerland

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Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) promotes skeletal muscle lipid refueling *in vivo* by activating *de-novo* lipogenesis and the pentose phosphate pathway

Serge Summermatter[1,2], Oliver Baum[3], Gesa Santos[1], Hans Hoppeler[3], and Christoph Handschin[1,2]

[1]Biozentrum, Department of Pharmacology/Neurobiology, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland

[2]Institute of Physiology and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

[3]Institute of Anatomy, University of Bern, Baltzerstrasse 2, CH-3009 Bern, Switzerland

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Address correspondence to: Christoph Handschin, Division of Pharmacology/Neurobiology, Biozentrum, University of Basel, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland, *Phone*: +41 61 267 2378, *Fax*: +41 61 267 2208, *Email*: christoph.handschin@unibas.ch

Exercise induces a pleiotropic adaptive response in skeletal muscle, largely through the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α). PGC-1α enhances lipid oxidation and thereby provides energy for sustained muscle contraction. Its potential implication in promoting muscle refueling remains, however, unresolved. Here, we investigated a possible role of elevated PGC-1α levels in skeletal muscle lipogenesis *in vivo* and the molecular mechanisms that underlie PGC-1α-mediated *de-novo* lipogenesis. To this end, we studied transgenic mice with physiological overexpression of PGC-1α and human muscle biopsies pre- and post exercise. We demonstrate that PGC-1α enhances lipogenesis in skeletal muscle through liver x receptor α (LXRα) dependent activation of the fatty acid synthase (FAS) promoter and by increasing FAS activity. Using chromatin immunoprecipitation, we establish a direct interaction between PGC-1α and the LXR-responsive element in the FAS promoter. Moreover, we show for the first time that increased glucose uptake and activation of the pentose phosphate pathway provide substrates for RNA synthesis and cofactors for *de-novo* lipogenesis. Similarly, we observed increased lipogenesis and lipid levels in human muscle biopsies that were obtained post-exercise. Our findings suggest that PGC-1α coordinates lipogenesis, intramyocellular lipid accumulation and substrate oxidation in exercised skeletal muscle *in vivo*.

Over the last century, people have become increasingly sedentary. This development has resulted in a marked rise in the prevalence of metabolic disorders (1). Inversely, exercise is an excellent intervention in metabolic diseases. However, the molecular mechanisms that underlie the health beneficial effects of exercise are unclear.

Chronic endurance exercise elicits substantial adaptations in skeletal muscle, including changes in metabolic and myofibrillar properties (2). The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a crucial regulator of this adaptive response (3-5). Accordingly, exercise leads to a rapid and marked increase in PGC-1α levels in skeletal muscle (6,7). Subsequently, PGC-1α interacts with a broad range of different transcription factors (8) and thereby promotes adaptive processes (9,10). Chronic PGC-1α induction drives fiber-type switching from fast, glycolytic towards slow, oxidative fibers (11), increases aerobic work performance (12) and confers higher resistance to fatigue in skeletal muscle preparations *ex vivo* (11). Interestingly, muscle-specific over-expression of PGC-1α *per se*, even in the absence of physical activity or a functional motor neuron, is sufficient to drive these changes, which are typical of regular endurance training (11-13).

Higher intramyocellular lipid (IMCL) content is observed in endurance athletes and constitutes a typical adaptation to chronic endurance exercise (14). These lipids are recruited as energy source during prolonged physical activity (15,16). While a central role for PGC-1α in enhancing lipid catabolism is well established (9,10), it is currently not known whether elevated PGC-1α affects lipid anabolism in trained skeletal muscle *in vivo*. A recent *in-vitro* study in myoblasts suggested that adenoviral over-expression of PGC-1α is paralleled by increased gene

expression of fatty acid synthase (17). Thus, PGC-1α might play a role in muscle lipid refueling, at least in a cell culture model under hyperglycemic conditions (17). Currently, it is unresolved whether PGC-1α can induce FAS transcription *in vivo,* whether there is a direct relationship between activation of PGC-1α and lipogenesis, how this could be brought about mechanistically and how this process is fueled.

We therefore studied the effect of stably elevated muscle PGC-1α levels on *de-novo* lipogenesis *in vivo* in a transgenic mouse model that allows the dissociation between the consequences of elevated muscle PGC-1α and other confounding factors induced by exercise (11). In particular, we have tested the effect of PGC-1α on fatty acid synthase (FAS), the multifunctional enzyme that catalyzes all seven reactions of *de-novo* lipogenesis, as well as on glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose phosphate pathway. The latter produces NADPH, which serves as reducing agent for lipogenesis. Moreover, we investigated the molecular mechanisms that underlie these adaptations. We focused our efforts on the function of PGC-1α *in vivo* in the *extensor digitorum longus* muscle (EDL), which represents a typical glycolytic muscle that naturally expresses low levels of PGC-1α (7,11). Gain-of-function of PGC-1α in EDL muscle results in PGC-1α protein levels that are comparable to those normally observed in oxidative muscles (11). The main findings of this study in the PGC-1α muscle-specific transgenic mouse model were subsequently validated in human muscle biopsies that were obtained pre- and post-exercise.

Materials and Methods

Animals

MPGC-1α TG mice (11) and control littermates were maintained in a conventional facility with a fixed 12-h light/dark cycle on a commercial pelleted chow diet and free access to tap water. Studies were performed according to criteria outlined for the care and use of laboratory animals and with approval of the Swiss authorities.

Human muscle biopsies

Human biopsies were obtained from vastus lateralis muscle in the course of a previously published study (18). All volunteers gave their written consent to participate in the study and the experiments were approved by the ethics committee of the University of Bern. In brief, all subjects trained on an electrically braked cycle ergometer at a constant load during 30 minutes per day, 5 days per week for a total of 6 weeks with 85% of maximal heart rate after 10 minutes of exercise and lactate levels between 4-6mmol/l. Biopsies were taken from vastus lateralis muscle before and after the 6-wk training period using the Bergstrom technique. Muscle tissue was immediately frozen in liquid nitrogen, and stored at -80°C for later RNA extraction.

Lipid quantification by electron microscopy

Quantification of muscle lipids was performed as described previously (19).
Glutaraldehyde-fixed samples were dehydrated in increasing ethanol concentrations and embedded in Epon. After cutting ultrathin sections (50–70 nm) on a LKB Ultrotome III, uranyl acetate and lead citrate staining was performed. The sections were then photographed and examined. We analyzed 15 micrographs of one section per animal.

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 ABI Prism 7000 Sequence Detector. Relative expression levels for each gene of interest were calculated with the ΔΔCt method and normalized to the expression of the Tata box binding protein (TBP). Primer sequences are listed in *Supplementary Table T1*.

De-novo lipogenesis

De-novo lipogenesis was assayed according to the method of Stansbie *et al*. (20). In brief, $^{3}H_2O$ (25μCi/g bw) were injected intraperitoneally and 1h later the animals were sacrificed. Muscles were dissected, saponified in ethanolic KOH, and acidified with H_2SO_4. Fatty acids were extracted with light petroleum and radioactivity was quantified using a Beckman liquid scintillation counter. The contralateral muscle was used to assay the incorporation of tritium into different lipid classes as described by Fungwe *et al*. (21).

Glucose uptake

Glucose uptake was determined using tritium labeled 2-deoxyglucose (Amersham Biosciences) according to the protocol of Hansen *at al*. (22). Radioactivity was quantified using a Beckman liquid scintillation counter.
All values were corrected for initial tissue weight and expressed as fold change compared to control animals.

Muscle metabolites (NADP, NADPH, Glutathione, ROS, glucose-6-phosphate)
NADP and NADPH levels were assessed colorimetrically using EnzyChrom $NADP^+$/NADPH Assay Kit from BioAssay Systems. Reduced, oxidized and total glutathione levels were determined using Glutathione Assay Kit from BioVision Research products. Glucose-6-phosphate was determined as described by Wende *et al*. (23).

Enzymatic activities
Muscles were dissected, immediately frozen in liquid nitrogen and homogenized under liquid nitrogen. Fatty acid synthase (FAS) activity was measured photometrically according to the method described by Pénicaud *et al*. (24). Glucose 6-phosphate dehydrogenase (G6PDH) activity was determined according to the method of Ninfali *et al.* (25).

Incorporation of glucose into macromolecules
Incorporation of [6-^{3}H] glucose (Amersham Biosciences) into RNA and DNA was measured according to the method described by Glazer and Weber (26).

Cell culture experiments
Mouse C_2C_{12} myoblasts were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 in subconfluent culture. For reporter gene assays, C_2C_{12} myoblasts were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Renilla luciferase was used for normalization. Cells were harvested 24 hrs after transfection and luciferase activity was measured using Dual-Glo Luciferase assay (Promega). LXRα siRNA was purchased from Santa Cruz.
C_2C_{12} myoblasts were differentiated into myotubes and then infected with adenoviral vectors for murine green fluorescent protein (GFP) or GFP- PGC-1α.

Chromatin Immunoprecipitation (ChIP) assay
ChIP assays were performed using ChIP-IT Express (Active Motif) according to the manufacturer's instructions. Chromatin was subjected to immunoprecipitation using anti-PGC-1α antibodies (PGC-1 (H-300), Santa Cruz) or unrelated IgG bound to protein G-coupled magnetic beads. Precipitated DNA was recovered and amplified by RT-PCR with primers flanking the LXRE or SREBP binding site, respectively.

Data analysis and statistics
All data are presented as means ± SE. The data were analyzed by 2-tailed, unpaired Student's *t* test or Mann-Whitney test when the difference between the two SDs was significantly different. For the analyses of human samples, 2-tailed, paired Student's *t* test was used. Levels of significance are indicated as follows: *** $p<0.001$, ** $p< 0.01$, * $p< 0.05$.
For the analysis of reporter gene assays, two-factor ANOVA was performed. Post hoc pairwise comparison was done by unpaired Student's *t* test.

Results

Elevated de-novo lipogenesis and lipid accumulation in glycolytic muscle of MPGC-1α TG mice

We first tested whether PGC-1α affects gene expression and/or activity of skeletal muscle fatty acid synthase (FAS) *in vivo*. MPGC-1α TG mice show higher levels of FAS expression (+50%, $P<0.05$) (**Figure 1A**) and enzymatic activity (+131%, $P<0.001$) (**Figure 1B**) in skeletal muscle than their control littermates along with increased *de-novo* lipogenesis (+44%, $P<0.01$) (**Figure 1C**) and elevated intramyocellular lipid content (+157%, $P<0.01$) (**Figure 1D**). Detailed analysis of the different lipid components revealed higher levels of *de-novo* synthesized free fatty acids and triglycerides in skeletal muscle of MPGC-1α TG mice (**Supplementary Figure 1A** and **B**). Since muscle often contains interspersed adipose cells that express high levels of FAS and show high rates of lipogenesis, we validated FAS expression, activity and *de-novo* lipogenesis in cultured skeletal muscle cells overexpressing PGC-1α. As observed in muscle *in vivo*, PGC-1α also increases FAS mRNA expression, activity and *de-novo* lipogenesis directly in muscle cells (**Supplementary Figure 2A, B** and **C**).

To test whether mRNA levels of genes involved in lipid uptake are concomitantly altered in MPGC-1α TG mice, we quantified the mRNA levels of lipoprotein lipase (LPL), cluster of differentiation 36 (CD36), plasma membrane fatty acid binding protein (FABPpm) and different fatty acid transporter proteins (FATPs). Of those, only CD36, FABPpm and FATP4 are significantly elevated in transgenic animals (**Figure 1E**). Then, we determined the level of acetyl-CoA synthase (ACS), which activates imported lipids by linking them to Coenzyme A. ACS mRNA expression is not different between control and transgenic animals (**Figure 1E**). Finally, expression of genes implicated in fatty acid esterification, namely acetyl-CoA:diacylglycerol transferase 1 (DGAT1) and mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) are increased in MPGC-1α TG mice (+51% and +150% respectively, P<0.05) (**Figure 1E**) while other mediators of lipid synthesis, such as lipin, remain unaltered (**Supplementary Figure 3**).

PGC-1α directly regulates the FAS promoter through coactivation of the Liver X Receptor α/ Retinoid X Receptor α (LXRα/RXRα)

As a consequence of the elevated FAS transcription in MPGC-1α TG animals (**Figure 1A**), a possible regulatory effect of PGC-1α on the FAS proximal promoter was investigated. Our data indicate that PGC-1α increases FAS promoter activity and that this effect of PGC-1α is potentiated by the concomitant expression of LXRα/RXRα, but not of SREBP1c (**Figure 2A**). Silencing of LXRα or deletion of the LXR-responsive element in the FAS promoter (ΔLXRE) abrogate the effect of PGC-1α on FAS transcription (**Figure 2B**). In contrast, mutation of the SREBP1c binding site did not reduce the effect of PGC-1α on LXRα/RXRα-induced FAS transcription (**Supplementary Figure 4**). Moreover, LXRα, RXRα and RXRβ expression levels are significantly elevated, while the sterol responsive element binding protein 1c (SREBP1c) is unaltered in skeletal muscle of MPGC-1α TG animals (**Figure 2C**). Using chromatin immunoprecipitation (ChIP) followed by RT-PCR we demonstrate that in MPGC-1α TG animals PGC-1α is enriched in the LXRE of the FAS proximal promoter (**Figure 2D**). In contrast, PGC-1α was not recruited to the SREBP binding site in the FAS promoter of MPGC-1α TG and control animals (**Figure 2D**).

Increased glucose utilization in glycolytic muscle of MPGC-1α TG mice

Besides FAS activation, important cofactors for lipid biosynthesis need to be delivered through glucose metabolism and sustained glucose uptake is required for lipogenesis to proceed. We analyzed different key steps of glucose uptake at the transcriptional level as well as glucose uptake directly. Gene expression of GLUT4, the main insulin-sensitive glucose transporter in muscle, and hexokinase 2, the enzyme used to trap glucose in muscle, is higher in MPGC-1α TG mice compared to controls (+50% and +93% respectively, P<0.05) while expression of the second major glucose transporter in muscle (GLUT1) is unchanged (**Figure 3A**). Moreover, glucose uptake as measured by the intracellular accumulation of tritiated deoxyglucose is elevated in MPGC-1α TG animals (+48%, P<0.05) (**Figure 3B**).

Enhanced glucose-6-phosphate dehydrogenase activity in MPGC-1α TG mice

We speculated that increased glucose uptake in MPGC-1α TG mice leads to intracellular accumulation of glucose-6-phosphate (G6P). Indeed, MPGC-1α TG mice show higher levels of muscle G6P than their control littermates (**Supplementary Figure 5A**). G6P concomitantly serves as initial substrate and thereby as an activator of the pentose phosphate pathway. Thus, we measured the expression and enzymatic activity of glucose-6-phosphate dehydrogenase (G6PDH). PGC-1α gain-of-function does not affect G6PDH gene expression (**Figure 3C**), but leads to increased enzymatic activity (+158%, P<0.05) (**Figure 3D)**. To validate whether PGC-1α increases G6PDH activity in muscle cells, we assessed G6PDH in isolated muscle cells overexpressing PGC-1α. Similar to our findings in muscle *in vivo*, PGC-1α augments G6PDH activity directly in muscle cells (**Supplementary Figure 5B**).

Increased synthesis of NADPH, GSH and ribose-5-phosphate in MPGC-1α TG mice

The pentose phosphate pathway is the major anaplerotic pathway of NADPH. NADPH serves as reducing agent for glutathione and is furthermore required for *de-novo* lipogenesis. Thus, consistent with the higher G6PDH activity

in muscle of MPGC-1α TG mice, we found significantly elevated NADPH with a parallel reduction in $NADP^+$ concentration compared to controls (**Figure 4A** and **B**) ($P<0.05$ for both). Consequently, we also observed higher levels of total and reduced glutathione (**Figure 4C** and **D**) in MPGC-1α TG mice ($P<0.05$). In contrast, the amount of oxidized glutathione is similar in MPGC-1α TG and control animals (**Figure 4E**).

In addition to its role for NADPH and glutathione production, the pentose phosphate pathway plays a crucial role in nucleotide biosynthesis by producing ribose-5-phosphate. Therefore, tritium labeled glucose was used to test whether glucose diverted toward the pentose phosphate pathway is partially incorporated into nucleotides as ribose-5-phosphate. While tritium labeled glucose is used as a substrate for RNA synthesis in MPGC-1α TG mice (+65%, $P<0.05$) **(Figure 4F),** no changes are observed for incorporation of glucose metabolites into DNA (**Figure 4G)**. Furthermore, gene expression of ribonucleotide reductase, a transcriptionally regulated gene involved in converting ribonucleotides to deoxy-ribonucleotides, is not different between MPGC-1α TG mice and control littermates (**Figure 4H)**.

Improved capacity for de-novo lipogenesis in endurance athletes

Endurance athletes display high levels of intramyocellular lipids; however, the origin of these lipids is unknown. Similar to the MPGC-1α TG animals, FAS gene expression is elevated in human muscle post exercise (**Figure 5A**). Importantly, the induction of FAS gene expression is paralleled by an increased level of intramyocellular lipids (**Figure 5B**) after 6 weeks of endurance training. In contrast, ACS levels are not altered in human muscle post exercise (**Figure 5C**). Consistent with our mouse date (**Figure 2C**), we observed elevated LXRα and RXRα levels in human muscle biopsies post exercise (**Figure 5D** and **E**). RXRβ levels were not altered (**Figure 5F**). Moreover, in line with our mouse data, Glut4 and hexokinase 2 levels were elevated post-exercise in human muscle, while G6PDH levels were unchanged (**Supplementary Figure 6A, B** and **C**).

Discussion

Elevated intramyocellular lipids are a hallmark of type 2 diabetic muscle and contribute to harmful effects on glucose homeostasis and insulin sensitivity (27,28). However, endurance athletes, which are highly insulin sensitive, also display elevated IMCL levels, a phenomenon referred to as the "athlete's metabolic paradox" (29,30). The difference in the molecular mechanisms between the IMCL accumulation in exercise and diabetes is poorly defined. We now show that *de-novo* lipogenesis in skeletal muscle contributes to the elevated intramyocellular lipid content in muscle-specific PGC-1α transgenic mice, a genetic model for elevated endurance capacity, and importantly, also in human endurance athletes. We demonstrate *in vivo* that PGC-1α mediates accumulation of IMCL by recruiting the lipogenic machinery in muscle, specifically by inducing the expression and activity of FAS, a multifunctional enzyme that catalyzes all seven reactions required for *de-novo* lipid biosynthesis. In combination, PGC-1α increases the expression of genes encoding lipid esterification proteins (namely DGAT and mtGPAT) and thereby allows the coordinate synthesis and subsequent accumulation of intramyofibrillar lipids. Moreover, we provide novel insights into the molecular mechanisms by which PGC-1α directly promotes FAS transcription by inducing and coactivating the nuclear receptor LXRα on the proximal FAS promoter. Taken together, these findings demonstrate for the first time a direct role of PGC-1α in muscle *de-novo* lipogenesis and intramyocellular lipid accumulation *in vivo*.

The absence of transcriptional response of key genes for lipid uptake and activation implies that the uptake of fatty acids plays a minor role for accumulation of IMCL compared to *de-novo* lipogenesis. Of all the genes that we studied, only CD36 and FABPpm were significantly induced in the MPGC-1α TG mice. However, in addition to their function at the cell membrane, these two genes have been associated with mitochondrial lipid oxidation and mitochondrial aspartate metabolism, respectively (31,32) and thus might be more relevant as mitochondrial enzymes in this context. An important role for muscle *de-novo* lipogenesis in the accumulation of intramyocellular lipids as opposed to lipolysis of triglycerides from adipose tissue, transport of fatty acids to the muscle und subsequent lipid import is further substantiated by previous studies showing unaltered fat mass, dietary lipid

intake and circulating lipid levels in MPGC-1α TG mice on regular chow or high fat-containing diet (12,33).
Moreover, the mRNA levels of ACS, an enzyme which is indispensable to activate imported lipids, but which is not required for *de-novo* lipogenesis, are unaltered in MPGC-1α TG compared to wild-type mice and in human muscle biopsies pre *vs*. post exercise, respectively. Finally, a direct functional analysis performed in muscle cells revealed that over-expression of PGC-1α reduces lipid import (17). Together, these data suggest that *de-novo* lipogenesis takes precedence over lipid import for the generation of IMCL in muscle, at least in our experimental setting. Thus, the cause of IMCL accumulation in trained muscle might differ from that in type 2 diabetes, which could be more dominantly driven by lipid uptake (34).

According to the classic hypothesis by Randle, lipid and glucose oxidation mutually regulate each other in a negative manner (35,36). Consistently, it has been found that the elevation of β-oxidation by PGC-1α in muscle-specific transgenic animals is accompanied by a reduction in glycolysis and glucose oxidation. Moreover, the increase in glucose uptake mediated by PGC-1α can only partially be explained by glycogen synthesis, since even in muscles fully loaded with glycogen, muscle-specific PGC-1α transgenic mice retain higher glucose uptake (23). Here, we demonstrate that PGC-1α induces glucose uptake in order to enhance the glucose flux through the pentose phosphate pathway as a prerequisite to produce the NADPH that is required as the reducing agent for *de-novo* lipogenesis. Furthermore, ribose-5-phosphate is made for nucleotide synthesis, and the subsequent increase in mRNA transcription that is strongly promoted by PGC-1α. While previous studies observed elevated G6PDH activity in skeletal muscle following exercise (37), we now provide evidence that G6PDH activation and the parallel increase in FAS and IMCL are driven by PGC-1α.

Interestingly, PGC-1α muscle-specific transgenic animals exhibit a higher susceptibility to develop insulin resistance when fed a high-fat diet (33). This seems paradoxical, since these animals exhibit all features of trained animals (11,12) and exercise is known to improve insulin sensitivity in muscle (38). Our findings could now explain this ostensible discrepancy by the direct effect of elevated PGC-1α on *de-novo* lipogenesis and lipid accumulation. In MPGC-1α TG animals, lipids accumulate due to the high-fat diet and in addition, due to *de-novo* lipogenesis that is promoted by the ectopic PGC-1α. Moreover, despite their elevated oxidative capacity, these mice are sedentary when kept in standard animal cages and lipid turnover in skeletal muscle is accordingly low compared to an active animal. Similarly, athletes that undergo training cessation often experience perturbations in insulin sensitivity and glucose homeostasis (39-41). These periods of relative insulin resistance are preceded by a reduction in PGC-1α, CPT-1b and citrate synthase gene expression along with reduced mitochondrial lipid oxidation (42). Reduced muscle lipid oxidation in combination with elevated IMCL, i.e. a low turnover of lipids, mirrors the metabolic profile of type 2 diabetes (43). Our findings have highly relevant implications for the development of so-called exercise mimetics, pharmaceutical interventions aimed at mirroring the plastic adaptations of muscle to exercise (44,45). If these compounds are true exercise mimetics, they will also promote *de-novo* lipogenesis and thus increase the susceptibility for unwanted side effects, in particular with a high fat, Western diet and lack or cessation of actual exercise. Therefore, as a monotherapy, such pharmaceuticals are not likely to constitute a valid alternative to real exercise (46,47).

In conclusion, we provide new evidence that PGC-1α promotes lipid anabolism *in vivo* by stimulating pentose phosphate pathway activity, *de-novo* lipid biosynthesis and IMCL accumulation, which can serve as substrates for subsequent physical activity (**Figure. 6**). In addition, we provide first insights into the molecular mechanisms that underlie PGC-1α-driven *de-novo* lipogenesis in skeletal muscle. These metabolic adaptations are highly advantageous for trained muscle due to the tight coordination of energy demand and supply. Our data highlight novel approaches as well as pitfalls for the therapy of metabolic diseases that are associated with a sedentary life-style.

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Figure Legends

Figure 1. Enhanced lipid anabolism in EDL muscle of MPGC-1α TG animals.
(**A-D**) FAS gene expression (**A**), activity (**B**), *de-novo* lipogenesis (**C**) and accumulation of intramyofibrillar lipids (**D**) in glycolytic muscle of MPGC-1α TG *vs.* wild type animals. Abbreviations: FAS, fatty acid synthase.
(**E**) Relative expression of genes involved in fatty acid uptake (LPL, CD36, FABPpm and FATPs), fatty acid activation (ACS) and esterification (DGAT and mGPAT). Abbreviations: LPL, lipoprotein lipase; CD36, cluster of differentiation 36; FABPpm, plasma membrane fatty acid binding protein; FATP, fatty acid transporter protein; ACS, acetyl-CoA synthase; DGAT, acetyl-CoA:diacylglycerol transferase; mtGPAT, mitochondrial glycerol-3-phosphate acyltransferase.
All values are expressed as means ±SE (n =6-8 per group); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Figure 2. LXRα-dependent activation of the FAS promoter.
(**A**) FAS promoter activity following transfection of myoblasts with pcDNA (empty vector) (black bars) or PGC-lα (grey bars) and in response to cotransfection with LXRα/RXRα or SREBP1c expression plasmids.
Values are expressed as means ±SE (n =6 per group); Effect of PGC-lα: @@@ $p<0.001$; effect of LXRα/RXRα or SREBP1c: ### $p<0.001$; interaction: §§ $p<0.01$, as assessed by two-factor ANOVA.
(**B**) FAS promoter activity following transfection of myoblasts with pcDNA (empty vector) (black bars) or PGC-lα (grey bars) and in response to silencing of LXRα or deletion of the LXR-responsive element (ΔLXRE).
(**C**) Relative expression of genes involved in FAS promoter activation in muscle of MPGC-1α TG mice *vs.* controls. All values are expressed as means ±SE (n =6-8 per group); * $p<0.05$; ** $p<0.01$.
(**D**) ChIP assays on mouse skeletal muscle. Recruiting of PGC-lα to LXRE and SRE binding sites in the FAS promoter of MPGC-1α TG mice and control animals, respectively. All values are expressed as means ±SE (n =4 per group); ** $p<0.01$.
Abbreviations: LXR, liver X receptor; RXR, retinoid X receptor; SREBP1c, Sterol responsive element binding protein 1c; LXRE, LXR responsive element; SRE, SREBP responsive element.

Figure 3. Enhanced glucose uptake and pentose phosphate metabolism in EDL muscle of MPGC-1α TG mice.
(**A**) Relative gene expression of GLUT1 (glucose transporter 1), GLUT4 (glucose transporter 4) and HK2 (hexokinase 2) in EDL as measured by RT-PCR and expressed as fold change over controls.
(**B**) Glucose uptake in EDL muscle.
(**C** and **D**) Determination of glucose 6-phosphate dehydrogenase (G6PDH) mRNA levels (**C**) and activity (**D**) in glycolytic muscle of MPGC-1α TG and control mice.
All values are means ±SE (n =6-8 per group). * $p<0.05$.

Figure 4. Increased pentose phosphate pathway products in MPGC-1α TG mice.
(**A** and **B**) Concentrations of NADPH (**A**) and $NADP^+$ (**B**) in glycolytic muscle of MPGC-1α TG mice *vs.* controls.
(**C-E**) Amounts of total (**C**), reduced (GSH) (**D**) and oxidized (GSSG) (**E**) glutathione extracted from glycolytic muscle.
(**F** and **G**) Incorporation of tritium labeled glucose into RNA (**F**) and DNA (**G**) in EDL of MPGC-1α TG mice and control animals.
(**H**) Determination of ribonucleotide reductase (RiboRed) mRNA levels.
All values are means ±SE. (n=6-8 per group). * $p<0.05$.

Figure 5. Higher FAS expression and IMCL in endurance athletes.
(**A-C**) FAS mRNA expression (**A**), intramyocellular lipids (**B**) and ACS mRNA expression (**C**) in human endurance athletes before and after 6 weeks of high intensity endurance exercise.
(**D-F**) Relative expression of LXRα, RXRα and RXRβ in human muscle biopsies pre- and post-exercise. Mean values are expressed as bars with SE (n=6). * $p<0.05$ as assessed by paired *t*-test.

Figure 6. PGC-1α coordinates anabolic and catabolic pathways in skeletal muscle.
A model integrating the findings of this study and showing the PGC-1α-mediated coordination of *de-novo* lipogenesis, lipid accumulation and lipid oxidation. Enzymes being activated by PGC-1α and regulating metabolic key steps are indicated in grey circles (G6PDH, FAS, CPT-1b). PGC-1α coordinates anabolic processes (lipogenesis and IMCL accumulation) and catabolic processes (β-oxidation, Krebs cycle and OXPHOS) in skeletal muscle. PGC-1α enhances glucose uptake and flux through the pentose phosphate pathway. Concomitantly, $NADP^+$ is reduced to NADPH, which serves as reducing agent for lipogenesis. *De-novo* synthesized fatty acids are then stored as IMCL and serve as energy substrate during endurance exercise. By metabolizing lipids through β-oxidation, Krebs cycle and OXPHOS, ATP for muscle contraction is produced. Fluxing glucose towards the pentose phosphate pathway also generates ribose-5-phosphate with constitutes a structural element of ATP and other nucleotides. Abbreviations: Glu, glucose; GLUT, glucose transporter; G6PDH, glucose 6-phosphate dehydrogenase; NTP, nucleotide triphosphate; ATP, adenosine triphosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; GSH, reduced glutathione, GSSG, oxidized glutathione; FAS, fatty acid synthase; FA, fatty acids; IMCL, intramyofibrillar lipids; CPT-1b, carnitine palmitoyl transferase 1b; OXPHOS, oxidative phosphorylation.

Figure 1

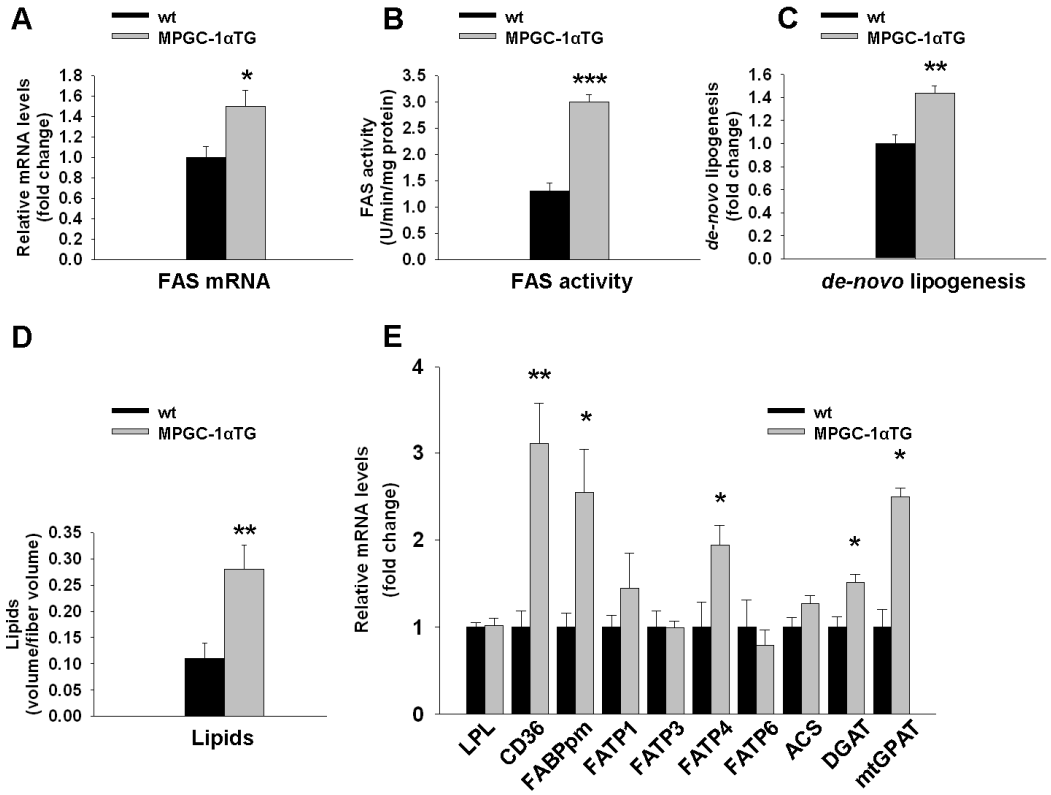

A
wt
MPGC-1αTG
Relative mRNA levels (fold change)
FAS mRNA
B
FAS activity (U/min/mg protein)
FAS activity
C
de-novo lipogenesis (fold change)
de-novo lipogenesis
D
Lipids (volume/fiber volume)
Lipids
E
Relative mRNA levels (fold change)
LPL
CD36
FABPpm
FATP1
FATP3
FATP4
FATP6
ACS
DGAT
mtGPAT

Figure 2

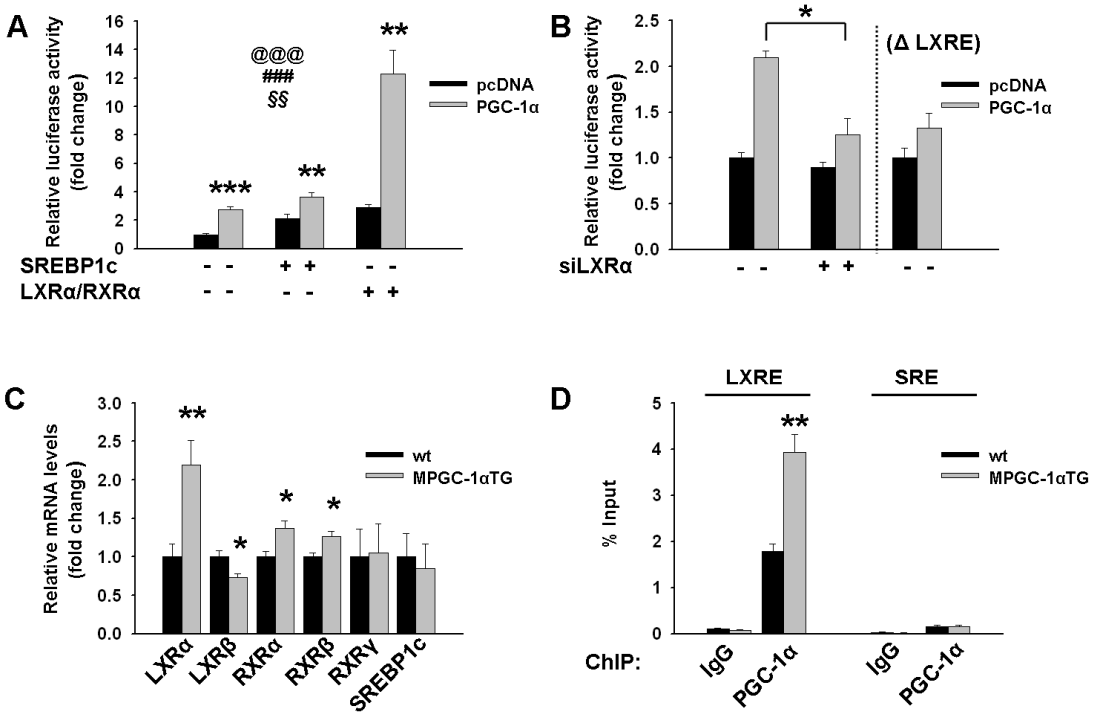

A
Relative luciferase activity (fold change)
pcDNA
PGC-1α
@@@
###
§§
***
**
**
SREBP1c
LXRα/RXRα
B
Relative luciferase activity (fold change)
*
(Δ LXRE)
pcDNA
PGC-1α
siLXRα
C
Relative mRNA levels (fold change)
wt
MPGC-1αTG
LXRα
LXRβ
RXRα
RXRβ
RXRγ
SREBP1c
D
LXRE
SRE
% Input
wt
MPGC-1αTG
ChIP:
IgG
PGC-1α
IgG
PGC-1α

Figure 3

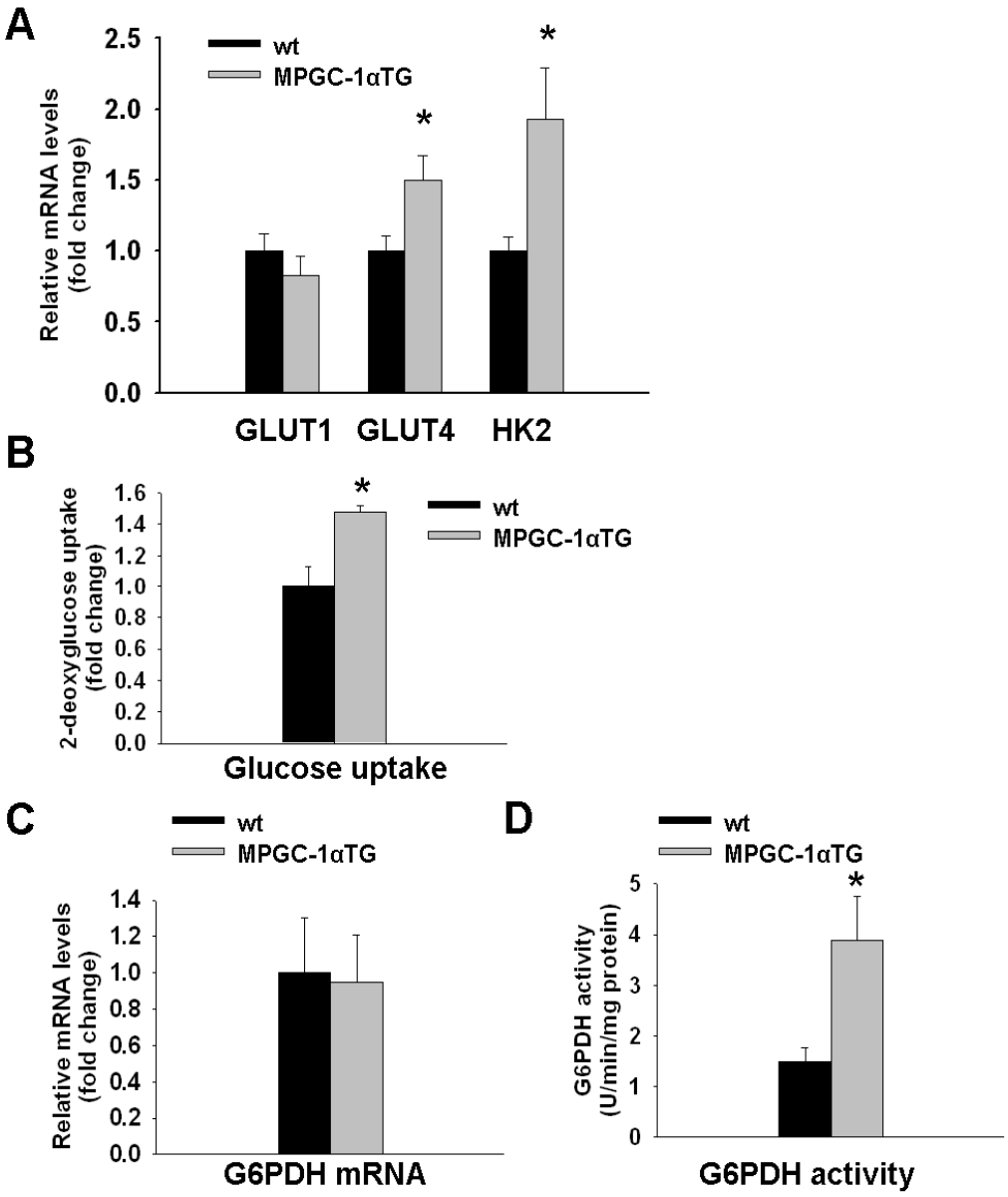

A
wt
MPGC-1αTG
Relative mRNA levels (fold change)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
GLUT1
GLUT4
HK2
B
2-deoxyglucose uptake (fold change)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
wt
MPGC-1αTG
Glucose uptake
C
wt
MPGC-1αTG
Relative mRNA levels (fold change)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
G6PDH mRNA
D
wt
MPGC-1αTG
G6PDH activity (U/min/mg protein)
5
4
3
2
1
0
*
G6PDH activity

Figure 4

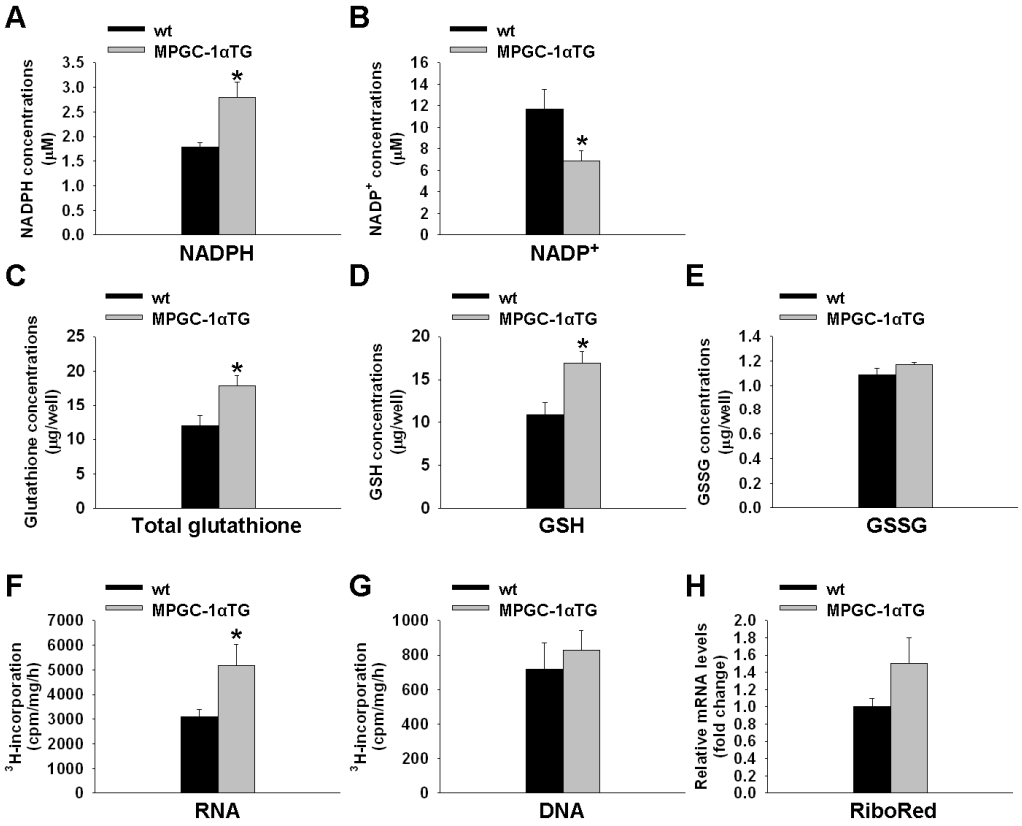

A
wt
MPGC-1αTG
NADPH concentrations (μM)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
NADPH
B
wt
MPGC-1αTG
NADP+ concentrations (μM)
16
14
12
10
8
6
4
2
0
*
NADP+
C
wt
MPGC-1αTG
Glutathione concentrations (μg/well)
25
20
15
10
5
0
*
Total glutathione
D
wt
MPGC-1αTG
GSH concentrations (μg/well)
20
15
10
5
0
*
GSH
E
wt
MPGC-1αTG
GSSG concentrations (μg/well)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
GSSG
F
wt
MPGC-1αTG
3H-incorporation (cpm/mg/h)
7000
6000
5000
4000
3000
2000
1000
0
*
RNA
G
wt
MPGC-1αTG
3H-incorporation (cpm/mg/h)
1000
800
600
400
200
0
DNA
H
wt
MPGC-1αTG
Relative mRNA levels (fold change)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
RiboRed

Figure 5

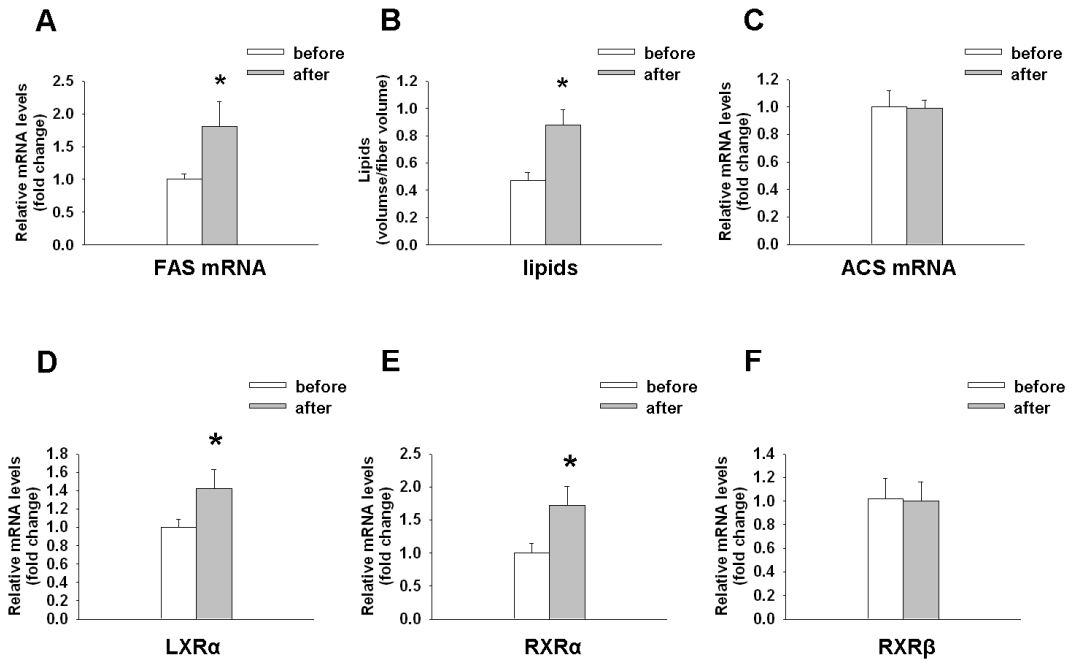
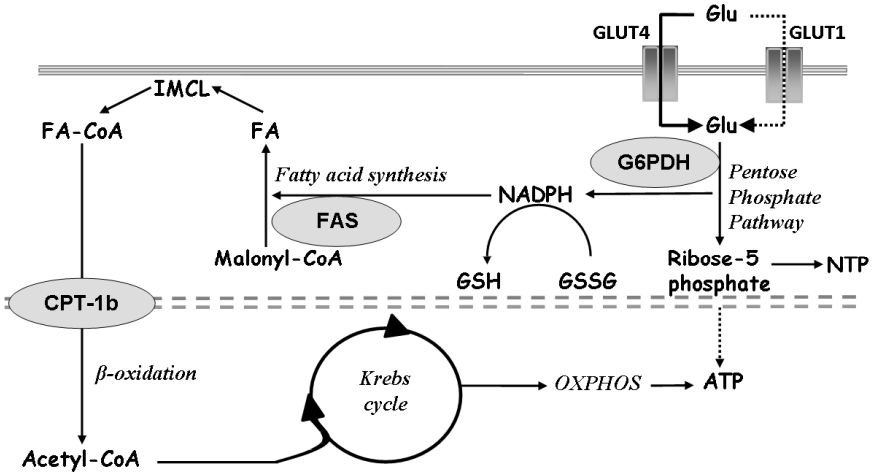

A
before
after
Relative mRNA levels (fold change)
2.5
2.0
1.5
1.0
0.5
0.0
*
FAS mRNA
B
before
after
Lipids (volumse/fiber volume)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
lipids
C
before
after
Relative mRNA levels (fold change)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ACS mRNA
D
before
after
Relative mRNA levels (fold change)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
LXRα
E
before
after
Relative mRNA levels (fold change)
2.5
2.0
1.5
1.0
0.5
0.0
*
RXRα
F
before
after
Relative mRNA levels (fold change)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
RXRβ

Figure 6


Glu
GLUT4
GLUT1
IMCL
FA-CoA
FA
Glu
G6PDH
Fatty acid synthesis
NADPH
Pentose
Phosphate
Pathway
FAS
Malonyl-CoA
GSH
GSSG
Ribose-5
phosphate
NTP
CPT-1b
β-oxidation
Krebs
cycle
OXPHOS
ATP
Acetyl-CoA