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PGC-1α improves glucose homeostasis in skeletal muscle in an activity-dependent manner

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Running title: PGC-1α improves glucose homeostasis in exercise

Abstract

Metabolic disorders are a major burden for public health systems globally. Regular exercise improves metabolic health. Pharmacological targeting of exercise mediators might facilitate physical activity or amplify the effects of exercise. The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) largely mediates musculoskeletal adaptions to exercise, including lipid refueling, and thus constitutes such a putative target. Paradoxically, forced expression of PGC-1α in muscle promotes diet-induced insulin resistance in sedentary animals. We now show that elevated PGC-1α in combination with exercise preferentially improves glucose homeostasis, increases Krebs cycle activity, and reduces the levels of acylcarnitines and sphingosine. Moreover, patterns of lipid partitioning are altered in favor of enhanced insulin sensitivity in response to combined PGC-1α and exercise. Our findings reveal how physical activity improves glucose homeostasis. Furthermore, our data suggest that the combination of elevated muscle PGC-1α together with exercise constitutes a promising approach for the treatment of metabolic disorders.

Keywords: PGC-1α; exercise mimetics; insulin resistance; metabolic syndrome; skeletal muscle

Introduction

Metabolic disorders are major threats to public health. Currently, almost two thirds of adult Americans are overweight (1). Importantly, excessive body weight fosters the development of comorbidities such as hypertension, dyslipidemia, cardiovascular disease and diabetes (2; 3).

Regular exercise improves metabolic parameters (4-6), promotes weight loss (6) and prevents adiposity relapse after successful weight loss (7). Problematically, people suffering from metabolic disorders are often unable or unwilling to achieve the levels of physical activity that are required to elicit health benefits. The auxiliary use of substances that mimic the plastic adaptations to exercise, so-called exercise mimetics (8), constitutes a seemingly attractive therapeutic approach to ease and support physical activity, or amplify the effects of exercise, at least when potential drawbacks and limitations are ignored (9; 10). As a key regulator of muscle plasticity (11; 12), the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) constitutes a potential target for such drugs (13).

Elevated expression of PGC-1α in skeletal muscle increases endurance performance (14). PGC-1α acts as a transcriptional coactivator that promotes the expression of several transcription factors, some of which it subsequently co-activates (15). PGC-1α then induces mitochondrial biogenesis (16; 17), promotes angiogenesis (18) and increases peak oxygen consumption and fatigue resistance (14; 17; 19). Moreover, PGC-1α drives slow fiber type-specific calcium handling (17) and switching from fast, glycolytic towards slow, oxidative fibers (19). PGC-1α also increases metabolic flexibility (20). Importantly, PGC-1α promotes lipid (21) and glucose refueling in skeletal muscle (22) and thereby ensures the provision of substrates during exercise.

Paradoxically, despite the strong promotion of an exercised muscle phenotype, elevated expression of PGC-1α in sedentary mice exacerbates diet-induced insulin resistance as indicated by impairments in glucose disposal rates and muscle glucose uptake under hyperinsulinemic-euglycemic clamp conditions (23). Elevated lipid refueling in muscle-specific PGC-1α transgenic (MPGC-1α TG) animals, in combination with a sedentary lifestyle, likely underlies the pronounced detrimental effects of a high-fat diet (21). We hypothesized that the metabolic impairments in sedentary MPGC-1α TG animals fed a high-fat diet is improvable in response to exercise. The aim of the current study was to longitudinally explore the potential therapeutic use of elevation of PGC-1α in combination with exercise in the treatment of diet-induced metabolic syndrome.

Materials and Methods

Animals

Male muscle-specific PGC-1α transgenic mice (MPGC-1α TG) (19) and control littermates were maintained in a fixed 12-h light/dark cycle on a pellet chow diet and free access to water. To induce insulin resistance, animals were administered a high-fat diet consisting, by energy, of 60% fat for two weeks. This time span was sufficient to induce impairments in glucose and insulin tolerance, which were more pronounced in MPGC-1α TG animals compared to control littermates (*data not shown*). Thereafter, all animals continued to consume the high-fat diet for another 3.5 weeks. During these 3.5 weeks, half of the animals remained sedentary (8 wild-type and 8 MPGC-1α TG mice) while the other half (8 wild-type and 8 MPGC-1α TG mice) underwent treadmill training three times per week separated by an intermittent day of recovery. All animals were sacrificed 24hrs after the last bout of exercise. Studies were performed according to criteria outlined for the care and use of laboratory animals and with approval of the Swiss authorities.

Body composition

Body composition was determined by EchoMRI qNMR (Echo Medical Systems).

Locomotor activity, endurance training and muscle strength

Locomotor activity was assessed by CLAMS (Columbus instruments). Endurance training was performed on a motorized treadmill (Columbus Instruments). After acclimatization, maximal endurance capacity was determined. The detailed protocol was as follows: 10m/min for 5min, then increase by 2m/min every 5 minutes up to 26m/min. The speed of 26m/min was then kept until exhaustion. Mice subsequently trained 3 days per week for 3.5 weeks. This training period started at 75% of maximal endurance capacity and exercise levels were

then gradually increased to reach 115% by the end of the training period. Maximal force was tested *in vivo* using a grip strength meter (Chatillon) as described (17).

RNA extraction and RT-PCR

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

Enzymatic activities

Citrate synthase (CS) activity was assessed according to the protocol by Srere et al (24).

Fatty acid synthase (FAS) and Glucose 6-phosphate dehydrogenase (G6PDH) activity were measured as described (21).

Glucose uptake

Isolated EDL muscles were blotted on filter paper, weighed and washed in Krebs-Ringer bicarbonate buffer (KRBB: 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.6 mM $NaHCO_3$, pH 7.4). 2-deoxyglucose uptake was assessed in fresh 2 ml KRBB containing 1 mmol/l 2-deoxy-D[1,2-^{3}H]glucose (1.5 μCi/ml) and 7 mmol/l D-[^{14}C]mannitol (0.45 μCi/ml) (Amersham Biosciences) at 30°C for 10 min (25). To terminate the transport, muscles were dipped into ice-cold KRBB buffer containing 80 μM cytochalasin B. Muscles were further processed by incubating in 300 μl 1N NaOH at 80°C for 10 min, neutralized with 300 μl 1N HCl, and particules were precipitated by centrifugation at 12,000 × g for 5 min (26). Radioactivity in the lysates 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 ROS

To monitor intracellular generation of ROS, 2′,7′-dichlorofluorescein diacetate (H_2DCFDA) (Sigma) was used as previously described (20).

Blood acylcarnitine and muscle lipid determination

Acylcarnitine concentrations were measured as described (20). Briefly, aylcarnitines were extracted from dried blood spots using methanol containing eight isotopically labeled internal standards (Cambridge Isotopes Laboratories) and analyzed without prior sample derivatization. Precursor ions of m/z 85 in the mass range of m/z 150 to 450 are acquired on a PerkinElmer API 365 LC-ESI-MS/MS instrument.

Analyses of lipids were carried out using methods described (27; 28). Lipid extracts were prepared using a modified Bligh/Dyer extraction procedure, spiked with appropriate internal standards and analyzed using an Agilent 1200 HPLC system coupled with an Applied Biosystem Triple Quadrupole/Ion Trap mass spectrometer (3200Qtrap). Briefly, separation of individual lipid classes of polar lipids by normal phase HPLC was carried out using a Phenomenex Luna 3u silica column (i.d. 150x2.0 mm) with the following conditions: mobile phase A (chloroform:methanol:ammonium hydroxide, 89.5:10:0.5), B (chloroform:methanol: ammonium hydroxide:water, 55:39:0.5:5.5); flow rate of 300 µl/min with gradient elution as described previously (28). Individual lipid species were quantified by referencing to spiked internal standards. PC-14:0/14:0, PE-14:0/14:0, Sphingosine-17:0 and C17-Cer were obtained from Avanti Polar Lipids. TAG, DAG and CE were analyzed using a modified version of reverse phase HPLC/ESI/MS described previously (27). Briefly, separation of TAG and CE from polar lipids was carried out on an Phenomenex Kinetex C18 column (i.d. 4.6X100mm)

using an isocratic mobile phase chloroform:methanol:0.1M ammonium acetate (100:100:4) at a flow rate of 150 µl/min. TAG were calculated as relative contents to the spiked d5-TAG 48:0 internal standard (CDN isotops), while cholesterol esters were normalized to corresponding d6-C18 cholesterol ester (CDN isotops). DAG species were quantified using 4ME 16:0 Diether DG as an internal standard (Avanti Polar Lipids).

Glucose and insulin tolerance tests

Animals were fasted for 16 h and 4 h before i.p. injection of 2 g/kg glucose and 0.8 U/kg insulin, respectively. Blood was obtained at intervals of 15 minutes from the tail vein, and glucose levels were determined using a standard glucometer.

Histology

Muscles were embedded in OCT and frozen in cooled isopentane. SDH stainings were performed on 8µm cryosections by exposing the sections to succinate (27mg/ml) and nitro-blue tetrazolium (1mg/ml). Cox staining was similarly performed with cytochrome c (0.1mg/ml) and diaminobenzidine (0.5mg/ml). Mitotracker staining was performed as described (20).

Cell culture experiments

Mouse C_2C_{12} myoblasts were maintained in DMEM supplemented with 10% fetal bovine serum in a subconfluent culture. C_2C_{12} myoblasts were fused into myotubes by using DMEM with 2% horse serum. On the third day of differentiation, myotubes were infected with adenoviral vectors for GFP or PGC-1α. Glucose uptake was determined two days following adenoviral infection in the presence of vehicle, 50 µM cerulenin or sphingosine and 100 nM insulin. Both drugs were added 3 mins prior to insulin stimulation. Glucose transport was assessed in 10µM 2-deoxy-glucose (0.5µCi/ml 2-deoxy-D[1,2-^{3}H]glucose) in Hepes buffer

(140mM NaCl, 20mM Hepes, pH 7,4, 5mM KCl, 2.5 mM $MgSO_4$ and 1mM $CaCl_2$). After 10 mins, glucose uptake was stopped by addition of ice cold PBS, cells were washed thrice and immediately lysed by addition of 0.05M NaOH. Radioactivity in the lysates was quantified using a Beckman liquid scintillation counter. All values were corrected for total protein and expressed as fold change compared to control animals.

Data analysis and statistics

All data are presented as means ± SE. The data were analyzed by factorial ANOVA for the main effects of genotype (wild-type *versus* MPGC-1α TG mice)(@), treatment (sedentary *versus* exercised)(#), and group-times-treatment interactions (x). Student *t*-test were applied to assess the effects of treatment (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) between two individual groups. Levels of significance are indicated as follows: single symbol $p< 0.05$; double symbols $p< 0.01$; triple symbols $p<0.001$.

Results

General parameters

MPGC-1α TG animals (sedentary and exercised) were significantly heavier than control littermates and tended to display an elevated fat, but unaltered lean mass (Table 1). Tibialis anterior and epididymal fat pad mass was significantly increased in MPGC-1α TG animals.

The level of exercise in our study was insufficient to counteract many of the detrimental effects of the high-fat diet and no general body weight loss, changes in body composition or reductions in specific tissue weights occurred in response to exercise in MPGC-1α TG and/or control mice (Table 1). We can therefore exclude that any metabolic changes observed in our study are related to body weight or fat mass loss.

The effect of training is amplified by elevated PGC-1α

Muscle function was assessed under different experimental settings. No changes in maximal force generation and spontaneous locomotor activity were observed across the 4 groups (Table 1).

MPGC-1α TG animals display an elevated exercise performance on standard chow diet (14). Interestingly, the two initial weeks of high-fat feeding completely abrogated this effect (Wild-type and MPGC-1α TG ran 445±33m and 449±39m, respectively). During the subsequent 3.5-week training period, MPGC-1α TG and wild-type animals exercised in parallel at exactly the same relative workload to ascertain maximal comparability between the exercising groups. At the end of the training period, the exercise benefit was more pronounced in MPGC-1α TG animals compared to control littermates and thereby again resembled the improved endurance of PGC-1α TG mice on regular chow diet (14). In fact, the maximal endurance performance in response to training increased by 43% and 102% in wild-type and

MPGC-1α TG animals, respectively (Table 1). The impact of exercise in wild-type controls resembled previously published data in the same mouse strain on chow diet (29).

Endurance exercise preferentially improves glucose homeostasis at elevated PGC-1α levels

We have reported that overall glucose homeostasis is unaltered in MPGC-1α TG animals under standard laboratory conditions (20). Paradoxically, when challenged by a high-fat diet and under hyperinsulinemic-euglycemic clamp conditions, MPGC-1α TG animals display an accelerated development of insulin resistance compared to control littermates (23). Exercise preferentially improved glucose (Fig. 1*A* and *B*) and insulin (Fig. 1*C* and *D*) tolerance in MPGC-1α TG by 28% and 17%, respectively. In comparison, glucose tolerance improved only by 6% (Fig. 1*A* and *B*) and insulin tolerance (Fig. 1*C* and *D*) was unaltered in response to exercise in wild-type animals. Thus, the beneficial effects of exercise on whole body glucose homeostasis are amplified by elevated PGC-1α levels.

Since skeletal muscle is a major contributor to overall glucose homeostasis, we assessed the expression of genes involved in muscle glucose uptake. We primarily focused on gene transcription as exercise alters the transcriptional profile of skeletal muscle and since PGC-1α acts as a transcriptional co-activator. Glucose transporter 4 (GLUT4) mRNA expression levels were reduced in tibialis anterior of sedentary, high fat diet-fed MPGC-1α TG animals and restored by exercise (Fig. 1*E*). Moreover, the mRNA expression of hexokinase II (HK II) was highest in trained MPGC-1α TG animals (Fig. 1*E*). In line with these findings, muscle glucose uptake was decreased in sedentary, but preferentially improved by 93% in trained MPGC-1α TG animals (Fig. 1*F*). In contrast, exercise increased glucose uptake in wild-type animals by only 22% (Fig. 1*F*). Intriguingly, muscle PI3K activity, an early marker of insulin sensitivity and key element in insulin signaling, was higher in MPGC-1α TG animals, but

unaltered by exercise (Fig. 1*G*). This absence of an effect of exercise on proximal insulin signaling is further underlined by the fact that the insulin responsiveness within the first 30 mins following insulin injection was similar between sedentary and exercised animals (Fig. 1*C* and *D*) and (supplemental Fig. S1).

Elevated PGC-1α potentiates the effect of exercise on Krebs cycle activity

We next assessed the capacity for muscle lipid uptake. MPGC-1α TG mice showed elevated levels of lipoprotein lipase (LPL) and cluster of differentiation 36 (CD36), which are involved in lipid uptake (Fig. 2*A* and *B*).

We then examined genes regulating lipid oxidation and Krebs cycle, namely carnitine palmitoyl transferase 1b (CPT-1b), malonyl CoA decarboxylase (MCD), acetyl CoA carboxylase 2 (ACC2) and citrate synthase (CS). While all of the genes were elevated in MPGC-1α TG animals, the majority of them were not significantly altered by exercise (Fig. 3*A*). In contrast, the enzymatic activity of citrate synthase, which is a marker of mitochondrial oxidation and central to both glucose and lipid oxidation, was further boosted by exercise at elevated PGC-1α levels (~28% compared to sedentary MPGC-1α TG animals) (Fig. 3*B*). In control littermates, the exercise regimen had no effect on citrate synthase activity in accordance with previous studies under high-fat feeding (30; 31).

Exercise restores acylcarnitine profiles in MPGC-1α TG animals to wild-type levels

In sedentary, high fat-fed MPGC-1α TG animals higher amounts of palmitoylcarnitine (C16:0) were released into the circulation, while exercise diminished the levels of palmitoylcarnitine in MPGC-1α TG animals (Fig. 3*C*). No major changes were observed across the 4 groups for the patterns of mono- (Fig. 3*D*) and poly- (Fig. 3*E*) unsaturated acylcarnitines. Total carnitine and acetylcarnitine (C2) levels were elevated in sedentary

MPGC-1α TG animals and returned to wild-type levels in response to exercise (Fig. 3*F* and *G*).

Unaltered OXPHOS activity in response to exercise

The increased levels of acetylcarnitine in sedentary MPGC-1α TG animals suggest that the amount of lipids taken up and fuelled into β-oxidation exceeded the energetic demand of the Krebs cycle and oxidative phosphorylation. We thus tested whether elements of the electron transport chain are altered in response to exercise on a high-fat diet. While all of these genes were elevated in MPGC-1α TG animals, gene expression patterns were not affected by exercise (Fig. 4*A*). Exercise increased the activity of succinate dehydrogenase (SDH), an enzyme that contributes to the Krebs cycle and oxidative phosphorylation and thereby links these processes (Fig. 4*B* and supplemental Fig. S2). In MPGC-1α TG animals, exercise resulted in the highest activity of succinate dehydrogenase. In contrast, the activity of cytochrome c oxidase (COX), which constitutes an element of OXPHOS, but not of the Krebs cycle, was elevated in MPGC-1α TG animals, while no additional effect of exercise could be observed (Fig. 4*B* and supplemental Fig. S2). These data suggest that OXPHOS activity is not rate-limiting within our experimental context.

We next determined the relative mRNA expression of genes implicated in reactive oxygen species (ROS) generation and detoxification since ROS can impair insulin sensitivity (32; 33) (Fig. 4*C*). The mitochondrial superoxide dismutase 2 (SOD2), which transforms superoxides into H_2O_2, was elevated in MPGC-1α TG animals. Moreover, catalase (CAT), which detoxifies H_2O_2, was simultaneously increased. Overall H_2O_2 was therefore not different (Fig. 4*D*).

Additive increases in *de-novo* lipogenesis by elevated PGC-1α and exercise

We have previously demonstrated that PGC-1α promotes *de-novo* lipogenesis and pentose phosphate activity (rate-limited by glucose-6-phosphate dehydrogenase (G6PDH)) and that this partially drives glucose uptake into trained muscle (21). Interestingly, the activities of G6PDH and fatty acid synthase (FAS) remained higher in MPGC-1α TG animals on a high-fat diet and increased even further with exercise (Fig. 5*A* and *B*).

Given the elevated lipogenesis in MPGC-1α TG animals, we next investigated whether lipid partitioning in skeletal muscle is altered. Triglyceride (TAG) levels were comparable between sedentary wild type and MPGC-1α TG animals on a high fat diet and increased to a similar extent in both genotypes following chronic exercise (Fig. 5*C*).

We found elevated levels of diacylglycerols (DAG) (Fig. 5*D*) in the MPGC-1α TG animals, while the ceramide content was unaltered (Fig. 5*E*). Moreover, phosphatidylcholine (Fig. 5*F*) and phosphatidylethanolamine (Fig. 5*G*) levels were elevated in MPGC-1α transgenic animals. Exercise had no additional effect on the levels of these lipid species. In contrast, sphingosine levels were significantly increased in MPGC-1α TG animals and markedly reduced by exercise (Fig. 5*H*).

Inhibition of FAS or high sphingosine prevent glucose uptake in muscle cells *in vitro*

Given the elevated FAS activity in MPGC-1α TG animals that even persists during high-fat feeding, we investigated the general role of FAS in glucose uptake into isolated muscle cells free of interspersed adipose tissue. Pharmacological inhibition of FAS by cerulenin blunted glucose uptake in muscle cells and this inhibitory effect was more pronounced following over-expression of PGC-1α (Fig. 6*A*).

The accumulation of sphingosine in muscle of MPGC-1α TG animals prompted us to investigate the impact of sphingosine on glucose homeostasis. Sphingosine diminished glucose uptake in muscle cells and completely abrogated the stimulating effect of insulin at high levels of PGC-1α (Fig. 6*B*).

Discussion

The role of skeletal muscle mitochondrial function and in particular, that of its key regulator PGC-1α in the etiology of type 2 diabetes remains controversial (34; 35). Studies in animal models for PGC-1α in skeletal muscle have not conclusively revealed how modulation of this coactivator affects peripheral insulin sensitivity so far (20; 23; 36). We have now demonstrated in a model of continuous high-fat feeding that elevated expression of PGC-1α preferentially improves whole body and muscle glucose homeostasis when combined with exercise. In stark contrast, elevated expression of PGC-1α favors the development of diet-induced insulin resistance in the sedentary state. The beneficial metabolic effects of concerted exercise and PGC-1α expression comprise augmented Krebs cycle activity, reduction in acetylcarnitine levels, elevated glucose uptake for *de-novo* lipogenesis and altered lipid partitioning in favor of enhanced insulin sensitivity in skeletal muscle (Fig. 7).

In sedentary MPGC-1α TG mice fed a high-fat diet, the high lipid provision exceeds the energetic demand of β-oxidation as indicated by the release of palmitoylcarnitine (C16:0) into the circulation. The parallel increase in citrate synthase activity is insufficient to cope with the metabolites deriving from lipid oxidation and, as a consequence, acetylcarnitine (C2) levels rise, which might impair glucose homeostasis (37; 38) (Fig. 7) possibly by activation of insulin de-sensitizing nuclear factor κB (NFκB). NFκB activation is sufficient to impair glucose homeostasis by interference with insulin receptor substrate and protein kinase B (PKB) phosphorylation, as well as GLUT4 translocation (39). Acylcarnitine species potently activate NFκB to a degree which is even higher than saturated fatty acids (37), a classical stimulus of NFκB. Whether this effect of acylcarnitines is mechanistically brought about by direct interference with NFκB or activation of upstream signaling events, such as toll-like receptor stimulation, remains unresolved.

In the sedentary state, Krebs cycle activity is less required for ATP regeneration and citrate is exported into the cytoplasm for *de-novo* lipogenesis. An increase in lipogenesis is indeed corroborated by the augmented phosphate pathway and FAS activity, which persist in sedentary transgenic animals in spite of abundant dietary fat. In MPGC-1α TG mice the increased mitochondrial biogenesis is associated with elevated levels of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which together account for more than 75% of total membrane phospholipids (40), and of diacylglycerol (DAG), which constitutes another membrane component. Moreover, sedentary MPGC-1α TG animals display markedly higher levels of sphingosine, which is implicated in PI3K-independent insulin resistance in adipocytes (41-43). We have now analogously demonstrated a direct effect of sphingosine on glucose uptake in skeletal muscle cells *in vitro*. Consistently, our *in vivo* data on skeletal muscle show markedly elevated sphingosine levels along with reduced muscle glucose uptake, but higher PI3K activity in sedentary MPGC-1α TG animals.

The mechanisms that underlie the detrimental effect of sphingosine remain obscure. In non-muscle cells, sphingosine exerts pleiotropic effects on insulin signaling. It inhibits Protein Kinase C (PKC) isoforms required for proper insulin signaling (42), but activates a truncated form of PKCδ, which is implicated in the development of insulin resistance (44). Moreover, sphingosine interferes with the MAPK pathway and activates protein phosphatases which target PKB, a downstream target of PI3K (44). Thus, sphingosine seems to target multiple mediators of insulin signaling.

In stark contrast to sedentary, high fat-fed mice, regular endurance exercise decreased the levels of C16:0 and C2 in trained MPGC-1α TG animals. Exercise accordingly increased β-oxidation and Krebs cycle activity in MPGC-1α TG animals, allowing complete oxidation.

Interestingly, an additional elevation in FAS activity occurs in skeletal muscle of exercised MPGC-1α TG animals. Regular exercise and exercise-independent activation of PGC-1α both promote FAS transcription in skeletal muscle (21). Concomitantly, PGC-1α increases glucose uptake (21; 22), but impedes glycolytic fluxes (22) thereby shunting glucose towards glycogen storage (22) and the pentose phosphate pathway for the generation of NADPH (21), a prerequisite for *de-novo* lipogenesis. In adipocytes, metabolic flux through *de-novo* lipogenesis determines insulin sensitivity and glucose uptake to a significant extent (45; 46). We have now shown by pharmacological intervention that in isolated muscle cells, FAS activity similarly plays a role in regulating glucose uptake. The possibility thus arises that the elevated pentose phosphate pathways activity and *de-novo* lipogenesis in exercised animals on a high fat diet might similarly be implicated in driving glucose uptake in skeletal muscle.

Trained transgenic animals subsequently display a different pattern of lipid partitioning. In addition to the elevated levels of membrane components (DAG, PC and PE), triglycerides (TG) are increased in response to exercise. According to the "athlete's paradox", elevated lipogenesis and high levels of intramyocellular triglycerides and even diacylglycerides co-exist with improved insulin sensitivity and thus *per se* do not seem to constitute predictors of muscle insulin sensitivity (47; 48).

Importantly, exercise reduced the levels of sphingosine in MPGC-1α TG mice and thus relieved its potential inhibitory effect on glucose uptake. The negative effect of sphingosine on glucose uptake is further counteracted in exercised MPGC-1α TG mice by elevated levels of triglycerides, which have been shown to inhibit sphingosine activity in a dose-dependent manner (49). Thus, exercise at elevated muscle levels of PGC-1α decreased palmitoylcarnitine and acetylcarnitine levels and diverted *de-novo* synthesized lipids away from sphingosine biosynthesis towards triglyceride storage. Combined elevated expression of PGC-1α and exercise therefore promote lipid partitioning in favor of enhanced insulin sensitivity (Fig. 7).

In our study, exercise exerts only moderate effects on glucose homeostasis in the context of continuous high-fat feeding similar to other studies where daily high fat-feeding largely antagonized the beneficial effects of exercise (50-52). We have specifically chosen a mild exercise regimen to avoid potentially confounding effects of reduced adiposity. Nevertheless, during the development of insulin resistance (two weeks of high-fat feeding in the sedentary state), the transgenic elevation of PGC-1α levels led to a more severe starting (pre-exercise) insulin resistance in MPGC-1α TG animals. This was unavoidable in our model, but partially compromises the interpretation of our data. Nonetheless however, the strong interactive effect of exercise and PGC-1α clearly demonstrates that exercise exerts its beneficial effects preferentially at elevated muscle PGC-1α levels and that the significant differences in exercise responses are unlikely related to differences in the absolute amount of muscle work due to the slightly higher body weight in MPGC-1α TG animals. Timely coordination of the expression of PGC-1α and dietary intervention would therefore presumably culminate in an exclusively positive outcome on metabolic health.

In conclusion, we demonstrate that exercise preferentially improves exercise capacity and metabolic parameters at high levels of muscle PGC-1α in the context of energy-dense nutrition. Moreover, we provide novel insights into metabolic alterations that affect glucose homeostasis in the insulin-resistant and trained muscle, respectively. Although our data suggest that elevation of PGC-1α, as a mono-therapy, is detrimental in sedentary patients exposed to a Western diet, targeting PGC-1α to further amplify the effects of exercise regimens might represent a novel avenue to improve skeletal muscle function and to achieve metabolic benefits. In light of the growing prevalence of metabolic disorders, which are favored by a sedentary lifestyle, the use of adjuvants to exercise to ease physical activity and

enhance exercise effects in people with a low drive to move, might gain profound medical and economical interest in the coming years.

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Dr. Serge Summermatter is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis.

The authors declare no conflict of interest.

Author contributions

S.S., G.SH., D.M. and G.SA. performed experiments and analyzed data. S.S. and C.H. wrote the manuscript. S.S., G.SH., D.M., M.R.W and C.H. contributed to discussion and edited/reviewed the manuscript.

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Table 1 ***General parameters***

Body weight, body composition, individual tissue weights and performance parameters in MPGC-1α TG mice and control littermates.

Values are expressed as means ±SE (n =8 per group); Overall effect of genotype (wild-type *vs*. MPGC-1α TG): @; Effect of training (sedentary *vs*. exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: p<0.05; double symbols: p<0.01; triple symbols: p<0.001.

	wild-type		**MPGC-1α TG**		**ANOVA**		
	sedentary	**exercised**	**sedentary**	**exercised**	**genotype**	**training**	**interaction**
body weight (g)	**30.1±0.5**	**31.0±0.6**	**32.4±0.8§**	**32.8±0.6§**	***@@***	***N.S***	***N.S***
fat mass (g)	**5.2±0.3**	**5.5±0.3**	**5.6±0.3**	**6.4±0.5**	***p=0.065***	***N.S***	***N.S***
lean mass (g)	**22.5±0.5**	**22.9±0.5**	**23.0±0.4**	**22.8±0.4**	***N.S***	***N.S***	***N.S***
heart (mg)	**121±6**	**122.1±2.3**	**128±5.6**	**134.1±3.7**	***p=0.055***	***N.S***	***N.S***
tibialis (mg)	**39.5±1.2**	**43.1±1.4**	**46.7±2§§**	**43.9±1.1**	***@***	***N.S***	***N.S***
EDL (mg)	**9.9±0.6**	**10.7±1.1**	**11.9±0.7**	**10.9±0.2**	***N.S***	***N.S***	***N.S***
soleus (mg)	**9.3±0.6**	**10.8±0.5**	**10.8±0.5**	**10.2±0.5**	***N.S***	***N.S***	***N.S***
gastrocnemius (mg)	**159.9±9.7**	**157.9±5.2**	**157.4±6**	**153.2±3.5**	***N.S***	***N.S***	***N.S***
epididymal fat (mg)	**403.1±39.7**	**453.9±38.6**	**550.7±66.3**	**527.5±60.3**	***@***	***N.S***	***N.S***
maximal force (front) (N)	**0.91±0.04**	**0.99±0.04**	**0.91±0.05**	**0.91±0.02**	***N.S***	***N.S***	***N.S***
maximal force (hind) (N)	**1.98±0.1**	**2.03±0.14**	**1.99±0.08**	**2.02±0.06**	***N.S***	***N.S***	***N.S***
locomotor activity (counts/24hrs)	**14000±1844**	**14303±2859**	**15034±3548**	**14268±1673**	***N.S***	***N.S***	***N.S***
endurance (distance) (m)	**435±22**	**624±30**	**487±36*****	**985±39***,§§§**	***@@@***	###	***xxx***

Figure legends

Fig. 1 ***Whole body and muscle glucose homeostasis***
(A and B) Glucose tolerance test (GTT) excursion curves (A) and corresponding area under the curve (B)
(C and D) Insulin tolerance test (ITT) excursion curves (C) and corresponding area under the curve (D)
(E) Relative gene expression of mediators of glucose uptake in skeletal muscle assessed by RT-PCR
(F) Glucose uptake into isolated skeletal muscle measured by the 2-deoxglucose technique
(G) IRS-1-associated muscle PI3K activity
All values are expressed as means ±SE (n =8 per group); Effect of genotype (wild-type *vs*. MPGC-1α TG): @; Effect of training (sedentary *vs*. exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. 2 ***Lipid uptake***
Relative mRNA expression of LPL (A) and CD36 (B) in MPGC-1α TG mice and control littermates. All values are expressed as means ±SE (n =8 per group); Overall effect of genotype (wild-type *vs*. MPGC-1α TG): @; Effect of training (sedentary *vs*. exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. 3 ***β-oxidation, TCA cycle and acylcarnitines***
(A) Relative gene expression of regulators of lipid oxidation and TCA cycle.
(B) Citrate synthase activity
(C) Levels of saturated acylcarnitines (SFA)
(D) Levels of mono-saturated acylcarnitines (MUFA)
(E) Levels of poly-saturated acylcarnitines (PUFA)
(F) Total carnitine levels
(G) Acetylcarnitine levels
All values are expressed as means ±SE (n =8 per group); Overall effect of genotype (wild-type *vs*. MPGC-1α TG): @; Effect of training (sedentary *vs*. exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. 4 ***Oxidative phosphorylation and reactive oxygen species***
(A) Relative gene expression of elements of oxidative phosphorylation.
(B) Succinate dehydrogenase (SDH) (*upper panel*), cytochrome c oxidase (Cox) (*middle panel*) and mitotracker green staining (*lower panel*).
(C) Relative gene expression of ROS detoxifying enzymes.

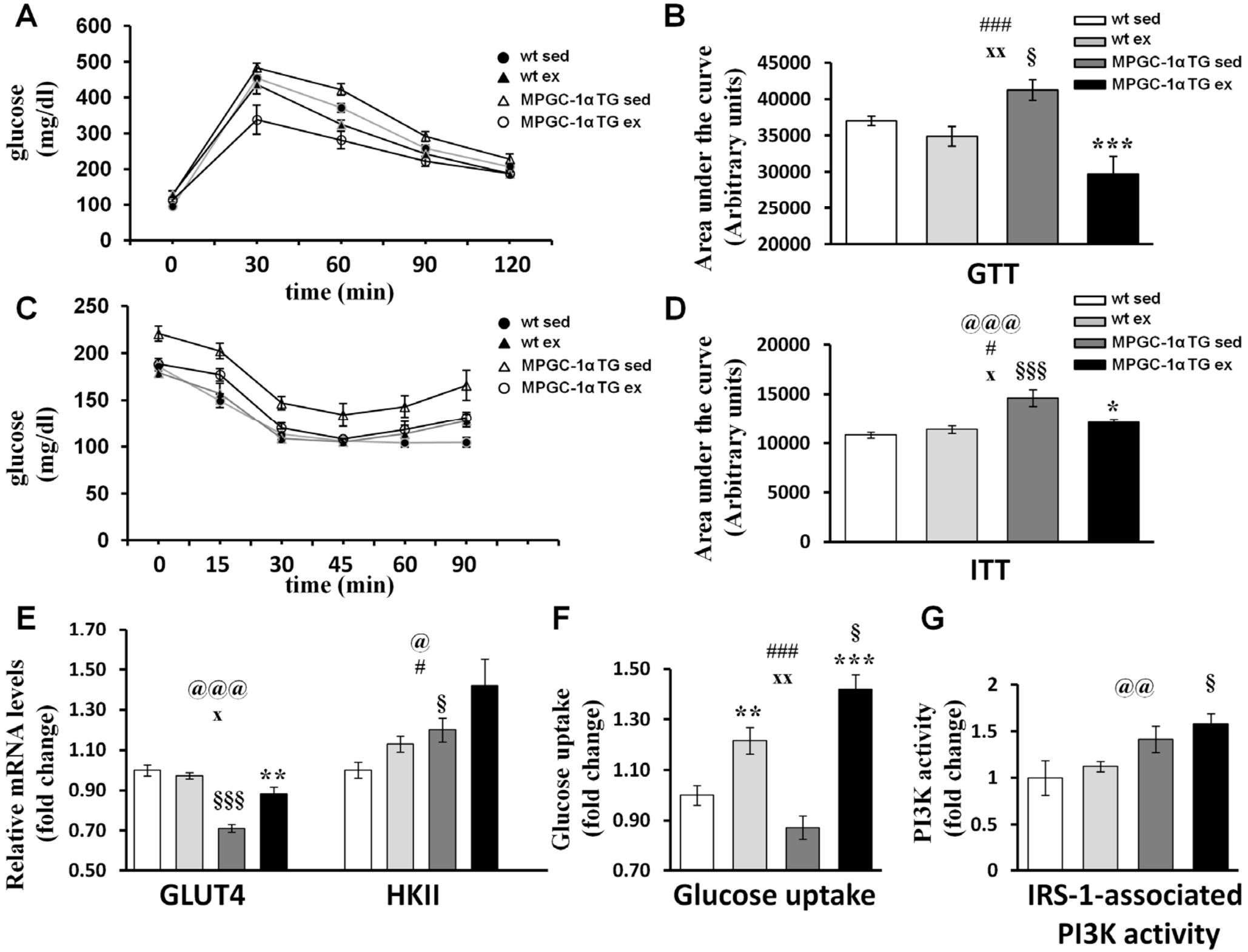
(D) H_2O_2 levels in skeletal muscle.
All values are expressed as means ±SE (n =8 per group); Overall effect of genotype (wild-type *vs*. MPGC-1α TG): @; Effect of training (sedentary *vs*. exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

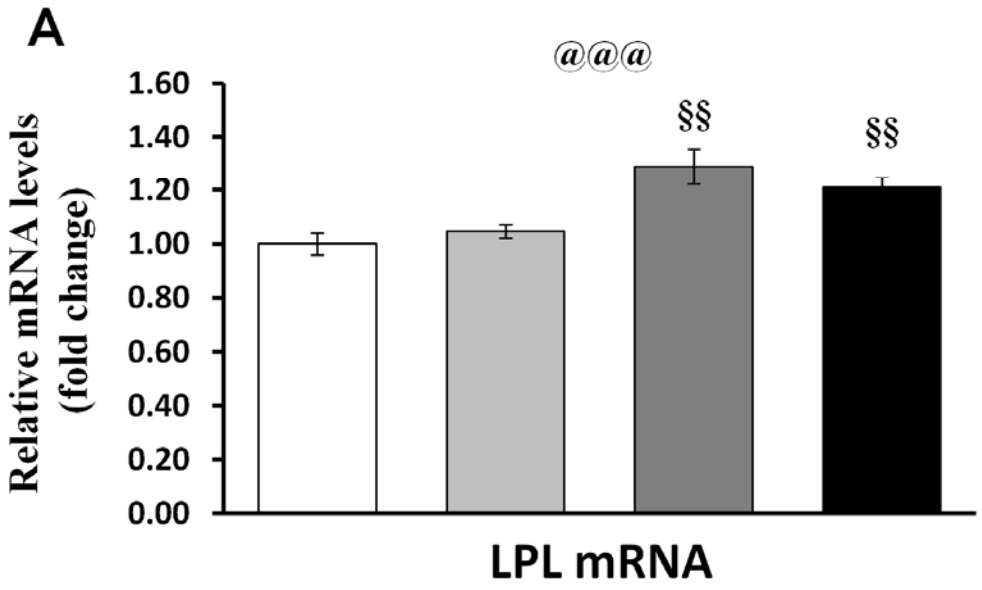
Fig. 5 ***Muscle lipid species***
(A) G6PDH activity and (B) FAS activity
(C) Triglycerides (TAG)
(D) Diacylglycerides (DAG)
(E) Ceramide
(F) Phosphatidylcholine
(G) Phosphatidylethanolamine
(H) Sphingosine
All values are expressed as means ±SE (n =8 per group); Overall effect of genotype (wild-type *vs*. MPGC-1α TG): @; Effect of training (sedentary *vs*. exercised): #; genotype times training interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. 6 ***Metabolic regulation of glucose uptake in skeletal muscle***
(A) Effect of the FAS inhibitor cerulenin on glucose uptake in the absence (white bars) or presence (black bars) of insulin.
(B) Effect of the sphingosine on glucose uptake in the absence (white bars) or presence (black bars) of insulin.
All values are expressed as means ±SE (n =6 per group); Overall effect of PGC-1α: @; Effect of drug: #; PGC-1α times drug interaction: x were assessed by ANOVA. Comparison between two individual groups: effects of training (sedentary *versus* exercised)(*) and genotype (wild-type *versus* MPGC-1α TG mice)(§) were assessed by *t*-test. Single symbol: $p<0.05$; double symbols: $p<0.01$; triple symbols: $p<0.001$.

Fig. 7 ***Integrative theoretical model***
Schematic theoretical interpretation of the findings on metabolic profiles predominating in response to training and PGC-1α levels.
Sedentary state: At elevated levels of PGC-1α the increase in β-oxidation relatively exceeds the increase in citrate synthase activity. Consequently, acetylcarnitine accumulates and impairs insulin sensitivity. Citrate, produced by citrate synthase, is fuelled into OXPHOS to generate ATP. In the absence of physical activity, the low ATP turnover ultimately limits OXPHOS activity and directs metabolic fluxes away from catabolism. The elevated citrate synthase activity, without an elevated need to produce ATP for muscle contraction, overloads the Krebs cycle. Citrate is consequently exported into the cytoplasm and promotes *de-novo* lipogenesis through FAS. *De-novo* synthesized fatty acids are subsequently fuelled back into lipid oxidation or incorporated into higher lipid species. They then exert distinct effects on glucose homeostasis. DAG, PC and PE are used to expand mitochondrial mass, without impact on glucose homeostasis, while sphingosine inhibits glucose uptake.

Exercised state: At elevated levels of PGC-1α citrate synthase activity further increases and the levels of acetylcarnitine diminish. Citrate is shunted towards catabolic and anabolic pathways.
Acutely, the high turn-over of ATP during muscle contraction drives further flux of lipids into the catabolic system. Chronically, endurance training further promotes lipid synthesis and storage as triglycerides. The combination of exercise and elevated muscle PGC-1α consequently results in an elevated activity of lipid synthesis and a concomitant increase in TG, DAG, PC and PE. Lipid fluxes are thus diverted away from sphingosine towards triglyceride biosynthesis. Moreover, the elevated levels of TG inhibit the action of sphingosine and relieve any inhibitory effect. The high FAS activity will drive glucose uptake into skeletal muscle and thus removal of glucose from the circulation.
Arrows/lines: dashed, basal conditions; straight, altered at elevated PGC-1α levels or exercise; light, chronic elevation; dark, additional elevation during acute exercise.
Boxes/lines: red, insulin-desensitizing metabolites; blue, neutral metabolites in respect to insulin sensitivity.


A
glucose (mg/dl)
time (min)
wt sed
wt ex
MPGC-1α TG sed
MPGC-1α TG ex
B
Area under the curve (Arbitrary units)
GTT
C
D
ITT
E
Relative mRNA levels (fold change)
GLUT4
HKII
F
Glucose uptake (fold change)
Glucose uptake
G
PI3K activity (fold change)
IRS-1-associated PI3K activity


A
@@@
§§
§§
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1.40
1.20
1.00
0.80
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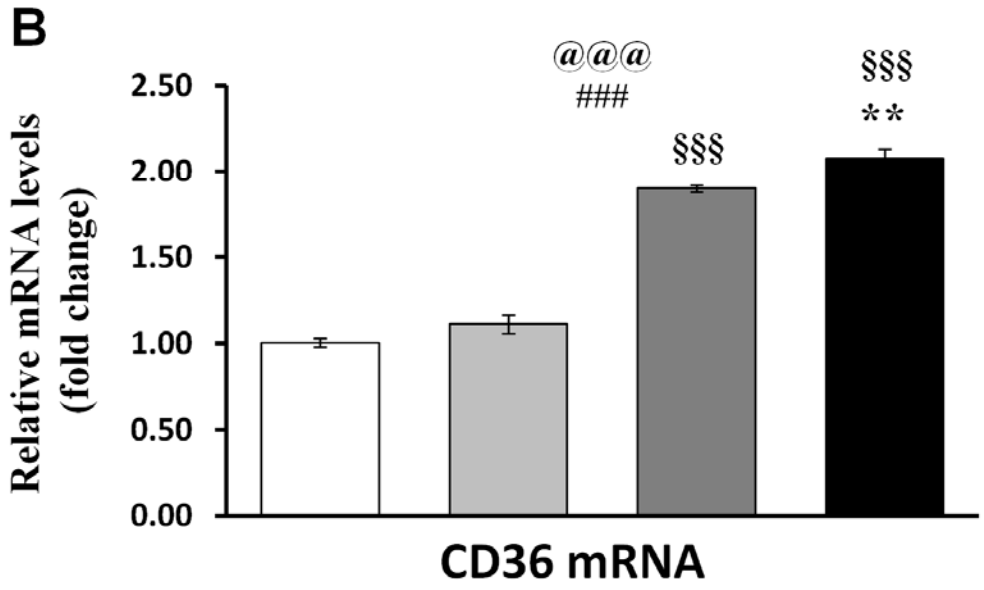
0.40
0.20
0.00
Relative mRNA levels
(fold change)
LPL mRNA


B
@@@
###
§§§
§§§
**
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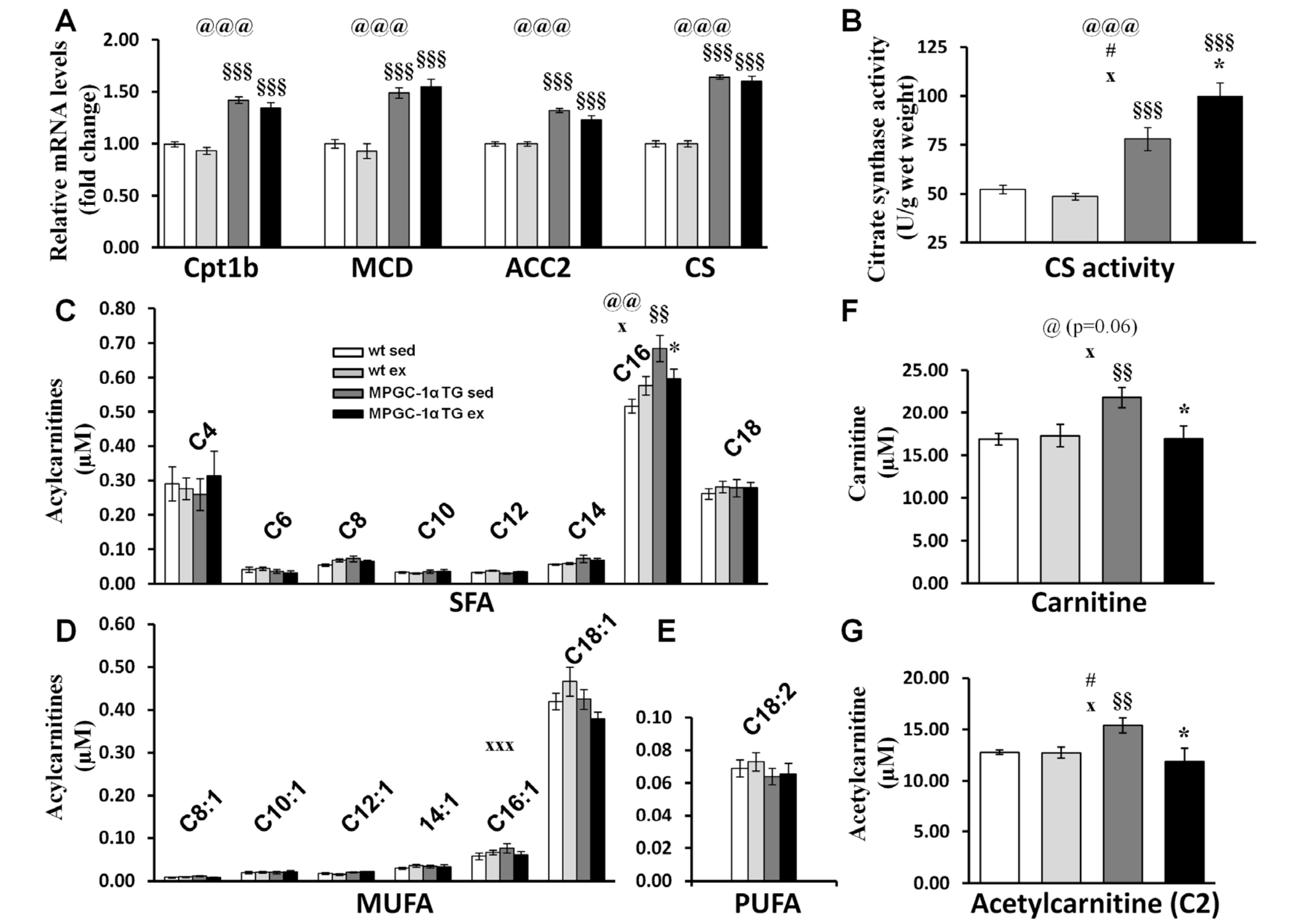
2.00
1.50
1.00
0.50
0.00
Relative mRNA levels
(fold change)
CD36 mRNA


A
Relative mRNA levels (fold change)
2.00
1.50
1.00
0.50
0.00
@@@
§§§
Cpt1b
MCD
ACC2
CS
B
Citrate synthase activity (U/g wet weight)
125
100
75
50
25
#
x
*
CS activity
C
Acylcarnitines (µM)
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
wt sed
wt ex
MPGC-1α TG sed
MPGC-1α TG ex
@@
§§
C4
C6
C8
C10
C12
C14
C16
C18
SFA
D
C8:1
C10:1
C12:1
14:1
C16:1
C18:1
xxx
MUFA
E
C18:2
PUFA
F
@ (p=0.06)
Carnitine (µM)
25.00
20.00
15.00
10.00
5.00
0.00
Carnitine
G
Acetylcarnitine (µM)
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10.00
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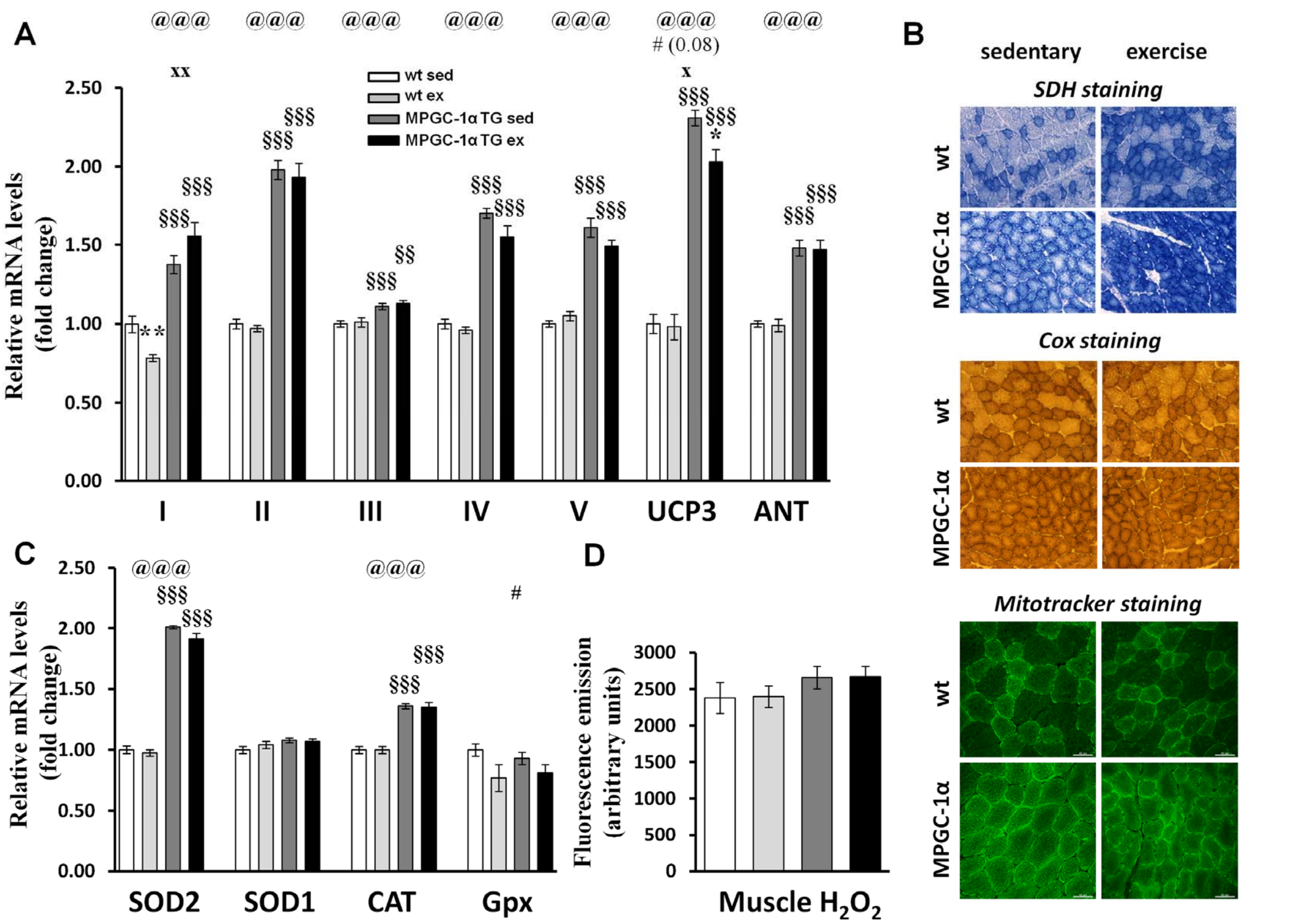
Acetylcarnitine (C2)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# (0.08)
xx
x
wt sed
wt ex
MPGC-1α TG sed
MPGC-1α TG ex
Relative mRNA levels (fold change)
2.50
2.00
1.50
1.00
0.50
0.00
§§§
§§
**
*
I
II
III
IV
V
UCP3
ANT
B
sedentary
exercise
SDH staining
wt
MPGC-1α
Cox staining
wt
MPGC-1α
Mitotracker staining
wt
MPGC-1α
C
@@@ @@@ #
§§§
Relative mRNA levels (fold change)
2.50
2.00
1.50
1.00
0.50
0.00
SOD2
SOD1
CAT
Gpx
D
Fluorescence emission (arbitrary units)
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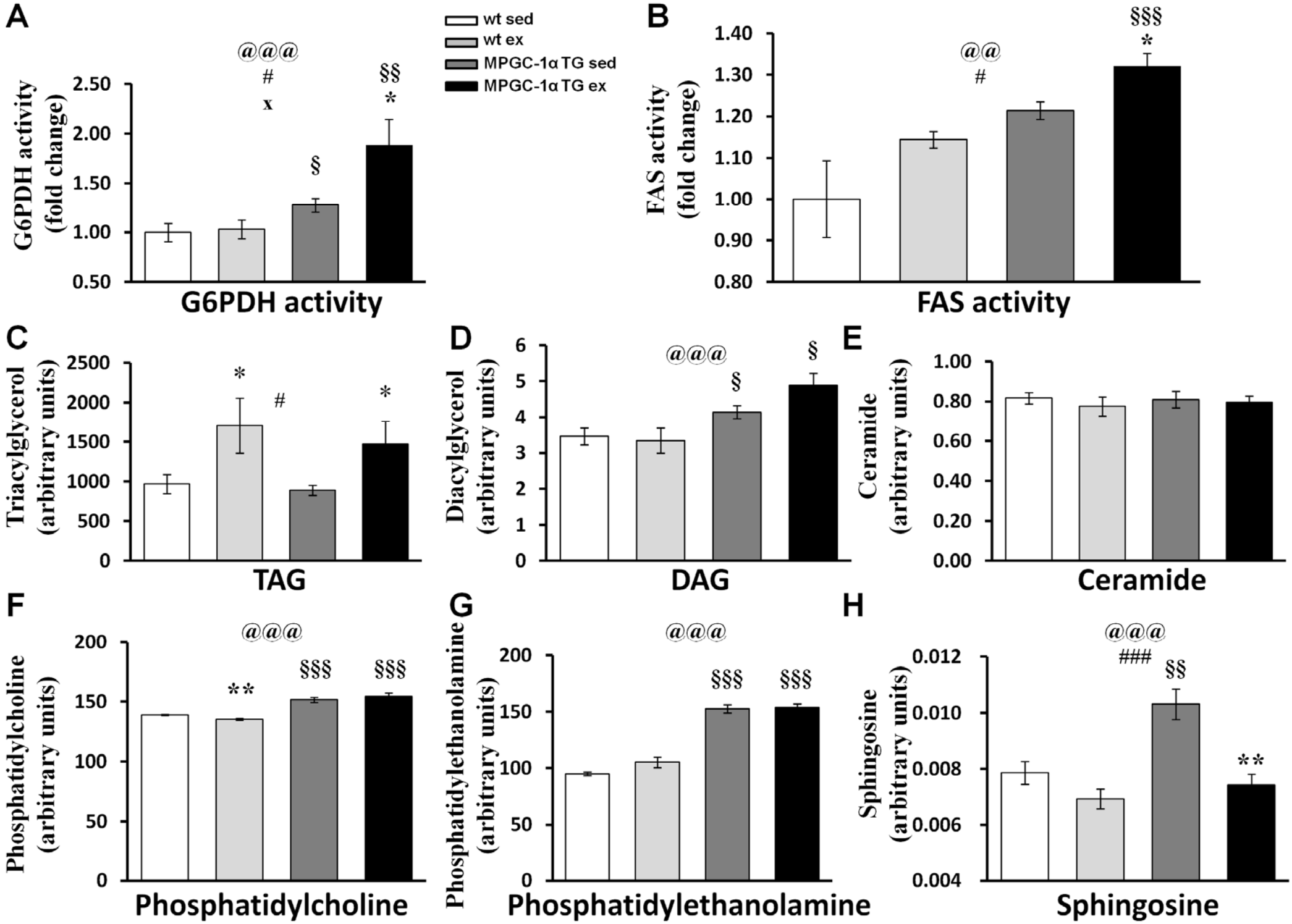
2500
2000
1500
1000
500
0
Muscle H_2O_2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wt sed
wt ex
MPGC-1α TG sed
MPGC-1α TG ex
G6PDH activity (fold change)
G6PDH activity
@@@
#
x
§
§§
*
B
FAS activity (fold change)
FAS activity
@@
#
§§§
*
C
Triacylglycerol (arbitrary units)
TAG
*
#
*
D
Diacylglycerol (arbitrary units)
DAG
@@@
§
§
E
Ceramide (arbitrary units)
Ceramide
F
Phosphatidylcholine (arbitrary units)
Phosphatidylcholine
@@@
**
§§§
§§§
G
Phosphatidylethanolamine (arbitrary units)
Phosphatidylethanolamine
@@@
§§§
§§§
H
Sphingosine (arbitrary units)
Sphingosine
@@@
###
§§
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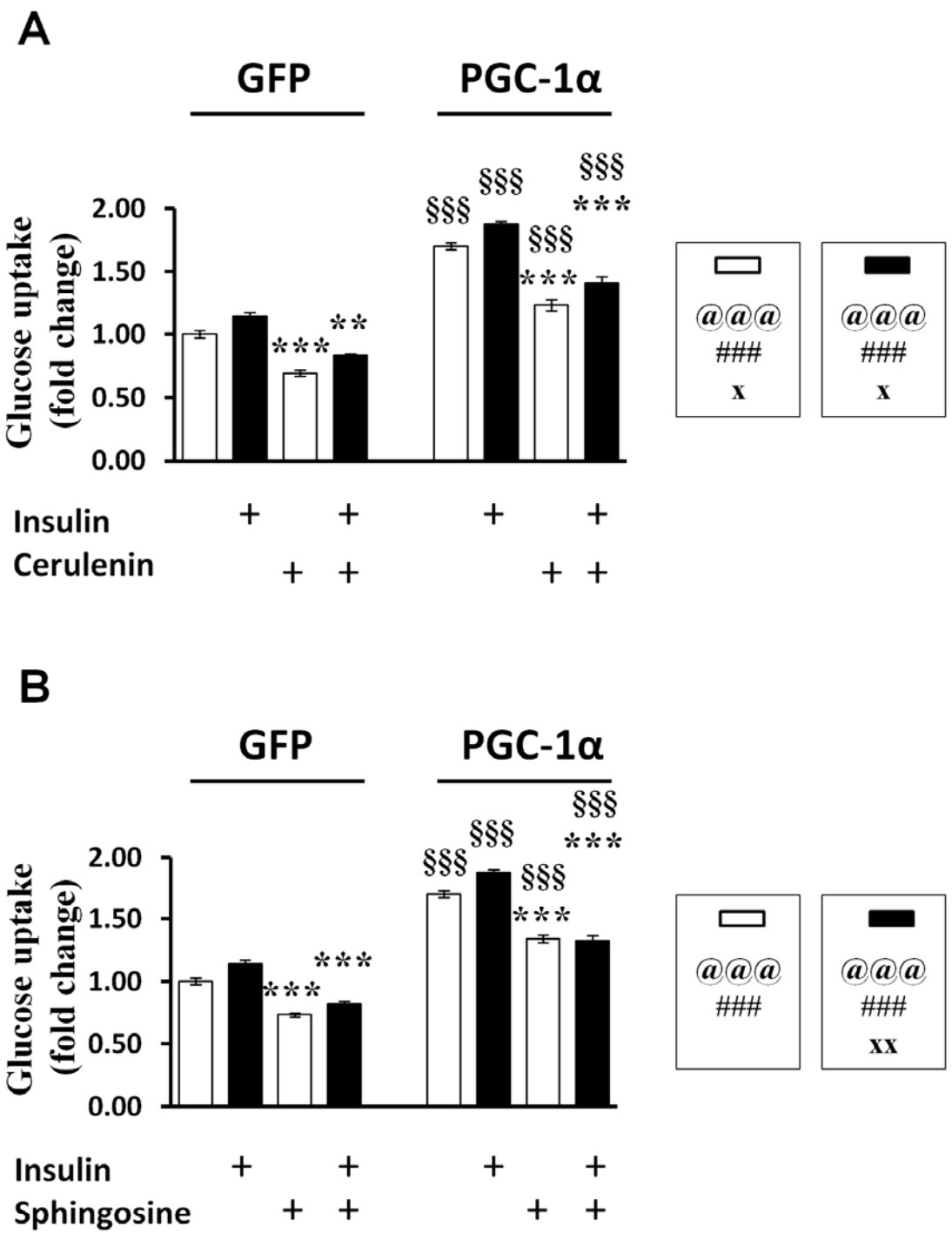
A
GFP
PGC-1α
Glucose uptake (fold change)
2.00
1.50
1.00
0.50
0.00
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§§§
§§§
§§§
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Insulin
Cerulenin
@@@
###
x
@@@
###
x
B
GFP
PGC-1α
Glucose uptake (fold change)
2.00
1.50
1.00
0.50
0.00
§§§
§§§
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Insulin
Sphingosine
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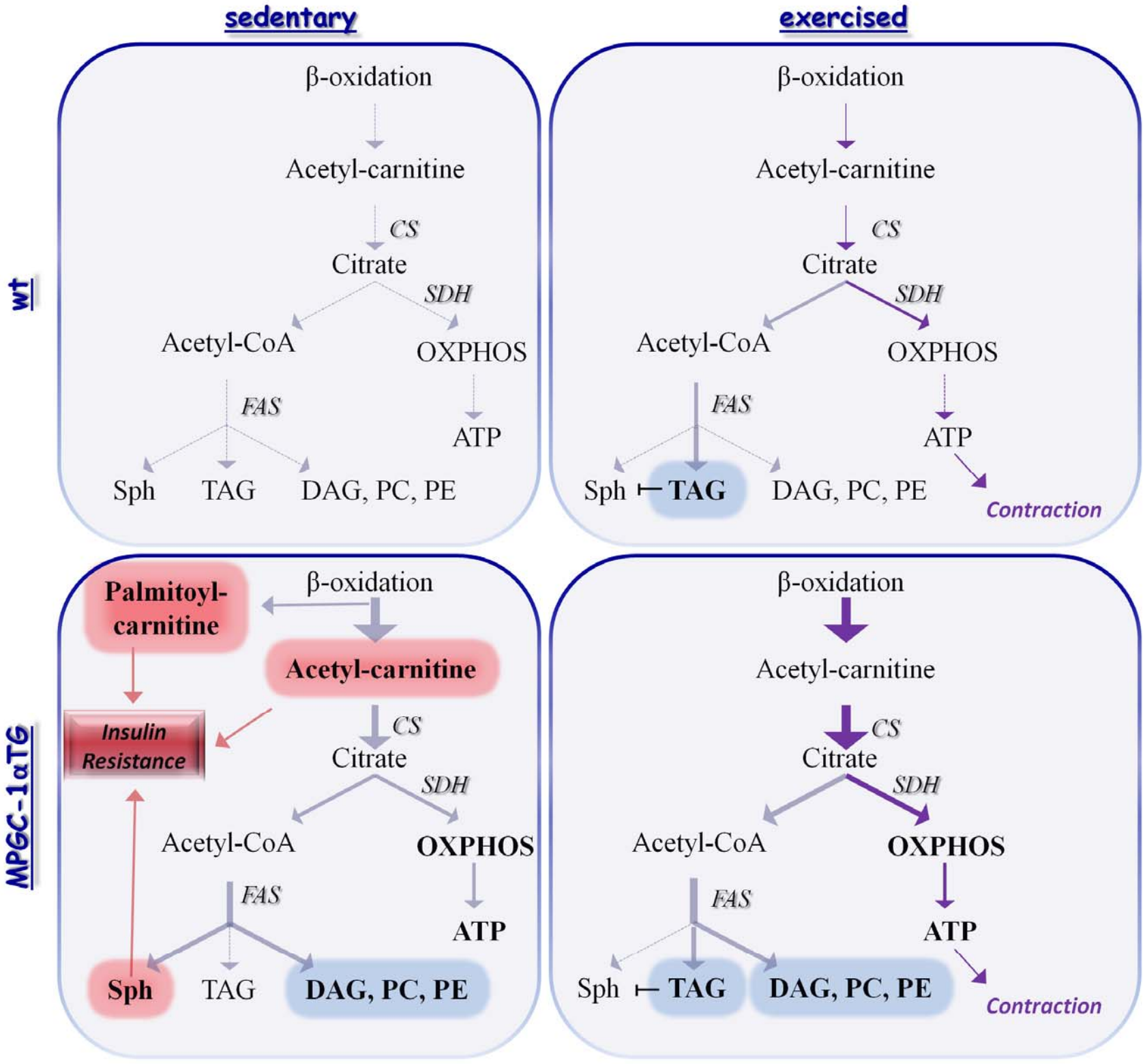
xx


sedentary
exercised
wt
MPGC-1αTG
β-oxidation
Acetyl-carnitine
CS
Citrate
SDH
Acetyl-CoA
OXPHOS
FAS
ATP
Sph
TAG
DAG, PC, PE
Contraction
Palmitoyl-carnitine
Insulin Resistance