Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake

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Calvo JA, Daniels TG, Wang X, Paul A, Lin J, Spiegelman BM, Stevenson SC, Rangwala SM. Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake. *J Appl Physiol* 104: 1304–1312, 2008. First published January 31, 2008; doi:10.1152/japplphysiol.01231.2007.—The induction of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), a key regulator of mitochondriogenesis, is well-established under multiple physical exercise regimens, including, endurance, resistance, and sprint training. We wanted to determine if increased expression of PGC-1α in muscle is sufficient to improve performance during exercise in vivo. We demonstrate that muscle-specific expression of PGC-1α improves the performance during voluntary as well as forced exercise challenges. Additionally, PGC-1α transgenic mice exhibit an enhanced performance during a peak oxygen uptake exercise test, demonstrating an increased peak oxidative capacity, or whole body oxygen uptake. This increased ability to perform in multiple exercise paradigms is supported by enhanced mitochondrial function as suggested by increased mitochondrial gene expression, mitochondrial DNA, and mitochondrial enzyme activity. Thus this study demonstrates that upregulation of PGC-1α in muscle in vivo is sufficient to greatly improve exercise performance under various exercise paradigms as well as increase peak oxygen uptake.

exercise capacity; peak oxygen uptake; peroxisome proliferator-activated receptor γ-coactivator-1α

PEROXISOME proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) and its closely related family member PGC-1β are coactivators essential for various cellular processes, including mitochondrial biogenesis, oxidative metabolism, and energy homeostasis (24, 26, 46). The PGC-1 proteins partner with a variety of transcription factors, including nuclear receptors such as the PPARs, estrogen-related receptors (ERRs), and hepatocyte nuclear factor 4α, as well as the nuclear respiratory factors (NRFs) and myocyte enhancer factor 2 (MEF2) (23, 27, 29, 40, 42, 46). Several experimental approaches in vitro as well as in vivo have established the role of PGC-1s as key regulators of mitochondrial gene expression, function, and biogenesis (40, 46). Both PGC-1α- and PGC-1β-deficient mice have been generated, and although both models exhibit impaired mitochondrial gene expression, and decreased mitochondrial area, they exhibit only slight changes in mitochondrial respiration during basal conditions (2, 21, 22, 25, 41). Conversely, they exhibit significantly decreased mitochondrial function during states that require increased energy, such as exercise and cold challenge (21, 22, 25, 41). Muscle-specific PGC-1α transgenic mice exhibit an increased expression of multiple markers of slow fiber-type muscle in type 2 muscle fibers, whereas muscle-specific PGC-1α knock-out mice display the converse shift in muscle fibers (11, 23). Furthermore, skeletal muscle from the PGC-1α transgenic mice exhibit a decreased fatiguability ex vivo, while muscle-specific PGC-1α knock-out mice demonstrate decreased exercise capacity (11, 23). Similarly, muscle-specific PGC-1β transgenic mice demonstrate increased mitochondrial gene expression, a fiber-type switch to oxidative myosin heavy chain type IIX fibers, and an improvement in exercise capacity (3). Together, these in vivo models indicate that PGC-1α and PGC-1β are important modulators of mitochondrial function.

Physical training induces muscle remodeling as well as mitochondrial biogenesis within skeletal muscle, resulting in multiple physiological changes, including fiber-type conversion from glycolytic to oxidative fibers, enhanced mitochondrial function, increased oxidative capacity, and improved metabolic flexibility (6, 10, 13). The expression of PGC-1α is induced in muscle following both an acute exercise challenge or following long-term exercise training (4, 18, 32–34, 37). Furthermore, inactivity and denervation are associated with decreased PGC-1α expression (17, 39). However, it is not known if an upregulation of PGC-1α and the subsequent mitochondrial biogenesis is sufficient to improve exercise capacity in vivo. It was recently demonstrated that an inducible, muscle-specific PGC-1α transgenic mouse model exhibited diminished exercise capacity as a result of decreased glycogen utilization (44). Using an independent mouse model, we wanted to investigate if an upregulation of PGC-1α in skeletal muscle of PGC-1α transgenic mice is sufficient to improve exercise capacity during voluntary and forced exercise paradigms. We demonstrate that increased PGC-1α expression in muscle results in an enhanced ability to exercise under various regimens and improves peak oxygen uptake.

MATERIALS AND METHODS

Animal studies. All mice used were maintained on 12:12-h light-dark cycle and cared for in accordance with animal welfare regulations under an approved institutional Animal Care and Use Committee protocol in the Novartis Institute for BioMedical Research animal facility, which is accredited by the Association for Assessment and Accreditation of Lab Animal Care. PGC-1α transgenic mice were rederived before use, and male transgenic and wild-type littermates were used for all experiments (23).

Male PGC-1α and wild-type mice were individually housed and fed a standard chow diet (LabDiet, St. Louis, MO) ad libitum. Body

weight and food intake were measured twice weekly. Body composition was determined using MRI, and fat and lean mass measurements were normalized to body weight. Fasting and fed blood glucose concentrations were determined using the OneTouch Ultra Glucometer (Lifespan, Milpitas, CA). To examine glucose tolerance, an oral glucose tolerance test was performed. Mice were fasted for 16 h, orally gavaged with a glucose solution (2 g/kg), and blood glucose readings were measured at baseline, 15, 30, 60, and 120 min following gavage. To examine insulin tolerance, an insulin tolerance test was performed. Mice were fasted for 4 h, injected intraperitoneally with Vetsulin (0.5 U/kg) (Intervet, Millsboro, DE), and blood glucose readings were measured at baseline, 15, 30, 60, and 120 min.

To collect insulin levels, tail blood was collected in EDTA-blood collection vials during a fed state or following a 16-h fast, and insulin was quantitated using an Ultra Sensitive Rat Insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL).

Determination of exercise capacity. To examine voluntary exercise capacity, 4-mo-old male mice were housed in cages equipped with exercise wheels. Mice were placed in a mouse single activity wheel system (Lafayette Instrument, Lafayette, IN), voluntary wheel-running was measured for 72 h, and the data were analyzed using the Activity Wheel Software (Lafayette Instrument).

Before exercise testing, the mice were acclimatized to the treadmill by running the day before the test for 10 min at 10 m/min followed by

2 min at 20 m/min. To determine exercise capacity under an endurance paradigm, 4-mo-old male mice were placed on a six-lane treadmill (Columbus Instruments, Columbus, OH) and run with a fixed slope of 10°. During the first 30 min, the speed was 10 m/min and increased 2 m/min every 15 min thereafter. The protocol is shown schematically in Fig. 1C and is similar to that previously performed (44). Animals ran until exhaustion, which is defined as remaining on the shocker plate for more than 10–15 s. Work performed (J) was calculated as previously described (31).

Exercise capacity during a higher-speed challenge was determined during the peak oxygen uptake ($\dot{V}o_2$) challenge. To determine peak $\dot{V}o_2$, 6-mo-old male mice were placed in an enclosed treadmill (Columbus Instruments) for 5 min at a 0° incline and 0 m/min. The mice were then challenged with 1.5-min intervals of increasing speed at a 15° incline. The protocol is shown schematically in Fig. 1E. The increasing speeds used in the protocol were 10, 14, 18, 22, 26, 28, 30, 32, 34, 36, 38, and 40 m/min. The protocol was performed until exhaustion, as defined above. Relative exercise intensity was derived by calculating the treadmill speed as a percentage of the maximal speed achieved by that genotype at peak $\dot{V}o_2$.

To examine the peak $\dot{V}o_2$ and respiratory exchange ratio (RER) values during exercise, 6-mo-old male mice were subjected to the peak $\dot{V}o_2$ test described above in an enclosed treadmill attached to the Comprehensive Laboratory Animal Monitoring System (CLAMS)
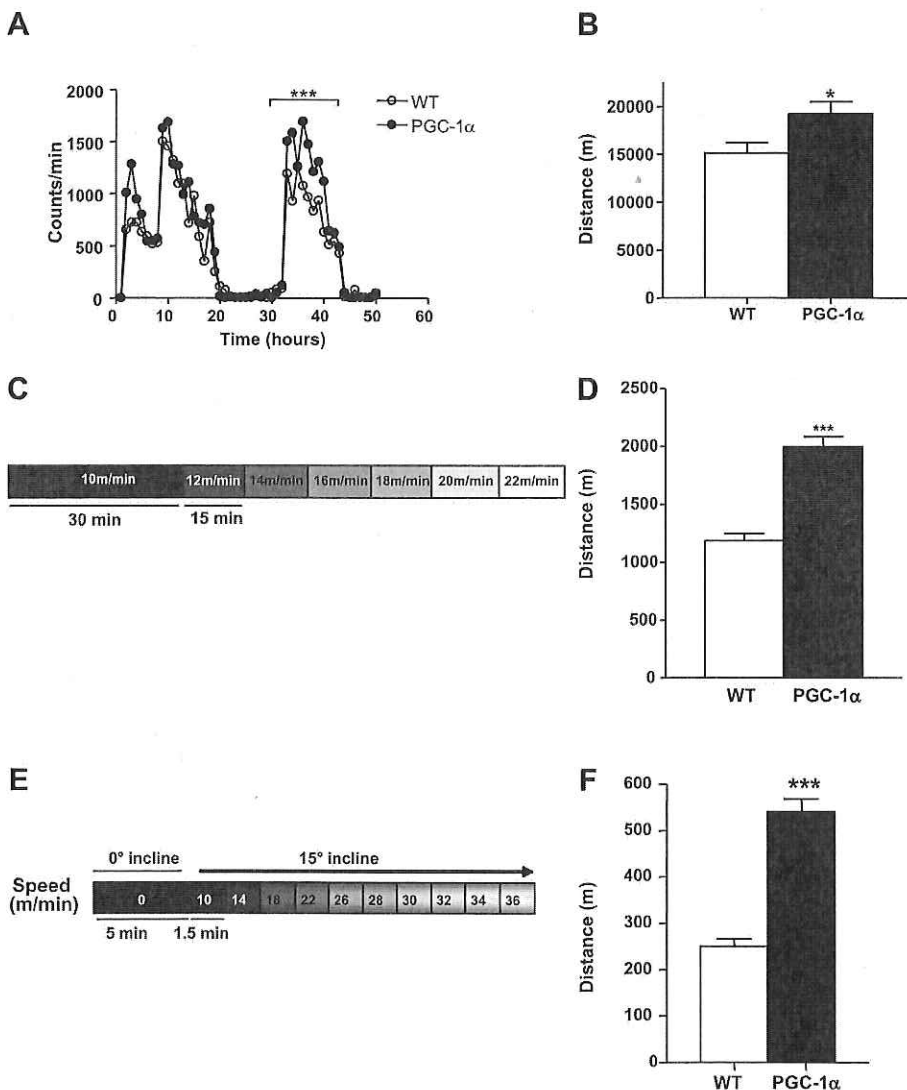

Fig. 1. Peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) transgenic mice exhibit greater exercise capacity during multiple exercise paradigms. *A*: voluntary exercise was measured by performing a wheel running experiment on 4-mo-old male PGC-1α transgenic and wild-type (WT) control mice (*n* = 5 each). Mice were housed in cages with running wheels for 72 h, and the speed (counts/min) was measured. The average speed for all mice during the for the first 50 h was plotted. *B*: total distance traveled during voluntary exercise test. Average distance traveled for PGC-1α transgenic and WT control mice is shown. *C*: schematic representation of the treadmill protocol used to determine the ability to perform endurance exercise, as modified from Ref. 44. *D*: average total distance traveled for 4-mo-old PGC-1α transgenic and WT control mice (*n* = 19) during the endurance exercise test is shown. *E*: schematic representation of the treadmill protocol used to examine peak oxygen uptake ($\dot{V}o_2$) values. Four-month-old male PGC-1α transgenic and WT control mice (*n* = 8 each) were exercised on an enclosed treadmill. *F*: total distance traveled by PGC-1α transgenic and WT control mice (*n* = 8 each) during the forced exercise challenge is shown. *P* < 0.05, ***P* < 0.001.

(Columbus Instruments). Measurements were collected for 5 min before the exercise challenge, throughout the challenge, and following failure until values approached baseline. Air flow through the treadmill was 0.6 l/min, and $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) readings were acquired every 15 s. Data analysis was performed using the Oxymax software (Columbus Instruments).

Glycogen content quantification. The gastrocnemius muscle and liver were harvested from 6-mo-old male mice in an ad libitum state, flash-frozen in liquid nitrogen, and pulverized, and approximately 50–100 mg was used to isolate glycogen. Briefly, samples were incubated with 500 μl of 30% sodium hydroxide and incubated for 30 min at 75°C (liver) or 86°C (gastrocnemius). The samples were cooled on ice, 1 ml of absolute ethanol was added, and incubated overnight at −20°C. The precipitated glycogen was recovered by centrifugation for 20 min at 840 g, washed with absolute ethanol, and incubated for 2 h at −20°C. Following centrifugation for 20 min at 5,000 g, the glycogen was resuspended in 250 μl distilled sterile water, hydrolyzed with an equal volume of 1 N HCl for 2 h at 99°C, and cooled to room temperature. The sample pH was neutralized by the addition of 23 μl of 10 N NaOH, and the glucose concentration was determined using the Amplex red kit (Molecular Probes, Carlsbad, CA).

Analysis of mRNA expression and mitochondrial DNA content. Six-month-old male wild-type and PGC-1α transgenic mice were euthanized in an ad libitum state, and gastrocnemius and heart muscles were harvested. Gastrocnemius muscle samples were pulverized, and total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) and quantified using a spectrophotometer at OD 260 nm. Reverse transcription was performed using the BD Sprint PowerScript kit (Clontech, Mountain View, CA), and quantitative real-time PCR (Q-PCR) was performed using Assay-on-Demand primer probes (Applied Biosystems, Foster City, CA). Average relative mRNA expression values were calculated for each group using $2^{-\Delta\Delta Ct}$, where Ct is cycle threshold (as described by Applied Biosystems, Foster City, CA) using β-2-microglobulin (B2M) as an endogenous control, and with the expression of wild-type mice normalized to a value of 1. The mRNA expression of B2M is similar for both wild-type and PGC-1α transgenic mice, and the Assay-on-Demand primer probes exhibit similar efficiencies. Statistical significance for each gene was determined using the Student's t-test comparing the transgenic mice with the wild-type controls.

To determine mitochondrial DNA (mtDNA) content, genomic DNA was isolated from 50 mg of pulverized gastrocnemius muscle using the DNAeasy tissue kit (Qiagen, Valencia, CA). DNA was quantitated using a spectrophotometer at OD 260 nm, and 40 ng of genomic DNA was used to amplify cytochrome b (mitochondrial encoded gene) and β-actin (nuclear-encoded gene) using Q-PCR. The β-actin primer probe set was obtained as an Assay-on-Demand, and the cytochrome b primer probe was generated by using the following sequences: forward primer 5'-TTC ACA CCT CAA AGC AAC GAA-3', reverse primer 5'-GCC CCC AAT TCA GGT TAA GAT AA-3', and probe 5'-CGC CCA ATC ACA CAA ATT TTG TAC TGA ATC C-3'.

Citrate synthase activity assay. Gastrocnemius and tibialis anterior muscles isolated from 6-mo-old male mice were pulverized and lysed in RIPA lysis buffer supplemented with a complete protease inhibitor cocktail (Roche, Indianapolis, IN). Total protein content was assessed using the BCA protein assay kit (Pierce, Rockford, IL). Citrate synthase (CS) activity was determined using 2 μg of total protein using the CS assay kit (Sigma, St. Louis, MO).

Data analysis. Data are presented as means ± SE. All statistical analysis was performed using Prism 4.0 (GraphPad 4, San Diego, CA). Differences between a two-tailed Student's t-test or ANOVA were considered statistically significant if $P < 0.05$. To determine statistical significance of RER values at relative exercise intensity, an area under the curve (AUC) was performed using the mean RER at rest for each genotype as baseline.

RESULTS

PGC-1α transgenic mice demonstrate increased exercise capacity. To determine if increased PGC-1α expression in skeletal muscle impacts the ability of mice to perform exercise, we investigated the exercise capacity of PGC-1α transgenic mice under various exercise paradigms. We evaluated the ability of these mice to perform voluntary exercise during a 3-day wheel-running experiment. During the first day housed in the treadmill cages, the mice experienced an acclimation period, resulting in high activity for both genotypes. During the second day, the PGC-1α transgenic mice exhibited greater voluntary exercise, as observed in a significant increase in speed (counts/min) (Fig. 1A). Furthermore, during the course of the experiment, PGC-1α transgenic mice traveled 27% further on the voluntary wheel compared with wild-type mice (Fig. 1B).

To determine if the increased voluntary exercise observed in the PGC-1α transgenic mice translated into increased performance during a forced exercise challenge, we examined their ability to perform a forced treadmill run. We examined the exercise capacity of PGC-1α transgenic mice during a slow, endurance-type running challenge. This challenge consisted of running the mice at a constant speed of 10 m/min for 30 min and then increasing the speed 2 m/min every 15 min (Fig. 1C). The mice were run until failure, which was defined as remaining on the shock-grid for greater than 10 consecutive seconds. During this endurance exercise paradigm, the PGC-1α transgenic mice ran significantly longer distances (Fig. 1D). Similarly, during the exercise challenge, the PGC-1α transgenic mice ran for a duration of 121.9 ± 3.4 min, a significant increase compared with the 88.2 ± 3.3 min the wild-type mice ran ($P < 0.0001$). Further, the PGC-1α transgenic mice attained a maximum speed of 23.4 ± 0.44 m/min, significantly faster than the 18.85 ± 0.47 m/min that the wild-type mice reached ($P < 0.0001$). Together, this indicates that increased PGC-1α expression in skeletal muscle results in greater performance during an oxidative challenge.

To determine if PGC-1α expression in skeletal muscle also improves performance during a shorter, more intense exercise paradigm, the PGC-1α transgenic mice and littermate controls were subjected to a treadmill exercise challenge designed to measure peak $\dot{V}O_2$ value. This peak $\dot{V}O_2$ challenge consisted of 1.5-min intervals of increasing speed at a fixed 15° incline (Fig. 1E). Consistent with the endurance challenge, the PGC-1α transgenic mice ran longer distances during this exercise test (Fig. 1F). The PGC-1α transgenic mice also reached a higher maximum speed, 39.25 ± 0.99 m/min compared with the speed wild-type mice achieved, 29.25 ± 0.84 m/min ($P < 0.0001$). Furthermore, the PGC-1α mice ran for 24.46 ± 0.78 min, whereas wild-type mice ran for 16.88 ± 0.513 min ($P < 0.0001$). This increased exercise capacity during these different exercise paradigms is consistent with the finding that isolated muscle from PGC-1α transgenic mice exhibits decreased fatigability in response to electrical stimulation ex vivo (23).

PGC-1α transgenic mice demonstrate increased peak oxygen uptake and reduced RER values during exercise challenge. To investigate the peak oxygen consumption, or oxygen uptake at a whole body level, in PGC-1α transgenic mice, we per-

formed a peak $\dot{V}O_2$ exercise challenge in an enclosed treadmill and acquired real-time respiratory measurements using indirect calorimetry (Fig. 1E). In both groups, $\dot{V}O_2$ values increased linearly as speed increased until the point of failure (Fig. 2A). As mice fail during the exercise challenge, the treadmill is stopped and $\dot{V}O_2$ measurements continue to be taken, resulting in a decrease in $\dot{V}O_2$ values. The curves in Fig. 2A represent the mean $\dot{V}O_2$ profiles and the inflection point corresponds to the mean peak $\dot{V}O_2$ value on failure. Figure 2B illustrates the mean peak $\dot{V}O_2$ during this exercise challenge for PGC-1α transgenic and wild-type mice. The PGC-1α transgenic mice demonstrated a 24% increase ($P <$ 0.0001) in their peak oxygen uptake compared with their control littermates. Comparatively, an 8-wk-long endurance training regimen induces only a 15% increase in peak oxygen uptake in humans (9, 12).

We examined the RER ($\dot{V}CO_2/\dot{V}O_2$) values during the peak $\dot{V}O_2$ exercise challenge to investigate fuel utilization during exercise. During the beginning of the capacity test, while the mice were either at rest or running at low speed, both wild-type and PGC-1α transgenic mice exhibited low RER values (0.799 ± 0.013 for wild-type mice and 0.806 ± 0.011 for PGC-1α transgenic mice), indicating the utilization of fatty acids for fuel (Fig. 2C). However, as the treadmill speed increases during the capacity test, RER values increase, indicating a switch from fatty acid oxidation to glucose oxidation as the predominate fuel source. In wild-type mice, this increase in RER values occurred at low speeds during the exercise challenge (Fig. 2C). Conversely, the PGC-1α transgenic mice were able to exercise at higher speeds before an increase in RER values was observed. Consequently, at speeds above 10 m/min, the PGC-1α transgenic mice exhibited significantly lower RER values (Fig. 2C). This suggests that PGC-1α

transgenic mice were capable of utilizing fatty acids and sparing carbohydrate stores at higher speeds during this exercise challenge. We normalized for the increased exercise performance by PGC-1α mice by examining RER values at similar relative exercise intensities. When the data are presented as the percentage of speed achieved at maximal peak $\dot{V}O_2$, the PGC-1α transgenic mice continue to exhibit significantly lower RER values (Fig. 2D). Together, these data indicate that PGC-1α transgenic mice exhibit lower RER values at either the same absolute exercise intensity (speed), or the same relative exercise intensities (percentage of maximum speed at peak $\dot{V}O_2$).

PGC-1α transgenic mice show no alterations in glucose tolerance or insulin sensitivity. Poor physical fitness is a known risk factor for developing Type 2 diabetes, whereas exercise training has been shown to improve insulin sensitivity (7, 8, 43). Therefore, we investigated if the increased exercise performance and peak oxygen uptake observed in the PGC-1α transgenic mice influenced whole body glucose homeostasis or insulin sensitivity. Glucose and insulin levels under fed and fasting conditions in PGC-1α transgenic mice did not differ compared with wild-type mice (Fig. 3A and Table 1). During an insulin tolerance test (ITT), PGC-1α transgenic mice exhibit similar insulin sensitivity compared with wild-type mice (Fig. 3B). Further, we also performed an oral glucose tolerance test (OGTT) to examine glucose homeostasis and observed similar glucose excursion curves in PGC-1α transgenic mice compared with their wild-type littermates (Fig. 3C).

To address the impact of enhanced mitochondrial content on additional metabolic parameters, we examined PGC-1α transgenic mice at both 2 mo and at 7 mo of age. We observed no differences in body weight, body composition, or body core temperature between the transgenic and wild-type mice (Table
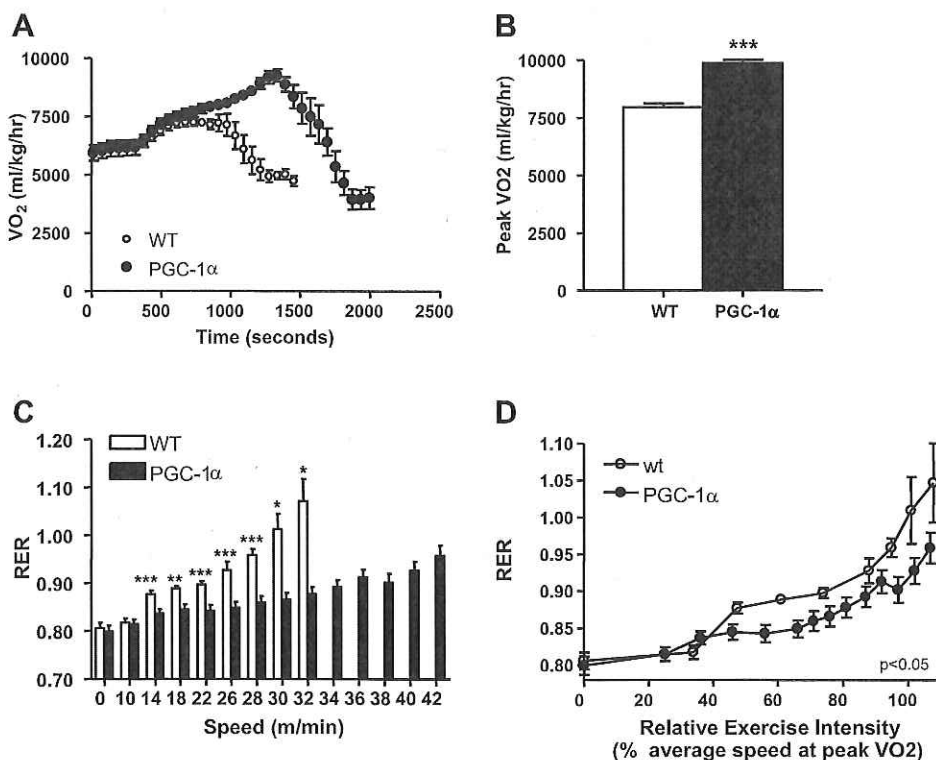

Fig. 2. PGC-1α transgenic mice exhibit greater peak $\dot{V}O_2$ and decreased respiratory exchange ratio (RER) values during exercise. A: during peak $\dot{V}O_2$ exercise challenge, indirect calorimetry measurements were acquired, and the $\dot{V}O_2$ values (means ± SE) are shown as a function of time. B: peak $\dot{V}O_2$, as defined by the $\dot{V}O_2$ measurement at the point of failure, is shown (mean ± SE) for the 4-mo-old male PGC-1α transgenic and WT control mice ($n = 8$ each). C: during peak $\dot{V}O_2$ exercise challenge, indirect calorimetry measurements were acquired, and RER (carbon dioxide production/$\dot{V}O_2$) values (means ± SE) for 4-mo-old male PGC-1α transgenic and WT control mice ($n = 8$ each) are plotted against speed. D: RER values (mean ± SE) were plotted against relative exercise intensity, as estimated by the percentage of mean speed at which peak $\dot{V}O_2$ occurred for each genotype. Eight 4-mo-old male PGC-1α transgenic and 8 male WT mice are represented. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ as determined by unpaired t-test (B, C). For D, statistical significance was calculated as area under the curve using the mean RER at rest for each genotype as baseline.
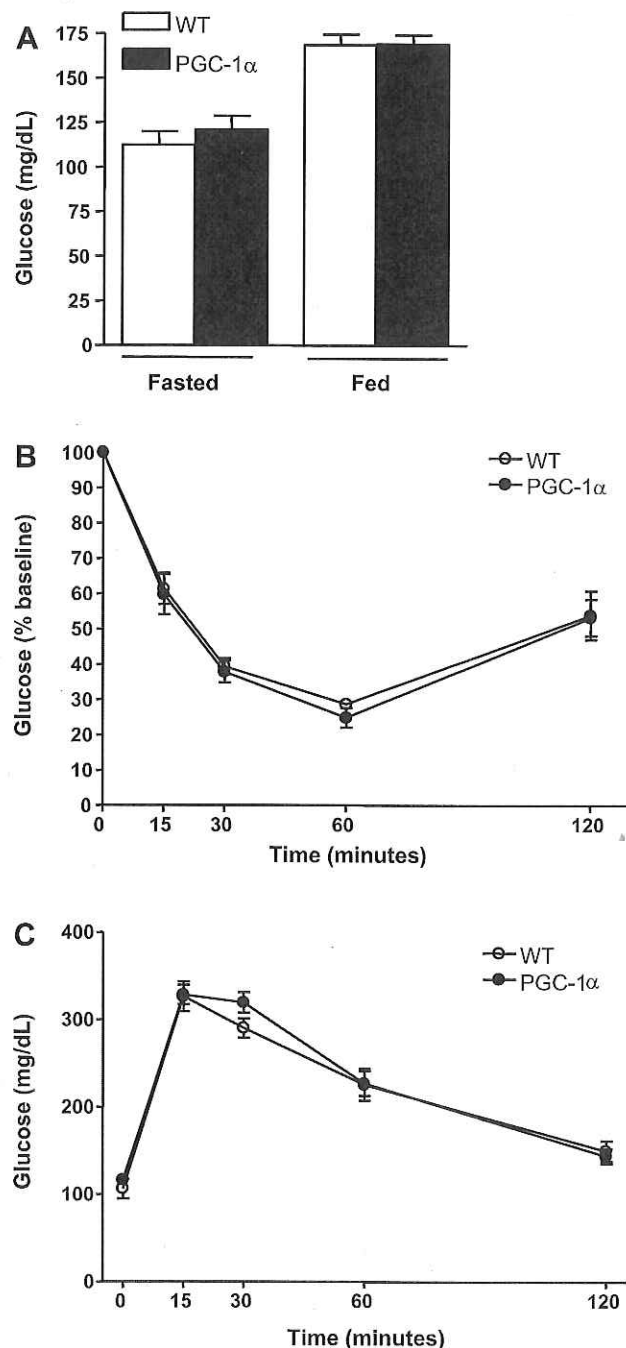
Fig. 3. PGC-1α transgenic mice exhibit no alteration in glucose tolerance or insulin sensitivity. A: blood glucose levels (means ± SE, $n = 9$–10 each) were measured in a fed state and following a 16-h fast in male PGC-1α transgenic and WT control mice at 6 mo of age. B: an insulin tolerance test performed in 4-mo-old male PGC-1α transgenic and WT control mice ($n = 9$–10 each) and the average plasma glucose values are shown as percentage of baseline. C: an oral glucose tolerance test was performed in 4-mo-old male PGC-1α transgenic and WT control mice ($n = 9$–10 each), and the average glucose excursion curves are shown.

1). Furthermore, analysis of energy expenditure ($\dot{V}O_2$) and activity revealed no differences between the transgenic and wild-type mice (Table 1). Thus the PGC-1α transgenic mice do not exhibit any differences in multiple metabolic parameters under basal conditions.

Expression of PGC-1α in skeletal muscle induces mitochondrial DNA levels, mRNA expression, and enzyme function. We performed quantitative real-time PCR (Q-PCR) to examine the expression of the PGC-1α transgene in gastrocnemius and heart muscles. In agreement with a previously published report, we observed a 16-fold increase in PGC-1α mRNA expression in gastrocnemius tissue (see Supplemental Fig. S1, available with the online version of this article) (23). Furthermore, we determined that the PGC-1α transgene is expressed at low levels in the heart (Supplemental Fig. S1). Next, we investigated the effect of increased PGC-1α expression on various mitochondrial pathways. Previous studies have illustrated increased expression of cytochrome c (CYCS), cytochrome oxidase II (COXII), and cytochrome oxidase IV (COXIV) in this mouse model (23). In gastrocnemius muscle of PGC-1α transgenic mice, we observed increased mRNA expression of genes important for the fatty acid transport and oxidation, OXPHOS genes, as well as genes important for the tricarboxylic acid (TCA) cycle, including fatty acid binding protein 3 (FABP3), fatty acid translocase (FAT/CD36), fatty acid transport protein 1 (FATP1), carnitine palmitoyltransferase 1b (CPT1b), medium-chain acyl dehydrogenase (MCAD), long-chain acyl dehydrogenase (LCAD), very long chain acyl dehydrogenase (ACADVL), CYCS, COXIV, ubiquinol-cytochrome c reductase binding protein (UQCRB), and isocitrate dehydrogenase (IDH3a) (Fig. 4A). We also observed alterations in select markers of the muscle fiber type in PGC-1α transgenic mice, consistent with a previously published report (Supplemental Fig. 1) (3). Furthermore, mRNA expression of the reactive oxygen species (ROS)-scavenging genes, superoxide dismutase-2 (SOD2), thioredoxin reductase-2 (TXNRD2) and catalase, were also increased (Supplemental Fig. 1), consistent with the recent finding that PGC-1α null mice exhibit decreased expression of ROS-detoxifying enzymes glutathione peroxidase-1 (GPX1) and SOD2 and are sensitive to multiple oxidative stressors (38). Increased mRNA expression of transcription factors essential for mitochondrial function, ERRα and mitochondrial transcription factor A (TFAM), was also observed (Supplemental Fig. 1).

We investigated mtDNA content by examining the ratio of cytochrome *b* to β-actin. We found that PGC-1α transgenic mice exhibited a 166% increase in mtDNA compared with wild-type controls (Fig. 4B). We next inspected the activity of CS, an enzyme of the TCA cycle and a well-validated marker of mitochondrial activity. Figure 4C demonstrates that the PGC-1α transgenic mice exhibited greater than twofold increases in CS activity in both the gastrocnemius and the tibialis anterior muscles. To further examine mitochondrial enzyme activity, we investigated the expression of pyruvate dehydrogenase kinase 4 (PDK4), a mitochondrial enzyme that controls metabolic substrate utilization. PDK4 induces fatty acid oxidation at the expense of glycolysis through the phosphorylation and inactivation of the PDH enzyme complex (16, 32). We investigated expression of PDK4 in the gastrocnemius muscle of untrained PGC-1α transgenic and wild-type littermates and observed an increase in PDK4 mRNA (Fig. 4A).

Expression of PGC-1α in vitro has been shown to increase complete fatty acid oxidation, resulting in an increased storage of glycogen (17, 30). Consistent with these experiments, PGC-1α transgenic mice exhibited a 57.6% increase in glycogen stores in their skeletal muscle in the fed state (Fig. 4D).

Table 1. *Metabolic parameters of PGC-1α transgenic mice*

	2 Mo Old			7 Mo Old		
	WT	PGC-1α	P Value	WT	PGC-1α	P Value
Body weight, g	19.48 (0.209)	19.35 (0.35)	NS	30.21 (0.91)	29.28 (0.73)	NS
%Lean mass	81.18 (1.00)	81.79 (1.05)	NS	70.36 (1.561)	73.84 (0.99)	NS
%Fat mass	16.34 (0.52)	16.05 (0.50)	NS	24.35 (1.646)	21.6 (1.039)	NS
Body core temperature, °C	ND	ND		36.91 (0.138)	37.21 (0.092)	NS
Triglycerides (fasted), mg/dl	92.92 (3.98)	94.94 (3.17)	NS	82.97 (2.79)	85.64 (2.30)	NS
Triglycerides (fed), mg/dl	ND	ND		163.6 (10.83)	149.8 (7.88)	NS
Insulin (fasted), ng/μl	ND	ND		0.5382 (0.064)	0.588 (0.073)	NS
Insulin (fed), ng/μl	ND	ND		1.141 (0.128)	1.395 (0.206)	NS
Glucose (fasted), mg/dl	163.3 (3.06)	159.1 (4.07)	NS	112.4 (7.49)	121.4 (8.48)	NS
Lactate (fasted), mg/dl	1.461 (0.113)	1.338 (0.080)	NS	ND	ND	
Activity*, total beam breaks	33,159 (2,562)	34,294 (2,435)	NS	38,194 (8,768)	31,955 (3,695)	NS
$\dot{V}O_2$*, ml·kg^{-1}·h^{-1}	251,781 (6,396)	262,576 (3,566)	NS	212,792 (5,614)	219,462 (7,494)	NS

Values are means (SE). PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; WT, wild type; NS, not significant; $\dot{V}O_2$, oxygen consumption; ND, not determined. *Denotes area under curve values during a 3-day experiment.

There was no difference in liver glycogen content, consistent with the muscle-specific expression of PGC-1α in this model (data not shown). We performed Q-PCR to determine a possible mechanism by which the PGC-1α transgenic mice exhibited increased glycogen content. We demonstrate that the transgenic mice exhibited decreased expression of glycogen phosphorylase but no change in mRNA for other genes important in glucose utilization (Fig. 4E). These data are consistent with a recent report demonstrating that inducible, muscle-specific expression of PGC-1α results in increased glycogen stores through multiple mechanisms (44).

DISCUSSION

It is well-established that various types of exercise induce PGC-1α expression in rodents and humans (1, 4, 15, 32, 34). This occurs coincident with increases in many aspects of mitochondrial function, including mitochondrial number, enzyme function, and expression of genes critical for mitochondrial function (5, 6, 14). However, at least one study demonstrates the increase in mitochondrial number/function occurs before the induction of PGC-1α (45). Further, skeletal muscle-specific expression of PGC-1α in an inducible manner results in decreased exercise capacity as a result of decreased glycogen utilization (44). Therefore, it remains unclear whether the PGC-1α induction observed on exercise training is sufficient to improve exercise capacity in vivo.

Previous work in PGC-1α transgenic mice demonstrates a fiber-type switch from glycolytic to oxidative muscle fibers and decreased fatigability during an ex vivo electrical stimulation assay (23). Consistent with these data, we demonstrate that these mice exhibit an increase in exercise ability during multiple exercise challenges. Further, the peak $\dot{V}O_2$ challenge demonstrated that expression of PGC-1α in skeletal muscle is sufficient to increase the aerobic capacity by 24% compared with their control littermates; the magnitude of this change is greater than what is observed following an intense exercise training regimen in humans (9, 12). Interestingly, both whole body and skeletal muscle-specific PGC-1α knock-out mice exhibit decreases in both voluntary physical activity levels and exercise capacity during forced treadmill training (11, 22). Furthermore, it has been recently demonstrated that activators of SIRT1, such as resveratrol, increase skeletal muscle mito-

chondrial content, oxidative capacity, and exercise capacity, through the deacetylation and activation of PGC-1α (19). Together, these data suggest that increased PGC-1α expression and/or activity in skeletal muscle is necessary and sufficient to improve exercise performance and peak oxygen uptake.

The magnitude of improvement in peak $\dot{V}O_2$ in the PGC-1α transgenic mice is suggestive that central factors may contribute to the improved exercise performance. We demonstrate that the PGC-1α transgenic mice exhibit a large increase of PGC-1α mRNA expression in gastrocnemius skeletal muscle, as well as a small increase in the heart (Supplemental Fig. S1). It is unknown if this twofold increase in PGC-1α expression contributes to the improved exercise capacity, as no analysis has been performed to examine cardiac structure or function in this transgenic mouse model. Multiple mouse models demonstrate that high expression of PGC-1α in cardiac muscle results in cardiomyopathy (20, 35). However, until additional experiments are performed to examine cardiac function in these mice, it is not possible to exclude the contribution of twofold increase in PGC-1α expression in heart to the improvements observed in peak oxygen uptake and exercise performance.

A recent report by Wende et al. (44) demonstrates a diminished capacity during a high-intensity exercise challenge in a different PGC-1α transgenic mouse model. Their paper used an inducible transgenic model to examine the consequences of PGC-1α induction for 3–4 wk, whereas the mouse model we utilized expressed PGC-1α constitutively (44). In addition to these temporal differences in transgene expression, the two mouse models likely exhibit differences due to expression levels (23, 44). The unexpected decrease in exercise capacity in the inducible PGC-1α transgenic mouse model is explained by a decreased ability to utilize glycogen during exercise. Wende et al. used forced exercise paradigms at significantly higher speeds to elicit these differences. In comparison, in our mouse model, we demonstrate increased performance both in a voluntary as well as in two forced exercise paradigms. Additionally, we challenged the mice at a slightly older age, and the overall decreased exercise capacity in the control animals at this age may have allowed us to detect a difference in exercise capacity between the genotypes. It is not unprecedented that two independent PGC-1α transgenic mouse models exhibit different phenotypes; the mouse model we examined prevents

Fig. 4. PGC-1α transgenic mice demonstrate increased mitochondrial gene expression, mitochondrial DNA content, and mitochondrial enzyme activity. A: real-time quantitative PCR was performed on gastrocnemius muscle from 6-mo-old male PGC-1α transgenic and WT control mice ($n = 8$ each). Data were normalized to β-2-microglobulin (B2M). MCAD, medium-chain acyl dehydrogenase; LCAD, long-chain acyl dehydrogenase; ACADVL, very long chain acyl dehydrogenase; CPT1b, carnitine palmitoyltransferase 1b; FABP3, fatty acid binding protein 3; CD36, fatty acid translocase; FATP1, fatty acid transport protein 1; CYCS, cytochrome c; COXIV, cytochrome oxidase IV; UQCRB, ubiquinol-cytochrome c reductase binding protein; PDK4, pyruvate dehydrogenase kinase 4; IDH3a, isocitrate dehydrogenase; CHO, carbohydrate; TCA, tricarboxylic acid. B: real-time quantitative PCR was performed on genomic DNA isolated from gastrocnemius muscle from PGC-1α transgenic and WT control mice ($n = 8$ each) to examine cytochrome b content. Data were normalized to β-actin. C: citrate synthase activity was quantitated from gastrocnemius (Gastroc) and tibialis anterior (TA) muscle protein lysates from the PGC-1α transgenic and WT control mice ($n = 8$ each). D: glycogen content (means ± SE) of gastrocnemius muscle samples from 6-mo-old male PGC-1α transgenic and WT control mice ($n = 8$ each) are shown. E: real-time quantitative PCR performed on gastrocnemius muscle from the PGC-1α transgenic and WT control mice ($n = 8$ each). $**P < 0.01$, $***P < 0.0001$ as determined by an unpaired t-test.
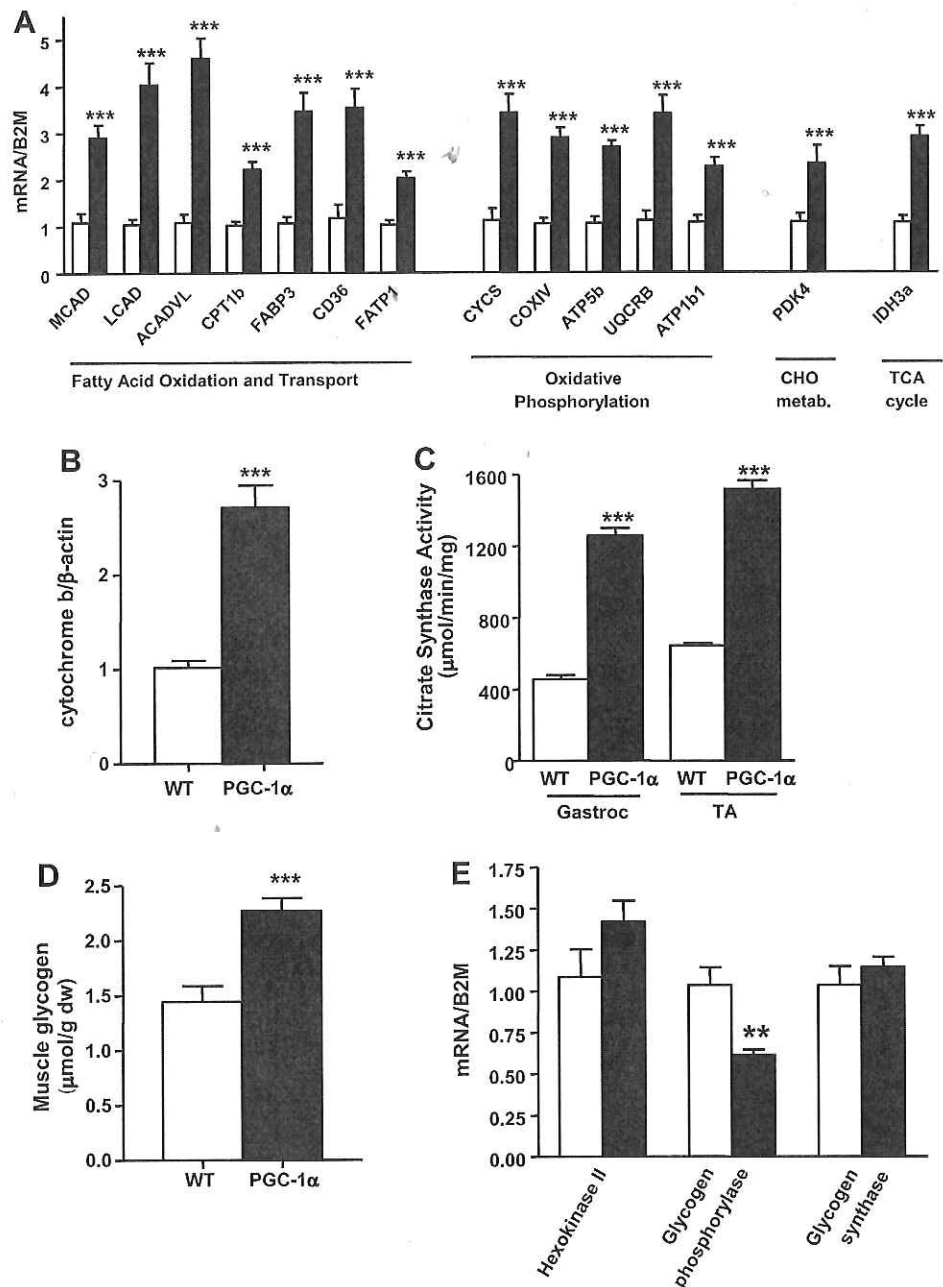
denervation and fasting-induced skeletal muscle atrophy whereas an independent model results in increased muscular atrophy (28, 36).

Another interesting finding illustrated by this work is that the PGC-1α transgenic mice exhibit lower RER values during a peak V̇o$_2$ challenge. This may be explained by the reduced relative workload (percentage of peak V̇o$_2$) experienced by the PGC-1α transgenic mice compared with wild-type mice. Since the PGC-1α transgenic mice exhibited an increase in peak V̇o$_2$, they experienced a reduced percentage of their peak V̇o$_2$ throughout the challenge compared with wild-type mice. To address this possibility, we examined RER values at the same relative exercise intensity. After comparing the RER values at the same percentage of maximal speed achieved during the exercise test, we demonstrate that the PGC-1α transgenic mice continue to exhibit lower RER values. Therefore, the PGC-1α transgenic mice exhibit lower RER values when examined at either absolute exercise intensity or relative exercise intensity. Although normalizing for relative exercise intensity decreases the divergence, the result is suggestive that the PGC-1α transgenic mice are capable of utilizing fatty acid as fuel for longer periods of time during exercise. Additionally, the RER values in PGC-1α transgenic mice never exceed 1.0 during the peak V̇o$_2$ challenge, suggesting that these mice achieve a more complete oxidation of fuel during exercise. In fact, the PGC-1α transgenic mice may exhibit a greater capacity for utilizing fatty acids as fuel at a molecular level. Consistent with this, we demonstrate increased expression of genes

necessary for lipid transport and oxidation, indicating that PGC-1α has pleiotropic effects on lipid utilization. These in vivo results are consistent with in vitro findings that PGC-1α expression stimulates fatty acid oxidation and induces complete fatty acid oxidation at the expense of incomplete fatty acid oxidation (17, 40). However, further studies are required to investigate lipid utilization and lipid flux in a dynamic setting to determine if the PGC-1α transgenic mice demonstrate greater fatty acid utilization in vivo.

Patients with Type 2 diabetes exhibit decreased mitochondrial function and impaired peak oxygen uptake. We demonstrate that the induction of PGC-1α in skeletal muscle is sufficient to increase both mitochondrial function, exercise ability, and peak oxygen uptake in vivo. However, under these circumstances, we were not able to detect any differences in glucose homeostasis or insulin sensitivity in the PGC-1α transgenic mice. It is possible challenging the PGC-1α transgenic mice with a high-fat diet or utilizing a more sensitive detection method such as the euglycemic hyperinsulinemic clamp may be necessary to reveal improvements in insulin sensitivity.

We advanced the characterization of mitochondrial function in the skeletal muscle of PGC-1α transgenic mice by demonstrating increased gene expression of various key mitochondrial pathways, including those involved in oxidative phosphorylation, TCA cycle, fatty acid oxidation, and ROS scavenging. We further demonstrate increased mitochondrial function by illustrating increased mtDNA, as well as enhanced CS activity and mitochondrial gene expression. The increased mitochondrial function shown here suggests that the PGC-1α transgenic mice may be better prepared to handle a metabolic challenge. This is exemplified by the ability of these mice to sustain fatty acid oxidation during the metabolic stress of an exercise challenge. Therefore, as patients afflicted with obesity or Type 2 diabetes are often unable to cope with metabolic stresses, increasing PGC-1α function in skeletal muscle may be a viable therapeutic strategy for the treatment of these patients.

ACKNOWLEDGMENTS

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