Overexpression of Peroxisome Proliferator-Activated Receptor γ Co-Activator-1 α Leads to Muscle Atrophy with Depletion of ATP

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Peroxisome proliferator-activated receptor- γ co-activator-1 α (PGC-1 α) is a key nuclear receptor co-activator for mitochondrial biogenesis. Here we report that overexpression of PGC-1 α in skeletal muscles increased mitochondrial number and caused atrophy of skeletal muscle, especially type 2B fiber-rich muscles (gastrocnemius, quadriceps, and plantaris). Muscle atrophy became evident at 25 weeks of age, and a portion of the muscle was replaced by adipocytes. Mice showed increased energy expenditure and reduced body weight; thyroid hormone levels were normal. Mitochondria exhibited normal respiratory chain activity per mitochondrion; however, mitochondrial respiration was not inhibited by an ATP synthase inhibitor, oligomycin, clearly indicating that oxidative phosphorylation was uncoupled. Accordingly, ATP content in gastrocnemius was markedly reduced. A similar phenotype is observed in Luft's disease, a mitochondrial disorder that involves increased uncoupling of respiration and muscle atrophy. Our results indicate that overexpression of PGC-1 α in skeletal muscle increases not only mitochondrial biogenesis but also uncoupling of respiration, resulting in muscle atrophy. (*Am J Pathol* 2006, 169:1129–1139; DOI: 10.2353/ajpath.2006.060034)

vator of nuclear receptors, is expressed in brown adipose tissue, skeletal muscle, heart, kidney, and brain and is markedly up-regulated in brown adipose tissue and skeletal muscle after acute exposure to cold stress.¹ Increased expression of PGC-1 α as part of activated adaptive thermogenesis occurs primarily in the mitochondria of brown adipose tissue and skeletal muscle through stimulation of mitochondrial biogenesis and respiration.² PGC-1 α is also believed to be a key molecule involved in fatty acid oxidation because it was found to interact with PPAR α to promote transcription of nuclear genes encoding mitochondrial fatty acid oxidation enzymes.^{3,4} In *in vivo* studies of muscles overexpressing PGC-1 α , the skeletal muscles showed a red color characteristic of oxidative muscle and elevated levels of enzymes related to mitochondrial oxidative phosphorylation and fatty acid oxidation.⁵ These same muscles showed reduced GLUT4 mRNA expression and impaired insulin tolerance.⁶ In brown adipose tissue mitochondria, as observed during prolonged exposure to cold, PGC-1 α promotes uncoupling of respiration through induction of uncoupling protein 1 (UCP1).¹ When respiration is uncoupled, the membrane potential energy is channeled to heat production rather than to adenosine triphosphate (ATP) production, which may cause functional abnormalities and cell death. In C2C12 myocytes, increased uncoupling of respiration driven by PGC-1 α was observed.⁷ Overexpression of PGC-1 α promoted mitochondrial biogenesis and resulted in dilated cardiomyopathy.⁴ These data suggest that PGC-1 α overexpression in skeletal muscles increases uncoupling of respiration, decreases

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Peroxisome proliferator-activated receptor (PPAR)- γ co-activator-1 α (PGC-1 α), which was identified as co-acti-

ATP content, and results in muscle atrophy. However, this hypothesis has not been verified. In the present study, we examined mitochondrial respiratory function and morphological changes in the skeletal muscles of mice overexpressing PGC-1 α and found that PGC-1 α transgenic mice had markedly decreased ATP content in skeletal muscles and developed myopathy at 25 weeks of age, which recapitulates the phenotype of Luft's disease.^{8–10}

Materials and Methods

Animals and Treatments

D-line mice expressed 10-fold higher PGC-1 α mRNA and E-line mice expressed 13-fold higher PGC-1 α mRNA in skeletal muscle compared to wild-type mice, as described in our previous study.⁶ Male chimeras harboring the PGC-1 transgene were mated with pure C57BL/6J females (Tokyo Laboratory Animals Science, Tokyo, Japan) to obtain F₁ offspring. The heterozygous F₁ male offspring from this breeding were then backcrossed with purebred C57BL/6J females to obtain F₂ offspring, and this process was continued until the F₃ generation of mice was obtained. Heterozygous transgenic mice were used for the following studies.

Mice were fed a standard laboratory chow diet (CE2; Clea, Tokyo, Japan). Mice were exposed to a 12-hour light/dark cycle and maintained at a constant temperature of 22°C. The mice were cared for in accordance with "Principles of Laboratory Animal Care" (National Institutes of Health publication no. 85-23, revised 1985: <http://grants1.nih.gov/grants/olaw/references/phspol.htm>) and our institutional guidelines.

Oxygen consumption was measured with a metabolic chamber as described previously.¹¹ For estimation of running wheel activity, mice were housed individually in cages (9 × 22 × 9 cm) equipped with a running wheel (20-cm diameter; Shinano Co., Tokyo, Japan). Each wheel revolution was registered by a magnetic switch connected to a counter. The number of revolutions was recorded daily for 10 days.

Histological Analyses

Samples of the tibialis anterior muscle at 16 weeks of age and hindlimb at 25 weeks of age were frozen in liquid nitrogen-cooled isopentane, and transverse serial sections were stained with hematoxylin and eosin (H&E), modified Gomori trichrome, and Oil red O.¹² These sections were also analyzed by enzyme histochemistry to evaluate cytochrome c oxidase (COX)¹³ and succinate dehydrogenase (SDH) activities.¹⁴ For electron microscopy, extensor digitorum longus muscles from 16-week-old mice were fixed in buffered 2% isotonic glutaraldehyde (pH 7.4), postfixed in osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead nitrate and examined with an H-7000 electron microscope (Hitachi, Tokyo, Japan).

Preparation of the Mitochondrial Fraction from Skeletal Muscle

Skeletal muscles (gastrocnemius and quadriceps) from both 14-week-old wild-type and PGC-1 α transgenic male mice were pooled from three mice in each group. Tissues were cut with scalpels and treated with 1 ml of protease buffer containing 0.1 mol/L KCl, 50 mmol/L Tris-HCl (pH 7.5), 5 mmol/L MgSO₄, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 5 mg/ml bovine serum albumin, 1 mmol/L ATP, and 2 mg/ml proteinase K for 2 minutes on ice. Tissues were then washed with ATP buffer containing 0.1 mol/L KCl, 50 mmol/L Tris-HCl (pH 7.5), 5 mmol/L MgSO₄, 1 mmol/L EDTA, 5 mg/ml bovine serum albumin, and 1 mmol/L ATP. The washed tissues were homogenized in 10 ml of ATP buffer with a glass/Teflon homogenizer in a power-driven Potter-Elvehjem homogenizer (Kadoguchi-Keiki, Tokyo, Japan). The homogenate was brought to 15 ml and centrifuged at 600 × *g* for 10 minutes to remove cell debris and nuclei. The supernatant was then centrifuged at 4500 × *g* for 15 minutes. The pellet was washed with KCl buffer containing 0.1 mol/L KCl, 50 mmol/L Tris-HCl (pH 7.5), 5 mmol/L MgSO₄, 1 mmol/L EDTA, and 5 mg/ml bovine serum albumin and centrifuged at 7000 × *g* for 15 minutes. The mitochondrial pellet was resuspended in mitochondrial buffer containing 225 mmol/L mannitol and 75 mmol/L sucrose.¹⁵ Protein concentrations were estimated by Lowry method.¹⁶

Measurement of Mitochondrial Respiration

Mitochondrial respiration was measured with Biological Oxygen Monitor 5300 (Yellow Springs Instrument, Yellow Springs, OH) was performed using Clark-type oxygen electrodes.¹⁷ Respiration buffer containing 225 mmol/L mannitol, 75 mmol/L sucrose, 10 mmol/L Tris-HCl (pH 7.2), 5 mmol/L potassium phosphate (pH 7.2), and 10 mmol/L KCl was used. Five mmol/L succinate as substrate and 100 μ g of mitochondria and 5 μ g/ml rotenone were added, and respiration was started by the addition of 5 mmol/L potassium succinate. State 3 respiration was started by the addition of 200 μ mol/L ADP (Sigma, St. Louis, MO). Finally, 2.5 μ g/ml oligomycin (Sigma) was added as an inhibitor of ATP synthetase to block all phosphorylation-related respiration.²

Enzymatic Activity of the Respiratory Complex

SDH activity was measured by monitoring the change in absorbance 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2,4-tetrazolium bromide (MTT) at 570 nm in the presence of phenazine methosulfate with the 17 mmol/L⁻¹cm⁻¹ extinction coefficient for MTT.¹⁸ Succinate-ubiquinone oxidoreductase activity was measured in the presence of 2,3,2,6-dichlorophenolindophenol (DCIP) with the 21 mmol/L⁻¹cm⁻¹ extinction coefficient for DCIP.¹⁸ NADH-ubiquinone reductase activity was assayed in 50 mmol/L potassium phosphate buffer (pH 7.7), 200 μ mol/L NADH, 2 mmol/L KCN, and 90 μ mol/L ubiquinone-1. Oxidation of NADH was monitored at 340 nm with a mmol/L⁻¹ extinc-

Figure 1. PGC-1 α overexpression increases energy expenditure. **A:** Oxygen consumption in PGC-1 α mice. Oxygen consumption by 10-week-old female mice housed individually was monitored during the dark cycle (7 p.m. to 7 a.m.) and the light cycle (7 a.m. to 7 p.m.). Each column shows the mean $\pm$ SEM values. Open column, wild type; closed column, PGC-1 α mice. Wild type, $n = 3$; PGC-1 α mice, $n = 6$ (a mixture of three D-line and three E-line mice). * $P < 0.05$ versus wild-type mice. **B:** Running wheel activities of PGC-1 α mice. The number of revolutions was recorded daily for 10 days, and cumulative values are shown. Open column, wild type; closed column, PGC-1 α mice. Mice were 8-week-old females. Wild type, $n = 3$; PGC-1 α mice, $n = 6$ (a mixture of three D-line and three E-line mice). * $P < 0.05$ versus wild-type mice.

tion coefficient of 6.2 for NADH.¹⁹ NADH oxidase activity was assayed in 50 mmol/L potassium phosphate buffer (pH 7.7) and 200 μ mol/L NADH in the presence or absence of 100 nmol/L antimycin A and 2 mmol/L KCN. The oxidation of NADH was monitored at 340 nm with a mmol/L⁻¹ extinction coefficient of 6.2 for NADH.²⁰ NADH-cytochrome c reductase, succinate-cytochrome c reductase, ubiquinol-cytochrome c reductase, and COX activities were measured as described previously.^{17,21} Citrate synthase activity was measured as described previously.²² Each measurement was performed in three times.

Northern Blot Analysis

Northern blot analysis was performed as described previously.²³

Southern Blot Analysis

To measure mitochondrial DNA (mtDNA) copy number, cDNA probes for mtDNA-COX II and nuclear genome gene-COX IV were generated as described previously.⁶ For each sample, 10 μ g of total DNA (containing both genomic and mitochondrial DNAs) from gastrocnemius were digested with *Eco*RI and *Nco*I, separated by electrophoresis on 0.8% agarose gels, and transferred overnight to nylon membranes.¹ Blots were then hybridized to COX II and COX IV probes radiolabeled with ³²P-dCTP. The blots were washed, and DNA were quantified with an image analyzer (BAS 1800; Fuji Film, Tokyo, Japan) and expressed as the intensity of phosphostimulated luminescence.

Real-Time Polymerase Chain Reaction (PCR) Analysis

Reactions were performed in the 96-well format with SYBR Green PCR Master Mix and a 7500 real-time PCR system (Applied Biosystems, Foster City, CA) with 3 ng of total genomic DNA as template. As a preliminary experiment, we determined appropriate amounts of DNA for the assay. Copy number of COX II was normalized to those of COX IV and 36B4. 36B4 encodes acidic ribosomal phosphoprotein PO,²⁴ and the 36B4 cDNA probe is widely used as a control in RNase protection experiments to study regulation of the transcription of several genes by estradiol. Mouse-specific primer pairs were: COX II forward, 5'-CCGACTAAATCAAGCAACAGTAACA-3' and COX II reverse, 5'-AAATTTTCAGAGCATTGGC-CATAG-3'; COX IV forward, 5'-CTATGTGTATGGCCCC-ATCC-3' and COX IV reverse, 5'-AGCGGGCTCTCACT-TCTTC-3'; and 36B4 forward, 5'-GGCCCTGCACTCTC-GCTTTC-3' and 36B4 reverse, 5'-TGCCAGGACG-CGCTTGT-3'.

Measurement of mtDNA Copy Number

The mtDNA content is the mtDNA copy number normalized to the copy number of a gene contained in the nuclear genome. The mtDNA copy number in PGC-1 α transgenic mice was expressed by percentages of those in control wild-type mice with the formula: [(mtDNA in PGC-1 α transgenic mice/nuclear genome gene in PGC-1 α transgenic mice)/(mtDNA in wild-type mice/nuclear genome gene in wild-type mice)] $\times$ 100%. Southern

Wild-type

50 μ m

50 μ m

50 μ m

50 μ m

2.5 μ m

PGC-1 α

50 μ m

50 μ m

50 μ m

50 μ m

2.5 μ m

blotting and real-time PCR were used to estimate the copy number of specific genes in skeletal muscles. The mitochondrial gene used for mtDNA copy estimation was COX II, and the copy number of COX II was normalized to the copy number of one of two genes, COX IV or 36B4, contained in the nuclear genome.

Measurements of ATP and AMP Contents

Gastrocnemius was homogenized with 1.0 N perchloric acid and centrifuged at $10,000 \times g$ for 15 minutes. After neutralization of the supernatant with calcium carbonate, ATP and AMP concentrations were determined by high performance liquid chromatogram (Phenomenex Luna 5 μ NH₂; mobile phase, sodium phosphate buffer; detector, 260 nm; Torrance, CA).^{25,26}

Body Composition Analysis

Mice were anesthetized with pentobarbital sodium, Nembutal (0.08 mg/g body weight; Abbot Laboratories, Chicago, IL), and scanned with a Lunar PIXI mus2 densitometer (Lunar Corp., Madison, WI), equipped for dual-energy X-ray absorptiometry (DEXA).²⁷

Statistical Analysis

All data are presented as mean $\pm$ SEM. Data from multiple groups were compared by one-way analysis of variance (StatView 5.0; Abacus Concepts, Berkeley, CA). When differences were significant, data were compared between groups by Fisher's protected least significant difference test. Data from two experimental groups were compared by unpaired Student's *t*-test. Statistical significance was defined as $P < 0.05$.

Results

PGC-1 α Transgenic Mice Show Increased Energy Expenditure

In our previous study, skeletal muscles of PGC-1 α transgenic mice showed the red color characteristic of oxidative muscle and increased levels of enzymes related to mitochondrial oxidative phosphorylation. In the same mice, expression of GLUT4 mRNA was reduced, and the glucose-lowering effects of insulin were impaired.⁶ To determine whether PGC-1 α transgenic mice show increased energy expenditure, whole-body oxygen consumption was measured in 10-week-old mice (Figure 1A). During both the dark (active) and light (sleeping)

phases, PGC-1 α transgenic mice showed 1.1-fold higher oxygen consumption than control mice. Because the cumulative number of wheel revolutions was 20% less in PGC-1 α transgenic mice (Figure 1B), the increased oxygen consumption in PGC-1 α transgenic mice was not attributable to increased voluntary exercise.

Blood triiodothyronine (T3) and thyroxine (T4) concentrations were not altered in 10-week-old PGC-1 α transgenic mice. Blood T3 concentrations in wild-type mice, D-line transgenic mice, and E-line transgenic mice were 1.16 ± 0.07 ng/ml, 1.14 ± 0.04 ng/ml, and 1.06 ± 0.01 ng/ml ($n = 3$ each group), respectively. Blood T4 concentrations in wild-type mice, D-line transgenic mice, and E-line transgenic mice were 5.89 ± 0.63 μ g/dl, 5.78 ± 0.58 μ g/dl, and 5.55 ± 0.29 μ g/dl ($n = 3$ each group), respectively.

Numbers of Normal-Sized Mitochondria Were Increased in Skeletal Muscles from PGC-1 α Transgenic Mice

We examined whether biogenesis of mitochondria and respiratory chain activity were increased in skeletal muscles from PGC-1 α transgenic mice using histochemical staining of tibialis anterior muscle (type 2B-rich fiber muscle) in 16-week-old D-line transgenic mice (Figure 2). H&E staining revealed increased variability in muscle fiber size and increased interstitial cell number and connective tissues in PGC-1 α transgenic mice (Figure 2E). Modified Gomori trichrome staining revealed a marked increase in the number of mitochondria in PGC-1 α transgenic mice (Figure 2F). In addition, increased COX (Figure 2G) and SDH (Figure 2H) activities in transgenic muscle indicated that the mitochondrial respiratory chain was functional and active. The pathological hallmarks of mtDNA disease, such as ragged-red fibers and COX deficiency,²⁸ were not observed.

To examine the subcellular structure of myocytes in PGC-1 α transgenic mice in detail, extensor digitorum longus skeletal muscle in 16-week-old E-line transgenic mice was examined by electron microscopy (Figure 2J). An increase in the thickness of the Z-band that is seen in type-1 fibers and a marked increase in the number of mitochondria in the subsarcolemmal region of muscle fibers were observed. Mitochondrial size was normal, and the alignment of myofibrils was intact in transgenic mice.

To estimate numbers of mitochondria in gastrocnemius from PGC-1 α transgenic mice, citrate synthase activity, the most commonly used marker of mitochondrial number,¹⁷ and mtDNA (COX II) copy number relative to those

Figure 2. Morphological analysis of skeletal muscles. Tissue sections from tibialis anterior muscle from 16-week-old wild-type (A–D) and PGC-1 α transgenic (D-line) mice (E–H) are presented. **A** and **E:** H&E staining of tibialis anterior muscle. There are scattered atrophic fibers in PGC-1 α transgenic muscle. **B** and **F:** Modified Gomori trichrome staining. An increase in the number of mitochondria was observed in PGC-1 α transgenic muscle. **C** and **G:** Staining to detect COX activity. **D** and **H:** Staining to detect SDH activity. Activities of these enzymes were greatly increased in myocytes from PGC-1 α transgenic mice. Similar results were obtained with E-line transgenic mice (data not shown). **I** and **J:** Electron microscopy of extensor digitorum longus muscles from wild-type control mice (16 weeks of age) (**I**) and PGC-1 α transgenic mice (16 weeks of age, E-line) (**J**). Numbers of mitochondria were markedly higher in transgenic mice, especially in the subsarcolemmal region, than in wild-type mice. Similar results were obtained with D-line transgenic mice (data not shown). Scale bars: 50 μ m (A–H); 2.5 μ m (I, J).

Table 1. Estimated Numbers of Mitochondria in Gastrocnemius from 14-Week-Old Mice

	Wild type	PGC-1 α /D line	PGC-1 α /E line
Citrate synthase activity (μ mol/minute/mg protein, %)	0.56 (100)	1.75 (313)	1.66 (296)
COX II/COX IV (%; determined by Southern blot)	100 $\pm$ 4 (6)	202 $\pm$ 21 (3) [‡]	165 $\pm$ 15 (3) [‡]
COX II/COX IV (%; determined by real-time PCR)	100 $\pm$ 5 (6)	178 $\pm$ 10 (3) [‡]	214 $\pm$ 29 (3) [‡]
COX II/36B4 (%; determined by real-time PCR)	100 $\pm$ 5 (6)	203 $\pm$ 8 (3) [*]	292 $\pm$ 58 (3) [‡]

For measurement of citrate synthase activity, tissues from each group were pooled from three animals before mitochondrial isolation, and the citrate synthase activities of pooled samples were measured. For estimation of mtDNA copy number in skeletal muscle, the relative mtDNA copy number in skeletal muscle from individual mice in each group was calculated as the ratio of COX II (mitochondrial) to COX IV or 36B4 (nuclear) genes as determined by Southern blotting and/or real-time PCR. The relative mtDNA copy number was expressed as the percentage of the ratio in wild type. Numbers of mice were indicated. * $P < 0.05$, [‡] $P < 0.01$, and [‡] $P < 0.001$ versus wild-type mice.

of nuclear genome genes (COX IV and 36B4), were measured (Table 1). The copy number of COX IV was estimated by two different methods, Southern blotting and real-time PCR. Citrate synthase activity in PGC-1 α transgenic mice was threefold higher than that in wild-type mice. The ratio of COX II copy number to genomic DNA copy number in PGC-1 α transgenic mice was twofold to threefold higher than that in wild-type mice, regardless of the detection method or nuclear genome reference gene. These data suggested that if we assume no significant differences in citrate synthase activity and mtDNA copy number in mitochondria between PGC-1 α transgenic and wild-type mice, the number of mitochondria in gastrocnemius from PGC-1 α transgenic mice was twofold to threefold larger than that in wild-type mice.

Increased Uncoupling of Oxidative Phosphorylation in Skeletal Muscle Mitochondria from PGC-1 α Transgenic Mice

To study the function of skeletal muscle mitochondria, we compared the respiration rate (=oxygen consumption) in isolated mitochondria from gastrocnemius and quadriceps of PGC-1 α transgenic mice with that in wild-type mice in the absence and presence of oligomycin, an inhibitor of the F_1F_0 -ATP synthase.²⁹ First, the respiration rate of mitochondria was measured under conditions in which mitochondrial substrate and ADP were not limiting to mimic state 3 respiration. To measure uncoupled respiration, oligomycin was added to inhibit oxidative phosphorylation before measurement of respiration rates. In the absence of oligomycin, both lines of PGC-1 α transgenic mice showed a twofold to threefold increase of respiration rate on a protein basis of mitochondria-rich fraction (Figure 3A). Because the increased respiration rate observed in PGC-1 α transgenic mice was likely attributable to an increased mitochondrial number, we divided the respiration rate by the citrate synthase activity to express respiration rate on a per mitochondrion basis.¹⁷ To normalize the respiration rate to the mitochondrial number, citrate synthase activity rather than mtDNA copy number was used, because the same method for preparation of mitochondria was used for both respiration rate and citrate synthase activity assays. When expressed per unit of citrate synthase activity, the respiration rate was similar between PGC-1 α transgenic mice and wild-type mice (Figure 3B), suggesting that respiration was comparable in each mitochondrion in PGC-1 α

Figure 3. Increased uncoupling of oxidative phosphorylation and UCP2 in skeletal muscle from PGC-1 α transgenic mice. Skeletal muscles (gastrocnemius, quadriceps) from both wild-type and PGC-1 α male transgenic mice (D-line and E-line) at 14 weeks of age ($n = 3$ each group). Mitochondrial fraction was prepared as described under Materials and Methods. State 3 respiration rate of the mitochondrial fraction was measured in the presence and absence of 2.5 μ g/ml oligomycin. Each measurement was performed in triplicate. Each data point is the mean value of the measurements normalized to the protein content (A) or to the citrate synthase activity of the fraction (B). C: Total RNA extracted from gastrocnemius was probed with ³²P-labeled human UCP2 and UCP3 cDNA probes. Ribosomal 18S RNA is shown as a loading control. The average densitometric ratios (wild type was set as 100) are under the blot. * $P < 0.05$, ** $P < 0.01$ versus wild-type mice.

Table 2. Enzymatic Activity of Respiratory Chain Complexes in Mitochondria from 14-Week-Old Mice

	Enzyme complex (enzyme activity/citrate synthase activity)						
	I	I + III	I + III + IV	II (SDH)	II (SQR)	II + III	IV
Wild type	0.217	0.238	0.279	0.132	0.150	0.177	0.415
PGC-1 α /D line	0.201	0.315	0.478	0.102	0.100	0.127	0.868
PGC-1 α /E line	0.181	0.225	0.417	0.105	0.099	0.129	0.832

For each group, tissues were pooled from three animals before mitochondrial isolation.

transgenic and wild-type mice. However, the mechanism of respiratory control was different; in wild-type mice, oligomycin-insensitive respiration (=uncoupling) constituted 40% of total respiration, whereas in PGC-1 α transgenic mice, most respiration was oligomycin-insensitive. Therefore, PGC-1 α transgenic mice contained more mitochondria, but these mitochondria showed lower ATP synthesis (=coupling) and more proton leaks (=uncoupling).

To clarify the mechanism that underlies the increased uncoupling of respiration and ATP synthesis in skeletal muscles of PGC-1 α transgenic mice, we measured expression of mRNAs for uncoupling protein UCP1, UCP2, and UCP3. UCP1 mRNA was not expressed in gastrocnemius from PGC-1 α transgenic mice. In contrast, expression of UCP2 increased threefold, and that of UCP3 mRNA was decreased slightly (Figure 3C). In liver brown and white adipose tissues, there was no change in levels of UCP1, UCP2, and UCP3 mRNAs (data not shown). Although the role of UCP2 in uncoupling respiration has not been established,³⁰ the elevated expression of UCP2 might contribute, at least in part, to the uncoupling of respiration observed in PGC-1 α transgenic mice.

We then measured the enzymatic activity of respiratory chain complex in mitochondria. Complex I, II, and III had similar activities in PGC-1 α transgenic mice and wild-type mice; however, complex IV activity was increased twofold in the transgenic mice when expressed on a per citrate synthase activity basis (=mitochondrial number) (Table 2). An increase in levels of complex IV was confirmed by measurements of COX IV protein, a component of complex IV; COX IV levels in both PGC-1 α transgenic mice lines were ~1.5-fold higher than those in wild-type mice, when expressed on a per citrate synthase activity basis (data not shown). Thus, the function of respiratory complexes was not impaired, but the ratio of the complexes was altered in PGC-1 α transgenic mice.

ATP Content Was Markedly Decreased in PGC-1 α Transgenic Mice

Because mitochondrial respiration in PGC-1 α transgenic mice was attributable to proton leak rather than ATP synthesis, we hypothesized that PGC-1 α transgenic mice would show reduced ATP levels in their skeletal muscle. To examine ATP levels, we measured the ATP and AMP contents of gastrocnemius (Figure 4). As expected, the ATP concentration in gastrocnemius from both lines of PGC-1 α transgenic mice was ~80% lower than that in wild-type mice, whereas the AMP concentration was increased by fivefold to sevenfold. The alteration of the

AMP/ATP ratio affects AMP-activated protein kinase (AMPK) activity.³¹ Gastrocnemius from PGC-1 α transgenic mice showed 4.7-fold higher α 1 AMPK activity than control mice, whereas α 2 AMPK activity was unchanged (data not shown), confirming that ATP depletion occurred *in vivo*.

PGC-1 α Transgenic Mice Develop Skeletal Muscle Atrophy and Adipocyte Proliferation

A marked morphological change was observed in PGC-1 α transgenic mice at 25 weeks of age compared with that of mice at 16 weeks of age (Figure 5, B and C). Quadriceps and gastrocnemius muscle (fastest and glycolytic type-2B fiber-rich muscle) from 25-week-old transgenic mice were paler in color than those of wild-type mice (Figure 5, A and C). This change was not observed in soleus muscle (type-1 fiber-rich muscle) (data not shown).

Histological analysis was performed to examine whether the pale, fat-like appearance of the gastrocnemius muscle in transgenic mice was attributable to increased lipid accumulation within myocytes or to an increased adipocyte numbers in skeletal muscles (Figure 5, D–I). Severe muscle atrophy was observed in PGC-1 α transgenic mice, and myocytes were replaced by white

Figure 4. ATP content was markedly decreased in PGC-1 α transgenic mice. ATP and AMP contents of gastrocnemius from wild-type, D-line, and E-line transgenic mice were measured at 10 weeks of age. Data are mean $\pm$ SEM values ($n = 3$). *** $P < 0.001$ versus wild-type mice.

Table 3. Physiological Characteristics of 25-Week-Old Wild-Type and PGC-1 α Transgenic Male Mice

	Wild type (n = 4)	PGC-1 α transgenic (D-line) (n = 3)	PGC-1 α transgenic (E-line) (n = 4)
Body weight (g)	41.7 $\pm$ 0.6	32.0 $\pm$ 3.7*	30.7 $\pm$ 1.8 [†]
Body fat (g)	10.4 $\pm$ 0.7	6.9 $\pm$ 1.1	6.9 $\pm$ 0.7*
Body fat (%)	30.4 $\pm$ 1.8	26.0 $\pm$ 3.7	26.0 $\pm$ 1.7
Lean body mass (g)	23.6 $\pm$ 0.8	19.4 $\pm$ 0.8 [†]	19.5 $\pm$ 0.3 [†]
Bone mineral density (g/cm ²)	0.051 $\pm$ 0.001	0.046 $\pm$ 0.000 [‡]	0.043 $\pm$ 0.001 [‡]
White adipose tissue weight (g)			
Epididymal	1.78 $\pm$ 0.15	0.88 $\pm$ 0.27 [†]	0.66 $\pm$ 0.17 [‡]
Retroperitoneal	0.46 $\pm$ 0.04	0.38 $\pm$ 0.12	0.31 $\pm$ 0.09
Subcutaneous	1.64 $\pm$ 0.15	0.93 $\pm$ 0.29	0.69 $\pm$ 0.14*
Brown adipose tissue weight (g)	0.28 $\pm$ 0.03	0.13 $\pm$ 0.01 [‡]	0.11 $\pm$ 0.02 [‡]
Gastrocnemius weight (g)	0.32 $\pm$ 0.01	0.17 $\pm$ 0.00 [‡]	0.16 $\pm$ 0.00 [‡]
Quadriceps weight (g)	0.34 $\pm$ 0.01	0.22 $\pm$ 0.05*	0.21 $\pm$ 0.01 [†]
Liver weight (g)	1.70 $\pm$ 0.13	1.17 $\pm$ 0.09	1.30 $\pm$ 0.05
Spleen weight (g)	0.10 $\pm$ 0.00	0.06 $\pm$ 0.01	0.08 $\pm$ 0.01
Kidney weight (g)	0.48 $\pm$ 0.02	0.37 $\pm$ 0.02*	0.39 $\pm$ 0.01*
Heart weight (g)	0.17 $\pm$ 0.01	0.17 $\pm$ 0.01	0.17 $\pm$ 0.01
Food intake (g/g body weight/day)	0.156 $\pm$ 0.014	0.195 $\pm$ 0.017	0.192 $\pm$ 0.017

**P* < 0.05, [†]*P* < 0.01, and [‡]*P* < 0.001 versus wild-type mice.

adipose and connective tissues (Figure 5, F and G). Oil red O staining confirmed that the lipid accumulation was attributable not to myocytes but to adipocytes (Figure 5, H and I). Expression of mRNAs typically observed in adipose tissues, such as aP2, HSL, and perilipin, was significantly higher in gastrocnemius from PGC-1 α transgenic mice than in gastrocnemius from wild-type mice at 25 weeks of age (data not shown). At 16 weeks of age, increases in the interstitial cell number and amount of connective tissue were observed, but lipid accumulation was absent (Figure 2). These data indicate that, in skeletal muscle tissues from PGC-1 α transgenic mice, infiltration of adipocytes occurs rapidly from 4 to 6 months of age, concomitant with the progression of muscle atrophy.

PGC-1 α Transgenic Mice Were 25% Smaller than Wild-Type Mice at 25 Weeks of Age

To further characterize PGC-1 α transgenic mice, body composition and tissue weights were measured in two independent lines of PGC-1 α transgenic mice at 25 weeks of age (Table 3). Although food intake was not different from controls, mean body weight of PGC-1 α transgenic mice in both lines was ~25% lower than that of wild-type mice. DEXA scan revealed that both fat mass and lean body mass were decreased in transgenic mice. Bone mineral density was also significantly lower in PGC-1 α transgenic mice. The tissue weights of white adipose tissue, brown adipose tissue, skeletal muscles, liver, spleen, and kidney in transgenic mice were 30 to 50% lower than those in wild-type mice. Thus, PGC-1 α transgenic mice showed growth retardation with reductions in both lean body mass and fat mass.

Discussion

In the present study, we found that overexpression of PGC-1 α in mouse skeletal muscle increased mitochondria numbers and energy expenditure, and eventually caused muscle atrophy, especially of type 2B fiber-rich muscles. PGC-1 α transgenic mice showed normal mitochondrial COX and SDH activities (Figure 2, G and H) and normal respiratory chain enzymatic activities (Table 2); however, ATP content was reduced by increased uncoupling.

The marked decrease in the ATP content of type-2B fiber-rich muscles suggests that ATP depletion might be a major cause of muscle atrophy in PGC-1 α transgenic mice (Figures 4 and 5). Consistent with our present findings, overexpression of PGC-1 α in heart promotes mitochondrial biogenesis but leads to dilated cardiomyopathy.⁴ ATP depletion due to impairment of mitochondrial functions is involved in the pathogenesis of a wide variety of inherited and acquired human diseases, including cardiomyopathy, neuromuscular dysfunction, and diabetes mellitus.³² In skeletal muscles, inherited mitochondrial myopathy is due to mutations of genes encoding enzymes responsible for ATP synthesis in mitochondria.²⁸ Thus, their mitochondria show impaired mitochondrial respiratory function (=ATP synthesis). These mitochondria are increased in number and size and occasionally show abnormal structures, possibly as adaptation against decreased ATP content.³³ In acquired cases of muscle atrophy, aging-mediated muscle atrophy, namely sarcopenia, was mostly due to loss of type-2 fibers.^{34,35} In sarcopenia, an increase in intramuscular adipose tissue was accompanied by a reduction in muscle fibers,³⁶

Figure 5. Morphological and histological changes in skeletal muscles at 25 weeks of age. **A–C:** Lateral view of hindlimbs from wild-type (**A**: 25 weeks of age) and E-line transgenic (**B**: 16 weeks of age, **C**: 25 weeks of age) mice. Morphological differences were not observed in wild-type muscle between 16 and 25 weeks of age (data not shown). **D–G:** H&E staining of tissue sections from gastrocnemius of wild-type (**D**, **E**) and E-line transgenic mice (**F**, **G**). Specimens from transgenic mice showed atrophy of myocytes and increased adipose and interstitial connective tissues. **H** and **I:** Oil red O staining of tissue sections from gastrocnemius of E-line transgenic mice (**H**, **I**). Staining of lipid was observed in adipose tissue that surrounded the atrophic myocytes (**H**). No positive staining was observed inside the muscle fiber (**I**). Similar results were obtained for D-line transgenic mice (data not shown).

as was observed in our PGC-1 α transgenic mice. *In vivo* proton magnetic resonance spectroscopy studies revealed that the mitochondrial ATP synthesis rate in skeletal muscle from elderly people was 46% lower than that in muscles from young patients.³⁷ Isolated mitochondria from elderly participants showed decreased state 3 (activated) mitochondrial respiration.³⁸ Taken together, these data indicate that ATP depletion caused by mitochondrial dysfunction might be a cause of sarcopenia. Therefore, ATP depletion in skeletal muscle might be a common feature of muscle atrophy.

However, reported clinical cases of ATP depletion attributable to increased uncoupling of respiration are very rare. Uncoupling-mediated muscle atrophy in humans was first described by Luft and colleagues^{8,9} in 1959 and was called Luft's disease. This disease is important not only because it was the first identified human mitochondrial disorder but also because Luft's disease is still the only mitochondrial disease caused by uncoupling of oxidative phosphorylation. The cause of Luft disease is unknown because this disease is extremely rare; only one patient has been reported by DiMauro and colleagues¹⁰ and in addition to the original case described by Luft and colleagues. To date, no animal model has been available. Patients with Luft's disease show increased energy expenditure and reduced body weight; however, thyroid hormone levels are normal. PGC-1 α transgenic mice also exhibit these cardinal features with biochemical evidence of uncoupling of oxidative phosphorylation. Therefore, our mice recapitulate the phenotype of Luft's disease, although there is some difference in mitochondrial morphology. Mitochondria in Luft's disease were highly variable in size and shape with a range of abnormal internal structures, including densely packed cristae, tubular structures, electron-dense regularly layered cores, and concentric structures.^{9,10,39} In PGC-1 α transgenic mice, such morphological abnormalities in mitochondria were not observed. The reasons for these differences are unknown. The mechanism that underlies increased uncoupling of respiration by PGC-1 α overexpression is also not clear. Increased expression of UCP2 may partially contribute to uncoupling (Figure 3C), but other unknown uncoupling proteins might also be involved.

The hypothesis that ATP depletion induces muscle atrophy may be supported by the fact that overexpression of UCP1 in skeletal muscle also reduces the masses of type-2B fiber-rich muscles (gastrocnemius and plantaris), which are vulnerable to ATP depletion, but not those of heart and soleus muscle, which undergo constant repeated contractions with high ATP supply.⁴⁰ Similar phenotype of mice overexpressing UCP1, the soleus muscle in PGC-1 α transgenic mice is not atrophic, suggesting a close relation between ATP content and muscle mass. In fact, after exercise training, the number of functional mitochondria and glucose and fatty acid oxidation are increased in skeletal muscle, possibly because of an increase of PGC-1 α ; however, muscle weight is increased.⁴¹ Thus, when myocyte energy expenditure is increased, a large supply of substrate from blood fatty acids and glucose might be required for maintenance of skeletal muscle integrity. Thus, mismatch between en-

ergy demand and supply may precipitate organ dysfunction.

Although the respiratory functions of complexes I, II, III, and IV in PGC-1 α transgenic mice were not impaired, the activity of complex IV activity was much higher than those of complexes I, II, and III (Table 2). Conversely, in humans, a significant age-related decline in COX activity (complex IV of the respiratory chain) has been observed, but SDH activity (complex II of the respiratory chain) is not altered.⁴² These data further indicate that aging might reduce PGC-1 α activity in skeletal muscle. It is not clear why complex IV was preferentially activated by PGC-1 α overexpression. Complexes I, II, and III are the significant sources of reactive oxygen species, namely superoxide and hydrogen peroxide, whereas complex IV is not.⁴³ We observed increased superoxide anion production in mitochondria from PGC-1 α transgenic mice, when isolated mitochondria were incubated with either NADH (twofold increase per mg protein) or succinate (fourfold increase per mg protein) (data not shown). To prevent harmful increases in reactive oxygen species, adaptive mechanisms might suppress increases in expression of complexes I, II, and III in transgenic mice.

In this study, we demonstrated that overexpression of PGC-1 α increased uncoupled oxidative phosphorylation and caused ATP deprivation, resulting in muscle atrophy, the phenotype of the first human mitochondrial disease described by Luft and colleagues,^{8,9} more than 50 years ago. This mouse model confirms Luft's historical observation that uncoupling of oxidative phosphorylation causes myopathy. Furthermore, our results suggest that the genetic cause of Luft's disease may be a defect in the PGC-1 α -related pathway. However, this is impossible to confirm until a new case can be found. Development of a mouse model of Luft's disease prompts further studies to understand the molecular mechanisms by which uncoupled oxidative phosphorylation leads to muscle atrophy.

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References

1. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM: A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998, 92:829–839
2. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM: Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999, 98:115–124
3. Vega RB, Huss JM, Kelly DP: The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 2000, 20:1868–1876
4. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP: Peroxisome proliferator-activated receptor γ coactivator-1 pro-

- motes cardiac mitochondrial biogenesis. *J Clin Invest* 2000, 106:847–856
5. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM: Transcriptional co-activator PGC-1 α drives the formation of slow-twitch muscle fibres. *Nature* 2002, 418:797–801
6. Miura S, Kai Y, Ono M, Ezaki O: Overexpression of peroxisome proliferator-activated receptor gamma coactivator-1 α down-regulates GLUT4 mRNA in skeletal muscles. *J Biol Chem* 2003, 278:31385–31390
7. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM: Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1 α and 1 β (PGC-1 α and PGC-1 β) in muscle cells. *J Biol Chem* 2003, 278:26597–26603
8. Ernster L, Ikkos D, Luft R: Enzymic activities of human skeletal muscle mitochondria: a tool in clinical metabolic research. *Nature* 1959, 184:1851–1854
9. Luft R, Ikkos D, Palmieri G, Ernster L, Afzelius B: A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study. *J Clin Invest* 1962, 41:1776–1804
10. DiMauro S, Bonilla E, Lee CP, Schotland DL, Scarpa A, Conn Jr H, Chance B: Luft's disease. Further biochemical and ultrastructural studies of skeletal muscle in the second case. *J Neurol Sci* 1976, 27:217–232
11. Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto S, Ezaki O: Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* 2000, 49:1534–1542
12. Black JT, Judge D, Demers L, Gordon S: Ragged-red fibers. A biochemical and morphological study. *J Neurol Sci* 1975, 26:479–488
13. Seligman AM, Karnovsky MJ, Wasserkrug HL, Hanker JS: Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). *J Cell Biol* 1968, 38:1–14
14. Dubowitz V, Brooke M: *A Modern Approach, Muscle Biopsy*. London, Saunders, 1973
15. Pallotti F, Lenaz G: Isolation and subfractionation of mitochondria from animal cells and tissue culture lines. *Methods Cell Biol* 2001, 65:1–35
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, 193:265–275
17. Trounce IA, Kim YL, Jun AS, Wallace DC: Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmittochondrial cell lines. *Methods Enzymol* 1996, 264:484–509
18. Kita K, Vibat CR, Meinhardt S, Guest JR, Gennis RB: One-step purification from *Escherichia coli* of complex II (succinate: ubiquinone oxidoreductase) associated with succinate-reducible cytochrome b556. *J Biol Chem* 1989, 264:2672–2677
19. Miyadera H, Amino H, Hiraishi A, Taka H, Murayama K, Miyoshi H, Sakamoto K, Ishii N, Hekimi S, Kita K: Altered quinone biosynthesis in the long-lived clk-1 mutants of *Caenorhabditis elegans*. *J Biol Chem* 2001, 276:7713–7716
20. Ventura B, Genova ML, Bovina C, Formiggini G, Lenaz G: Control of oxidative phosphorylation by complex I in rat liver mitochondria: implications for aging. *Biochim Biophys Acta* 2002, 1553:249–260
21. Birch-Machin MA, Turnbull DM: Assaying mitochondrial respiratory complex activity in mitochondria isolated from human cells and tissues. *Methods Cell Biol* 2001, 65:97–117
22. Srere PA: Citrate synthase. *Methods Enzymol* 1969, 13:3–11
23. Tsuboyama-Kasaoka N, Tsunoda N, Maruyama K, Takahashi M, Kim H, Ikemoto S, Ezaki O: Up-regulation of uncoupling protein 3 (UCP3) mRNA by exercise training and down-regulation of UCP3 by denervation in skeletal muscles. *Biochem Biophys Res Commun* 1998, 247:498–503
24. Laborda J: 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. *Nucleic Acids Res* 1991, 19:3998
25. Watanabe A, Tsuneishi E, Takimoto Y: Analysis of ATP and its breakdown products in beef by reversed-phase HPLC. *J Food Sci* 1989, 54:1169–1172
26. Scott MD, Baudendistel LJ, Dahms TE: Rapid separation of creatine, phosphocreatine and adenosine metabolites by ion-pair reversed-phase high-performance liquid chromatography in plasma and cardiac tissue. *J Chromatogr* 1992, 576:149–154
27. Nagy TR, Clair AL: Precision and accuracy of dual-energy X-ray absorptiometry for determining in vivo body composition of mice. *Obes Res* 2000, 8:392–398
28. Larsson NG, Clayton DA: Molecular genetic aspects of human mitochondrial disorders. *Annu Rev Genet* 1995, 29:151–178
29. Brand M: *Mitochondria: A Practical Approach*. Edited by G Brown, C Cooper Oxford University Press, 1995, pp 39–62
30. Esteves TC, Brand MD: The reactions catalysed by the mitochondrial uncoupling proteins UCP2 and UCP3. *Biochim Biophys Acta* 2005, 1709:35–44
31. Hardie DG, Carling D, Carlson M: The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? *Annu Rev Biochem* 1998, 67:821–855
32. Wallace DC: Mitochondrial diseases in man and mouse. *Science* 1999, 283:1482–1488
33. Heddi A, Lestienne P, Wallace DC, Stepien G: Mitochondrial DNA expression in mitochondrial myopathies and coordinated expression of nuclear genes involved in ATP production. *J Biol Chem* 1993, 268:12156–12163
34. Larsson L, Sjödin B, Karlsson J: Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22–65 years. *Acta Physiol Scand* 1978, 103:31–39
35. Tomonaga M: Histochemical and ultrastructural changes in senile human skeletal muscle. *Am J Geriatr Soc* 1977, 25:125–131
36. Song MY, Ruts E, Kim J, Janumala I, Heymsfield S, Gallagher D: Sarcopenia and increased adipose tissue infiltration of muscle in elderly African American women. *Am J Clin Nutr* 2004, 79:874–880
37. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI: Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 2003, 300:1140–1142
38. Trounce I, Byrne E, Marzuki S: Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet* 1989, 1:637–639
39. Sjöstrand FS: Molecular pathology of Luft disease and structure and function of mitochondria. *J Submicrosc Cytol Pathol* 1999, 31:41–50
40. Couplan E, Gelly C, Goubern M, Fleury C, Quesson B, Silberberg M, Thiaudiere E, Mateo P, Lonchamps M, Levens N, De Montron C, Ortmann S, Klaus S, Gonzalez-Barroso MD, Cassard-Doulcier AM, Ricquier D, Bigard AX, Dolez P, Bouillaud F: High level of uncoupling protein 1 expression in muscle of transgenic mice selectively affects muscles at rest and decreases their IIb fiber content. *J Biol Chem* 2002, 277:43079–43088
41. Holloszy JO, Kohrt WM, Hansen PA: The regulation of carbohydrate and fat metabolism during and after exercise. *Front Biosci* 1998, 3:D1011–D1027
42. Müller-Höcker J: Cytochrome c oxidase deficient fibres in the limb muscle and diaphragm of man without muscular disease: an aged-related alteration. *J Neurol Sci* 1990, 100:14–21
43. Jezek P, Hlavatá L: Mitochondria in homeostasis of reactive oxygen species in cell, tissue, and organism. *Int J Biochem Cell Biol* 2005, 37:2478–2503