PGC-1β Down-Regulation Is Associated With Reduced ERRα Activity and MCAD Expression in Skeletal Muscle of Senescence-Accelerated Mice

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Mitochondrial dysfunction is involved in the development of aging. Here, we examined the effect of aging on the mitochondrial expression of two isoforms of the transcriptional peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 (PGC-1) in an experimental murine model of accelerated aging, the senescence-accelerated mouse (SAM). The senescence-accelerated prone mice (SAM-P8) showed no changes in PGC-1α, but a decrease in PGC-1β expression (52% reduction, \( p < .001 \)) was observed compared to the senescence-accelerated resistant mice (SAM-R1). In agreement with the proposed role of PGC-1β as an estrogen-related receptor (ERR) protein ligand, the expression of the ERRα target gene medium-chain acyl-coenzyme A dehydrogenase was strongly suppressed (85%, \( p < .001 \)) in SAM-P8. The decrease in the expression of medium-chain acyl-coenzyme A dehydrogenase was consistent with the reduction in ERRα DNA-binding activity of SAM-P8. These findings indicate that the age-mediated decrease in PGC-1β expression in SAM-P8 skeletal muscle affects the expression of genes involved in mitochondrial fatty acid oxidation.

Recent studies have implicated the mitochondrion in the development of aging. In fact, mitochondrial dysfunction has been found in old men (1). Interestingly, the expression of many genes coding for proteins in mitochondria are regulated by the transcriptional peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 (PGC-1) [for review, see (2)]. This coactivator was initially identified as a protein that interacted with one transcription factor involved in the control of glucose and lipid metabolism, PPARγ (3). Several isoforms of PGC-1 have been cloned, including PGC-1α, PGC-1β, and the PGC-related coactivator (4–6). PGC-1α and -β are key regulators of genes involved in oxidative phosphorylation in skeletal muscle. The former activates a large number of nuclear receptors and transcription factors in muscle cells, suggesting that it can function as a wide-range nuclear-receptor coactivator, whereas PGC-1β specifically coactivates estrogen-related receptor α (ERRα) (7). When activated, this nuclear receptor regulates the expression of its target genes, such as the medium-chain acyl-coenzyme A dehydrogenase (MCAD; a pivotal enzyme of mitochondrial β-oxidation in skeletal muscle) through its binding to the nuclear receptor response element 1 (NRRE-1) located in the promoter region of this gene (8). Thus, PGC-1β transgenic mice, with enhanced PGC-1β expression in skeletal muscle, exhibit increased muscle expression of MCAD (7). Interestingly, PGC-1α and β messenger RNA (mRNA) levels decrease with age in skeletal muscle in humans and the latter has been positively associated with lipid oxidation (7). The mechanisms underlying the association between PGC-1β and the expression of genes involved in lipid metabolism in skeletal muscle during aging remain unknown, however. We examined the expression of PGC-1α and -β in the skeletal muscle of the senescence-accelerated mouse (SAM), an experimental model of accelerated aging that has been used extensively to examine the mechanisms responsible for aging. There are two strains: the senescence-accelerated prone mouse (SAM-P), which consists of nine substrains (SAM-P1–3 and 6–11) and the senescence-accelerated resistant mouse (SAM-R), which consists of three substrains (SAM-R1, 4, and 5). The SAM-R series age normally, whereas the SAM-P series show an accelerated aging with a number of common characteristics, such a loss of skin glossiness, increased skin coarseness, hair loss, periophthalmic lesions, and increased lordokyphosis of the spine (9). In this study, we used 5-month-old SAM-P8, which are characterized by both accelerated aging and neuronal dysfunctions (10,11), which appear from age 2 to 8 months (12). Our data reveal changes in the expression of PGC-1α and -β in skeletal muscle of SAM-P8 compared to SAM-P1 in the early states of aging. Whereas the slight increase in PGC-1α mRNA levels in SAM-P8 did not affect the expression of several genes regulated by this coactivator, the strong reduction in PGC-1β expression was associated with a decrease in ERRα DNA-binding activity and a decrease in the expression of its target gene MCAD. These findings indicate that changes in the expression of PGC-1β may lead to metabolic disturbances linking skeletal muscle mitochondrial efficiency and aging.
METHODS

Animals

SAM-P8 and SAM-R1 were bred under standard conditions with free access to food and water. Male mice at the age of 5 months were used in this study. Animal handling and disposal were performed in accordance with law 5/1995, July 21, of the Generalitat de Catalunya. Mice were killed under pentobarbital anesthesia, blood samples were collected, and soleus skeletal muscle was excised.

Plasma Determinations

Plasma triglycerides (Sigma, St. Louis, MO), nonesterified fatty acids (Wako, Neuss, Germany), and glucose (Sigma) concentrations were measured by colorimetric tests. Insulin levels were assayed by enzyme-linked immunosorbent assay (ELISA; Wako).

RNA Preparation and Analysis

Total RNA was isolated by using ULTRASPEC reagent (Biotech, Houston, TX). The total RNA isolated by this method is undegraded and free of protein and DNA contamination. Relative levels of specific mRNAs were assessed by reverse transcription–polymerase chain reaction (RT–PCR) as previously described (13). Complementary RNA (cDNA) was synthesized from RNA samples by mixing 0.5 μg of total RNA, 125 ng of random hexamers as primers in the presence of 50 mM Tris-HCl buffer (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, 200 U Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies), and 0.5 mM of each deoxynucleotide triphosphate (dNTP; Sigma) in a total volume of 20 μL. Samples were incubated at 37°C for 60 minutes. A 5 μL aliquot of the RT reaction was then used for subsequent PCR amplification with specific primers.

Each 25-μL PCR contained 5 μL of the RT reaction, 1.2 mM MgCl₂, 200 μM dNTPs, 1.25 μCi [³²P]-deoxyadenosine triphosphate (ATP, 3000 Ci/mmol; Amersham Biosciences, Piscataway, NJ), 1 U Taq polymerase (Invitrogen Life Technologies), and 0.5 mM of each primer, and 20 mM Tris-HCl, pH 8.5. To avoid unspecific annealing, cDNA and Taq polymerase were separated from primers and dNTPs by using paraffin fuses, at 60°C. The sequences of the sense and antisense primers used for amplification were: PPARα, 5′-GGCTGGAGGGCTCCTGTCATC-3′ and 5′-ACATGCACACTGGCAGTTGAA-3′; muscle-type carnitine palmitoyltransferase I (M-CPT-I), 5′-TTCACTGTGACCGCAGAGATG-3′ and 5′-AATTGGCAAATAGCAAGCAG-3′; and 5′-CACCAGCTTTCGGGAGAT-3′; nuclear respiratory factor (NRF)-1, 5′-CTAACCTGACCGACGTCATG-3′ and 5′-ATCTTACGAGGCTGTA-3′; and adenosine deaminase (ADA), 5′-CCACGGAAGCATCAAGG-3′ and 5′-CCTTGAAGGCCATTCCACTGTCGAA-3′; pyruvate dehydrogenase kinase 4 (PDK-4), 5′-AGGTCGAGGTGTTCCTCCGCT-3′ and 5′-GCCGGTCAGGCAGGGATGTTCAA-3′; NADH dehydrogenase subunit 1 (ND1), 5′-CGGCCATTCCTGATTCCCT-3′ and 5′-TGATTCGTAACGGAAGCTGTA-3′; mitochondrial transcription factor A (mtTFA), 5′-ATTTGGCTGGTCCACTGTC-3′ and 5′-ATCTTACGAGGCTGTA-3′; nuclear respiratory factor (NRF)-1, 5′-CTAACCTGACCGACGTCATG-3′ and 5′-ATCTTACGAGGCTGTA-3′; and adenosine deaminase (ADA), 5′-CCACGGAAGCATCAAGG-3′ and 5′-CCTTGAAGGCCATTCCACTGTCGAA-3′. PCR was performed in an MJ Research Thermocycler (Waltham, MA) equipped with a Peltier system and temperature probe. After initial denaturation for 1 minute at 94°C, PCR was performed for 18 (ND1, PDK-4), 20 (GLUT4, CD36), 21 (manganese superoxide dismutase; MnSOD), 22 (PGC-1α, ERα), 23 (PGC-1β, NRF-1, NRF-2, PPARβ/α, and M-CPT-I), 24 (PPARα), 25 (MCAD), and 29 (CTE) cycles. Each cycle consisted of denaturation at 92°C for 1 minute, primer annealing at 60°C, and primer extension at 72°C for 1 minute and 50 seconds. A final 5-minute extension step at 72°C was performed. Five microliters of each PCR sample was separated on a 1 mm-thick 5% polyacrylamide gel. The gels were dried and subjected to autoradiography using Kodak x-ray films to show the amplified DNA products. Amplification of each gene yielded a single band of the expected size (PPARα: 645 bp, M-CPT-I: 222 bp, PGC-1α: 228 bp, PGC-1β: 185 bp CTE: 224 bp, CD36: 256; MCAD: 216 bp, ERα: 210 bp, GLUT4: 232, PDK-4: 167 bp, ND1: 229 bp, PGC-1: 228 bp, NRF-1: 218 bp, NRF-2: 194, mtTFA: 160 bp, MnSOD: 191 bp, CTE: 224 bp, and APRT: 339 bp). Preliminary experiments were carried out with various amounts of cDNA to determine non-saturating conditions of PCR amplification for all the genes studied. Therefore, under these conditions, relative quantification of mRNA was assessed by the RT–PCR method used in this study (14). The results for the expression of specific mRNAs are always presented relative to the expression of the control gene (aprt).

Immunoblotting

Soleus muscles were weighed and homogenized in cold lysis buffer (5 mM Tris-HCl [pH 7.4], 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and aprotinin at 5.4 μg/mL). The homogenate was centrifuged at 10,000 g for 30 minutes at 4°C. Protein concentration was measured by using the Bradford method. Proteins (50 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) on 10% separation gels and transferred to Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). Samples were analyzed using antibodies that recognize PPARα (Santa Cruz Biotechnology, Santa Cruz, CA), PPARβ/δ (gift from Dr. Walter Wahli, University of Lausanne, Switzerland), chicken ovalbumin upstream promoter transcription factor II (COUP-TF II; Santa Cruz Biotechnology),...
Table 1. Plasma Levels of Glucose, NEFA, Triglycerides, Cholesterol, and Insulin in 5-Month-Old SAM-R1 and SAM-P8 Mice

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<td>Glucose, mg/dL</td>
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<td>NEFA, mmol/L</td>
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Notes: Values are means ± standard deviations of five mice.
*p < .01.
NEFA = nonesterified fatty acids; SAM-R1 = senescence-accelerated resistant mice; SAM-P8 = senescence-accelerated prone mice.

RESULTS

Plasma Parameters in SAM-R1 and SAM-P8

First, we determined the levels of several plasma parameters in SAM. Plasma glucose, nonesterified fatty acids, cholesterol, and insulin were not significantly different between SAM-R1 and SAM-P8 (Table 1). In contrast, the levels of plasma triglycerides in SAM-P8 were 1.6-fold (p < .01) higher than in SAM-R1.

PGC-1β Expression Is Down-Regulated in Skeletal Muscle of SAM-P8

We next analyzed the expression of PGC-1α and PGC-1β in the soleus skeletal muscle of SAM-R1 and SAM-P8. As it is shown in Figure 1A, SAM-P8 showed a slight increase (1.4-fold) in PGC-1α mRNA levels that did not reach statistical significance. In contrast, the mRNA expression of PGC-1β was 52% (p < .001) lower in the skeletal muscle of SAM-P8 than in SAM-R1 (Figure 1B). PGC1α coactivates several transcription factors, including NRF-1 and -2 (16), which, in turn, stimulate the expression of mtTFA, a mitochondrial protein essential for the replication and transcription of DNA mitochondrial-encoded genes, such as the subunit 1 of complex I (NADH dehydrogenase subunit 1, ND1). Furthermore, suppression of PGC-1α expression in vascular endothelial cells may lead to the reduction of mRNA levels of mitochondrial antioxidant genes, including MnSOD (17). Despite the changes in the expression of PGC-1α in skeletal muscle of SAM-P8, NRF-1, NRF-2, ND1, and MnSOD, expression in the skeletal muscle was the same in both strains (Figure 1C–F).

Table 1. Plasma Levels of Glucose, NEFA, Triglycerides, Cholesterol, and Insulin in 5-Month-Old SAM-R1 and SAM-P8 Mice

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Electrophoretic Mobility Shift Assay

Isolation of nuclear extracts and electrophoretic mobility shift assay (EMSA) were performed as previously described (15).

Statistical Analyses

Results were obtained from five animals and presented as mean ± standard deviation. Student’s t test was used to determine statistical significance (GraphPad Instat; GraphPad Software Inc., San Diego, CA). Differences were considered significant at p < .05.

Figure 1. Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 (PGC-1) messenger RNA (mRNA) levels are down-regulated in skeletal muscle of senescence-accelerated prone mice (SAM-P8). Analysis of the mRNA levels of PGC-1α (A), PGC-1β (B), nuclear respiratory factor-1 (NRF-1; C), NRF-2 (D), NADH dehydrogenase subunit 1 (ND1) (E), and manganese superoxide dismutase (MnSOD) (F) in skeletal muscle of senescence-accelerated resistant mice (SAM-R1) and SAM-P8. Representative autoradiograph and the quantification normalized to the adenosyl phosphoribosyl transferase (APRT) mRNA levels are shown. Data are expressed as mean ± standard deviation of five different animals. ***p < .001.
Lack of Changes in the Expression of Genes Involved in Fatty Acid and Glucose Metabolism in Skeletal Muscle of SAM-R1 and SAM-P8

Given that ectopic expression of PGC-1α affects the expression of genes involved in lipid (17) and glucose metabolism (18), we next compared the expression of these genes in SAM-R1 and SAM-P8. No changes were observed in the transcript levels of the nuclear receptors PPARα and PPARβ/δ (Figure 2A and B). Furthermore, the skeletal muscle expression of several PPAR-target genes involved in fatty acid metabolism (M-CPT-I, CD36, and CTE) (Figure 2C–E) was not significantly different between SAM-R1 and SAM-P8. Only the expression of PDK-4, which suppresses glucose oxidation by its inhibitory effect on the pyruvate dehydrogenase complex leading to an increase in fatty acid utilization (19), was 37% lower in skeletal muscle of SAM-P8 compared to SAM-R1, although differences did not reach statistical significance (Figure 2F). Finally, the mRNA levels of the glucose transporter GLUT4 were similar in skeletal muscle of SAM-P8 and SAM-R1 (Figure 2G).

Moreover, we examined whether the protein expression of PPARα and PPARβ/δ was different between SAM-P8 and SAM-R1. As it is shown in Figure 3, no differences were found in the protein expression of these transcription factors between the two SAM strains. We also evaluated the protein expression of COUP-TF-II (also called apolipoprotein A1 regulatory protein, ARP-1), which increases with age (20) and acts as a PPAR transcriptional repressor (21) through its ability to bind the peroxisome proliferator-response element (PPRE). The protein expression of COUP-TF II was higher in skeletal muscle of SAM-P8 than in SAM-R1 (Figure 3).

Figure 2. Lack of changes in the messenger RNA (mRNA) levels of several genes involved in fatty acid and glucose metabolism in senescence-accelerated prone mice (SAM-P8). Analysis of the mRNA levels of peroxisome proliferator-activated receptor (PPAR) α (A), PPARβ/δ (B), muscle-type carnitine palmitoyltransferase I (M-CPT-I) (C), CD36 (D), cytosolic acyl-coenzyme A thioesterase (CTE) (E), pyruvate dehydrogenase kinase 4 (PDK-4) (F), and glucose transporter 4 (G) in skeletal muscle of senescence-accelerated resistant mice (SAM-R1) and SAM-P8. Representative autoradiograph and the quantification normalized to the adenosyl phosphoribosyl transferase (APRT) mRNA levels are shown. Data are expressed as mean ± standard deviation of five different animals.

Figure 3. Increased expression of chicken ovalbumin upstream promoter transcription factor II (COUP-TF II) protein levels in skeletal muscle of senescence-accelerated prone mice (SAM-P8). Nuclear extracts from soleus skeletal muscle from senescence-accelerated resistant mice (SAM-R1) and SAM-P8 were subjected to immunoblot analysis as described in Methods. Representative immunoblots using antiperoxisome proliferator-activated receptor (PPAR)α, anti-PPARβ/δ, COUP-TF II, and β-tubulin antibodies are shown. Autoradiograph data are representative of three animals.
SAM-P8 Show Decreased PPAR and Enhanced Nuclear Factor-κB Binding Activity in Skeletal Muscle Compared to SAM-R1

Because PGC-1α and β are coactivators for PPARs enhancing their transcriptional activity (2), we next examined PPAR DNA-binding activity using a 32P-labeled PPRE probe and skeletal muscle nuclear extracts from SAM-P8 and SAM-R1. The PPRE probe formed two complexes with nuclear proteins (complexes I and II, Figure 4A). Competition studies performed with a molar excess of unlabeled probe revealed that the complexes represented specific PPRE–protein interactions. A reduction in the binding of nuclear proteins to the PPRE cis-element was observed (Figure 4A) in SAM-P8. Given that the PPRE interacts in vitro with multiple nuclear receptors, including homodimers of the transcriptional repressor COUP-TF II, we determined whether the COUP-TF II protein interacted with the PPRE probe (Figure 4B). When supershift assays were performed with COUP-TF II antibody and skeletal muscle nuclear extracts from SAM-R1 and SAM-P8, only

![Figure 4A](image1.png)

**Figure 4A.** Autoradiograph of electrophoretic mobility shift assay (EMSA) performed with a 32P-labeled PPRE nucleotide and crude nuclear protein extract (NE) shows two specific complexes (I and II), based on competition with a molar excess of unlabeled probe. Autoradiograph of EMSA performed with a 32P-labeled PPRE nucleotide and NE from skeletal muscle of senescence-accelerated resistant mice (SAM-R1) and SAM-P8.

![Figure 4B](image2.png)

**Figure 4B.** Identification of chicken ovalbumin upstream promoter transcription factor II (COUP-TF II) in skeletal muscle nuclear protein complexes by antibody recognition experiments.

![Figure 4C](image3.png)

**Figure 4C.** Autoradiograph of EMSA performed with a32P-labeled nuclear factor-κB (NF-κB) nucleotide and NEs. One specific complex (I), based on competition with a molar excess of unlabeled probe, is shown. Autoradiograph data are representative of three separate experiments.
a supershifted band was observed in the second, indicating that COUP-TF II was bound to the PPRE probe in skeletal muscle nuclear extracts from SAM-P8.

As reductions in PPAR expression and activity with aging have been associated with enhanced nuclear factor-κB (NF-κB) activity in splenocytes (22), we subsequently investigated whether the decrease in PPAR activity in SAM-P8 led to changes in NF-κB binding activity. As shown in Figure 4C, EMSA showed that the NF-κB probe formed one complex with nuclear proteins (Figure 4C). Specificity of the DNA-binding complex was assessed in competition experiments by adding an excess of unlabeled NF-κB oligonucleotide. NF-κB DNA-binding activity increased in nuclear extracts from SAM-P8 compared to SAM-R1; this finding is consistent with enhanced activation of this redox transcription factor with aging (23).

**SAM-P8 Show Reduced MCAD mRNA Levels and ERRα DNA Binding Activity in Skeletal Muscle Compared to SAM-R1**

Because it has been reported that PGC-1β functions as an ERRα ligand leading to enhanced ERR-mediated transcription of the MCAD gene (7), which plays a pivotal role in mitochondrial β-oxidation, we assessed whether the decrease in the expression of PGC-1β in the skeletal muscle of SAM-P8 affected the activity of this nuclear receptor. First, we examined the mRNA levels of the MCAD gene in skeletal muscle. Interestingly, SAM-P8 showed an 85% reduction (p < .01) in the expression of this gene compared to SAM-R1 (Figure 5A). In contrast, no changes were observed in the mRNA levels of ERRα in skeletal muscle from SAM-P8 (Figure 5B), suggesting that a reduction in the expression of this transcription factor was not involved in the decrease of MCAD expression. The reduction in MCAD expression was specific for skeletal muscle, as no changes were observed in the transcript levels of this gene in liver (data not shown). MCAD expression is regulated by ERRα through its binding to the NRRE-1, located in the proximal region of the MCAD gene promoter (24). Thus, we examined the ERRα DNA-binding activity using the NRRE-1 probe and skeletal muscle nuclear proteins from SAM-R1 and SAM-P8. The NRRE-1 probe formed two complexes with nuclear proteins (complexes I and II, Figure 5C). Competition studies performed with a molar excess of unlabeled probe revealed that the complexes represented specific NRRE-1-protein interactions (data not shown). SAM-P8 showed a reduction in the binding of nuclear proteins to the NRRE-1 probe (Figure 5C). Characterization of ERRα was performed by incubating nuclear extracts with antibody directed against this protein. Addition of anti-ERRα reduced the formation of both complexes, showing that the incubation mixtures contained ERRα, whereas an unrelated antibody (Oct-1) did not affect the formation of the complexes (Figure 5D). Overall, these data indicate that PGC-1β down-regulation in skeletal muscle of SAM-P8 leads to reduced DNA-binding activity of ERRα and, subsequently, to a decrease in the expression of its target gene MCAD.
DISCUSSION

PGC-1α and β are key regulators of mitochondrial number and function and lipid metabolism. They function as transcriptional coactivators, regulating glucose and fat oxidation in muscle (25). Interestingly, it has been recently reported that the mRNA expression of PGC-1α and β is reduced in skeletal muscle with age in humans (26), although the contribution of their reduction to the activity of the nuclear receptors they coactivate remains unknown. Furthermore, in the same study PGC-1β expression in skeletal muscle was positively associated with lipid oxidation, although no clues were given about the mechanisms involved. In the present study we show that PGC-1β expression is down-regulated in skeletal muscle of SAM-P8. Because this coactivator is an ERRα protein ligand (7) that regulates MCAD expression, we determined the expression of this gene, which catalyzes a rate-limiting step in the mitochondrial β-oxidation of medium-chain fatty acids. The mRNA expression of this gene was strongly reduced, probably due to the decrease in ERRα DNA-binding activity. The relationship between PGC-1β and MCAD expression has been clearly defined in the PGC-1β transgenic mice. In these mice, enhanced expression of PGC-1β in skeletal muscle led to increased MCAD mRNA expression (7). In addition, the PGC-1β transgenic mice had low plasma triglyceride levels compared to wild-type mice. This finding may indicate that the high levels of plasma triglycerides in the SAM-P8 compared to SAM-R1 may result from the reduced expression of PGC-1β in the skeletal muscle of these mice. Furthermore, these findings provide clues about the mechanisms through which reduced PGC-1β expression in skeletal muscle regulates lipid oxidation, as both have been positively associated in human skeletal muscle (7).

Finally, the decrease in the expression of MCAD may lead to reduced mitochondrial β-oxidation, which in turn increases the levels of fatty acid derivatives (a source of reactive oxygen species [ROS]) that may activate the redox transcription factor NF-kB. In agreement with this hypothesis, when we performed EMSAs we observed enhanced NF-κB DNA-binding activity in SAM-P8 compared to SAM-R1. This finding links the decrease in mitochondrial fatty acid oxidation during aging with NF-κB activation, a relationship that has been previously reported in other organs (23).

In contrast, analysis of PGC-1α expression in skeletal muscle of 5-month-old SAM-P8 showed a slight increase. This finding is in conflict with those in previous studies on aged humans (26). We do not know the reasons for these differences between humans and SAM-P8 although the expression of PGC-1α may be down-regulated in older SAM-P8, as the mice used in this study were 5 months old and they show accelerated aging from age 2 to 8 months. In any case, the enhanced expression of PGC-1α in skeletal muscle of SAM-P8 seems negligible, as no changes were observed in the expression of several genes and transcriptional factors regulated by this coactivator. Similarly, the change in the content of PGC-1α in skeletal muscle did not affect the expression of genes involved in fatty acid and glucose metabolism.

In the skeletal muscle of SAM-P8 we also found that the protein expression of the transcriptional repressor COUP-TF II was strongly enhanced. An age-related increase in the expression of this transcriptional repressor in liver has been reported to reduce the expression of the PPARα-target gene apo AI (20). In addition, we have reported that in the heart of rats treated with troglitazone (an insulin-sensitizing drug that enhances the use of glucose in skeletal muscle; 21), the enhanced expression of COUP-TF II reduced the expression of the PPARα-target gene acyl-CoA oxidase, which codes for a key enzyme involved in peroxisomal fatty acid β-oxidation. Therefore, all these data suggest that when fatty acid oxidation is reduced or impaired, skeletal muscle energy demand relies on glucose. In agreement with this idea it has been reported that 4- to 8-week-old SAM-P8 are more sensitive to insulin than are SAM-R1 and the reduction of blood glucose levels in SAM-P8 after insulin injection was higher than in SAM-R1 (27). Moreover, the shift in the source of energy from fatty acids to glucose observed in cardiac hypertrophy, which is a reversion to fetal metabolism, is accompanied by an induction in the expression of COUP-TF II (28), suggesting that this transcriptional repressor is involved in the adaptation from fatty acids to glucose. In fact, we found that COUP-TF II was bound to the PPRE probe in nuclear extracts from SAM-P8 but not from SAM-R1. Although the PPAR binding activity was reduced in SAM-P8, this reduction did not affect the expression of PPAR target genes, suggesting that this reduction in PPAR binding does not affect basal expression of genes involved in fatty acid metabolism. It remains to be seen whether under stress metabolic conditions (such as fasting, during which fatty acid metabolism is enhanced through PPAR activation) the expression of PPAR target genes would be reduced. Furthermore, it is conceivable that, in addition to the increase in COUP-TF II, a decrease in the protein levels of PPARs may also be necessary to affect the expression of PPAR target genes as has been reported in cardiac hypertrophy (29).

Summary

In the present study we show decreased expression of PGC-1β in skeletal muscle of SAM-P8. The reduction in the expression of this coactivator correlates with reduced binding activity of the transcription factor ERRα and a decrease in the expression of the MCAD gene, which is involved in mitochondrial β-oxidation of fatty acids. These findings may confirm previous links between mitochondrial dysfunction and the development of aging.

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