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The PGC-1α-related coactivator promotes mitochondrial and myogenic adaptations in C2C12 myotubes

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Philp A, Belew MY, Evans A, Pham D, Sivia I, Chen A, Schenk S, Baar K. The PGC-1α-related coactivator promotes mitochondrial and myogenic adaptations in C2C12 myotubes. Am J Physiol Regul Integr Comp Physiol 301: R864–R872, 2011. First published July 27, 2011; doi:10.1152/ajpregu.00232.2011.—The transcriptional coactivator PGC-1α is a potent regulator of skeletal muscle metabolism. Less is known about the structurally similar PGC-1α-related coactivator (PRC) that is enriched in myoblasts and adult skeletal muscle. The present study was designed to determine the effect of PRC on the metabolic profile of C2C12 myotubes. Overexpression of full-length PRC increased PRC gene expression by 2.7 ± 0.3-fold and protein content by 108 ± 5.3%. This modest elevation in PRC resulted in an increased rate of myoblast proliferation (61.5 ± 2.7%) and resulted in myotubes characterized by increased MyOD (18.2 ± 0.52%) and myosin heavy chain (15.4 ± 3.13%) protein. PRC overexpressing myotubes showed increases in mRNA for some—COX4 (2.6 ± 0.18-fold), ATP5B (2.7 ± 0.34-fold) cytochrome c (5.1 ± 0.68-fold)—but not all, MTCO1 (0.61 ± 0.18-fold) and HAD (0.98 ± 0.36-fold) mitochondrial genes, as well as a significant increase in cytochrome-c (28.7 ± 7.02%) protein content. The enzyme activity of the electron transport chain (ETC) complex IV (3.7 ± 0.01-fold) and citrate synthase (2.1 ± 0.14-fold) was increased by PRC, as was the mtDNA:nucDNA ratio (11 ± 0.3%). PRC increased cellular respiration (142%), basal (197%) and insulin-stimulated (253%) glucose uptake, as well as palmitate uptake (28.3 ± 3.31%) and oxidation (31.1 ± 2.17%). Associated with these changes in function, PRC overexpression increased GLUT4 mRNA (4.5 ± 0.22-fold) and protein (13.8 ± 2.08%) and CPT1 protein (28.9 ± 4.23%). Electrical stimulation of C2C12 myotubes resulted in a transient increase in PRC mRNA that was smaller (2.1 ± 0.33-fold vs. 4.4 ± 0.23-fold) and occurred earlier (3 h vs. 6 h) than PGC-1α. Collectively, our data show that PRC promotes skeletal muscle myogenesis and metabolism in vitro, thus identifying PRC as a functional skeletal muscle coactivator capable of regulating mitochondrial substrate utilization and respiration.

exercise; glucose uptake; fatty acid oxidation; muscle; metabolism

TRANSCRIPTIONAL COACTIVATORS have a wide range of metabolic effects due to their ability to amplify transcription factor activity toward target genes (21). The best characterized coactivator in skeletal muscle is the peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α), which is regarded as a principal regulator of mammalian mitochondrial biogenesis (13). Consistent with this role, PGC-1α is activated in skeletal muscle in response to exercise (4, 27), and PGC-1α transgenic mice display increased skeletal muscle oxidative capacity (8) and an increase in mitochondrial protein (20). In contrast, aberrant PGC-1α activity has been implicated in the etiology of insulin resistance, chronic inflammation, and mitochondrial myopathy (12, 13).

A second PGC isoform, subsequently termed PGC-1β was identified by Lin and coworkers (19). Like PGC-1α, PGC-1β overexpression leads to mitochondrial biogenesis and an increase in cellular respiration (29). PGC-1β is abundantly expressed during myogenesis (16) and has been implicated in the development of type IIX fibers in vivo (2). Recently, Liesa et al. (18) identified PGC-1β as a regulator of mitochondrial fusion through its control of mitofusin-2 (mfn2). Unlike PGC-1α, PGC-1β is insensitive to exercise (24), suggesting that PGC-1β is important during muscle development and maturation, with a limited role in phenotype plasticity in mature skeletal muscle.

A third, less-characterized member of the PGC family was identified a decade ago by Andersson and Scarpulla (1). The PGC-1α-related coactivator (PRC) is a 177-kDa protein with structural similarities to PGC-1α, including an acidic NH2 terminal region; a potent transcriptional activation domain; an LXXLL transcription factor recognition motif; and a central proline-rich region (1). PRC is enriched in dividing cells and is activated by serum to assist with the provision of ATP for cell cycle progression (1), whereas shRNA mediated knock-down of PRC decreases ATP production and leads to abnormal mitochondria (31). PRC gene expression is increased in human skeletal muscle following exercise (28, 24), however, the precise function of this adaptive response is currently unknown. Given that PGC-1β is unresponsive to exercise, this raises the possibility that PRC, like PGC-1α, may play an adaptive role in regulating skeletal muscle adaptation to exercise and suggests that PRC may be an important mitochondrial coactivator regulating mitochondrial respiratory chain subunit expression and ATP production (1, 11, 30–32).

The aim of the present study was to determine whether PRC has a functional role in metabolic provision and mitochondrial adaptation during myogenesis and in differentiated myotubes. Given the respiratory chain deficiencies when PRC is reduced and its similarity to PGC-1α, we hypothesized that PRC overexpression in C2C12 myoblasts would promote myogenesis and mitochondrial biogenesis.

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MATERIALS AND METHODS

Materials. C2C12 cells were from American Type Culture Collection (Manassas, VA). All materials were from Sigma Aldrich (St. Louis, MO) unless otherwise stated. The FL-PRC/pSV-SPORT PRC expression plasmid was generously donated by R. Scarpulla, Northwestern University and has been described in full previously (1).

Cell culture. C2C12 myoblasts were cultured in 100-mm or 35-mm plates (high glucose DMEM, 10% FBS, 1% penicillin/streptomycin) until 90% confluent when they were differentiated (high glucose DMEM, 2% horse serum, 1% penicillin/streptomycin). All metabolic measurements took place after 5 days of differentiation on fully formed myotubes unless stated otherwise.

PRC overexpression. Transient transfection of full-length PRC was performed on subconfluent C2C12 myoblasts using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). 2 × 10⁶ cells were seeded on 6-well plates in serum and antibiotic-free DMEM. Three hours after plating, 2 μg of plasmid DNA was complexed with Lipofectamine at a 1:1 ratio in 500 μL OptiMEM transfection media (Invitrogen, Carlsbad, CA) and added to each well. Twelve hours posttransfection, cells were washed twice with PBS, and 2 μl growth media (high-glucose DMEM, 10% FBS, 1% penicillin/streptomycin) added to each plate.

Cell growth assay. Cell proliferation was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) incorporation assay (Cayman Chemicals, Ann Arbor, MI), according to manufacturer’s instructions. Briefly, 5 × 10⁵ cells/well were plated on 96-well plates, and PRC transient transfections were carried out as previously described. Twelve and thirty-six hours after transfection, the MTT assay was performed by incubating cells for 4 h with 10 μl MTT reagent at 37°C. Following the incubation period, culture media were aspirated, and 100 μl of crystal dissolving solution added to each well. Absorbance was read at 570 nm using an Epoch spectrophotometer (BioTek, Woburn, MA).

Western blot. Following differentiation, cells were collected in lysis buffer (50 mM Tris pH 7.5; 250 mM sucrose; 1 mM EDTA; 1 mM EGTA; 1% Triton X-100; 1 mM sodium orthovanadate; 50 mM sodium fluoride; 0.1% dithiothreitol; 0.5% protease inhibitor cocktail), shaken at 4°C for 20 min, centrifuged for 5 min at 12,000 g, and the supernatant was removed for protein determination. Protein concentration was determined using the DC protein assay (Bio-Rad, Hercules, CA). Equal aliquots of protein were boiled in Laemmli sample buffer (250 mM Tris-HCl, pH 6.8; 2% SDS; 10% glycerol; 0.01% bromophenol blue; 5% β-mercaptoethanol and separated on SDS polyacrylamide gels (7.5–10%) for 1 hr. Following electrophoresis; proteins were transferred to a Protran nitrocellulose membrane (Whatman, Piscataway, NJ) at 100 V for 1 h. The membranes were incubated overnight at 4°C with appropriate primary antibody [1:1,000 dilution in Tris-buffered saline Tween-20 (TBS-T)]. The primary antibodies used were PRC (135516; Santa Cruz Biotechnology, Santa Cruz, CA), PGC-1α (AB3242; Millipore, Billerica, MA), mtTFA (23588; Santa Cruz Biotechnology), GLUT1 (7903; Santa Cruz Biotechnology), GLUT4 (7938; Santa Cruz Biotechnology), carnitine palmitoyltransferase (CPT1, CPT1M11-S; Alpha Diagnostics, Owings Mills, MD), succinate dehydrogenase (SDH; A11142; Molecular Probes, Eugene, OR), cytochrome oxidase I (COX I; 6403; Molecular Probes), cytochrome oxidase IV (COX IV; 21384; Molecular Probes), ATP synthase-β (A21351; Molecular Probes), cytochrome c (556433; BD Pharmingen, Franklin Lakes, NJ), 3-hydroxyacyl-CoA dehydrogenase (HAD; 37673; Abcam, Cambridge, MA), myoD (554130; BD Pharmingen). Total myosin heavy chain (MF20) and slow/fast myosin antibodies were used at a 1:1 dilution. All MHC antibodies were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences (Iowa City, IA). Protein content was normalized to eukaryotic initiation factor 2 (eEF2; 2332; Cell Signaling). Antibody binding was detected using enhanced chemiluminescence (Millipore, Billerica, MA). Imaging and band quantification were carried out using a Chemi Genius Bioimaging Gel Doc System (Syngene, Cambridge, UK).

Fig. 1. Overexpression of PGC-1α related coactivator (PRC) in C2C12 myotubes increased the protein content (A) and gene expression of PRC (B) with no effect on PGC-1α protein content (C). PRC increased cellular proliferation 12 and 36 h after transfection (D). Compared with empty vector (EV) controls (E), myotubes overexpressing PRC (F) displayed an altered phenotype associated with PRC overexpression. Data are representative (n = 8) of three independent transfection experiments. *Significantly different than EV control and †significantly different from 12 h (P < 0.0001).
RNA extraction and cDNA synthesis. RNA was extracted from cells using the phenol/chloroform method, as previously described (26). RNA was quantified using an Epoch spectrophotometer (BioTek) at 260 and 280 nm. First-strand cDNA was synthesized on an Eppendorf thermocycler (Eppendorf, Hauppauge, NY) from 1 μg of RNA using the reverse transcription system (Promega, Madison, WI), according to the manufacturer’s instructions.

Quantitative RT-PCR. Real-time PCR was performed to measure relative mRNA expression using an Eppendorf Light Cycler PCR machine (Eppendorf), SYBR Green PCR plus reagents (Sigma Aldrich) and custom-designed primers. Ten-microliter PCR reactions were assayed in triplicate on a 96-well plate. Each reaction contained 5 μl SYBR Green Taq, 1 μl of forward and reverse primers and 3 μl of cDNA (1:10 dilution). The target gene expression was calculated relative to values from glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and expressed normalized to pretreatment values. Absolute cycle threshold for GAPDH was unchanged by any of the treatments. Primers for real-time PCR were designed to span exon-exon boundaries to avoid amplification of genomic DNA. All primer sequences used have been described previously (26).

Cellular respiration. Cells were seeded in XF 24-well cell culture microplates (Seahorse Bioscience, North Billerica, MA) at 2.0 – 3.0 × 10^4 cells/well (0.32 cm^2) in 500 μl of growth medium and incubated at 37°C and 5% CO2 for 24 h. The cells were then differentiated for 5 days with or without PRC transient transfection. Assays were initiated by removing the growth medium from each well and replacing it with 600 – 900 μl of assay medium prewarmed to 37°C. The cells were incubated at 37°C for 30 min to allow media temperature and pH to reach equilibrium before the first rate measurement. Prior to each rate measurement, the XF24 Analyzer gently mixed the assay media in each well for 10 min to allow the oxygen partial pressure to reach equilibrium. Following mixing, basal oxygen consumption rate (OCR) was measured for 3–5 min. The assay medium was gently mixed for 3–5 min between each rate measurement to restore normal oxygen tension and pH in the microenvironment surrounding the cells. A total of 10 measurements were made throughout the testing period. On completion of the OCR assay, cells were collected and analyzed for total protein content as previously described. OCR is reported as oxygen consumption rate relative to cell protein content.

2-DG uptake. Following transfection, C2C12 myotubes were treated with either 100 nM insulin or ETH for 30 min and then washed three times with HEPES-buffered saline (HBS; 140 mM sodium chloride, 20 mM HEPES, 5 mM potassium chloride, 2.5 mM magnesium sulfate, 1 mM calcium chloride, pH 7.4). Glucose uptake was assayed by incubation with 10 μM 2-deoxy-[3H] glucose (2DG; 1 μCi/ml) for 10 min (Perkin Elmer-NET549). Nonspecific binding was determined by quantifying cell-associated radioactivity in the presence of 10 μM cytochalasin B (9). Media were aspirated prior to rapidly washing adherent cells three times with 0.9% (w/v) ice-cold saline. Cells were subsequently lysed in 50 mM sodium hydroxide and radioactivity quantified using a Beckman LS 6000IC scintillation counter. Protein concentration in cell lysates was determined using Bradford reagent, as described above.

Palmitate uptake and oxidation. Transfected myotubes were washed with HBS and subsequently incubated with media containing 0.75 mM palmitate (conjugated to 2% fatty acid free BSA)/[14C] palmitate at 2 μCi/ml (CFA23; GE Healthcare, New York, NY) for 2 h. Following this incubation period, 1 ml of the culture medium was carefully transferred to a sealable tube, the cap of which housed a Whatman (GF/B) filter paper disc that had been presoaked with 1 M potassium hydroxide. 14CO2 trapped in the media was then released by acidification of media using 60% (vol/vol) perchloric acid and
gently agitating the tubes at 37°C for 2 h. Radioactivity that had become adsorbed onto the filter discs was subsequently quantified by liquid scintillation counting. For measurement of palmitate uptake, cells were lysed, and 14C uptake was measured as previously described for glucose uptake.

Mitochondrial enzyme activity assays. To isolate mitochondria, myotubes were collected in 75 μl of buffer A (210 mM mannitol, 70 mM sucrose, 1 mM EGTA, 0.5% BSA, 5 mM HEPES). The homogenate was centrifuged at 1,000 g for 10 min (4°C), the resulting supernatant transferred to a separate microtube and recentrifuged at 10,000 g for 20 min (4°C). Following aspiration, the remaining pellet was resuspended in 50 μl buffer B (in mM): 210 mannitol, 70 sucrose, 1 EGTA, 5 HEPES; pH 7.2, and it was frozen at −80°C. Complex III and IV and citrate synthase (CS) enzyme activity assays were performed using standard methods, as previously described (32) with each experiment repeated in triplicate.

Quantification of mitochondrial DNA (mtDNA). Myotubes were collected in 200 μl of DNAzol genomic DNA isolation reagent ( Molecular Research Center, Cincinnati, OH) and centrifuged at 10,000 g for 10 min (4°C) to remove any insoluble material. DNA in the resulting supernatant was precipitated using 100 μl ethanol and incubated at room temperature for 3 min. The sample was centrifuged at 5,000 g for 5 min (25°C), and the supernatant was aspirated. The resulting DNA pellet was washed twice with 75% ethanol and resuspended without drying using 100 μl of 8 mM NaOH. DNA was quantified using an Epoch spectrophotometer (BioTek), and samples were diluted to a concentration of 20 ng/μl. Samples were analyzed in triplicate using 3 μl of DNA per reaction. qRT-PCR was performed using primers specific to a mitochondrial (cytochrome b Fwd: 5’-TTC GCA GTC ATA GCC ACA AG-3’, Rev: 5’-AGA TGA AGT GGA ATC ACA AG-3’) or nuclear (β-globulin Fwd: 5’-TGC CAT CCC ATC ACA ACA AG-3’, Rev: 5’-GCC AAT ACA CAG GTC ACA GAG-3’) encoded gene using an Eppendorf Light Cycler PCR machine.

In vitro electrical contraction. Fully differentiated myotubes were electrically stimulated for 3 h with a continuous 1-Hz stimulation pattern using a pulse width of 0.3 ms and pulse amplitude of 40 V, as previously described (10). Briefly, following 5 days of differentiation, the standard 6-well tissue culture lid was replaced with a lid containing parallel U-shaped stainless-steel electrodes embedded 20 mm apart. 0.3-ms pulses from the stimulator were amplified to 40 V using a commercially available amplifier (Rolls RA2100b; Direct Pro Audio, Omaha, NE). Myotubes were collected either before or after (1, 3, and 6 h) the 3-h contraction period, and processed for qRT-PCR, as previously described.

Statistical analysis. A Student’s t-test or two-way repeated-measures ANOVA (SigmaPlot 11.2; Systat Software, San Jose, CA) was used to determine differences between groups and experimental conditions and Tukey post hoc was used where appropriate. Values are displayed as means ± SE, with statistical significance set at P < 0.05.

RESULTS

Physiological overexpression of PRC. Transient transfection of full-length PRC resulted in an increase in PRC gene expression (180% P < 0.01; Fig. 1A) and protein content (107% P < 0.01; Fig. 1B). PRC overexpression had no effect on PGC-1α protein content (Fig. 1C).

PRC overexpression increases C2C12 myoblast proliferation. PRC resulted in a significant increase in the rate of proliferation compared with an empty vector (EV) control (Fig. 1D). Twelve hours after transfection, there were significantly more cells (EV = 0.35 ± 0.019; PRC = 0.56 ± 0.023; P < 0.0001) in the PRC group, and this was maintained at 36 h (EV = 0.70 ± 0.027; PRC = 1.09 ± 0.06; P < 0.0001).

PRC transfected myotubes have altered morphology and contractile apparatus. Concomitant with the increase in proliferation, PRC overexpression resulted in a greater abundance of smaller myotubes compared with control (Fig. 1, E and F). The increase in cellularity was paralleled by an increase in MyoD (EV = 89.1 ± 0.6; PRC = 105.3 ± 1.07 arbitrary units (AU)) and total myosin heavy chain (MHC; EV = 96.7 ± 2; PRC = 111.9 ± 5 AU; Fig. 2, A–D) protein content. The increase in total MHC appeared to be, in part, due to an increase in the slow MHC isoform (EV = 95 ± 3; PRC = 115.3 ± 7.4 AU), whereas the fast and isoforms of MHC were unchanged between PRC control samples. Specific assessment of developmental MHC isoforms was not addressed, and so it is currently not possible to conclude that the MHC alterations were solely due to increases in slow MHC content.

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![Fig. 3. PRC overexpression increases mitochondrial gene expression but not protein content. A: PRC increases cytochrome oxidase (COX) 4, β ATP synthase (ATP5B) and cytochrome-c (CytC) mRNA expression, whereas the mitochondrial encoded cytochrome oxidase 1 (MTOC1), citrate synthase (CS), and 3-hydroxyacyl-CoA dehydrogenase (HAD) mRNA expression was unchanged. B: PRC significantly increased CytC and tended to increase mitochondrial transcription factor-A (mtTFA) and succinate dehydrogenase (SDH) protein content but had modest effects on COX IV and β ATP synthase (ATP Synβ) protein content. Eukaryotic initiation factor 2 (eEF2) was used as a loading control for each protein. Data are representative (n = 6) of two independent experiments. *Significantly different than EV controls (P < 0.05).](http://ajpregu.physiology.org/ by 10.220.33.3 on July 6, 2017)
PRC overexpression induces mitochondrial gene expression. Overexpression of PRC significantly increased COX4 (2.62 ± 0.05 AU), ATP5B (2.73 ± 0.3 AU) and cytochrome c (5.06 ± 0.67 AU; P < 0.05; Fig. 3) mRNA. In contrast, the mitochondrially encoded MTCO1 showed a trend to be reduced (0.61 ± 0.18 AU). The tricarboxylic acid (TCA) cycle enzyme, CS drially encoded MTCO1 showed a trend to be reduced (0.61 ± 0.31 AU; P < 0.05) glucose uptake was higher in the PRC-transfected cells.

PRC increases palmitate uptake and oxidation. Similar to its effect on glucose uptake, PRC overexpression increased 14C palmitate uptake (EV = 2.78 ± 0.08; PRC = 3.57 ± 0.13 pmol·mg⁻¹·min⁻¹; P < 0.05; Fig. 6) and oxidation (EV = 0.06 ± 0.003; PRC = 0.08 ± 0.005 pmol·mg⁻¹·min⁻¹; P < 0.05) concomitant with an increase in carnitine palmitoyltransferase (CPT1) protein content (EV = 215.2 ± 9.9; PRC = 271.6 ± 21.1).

Contraction increased expression of PRC. To determine whether PRC could be induced by contractile activity, C2C12 myotubes were electrically stimulated (1 Hz) for 3 h and PRC and PGC-1α mRNA were measured in the 6 h recovery period. Chronic low-frequency electrical stimulation resulted in a transient increase in PRC mRNA 3 h post stimulation (2.06 ± 0.33-fold; P < 0.05; Fig. 6). In contrast, PGC1α mRNA increased more (4.37 ± 0.23-fold; P < 0.05) and at a later time point (6 h) in response to this low-frequency signal.

DISCUSSION

Transient overexpression of PRC, at physiological levels, results in myogenic and metabolic alterations in C2C12 myotubes. Myotubes overexpressing PRC display increased mitochondrial gene expression, activity, and mass, coupled to elevated rates of oxygen consumption and an enhanced ability to utilize carbohydrate and lipid substrates (increased glucose uptake, and palmitate utilization). Collectively, these data suggest that PRC can regulate the myogenic program and alter mitochondrial dynamics, metabolism, and function in differentiated myotubes.

Like both PGC-1α (33) and PGC-1β (29), overexpression of PRC results in a significant increase in respiration. Even though the increase in respiration and the metabolic phenotype of myotubes overexpressing PRC is similar than that observed for PGC-1α (33), there appear to be a some subtle differences between the effects of PRC, PGC-1α, and PGC-1β overexpression. First, PGC-1α and PGC-1β overexpression results in a more complete mitochondrial biogenesis, including dramatic upregulation of the mitochondrial transcription factor-A (mtTFA), mitochondrial DNA, and enhanced expression and function of all of the electron transport chain subunits (29, 33). In contrast, the phenotype of the PRC cells was much more...
akin to that of NRF1 overexpression: a large increase in GLUT4 and cytochrome c, smaller changes in SDH and COX IV, and modest increases in mtTFA protein content and the mtDNA:nucDNA ratio (3). This suggests the possibility that, in muscle cells, PRC may signal primarily through NRF1 and in
a more limited manner with NRF2/GABP. Consistent with this hypothesis, PRC overexpression had a limited effect on COX4 mRNA (controlled by NRF2/GABP) and genes that require both NRF1 and NRF2/GABP (mtTFA) were modestly increased by PRC overexpression. Together, these data suggest that PRC affects the expression of a subset of nuclear encoded mitochondrial genes in skeletal muscle through the coactivation of NRF1. However, Gleyzer et al. (11) and Vercauteren et al. (30–32) have demonstrated that in U2OS and BALB/3T3 cells, PRC can complex with both NRF1 and NRF2/GABP. The reason for the disparity between the current work and that previously reported is unclear, but may reflect a difference in the cell types used. Clearly, further studies are required to assess PRC interaction with target gene promoter regions in skeletal muscle, both in vitro and in vivo to fully address this question.

PRC overexpression had a dramatic effect on both basal and insulin-stimulated glucose uptake. Basal glucose uptake was elevated 3.2-fold in cells overexpressing PRC. In addition, there was a similar three-fold increase in insulin-stimulated glucose uptake. Consistent with the improved insulin-stimulated glucose uptake, there was an increase in GLUT4 mRNA and protein in the PRC cells, independent of changes in regulated glucose uptake, there was an increase in GLUT4 mRNA (controlled by NRF2/GABP) and genes that require both NRF1 and NRF2/GABP (mtTFA) were modestly increased by PRC overexpression. Together, these data suggest that PRC affects the expression of a subset of nuclear encoded mitochondrial genes in skeletal muscle through the coactivation of NRF1. However, Gleyzer et al. (11) and Vercauteren et al. (30–32) have demonstrated that in U2OS and BALB/3T3 cells, PRC can complex with both NRF1 and NRF2/GABP. The reason for the disparity between the current work and that previously reported is unclear, but may reflect a difference in the cell types used. Clearly, further studies are required to assess PRC interaction with target gene promoter regions in skeletal muscle, both in vitro and in vivo to fully address this question.

The positive effect of PRC on insulin-stimulated glucose uptake is similar to that reported for PGC-1α (6, 25). Ectopic expression of PGC-1α increases insulin sensitivity in C2C12 myoblasts and in skeletal muscle in vivo; however, the precise mechanism for this effect is currently unknown. Pagel-Langenickel et al. (25) speculated that PGC-1α activation may restore insulin signaling through reduction of reactive oxygen species or the lipid derivatives, ceramide, and diacylglycerol, each independently shown to blunt the effect of insulin on the classical insulin signaling cascade. Consistent with this hypothesis, PRC overexpression also increased CPT1 and palmitate oxidation. PGC-1α has previously been implicated in regulating CPT1 and fatty acid metabolism in skeletal muscle via PPARα/δ, and it is possible that PRC can replicate this effect (5). In vivo, overexpression of CPT1 is sufficient to increase fat oxidation and decrease other lipid fates (7). Together, these data suggest that PRC may improve insulin sensitivity by increasing fat oxidation via a CPT1-dependent mechanism. An alternative explanation for the accelerated insulin-stimulated glucose uptake might be that the increase in GLUT4 with PRC overexpression results in more of the transporter associated with the cell membrane during insulin stimulation.

The ability of PRC to increase myoblast proliferation and alter MHC protein content highlights a novel function for PRC in the regulation of myogenesis. A similar role in regulating the slow MHC levels has previously been suggested for PGC-1α in vivo (9), in which PGC-1α transgenic mice demonstrated a modest increase of slow MHC expression of ~7%. Parallel in vitro promoter-based assays suggested that the PGC-1α-mediated myogenic program appeared to function through MEF2 transcription factors in a calcineurin-dependent manner. Even though PRC interacts with NRF transcription factors (18, 19) and the cyclic-AMP response element binding protein (CREB; Ref. 19), a PRC-MEF2 interaction has not previously been demonstrated. On the basis of our data, future research examining PRC’s ability to coactivate transcription factors involved in myogenesis and MHC development is warranted.

PRC gain-of-function experiments were used exclusively throughout the present study, and as such, this study does not demonstrate a requirement for PRC in skeletal muscle cell myogenesis or mitochondrial adaptation. With any forced expression study, overexpressing a protein at high abundance can produce an aberrant phenotype as an artifact of supraphysiological gene expression. The transient transfection approach employed herein resulted in a moderate increase in PRC protein content and gene expression similar to the induction previously reported in human skeletal muscle in response to
exercise (28, 24). Therefore, we propose that the data reported herein is physiological with regard to the effects of PRC. Clearly, given the widespread metabolic effects of PRC action that we demonstrate, further studies performing PRC overexpression, knock-down, and ablation in skeletal muscle in vivo are required.

**Perspectives and Significance**

Here, we have demonstrated a number of novel, functional roles for PRC in skeletal muscle. In vitro, PRC regulates the rate of proliferation, cell respiration, glucose and fatty acid metabolism, and contractile protein content. As such, PRC mirrors many, but not all, of the previously described functions for PGC-1α. We propose that PRC is a functional coactivator in skeletal muscle, and even though PRC activation by contraction is not as potent as PGC-1α, PRC may be involved in the metabolic adaptation to exercise. Further, whether PRC can serve a compensatory role (26) to protect the cell from external stresses when PGC-1α expression is compromised (23) or can substitute for PGC-1α at times (17) is a question warranting further investigation.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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