

# Oleic Acid Stimulates Complete Oxidation of Fatty Acids through Protein Kinase A-dependent Activation of SIRT1-PGC1 $\alpha$ Complex\*

Received for publication, September 4, 2012, and in revised form, December 30, 2012. Published, JBC Papers in Press, January 17, 2013, DOI 10.1074/jbc.M112.415729

Ji-Hong Lim<sup>#1</sup>, Zachary Gerhart-Hines<sup>#2</sup>, John E. Dominy<sup>#3</sup>, Yoonjin Lee<sup>‡</sup>, Sungjin Kim<sup>§</sup>, Mitsuhsa Tabata<sup>#2</sup>, Yang K. Xiang<sup>§</sup>, and Pere Puigserver<sup>#4</sup>

From the <sup>#</sup>Department of Cancer Biology, Dana-Farber Cancer Institute and Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115 and <sup>§</sup>Department of Pharmacology, University of California, Davis, California 95616

**Background:** Different fatty acids signal to control intracellular lipid dynamic metabolism.

**Results:** In skeletal muscle cells, oleic acid induces cAMP/PKA and SIRT1 Ser-434 phosphorylation and activity, causing PGC1 $\alpha$  deacetylation and increased fatty acid oxidation.

**Conclusion:** Oleic acid signals to the SIRT1-PGC1 $\alpha$  complex to increase rates of fatty acid oxidation.

**Significance:** Pharmacological manipulation of the oleic acid signaling pathway might be a therapeutic option for conditions of lipid dysregulation.

Fatty acids are essential components of the dynamic lipid metabolism in cells. Fatty acids can also signal to intracellular pathways to trigger a broad range of cellular responses. Oleic acid is an abundant monounsaturated omega-9 fatty acid that impinges on different biological processes, but the mechanisms of action are not completely understood. Here, we report that oleic acid stimulates the cAMP/protein kinase A pathway and activates the SIRT1-PGC1 $\alpha$  transcriptional complex to modulate rates of fatty acid oxidation. In skeletal muscle cells, oleic acid treatment increased intracellular levels of cyclic adenosine monophosphate (cAMP) that turned on protein kinase A activity. This resulted in SIRT1 phosphorylation at Ser-434 and elevation of its catalytic deacetylase activity. A direct SIRT1 substrate is the transcriptional coactivator peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (PGC1 $\alpha$ ), which became deacetylated and hyperactive after oleic acid treatment. Importantly, oleic acid, but not other long chain fatty acids such as palmitate, increased the expression of genes linked to fatty acid oxidation pathway in a SIRT1-PGC1 $\alpha$ -dependent mechanism. As a result, oleic acid potently accelerated rates of complete fatty acid oxidation in skeletal muscle cells. These results illustrate how a single long chain fatty acid specifically controls lipid oxidation through a signaling/transcriptional pathway. Pharmacological manipulation of this lipid signaling pathway might provide therapeutic possibilities to treat metabolic diseases associated with lipid dysregulation.

Fatty acids are central structural and functional components of lipid metabolism, forming parts of cellular membranes, lipid droplets, or lipoproteins (1–3). Fatty acids can be obtained from the diet or synthesized in cells through metabolic pathways such as *de novo* lipogenesis (4). Depending on the nutrient and energy conditions, fatty acids can be rapidly oxidized in peroxisomes and mitochondria to maintain the cellular bioenergetic homeostasis (5). This ability to completely oxidize fatty acids to generate energy occurs in most mammalian cells except in neurons and red blood cells that strictly depend on glucose utilization to produce sufficient quantities of ATP for survival (6). There are different types of fatty acids with specific biophysical properties based on chain length and degree of saturation, including saturated long chain fatty acids (e.g. palmitic acid), monounsaturated long chain fatty acids (e.g. oleic acid), and polyunsaturated fatty acids (e.g. docosahexaenoic acid or linoleic acid). Interestingly, whereas saturated fatty acids cause inflammation that is associated with insulin-resistant states and atherosclerosis, oleic acid and polyunsaturated omega-3 fatty acids are anti-inflammatory and protective against these metabolic diseases. In the case of omega-3 fatty acids, the mechanisms of inflammatory suppression and insulin sensitivity have been recently elucidated and involve the GPR120 receptor signaling (7). However, the molecular regulatory mechanisms by which oleic acid reduces inflammation and dyslipidemias as well as improves insulin sensitivity (8–10) are not completely understood.

The regulatory control of fatty acid oxidation rates occurs at multiple levels involving transcriptional and non-transcriptional components. For example, among the non-transcriptional mechanisms increases in intracellular malonyl-CoA (the product of the fatty acid synthesis enzyme, acetyl-CoA carboxylase) potently suppress fatty acid oxidation by inhibiting the mitochondrial fatty acid transporter, carnitine-palmitoyltransferase-1 (11–13). At the transcriptional level, several hormone nuclear receptors (e.g. peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ), estrogen related receptor  $\alpha$  (ERR $\alpha$ )) target sets

\* This work was supported by the Dana-Farber Cancer Institute, the American Diabetes Association, the Department of Defense, National Institutes of Health/NIDDK Grant RO1 069966 (to P. P.) as well as National Institutes of Health Grant RO1-HL82846 and American Heart Association Grant 12EIA841007 (to Y. K. X.).

<sup>1</sup> Supported by an American Diabetes Association mentor supported post-doctoral fellowship.

<sup>2</sup> Supported in part by a fellowship from the American Heart Association.

<sup>3</sup> Supported in part by an National Research Service Award Kirschstein fellowship from the National Institutes of Health.

<sup>4</sup> To whom correspondence should be addressed: Dana Farber Cancer Institute, 450 Brookline Ave., CLSB-11144, Boston, MA 02215. Tel.: 617-582-7977; Fax: 617-632-5363; E-mail: pere\_puigserver@dfci.harvard.edu.

## Oleic Acid Activates the SIRT1-PGC1 $\alpha$ Complex

of genes encoding fatty acid oxidation enzymes (14–16). The potency of these transcription factors largely depends on the assembly of co-activators such as PGC1 $\alpha$ , which strongly increase the ability of these hormone nuclear receptors to drive transcriptional programs (17–20). PGC1 $\alpha$  is controlled through reversible lysine side chain hyperacetylation that depends on the enzymatic activities of the acetyltransferase General Control of Amino-acid Synthesis 5-like 2 (GCN5) and the deacetylase SIRT1 (21, 22). SIRT1 deacetylase activity is increased through different nutrient and signaling pathways, including glucose restriction, AMPK activation, and cAMP/PKA<sup>5</sup> activation; collectively, induction of these pathways promotes fatty acid oxidation (23–26). It is unknown, however, whether lipid signaling can modulate SIRT1 and, as a consequence, increase oxidation rates of fatty acids.

In these studies, we report that oleic acid, but not other long chain fatty acids, stimulates SIRT1 deacetylase activity through the elevation of cAMP intracellular levels and PKA signaling. As a result, the transcriptional coactivator PGC1 $\alpha$  is deacetylated and activated, leading to increases in the expression of genes linked to complete oxidation of fatty acids. Oleic acid specifically augmented rates of fatty acid oxidation in a SIRT1-PGC1 $\alpha$ -dependent manner. These results support a model in which oleic acid promotes fatty acid oxidation through signaling/transcriptional mechanisms providing a negative feedback loop that might explain, at least in part, some of the protective effects of this fatty acid against inflammation, dyslipidemias, and insulin resistance.

### EXPERIMENTAL PROCEDURES

**Reagents**—Oleic (O1383), palmitic (P0500), stearic (S4751), elaidic (E4637), linoleic acid (L1376), palmitoleic (P9417), H-89, and forskolin were purchased from Sigma. Oleoylethanolamide, GW9508, and EX527 were purchased from Cayman and Tocris.

**SIRT1 Phosphorylation, PGC1 $\alpha$  Acetylation, and Western Blot Analysis**—SIRT1 phosphorylation and PGC1 $\alpha$  acetylation were detected by immunoprecipitation of SIRT1 or PGC1 $\alpha$  followed by Western blot using phospho-PKA substrate or acetyl-lysine antibodies (Cell Signaling Technology) as described previously (26). Briefly, C<sub>2</sub>C<sub>12</sub> myotubes were infected with adenovirus expressing FLAG-tagged SIRT1 or PGC1 $\alpha$  as indicated. After 48 h post-infection, cells were lysed in a buffer that contained 1% IGEPAL, 150 mM NaCl, 20 mM HEPES (pH 7.9), 10 mM sodium fluoride, 10 mM glycerol 2-phosphate, 1 mM sodium orthovanadate, and protease inhibitor mixture. SIRT1 or PGC1 $\alpha$  was immunoprecipitated using anti-FLAG beads (Sigma), and immunocomplexes were electrophoresed on SDS-polyacrylamide gels and transferred to Immobilon-P membrane (Millipore). Membranes were incubated with primary antibodies in 5% bovine serum albumin containing 1 $\times$  TBS overnight at 4 °C. The membrane was then incubated with HRP-conjugated secondary antibodies for 1 h at room temper-

ature and visualized using a Super Signal West Dura or Super Signal West Femto Substrate (Pierce).

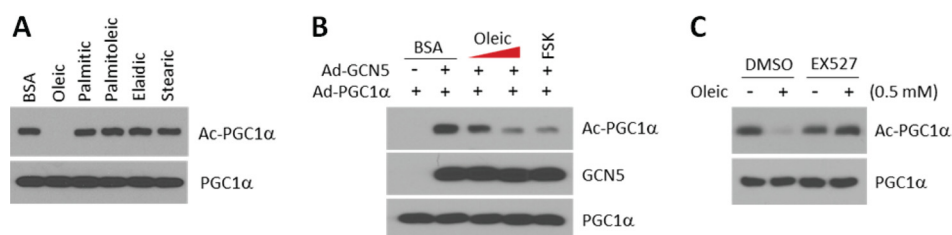
**Quantitative Real Time PCR and Primer Sequences**—Total RNA was isolated with TRIzol (Invitrogen), and 2  $\mu$ g of total RNA was used for cDNA synthesis using a high capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCRs were performed using SYBR Green PCR Master Mix (Applied Biosystems). Experimental Ct values were normalized to 36B4, and relative mRNA expression was calculated versus 36B4 expression. MCAD cDNA was amplified with sense primer (5'-GAAGGTTGAACTCGCTAGGC-3') and anti-sense primer (5'-GCTAGCTGATTGGCAATGTC-3'). PDK4 cDNA was amplified with sense primer (5'-CCGCTGTCCATGAAGCA-3') and antisense primer (5'-GCAGAAAAGCAAAGGACGTT-3'). ERR $\alpha$  cDNA was amplified with sense primer (5'-CAAGAGCATCCCAGGCTT-3') and antisense primer (5'-GCACTTCCATCCACACTC-3'). ACO cDNA was amplified with sense primer (5'-AGAACCATTGTCACACCTTG-3') and antisense primer (5'-AGCGTCCGTATCTTGAGTCCT-3'). ACAD9 cDNA was amplified with sense primer (5'-TTTCCAGAGGTCAGTCAACATGA-3') and antisense primer (5'-TGGTCAATTTTTCGAGAGTCAC-3'). ACADSB cDNA was amplified with sense primer (5'-CCCAACCTGCTTGTCTCCTTG-3') and antisense primer (5'-ATCCCTGGATCACCGATTTCT-3').

**Cell Culture and Adenoviral Infections**—C<sub>2</sub>C<sub>12</sub> myoblasts were cultured in 10% calf serum containing DMEM. C<sub>2</sub>C<sub>12</sub> myoblasts were differentiated at ~90% confluency by switching the medium to DMEM containing 2% horse serum (Invitrogen) for 72 h. Primary muscle cells were isolated and cultured from 1~2-week-old C57BL/6 mice. To induce differentiation, isolated myoblasts were grown to 80% confluence and then switched to the differentiation medium containing 5% horse serum for 72 h (23). Differentiated C<sub>2</sub>C<sub>12</sub> or primary myotubes were infected with adenovirus expressing FLAG-PGC1 $\alpha$ , FLAG-SIRT1-WT, or FLAG-SIRT1-S434A for 6 h as described previously (26). After 48 h post-infection, the infected myotubes were incubated with DMEM containing 5 mM low glucose and free fatty acid-free BSA-conjugated oleic, palmitic, stearic, or elaidic acid as indicated.

**Rates of Fatty Acid Oxidation**—Fatty acid oxidation assays were performed as described previously (23). Briefly, differentiated myotubes were incubated with BSA-conjugated unlabeled oleic (0.3 mM), palmitic (0.3 mM) acid in 5 mM glucose and 0.5% horse serum containing DMEM. After 6 h, myotubes were incubated with [<sup>14</sup>C]oleic acid (Perkin Elmer Life Sciences) or [<sup>14</sup>C]palmitic acid (Perkin Elmer Life Sciences) for 3 h. <sup>14</sup>C-labeled CO<sub>2</sub> was captured in phenylethylamine-soaked Whatman paper and measured on a scintillation counter.

**Measurement of NAD<sup>+</sup> Levels**—NAD<sup>+</sup> and NADH nucleotides were determined in cell lysates using NAD<sup>+</sup> and NADH determination kit (Bioassay Systems). Briefly, C<sub>2</sub>C<sub>12</sub> myotubes were lysed in 200  $\mu$ l of acid extraction buffer to measure the NAD<sup>+</sup> levels or 200  $\mu$ l of alkali extraction to determine NADH levels. Then, extracts were neutralized, and the concentration of NAD<sup>+</sup> or NADH was measured after an enzymatic cycling reaction using 5  $\mu$ l of sample. NAD<sup>+</sup>/NADH ratios were calcu-

<sup>5</sup> The abbreviations used are: PKA, protein kinase A; AKAR3, protein kinase A activity reporter; CREB, cAMP-response element-binding protein; PGC1 $\alpha$ , Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$ .



**FIGURE 1. Oleic acid, but not other long chain fatty acids, induces PGC1 $\alpha$  deacetylation through SIRT1 in skeletal muscle cells.** *A*, oleic fatty acid deacetylates PGC1 $\alpha$ . C<sub>2</sub>C<sub>12</sub> myotubes were infected with adenoviruses encoding for FLAG-PGC1 $\alpha$ . Two days after infection, cells were treated with the indicated fatty acids at 0.5 mM for 3 h. Acetylated PGC1 $\alpha$  was detected using the acetyllysine antibody after FLAG immunoprecipitation. *B*, oleic acid deacetylates PGC1 $\alpha$  in a concentration-dependent manner. Indicated adenoviruses were used to infect C<sub>2</sub>C<sub>12</sub> myotubes as indicated in *A*. Oleic acid (0.2 and 0.5 mM) or forskolin (FSK; 10  $\mu$ M) were treated for 3 h. *C*, oleic acid deacetylates PGC1 $\alpha$  in a SIRT1-dependent manner. C<sub>2</sub>C<sub>12</sub> myotubes were treated as indicated in *A*, but cells were pretreated with EX-527 (2  $\mu$ M) for 4 h before the addition of oleic acid. DMSO, dimethyl sulfoxide.

lated by comparing the ratios obtained from C<sub>2</sub>C<sub>12</sub> myotube-derived extracts.

**Measurement of cAMP Levels**—Intracellular cAMP levels were measured using a nonacetylation protocol and a cAMP enzyme-linked immunoassay, according to the manufacturer's instructions (GE Healthcare). Briefly, C<sub>2</sub>C<sub>12</sub> myoblasts were cultured and differentiated in 96-well culture plates, and then differentiated myotubes were treated with various fatty acids for 30 min in as indicated. Cells were washed and lysed, and each cAMP concentration was then measured by triplicate. cAMP concentrations were normalized to protein content of each sample by performing BCA assay.

**Sirt1 Enzymatic Activity**—SIRT1 enzymatic activity was measured using a fluorescent assay as described previously (26). Briefly, adenoviruses expressing either FLAG-tagged SIRT1-WT or SIRT1-S434A were used to infect C<sub>2</sub>C<sub>12</sub> myotubes. After 48 h post-infection, cells were incubated with BSA only or BSA-conjugated oleic acid for 30 min. Cells were lysed in a buffer containing 20 mM (pH 7.5), 175 mM NaCl, 0.5% IGEPAL, 0.1% CHAPS, 10 mM sodium fluoride, 10 mM glycerol 2-phosphate, and protease inhibitor mixture. FLAG-tagged SIRT1 was immunoprecipitated using FLAG beads from whole cell extracts from oleic acid-treated myotubes, and then immunoprecipitated SIRT1 was concentrated on a 30-kDa cut-off spin column (Millipore) with buffer exchange to remove excess 3 $\times$ FLAG peptide. The final eluted protein was incubated with two different concentrations (350 and 750  $\mu$ M) of NAD<sup>+</sup> and 70  $\mu$ M fluorescently labeled acetylated p53 peptide for 75 min at 37 °C according to the manufacturer's instructions (Enzo Life Sciences). The reaction was terminated by adding developer solution containing nicotinamide at a final concentration of 2 mM, and then SIRT1 activity was assessed by measuring the fluorescent emission at 460 nm, following excitation at 360 nm.

**PKA Activity Measurement through Fluorescent Resonance Energy Transfer (FRET) Assay**—Mouse skeletal C<sub>2</sub>C<sub>12</sub> cells were transfected with protein kinase A activity reporter (AKAR3) or Nuclear Localization Sequence (NLS)-AKAR3 cDNAs to express cytosolic or nuclear PKA activity reporter, respectively, as described previously (27). 24 h after transfection, cells were used for FRET assay. Briefly, living C<sub>2</sub>C<sub>12</sub> cells were imaged with a CCD camera with Metamorph software (Molecular Devices) on a Zeiss Axiovert 200 M microscope (Carl Zeiss, Thornwood, NY) with a  $\times$ 40/1.3 numerical aperture oil-immersion objective lens. Dual-emission recording of CFP (440/480 nm, excitation/emission) and FRET (440/535

nm, excitation/emission) were coordinated with Lambda 10-3 filter shutter wheels (Sutter Instrument Co., Novato, CA). Exposure time was 100 ms, and recording intervals were 20 s. The FRET/CFP ratio was normalized against the base-line levels.

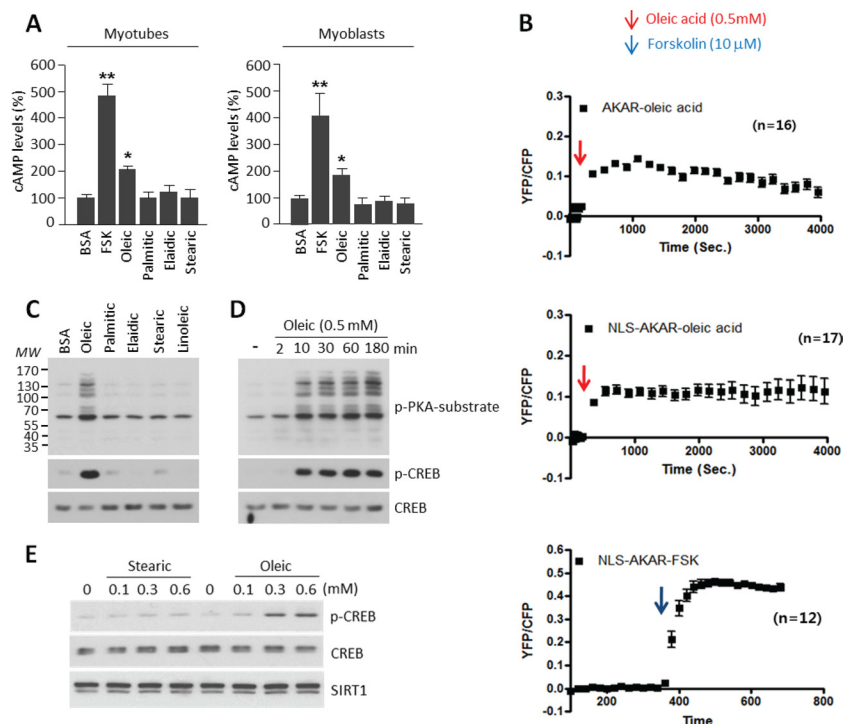
**Animal Studies**—All mouse studies were performed with an approved protocol from the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee. Six-week-old C57BL/6 mice were housed on a 12:12-h light/dark cycle. Oleic acid (1 g/kg body weight, conjugated with BSA, and suspended in phosphate-buffered saline (PBS)), palmitic acid (1 g/kg body weight), or BSA were administered to mice via oral gavage. After 3 or 6 h, mice were sacrificed, skeletal muscle was rapidly harvested, and mRNA was isolated by TRIzol reagent for measurement of gene expression.

**Statistics**—Data are expressed as mean  $\pm$  S.D. Statistical significance was evaluated by using Student's two-tailed *t* test. *p* < 0.05 was considered to be statistically significant.

## RESULTS

**Oleic Acid Induces PGC1 $\alpha$  Deacetylation in a SIRT1-dependent Manner in Skeletal Muscle Cells**—We have previously shown that low concentrations of glucose cause PGC1 $\alpha$  deacetylation and promote fatty acid oxidation in skeletal muscle cells (23). However, whether changes in the concentrations of fatty acids might also affect PGC1 $\alpha$  acetylation, thus establishing an auto-regulatory loop, was unknown. To test this, we treated skeletal muscle cells with different types of fatty acids and measured PGC1 $\alpha$  acetylation levels. Interestingly, among the fatty acids tested only oleic acid, but not other long chain saturated or polyunsaturated fatty acids, was sufficient to robustly induce PGC1 $\alpha$  deacetylation (Fig. 1*A*). In addition, oleic acid-mediated PGC1 $\alpha$  deacetylation occurred in a dose-dependent manner (Fig. 1*B*). Importantly, the concentrations of non-esterified oleic acid (0.2 and 0.5 mM) fall within the range of postprandial levels of this fatty acid in mice (28) and humans (29, 30). Steady-state levels of PGC1 $\alpha$  acetylation are dynamically controlled by endogenous SIRT1 deacetylase activity. Thus, to assess whether oleic acid-induced PGC1 $\alpha$  deacetylation was dependent on SIRT1, we used a specific SIRT1 inhibitor (EX-527). Fig. 1*C* shows that oleic acid-mediated PGC1 $\alpha$  deacetylation was completely blocked by the EX-527 inhibitor. Together, these results indicate that oleic acid, but not other long chain fatty acids, specifically induces PGC1 $\alpha$  deacetylation in skeletal muscle cells through SIRT1.

## Oleic Acid Activates the SIRT1-PGC1 $\alpha$ Complex

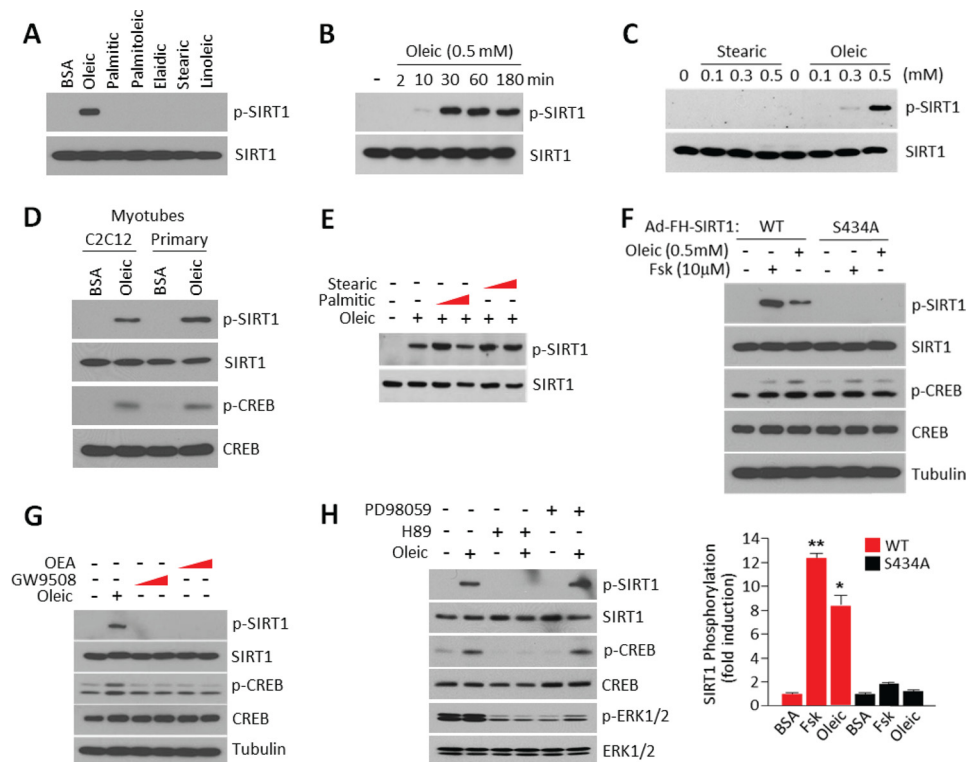


**FIGURE 2. Oleic acid increases intracellular levels of cAMP and PKA phosphorylation activity.** *A*, oleic acid increases cAMP levels. Forskolin (FSK; 10  $\mu$ M) and BSA-conjugated various fatty acids (0.5 mM) were treated for 30 min either in C<sub>2</sub>C<sub>12</sub> myotubes or myoblasts. Values represent mean  $\pm$  S.D. of three independent experiments in duplicate. Statistical analysis was performed using Student's *t* test. \*, *p* < 0.05 and \*\*, *p* < 0.01. *B*, oleic acid induces PKA activity. The PKA biosensors, cytosolic AKAR3 (*n* = 16, upper panel), and nuclear AKAR3 (*n* = 17, middle panel), were expressed in C<sub>2</sub>C<sub>12</sub> cells. Cells were treated with 0.6 mM oleic acid or 10  $\mu$ M forskolin as a positive control (*n* = 12, lower panel) at the indicated time points. Changes in PKA FRET ratio (an indication of PKA activity) were measured. *C*, oleic acid but not other long chain fatty acids induces phosphorylation of PKA substrates. Indicated fatty acids were used at 0.5 mM to treat C<sub>2</sub>C<sub>12</sub> myotubes. Phosphorylation of PKA substrates was detected using p-PKA substrate and p-CREB antibodies. *D*, oleic acid induces phosphorylation of PKA substrates at early time points. Experiments were performed as indicated described in *C*. *E*, concentration-dependent effects of oleic acid and stearic on p-CREB.

**Oleic Acid Increases Intracellular Levels of cAMP and Activates PKA**—We have recently identified that the cAMP/PKA pathway potently induces PGC1 $\alpha$  deacetylation (26). To test whether oleic acid could affect this pathway, we measured intracellular cAMP levels. Fig. 2*A* shows that oleic acid treatment, but not other long chain fatty acids, caused increases in cAMP levels. In addition, we indirectly measured cAMP levels using an established FRET system based on the different cellular localization of a genetically encoded AKAR3 (31). Oleic acid induced the YFP/CFP FRET signal of AKAR or NLS-AKAR reporters (Fig. 2*B*). This signal induction was approximately four times less compared with a forskolin (which directly activates adenylate cyclase) control. In agreement with oleic acid induction of PKA activity, CREB phosphorylation as well as other PKA targets increased after oleic acid treatment using different concentrations and times (Fig. 2, *C–E*). In the same set of experiments, addition of stearic acid or other long chain fatty acids did not affect CREB phosphorylation that is consistent with its lack of effect on PGC1 $\alpha$  acetylation levels (Fig. 2, *C* and *E*). Together, these results indicate that oleic acid treatment in skeletal muscle cells causes increases in cAMP levels and activates the PKA pathway.

**Oleic Acid Induces SIRT1 Phosphorylation at Ser-434**—We have recently shown that activation of PKA induces SIRT1 phosphorylation at Ser-434, a residue located in the catalytic site, increasing its deacetylase activity through changes in *K<sub>m</sub>* and *V* (26). The observations that the oleic acid effects on PGC1 $\alpha$  acetylation were dependent upon SIRT1 deacetylase

activity and that oleic acid activated PKA signaling in skeletal muscle cells prompted us to test whether oleic acid could induce SIRT1 phosphorylation at Ser-434. Fig. 3*A* shows that among the different long chain fatty acids tested, only oleic acid was sufficient to strongly induce SIRT1 phosphorylation using an antibody that recognizes phosphorylated PKA substrates. In addition, the concentrations and times of oleic acid treatment that caused phosphorylation of SIRT1 were in the same range as the ones that promoted PKA activation (Fig. 3, *B* and *C*). Oleic acid induced SIRT1 phosphorylation in C<sub>2</sub>C<sub>12</sub> and primary skeletal muscle myotubes (Fig. 3*D*). Interestingly, treatment of saturated fatty acids such as stearic and palmitic acid at different concentrations did not interfere with the ability of oleic acid to induce SIRT1 phosphorylation (Fig. 3*E*). The effects of oleic acid on SIRT1 phosphorylation were dependent on Ser-434 as indicated by the fact that the SIRT1 mutant (S434A) was not phosphorylated after oleic acid treatment (Fig. 3*F*). To test whether oleic acid induced SIRT1 phosphorylation was mediated through GPR120, a G Protein Coupled Receptor (GPCR) that binds long chain fatty acids such as omega-3, we used specific agonists of this receptor such as GW9508 or oleoylethanolamide. In contrast to oleic acid treatment, these two agonists were unable to induce SIRT1 phosphorylation (Fig. 3*G*), indicating that this receptor is not involved in the specific oleic acid signaling. Finally, to test whether SIRT1 phosphorylation on Ser-434 induced by oleic acid required PKA activity, we used the specific PKA inhibitor H-89 that completely blocked SIRT1 phosphorylation at this residue. As addi-



**FIGURE 3. SIRT1 phosphorylation at Ser-434 is induced by oleic acid treatment.** *A*, oleic acid stimulates SIRT1 phosphorylation.  $C_2C_{12}$  myotubes were infected with adenoviruses encoding FLAG-SIRT1 alleles. Fatty acids were used at 0.5 mM and treated for 3 h. Immunoprecipitated SIRT1 was used to detect phosphorylation using specific phospho-PKA substrate antibodies. *B*, time-dependent induction of SIRT1 phosphorylation by oleic acid. Experiments were performed as described in *A* but with the indicated times of oleic acid treatment. *C*, oleic acid concentration-dependent increases of SIRT1 phosphorylation. Experiments were performed as described in *A*, but different concentrations of oleic acid were used. *D*, oleic acid stimulates SIRT1 phosphorylation in primary skeletal muscle cells and  $C_2C_{12}$  myotubes. Experiments were performed as described in *A*. *E*, saturated fatty acids do not induce SIRT1 phosphorylation. Different concentrations and combinations of fatty acids were used as indicated in the panel. *F*, oleic acid induces SIRT1 phosphorylation at Ser-434.  $C_2C_{12}$  myotubes were infected with adenoviruses encoding FLAG-SIRT1 or FLAG-SIRT1-S434A and treated with oleic acid or forskolin at the indicated concentrations for 3 h. The *bottom panel* illustrates the quantification of SIRT1 phosphorylation. Statistical analysis was performed by Student's *t* test. \*,  $p < 0.05$  and \*\*,  $p < 0.01$ . *G*, GPR120 agonist does not cause SIRT1 phosphorylation.  $C_2C_{12}$  myotubes were treated with oleic acid or GPR120 agonists (oleoylethanolamide (OEA), 20  $\mu$ M or GW9508, 100  $\mu$ M) for 30 min, and phosphorylation of SIRT1 was analyzed. *H*, PKA, but not MAPK, inhibition blocks SIRT1 phosphorylation induced by oleic acid. MAPK inhibitor (PD98059, 20  $\mu$ M) or PKA inhibitor (H89, 10  $\mu$ M) were pretreated for 1 h before oleic acid treatment.

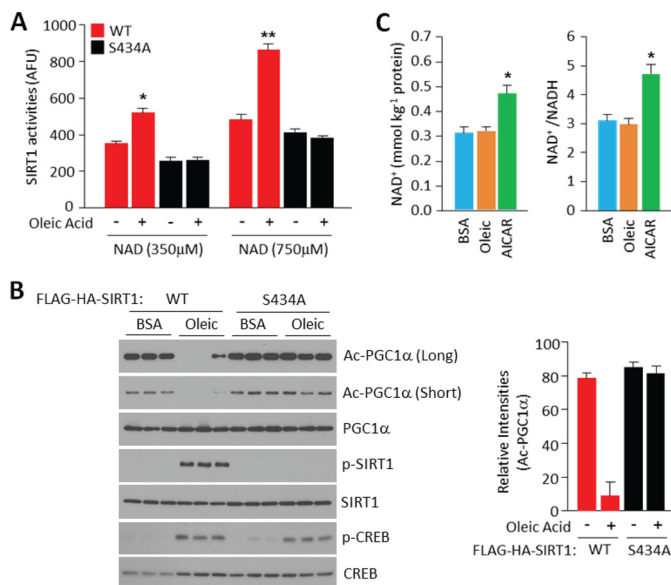
tional control, the Erk1/2 inhibitor PD98059 did not affect SIRT1 phosphorylation (Fig. 3H). The results in this section indicate that oleic acid promotes an increase in the intracellular cAMP pool, which activates PKA. The activation of PKA subsequently results in phosphorylation of SIRT1 at serine residue 434.

**Oleic Acid Promotes SIRT1 Deacetylase Enzymatic Activity**—Next, we assessed whether oleic acid treatment induced SIRT1 deacetylase activity through PKA-dependent phosphorylation at residue 434 using both *in vitro* SIRT1 deacetylase assays as well as measurements of PGC1 $\alpha$  acetylation levels in skeletal muscle cells. In the *in vitro* assays, ectopically expressed SIRT1 was immunoprecipitated from cells treated with oleic acid or vehicle, and the deacetylase activity was measured using a p53-acetylated substrate. Oleic acid specifically induced the deacetylase activity of wild type SIRT1, but not mutant SIRT1 S434A, at two different concentrations of NAD<sup>+</sup> tested (Fig. 4A). In agreement with our previous observations (26), SIRT1 S434A exhibited a slightly lower deacetylase activity, compared with wild type SIRT1, which was sensitive to NAD<sup>+</sup> concentrations but not to oleic acid treatment. In similar sets of experiments, oleic acid specifically induced PGC1 $\alpha$  deacetylation in muscle cells expressing wild type SIRT1, but not mutant SIRT1

S434A (Fig. 4B). Interestingly, consistent with our previous data that cAMP/PKA induces SIRT1 deacetylase activity, independent of changes in NAD<sup>+</sup>, oleic acid treatment in skeletal muscle cells did not affect NAD<sup>+</sup> intracellular concentrations (Fig. 4C). As a positive control, we used AICAR, an AMPK activator known to increase NAD<sup>+</sup> levels (25). These results indicate that oleic acid activates SIRT1 deacetylase through phosphorylation of SIRT1 at Ser-434.

**Oleic Acid Increases the PGC1 $\alpha$  Transcriptional Activity of Genes Linked to Fatty Acid Oxidation *In Vitro* and *In Vivo***—PGC1 $\alpha$  acetylation is a chemical enzymatic modification associated with repression of its ability to induce expression of target genes (32). We and others (23, 25, 26) have previously shown in skeletal muscle cells that SIRT1 activation induced by low concentrations of glucose, AMPK, or cAMP/PKA is sufficient to increase the expression of PGC1 $\alpha$  target genes that are linked to the fatty acid oxidation pathway. Given that oleic acid produced similar effects as these different signals on PGC1 $\alpha$  acetylation and in certain contexts induce PGC1 $\alpha$  mRNA (33), we tested the ability of oleic acid to modulate PGC1 $\alpha$  target gene expression, with particular focus on those that promote fatty acid oxidation. We first treated skeletal muscle cells with oleic, palmitic, and stearic fatty acids. Oleic acid was sufficient to significantly increase a broad

## Oleic Acid Activates the SIRT1-PGC1 $\alpha$ Complex



**FIGURE 4. Oleic acid promotes SIRT1 deacetylase enzymatic activity independently of changes in NAD<sup>+</sup> levels.** *A*, oleic acid treatment increases SIRT1 activity. Immunoprecipitated FLAG-SIRT1 or FLAG-SIRT1 S434A from infected C<sub>2</sub>C<sub>12</sub> myotubes were used to measure SIRT1 activity using the Fluor-de Lys<sup>®</sup> fluorimetric kit that uses acetylated p53 peptides. SIRT1 activity is represented as arbitrary fluorescence units (AFU). Values represent mean  $\pm$  S.D. of one independent experiment in triplicate. *B*, oleic acid induces PGC1 $\alpha$  deacetylation mediated by SIRT1. C<sub>2</sub>C<sub>12</sub> myotubes infected with PGC1 $\alpha$  and SIRT1 alleles were treated with oleic acid. Cellular extracts or immunoprecipitated proteins were used to detect indicated acetylated and phosphoproteins. The *right panel* illustrates the quantification of PGC1 $\alpha$  acetylation. Values represent mean  $\pm$  S.D. of three independent experiments. *C*, oleic acid does not change intracellular NAD<sup>+</sup> levels. C<sub>2</sub>C<sub>12</sub> myotubes treated with BSA, oleic acid (0.5 mM for 3 h), or AICAR (0.5 mM for 8 h) extracts were used to measure NAD<sup>+</sup> and NADH levels. Values were normalized by total protein and represented as mean  $\pm$  S.D. of two independent experiments in triplicate. Statistical analysis was performed by Student's *t* test. \*, *p* < 0.05.

range of genes associated with fatty acid oxidation that included the following: (i) transcriptional regulatory genes ERR $\alpha$  and NOR1; (ii) fatty acid transport genes CD36/FAT; and (iii) mitochondrial  $\beta$ -oxidation genes CPT1 $\beta$ , MCAD, and ACO. In contrast to these effects elicited by oleic acid, neither stearic nor palmitic acid caused a complete change in expression of all these genes (Fig. 5A). In some genes such as CD36 and ACAD5B, palmitic and stearic acids were sufficient to cause an increase, suggesting that these fatty acids might signal independently of cAMP/PKA.

To assess whether oleic acid was able to induce this genetic program of fatty acid oxidation in skeletal muscle in whole animals, we administered oleic acid via oral gavage to mice and quantified changes in expression of these genes. As shown in Fig. 5, *B* and *C*, at 3 and 6 h after oleic but not palmitic acid treatment, genes linked to fatty acid oxidation were significantly increased in skeletal muscle. Consistent with oleic acid-induced PKA activation changes observed in cultured muscle cells, administration of oleic acid in mice increased CREB phosphorylation (Fig. 5D). Together, the results in this section indicate that oleic acid is sufficient to increase PKA activation and PGC1 $\alpha$  target genes in cultures myotubes as well as skeletal muscle.

**Oleic Acid Increases Fatty Acid Oxidation Genes in a SIRT1- and PGC1 $\alpha$ -dependent Manner**—Next, we performed a set of experiments to evaluate whether the oleic acid and PKA nutri-

ent and signaling mechanisms activated fatty acid oxidation gene expression through the SIRT1-PGC1 $\alpha$  transcriptional complex. Inhibition with SIRT1 with EX-527 attenuated the ability of oleic acid to induce fatty acid oxidation gene expression (Fig. 6A). Similarly, oleic acid effects were blocked in cells expressing the non-phosphorylated SIRT1 S434A mutant allele (Fig. 6B). These results indicate that the actions of oleic acid on gene expression linked to fatty acid oxidation are dependent on SIRT1 and, more specifically, on oleic acid-induced phosphorylation of SIRT1 S434.

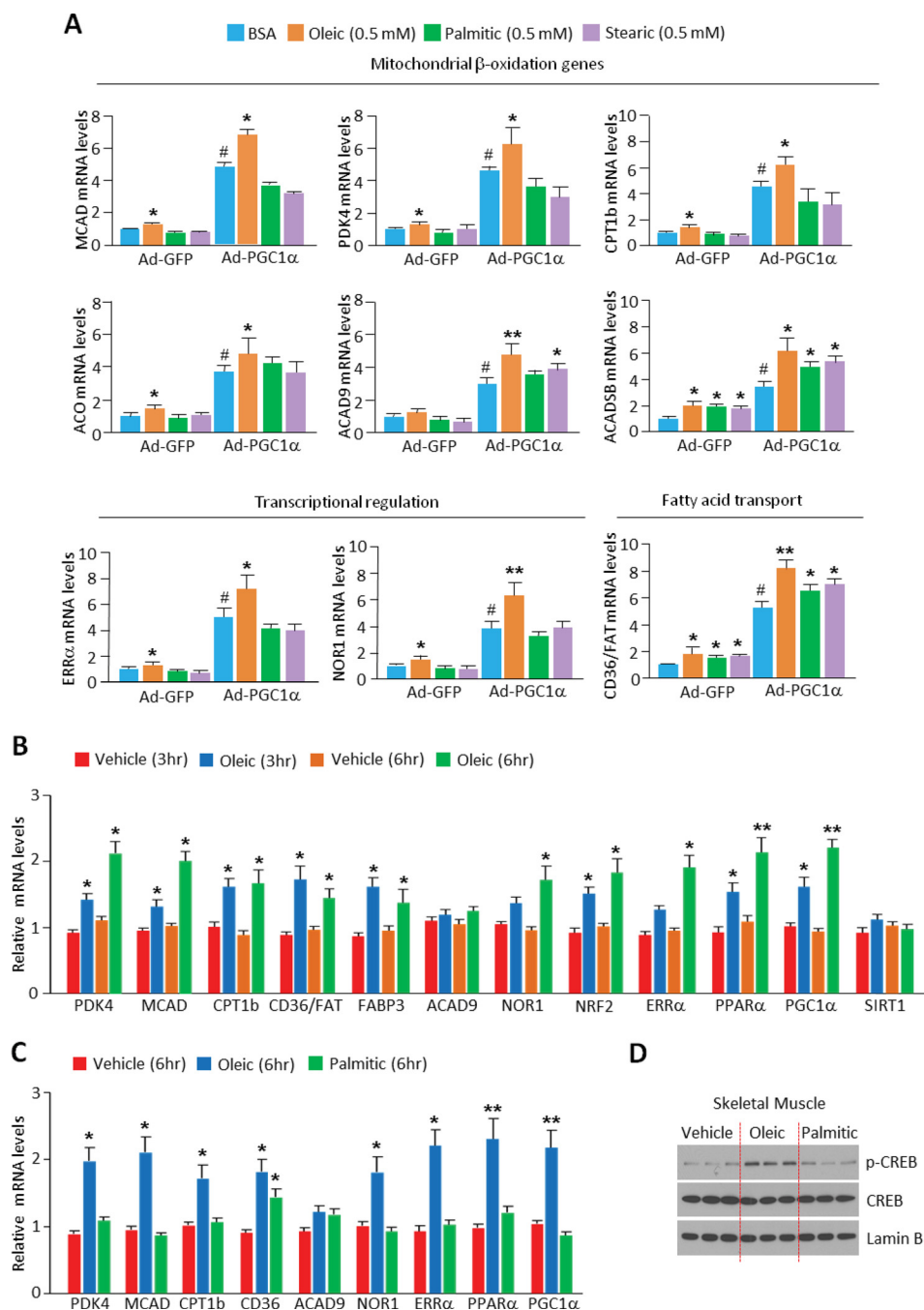
The dependence upon PGC1 $\alpha$  was determined through shRNA-mediated PGC1 $\alpha$  depletion in C<sub>2</sub>C<sub>12</sub> and primary skeletal muscle myotubes. Notably, all the oleic acid increased fatty acid oxidation gene expression was completely absent in differentiated myotubes in which PGC1 $\alpha$  was knocked down (Fig. 6C). In agreement with oleic acid functioning through PKA, many of the same fatty acid oxidation gene targets induced by oleic acid in cultured myotubes were also induced by the PKA agonist forskolin (Fig. 6D). Collectively, these results strongly support that oleic acid signaling through PKA utilizes the transcriptional complex SIRT1-PGC1 $\alpha$  to activate genes associated to fatty acid oxidation.

**Oleic Acid Increases Rates of Fatty Acid Oxidation in a SIRT1-dependent Manner**—Finally, to assess the functional consequences of the oleic acid-mediated changes in fatty acid oxidation gene expression, we measured complete rates of fatty acid oxidation in the presence and absence of oleic fatty acid. Consistent with the increases in fatty acid gene expression induced by oleic acid in skeletal muscle cells (Fig. 5), cellular fatty acid oxidation rates were markedly increased following treatment with oleic acid as measured by radioactive CO<sub>2</sub> derived from radiolabeled [<sup>14</sup>C]palmitate or [<sup>14</sup>C]oleic acid (Fig. 7A). Ectopic expression of PGC1 $\alpha$  synergized with oleic acid treatment to further magnify the increase in fatty acid oxidation. Importantly, these effects were both SIRT1- and PKA-dependent as the inhibitors EX-527 or H-89 and the SIRT1 S434A mutant (Fig. 7B) largely abolished oleic acid-induced lipid utilization. In contrast, palmitic acid did not substantially induce rates of fatty acid oxidation compared with vehicle controls (Fig. 7B). In short, these results indicate that oleic acid induces a signal transduction through the PKA pathway and SIRT1-PGC1 $\alpha$  to accelerate rates of fatty acid oxidation.

## DISCUSSION

In these studies, we have identified a new lipid signaling/transcriptional cellular route by which oleic fatty acid communicates to increase rates of fatty acids and contribute to lipid homeostasis. This biochemical route relies upon the activation of the cAMP/PKA pathway that phosphorylates SIRT1 Ser-434 and increases its deacetylase activity. This activated SIRT1 deacetylates PGC1 $\alpha$  and increases the expression of fatty acid oxidation genes, leading to increases in complete oxidation of fatty acids.

Specific activation of PGC1 $\alpha$  in skeletal muscle through transgenic expression exerts strong beneficial effects and protection against age-associated diseases (34, 35). For example, lipid dysregulation and inflammation linked to aging are

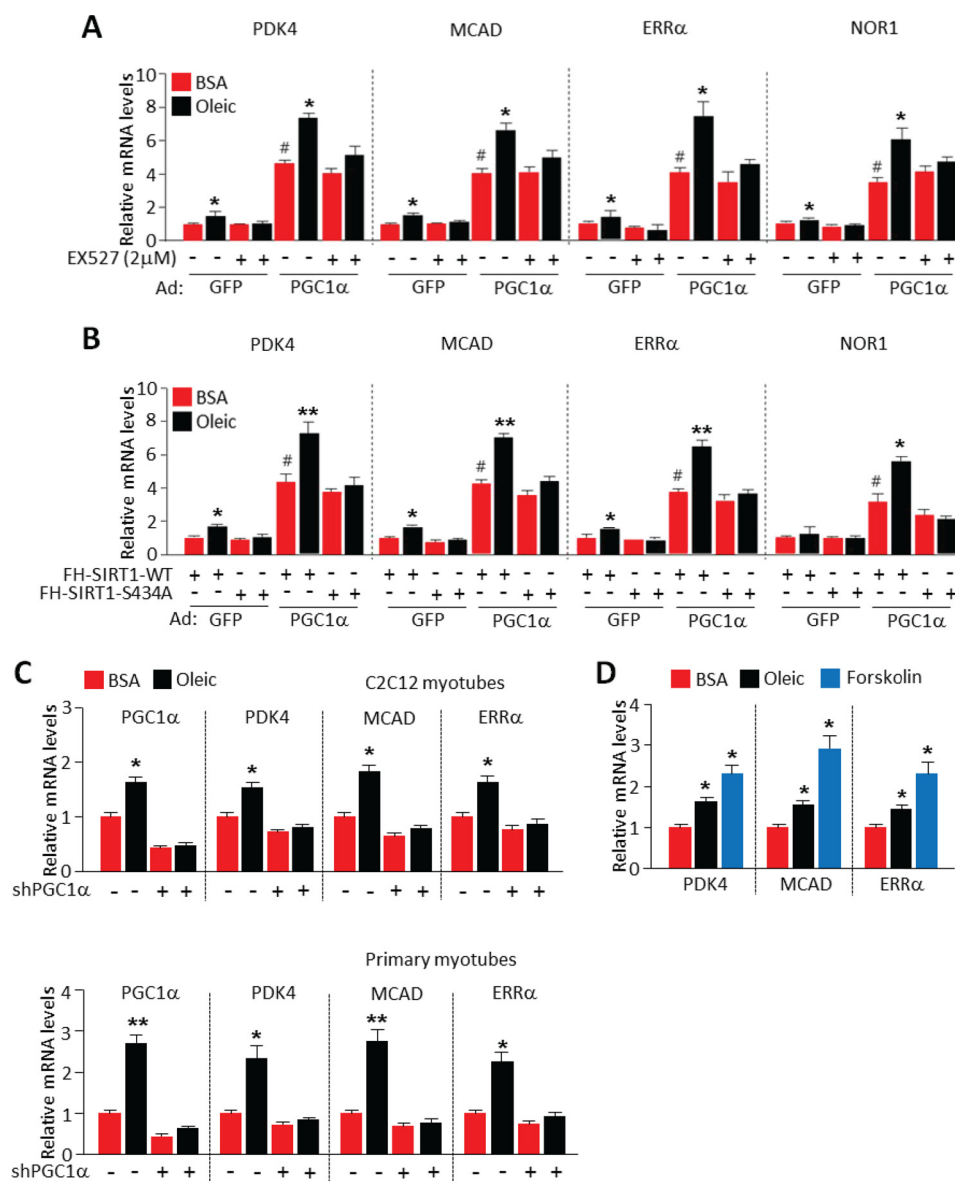


**FIGURE 5. Oleic acid increases expression of genes linked to fatty acid oxidation in cultured muscle cells and skeletal muscle *in vivo*.** A, Oleic acid increases gene expression of the fatty acid oxidation in  $C_2C_{12}$  myotubes. Total RNA extracted from myotubes infected with control or PGC1 $\alpha$  adenoviruses was used to measure the indicated genes using quantitative PCR. Indicated fatty acids were treated at 0.5 mM for 12 h. Values represent mean  $\pm$  S.D. of three independent experiments in triplicate. Statistical analysis was performed using Student's *t* test. \*, *p* < 0.05; \*\*, *p* < 0.01, BSA versus fatty acids treatment; and #, *p* < 0.01, Ad-GFP versus Ad-PGC1 $\alpha$ . B, oleic acid increases fatty acid oxidation gene expression in mouse skeletal muscle. BSA-conjugated oleic or palmitic acid (C) at 1g/kg was administered by oral gavage. Statistical analysis was performed using Student's *t* test (*n* = 4 mice). \*, *p* < 0.05; \*\*, *p* < 0.01, BSA vehicle versus fatty acid treatment. D, oleic acid induces CREB phosphorylation. Skeletal muscle from oleic or palmitic acid treated mice was homogenized and nuclear extracts were used to detect phosphorylation of CREB using specific phospho-CREB antibodies.

strongly attenuated in mice with elevated PGC1 $\alpha$  function in skeletal muscle. These studies suggest that increasing PGC1 $\alpha$  activity could be a valid therapeutic target to ameliorate these metabolic diseases. PGC1 $\alpha$  functions as a scaffold protein that is recruited to specific transcription factors and potently induces expression of genes linked to oxidative metabolism (20, 36). Although not entirely impossible, it might prove difficult to identify small molecules that directly bind to and activate

PGC1 $\alpha$ . Alternatively, it might be more feasible to target pathways that lead to PGC1 $\alpha$  activation. We and others (32, 37, 38) have shown that changes in PGC1 $\alpha$  acetylation are linked to PGC1 $\alpha$  transcriptional function: hyperacetylation through the GCN5 acetyl transferase is inhibitory, whereas deacetylation through the NAD<sup>+</sup>-dependent SIRT1 deacetylase is activation. In fact, increases of SIRT1 activity by different means, such as decreases of glucose concentrations, increases in

## Oleic Acid Activates the SIRT1-PGC1 $\alpha$ Complex



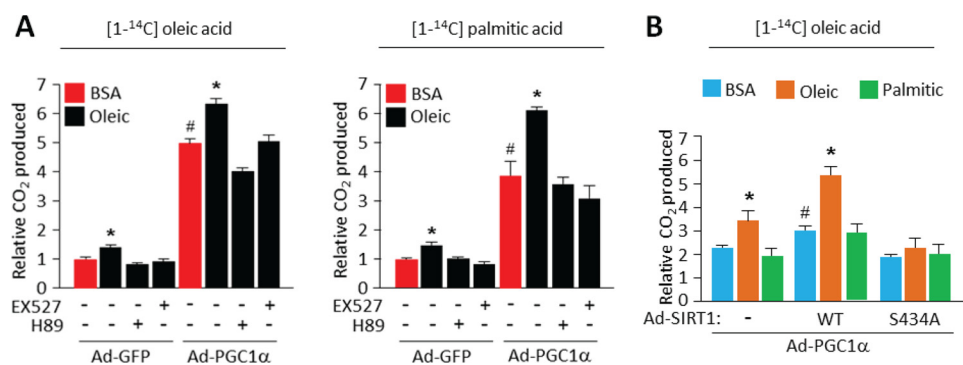
**FIGURE 6. Oleic acid-mediated increases in fatty acid oxidation gene expression depend on SIRT1 and PGC1 $\alpha$  transcriptional complex.** SIRT1 inhibition by EX-527 (A) or SIRT1 S434A mutant (B) blocks the increase of fatty acid oxidation genes induced by oleic acid. Experiments were performed as described in Fig. 5A, EX-527 was treated for 13 h (added 1 h prior to oleic acid addition). Values represent mean  $\pm$  S.D. of three independent experiments in triplicate. Statistical analysis was performed using Student's *t* test. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ , BSA versus oleic acid treatment; and #,  $p < 0.01$ , Ad-GFP versus Ad-PGC1 $\alpha$ . C, PGC1 $\alpha$  is necessary for oleic acid-induced gene expression linked to fatty acid oxidation. C<sub>2</sub>C<sub>12</sub> or primary myotubes were infected with adenoviruses expressing either control shRNA or PGC1 $\alpha$  targeting shRNA for 48 h. After post infection, cells were incubated with 0.5 mM oleic acid for 12 h. Values represent mean  $\pm$  S.D. of three independent experiments in triplicate. Statistical analysis was performed using Student's *t* test. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ , BSA versus oleic acid treatment. D, effect of oleic acid is less than forskolin upon metabolic gene expression. C<sub>2</sub>C<sub>12</sub> myotubes were incubated with oleic acid (0.5 mM) or forskolin (10  $\mu$ M) for 12 h. Values represent mean  $\pm$  S.D. of three independent experiments in triplicate. Statistical analysis was performed using Student's *t* test. \*,  $p < 0.05$ .

AMPK or cAMP/PKA, or activators such as resveratrol, can cause deacetylation and activation of PGC1 $\alpha$  and ultimately promote downstream target functions, including mitochondrial oxidative metabolism (23–26, 39). The study reported here suggests that oleic acid and/or small molecules that target components of this lipid signaling pathway could be used to activate PGC1 $\alpha$  in skeletal muscle. Whereas an obvious possibility is to increase intake of oleic acid, alternative ways will need to be further explored in the future. For example, what cAMP/PKA upstream components are mediating oleic acid cellular effects? It is clear that in the case of polyunsaturated omega-3 fatty acids, direct binding to the GPCR transmem-

brane receptor GPR120 mediates most of the beneficial effects on inflammation and insulin resistance. It is, however, unknown whether oleic acid can bind to similar types of receptors to activate cAMP signaling but, if this was the case, agonism of this pathway in skeletal muscle would be an attractive therapeutic avenue.

Oleic acid, in complete opposition to saturated fatty acids, protects against inflammation and insulin resistance (8–10, 40). We propose here that at least one of the mechanisms by which this monounsaturated  $\omega$ -9 fatty acid might elicit these beneficial effects is, at least in part, through increasing lipid oxidation rates in skeletal muscle. As a result, oleic acid will help to





**FIGURE 7. Oleic acid accelerates rates of complete fatty acid oxidation via a SIRT1-dependent mechanism.** Pharmacological SIRT1 inhibition with EX527 (A) or ectopic expression of a SIRT1 S434A mutant (B) blocks rates of fatty acid oxidation induced by oleic acid. C<sub>2</sub>C<sub>12</sub> myotubes infected with the indicated adenoviruses and treatments were used to measure fatty acid oxidation as described under "Experimental Procedures." Values represent mean  $\pm$  S.D. of two independent experiments performed in triplicate. Statistical analysis was performed using Student's *t* test. \*, *p* < 0.05, BSA versus oleic acid treatment; and #, *p* < 0.01, Ad-GFP versus Ad-PGC1 $\alpha$  (A) or Ad-GFP versus Ad-SIRT1-WT (B).

increase clearance of excess of saturated fatty acids counteracting inflammation and insulin resistance. In addition, it is conceivable that increased rates of oxidation in skeletal muscle caused by oleic acid might also participate to boost energy expenditure and balance body weight in overnutrition states. These results might also have important therapeutic dietary implications in obesity management as indicated previously. It is, however, plausible that in addition to the effects on mitochondrial lipid oxidation other unknown cellular mechanisms could also contribute to the biological actions driven by oleic acid.

Why does there exist a big divergence in the metabolic/energetic effects of different types of fatty acids? Although at this point this is mainly speculative, it might be that a mixed diet with different type of fatty acids (saturated and unsaturated) has evolutionary advantages due to an adequate balance between energy storage and expenditure. In the same vein, unsaturated fatty acids, including oleic acid, could act as fatty acid sensors to maintain cellular and systemic levels of these lipids. Thus, oleic acid may function as a signal to trigger a negative feedback loop to deal with an excess of fatty acids and maintain lipid homeostasis. The metabolic advantage and beneficial effects triggered by oleic acid in skeletal muscle cells signaling to SIRT1-PGC1 $\alpha$  complex and increasing levels of lipid oxidation could eventually be pharmacologically exploited. Future identification of new components in this pathway will provide therapeutic possibilities to treat diseases associated with lipid dysregulation.

*Acknowledgments*—We thank all members of the Puigserver laboratory for important discussions about this project.

## REFERENCES

- Dowhan, W. (1997) Molecular basis for membrane phospholipid diversity: why are there so many lipids? *Annu. Rev. Biochem.* **66**, 199–232
- Nohturfft, A., and Zhang, S. C. (2009) Coordination of lipid metabolism in membrane biogenesis. *Annu. Rev. Cell Dev. Biol.* **25**, 539–566
- Walther, T. C., and Farese, R. V., Jr. (2012) Lipid droplets and cellular lipid metabolism. *Annu. Rev. Biochem.* **81**, 687–714
- Postic, C., and Girard, J. (2008) The role of the lipogenic pathway in the development of hepatic steatosis. *Diabetes Metab.* **34**, 643–648
- Van den Branden, C., and Vamecq, J. (2003) Metabolic regulation of peroxisomal and mitochondrial fatty acid oxidation. *Adv. Exp. Med. Biol.* **544**, 307–314
- Cahill, G. F., Jr. (2006) Fuel metabolism in starvation. *Annu. Rev. Nutr.* **26**, 1–22
- Oh, D. Y., Talukdar, S., Bae, E. J., Imamura, T., Morinaga, H., Fan, W., Li, P., Lu, W. J., Watkins, S. M., and Olefsky, J. M. (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* **142**, 687–698
- Tardif, N., Salles, J., Landrier, J. F., Mothe-Satney, I., Guillet, C., Boue-Vaysse, C., Combaret, L., Giraudet, C., Patrac, V., Bertrand-Michel, J., Migné, C., Chardigny, J. M., Boirie, Y., and Walrand, S. (2011) Oleate-enriched diet improves insulin sensitivity and restores muscle protein synthesis in old rats. *Clin. Nutr.* **30**, 799–806
- Coll, T., Eyre, E., Rodríguez-Calvo, R., Palomer, X., Sánchez, R. M., Merlos, M., Laguna, J. C., and Vázquez-Carrera, M. (2008) Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells. *J. Biol. Chem.* **283**, 11107–11116
- Kien, C. L. (2009) Dietary interventions for metabolic syndrome: role of modifying dietary fats. *Curr. Diab. Rep.* **9**, 43–50
- Rasmussen, B. B., and Wolfe, R. R. (1999) Regulation of fatty acid oxidation in skeletal muscle. *Annu. Rev. Nutr.* **19**, 463–484
- Schreurs, M., Kuipers, F., and van der Leij, F. R. (2010) Regulatory enzymes of mitochondrial beta-oxidation as targets for treatment of the metabolic syndrome. *Obes Rev.* **11**, 380–388
- Foster, D. W. (2012) Malonyl-CoA: the regulator of fatty acid synthesis and oxidation. *J. Clin. Invest.* **122**, 1958–1959
- Sladek, R., Bader, J. A., and Giguère, V. (1997) The orphan nuclear receptor estrogen-related receptor  $\alpha$  is a transcriptional regulator of the human medium-chain acyl coenzyme A dehydrogenase gene. *Mol. Cell. Biol.* **17**, 5400–5409
- Pyper, S. R., Viswakarma, N., Yu, S., and Reddy, J. K. (2010) PPAR $\alpha$ : energy combustion, hypolipidemia, inflammation and cancer. *Nucl. Recept. Signal.* **8**, e002
- Narkar, V. A., Downes, M., Yu, R. T., Emblar, E., Wang, Y. X., Banayo, E., Mihaylova, M. M., Nelson, M. C., Zou, Y., Juguilon, H., Kang, H., Shaw, R. J., and Evans, R. M. (2008) AMPK and PPAR $\delta$  agonists are exercise mimetics. *Cell* **134**, 405–415
- Puigserver, P., and Spiegelman, B. M. (2003) Peroxisome proliferator-activated receptor- $\gamma$  coactivator 1  $\alpha$  (PGC-1  $\alpha$ ): transcriptional coactivator and metabolic regulator. *Endocr. Rev.* **24**, 78–90
- Kelly, D. P., and Scarpulla, R. C. (2004) Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev.* **18**, 357–368
- Hock, M. B., and Kralli, A. (2009) Transcriptional control of mitochondrial biogenesis and function. *Annu. Rev. Physiol.* **71**, 177–203
- Scarpulla, R. C., Vega, R. B., and Kelly, D. P. (2012) Transcriptional integration of mitochondrial biogenesis. *Trends Endocrinol. Metab.* **23**, 459–466

## Oleic Acid Activates the SIRT1-PGC1 $\alpha$ Complex

- Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M., and Puigserver, P. (2005) Nutrient control of glucose homeostasis through a complex of PGC-1 $\alpha$  and SIRT1. *Nature* **434**, 113–118
- Lerin, C., Rodgers, J. T., Kalume, D. E., Kim, S. H., Pandey, A., and Puigserver, P. (2006) GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1 $\alpha$ . *Cell Metab.* **3**, 429–438
- Gerhart-Hines, Z., Rodgers, J. T., Bare, O., Lerin, C., Kim, S. H., Mostoslavsky, R., Alt, F. W., Wu, Z., and Puigserver, P. (2007) Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1 $\alpha$ . *EMBO J.* **26**, 1913–1923
- Fulco, M., Schiltz, R. L., Iezzi, S., King, M. T., Zhao, P., Kashiwaya, Y., Hoffman, E., Veech, R. L., and Sartorelli, V. (2003) Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. *Mol. Cell* **12**, 51–62
- Cantó, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., Elliott, P. J., Puigserver, P., and Auwerx, J. (2009) AMPK regulates energy expenditure by modulating NAD<sup>+</sup> metabolism and SIRT1 activity. *Nature* **458**, 1056–1060
- Gerhart-Hines, Z., Dominy, J. E., Jr., Blättler, S. M., Jedrychowski, M. P., Banks, A. S., Lim, J. H., Chim, H., Gygi, S. P., and Puigserver, P. (2011) The cAMP/PKA pathway rapidly activates SIRT1 to promote fatty acid oxidation independently of changes in NAD(+). *Mol. Cell* **44**, 851–863
- De Arcangelis, V., Liu, S., Zhang, D., Soto, D., and Xiang, Y. K. (2010) Equilibrium between adenylyl cyclase and phosphodiesterase patterns adrenergic agonist dose-dependent spatiotemporal cAMP/protein kinase A activities in cardiomyocytes. *Mol. Pharmacol.* **78**, 340–349
- Gonçalves de Albuquerque, C. F., Burth, P., Younes Ibrahim, M., Garcia, D. G., Bozza, P. T., Castro Faria Neto, H. C., and Castro Faria, M. V. (2012) Reduced plasma nonesterified fatty acid levels and the advent of an acute lung injury in mice after intravenous or enteral oleic acid administration. *Mediators Inflamm.* 601032
- Raatz, S. K., Bibus, D., Thomas, W., and Kris-Etherton, P. (2001) Total fat intake modifies plasma fatty acid composition in humans. *J. Nutr.* **131**, 231–234
- Tholstrup, T., Sandström, B., Bysted, A., and Hølmer, G. (2001) Effect of 6 dietary fatty acids on the postprandial lipid profile, plasma fatty acids, lipoprotein lipase, and cholesterol ester transfer activities in healthy young men. *Am. J. Clin. Nutr.* **73**, 198–208
- Allen, M. D., and Zhang, J. (2006) Subcellular dynamics of protein kinase A activity visualized by FRET-based reporters. *Biochem. Biophys. Res. Commun.* **348**, 716–721
- Dominy, J. E., Gerhart-Hines, Z., and Puigserver, P. (2011) Nutrient-dependent acetylation controls basic regulatory metabolic switches and cellular reprogramming. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 203–209
- Zhang, P., Liu, C., Zhang, C., Zhang, Y., Shen, P., Zhang, J., and Zhang, C. Y. (2005) Free fatty acids increase PGC-1 $\alpha$  expression in isolated rat islets. *FEBS Lett.* **579**, 1446–1452
- Wenz, T., Rossi, S. G., Rotundo, R. L., Spiegelman, B. M., and Moraes, C. T. (2009) Increased muscle PGC-1 $\alpha$  expression protects from sarcopenia and metabolic disease during aging. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 20405–20410
- Boström, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., Rasbach, K. A., Boström, E. A., Choi, J. H., Long, J. Z., Kajimura, S., Zingaretti, M. C., Vind, B. F., Tu, H., Cinti, S., Höjlund, K., Gygi, S. P., and Spiegelman, B. M. (2012) A PGC1- $\alpha$ -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* **481**, 463–468
- Lin, J., Handschin, C., and Spiegelman, B. M. (2005) Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab.* **1**, 361–370
- Nemoto, S., Fergusson, M. M., and Finkel, T. (2005) SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1 $\alpha$ . *J. Biol. Chem.* **280**, 16456–16460
- Cantó, C., and Auwerx, J. (2009) PGC-1 $\alpha$ , SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr. Opin. Lipidol.* **20**, 98–105
- Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de Cabo, R., and Sinclair, D. A. (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337–342
- Gao, D., Griffiths, H. R., and Bailey, C. J. (2009) Oleate protects against palmitate-induced insulin resistance in L6 myotubes. *Br. J. Nutr.* **102**, 1557–1563