Peroxisome Proliferator–Activated Receptor-γ Coactivator-1α Overexpression Increases Lipid Oxidation in Myocytes From Extremely Obese Individuals

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OBJECTIVE—To determine whether the obesity-related decrement in fatty acid oxidation (FAO) in primary human skeletal muscle cells (HSkMC) is linked with lower mitochondrial content and whether this deficit could be corrected via overexpression of peroxisome proliferator–activated receptor-γ coactivator-1α (PGC-1α).

RESEARCH DESIGN AND METHODS—FAO was studied in HSkMC from lean (BMI 22.4 ± 0.9 kg/m^2; $N = 12$) and extremely obese (45.3 ± 1.4 kg/m^2; $N = 9$) subjects. Recombinant adenovirus was used to increase HSkMC PGC-1α expression (3.5- and 8.0-fold), followed by assessment of mitochondrial content (mtDNA and cytochrome C oxidase IV [COXIV]), complete ($^{14}CO_2$ production from labeled oleate), and incomplete (acid soluble metabolites [ASM]) FAO, and glycerolipid synthesis.

RESULTS—Obesity was associated with a 30% decrease ($P < 0.05$) in complete FAO, which was accompanied by higher relative rates of incomplete FAO ([^{14}C]ASM production/$^{14}CO_2$), increased partitioning of fatty acid toward storage, and lower ($P < 0.05$) mtDNA (−27%), COXIV (−35%), and mitochondrial transcription factor (mtTFA) (−43%) protein levels. PGC-1α overexpression increased ($P < 0.05$) FAO, mtDNA, COXIV, mtTFA, and fatty acid incorporation into triacylglycerol in both lean and obese groups. Perturbations in FAO, triacylglycerol synthesis, mtDNA, COXIV, and mtTFA in obese compared with lean HSkMC persisted despite PGC-1α overexpression. When adjusted for mtDNA and COXIV content, FAO was equivalent between lean and obese groups.

CONCLUSION—Reduced mitochondrial content is related to impaired FAO in HSkMC derived from obese individuals. Increasing PGC-1α protein levels did not correct the obesity-related absolute reduction in FAO or mtDNA content, implicating mechanisms other than PGC-1α abundance. ***Diabetes*** **59:1407–1415, 2010**

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The skeletal muscle of obese individuals typically exhibits an inability to effectively oxidize lipid. Using arteriovenous difference measurements across a skeletal muscle bed, Kelly et al. (1) observed a significant reduction of in vivo fatty acid oxidation (FAO) in obese versus lean subjects. Our laboratory has reported a consistent reduction in FAO in skeletal muscle from individuals with extreme or class III (BMI ≥40 kg/m^2) obesity in a variety of preparations such as muscle homogenates from the vastus lateralis (2), intact muscle strips from the rectus abdominus (3), and in vivo when examining substrate utilization (indirect calorimetry) during exercise (4) or when determining the fate of infused lipid (5). While the specific mechanism(s) responsible for the impairment remains unknown, it has been hypothesized that decreased mitochondrial content (6) or function (7) contributes to this obesity-related phenotype. A reduction in skeletal muscle mitochondrial DNA (mtDNA) (7), altered mitochondria morphology (8), and decrements in mitochondrial enzyme activity (2,9) have all been associated with obesity/diabetes and may contribute to the decreased capacity for FAO.

The depression of FAO in skeletal muscle with obesity is of concern as this defect may contribute to lipid accumulation within the myocyte and the onset of insulin resistance (3,10–12); a reduced capacity for lipid oxidation is also associated with weight gain (2). In terms of intervention, weight loss does not appear to reverse the obesity-associated reduction in skeletal muscle FAO (5,13). In contrast, we recently reported that only 10 days of exercise training (60 min/day) increased FAO in the skeletal muscle of previously extremely obese subjects; a novel finding was that physical activity overcame the initial decrement in FAO with obesity and elevated FAO to an equivalent absolute value in both lean and obese individuals (13). These data suggest that contractile activity, through a yet undefined mechanism is an effective intervention for the decrement in FAO reported with obesity.

Peroxisome proliferator–activated receptor-γ coactivator-1α (PGC-1α) is a metabolic coactivator that binds to transcription factors stimulating mitochondrial biogenesis (14) and lipid oxidation (15). PGC-1α has also been shown to be upregulated in response to exercise training (13,16,17), making it an attractive candidate for explaining improvements in FAO with physical activity in obese individuals (13) or as a target for the development of antiobesity or antidiabetic drugs. The objectives of the present study were as follows: *1*) to determine whether obesity-related impairments in FAO were associated with a reduction in myocyte mitochondrial content and *2*) to

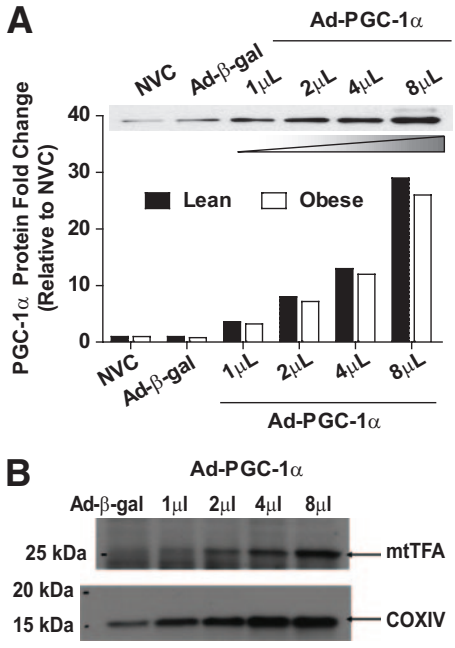

FIG. 1. Ad-PGC-1α overexpression in cultured myotubes (HSkMC) from lean and obese donors. *A*: PGC-1α protein content in no-virus controls (NVC), Ad-β-gal controls, and Ad-PGC-1α–treated HSkMC. PGC-1α protein content increased dose dependently in HSkMC from lean and obese donors. *B*: mtTFA and COXIV protein content increased dose dependently with increasing PGC-1α viral titer in HSkMC.

determine whether increasing the expression of PGC-1α in the myocytes of obese individuals could normalize absolute rates of FAO in a manner similar to that associated with exercise training (13). We have previously reported that primary human skeletal muscle cell cultures (HSkMC) display the phenotype of skeletal muscle from obese individuals with respect to a reduction in FAO (11); we thus utilized this model to examine mechanisms controlling FAO and to specifically manipulate PGC-1α.

RESEARCH DESIGN AND METHODS

Muscle biopsies (~50–100 mg) were obtained from the vastus lateralis of lean (BMI 22.4 ± 0.9 kg/m^2; N = 12) and extremely obese (BMI 45.3 ± 1.4 kg/m^2; N = 9) women with the percutaneous needle biopsy technique. Satellite cells were isolated and cultured into myoblasts as previously described (18,19). After reaching ~70% confluency, cells were subcultured to examine the recombinant adenoviral overexpression of PGC-1α on FAO, markers of mitochondrial content, and lipid accumulation as described below. All procedures were approved by the East Carolina University Institutional Review Board.

Recombinant adenovirus. Recombinant adenoviruses encoding mouse PGC-1α (Ad-PGC-1α) or β-galactosidase (Ad-β-gal) were constructed, amplified, and purified as described previously (17). Ad-β-gal was used to control for nonspecific effects of virus treatment.

Overexpression of PGC-1α in HSkMC. Myoblasts were subcultured onto 6- and 24-well type I collagen-coated plates at densities of 80 and 20 × 10^3 cells per well, respectively. Upon reaching 70–80% confluence, differentiation to myotubes was induced by switching the growth media to differentiation media (Dulbecco's Modified Eeagle's Medium supplemented with 2% horse serum, 0.5 mg/ml BSA, 0.5 mg/ml fetuin, and 50 μg/ml gentamicin/amphotericin B). On day five, myotubes were given fresh differentiation media (no-virus control) or transfected with either Ad-PGC-1α or Ad-β-gal (control virus). To determine the appropriate adenoviral titer to use for metabolic experiments, we initially performed a protein (Fig. 1) and mRNA (not shown) dose-response curve.

For all subsequent experiments, a "low" Ad-PGC-1α dose (5 × 10^9 plaque-forming units/ml) was used to mimic the effects of endurance exercise (~3.5-fold increase in PGC-1α protein over controls) (20) and a "high" Ad-PGC-1α dose (1 × 10^{10} plaque-forming units/ml) used to represent a supraphysiological increase in PGC-1α (approximately eightfold increase in PGC-1α protein over control). Twenty-four hours after transfection, the medium was removed and replaced with fresh differentiation media. Myotubes were harvested for respective experiments on day eight based on previous research (19). There were no obvious differences in the extent of myotube differentiation between lean and obese HSkMC.

Determination of FAO and lipid esterification. On day eight of differentiation, myotubes were incubated at 37°C in sealed 24-well plates containing differentiation media, 12.5 mmol/l HEPES, 0.5% BSA, 1 mmol/l carnitine, either 100 μmol/l or 500 μmol/l sodium oleate (Sigma-Aldrich, St. Louis, MO), and 1 μCi/ml [^{14}C] oleate (PerkinElmer, MA) for 3 h. Following incubation, medium was assayed for $^{14}CO_2$ (measure of complete oxidation), and radiolabeled acid soluble metabolites (ASM) (measure of incomplete oxidation) as previously described (19). Cells were washed twice with PBS, harvested in 600 μl of 0.05% SDS lysis buffer, and stored at −80°C for subsequent determination of protein concentration and lipid esterfication.

For determination of lipid esterfication, 500 μl cell lysate was added to 1:2 chloroform:methanol (vol/vol). After vortexing, 625 μl chloroform was added, followed by the addition of 625 μl deionized H_2O. Samples were vortexed and centrifuged at 1,000 RPM for 5 min at room temperature. The chloroform phase (containing total lipids extracted) was transferred to a clean glass tube and evaporated under a stream of 100% N_2. Samples were resuspended in 500 μl of 2:1 chloroform:methanol. For the quantification of total lipids, 50 μl of the sample was added to scintillation fluid for counting. For determination of specific lipid fractions, 50 μl of each sample was spotted onto oven-dried silica plates (Silica Gel GF; Analtech, Newark, DE), and placed in a sealed tank containing solvent (60:40:3 heptane:isopropyl ether:acetic acid) for 45 min. Plates were air-dried and scanned for the visualization of the bands representing triacylglycerol (TAG), diacylglycerol (DAG), and phospholipid (PL).

DNA isolation and mtDNA quantification. Cells were washed twice with PBS and trypsinized with trypsin-EDTA (0.05% trypsin and 0.02% EDTA). Total DNA (mitochondrial and nuclear) was extracted from cells using a QIAamp DNA minikit (Qiagen, Valencia, CA) and total DNA quantified using the PicoGreen DNA quantification kit (Molecular Probes, Eugene,OR). mtDNA content was measured as relative copy number of mtDNA per diploid nuclear genome using real-time PCR. As recommended by Miller et al. (21), detection of a 69-bp fragment of mtDNA (nucleotides 14918–14986) and a 77-bp fragment of β-globin were used as markers of mtDNA and nuclear DNA, respectively. Primer and probe sets were purchased from Applied Biosystems (Foster City, CA) using sequences previously reported by Menshikova et al. (22). Real-time PCR was conducted using an ABI Prism 7900HT sequence detection system under conditions previously described (22). The threshold cycle number (Ct) was calculated using SDS software (version 2; Applied Biosystems). mtDNA was expressed as a relative copy number (Rc) by expressing Ct differences between β-globin and mtDNA as previously described (23,24) and based on the following calculations: $Rc = 2^{\Delta Ct}$ and $\Delta Ct = Ct_{\beta\text{-globin}} - Ct_{mtDNA}$.

Western blot Analyses. Cells were washed twice with ice-cold PBS and harvested in 150 μl lysis buffer (50 mmol/l HEPES [pH 7.4], 1% Triton X-100, 10 mmol/l EDTA, 100 mmol/l NaFl, and 12 mmol/l Na pyrophosphate) supplemented with protease and phosphatate inhibitors (Sigma-Aldrich). Samples were sonicated and centrifuged at 20,000*g* for 20 min at 4°C. Protein concentrations were determined from cell extracts using the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Thirty micrograms of cellular protein were separated by SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA) and probed overnight for either PGC-1α (1:500; Cell Signaling, Beverly, MA), mitochondrial transcription factor (mtTFA) (1:500; Santa Cruz Biotechnologies, Santa Cruz, CA), or cytochrome C oxidase (COX)IV (1:8,000; Cell Signaling). Samples were normalized to a control sample on each gel.

Statistics. Comparisons between HSkMC from lean and obese donors were performed with repeated-measures ANOVA. Significant main effects and interactions were further analyzed using contrast-contrast tests when appropriate. Statistical significance was defined as a P value <0.05, and data are presented as means ± SE.

RESULTS

PGC-1α overexpression. Findings from the dose-response experiment for Ad-PGC-1α are presented in Fig. 1. Treatment of myotubes with Ad-β-gal (control virus) had no effect on PGC-1α protein content compared with that on no-virus controls (Fig. 1*A*). In HSkMC derived from lean and obese individuals, PGC-1α protein (Fig. 1*A*) and mRNA (data not shown) increased in a dose-dependent manner with increasing PGC-1α adenovirus titer; there were no differences between PGC-1α protein content in

HSkMC from lean and obese individuals with or without overexpression. Based on the findings presented in Fig. 1*A*, for subsequent experiments we selected the 1-ul dose (5×10^9 plaque-forming units/ml) as PGC-1α protein content was increased by a magnitude similar to that reported with endurance-oriented exercise training (~3.5-fold increase in PGC-1α protein over control) (20) and the 2-μl dose (1×10^{10} plaque-forming units/ml) to represent a supraphysiological increment (approximately eightfold increase in PGC-1α protein over controls) in protein content. These doses are referred to as low and high Ad-PGC-1α, respectively. Protein levels of mtTFA and COXIV, indicators of mitochondrial biogenesis, also increased in a dose-dependent manner with PGC-1α adenovirus (Fig. 1*B*).

FAO. To evaluate the role of PGC-1α in regulating skeletal muscle lipid oxidation, we treated myotubes from lean and obese donors with either low or high Ad-PGC-1α for 24 h, followed by 3 h incubation with either 100 or 500 μmol/l oleate. In all experiments, FAO from the no-virus controls did not differ from that in the Ad-β-gal controls (data not shown). Data obtained from the experiments determining FAO are presented in Fig. 2. Under control conditions (low and high β-gal), complete FAO ($^{14}CO_2$ production) was consistently depressed by ~30% in HSkMC from obese individuals with either the 100 or 500 μmol/l oleate incubation (Fig. 2*A* and *B*). As presented in Fig. 2*A*, overexpression of PGC-1α by ~3.5-fold (low dose) resulted in an ~30% increase in complete FAO (100 μmol/l oleate) in HSkMC derived from lean and obese donors. The eightfold increase in PGC-1α protein (high dose) also increased complete FAO in cells from both lean (64%) and obese (70%) donors at the 100 μmol/l oleate concentration ($P < 0.05$) (Fig. 2*A*). Although PGC-1α overexpression increased FAO regardless of the category of the donor, absolute values for complete FAO remained depressed in HSkMC derived from obese compared with those in lean subjects under all conditions (Fig. 2*A*). Similar findings were obtained in response to the higher oleate concentration (500 μmol/l) with respect to differences between cell types and the effects of PGC-1α overexpression (Fig. 2*B*). Total FAO, determined as the sum of incomplete ([^{14}C]ASM) and complete ([$^{14}CO_2$]) oxidation did not differ between cell type under any conditions (Fig. 2*C* and *D*), and PGC-1α overexpression increased total oxidation in a dose-dependent manner. Total and complete FAO were significantly higher at 500 μmol/l oleate compared with 100 μmol/l oleate in control and PGC-1α–treated cells ($P < 0.05$) (Fig. 2*A*–*D*).

A ratio of incomplete ([^{14}C]ASM) to complete ($^{14}CO_2$) oleate oxidation was calculated as an index of FAO efficiency (14). In control cells treated with 100 μmol/l [^{14}C]oleate, radiolabel incorporation into ASM relative to CO_2 was approximately twofold higher in myotubes from obese compared with lean individuals ($P < 0.05$) (Fig. 2*E*). The high relative rate of incomplete oxidation in the obese compared with the lean group was retained regardless of the level of PGC-1α overexpression or oleate concentration (Fig. 2*E* and *F*). In cells from lean individuals, PGC-1α overexpression increased the ASM-to-CO_2 ratio by ~55% ($P < 0.05$); PGC-1α overexpression in cells from obese subjects had no effect on this ratio (Fig. 2*E* and *F*). The ASM/CO_2 ratio was significantly higher at 500 μmol/l oleate compared with 100 μmol/l oleate in control and PGC-1α–treated cells ($P < 0.05$) (Fig. 2*E* and *F*).

Intramyocellular lipid content. Oleate incorporation into glycerolipid, TAG, and DAG pools are presented in Fig. 3. Control cells (β-gal) from obese individuals had a greater rate of oleate incorporation into the glycerolipid pool in response to low (~64%) and high (~42%) oleate concentrations (Fig. 3*A* and *B*). Cells from obese individuals, regardless of treatment, consistently incorporated more lipids into the glycerolipid pool compared with HSkMC from lean subjects (Fig. 3*A* and *B*). PGC-1α overexpression increased esterification into the glycerolipid pool at both concentrations of oleate in both groups of subjects.

Incorporation of lipid into TAG and DAG are presented in Fig. 3*C*–*F*. Under control conditions, cells from obese subjects had increased oleate incorporation into TAG ($P < 0.05$) (Fig. 3*C* and *D*) when exposed to low and high oleate conditions. Lipid incorporation into DAG was higher ($P < 0.05$) (Fig. 3*E*) in obese cells compared with lean cells when exposed to low oleate conditions. Oleate incorporation into PL did not differ between lean and obese control cells regardless of oleate concentration (data not shown; $P > 0.05$). Compared with the control condition (β-gal), PGC-1α overexpression increased oleate incorporation into TAG ($P < 0.05$) (Fig. 3*C* and *D*) and PL (data not shown; $P < 0.05$) without affecting DAG. This was evident in HSkMC from both lean and obese subjects (Fig. 3*C*–*F*). PGC-1α overexpression did not normalize intramyocellular lipid incorporation into storage in HSkMC from obese subjects to that of the lean individuals under the same treatment (Fig. 3*C* and *E*). The rate of oleate incorporation into glycerolipids (specifically, TAG and DAG) was higher when cells were exposed to the higher oleate concentration ($P < 0.05$) (Fig. 3*A*–*F*).

Lipid partitioning. Lipid partitioning was estimated by determining the rate of lipid esterification relative to complete FAO; a higher ratio is indicative of increased partitioning toward storage. Myotubes from obese donors had a higher partitioning index compared with HSkMC from lean donors ($P < 0.05$) (Fig. 4*A* and *B*) regardless of the oleate concentration. Overexpression of PGC-1α at the low dose did not alter this ratio from the control condition, whereas high-dose PGC-1α overexpression (approximately eightfold increase in PGC-1α protein) decreased this ratio under low oleate conditions ($P < 0.05$) (Fig. 4*A*).

Indexes of mitochondrial content. mtDNA, COXIV, and mtTFA protein content data are presented in Fig. 5. mtDNA, COXIV, and mtTFA protein content were depressed (−27, −35, and −43%, respectively; $P < 0.05$) (Fig. 5*A*–*C*) in control myotubes from obese compared with lean donors. The overexpression of PGC-1α protein by ~3.5-fold (low dose) increased mtDNA content by 27% in HSkMC from lean and obese subjects, whereas increasing PGC-1α protein approximately eightfold increased mtDNA content by 66 and 72% in myotubes from lean and obese individuals, respectively ($P < 0.05$) (Fig. 5*A*). Overexpression of PGC-1α increased COXIV and mtTFA protein content in a dose-dependent manner ($P < 0.05$) (Fig. 5*B* and *C*). However, despite PGC-1α overexpression, mtDNA, COXIV, and mtTFA protein content still remained depressed in the cells from obese compared with lean donors under the same experimental treatment ($P < 0.05$) (Fig. 5*A*–*C*).

FAO and mitochondrial content. As presented in Fig. 6, when complete FAO ($^{14}CO_2$ production) at either 100 or 500 μmol/l oleate was expressed relative to mtDNA

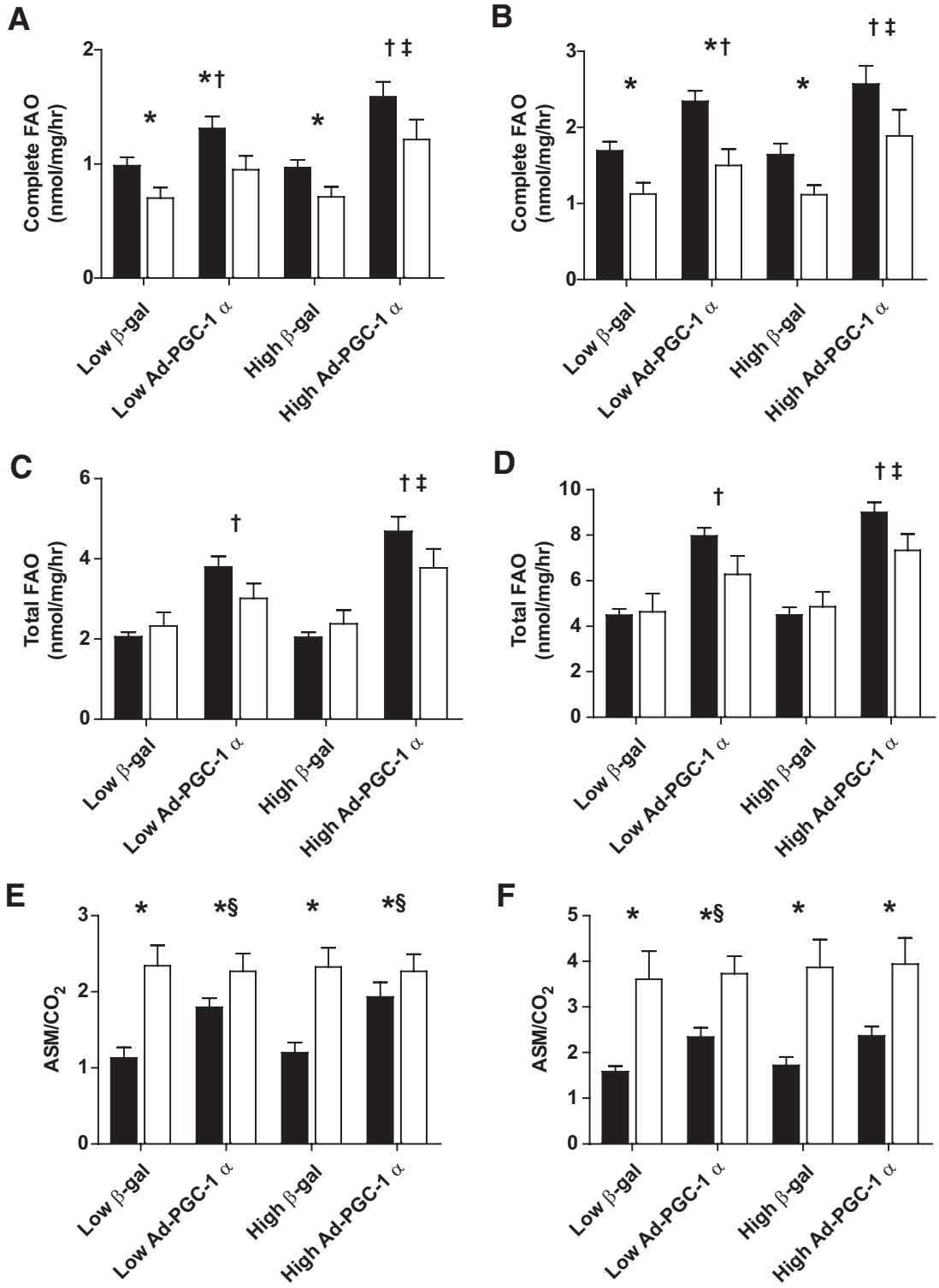

FIG. 2. Effect of PGC-1α overexpression on FAO and oxidation efficiency in HSkMC from lean (■) and obese (□) donors. HSkMC cultured from lean (n = 12) and obese (n = 9) donors was treated with either low- or high-dose recombinant Ad-β-gal or PGC-1α and incubated with either 100 μmol/l (*A*, *C*, and *E*) or 500 (*B*, *D*, and *F*) μmol/l [^{14}C] oleate. Complete FAO was measured from ^{14}C-labeled incorporation into CO_2 (*A* and *B*). Total FAO (*C* and *D*) was measured as the sum of ^{14}C-labeled incorporation into CO_2 and ^{14}C-labeled incorporation into ASMs, with ASM serving as an index of incomplete FAO. Oxidation efficiency was determined as the ratio of ASM to complete FAO, represented as ASM/CO_2 (*E* and *F*), with higher values indicative of reduced efficiency. Data are expressed as means ± SE and significant differences denoted at the $P \leq 0.05$ level. *Significant difference between lean and obese for that treatment. †Significant main effect comparing control (β-gal) and PGC-1 α overexpression (Ad-PGC-1α) at the respective adenoviral doses. ‡Significant difference between the high and low Ad-PGC-1α doses. §Significant increase in lean subjects with PGC-1α overexpression compared with control at the respective adenoviral doses.

(Fig. 6*A* and *B*) or COXIV (Fig. 6*C* and *D*) protein content, the decrement in FAO evident with obesity (Fig. 2) was abolished. PGC-1α overexpression had no effect on FAO relative to mtDNA ($P > 0.05$) and decreased FAO relative to COXIV protein content by ~50% ($P < 0.05$) (Fig. 6).

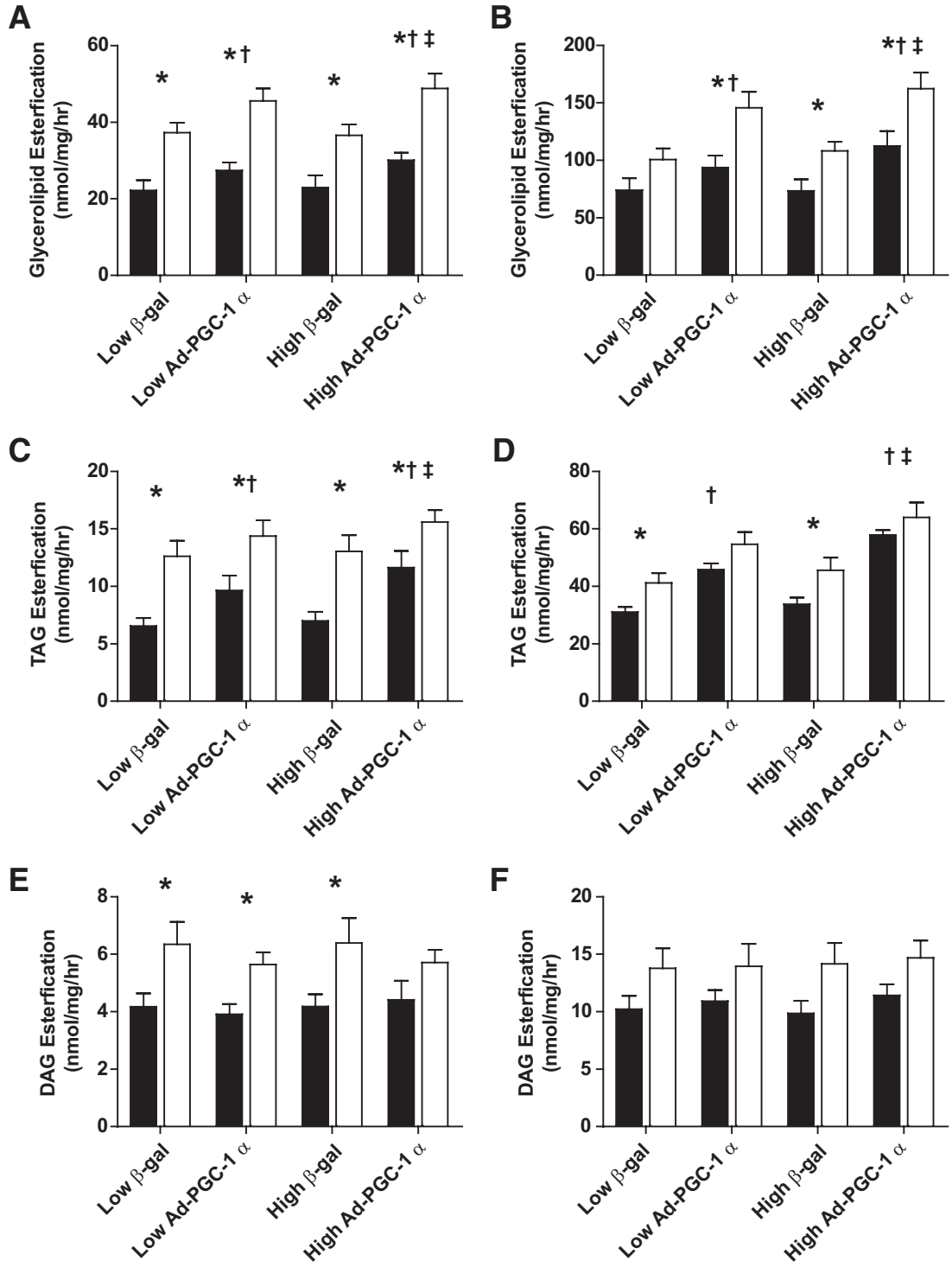

FIG. 3. Effect of PGC-1α overexpression on fatty acid incorporation in HSkMC from lean (■) and obese (□) donors. HSkMC cultured from lean (n = 8) and obese (n = 8) donors were incubated with either 100 μmol/l (*A*, *C*, and *E*) or 500 μmol/l (*B*, *D*, and *F*) [^{14}C] oleate, and ^{14}C-labeled incorporation into glycerolipid (*A* and *B*), TAG (*C* and *D*), and DAG (*E* and *F*) was determined. Data are expressed as means ± SE and significant differences denoted at the $P \leq 0.05$ level. *Significant difference between lean and obese for that treatment. †Significant main effect comparing control (β-gal) and PGC-1α overexpression (Ad-PGC-1α) at the respective adenoviral doses. ‡Significant difference between the high and low Ad-PGC-1α doses.

DISCUSSION

The intent of the current study was to determine whether the low rates of skeletal muscle FAO observed in obese humans could be linked to a lower mitochondrial content in skeletal muscle and, given the role of PGC-1α in stimulating mitochondrial biogenesis, to examine whether increasing PGC-1α by a physiologically relevant increment could mitigate the foregoing deficits. HSkMC was selected as the experimental model because myotubes established in culture from extremely obese donors display a reduction in FAO that is quantitatively similar to that reported in skeletal muscle strips (60%) (3), muscle homogenates (50%) (2), and in vivo studies using ^{13}C tracers (22%) (5) or indirect calorimetry (40%) (4). Other reports have likewise

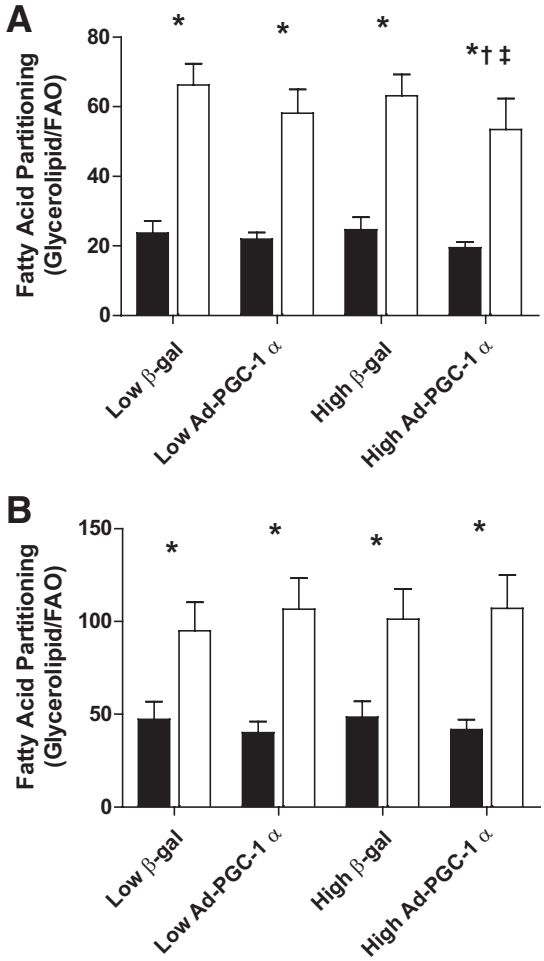

FIG. 4. PGC-1α overexpression does not normalize lipid partitioning rates between HSkMC from lean (■) and obese (□) donors. The partitioning of fatty acids between oxidative and storage pathways was evaluated by dividing the rate of oleate esterified into glycerolipid by the rate completely oxidized in response to either 100 μmol/l (*A*) or 500 μmol/l (*B*) [^{14}C] oleate in HSkMC from lean (n = 8) and obese (n = 8) donors. Data are means ± SE and significant differences denoted at the $P \leq 0.05$ level. *Significant difference between lean and obese for that treatment. †Significant main effect comparing control (β-gal) and PGC-1α overexpression (Ad-PGC-1α) at the respective adenoviral doses. ‡Significant difference between the high and low Ad-PGC-1α doses.

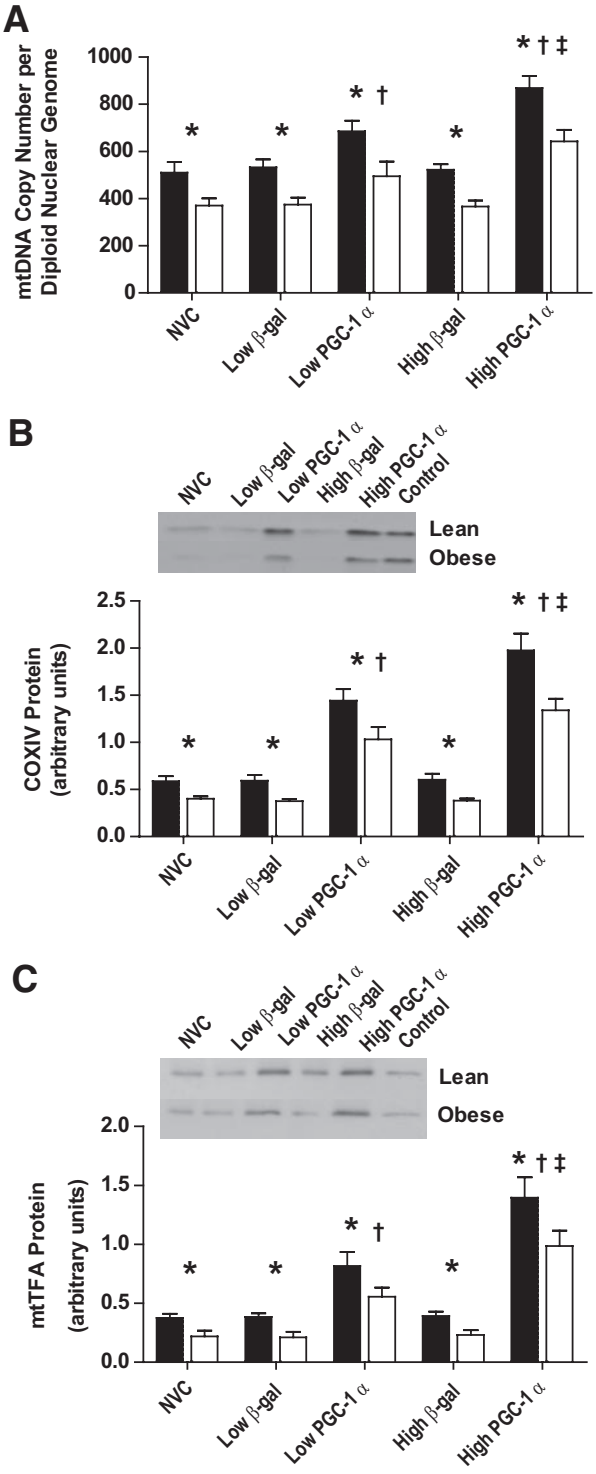

FIG. 5. PGC-1α increases mtDNA (*A*), COXIV protein (*B*), and mtTFA protein (*C*) in HSkMC from lean (■) and obese (□) donors. mtDNA, n = 9 for lean and obese; COXIV and mtTFA, n = 8 for lean and obese. Data are expressed as means ± SE and significant differences denoted at the $P \leq 0.05$ level. *Significant difference between lean and obese for that treatment. †Significant main effect comparing control (β-gal) and PGC-1α overexpression (Ad-PGC-1α) at the respective adenoviral doses. ‡Significant difference between the high and low Ad-PGC-1α doses. NVC, no-virus control.

demonstrated that interindividual variability in fat oxidation assessed in vivo in healthy young men was preserved in HSkMC (25). Our present findings (Fig. 2) further establish the utility of HSkMC as a model for studying fuel metabolism in human skeletal muscle.

There is controversy whether the reduction in complete FAO in human skeletal muscle observed with obesity can be attributed to the existing mitochondria being dysfunctional (i.e., altered morphology), the existing mitochondria being fully functional but expressed at a lower concentration with obesity, or a combination of both of these conditions (26). An important finding of the present study was that although complete FAO and mitochondrial content were reduced in HSkMC from extremely obese donors (Figs. 2 and 5), when complete FAO was normalized to indexes of mitochondrial content, the differences between lean and obese subjects were abolished (Fig. 6). The finding that the reduced complete FAO in myocytes from obese donors is associated with reduced mitochondrial content (Fig. 6) suggests that the impairment in complete FAO in human skeletal muscle with obesity may be attributed, at least in part, to a reduction in mitochondrial content (Fig. 5). This obesity phenotype is akin to characteristics exhibited by type 2 muscle fibers; in support, a greater proportion of type 2 fibers has been reported with

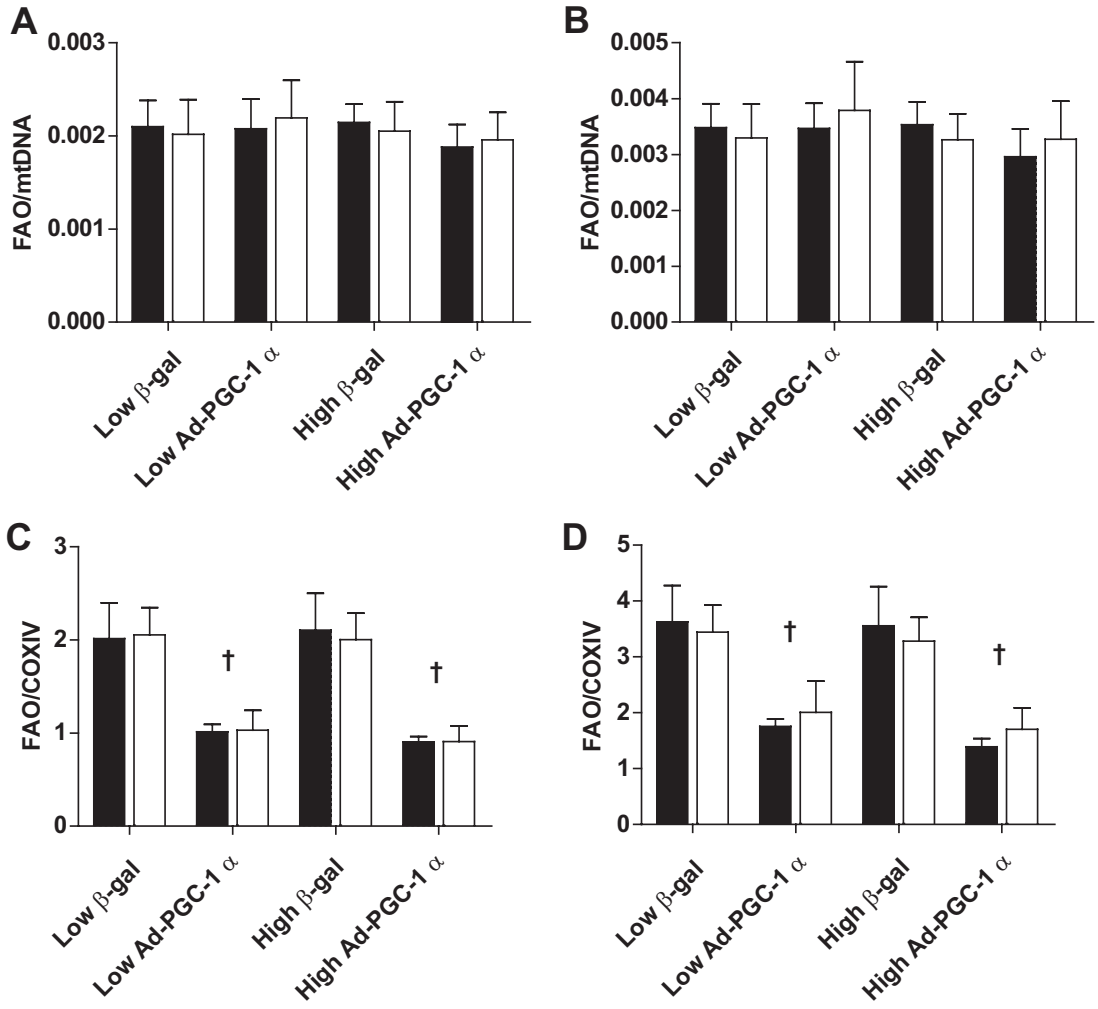

FIG. 6. FAO does not differ between HSkMC from lean (■) and obese (□) donors when normalized to indexes of mitochondrial content. FAO normalization to indexes of mitochondrial content were evaluated by dividing the rate of complete oleate oxidation from ^{14}C-labeled incorporation into CO_2 (FAO) by mtDNA copy number per diploid nuclear genome (*A* and *B*) or by COXIV protein expression in arbitrary units (*C* and *D*) under both 100 umol/l (*A* and *C*) and 500 umol/l (*B* and *D*) oleate conditions. FAO/mtDNA, $n = 9$ for lean and obese; FAO/COXIV, $n = 8$ for lean and obese. *Significant difference between lean and obese for that treatment. †Significant main effect comparing control (β-gal) and PGC-1α overexpression (Ad-PGC-1α) at the respective adenoviral doses.

extreme obesity (27), although it is not evident whether all traits of glycolytic tissue (i.e., contractile properties) are retained in cell culture. While our data suggest that mitochondrial functionality remains intact with obesity, more extensive mitochondrial characterization (i.e., respiration data) is needed to provide definitive conclusions.

In support of our findings, Holloway et al. (6) have reported that FAO in mitochondria isolated from muscle biopsies was reduced with obesity when calculated on a whole-muscle basis; however, when normalized to mitochondrial protein content, FAO was equivalent in lean and obese. The present findings add to the existing data (6) in two novel ways. First, the absolute decrement in complete FAO (Fig. 2) and associated reduction in mitochondrial content (Figs. 5 and 6) with obesity were evident even when mitochondrial content was manipulated via PGC-1α overexpression, indicating consistency. Second, to our knowledge this is the first study to demonstrate a reduction in mitochondrial content in primary human muscle cell cultures from obese donors. It has been proposed that characteristics evident in HSkMC have a genetic origin, given that any phenotype is retained as the cells proliferate and differentiate independently of in vivo influences (11,25,28). The current data thus provide the novel information that the reduction in mitochondrial content and the accompanying decrement in FAO in skeletal muscle from extremely obese subjects consist of a heritable or imprinted component.

PGC-1α is considered a "master regulator" coordinating mitochondrial biogenesis because elevating PGC-1α content activates critical downstream transcription factors, which effectively remodel the muscle cell to favor oxidative metabolism (17,29–32). Accordingly, in L6 muscle cells the overexpression of PGC-1α enhanced FAO and increased the expression of genes involved in oxidative processes (17); an elevation in PGC-1α is also believed to be a critical factor accounting for the increased FAO and mitochondrial content in skeletal muscle with exercise training (16,17). In the present study, while PGC-1α overexpression increased FAO and mitochondrial content independently of body composition, absolute values for complete FAO and mitochondrial content remained depressed in the myocytes from obese subjects at both the physiological and supraphysiological PGC-1α doses (Figs. 2 and 5). This novel result indicates that skeletal muscle of obese individuals responds to signals triggered by PGC-1α;

however, the obese state appears to limit both mitochondrial biogenesis and oxidative capacity via mechanisms that are independent of PGC-1α abundance (Figs. 2 and 5). The differences between the lean and obese myocytes in indexes of mitochondrial content (Fig. 5) and FAO (Fig. 2) were relatively consistent with and without (control) PGC-1α overexpression; this suggests a possible reduction in another coactivator or mechanism involved with mitochondrial proliferation. mtTFA protein content, a transcription factor downstream of PGC-1α critical for mtDNA replication (33), mirrored the pattern of change seen in mitochondrial content (Fig. 5), suggesting that the defect with obesity may involve this arm of PGC-1α coordination. A combination of variables such as PGC-1α cellular location (cytoplasm vs. nucleus), posttranslational modifications (i.e., acetylation and phosphorylation), and PGC-1α binding to or activation of transcription factors could contribute to the mitochondrial phenotype of obese HSkMC. Given the complexity of mitochondrial biogenesis, mechanisms independent of PGC-1 could also explain the obesity-associated decrement in FAO and mitochondrial content (34). Although the precise mechanism(s) are yet unknown, the present data indicate that extreme human obesity involves an intrinsic impairment in skeletal muscle mitochondrial biogenesis and content.

We previously reported that exercise training increased complete FAO and improved oxidation efficiency (CO_2 production/ASM) to equivalent absolute values in lean and formerly extremely obese individuals despite an initial decrement in FAO and elevated ASM production in the obese subjects (13). As presented in Fig. 2, increasing PGC-1α content by both physiological and supraphysiological increments did not abolish the difference in complete FAO and oxidation efficiency between lean and obese subjects as opposed to the total normalization seen with exercise training (13). This finding further suggests that other exercise factors, in addition to an increase in PGC-1α content, account for the improvement in skeletal muscle FAO with physical activity in obese individuals (13).

Despite the reduced ability to completely oxidize lipid, obese cells exhibited similar rates of total oxidation, suggesting a downstream defect in the lipid oxidation pathway. Koves et al. (17) suggested that an elevated ASM/CO_2 ratio, as observed in cells derived from obese donors (Fig. 2), signifies a mismatch between β-oxidation relative to trichloroacetic acid cycle activity. It is plausible that this occurred in the present study and that products of oxidative metabolism accumulated when downstream metabolic pathways could not adjust appropriately. In addition, obesity was linked to preferential partitioning of FA into the glycerolipid (TAG and DAG) pools (Fig. 3). These abnormalities are clinically relevant because both incomplete FAO and intramuscular lipid accumulation have been implicated as markers and perhaps mediators of insulin resistance in obese individuals (35,36).

Interestingly, PGC-1α overexpression increased fatty acid incorporation into TAG but not DAG (Fig. 3). This finding is consistent with a recent report showing that transgenic mice with muscle-specific overexpression of PGC-1α have increased muscle TAG when fed a high-fat diet (37). In this mouse model, PGC-1α overexpression resulted in upregulation of diacylglycerol aclytransferase and mitochondrial glycerol-3-PO4 acyl-transferase, two enzymes involved in TAG synthesis; we speculate that similar mechanisms might be operative in the HSkMC. PGC-1α overexpression had little effect on overall lipid partitioning (Fig. 4) because improved rates of FAO (Fig. 2) were matched by accelerated rates of glycerolipid and TAG synthesis (Fig. 3). Increasing the expression of PGC-1α thus did not rescue the cells from the obese subjects in terms of returning the indexes of lipid storage (Figs. 3 and 4) to values seen in the cells from lean donors.

In summary, skeletal muscle cells cultured from extremely obese donors exhibited depressed mitochondrial content, which could in turn be responsible for the diminished capacity to oxidize lipid and the preferential partitioning of lipid toward intramuscular storage with obesity. This phenotype may consist of a heritable or imprinted component because it is proposed that characteristics expressed in HSkMC have a genetic origin. When PGC-1α was overexpressed in HSkMC from lean and obese subjects, the mitochondrial phenotype of obesity persisted. This finding suggests a molecular impairment in mitochondrial proliferation that occurs independent or downstream of PGC-1α expression. Additionally, PGC-1α overexpression did not fully recapitulate the effects of exercise training on FAO in obese individuals, suggesting that additional mechanisms are involved with this intervention.

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