PGC-1α overexpression increases lipid oxidation in myocytes from extremely obese individuals

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**Objective** - To determine if 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 over-expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α).

**Research design and methods** - FAO was studied in HSkMC from lean (L) (BMI, 22.4 ± 0.9 kg/m², N = 12) and extremely obese (O) (45.3 ± 1.4 kg/m², N = 9) subjects. Recombinant adenovirus was used to increase HSkMC PGC-1α expression (3.5- and 8-fold), followed by assessment of mitochondrial content, (mtDNA, COXIV), complete (¹⁴CO₂ 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]CASM production /[^14]CO₂), increased partitioning of FA towards storage, and lower (P < 0.05) mtDNA (-27%), COXIV (-35%), and mtTFA (-43%) protein levels. PGC-1α overexpression increased (P < 0.05) FAO, mtDNA, COXIV, mtTFA, and fatty acid incorporation into triacylglycerol in both L and O. Perturbations in FAO, triacylglycerol synthesis, mtDNA, COXIV, and mtTFA in O compared to L HSkMC persisted despite PGC-1α overexpression. When adjusted for mtDNA and COXIV content, FAO was equivalent between L and O.

**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 and mtDNA content, implicating mechanisms other than PGC-1α abundance.
The skeletal muscle of obese individuals typically exhibits an inability to effectively oxidize lipid. Using arterio-venous 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 \( \geq 40 \text{ kg/m}^2 \)) obesity in a variety of preparations such as muscle homogenates from the vastus lateralis (2), intact muscle strips from the rectus abdominus (3), as well as 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) and/or function (7) contribute to this obesity-related phenotype. A reduction in skeletal muscle mtDNA (7), altered mitochondria morphology (8), as well as 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-\( \gamma \) coactivator-1\( \alpha \) (PGC-1\( \alpha \)) is a metabolic co-activator which binds to transcription factors stimulating mitochondrial biogenesis (14) and lipid oxidation (15). PGC-1\( \alpha \) has also been shown to be up-regulated in response to exercise training (13, 16, 17), making it an attractive candidate to explain improvements in FAO with physical activity in obese individuals (13) or as a target for the development of antiobesity and/or antidiabetic drugs. The objectives of the present study were to: 1) determine if obesity-related impairments in FAO were associated with a reduction in myocyte mitochondrial content and 2) determine if increasing the expression of PGC-1\( \alpha \) in the myocytes of obese individuals could normalize absolute rates of FAO in a manner similar to exercise training (13). We have previously reported that primary human skeletal muscle cell cultures (HSkMC) display the phenotype of skeletal muscle from obese individuals in respect to a reduction in FAO (11); we thus utilized this model to examine mechanisms controlling FAO and to specifically manipulate PGC-1\( \alpha \).

**EXPERIMENTAL PROCEDURES**

**Experimental Design.** 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-1\( \alpha \) 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 x 10^3 cells per well, respectively. Upon reaching 70-80% confluence, differentiation to myotubes was induced by switching the growth media to differentiation media (DMEM supplemented with 2% horse serum, 0.5 mg/ml BSA, 0.5 mg/ml fetuin, and 50 µg/ml gentamicin/amphotericin B). On day 5, 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 (see Figure 1) and mRNA (not shown) dose-response curve.

For all subsequent experiments, a “Low” Ad-PGC-1α dose (5x10^9 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 (1x10^10 plaque-forming units/ml) used to represent a supraphysiological increase in PGC-1α (~8-fold 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 8 based on previous research (19). There were no obvious differences in the extent of myotube differentiation between lean and obese HSkMC.

Determination of Fatty Acid Oxidation and Lipid Esterification. On day 8 of differentiation, myotubes were incubated at 37°C in sealed 24-well plates containing differentiation media, 12.5mM HEPES, 0.5% BSA, 1mM carnitine, either 100µM or 500µM sodium oleate (Sigma-Aldrich, St. Louis, MO) and 1 µCi/ml [14C] oleate (PerkinElmer, MA) for 3 hours. Following incubation, medium was assayed for 14CO2 (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 -80ºC for subsequent determination of protein concentration and lipid esterification.

For the determination of lipid esterification, 500µl of cell lysate was added to 1:2 chloroform:methanol (vol/vol). After vortexing, 625 µl of chloroform was added, followed by the addition of 625 µl of deionized H2O. Samples were vortexed and centrifuged at 1000 RPM for 5 minutes at room temperature. The chloroform phase (containing total lipids extracted) was transferred to a clean glass tube and evaporated under a stream of 100% N2. Samples were re-suspended in 100 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 minutes. 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, 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 v. 2.0 (Applied Biosystems). mtDNA was expressed as a relative copy number (Rc) by expressing Ct differences between β-globin and mtDNA as described previously (23, 24) and based on the calculation: \( Rc = 2^{\Delta Ct} \) and \( \Delta Ct = Ct_{\beta\text{-globin}} - Ct_{\text{mtDNA}} \).

**Western Blot Analyses.** Cells were washed twice with ice-cold PBS and harvested in 150 µl of lysis buffer (50 mM HEPES (pH 7.4), 1% Triton X-100, 10 mM EDTA, 100 mM NaF, and 12 mM Na pyrophosphate) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich). Samples were sonicated and centrifuged at 20,000 g for 20 minutes at 4°C. Protein concentrations were determined from cell extracts using the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Thirty µg of cellular protein was separated by SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA), and probed overnight for either PGC-1α (1:500; Santa Cruz, Santa Cruz, CA), or COXIV (1:8000; 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 analysis of variance (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 mean ± S.E.

**RESULTS**

**PGC-1α Overexpression.** Findings from the dose-response experiment for Ad-PGC-1α are presented in Figure 1. Treatment of myotubes with Ad-β-gal (control virus) had no effect on PGC-1α protein content compared to no virus controls (Figure 1A). In HSkMC derived from lean and obese individuals, PGC-1α protein (Figure 1A) and mRNA (data not shown) increased in a dose-dependent manner with increasing PGC-1α adenovirus titer; there was no difference between PGC-1α protein content in HSkMC from lean and obese individuals with or without overexpression. Based on the findings presented in Figure 1A, for subsequent experiments we selected the 1ul dose (5x10⁹ 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 ul dose (1x10¹⁰ plaque-forming units/ml) to represent a supraphysiological increment (~8-fold increase in PGC-1α protein over controls) in protein content. These doses are referred to as “Low” Ad-PGC-1α and “High” Ad-PGC-1α. Protein levels of mtTFA and COXIV, indicators of mitochondrial biogenesis, also increased in a dose-dependent manner with PGC-1α adenovirus (Figure 1B).
Fatty Acid Oxidation. 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-PCG-1α for 24 hours, followed by 3 hr incubation with either 100 µM or 500 µM oleate. In all experiments, FAO from the no virus controls did not differ from Ad-β-gal controls (data not shown). Data obtained from the experiments determining FAO are presented in Figure 2. Under control conditions (Low and High β-gal) complete FAO ([14]CO₂ production) was consistently depressed by ~30% in HSkMC from obese individuals with either the 100 or 500 µM oleate incubation (Figures 2A and 2B). As presented in Figure 2A, overexpression of PGC-1α by ~3.5-fold (Low dose) resulted in a ~30% increase in complete FAO (100 µM oleate) in HSkMC derived from lean and obese donors. The 8-fold increase in PGC-1α protein (High dose) also increased complete FAO in cells from both lean (64%) and obese (70%) donors at the 100 µM oleate concentration (P<0.05, Figure 2A). 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 to lean subjects under all conditions (Figure 2A). Similar findings were obtained in response to the higher oleate concentration (500 µM) with respect to differences between cell types and the effects of PGC-1α overexpression (Figure 2B). Total FAO, determined as the sum of incomplete ([14]CASM ) and complete ([14]CO₂) oxidation did not differ between cell type under any conditions (Figures 2C and 2D) and PGC-1α overexpression increased total oxidation in a dose dependent manner. Total and complete FAO were significantly higher at 500 µM oleate compared to 100 µM oleate in control and PGC-1α treated cells (P<0.05, Figures 2A-D).

A ratio of incomplete ([14]CASM) to complete ([14]CO₂) oleate oxidation was calculated as an index of FAO efficiency (14). In control cells treated with 100 µM [14]C oleate, radiolabel incorporation into ASM relative to CO₂ was approximately 2-fold higher in myotubes from obese compared to lean individuals (P<0.05, Figure 2E). The high relative rate of incomplete oxidation in the obese compared to the lean group was retained regardless of the level of PGC-1α overexpression or oleate concentration (Figures 2E and 2F). In cells from lean individuals, PGC-1α overexpression increased the ASM/CO₂ ratio by ~55% (P<0.05); PGC-1α overexpression in cells from obese subjects had no effect on this ratio (Figures 2E and 2F). The ASM/CO₂ ratio was significantly higher at 500 µM oleate compared to 100 µM oleate in control and PGC-1α treated cells (P<0.05, Figures 2E-2F).

Intramycellular Lipid Content. Oleate incorporation into glycerolipid, TAG, and DAG pools are presented in Figure 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 (Figures 3A and 3B). Cells from obese subjects, regardless of treatment, consistently incorporated more lipids into the glycerolipid pool compared to HSkMC from lean subjects (Figures 3A and 3B). Cells from obese individuals, regardless of treatment, consistently incorporated more lipids into the glycerolipid pool compared to HSkMC from lean subjects (Figures 3A and 3B). 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 Figures 3C-3F. Under control conditions, cells from obese subjects had increased oleate incorporation into TAG (P<0.05, Figures 3C and 3D) when exposed to low and high oleate conditions. Lipid incorporation into DAG was higher (P<0.05, Figure 3E) in obese cells compared to 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 to the control condition (β-gal), PGC-1α overexpression increased oleate incorporation into TAG (P<0.05, Figures 3C and 3D) and PL (data not shown, P<0.05) without affecting DAG. This was evident in HSkMC from both lean and obese subjects (Figures 3C – 3F). PGC-1α overexpression did not normalize intramyocellular lipid incorporation into storage in HSkMC from obese subjects to those of the lean individuals under the same treatment (Figures 3C and 3E). 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, Figures 3A-3F).

**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 towards storage. Myotubes from obese donors had a higher partitioning index compared to HSkMC from lean donors (P<0.05) (Figures 4A and 4B), 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 (~8-fold increase in PGC-1α protein) decreased this ratio under low oleate conditions (P<0.05, Figure 4A).

**Indices of Mitochondrial Content.** Mitochondrial DNA (mtDNA), COXIV protein content, and mtTFA protein content data are presented in Figure 5. mtDNA, as well as, COXIV and mtTFA protein content were depressed (-27%, -35%, and -43%, respectively, P<0.05, Figure 5A-C) in control myotubes from obese compared to lean donors. The overexpression of PGC-1α protein by ~3.5-fold (Low Dose) increased mtDNA content 27% in HSkMC from lean and obese subjects, whereas increasing PGC-1α protein ~8-fold, increased mtDNA content 66% and 72% in myotubes from lean and obese individuals, respectively (P<0.05, Figure 5A). Overexpression of PGC-1α increased COXIV and mtTFA protein content in a dose-dependent manner (P<0.05, Figure 5B and 5C). PGC-1α overexpression increased mtTFA protein content in a dose-response manner (P<0.05, Figure 5C). However, despite PGC-1α overexpression, mtDNA, COXIV and mtTFA protein content still remained depressed in the cells from obese compared to lean donors under the same experimental treatment (P<0.05, Figure 5A-C).

**FAO and Mitochondrial Content.** As presented in Figure 6, when complete FAO ($^{14}$CO$_2$ production) at either 100 or 500 µM oleate was expressed relative to mtDNA (Figures 6A and 6B) or COXIV protein content (Figures 6C and 6D) the decrement in FAO evident with obesity (Figure 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 (~50%, P<0.05, Figure 6).

**DISCUSSION**

The intent of the current study was to determine if 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, examine if increasing PGC-1α by a physiologically relevant increment could mitigate the foregoing deficits. HSkMC was selected as the experimental model as 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), as well as, in-vivo studies using $^{13}$C tracers (-22%) (5) and/or indirect calorimetry (-40%) (4). Other reports have likewise demonstrated that inter-
individual variability in fat oxidation assessed *in-vivo* in healthy, young men was preserved in HSkMC (25). Our present findings (Figure 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: 1) the existing mitochondria being dysfunctional (i.e. altered morphology); 2) the existing mitochondria being fully functional but expressed at a lower concentration with obesity or; 3) 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 (Figures 2 and 5), when complete FAO was normalized to indices of mitochondrial content, the differences between lean and obese subjects were abolished (Figure 6). The finding that the reduced complete FAO in myocytes from obese donors is associated with reduced mitochondrial content (Figure 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 (Figure 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 extreme obesity (27) although it is not evident if all traits of glycolytic tissue (i.e. contractile properties) are retained in cell culture. While our data suggests 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) 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 (Figure 2) and associated reduction in mitochondrial content (Figures 5 and 6) with obesity was evident even when mitochondrial content was manipulated via PGC-1α overexpression, indicating consistency. Secondly, 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, as any phenotype is retained as the cells proliferate and differentiate independently of *in-vivo* influences (11, 25, 28). The current data thus provides the novel information that the reduction in mitochondrial content and the accompanying decrement in FAO in skeletal muscle from extremely obese subjects consists of a heritable or imprinted component.

PGC-1α is considered a “master regulator” coordinating mitochondrial biogenesis as 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.
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(Figures 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 (Figures 2 and 5). The difference between the lean and obese myocytes in indices of mitochondrial content (Figure 5) and FAO (Figure 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 which is critical for mtDNA replication (33) mirrored the pattern of change seen in mitochondrial content (Figure 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), post-translational modifications (ie., acetylation, phosphorylation), as well as, PGC-1α binding to and/or activation of transcription factors could contribute to the mitochondrial phenotype of obese HSkMC. Due to 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 (CO₂ 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 Figure 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/CO₂ ratio, as observed in cells derived from obese donors (Figure 2), signifies a mismatch between β-oxidation relative to TCA 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 (Figure 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 (Figure 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 DGAT1 and mtGPAT, 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 (Figure 4) as improved rates of FAO (Figure 2) were
matched by accelerated rates of glycerolipid and TAG synthesis (Figure 3). Increasing the expression of PGC-1α thus did not rescue the cells from the obese subjects in term of returning the indices of lipid storage (Figures 3 & 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 towards intramuscular storage with obesity. This phenotype may consist of a heritable or imprinted component as 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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**Figure Legends**

**Figure 1.** Adenovirus-mediated PGC-1α overexpression in cultured myotubes (HSkMC) from lean and obese donors.

A) PGC-1α protein content in no virus controls (NVC), adenovirus β-galactosidase (Ad-βgal) controls, and adenovirus PGC-1α (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.

**Figure 2.** Effect of PGC-1α overexpression on fatty acid oxidation (FAO) and oxidation efficiency in HSkMC from lean and obese donors.

HSkMC cultured from lean (n = 12) and obese (n = 9) donors were treated with either low or high dose recombinant adenovirus encoding β-galactosidase (β-gal) or PGC-1α and incubated with either 100µM (Panels A, C, and E) or 500µM (Panels B, D, and F) [14C] oleate. Complete fatty acid oxidation (FAO) was measured from [14C] labeled incorporation into CO₂ (Panels A and B). Total FAO (Panels C and D) was measured as the sum of [14C] labeled incorporation into CO₂ and [14C] labeled incorporation into acid soluble metabolites (ASM), with ASM serving as an index of incomplete FAO. Oxidation efficiency was determined as the ratio of ASM to complete FAO, represented as ASM/CO₂ (Panels E and F), with higher values indicative of reduced efficiency. Data are expressed as mean ± S.E and significant differences denoted at the *P* ≤ 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 dose.
‡ Significant difference between the high Ad-PGC-1α and low Ad-PGC-1α dose.
§ Significant increase in lean subjects with PGC-1α overexpression compared to control at the respective adenoviral dose.

Figure 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 donors (n = 8) were incubated with either 100 µM (Panels A, C and E) or 500 µM (Panels B, D, F) [14C] oleate and 14C labeled incorporation into glycerolipid (Panels A and B), TAG (Panels C and D) and DAG (Panels E and F) determined. Data are expressed as mean ± S.E and significant differences denoted at the P < 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 dose.
‡ Significant difference between the high Ad-PGC-1α and low Ad-PGC-1α dose.

Figure 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 µM (Panel A) or 500 µM (Panel B) [14C] oleate in HSkMC from lean (n=8) and obese (n=8) donors. Data are expressed as mean ± S.E and significant differences denoted at the P < 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 dose.
‡ Significant difference between the high Ad-PGC-1α and low Ad-PGC-1α dose.

Figure 5. PGC-1α increases mtDNA (Panel A), COXIV protein (Panel B) and mtTFA protein (Panel 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 mean ± S.E and significant differences denoted at the P < 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 dose.
‡ Significant difference between the high Ad-PGC-1α and low Ad-PGC-1α dose.

Figure 6. FAO does not differ between HSkMC from lean and obese donors when normalized to indices of mitochondrial content. FAO normalization to indices of mitochondrial content were evaluated by dividing the rate of complete oleate oxidation from 14C labeled incorporation into CO2 (FAO) by mtDNA copy number per diploid nuclear genome (Panels A and B) or by COXIV protein expression in arbitrary units (Panels C and D) under both 100 µM (Panels A and C) and 500 µM (Panels 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 dose.
Figure 1

A. Ad-PGC-1α

B. Ad-PGC-1α
Figure 4

A. 

**Fatty Acid Partitioning (Glycerolipid/FAO)**

- Lean
- Obese

- Low β-gal
- Low Ad-PPC-1α
- High β-gal
- High Ad-PPC-1α

B. 

**Fatty Acid Partitioning (Glycerolipid/FAO)**

- Lean
- Obese

- Low β-gal
- Low Ad-PPC-1α
- High β-gal
- High Ad-PPC-1α
Figure 5

A. 

```latex
\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5a}
\caption{mtDNA Copy Number per Diploid Nuclear Genome}
\end{figure}
```

B. 

```latex
\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5b}
\caption{COXIV Protein (arbitrary units)}
\end{figure}
```

C. 

```latex
\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5c}
\caption{mtHIF Protein (arbitrary units)}
\end{figure}
```
Figure 6

A. Lean □ Obese

B. Lean □ Obese

C. Lean □ Obese

D. Lean □ Obese