HHS Public Access

Author manuscript

Metabolism. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Metabolism. 2019 September ; 98: 53–61. doi:10.1016/j.metabol.2019.06.009.

Peroxisomal gene and protein expression increase in response to a high-lipid challenge in human skeletal muscle

Tai-Yu Huang[a], **Donghai Zheng**[a,d], **Robert C. Hickner**[a,b,d,e,f], **Jeffrey J. Brault**[a,b,d], **Ronald N. Cortright**[a,b,c,d,*]

[a]Department of Kinesiology, East Carolina University, Greenville, NC, United States of America

[b]Department of Physiology, East Carolina University, Greenville, NC, United States of America

[c]Department of Surgery, East Carolina University, Greenville, NC, United States of America

[d]Diabetes and Obesity Institute, East Carolina University, Greenville, NC, United States of America

[e]College of Human Sciences, Florida State University, Tallahassee, FL, United States of America

[f]School of Health Sciences, University of KwaZulu-Natal, Westville, South Africa

Abstract

Peroxisomes are essential for lipid metabolism and disruption of liver peroxisomal function results in neonatal death. Little is known about how peroxisomal content and activity respond to changes in the lipid environment in human skeletal muscle (HSkM).

Aims: We hypothesized and tested that increased peroxisomal gene/protein expression and functionality occur in HSkM as an adaptive response to lipid oversupply.

Materials and methods: HSkM biopsies, derived from a total of sixty-two subjects, were collected for 1) examining correlations between peroxisomal proteins and intramyocellular lipid content (IMLC) as well as between peroxisomal functionality and IMLC, 2) assessing peroxisomal gene expression in response to acute- or 7-day high fat meal (HFM), and in human tissue derived primary myotubes for 3) treating with high fatty acids to induce peroxisomal adaptions. IMLC were measured by both biochemical analyses and fluorescent staining. Peroxisomal membrane protein PMP70 and biogenesis gene (*PEX*) expression were assessed using western blotting and realtime qRT-PCR respectively. 1-^{14}C radiolabeled lignocerate and palmitate oxidation assays were performed for peroxisomal and mitochondrial functionality respectively.

*Corresponding author at: Departments of Kinesiology, Physiology, Surgery and the Diabetes and Obesity Institute, East Carolina University, Greenville, NC 27834, United States of America. cortrightr@ecu.edu (R.N. Cortright).

Author contributions

R.N.C., T.-Y. H. and D. Z. conceived and designed the research study; T.-Y. H., R.C.H., D. Z. and J.J.B. performed the experiments; T.-Y. H. and D. Z. and J.J.B. analyzed the data; T.-Y. H., R.N.C., and D.Z. interpreted the results of experiments; T.-Y. H. and R.N.C. jointly drafted the manuscript; T.-Y.H., R.N.C., D.Z., and R.C.H. edited and revised the manuscript; T.-Y. H., R.N.C., D.Z., R.C.H., and J.J.B. approved the final version of manuscript; T.-Y. H. and J.J.B. prepared the figures.

Declaration of Competing Interest

The authors declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.06.009.

Results: 1) Under fasting conditions, HSkM tissue demonstrated a significant correlation ($P \ll 0.05$) between IMCL and the peroxisomal biogenesis factor 19 (PEX19) protein as well as between lipid content and palmitate and lignocerate complete oxidation. 2)Similarly, post-HFM, additional PEX genes (*Pex19, PEX11A, and PEX5*) were significantly ($P \ll 0.05$) upregulated. 3)Increments in *PMP70*, carnitine octanoyl transferase (CrOT), *PGC-1α*, and *ERRα* mRNA were observed post-fatty acid incubation in HSkM cells. PMP70 protein was significantly ($P \ll 0.05$) elevated 48-h post lipid treatment.

Conclusions: These results are the first to associate IMLC with peroxisomal gene/protein expression and function in HSkM suggesting an adaptive role for peroxisomes in lipid metabolism in this tissue.

Keywords

Lignoceric acid oxidation; Human primary myotubes; Mitochondrial β-oxidation; Lipotoxicity; Obesity

1. Introduction

Disparate opinions exist regarding mitochondrial lipid oxidation in the obese and diabetic state. Thus, there is still debate as to whether skeletal muscle mitochondrial fatty acid oxidation (FAO) rates are diminished [1,2], unchanged [3,4] or even increased with obesity [5]. However, it is well accepted that lipid metabolism is dysregulated in human skeletal muscle (HSkM) as evidenced by ectopic lipid accumulation which has been associated with insulin resistance [5]. Further, diabetic individuals with high intramyofibrillar lipid content (IMLC) possess reduced mitochondrial content and alterations in morphology [6]. This has encouraged researchers to focus on extra-mitochondrial factors that may be involved in lipid metabolism, such as pathways regulating lipid catabolism and trafficking [7]. However, an important factor that has largely been overlooked with respect to maintaining a healthy cellular lipid environment in skeletal muscle is the peroxisome.

The majority of our understanding of peroxisomal function has been derived from studies in hepatic tissue from animals [8], whereas the role of peroxisomes in maintaining lipid homeostasis in extra-hepatic tissues is less well understood. The early identification of peroxisomal function in humans was realized from the study of genetic diseases such as X-linked adrenoleukodystrophy [9]. Moreover, peroxisomes are indispensable for specific catabolic and anabolic reactions in lipid metabolism in mammals and can be found in most human tissues, especially in liver. For example, they are also involved in catabolizing other dietary fatty acids such as β-oxidation of long-chain fatty acids (LCFA) and very-long-chain fatty acids (VLCFA). Other aspects of lipid metabolism in hepatic tissue include synthesis of ether phospholipids and bile acids [10]. Previous studies indicted increases in PMP70, and other *PEXs* genes via PPARα expression following high fat diets in rodent hepatocytes [11,12]. It is still largely understudied regarding the potential contribution(s) for peroxisomes in lipid metabolism of human skeletal muscle. To our knowledge, reports are rare describing a peroxisomal presence in skeletal muscle tissue, and these come from studies in rodents [13-15] and one recent report in human primary myotubes [16].

It is well established that mitochondrial entry of fatty acids for full oxidation is mainly mediated by mitochondrial carnitine palmitoyltransferase 1 (CPT1) [17,18]. Our laboratory previously reported that CPT1 activity was reduced with obesity [1]. We have subsequently reported that peroxisomal activity and peroxisomal oxidation of very-long-chain fatty acid (VLCFA; e.g., C-24 lignocerate which must undergo peroxisomal partial β-oxidation prior to complete oxidation by mitochondria) was significantly elevated in skeletal muscle from obese fatty Zucker (fa/fa) rats containing high IMLC compared to lean counterparts [13].

The involvement of peroxisomal FAO may be especially crucial for cellular lipid homeostasis in obesity when muscle CPT1 activity is decreased [1]. In this regard, a more recent investigation utilizing a muscle-specific CPTβ knock-out mouse has shown that impaired mitochondrial fat oxidation induced peroxisomal β-oxidation to compensate for reductions in mitochondrial lipid disposal [14]. Despite these observations suggesting that peroxisomal FAO activity and conferred functional interactions with mitochondria might be crucial in FAO, studies describing cooperation between peroxisomes and mitochondria are nearly non-existent in HSkM. Therefore, the purpose of the present study was to determine if peroxisomal gene and protein expression regulating biogenesis/activity occurs under conditions of elevated IMLC in HSkM. The present findings are the first to report a peroxisomal presence in HSkM tissue that is associated with elevations in IMLC. Results support and encourage future research to determine whether peroxisomes may serve as a viable therapeutic target for enhancing skeletal muscle FAO in an effort to reduce the prevalence and metabolic consequences associated with high intramyocellular lipids as noted with obesity and Type 2 Diabetes [1,19,20].

2. Research design and methods

2.1. Human subjects

Subjects were recruited from the Greenville area of North Carolina. Inclusion criteria for the study were non-obese/lean (BMI ≤26 kg/m^2), obese (BMI ≥30 kg/m^2), non-diabetic (fasting glucose ≪110 mg/dl), and pre-menopausal, sedentary female subjects between the ages of 18–45 years. Individuals reported no recent history of substantial weight loss or gain [(±2 kg for the previous 12 months), hypertension, metabolic and/or musculoskeletal diseases (not taking prescribed medications for metabolic diseases). All procedures were approved by the East Carolina University Institutional Review Board.

2.2. Materials

Radiolabeled [1-^{14}C] palmitate was purchased from PerkinElmer (Boston MA), and [1-^{14}C] lignoceric acid was obtained from American Radiolabeled Chemicals (St. Louis, MO). Primary cell culture reagents and plates were purchase from Invitrogen, as described previously [16]. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2.3. High fat meal/diet intervention

The dietary phase of the study was divided into two phases as follows: A)High Fat Meal: On day 1, subjects reported to the laboratory following a 10 h, overnight fast. A blood sample was obtained from an antecubital vein to determine fasting glucose and insulin to confirm

non-insulin resistance and diabetic status (i.e., HOMA-IR) [21] and sex steroids (estradiol and progesterone) to confirm subjects were in the early follicular phase of the menstrual cycle [22] using a Synchron Access Clinical System, UniCel DxC600i, (Beckman-Coulter, Brea, CA). A vastus lateralis muscle biopsy was taken pre- and 4 h-post- high fat liquid meal of Pulmocare (Abbott Laboratories, Columbus, OH) and heavy cream (50% daily caloric requirements; ~70% calories from fat: SAT:MUFA:PUFA = 48:37:15). B) High Fat Diet (HFD): Subjects were subsequently placed on a HFD composed of 60% fat, 20% carbohydrate, and 20% protein over the remaining day following the second biopsy and 4 h post liquid meal. The subjects consumed a solid food HFD at ~40 kcal/kg body weight/day and were asked to keep a food consumption log for seven days. The diet components were provided by the study staff based on individual food preferences. The structured solid food diet was designed to reproduce the typical "Western Diet" with a lipid composition of 48% SFA, 15% PUFA, and 37% MUFA. On day 7, the third and final muscle biopsy and blood samples were obtained as on the day 1 visit.

2.4. Triacylglycerol assay (TAG)

To measure intramyocellular lipid content and for correlational analyses with functional measurements, we employed the method established and validated in our laboratory [16]. A commercial triglyceride assay (SIGMA, CAT. # TR0100) was used to measure glycerol as the indirect measure of total triglycerides in the muscle extracts.

2.5. BODIPY (493/503) dye

BODIPY was used to visualize intramuscular lipid droplets.—BODIPY (Life Technologies, Eugene, OR) assays were conducted to confirm differences in intramyocellular lipid content (mainly lipid droplets) and to correlate with PEX19 and palmitic and lignocerate oxidation. The staining and quantification procedure was based on previous publications [23-25]. Briefly, frozen human skeletal muscle was sectioned at 10 μm using a cryostat, mounted on slides (two cryosections per slide), rapidly fixed in ice-cold 4% paraformaldehyde solution for 1 h, and then rinsed with phosphate buffered solution (PBS) for 3 × 5 min. The sections were immersed in 10 μg/ml BODIPY working solution using Coplin jars for 30 min. Subsequently, the sections were rinsed in PBS 5 × 5 min, covered with coverslips using Vectashield mounting medium (Burlingame, CA) and sealed with transparent nail polish. Fluorescent images were captured with an optical microscope (Leica DM6000, Germany) through a 20× objective. A laser was used to excite BODIPY 493/503. Imaging settings were kept constant for all slides. Quantification of lipid droplets was performed using multi measurement plugin of ImageJ with a constant threshold to compare the fluorescent signal between subjects. Individual muscle fibers were manually delineated and measured.

2.6. Human skeletal muscle cell culture

Satellite cells were isolated from 50 to 80 mg of fresh muscle tissues from vastus lateralis and cultured into myotubes as previously described [16] and validated [26] in our laboratory. All assays were done using myoblasts of passage 4 or less. Regarding high fatty acid treatment, on day 5 of differentiation, mature myotubes were treated with differentiation

media supplemented with 1% bovine serum albumin (control) or a mixture of 500 μM palmitate:oleate (1:1 ratio; 250 μM each) bound to 1% BSA + 1 mM carnitine (i.e., high fatty acid incubation) for 48 h (for protein measures) or for 12 h (for mRNA measures).

2.7. Western blotting assessment

Western blotting assays were performed using PAGE-SDS procedures after whole cell homogenization/tissue extraction and BCA assays for total protein as described previously [16]. All information of primary and secondary antibodies was also described in previously published work [8].

2.8. RNA extraction and mRNA expression quantification

RNA extraction and mRNA expression quantification were performed as described previously [16]. Briefly, total RNA was isolated from human primary myotubes and HSkM tissues with RNeasy plus mini kit (Qiagen) and TRIzol reagent respectively. The extraction procedures were based on manufacturer's instructions. 2 μg RNA were used to perform the reverse transcription reaction. Subsequent qRT-PCR was performed with ViiA™ 7 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) using TaqMan Gene expression assay for Estrogen related receptor α (ERRα; Hs00607062_gH). All the other gene expression reagents were described in previous published work from our laboratory [16]. mRNA expression was determined using the comparative Ct method with an endogenous control (18S ribosomal RNA) and converted to a linear function by using a base 2 antilog transformation.

2.9. Long-chain (LCFA) and very-long-chain fatty acid (VLCFA) oxidations

Whole tissue homogenization and FAO assays were preformed according to our established methods [1,13,16]. Briefly, 40–100 mg muscle from vastus lateralis were quickly homogenized and kept on ice until oxidation experiments were performed. Our experiments employed [1-^{14}C] palmitate ± etomoxir (a CPT1 inhibitor at 100 μM) to examine fatty acid oxidation by peroxisomes and mitochondria, and [1-^{14}C] lignoceric acid (C24:0) to evaluate the influence of peroxisomal β-oxidation on oxidation capacity of mitochondria (lignoceric acid cannot be oxidized by the mitochondrion directly and requires a peroxisomal presence). Data were expressed as picomole (lignoceric acid) or nanomole (palmitate) of substrate oxidized per gram tissue wet weight per hour as published earlier by laboratory [16].

2.10. Statistical analysis

To examine relationships between serum TG, IMLC, and specific variables, Pearson Product Moment Correlation and linear regression analyses were used. Paired *t*-test was used to compare data from pre- and post-fat treatment (4-h HFM or 7-day HFD). Repeated measures ANOVA was used to examine group differences of the data in terms of interactions of body composition (lean vs. obese) × environmental stimulus (control vs. 4-h HFM/7-day HFD or fatty acid-treatment). Post hoc analyses were performed as appropriate. Statistical analyses were performed using SPSS Statistics for Windows version 20.0 (IBM, Armonk, NY). All group data collected are presented as means ± SEM, and an α-level for statistical significance was set a priori at $P \leq 0.05$.

3. Results

3.1. Subject profiles (Table 1)

Groups delineated as lean and obese are described for each figure. Collectively, results indicated that the lean/non-obese individuals had BMIs ≦25 kg/m^2 and obese subjects had BMIs ranging from 35 to 50 kg/m^2 indicating that target goals were obtained. Lean subjects were non-insulin resistant as defined by HOMA-IR obtained from fasting glucose and insulin measurements (non-insulin resistance: HOMA-IR ≪ 3.0). All subjects had fasting glucose levels ≪100 mg/dl and were non-diabetic. Furthermore, subjects were in the early follicular phase of their menstrual cycle as indicated by fasting estradiol and progesterone values and were sedentary but healthy as established by activity questionnaire responses and VO_2 peak measurements (15–32 ml/kg/min).

3.2. Correlations of serum triacylglycerides (TAG) and intramyocellular lipid content (IMLC) and peroxisomal biogenesis factor 19 (Pex19)

To determine if a relationship exists between IMLC (i.e., TAG/lipid droplets) and peroxisomal biogenesis in HSkM, muscle lipid content was quantified by TAG and BODIPY staining assays and were correlated with PEX19 from human biopsies in the pre-high fat meal and post-7d diet protocol conditions. The gene PEX19 encodes a protein involved in early peroxisomal biogenesis, which acts both as a cytosolic chaperone and as an import receptor for peroxisomal membrane proteins [27]. The amount of PEX19 protein was significantly, positively correlated to serum TAG level (Fig. 1A) ($r = 0.42$, $P = 0.05$) and to IMTG content (Fig. 1B) ($r = 0.47$, $P = 0.02$). To further demonstrate the association between lipid and PEX19, BODIPY staining was used to histochemically quantify and to visualize neutral lipid from HSkM (Fig. 1C & E). Similarly, lipid droplet fluorescence and PEX19 protein amount was also significantly correlated ($r = 0.67$, $P = 0.002$) (Fig. 1C). However, PEX19 protein content was not significantly different between the lean and obese subjects (Fig. 1D).

3.3. Peroxisomal-mitochondrial fatty acid oxidation is significantly correlated with IMLC in HSkM

To test whether IMLC is associated with peroxisomal activity, we performed BODIPY staining for lipid droplets using HSkM tissue cryosections obtained pre-high fat meal. A significant correlation ($r = 0.70$; $P = 0.01$) was demonstrated between lipid droplets and complete palmitate oxidation (Fig. 2A), palmitate oxidation in the presence of etomoxir ($r = 0.65$, $P = 0.02$) (Fig. 2B) and lignocerate oxidation ($r = 0.63$, $P = 0.02$) (Fig. 2C).

3.4. Lipid overload (high fat meal/high fat diet; HFM/HFD) induces peroxisomal biogenesis gene expression

To test whether acute lipid overload can induce the mRNA of peroxisomal biogenesis genes, an experimental design was employed on Day 1 of the study utilizing a single HFM with 70% of calories from dietary fat. The 70-kDa peroxisomal membrane protein (PMP70) is a peroxisomal specific ATP binding cassette transporter believed to import long chain and odd chain fatty acids into the peroxisomal matrix for partial β-oxidation [10] [28]. In response to

the HFM, *PMP70* gene expression was increased by 190% at 4 h post HFM ($P \leq 0.05$, Fig. 3A). Furthermore, there was a significant increase in *PEX19* mRNA expression (Fig. 3B) as well as *PEX11A* mRNA [involved in peroxisomal proliferation and regulation of peroxisomal division [29], (Fig. 3C)] and *PEX5* mRNA [(import receptor for peroxisomal targeting sequences containing peroxisomal matrix proteins) (Fig. 3D)] in both lean and obese subjects ($P \leq 0.05$).

To demonstrate whether a chronic HFD had an impact on the *PEX* gene expression, we fed lean and obese subjects a high fat diet (60% of calories) for 7 days. *PMP70* gene expression was significantly up-regulated ($P < 0.05$) in obese subjects (Fig. 4A). In contrast, no significant differences in the level of *PEX19* (Fig. 4B), *PEX11A* (Fig. 4C) *or PEX5* (Fig. 4D) mRNA expression were detected. Overall, when considering the data from both Figs. 3 and 4, it appears that except for PMP70 in the obese state, the peroxisomal mRNA levels are only observed to increase in the acute state (i.e., following a 4-hour HFM).

3.5. Peroxisomes respond to a high fatty acid treatment in human primary myotubes

To verify whether peroxisomal genes were responsive to high fatty acid environments, primary myotubes derived from human skeletal muscle biopsies were incubation with a 50:50 mixture of palmitate:oleate (250 μM each) for 12 h. The established peroxisomal marker, PMP70 [13,16], and other genes were measured by real-time RT-PCR assays. *PMP70* mRNA was 24% higher between control and lipid-treatment groups in obese individuals ($P = 0.07$) (Fig. 5A). Likewise, *CrOT* mRNA significantly increased in both groups ($P \ll 0.05$) (Fig. 5B) in response to lipid challenge. Additionally, *PGC-1α* mRNA expression was increased by 63% ($P = 0.08$) in the obese group, but not in the lean group (Fig. 5C). Interestingly, estrogen related receptor α (*ERRα*) mRNA increased significantly ($P < 0.05$) in primary myotubes following 12 h high fatty acid exposure (Fig. 5D).

3.6. PMP70 protein content is significantly elevated in human primary myotubes after 48 h lipid incubation

Following 48-h lipid treatment, PMP70 protein content was significantly elevated in primary myotubes derived from obese subjects ($P \ll 0.05$). In lean counterparts, PMP70 protein content was increased by 27% but did not reach statistical significance ($P = 0.09$) (Fig. 5E). This finding demonstrates that elevations in the peroxisomal marker PMP70 is inducible by high lipid exposure in human primary myotubes.

4. Discussion

This is the first report suggesting a peroxisomal presence in association with increased intramuscular lipid content (IMLC) in human skeletal muscle (HSkM). Our major findings were 1) peroxisomal gene and membrane protein levels are responsive to environmental stimuli (lipid overload) in HSkM following an acute HFM and a more chronic HFD in vivo or an acute, high fatty acid exposure in human primary myotubes in vitro and 2) IMLC is significantly correlated with peroxisomal VLCFA and LCFA oxidation [and with a condition of inhibited mitochondrial fatty acid entry (CPT1) by etomoxir] in HSkM.

In the present studies, we investigated the relationship between intramuscular triacylglycerol (IMTG) content and PEX19 protein content. PEX19 is a cytosolic chaperone responsible for imparting class 1 peroxisomal targeting signal (PTS1) proteins to the peroxisomal membrane to confer function. Moreover, PEX19 can mediate peroxisomal membrane protein formation by cooperating with other peroxins; for example, peroxisomal biogenesis factor 16 [30,31]. To expand, in the cytoplasm, PEX19 is believed to mediate peroxisomal membrane protein (PMP) assembly to facilitate peroxisomal membrane elongation at the early stage of peroxisomal biogenesis [15]. New insights into the distribution, protein abundance, and subcellular localization of the endogenous peroxisomal biogenesis proteins including PEX19 in different fiber types of the adult mouse are reported in the literature [15,32]. Therefore, it is reasonable to suggest that PEX19 protein elevations may be linked to peroxisomal biogenesis in HSkM. However, this association does not establish a cause-effect relationship between IMTG/lipid droplets and an induction of peroxisomal biogenesis or function, with future studies still required. It is also noteworthy that we did not observe differences between lean and obese subjects. Thus, it appears that obesity per se does not appear to drive peroxisomal induction in HSkM but rather the extent of the lipid milieu, which can be quite variable among both lean and obese individuals. Further studies that include lipidomic analyses along with high fat treatment to explain the relationship between specific intramyocellular lipid species and induction of peroxisomal β-oxidation function in HSkM may prove insightful.

Peroxisomal and mitochondrial functions are closely linked to perform lipid oxidation. Our laboratory demonstrated that *PEX19* gene expression was elevated following PGC-1α induction of peroxisomal activity [16]. Data also suggested that human primary myotubes with ad-PGC-1α overexpression increased mitochondrial *citrate synthesis, Tfam, and PMP70* mRNA as well as concomitant increase in IMTG [16]. Similarly, albeit in rodents, we observed a co-induction in peroxisomal and mitochondrial content in an obese Zucker rat (fa/fa) model [13]. Data indicated a significant elevation in mitochondrial citrate synthase in association with elevations in peroxisomal derived catalase activity, peroxisomal density, and functional oxidation of peroxisomal specific lignoceric acid oxidation by peroxisomes and mitochondria. Both observations occurred concomitantly with significant elevations in mitochondrial complete oxidation of acyl carnitines in the obese state with higher IMLC. Despite the clear connections, the relative contribution of peroxisomes and mitochondria to overall oxidative flux is currently unknown.

To test whether IMLC is associated with peroxisomal activity, we performed BODIPY staining for lipid droplets using skeletal muscle tissue cryosections. A significant correlation ($P \ll 0.05$) between lipid droplets and palmitate oxidation (Fig. 2A), palmitate plus etomoxir (CPT1 inhibitor) treatment (Fig. 2B) and lignocerate oxidation (Fig. 2C) was demonstrated. These data suggest that peroxisomes are inducible in association with elevations in the lipid environment in HSkM, presumably to facilitate complete FAO of LCFA and VLCFA by mitochondria. Collectively, the data demonstrate that VLCFA and LCFA oxidation by peroxisomes from HSkM homogenates is significantly correlated with measures of lipid droplets (Fig. 2B & C, $P = 0.02$ and $P = 0.02$ respectively). This supports the current hypothesis that peroxisomal abundance is elevated concurrently with IMLC to provide an

alternative route for lipid disposal and thus compensate for an excess in the lipid environment to reduce lipotoxicity.

A further novel aspect of the present study is the finding that a single high fat meal (HFM) induces peroxisomal gene expression in HSkM. Indeed, peroxisomal *PEX* genes were acutely up-regulated in humans in vivo (Fig. 3). In addition to *PEX 19*, we observed an upregulation of *PMP70* gene expression. PMP70 is involved not only in importation of long- and very long chain- fatty acids into the peroxisome matrix for partial oxidation but is also a common marker for peroxisomal biogenesis due to concomitant elevated expression of the *PMP70* gene. For example, Kozawa et al. [8] noted that the hepatic mRNA expression level of *Abcd2*, a subtype of ABC transporters in rodents, increases 6-fold in 20-weeks following a HFD fed in mice. Our studies also determined that there are two additional peroxisomal genes, *PEX11A* and *PEX5*, which were elevated in response to the HFM (Fig. 3C & D). Peroxisomal proteins encoded by these two genes are also involved in peroxisomal biogenesis and membrane elongation. PEX5 functions as a peroxisomal matrix protein importer. Matrix proteins associated with targeting sequences PTS1 and PTS2 can be recognized by PEX5 for peroxisomal matrix import. Caution should be exercised at this point, however, because relative to the current paper, the study by Kozawa et al. [8] utilized hepatic tissues and an extended dietary intervention protocol. This clearly justifies further investigations in HSkM to compare findings from rodent studies.

To simulate chronic high lipid consumption typical of many individuals consuming a “Western Diet”, the present studies also investigated the effects of a 7-day HFD intervention on induction of peroxisomal genes. Data obtained using qRT-PCR demonstrated that *PMP70* mRNA expression levels were significantly ($P < 0.05$) up-regulated after 7 days of a HFD in obese subjects (Fig. 4A). However, smaller changes in *PEX* genes (i.e., *PEX19, PEX11A*, and *PEX5*) were non-significant ($P > 0.05$) in both lean and obese subjects (Fig. 4B, C, & D). Compared to the previous literature with 20 weeks of diet intervention from Kozawa et al. [8], the results in present study offered the possibility that the 7-day diet would be insufficient to elicit accumulated adaptations for PEX genes expression. Second, decreases in *PEX11A* and *PEX5* mRNA in obese subjects suggested that a longer term of HFD would be needed to promote PEX gene induction. It is also possible that the obese subjects had less IMLC in the skeletal muscle based on the positive correlation data between PEX19 and lipid content in HSkM samples obtained in the pre-HFD status. These premises will need further investigations.

Accordingly, we utilized primary myotubes to test our hypothesis that peroxisomal gene and protein expression are inducible in response to elevations in the cellular lipid environment. The mRNA/gene expression data suggested that 12-h post fatty acid treatment, the peroxisomal marker PMP70 ($P = 0.07$) (Fig. 5A) and the peroxisomal acylcarnitine transporter CrOT mRNA ($P \ll 0.05$) were upregulated (Fig. 5B), presumably to facilitate lipid oxidation in response to a lipid challenge. In the primary myotubes, *PGC-1α* mRNA was also noted to be elevated following lipid incubation/overload (Fig. 5C) although statistical significance was not achieved. However, recently our group applied a gain of function approach to demonstrate the role of PGC-1α in inducing both peroxisomal activity

and mitochondrial biogenesis [16] and induced overexpression resulted in increased peroxisomal mRNA and lipid oxidation in human derived primary myotubes.

Linked with PGC1α induction, peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the superfamily of nuclear receptors. Three isoforms (α, δ, and γ) have been described. They act on DNA response elements as heterodimers with the nuclear retinoic acid receptor. Their natural activating ligands are fatty acids and lipid-derived substrates. PPAR-α is present in liver, heart, and, to a lesser extent, skeletal muscle. When activated, it promotes fatty acid oxidation, ketone body synthesis, and glucose sparing. Under the present experimental conditions *PPARα* mRNA was not elevated following lipid incubation (data not shown) suggesting that the peroxisomal proliferation signaling pathway(s) may differ in HSkM compared to other tissues such as the liver [11,12,33]. Alternatively, it is noteworthy that *ERRα* gene expression was significantly elevated ($P < 0.05$; Fig. 5D) following lipid incubation. ERRα would be a transcriptional factor partnered with PGC1α in tissues that rely primarily on mitochondrial oxidative metabolism for energy production such as heart and skeletal muscle [34-36]. As such, the present findings suggest that ERRα may act to potentiate peroxisomal biogenesis and concomitant enhance the mitochondria's ability to completely oxidize peroxisomal export products which include medium chain acyl-carnitines.

In conclusion, the evidence herein suggests that peroxisomal abundance by measures of mRNA, protein expression, and fatty acid, radioisotope activity assays are responsive to the lipid milieu. Thus, the current study provides the first evidence for a peroxisomal presence associated with lipid oversupply and IMTG accumulation in human skeletal muscle which may include a peroxisomal-mitochondrial functional cooperation regarding lipid oxidation, obesity and diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to Dr. Sanghee Park, Ph.D. (East Carolina University) for assistance with human skeletal muscle cell culture.

Funding agencies

This work was supported in part by a seed grant from the Diabetes Obesity Institute, East Carolina University (RNC), NIH R15HL113854 (RCH) and R01 AR070200 (JJB).

References

[1]. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle.Am J Physiol Endocrinol Metab 2000;279:E1039–44. [PubMed: 11052958]

[2]. Thyfault JP, Kraus RM, Hickner RC, Howell AW, Wolfe RR, Dohm GL. Impaired plasma fatty acid oxidation in extremely obese women. Am J Physiol Endocrinol Metab 2004;287:E1076–81. [PubMed: 15280153]

[3]. Fisher-Wellman KH, Weber TM, Cathey BL, Brophy PM, Gilliam LA, Kane CL, et al. Mitochondrial respiratory capacity and content are normal in young insulin-resistant obese humans. Diabetes 2014;63:132–41. [PubMed: 23974920]

[4]. Hulver MW, Berggren JR, Cortright RN, Dudek RW, Thompson RP, Pories WJ, et al. Skeletal muscle lipid metabolism with obesity. Am J Physiol Endocrinol Metab 2003;284:E741–7. [PubMed: 12626325]

[5]. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 2008;7:45–56. [PubMed: 18177724]

[6]. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002;51:2944–50. [PubMed: 12351431]

[7]. Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. Physiol Rev 2010;90:367–417. [PubMed: 20086080]

[8]. Kozawa S, Honda A, Kajiwara N, Takemoto Y, Nagase T, Nikami H, et al. Induction of peroxisomal lipid metabolism in mice fed a high-fat diet. Mol Med Rep 2011;4: 1157–62. [PubMed: 21850377]

[9]. Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, et al. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature 1993;361:726–30. [PubMed: 8441467]

[10]. Lodhi IJ, Semenkovich CF. Peroxisomes: a nexus for lipid metabolism and cellular signaling. Cell Metab 2014;19:380–92. [PubMed: 24508507]

[11]. Fiamoncini J, Turner N, Hirabara SM, Salgado TM, Marcal AC, Leslie S, et al. Enhanced peroxisomal beta-oxidation is associated with prevention of obesity and glucose intolerance by fish oil-enriched diets. Obesity (Silver Spring) 2013;21:1200–7. [PubMed: 23666909]

[12]. Leclercq S, Skrzypski J, Courvoisier A, Gondcaille C, Bonnetain F, Andre A, et al. Effect of dietary polyunsaturated fatty acids on the expression of peroxisomal ABC transporters. Biochimie 2008;90:1602–7. [PubMed: 18585430]

[13]. Noland RC, Woodlief TL, Whitfield BR, Manning SM, Evans JR, Dudek RW, et al. Peroxisomal-mitochondrial oxidation in a rodent model of obesity-associated insulin resistance. Am J Physiol Endocrinol Metab 2007;293:E986–1001. [PubMed: 17638705]

[14]. Wicks SE, Vandanmagsar B, Haynie KR, Fuller SE, Warfel JD, Stephens JM, et al. Impaired mitochondrial fat oxidation induces adaptive remodeling of muscle metabolism. Proc Natl Acad Sci U S A 2015;112:E3300–9. [PubMed: 26056297]

[15]. Colasante C, Chen J, Ahlemeyer B, Bonilla-Martinez R, Karnati S, Baumgart-Vogt E. New insights into the distribution, protein abundance and subcellular localisation of the endogenous peroxisomal biogenesis proteins PEX3 and PEX19 in different organs and cell types of the adult mouse. PLoS One 2017;12:e0183150. [PubMed: 28817674]

[16]. Huang TY, Zheng D, Houmard JA, Brault JJ, Hickner RC, Cortright RN. Overexpression of PGC-1alpha increases peroxisomal activity and mitochondrial fatty acid oxidation in human primary myotubes. Am J Physiol Endocrinol Metab 2017;312:E253–63. [PubMed: 28073778]

[17]. Wanders RJ, Ferdinandusse S, Brites P, Kemp S. Peroxisomes, lipid metabolism and lipotoxicity. Biochim Biophys Acta 2010;1801:272–80. [PubMed: 20064629]

[18]. Jakobs BS, Wanders RJ. Fatty acid beta-oxidation in peroxisomes and mitochondria: the first, unequivocal evidence for the involvement of carnitine in shuttling propionyl-CoA from peroxisomes to mitochondria. Biochem Biophys Res Commun 1995;213:1035–41. [PubMed: 7654220]

[19]. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005;307:384–7. [PubMed: 15662004]

[20]. Dagenais GR, Tancredi RG, Zierler KL. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm.J Clin Invest 1976;58:421–31. [PubMed: 956375]

[21]. Qu HQ, Li Q, Rentfro AR, Fisher-Hoch SP, McCormick JB. The definition of insulin resistance using HOMA-IR for Americans of Mexican descent using machine learning. PLoS One 2011;6:e21041. [PubMed: 21695082]

[22]. Kane DA, Lin CT, Anderson EJ, Kwak HB, Cox JH, Brophy PM, et al. Progesterone increases skeletal muscle mitochondrial H2O2 emission in nonmenopausal women. Am J Physiol Endocrinol Metab 2011;300:E528–35. [PubMed: 21189359]

[23]. Prats C, Gomez-Cabello A, Nordby P, Andersen JL, Helge JW, Dela F, et al. An optimized histochemical method to assess skeletal muscle glycogen and lipid stores reveals two metabolically distinct populations of type I muscle fibers. PLoS One 2013;8:e77774. [PubMed: 24204959]

[24]. Spangenburg EE, Pratt SJ, Wohlers LM, Lovering RM. Use of BODIPY (493/503) to visualize intramuscular lipid droplets in skeletal muscle. J Biomed Biotechnol 2011; 2011:598358. [PubMed: 21960738]

[25]. Teodoro BG, Sampaio IH, Bomfim LH, Queiroz AL, Silveira LR, Souza AO, et al. Long-chain acyl-CoA synthetase 6 regulates lipid synthesis and mitochondrial oxidative capacity in human and rat skeletal muscle. J Physiol 2017;595:677–93. [PubMed: 27647415]

[26]. Berggren JR, Tanner CJ, Houmard JA. Primary cell cultures in the study of human muscle metabolism. Exerc Sport Sci Rev 2007;35:56–61. [PubMed: 17417051]

[27]. Schrul B, Schliebs W. Intracellular communication between lipid droplets and peroxisomes: the Janus face of PEX19. Biol Chem 2018;399(7):741–9. [PubMed: 29500918]

[28]. Imanaka T, Aihara K, Takano T, Yamashita A, Sato R, Suzuki Y, et al. Characterization of the 70-kDa peroxisomal membrane protein, an ATP binding cassette transporter. J Biol Chem 1999;274:11968–76. [PubMed: 10207018]

[29]. Koch J, Pranjic K, Huber A, Ellinger A, Hartig A, Kragler F, et al. PEX11 family members are membrane elongation factors that coordinate peroxisome proliferation and maintenance. J Cell Sci 2010;123:3389–400. [PubMed: 20826455]

[30]. Jones JM, Morrell jC, Gould SJ. PEX19 is a predominantly cytosolic chaperone and import receptor for class 1 peroxisomal membrane proteins. J Cell Biol 2004;164: 57–67. [PubMed: 14709540]

[31]. Smith JJ, Aitchison JD. Peroxisomes take shape. Nat Rev Mol Cell Biol 2013;14: 803–17. [PubMed: 24263361]

[32]. Qian G, Fan W, Ahlemeyer B, Karnati S, Baumgart-Vogt E. Peroxisomes in different skeletal cell types during intramembranous and endochondral ossification and their regulation during osteoblast differentiation by distinct peroxisome proliferator-activated receptors. PLoS One 2015;10:e0143439. [PubMed: 26630504]

[33]. Lawrence JW, Li Y, Chen S, DeLuca JG, Berger JP, Umbenhauer DR, et al. Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) alpha. PPAR alpha fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expresson. J Biol Chem 2001;276:31521–7. [PubMed: 11418601]

[34]. Heard DJ, Norby PL, Holloway J, Vissing H. Human ERRgamma, a third member of the estrogen receptor-related receptor (ERR) subfamily of orphan nuclear receptors: tissue-specific isoforms are expressed during development and in the adult. Mol Endocrinol 2000;14:382–92. [PubMed: 10707956]

[35]. Sladek R, Bader JA, Giguere V. 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 1997;17:5400–9. [PubMed: 9271417]

[36]. Vega RB, Kelly DP. A role for estrogen-related receptor alpha in the control of mitochondrial fatty acid beta-oxidation during brown adipocyte differentiation. J Biol Chem 1997;272:31693–9. [PubMed: 9395511]

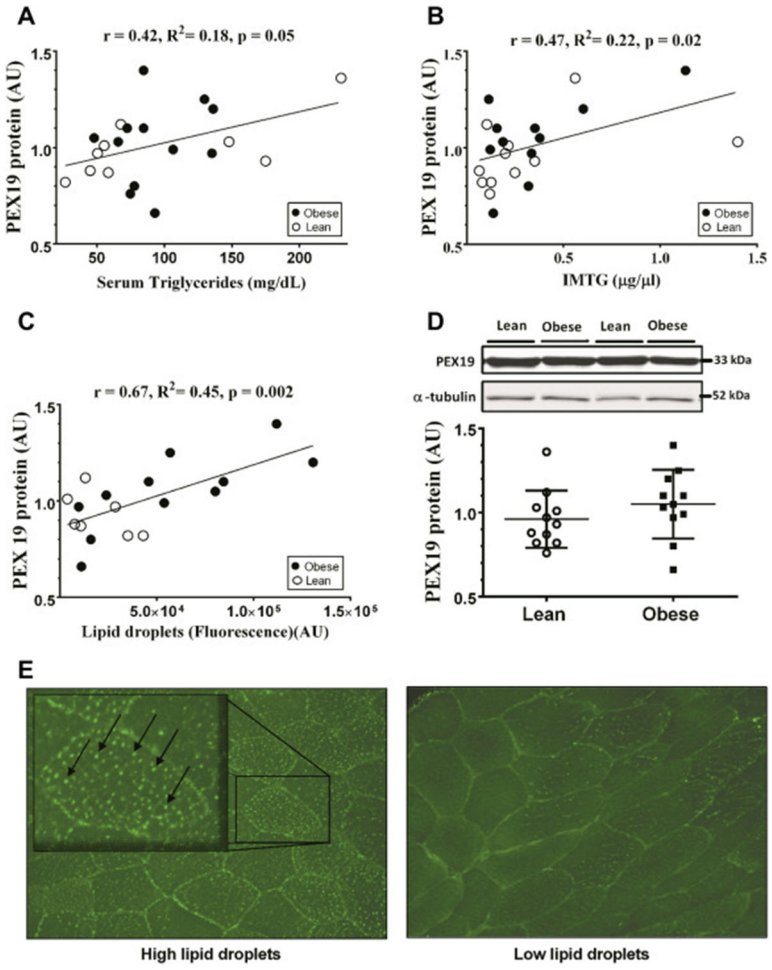

Fig. 1.

Intramyocellular lipid is significantly correlated with peroxisomal biogenesis factor 19 (PEX19) protein in human skeletal muscle. TAG assay, BODIPY staining, and western blot were performed to quantify the neutral lipid and PEX19 content respectively in human skeletal muscle tissue extracts in the pre-high fat meal and post-7d diet protocol conditions. Results are shown as a scatter plot of paired variables and regression analysis (A) Serum TG and PEX19 (n = 21) (B) IMTG and PEX19 (n = 21), (C) lipid droplets and PEX19 (n = 18), (D) western blots for PEX19 protein and α-tubulin (loading control); data are mean ± SEM expressed as arbitrary units, (E) BODIPY: high lipid and low lipid droplets.

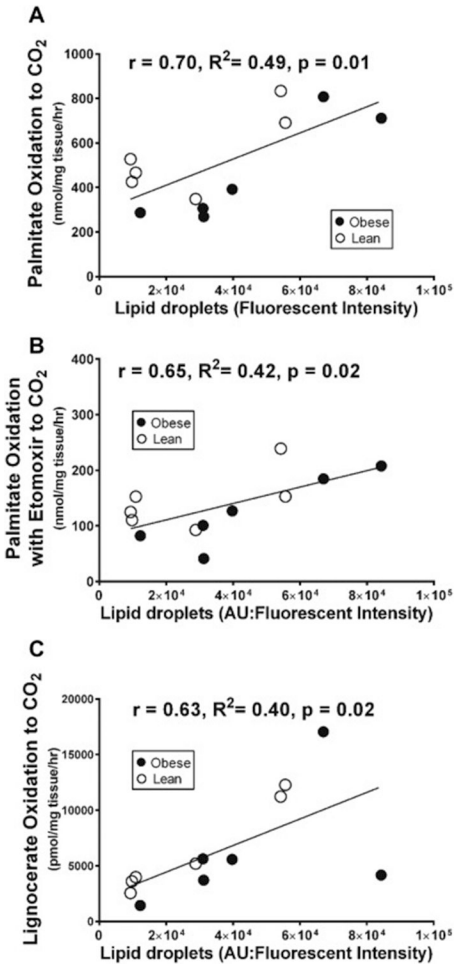

Fig. 2.

Mitochondrial-peroxisomal fatty acid oxidation is significantly correlated with intramyocellular lipid content in human skeletal muscle. Correlation between palmitate complete oxidation as $^{14}CO_2$ and lipid droplet (fluorescent intensity) (N = 12; $r = 0.70$; $P = 0.01$) (B) palmitate (C16:0) oxidation with etomoxir and lipid droplet (N = 12; $r = 0.65$; $P = 0.02$), (C) correlation scatter plot between intramyofiber lipid droplets by BODIPY assessment and complete lignocerate (C24:0) oxidation (N = 12; $r = 0.63$; $P = 0.02$).

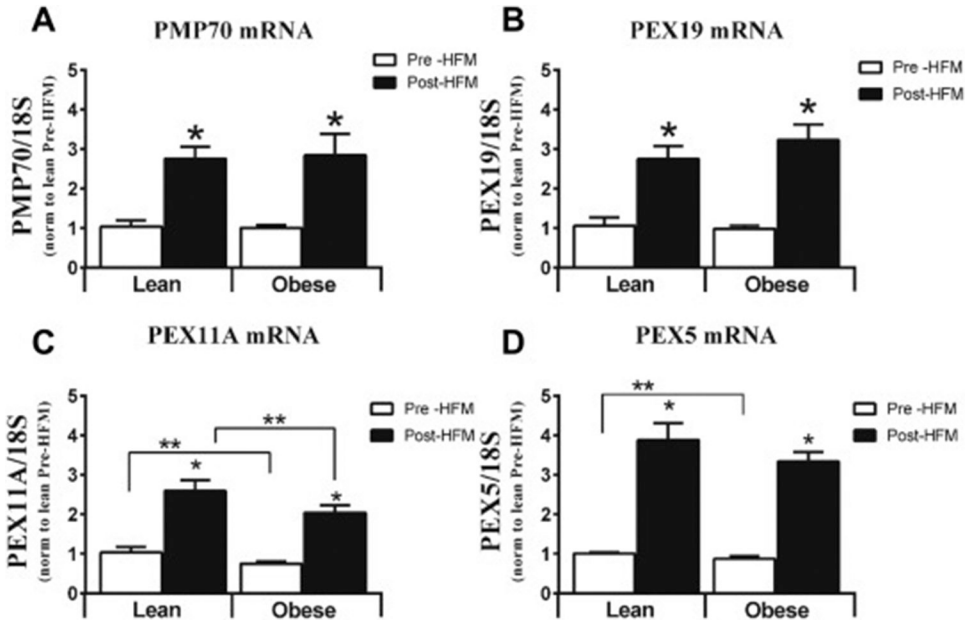

Fig. 3.

Peroxisomal mRNA is up-regulated in response to a high fat meal in human skeletal muscle in both lean and obese subjects. mRNA expression levels (A) PMP70, (B) PEX19, (C) PEX11A and (D) PEX5 were significantly up-regulated 4-h post - high fat meal. mRNA expression levels were quantified using RT-PCR. Data (mean ± SEM) were normalized to 18S and then compared to the lean subjects. *$P \leq 0.05$ vs. pre-HFM. ** $P < 0.05$ between lean and obese. HFM: high fat meal (n = 4/group).

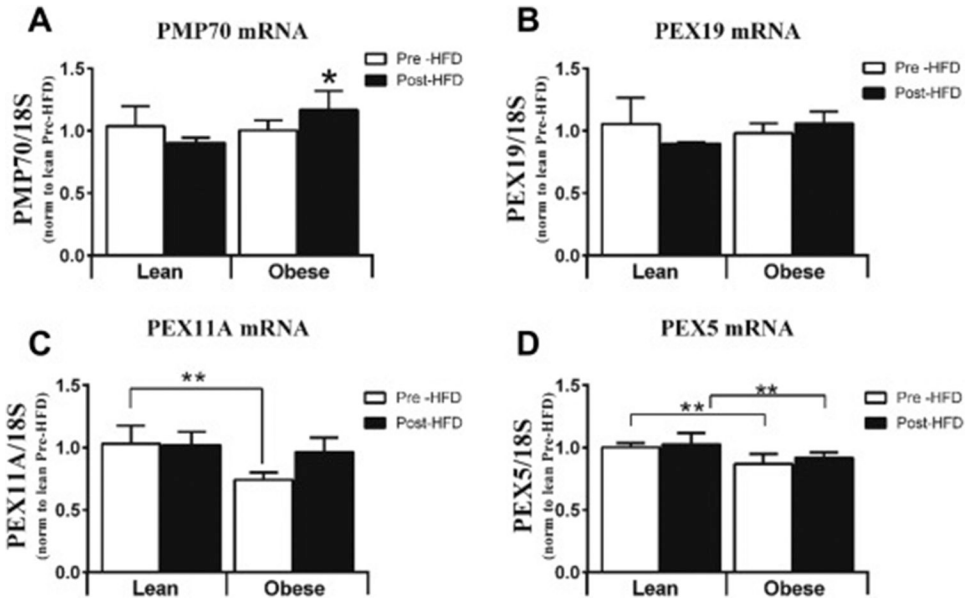

Fig. 4.

PMP70 mRNA is up-regulated after 7 days high fat diet in human skeletal muscle from obese subjects. mRNA levels for (A) *PMP70*, (B) *PEX19*, (C) *PEX11A* and (D) *PEX5* by RT-PCR. * $P \leq 0.05$ vs. pre-HFD. ** $P < 0.05$ between lean and obese. HFM: high fat meal. HFD: high fat diet. Data (mean ± SEM) were normalized to 18S and then compared to the lean subjects (n = 4/group).

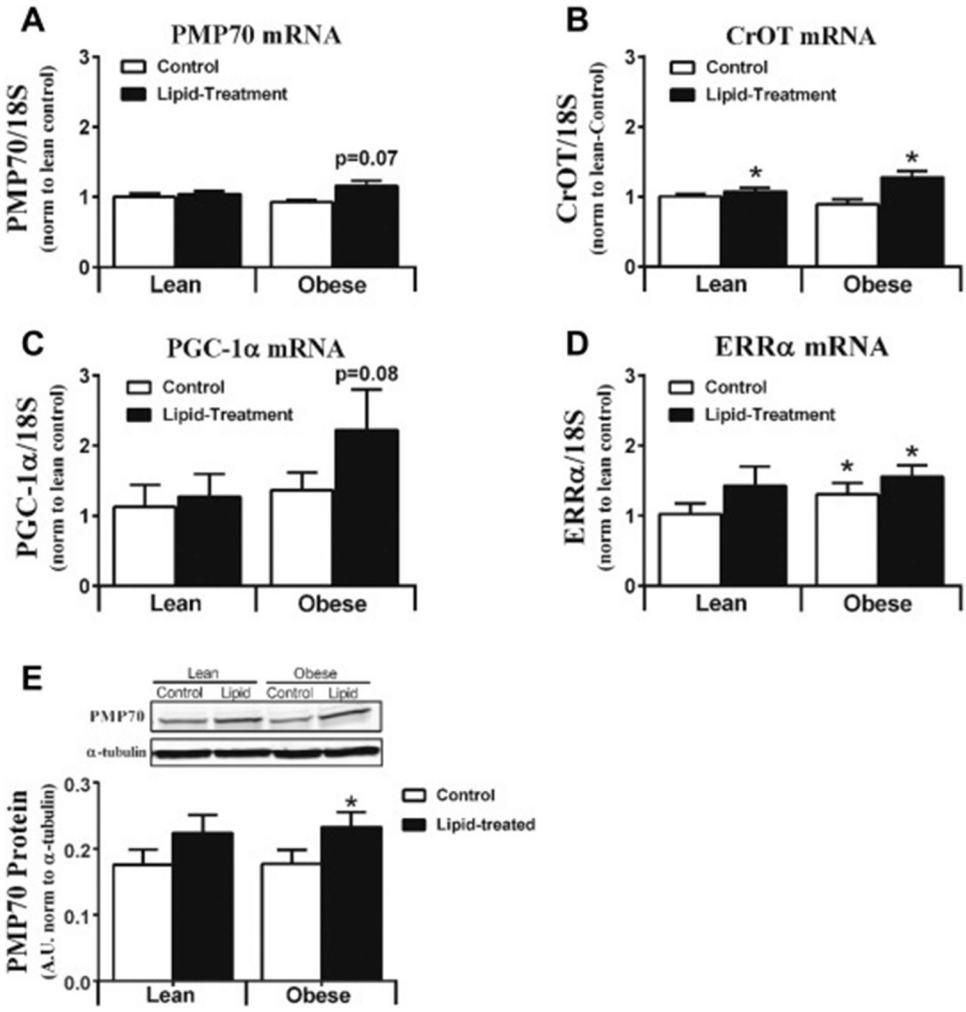

Fig. 5.

Peroxisomal mRNA and protein responses to a high fatty acid treatment in human primary myotubes. Quantification of (A) PMP70, (B) CrOT, (C) *PGC-1α, and* (*D*) ERRα gene expression by RT-PCR after 12 h high fat incubation on day 5 of differentiation in human primary myotubes (n = 4/group). All data were normalized to *18S*. (E) PMP70 protein content in human primary myotubes derived from lean vs. obese subjects after high fatty acid treatment for 48 h (n = 6/group). PMP70: peroxisomal membrane protein; *CrOT*: carnitine octanoyl transferase; *ERRα*: (estrogen related receptor α). Data are presented as mean ± SEM normalized to lean controls. **P* ≪ 0.05 vs. control.

Table 1

Subject profiles.

Group	Fig. 1		Fig. 2		Figs. 3, 4		Fig. 5		Fig. 5E	
	Lean (n = 11)	Obese (n = 11)	Lean (n = 6)	Obese (n = 6)	Lean (n = 4)	Obese (n = 4)	Lean (n = 4)	Obese (n = 4)	Lean (n = 6)	Obese (n = 6)
Age (years)	34.5 ± 2.3	32.0 ± 2.4	25.0 ± 1.8	34.7 ± 2.8 *	30.8 ± 4.1	36.4 ± 3.8	29.0 ± 2.5	37.0 ± 4.7	27.7 ± 2.9	33.5 ± 3.6
Height (cm)	166.7 ± 1.5	165.0 ± 1.2	165.5 ± 1.8	164.6 ± 3.0	169.5 ± 0.0	167.4 ± 0.0	165.7 ± 5.7	169.1 ± 3.2	161.7 ± 0.8	168.4 ± 2.6
Weight (kg)	65.3 ± 1.9	107.7 ± 3.1 **	61.7 ± 4.2	93.5 ± 6.6 **	71.9 ± 2.3	106.7 ± 7.0 **	67.3 ± 2.7	142.0 ± 8.9 *	58.7 ± 1.6	132.2 ± 9.9 **
BMI (km/m^2)	23.5 ± 0.6	39.6 ± 1.2 **	22.6 ± 1.5	34.6 ± 2.7 **	25.1 ± 1.1	38.1 ± 2.7 **	24.6 ± 0.8	49.6 ± 2.1 *	22.5 ± 0.7	46.4 ± 2.7 **
Body fat (%)	37.7 ± 1.8	50.7 ± 1.0 **	33.8 ± 2.3	42.9 ± 1.8 *	41.6 ± 1.3	48.5 ± 2.5 *				
Fasting glucose (mg/dL)	89.5 ± 1.4	90.9 ± 2.4	88.8 ± 5.2	94.8 ± 7.5	90.7 ± 2.9	96.7 ± 4.3	85.3 ± 1.5	89.8 ± 3.6	86.2 ± 4.6 (n = 5)	85.8 ± 0.9 (n = 5)
Insulin (μIU/mL)			5.8 ± 0.7	18.9 ± 8.6	7.6 ± 1.2	11.8 ± 2.1	6.5 ± 1.3	15.4 ± 1.5 **	8.8 ± 0.5 (n = 5)	16.1 ± 1.6 * (n = 5)
HOMA-IR			1.3 ± 0.2	4.9 ± 2.3	1.7 ± 0.3	2.8 ± 0.5	1.4 ± 0.3	3.4 ± 0.3 **	1.5 ± 0.4 (n = 5)	3.4 ± 0.3 ** (n = 5)
E2 (pM)			338.4 ± 99.6	325.1 ± 136.1	348.5 ± 101.2	201.7 ± 84.6				
P4 (nM)			3.1 ± 0.6	1.5 ± 0.3	2.3 ± 0.9	1.9 ± 0.6				
Triglyceride (mg/dL)	93.2 ± 21.3	98.6 ± 8.0	75.7 ± 12.7	106.6 ± 20.4	74.3 ± 19.2	66.2 ± 8.8	69.5 ± 11.8	73.3 ± 15.2	102.2 ± 24.0 (n = 5)	58.5 ± 4.6 (n = 5)
VO_2max (ml/kg/min)	20.3 ± 1.7	15.0 ± 0.6	32.3 ± 1.9	31.3 ± 0.0	28.9 ± 3.5	20.2 ± 1.6				

Data are mean ± SEM. Abbreviations: BMI: body mass index; HOMA-IR: homeostasis model assessment for insulin resistance; E2: estradiol; P4: progesterone.

* $P < 0.05$ vs. lean group within each figure.

** $P < 0.001$ vs. lean group within each figure.