Regulation of Mitochondrial Biogenesis by Lipoprotein Lipase in Muscle of Insulin-Resistant Offspring of Parents With Type 2 Diabetes

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Recent studies reveal a strong relationship between reduced mitochondrial content and insulin resistance in human skeletal muscle, although the underlying factors responsible for this association remain unknown. To address this question, we analyzed muscle biopsy samples from young, lean, insulin resistant (IR) offspring of parents with type 2 diabetes and control subjects by microarray analyses and found significant differences in expression of ~512 probe pairs. We then screened these genes for their potential involvement in the regulation of mitochondrial biogenesis using RNA interference and found that mRNA and protein expression of lipoprotein lipase (LPL) in skeletal muscle was significantly decreased in the IR offspring and was associated with decreased mitochondrial density. Furthermore, we show that LPL knockdown in muscle cells decreased mitochondrial content by effectively decreasing fatty acid delivery and subsequent activation of peroxisome proliferator-activated receptor (PPAR)-γ. Taken together, these data suggest that decreased mitochondrial content in muscle of IR offspring may be due in part to reductions in LPL expression in skeletal muscle resulting in decreased PPAR-γ activation.

Although reduced mitochondrial function, as a result of decreased mitochondrial content, is associated with increased intramyocellular lipid content and insulin resistance in human skeletal muscle (1–3), the underlying factors responsible for these changes remain unknown. Two previous microarray studies implicate downregulation of peroxisome proliferator activated–receptor (PPAR)-γ coactivator-1α (PGC-1α) responsive genes in patients with type 2 diabetes (T2D) (4,5) as being responsible for decreased mitochondrial biogenesis; however, these findings could not be replicated in lean, nondiabetic, insulin resistant (IR) offspring of parents with T2D (2).

To identify potential factors responsible for reduced mitochondrial content in IR offspring, we analyzed mRNA expression in skeletal muscle of young, lean IR offspring and control subjects using microarray analysis and found ~512 probe pairs, which were significantly different between the IR offspring and the insulin-sensitive control subjects. Consistent with our previous studies, we found no differences in the expression of PGC-1α or gene sets regulated by PGC-1α using Gene Enrichment Set Analysis (5). However, we did observe significant reductions in the expression of genes involved in mitochondrial fatty acid oxidation in the IR offspring group (Supplementary Table 2). We therefore screened these 250 genes for their potential role in the regulation of mitochondrial biogenesis by treating RC13 cells (human rhabdomyosarcoma cell line) with RNA interference (RNAi) reagents to knock down expression of these candidate genes and monitored mitochondrial content in these cells using a mitochondrial-specific dye. Using this approach, we identified reduced expression of lipoprotein lipase (LPL) as a potential factor that may be responsible in part for reduced muscle mitochondrial content in IR offspring.

RESEARCH DESIGN AND METHODS

All subjects (N = 22) were recruited by means of local advertising and were prescreened to confirm that they were in excellent health, lean, nonsmoking, and taking no medications. A birth weight >2.3 kg and a sedentary lifestyle, as defined by an activity index questionnaire (6), were also required. Qualifying subjects underwent a 3-h oral glucose tolerance test (with a 75-g oral glucose load) with calculation of an insulin sensitivity index (ISI) (7), after which two subgroups of subjects were selected to identify extreme phenotypes for insulin resistance and increased insulin sensitivity.

The group of IR subjects (n = 11) had at least one parent or grandparent with T2D, at least one other family member with T2D, and an ISI of <4.0 (7). The insulin-sensitive control subjects (n = 11) were defined by an ISI >7.0 and without a family history of T2D. The groups were matched for age, weight, BMI, and activity.

Written consent was obtained from each subject after the purpose, nature, and potential consequences of the study had been explained. The protocol was approved by the Yale Human Investigation Committee.

Diet and study preparation. Subjects were instructed to eat a regular, weight-maintenance diet containing at least 150 g carbohydrate and not to perform any exercise other than normal walking for the 3 days before the study. Subjects were admitted to the Yale–New Haven Hospital the evening before the study and fasted from 10 p.m. with free access to water until the completion of the study the following day.

Measurement of metabolites and hormones. Plasma glucose concentrations were measured with a YSI 2300 Analyzer. Plasma concentrations of insulin were measured using a double-antibody radioimmunoassay kit.

Muscle biopsy. The skin over the vastus lateralis muscle was steriley prepared with betadine, and 1% lidocaine was injected subcutaneously. A 2-cm incision was made using a scalpel, and a baseline punch muscle biopsy was extracted using a 5-mm Bergstrom biopsy needle (Warsaw, IN). A piece of muscle tissue was dissected with a scalpel and immediately fixed in glutaraldehyde buffer for

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DIABETES
from Dharmacon. RC13 and L6 myotubes were transfected with small interfering RNA (siRNA) reagents against either non-targeting or LPL (50 nmol); and incubated for 48 h. Cells were fixed with 3.8% formaldehyde and treated with both Mitotracker Green (1:1,000, Molecular Probe) for 30 min and DAPI (1:1,000; Molecular Probe) for 10 min. Fluorescence was detected with a multiple fluorescence reader and the mitochondria-to-nuclear ratio was calculated. When the mitochondria-to-nuclear ratios deviated >20% from the control samples, the candidate genes were further analyzed by Western blotting to confirm change in mitochondrial protein expression.

**Determination of mitochondrial activity by MTT assay.** RC13 and L6 were spread in 96-well plates and transfected with siRNA reagent against either non-targeting or LPL. Two days after the RNAi treatment, the cells were incubated with dye-free medium and incubated for 2 h with 0.5 mg/mL 3-(4,5-dimethyl-2-yl)-2,5- diphenyltetrazolium bromide (MTT) before 1-h lysis by addition of two volumes of 10% SDS in 0.1 mol/L HCl (Cell Proliferation Kit [MTT], Sigma-Aldrich). The lysates were shaken and then formazan dye produced by mitochondrial dehydrogenases was photometrically measured at 565 nm.

**Fatty acid stock preparation.** Fatty acids were bound to fatty acid-free BSA as previously described (11) with minor modifications. Fatty acids (200 mM in ethanol) were diluted 1:12 into 10% BSA. The fatty acid mixture was gently agitated at 37°C for 1 h. The control medium containing ethanol and BSA was prepared similarly. The stock solutions were stored at −20°C, and working solutions were prepared fresh by diluting stock solution in 2% FCS⁄α-MEM.

**Electron microscopy.** Individually labeled muscle supernatants were analyzed by electron microscopy by serial sectioning and two-dimensional reconstruction as previously described (12). In brief, a Reichert Ultracut ultramicrotome, collected on formvar- and carbon-coated grids, stained with 2% uranyl acetate and lead citrate, and examined in a Philips 410 electron microscope. Only cross-sections of skeletal muscle were examined for quantification of mitochondrial density. For each individual muscle, 15 random pictures were taken at a magnification of ×7,100 and performed at a final magnification of ×25,000. The volume density of mitochondria was estimated using the point-counting method. The average volume density was calculated for each individual muscle sample and was used to calculate the average volume density for each treatment.

**Oxygen consumption assay.** Basal oxygen consumption was measured as previously described (12). In brief, a Seahorse Bioscience XF24–3 Extracellular Flux Analyzer was used to measure the rate of O2 consumption of L6 cells cultured in a collagen I–coated XF24 V7 cell culture microplate (Seahorse Bioscience). L6 myoblasts were seeded in XF24-well microplates at 2.0 × 10^4 cells per well (area 0.32 cm²) in 100 μL growth medium and then incubated at 37°C with 5% CO2 overnight. The following day, an additional 100 μL growth medium was added; 1 day later, the medium was replaced with fresh medium. The RNAi experiment was performed against PPAR-γ 48 h before measurement of oxygen consumption. The basal oxygen consumption by PPAR-γ (1 μmol/L) treatment was performed 48 before measuring oxygen consumption at 50 μmol/L.

**Statistical analysis.** Statistical analyses were performed using ANOVA and unpaired Student t test with JMP software (SAS Institute). Fisher exact test was used in the microarray experiments, and Pearson correlation coefficients were calculated for correlation analyses. All data are expressed as means ± SE.

**RESULTS**

**Microarray in the offspring of T2D parents and matched control subjects.** To assess gene expression in muscle of IR offspring, we purified mRNA in muscle biopsy samples taken from vastus lateralis muscles and then performed microarray data analysis using Human Genome U133 Affymetrix plus 2.0 GeneChip. Out of 53,947 probe pairs, 512 showed significant differences in expression compared with control subjects (Fig. L1 and Supplementary Tables 1 and 2). Consistent with our previous studies, we found no differences in the expression of PGC-1α or gene sets regulated by PGC-1α using Gene Expression Set Analysis (5). However, we did observe significant reductions in the expression of genes involved in mitochondrial fatty acid oxidation in the IR offspring (Supplementary Table 2).
FIG. 1. Microarray in the offspring of T2D parents and age-matched control subjects. A: Heat-map representations of genes detected in expression analysis of skeletal muscle of the offspring of T2D parents and age-matched control subjects. The list shows the top 100 genes by P value between two groups. B: Screening experiments using Mitotracker-to-DAPI ratio were performed after treatment with RNAi against 250 candidate genes in RC13 human rhabdomyosarcoma cell line. After the screening experiments, 45 genes were tested by Western blotting with MTCOI, PDH, and SDHA antibodies, and 15 genes showed significant change compared with treatment of nontargeting RNAi. C: Heat-map representations of overlapped genes in first microarray and second microarray. D: Six genes were overlapped with first microarray, second microarray, and former screening (B). A total of 2 out of 6 genes showed significant difference determined by RT-qPCR using TaqMan probe in control subjects (n = 7) and IR offspring (n = 13).
Most of the genes on the list were orphan; so to identify a potential new regulator of mitochondrial biogenesis, we screened our genes with high throughput assays using cultured muscle cells by the ratio of mitochondrial- and nuclear-specific dye after RNAi treatment against the 250 candidate genes, which were selected based on stricter criteria. We therefore screened these 250 genes for their effect on mitochondrial density (mitochondria-to-nuclear ratio) and mitochondrial protein expression using Western blot against MTCOI, PDH, and SDHA. Out of these 250 candidate genes, we found 15 genes that changed mitochondrial density and mitochondrial protein expression (Fig. 1B). To confirm these observations in an independent group of young, lean IR offspring \((n = 6)\) and an age/weight/BMI–matched group of insulin-sensitive control subjects \((n = 5)\), we performed a second microarray study. In this study, two genes were consistently lower in the IR offspring compared with the insulin-sensitive control subjects; this finding was confirmed by real-time quantitative PCR (RT-qPCR) (Fig. 1C and D). To further confirm these screening data using RNAi, we measured several mitochondrial proteins by Western blot and found that expression of LPL mRNA and LPL protein were \(~45\%\) reduced in the IR offspring as compared with the age/weight/BMI/activity–matched control subjects (Fig. 2A and B). Protein expression of LPL was positively correlated with mitochondrial density (Fig. 2C).

**Suppression of the LPL gene by RNAi decreased mitochondrial density and protein expression in human rhabdomyosarcoma cell line RC13.** To test whether decreased expression of LPL can result in decreased mitochondrial density, we performed LPL knockdown by RNAi techniques in a human rhabdomyosarcoma cell line \((RC13)\). LPL RNAi treatment decreased LPL mRNA expression by \(~95\%\) after 48 h of RNAi treatment (Fig. 3A). Under this condition, we analyzed mitochondrial density using mitochondrial-specific dye and found that depletion of LPL mRNA decreased mitochondrial density (Fig. 3B and C). This phenomenon was observed only when cells were treated with media containing FBS, the only source of fat for culture cells, or the VLDL fraction of human blood (Fig. 2C and Supplementary Fig. 1A).

Furthermore, we found that several mitochondrial proteins, such as MTCOI, PDH, and SDH, which we have previously shown to be reduced in IR offspring (Fig. 3D). There were no significant changes in expression of cytochrome c, somatic or MnSOD. We also observed a strong decrease in porin expression, the main pathway for transport of metabolites across the mitochondrial outer membrane (Fig. 3D). Finally, we analyzed mitochondrial oxidative function using MTT dye and found that LPL depletion reduced mitochondrial function in the RC13 cell line (Fig. 3E).

**Suppression of LPL gene by RNAi decreased mitochondrial density and protein expression in L6 cell line.** To further evaluate the role of LPL in skeletal muscle, we analyzed LPL knockdown in L6 rat myotubes; in contrast to RC13, L6 differentiate into muscle fiber morphologically (Fig. 4A). The expression level of the mitochondrial proteins, such as porin, MnSOD, and cytochrome c, somatic, increased dramatically during L6 differentiation, but the others did not change (Fig. 4B). LPL RNAi treatment decreased LPL mRNA expression \(~90\%\) at 24, 48, and 96 h posttransfection (Fig. 4C). In both RC13 and L6 myotubes, we found that depletion of LPL decreased the mitochondrial proteins MTCOI and porin (Fig. 4D) and mRNA of MTCOI (data not shown) without apparent morphological change and differentiation. Finally, LPL depletion reduced mitochondrial

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**FIG. 2.** Lipoprotein lipase is decreased in skeletal muscle of young, lean IR offspring of T2D parents. A: mRNA expression of LPL determined by RT-qPCR using TaqMan probe in control subjects \((n = 7)\) and IR offspring \((n = 13)\). B: Protein expression of LPL measured by Western blotting in control subjects \((n = 7)\) and IR offspring \((n = 9)\). Results represent mean ± SEM. *\(P < 0.05\) vs. age/weight/BMI–matched healthy control subjects. C: Relationship between mitochondrial density measured by electron microscopy and LPL protein expression in skeletal muscle.
FIG. 3. LPL knockdown by RNAi in RC13 human rhabdomyosarcoma cell line. A: mRNA expression of LPL determined by RT-qPCR and Western blotting 72 h after RNAi treatment (50 μmol) in RC13. Represented LPL protein expression 48 h after RNAi treatment. B: Mitochondrial density measured by fluorescence dyes. RC13 cells were treated with RNAi reagents against either LPL or nontargeting control (50 nmol/L), maintained in 10% FBS RPMI for 48 h, fixed with 1% formaldehyde, stained with mitochondrial-specific Mitotracker Green (which stain mitochondria specifically), and scanned with multifluorescence reader. Nuclei were stained by DAPI. C: The intensity of Mitotracker dye was divided by DAPI signal to normalize cell number. RC13 cell were treated with LPL and PGC-1α RNAi reagent and maintained in either FBS-free RPMI or 10% FBS RPMI. D: Mitochondrial protein expression was determined by Western blotting 48 h after RNAi treatment (n = 8). Cyt-c, cytochrome c. E: MTT assay. RC13 cells were treated with LPL RNAi reagents (50 nmol/L) and maintained in 10% FBS RPMI for 48 h. Cells subsequently were incubated in phenol-red–free RPMI with and without MTT reagent (0.5 mg/mL) for 2 h, and mitochondrial oxidative activity was photometrically determined by measuring the formation of formazan from the MTT substrate (n = 16). Results represent mean ± SEM. *P < 0.05, ***P < 0.0005 vs. nontargeting control.
Fatty acid flux regulates mitochondrial biogenesis in L6 myotubes. We hypothesized that fatty acid flux into the skeletal muscle regulates mitochondrial biogenesis through a PPAR-dependent process. To test this hypothesis, we first analyzed three PPAR genes by RT-qPCR to clarify which isoform is dominant in human skeletal muscle and found that both PPAR-α and PPAR-δ are dominant compared with the PPAR-γ isoforms, as previously reported (Fig. 5A) (13).

We next knocked down PPAR-α and PPAR-δ in RC13 cells and measured mitochondrial density by mitochondrial-specific dye. As we expected, both PPAR-α and PPAR-δ RNAi decreased mitochondrial density (Fig. 5B).

To decrease fatty acid incorporation into the cell, we next knocked down CD36 (fatty acid translocase) by RNAi and found that this treatment decreased mitochondrial density by 17% (Fig. 5B). To confirm these data by protein expression level, we treated L6 myotubes with LPL, PPAR-δ, and CD36 RNAi and found that not only LPL RNAi but also both...
FIG. 5. Role of PPARs and CD36 in mitochondrial biogenesis. 

**A:** mRNA expression of PPAR isoforms determined by RT-qPCR using TaqMan probe in RC13 human rhabdomyosarcoma cell line \((n = 4)\) and human skeletal muscle \((n = 4)\). Data were normalized with 18S rRNA expression. 

**B:** RC13 cells were treated with CD36, PPAR-α, and PPAR-δ RNAi \((50 \text{ nmol/L})\), maintained in 10% FBS RPMI for 48 h, fixed with 1% formaldehyde, stained with Mitotracker Green \(\) which stain mitochondria specifically), and scanned with multilabel fluorescence reader. The quantity of Mitotracker Green dye was divided by DAPI signal to normalize cell number. Results represent mean ± SEM. * \(P < 0.05\), ** \(P < 0.005\), *** \(P < 0.0005\) vs. nontargeting control. 

**C:** Mitochondrial protein expressions in L6 myotubes measured by Western blotting in control \(\) nontargeting control 50 nmol/L; \(n = 6)\), CD36 RNAi \(\) CD36 RNAi 25 nmol/L + nontargeting control 25 nmol/L; \(n = 6)\), LPL RNAi \(\) LPL RNAi 25 nmol/L + nontargeting control 25 nmol/L; \(n = 3)\), combination of LPL and CD36 RNAi \(\) LPL RNAi 25 nmol/L + CD36 RNAi 25 nmol/L; \(n = 3)\), PPAR-δ RNAi \(\) PPAR-δ RNAi 25 nmol/L + nontargeting control 25 nmol/L; \(n = 3)\), combination of PPAR-δ RNAi and LPL \(\) PPAR-δ RNAi 25 nmol/L + LPL RNAi 25 nmol/L; \(n = 3)\), and CD36 RNAi \(\) CD36 RNAi 25 nmol/L + nontargeting control 25 nmol/L; \(n = 3)\). Cyt-c, cytochrome c. 

**D:** mRNA expression of MTCOI determined by RT-qPCR in L6 myotubes after RNAi treatment against control \(\) nontargeting control 50 nmol/L; \(n = 3)\), LPL RNAi \(\) LPL RNAi 25 nmol/L + nontargeting control 25 nmol/L; \(n = 3)\), and CD36 RNAi \(\) CD36 RNAi 25 nmol/L + nontargeting control 25 nmol/L; \(n = 3)\). Results represent mean ± SEM. * \(P < 0.05\), ** \(P < 0.005\), *** \(P < 0.0005\).
PPAR-δ and CD36 RNAi decreased mitochondrial protein expression (Fig. 5C). We also knocked down PPAR-α by RNAi but did not see the difference on the protein expression of MTCOI, SDHA, and PDH (data not shown). We used RT-qPCR to examine MTCOI mRNA expression and found that CD36 and PPAR-δ RNAi treatment decreased MTCOI mRNA expression (Fig. 5D and E). In contrast, fatty acid treatment increased several mitochondrial genes related to fatty acid oxidation and glucose metabolism. mRNA expression of CPT1b, UCP3, and PDK4 were significantly induced by EPA, docosahexaenoic acid (DHA), and bromopalmitate (a known PPAR activator), suggesting that fatty acid flux into the cell mediates fatty acid oxidation (Supplementary Fig. 2A–C).

Next, we measured known mitochondrial biogenesis genes by RT-qPCR and found that EPA, not DHA, increased PGC-1α and MTCOI mRNA expression, suggesting that polyunsaturated fatty acid (PUFA) stimulates mitochondrial biogenesis at least in part by PGC-1–dependent fashion (Supplementary Fig. 2D). In addition, we tested the effect of several fatty acids on the mitochondrial-to-nuclear ratio and found EPA to have the strongest effects (Supplementary Fig. 2). We hypothesized that PUFA may be rescuing mitochondrial gene expression during the LPL RNAi treatment and found that indeed, EPA rescued MTCOI protein during LPL knockdown but that MTCOI protein expression did not change during CD36 knockdown (Fig. 6). GW501516, a PPAR-δ agonist, and PUFA stimulate mRNA expression of CPT1b, UCP3, and PDK4 (Supplementary Figs. 2 and 3A–C). Thus, we tested GW501516 treatment on MTCOI protein expression during LPL knockdown and found a partial rescue of MTCOI protein expression after GW501516 treatment (Supplementary Fig. 3D).

**DISCUSSION**

Insulin resistance in skeletal muscle, as a result of decreased insulin-stimulated muscle glucose transport and muscle glycogen synthesis, is one of the earliest detected abnormalities in the pathogenesis of T2D (14–16). Previous studies show a strong relationship between increased intramyocellular lipid content and reduced insulin-stimulated muscle glucose uptake in both humans (14,17) and rodents (18–20), which has led to the hypothesis that an increase in intracellular lipid metabolites (e.g., diacylglycerol and ceramides), due to a mismatch between fatty acid delivery and mitochondrial oxidation or conversion to neutral lipid (triacylglycerol), leads to activation of novel protein kinase Cs (e.g. PKCα, PKCβ, and PKCδ) that result in decreased insulin signaling and insulin action (2,19,21–23). In this regard, recent studies show an ~35% reduction in resting muscle mitochondrial ATP production (3) and tricarboxylic acid activity (1) in young, lean IR offspring, which could be attributed to a 35% reduction in mitochondrial density (2). Taken together, these data suggest that reductions in mitochondrial biogenesis in muscle of IR offspring may be an important contributing factor that predisposes these individuals to intramyocellular lipid accumulation and muscle insulin resistance. To further examine the mechanism responsible for reduced muscle mitochondrial density in these individuals, we assessed gene expression in muscle by microarray analysis in a similar group of young, lean IR offspring and found that mRNA and protein expression of LPL were reduced by ~45% compared with age/weight/BMI–matched control subjects. This observation is consistent with previous studies that observe lower skeletal muscle LPL activity in overweight and T2D subjects (24).

Previous studies show that insulin per se stimulates mitochondrial biogenesis (25,26) and that mitochondrial morphology is altered in obese IR and T2D individuals (27). It is therefore possible that insulin resistance is the cause and not the effect of reduced mitochondrial content in the IR offspring. Insulin has been shown to upregulate expression of LPL mRNA in adipose tissue and in postheparin plasma (28), but in contrast, insulin does not appear to

![FIG. 6. EPA treatment rescued LPL RNAi–induced suppression of MTCOI protein expression. L6 myotubes were incubated with EPA (50 μmol/L), DHA (50 μmol/L), or 0.125% BSA for 48 h after LPL and CD36 RNAi treatment (50 nmol/L). MTCOI protein expressions were measured by Western blotting (n = 9). Results represent mean ± SEM. *P < 0.05, ***P < 0.0005.](image-url)
activate LPL gene expression in skeletal muscle (29–31). To assess the potential role of LPL in the regulation of mitochondrial biogenesis, we next investigated the effect of knocking down LPL gene expression by RNAi techniques and assessed mitochondrial density and mitochondrial protein expression. Using this approach, we found that knocking down LPL expression in both RC13 human rhabdomyosarcoma cells and L6 rat myotubes decreased mitochondrial density and protein expression as well as mitochondrial oxidative capacity. These observations are supported by previous studies that show increased mitochondria density and SDH activity in mice with overexpression of LPL in skeletal muscle (32).

Given these findings, we examined the hypothesis that fatty acid flux into the myocyte, working through activation of PPARs, may be an important intracellular regulator of mitochondrial biogenesis (Fig. 7) (33,34). Consistent with this hypothesis, we found that RNAi treatment of CD36 (fatty acid translocase) partially decreased mitochondrial protein expression, indicating that fatty acid uptake into the cell may be an important factor regulating mitochondrial biogenesis.

Both PPAR-α and PPAR-δ are highly expressed in skeletal muscle, and PPAR-δ activation has been shown to improve insulin sensitivity through increased fat oxidation and increased muscle mitochondrial biogenesis in rodents (35,36), although there was no significant difference in mRNA expression of PPAR-α and PPAR-δ between the IR offspring and control subjects (data not shown). In addition, polymorphisms in the PPAR-δ gene have been found to be associated with alterations in mitochondrial function, aerobic physical fitness, and insulin sensitivity in humans (37). Consistent with these findings, we found that knocking down PPAR-δ expression in L6 myotubes decreased mitochondrial density, mitochondrial protein expression, and oxygen consumption. Previous studies show that unsaturated fatty acids increase PGC-1α expression in cultured muscle cells (38) and that PUFA increases mitochondrial biogenesis in liver (39) and white adipose tissue (40). We therefore examined the possible role of PUFA as potential mediators of mitochondrial biogenesis in muscle cells and found that EPA, but not DHA, rescued decreased MTCO1 protein expression by LPL knockdown in L6 myotubes, suggesting that EPA may be a key fatty acid mediator by which LPL expression regulates mitochondrial biogenesis in skeletal muscle.

In summary, these results provide new insights into the earliest abnormalities that may be responsible for the pathogenesis of muscle insulin resistance in T2D. Taken together, these data suggest that decreased mitochondrial content in muscle of IR offspring may be due in part to reductions in LPL expression in skeletal muscle, resulting in decreased PPAR-δ activation. Furthermore, these data support the hypothesis that EPA influx into skeletal muscle is a potential molecular signal that regulates...
mitochondrial biogenesis in skeletal muscle via activation of PPAR-δ.

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K.M., K.F.P., and G.I.S. designed the study, performed the experiments, analyzed and researched data, and wrote, reviewed, and edited the manuscript. S.S., C.S.C., V.T.S., A.L., A.G., H.Z., and A.K. analyzed and researched data. I.G., H.W., R.H.E., and H.M. contributed reagents and reviewed and edited the manuscript. G.I.S. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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