Exercise training decreases activation of the mitochondrial fission protein dynamin-related protein-1 in insulin-resistant human skeletal muscle

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Fealy CE, Mulya A, Lai N, Kirwan JP. Exercise training decreases activation of the mitochondrial fission protein dynamin-related protein-1 in insulin-resistant human skeletal muscle. J Appl Physiol 117: 239–245, 2014. First published June 19, 2014; doi:10.1152/japplphysiol.01064.2013.—Defects in mitochondrial dynamics, the processes of fission, fusion, and mitochondrial autophagy, may contribute to metabolic disease including type 2 diabetes. DYNAMIN-related protein-1 (Drp1) is a GTPase protein that plays a central role in mitochondrial fission. We hypothesized that aerobic exercise training would decrease Drp1 Ser616 phosphorylation and increase fat oxidation and insulin sensitivity in obese (body mass index: 34.6 ± 0.8 kg/m2) insulin-resistant adults. Seventeen subjects performed supervised exercise for 60 min/day, 5 days/wk at 80–85% of maximal heart rate for 12 wk. Insulin sensitivity was measured by hyperinsulinenic-euglycemic clamp, and fat oxidation was determined by indirect calorimetry. Skeletal muscle biopsies were obtained from the vastus lateralis muscle before and after the 12-wk program. The exercise intervention increased insulin sensitivity 2.1 ± 0.2-fold (P < 0.01) and fat oxidation 1.3 ± 0.3-fold (P < 0.01). Phosphorylation of Drp1 at Ser616 was decreased (pre vs. post: 0.81 ± 0.15 vs. 0.58 ± 0.14 arbitrary units; P < 0.05) following the intervention. Furthermore, reductions in Drp1 Ser616 phosphorylation were negatively correlated with increases in fat oxidation (r = −0.58; P < 0.05) and insulin sensitivity (r = −0.52; P < 0.05). We also examined expression of genes related to mitochondrial dynamics. DYNAMIN-like protein (DNM1L; P < 0.01), the gene that codes for Drp1, and OPTIC atrophy 1 (OPA1; P = 0.05) were significantly upregulated following the intervention, while there was a trend towards an increase in expression of both mitofusin protein MFN1 (P = 0.08) and MFN2 (P = 0.07). These are the first data to suggest that lifestyle-mediated improvements in substrate metabolism and insulin sensitivity in obese insulin-resistant adults may be regulated through decreased activation of the mitochondrial fission protein Drp1.

mitochondrial fission; mitochondrial dynamics; insulin sensitivity; aerobic exercise; fat oxidation

INCREASED INTRAMYOCELULAR lipids (27, 41) and lipid intermediates, including diacylglycerols (DGs) and ceramides (36), combined with impaired fat oxidation (43) suggests the presence of mitochondrial dysfunction in the pathogenesis of insulin resistance. However, despite significant attention, no consensus has thus far emerged regarding a mitochondrial defect antecedent to the development of insulin resistance (for review, see Refs. 10 and 15).

One aspect of mitochondrial physiology that has heretofore received comparatively less attention is the potential role of altered mitochondrial dynamics in the pathogenesis of insulin resistance. Mitochondria are highly dynamic organelles that continually fuse and divide. In skeletal muscle, mitochondria form as a highly reticular branched network (23) with morphological characteristics that differ between muscle fiber type (39) and muscle mitochondrial subpopulations (45). It is the balance among the processes of fusion, fission, and mitophagy that define the reticular nature of the mitochondrial network (4, 54). Recently, greater knowledge has emerged regarding the protein machinery that regulates these processes. Outer mitochondrial membrane fusion is regulated by the activity of the mitofusins proteins (Mfn1 and Mfn2) (17, 53) while fusion of the inner mitochondrial membrane is coordinated by the activity of Optic atrophy 1 (OPA1) (53). For fission to occur, evidence suggests that Fission-1 (Fis1) (19, 62) binds to the outer mitochondrial membrane and recruits the dynamin-like GTPase protein dynamin-related protein-1 (Drp1) (62), which, when activated, pinches off a portion of the mitochondrial network (28, 49, 50) The excised mitochondrial fragment may then be tagged for autophagy by the recruitment of PTEN-induced putative kinase 1 (PINK1) and Parkin or may, after a period, rejoin the mitochondrial network by the action of the fusion proteins (59). Crucially, Drp1-mediated fission events are associated with a transient loss of mitochondrial membrane potential in the excised mitochondrial portion (59).

Dysregulation of mitochondrial dynamics has been implicated in an increasing number of disease states (4, 18, 57, 65), including type 2 diabetes (1, 16). However, data linking alterations in mitochondrial dynamics and skeletal muscle insulin resistance are limited. Muscle mitochondria from patients with type 2 diabetes, observed by electron microscopy, are smaller in size compared with nondiabetic controls (22), a morphological characteristic that is partially rescued by exercise training (58), while protein expression studies indicate a profragmentary environment in muscle from obese individuals (2) and in type 2 diabetes (21). Furthermore, Bach et al. (2) reported a 25% reduction in the mitochondrial network in skeletal muscle of obese Zucker rats, compared with wild type, in the absence of differences in mitochondrial mass. In the same study, fibroblasts treated with an Mfn2 antisense sequence exhibited decreased glucose oxidation and oxygen consumption. Mfn2 repression is also associated with decreased rates of pyruvate and palmitate oxidation in L6E9 muscle cells (46). A recent report by Jheng et al. (20) provides evidence that Drp1-mediated mitochondrial fission results in mitochondrial fragmentation, loss of mitochondrial membrane potential, increased oxidative stress, decreased ATP content, and insulin-mediated glucose uptake in C2C12 cells preincubated with palmitic acid. Moreover, Drp1 knockdown experiments reversed these alterations. These findings were duplicated in

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ob/ob mice. In this case, mice treated with Mdivi-1, a Drp1 inhibitor, recovered tubular mitochondrial percentage and membrane potential, decreased oxidative stress to baseline levels, and increased insulin-mediated glucose uptake. Collectively, these data provide strong evidence implicating Drp1-mediated mitochondrial fission as a likely contributing factor to insulin resistance. However, there are currently no data that link this process to insulin resistance in humans.

It has been postulated that alterations in energy balance may contribute to changes in mitochondrial network structure (44) such that increased energy demand/starvation leads to a more highly reticular mitochondrial network (13, 47), while increased energy supply results in a more highly fragmented network (20, 35, 60, 61). Since aerobic exercise interventions are an effective strategy for increasing energy demand and simultaneously enhancing insulin sensitivity and fat oxidation in humans, it is possible that a mitochondrial mechanism for improvement in insulin sensitivity may reside in alterations in the mitochondrial fission pathway. However, no studies have, hitherto, reported on the effect of exercise on activation of Drp1-mediated mitochondrial fission in insulin-resistant humans. Indeed, if Drp1-mediated mitochondrial fission is implicated in insulin resistance, we would expect to see reductions in Drp1 activation following an aerobic exercise intervention. Further, alterations in Drp1 activation would likely be related to changes in fat oxidation and insulin sensitivity. To test this hypothesis, we examined the effect of a 12-wk aerobic exercise intervention on skeletal muscle Drp1 activation at Ser616 phosphorylation (the form required for mitochondrial fission activity) (56), insulin sensitivity, and fat oxidation in obese insulin-resistant individuals.

METHODS

Participants. Seventeen (male/female: 10/7) older (age; 66 ± 1 yr), previously sedentary (individuals exercising for 20 min or more at least 2 times per wk were excluded), nonsmoking, obese (body mass index: 34.6 ± 0.8 kg/m²) adults were recruited from the Greater Cleveland community to undergo a 12 wk aerobic exercise intervention. Medical screenings excluded individuals with heart, kidney, liver, thyroid, intestinal, and pulmonary diseases as well as individuals taking medications known to affect the outcome variables of the study. Resting 12-lead electrocardiograms and submaximal exercise stress tests excluded individuals with any contraindication to physical activity. All women were postmenopausal and not using hormone replacement therapy. Participants had also been weight stable for at least the previous 6 mo. The Cleveland Clinic Institutional Review Board approved the study, and all subjects provided informed consent in accordance with guidelines on the protection of human subjects.

Intervention. Participants performed 60 min (20 min cycle ergometry and 40 min treadmill walking) of supervised aerobic exercise at ~80–85% maximum heart rate on 5 days/wk for 12 wk. Compliance with exercise intensity was monitored using a heart rate monitor (Polar Electro, Woodbury, NY), Caloric intake was monitored weekly, and average energy intake pre and post was 1984.1 ± 169.7 and 1,898.7 ± 175.7 kcal/day, respectively.

Inpatient control period. Pre- and postintervention measures were controlled during a 3-day in-patient stay in the Clinical Research Unit at the Cleveland Clinic. During the inpatient control periods, participants were provided with a weight maintenance isocaloric diet (total kcal/day = resting metabolic rate × 1.25; 55% carbohydrate, 35% fat, and 10% protein) derived from indirect calorimetry measurements conducted at the beginning of the inpatient control period. All metabolic measurements were conducted during the inpatient control period and within 24 h of the last exercise bout.

Body composition. Height and body weight were measured using a stadiometer and digital scale. Whole body adiposity was measured by dual energy X-ray absorptiometry (model iDXA; Lunar, Madison, WI).

Aerobic fitness. Each participant performed an incremental graded treadmill exercise test to determine maximal oxygen consumption (VO2max), as previously described (24). Exhaled air was continuously sampled online with the use of an automated system (Jaeger Oxycon Pro; Viasys, Yorba Linda, CA).

Insulin sensitivity and substrate metabolism. Insulin sensitivity measurements were obtained after an overnight fast using a 2-h euglycemic-hyperinsulinemic clamp (90 mg/dl, 40 mU·m⁻²·min⁻¹), as previously described (24). Briefly, a primed (3.28 mg/kg) continuous (0.036 mg·kg⁻¹·min⁻¹) infusion of [6,6-²H₂]glucose began at t = −120 min and continued throughout the procedure for estimation of endogenous glucose production. At t = 0, the insulin infusion commenced and glucose was infused at a variable rate. Arterialized venous blood was sampled at 5-min intervals (YSI 2300; STAT Plus, Yellow Springs, OH), and glucose infusion was adjusted according to the calculations of DeFronzo et al. (9). Insulin sensitivity was calculated as insulin-stimulated glucose disposal rate (GDR; mg·kg⁻¹·min⁻¹) divided by plasma insulin (µU/ml) during the last 40 min of the clamp (24). Homeostatic model assessment of insulin resistance (HOMA-IR) (fasting plasma glucose (FPG) × fasting plasma insulin (FPI)) / 405 (34) was also measured as an estimate of insulin resistance. Indirect calorimetry (Vmax Encore; Viasys) measurements were performed before the clamp procedure for determination of respiratory exchange rate (RER) and substrate metabolism. Furthermore, protein metabolism was estimated from overnight, timed measurements of urinary nitrogen excretion as previously described (52). Protein corrected fat oxidation was calculated using the Frayn equation (1.67 V0₂ + 1.67 VCO₂ − 1.92 m) (12). Plasma for glucose kinetics analyses was deproteinized, extracted and derivatized before analysis by gas chromatography-mass spectrometry. First, 1 ml 70% methanol was added to 200 µl plasma and centrifuged at 1,000 rpm for 10 min. The supernatant fluid was collected, dried under air, and reconstituted with 200 µl double-distilled H₂O. The sample was then applied to a glass column containing a cation exchange resin (AG50W-X8 200–400 mesh; Bio-Rad, Hercules, CA), eluted with 5 ml double-distilled H₂O (pH 8.0), and dried (Labconco, Kansas City, MO). Thirty microliters of pyridine and 15 µl acetic anhydride were added to the dried sample and incubated for 2 h at room temperature. Finally, 400 µl H₂O and 400 µl ethyl acetate were added. The sample was centrifuged for 5 min at 1,000 rpm, and the upper layer was collected for injection into a Hewlett-Packard 5985A gas chromatograph-mass spectrometer (Hewlett-Packard, Palo Alto, CA). Ion mass-to-charge ratios (m/z) 200 and 202 were selectively monitored. The isotopic enrichment (mol percent excess) of the samples were obtained by comparing their peak area percentage (m/z 202)/(m/z 202 + m/z 200) with that of a standard curve (51).

Skeletal muscle biopsy. Basal muscle specimens were obtained from the vastus lateralis before and after the intervention. The muscle was dissected free from visible fat and connective tissue. The tissue was immediately frozen and stored in liquid nitrogen until subsequent analysis. Biopsies and indirect calorimetry measurements were taken immediately before initiation of insulin infusion.

RNA extraction. RNA was extracted from human muscle with TRI Reagent (Sigma, St. Louis, MO). Briefly, 10–20 mg of muscle tissue were homogenized in 1 ml of TRI Reagent at 4°C with repetitive short bursts. Homogenized tissue was incubated at room temperature for 5–10 min, followed by centrifugation at 12,000 g for 10 min at 4°C. RNA was separated into an aqueous phase using 0.1 ml of 1-bromo-3-chloropropane and precipitated with 0.5 ml of isopropanol. Isolated RNA was washed with 1 ml of 75% ethanol, air dried, and dissolved in 40 µl nuclease free water. RNA concentration and purity were

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determined using a NanoDrop ND-1,000 Spectrophotometer (Thermo Scientific, Wilmington, DE). RNA integrity was also randomly assessed using an Agilent bioanalyzer (Agilent, Santa Clara, CA). Isolated RNA was aliquoted and stored at −80°C until further analysis.

cDNA synthesis. Before cDNA synthesis, RNA samples were treated with DNase I for 15 min at room temperature to remove any contaminating DNA (Invitrogen, Carlsbad, CA). One microgram of total RNA was reverse transcribed into cDNA (iScript cDNA synthesis kit; Bio-Rad) using a PX2 Thermal Cycler (Thermo Scientific). The reaction volume was 20 μl and synthesis was performed at 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min, respectively. cDNA samples were stored at −20°C until later analysis.

Quantitative RT-PCR primer pairs. Primer pairs for target genes were obtained from PrimerBank database (pga.mgh.harvard.edu/primerbank/; see Table 1). All primers were checked for specificity to the genes of interest by Blast analysis.

Semiquantitative real-time PCR analysis. Determination of relative mRNA expression was performed in duplicate on an MX3000P QPCR system (Agilent Technologies/Stratagene, La Jolla, CA) using 10 ng of cDNA as the template and the Brilliant II SYBR Green QPCR Master Mix (Stratagene). The human GAPDH gene was used as an internal standard (31). Relative change in mRNA abundance was calculated using the comparative ΔCt method (29). Briefly, the threshold cycle (Ct) for GAPDH was subtracted from the Ct for the gene of interest to adjust for variations in mRNA/cDNA generation efficiency to generate the ΔCt value. Preintervention was used as the baseline and the fold induction of the target gene at postintervention was calculated as an exponential of the negative value of the subtraction of the ΔCt at preintervention from ΔCt at postintervention (2−ΔΔCt) (29).

Tissue homogenization and Western blot analysis. Muscle homogenates were prepared by grinding muscle tissue with ice-cold lysis buffer (Invitrogen) in the presence of protease inhibitor cocktail, 5 mM phenylmethylsulfonyl fluoride (Sigma), and Phos-STOP (Roche homeostasis model assessment of insulin resistance; V˙O2max, maximal oxygen consumption.

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Preintervention</th>
<th>Postintervention</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (n/f)</td>
<td>17 (10/7)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age, yr</td>
<td>66 ± 1</td>
<td>—</td>
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</tr>
<tr>
<td>Weight, kg</td>
<td>101.5 ± 3.6</td>
<td>90.4 ± 3.1</td>
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<tr>
<td>BMI, kg/m²</td>
<td>34.6 ± 0.8</td>
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<td>FM, kg</td>
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<td>FFM, kg</td>
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<td>FPG, mg/dl</td>
<td>98.3 ± 1.9</td>
<td>94.4 ± 1.6</td>
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<td>FPI, μU/ml</td>
<td>13.3 ± 0.9</td>
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<td>HOMA-IR</td>
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<td>Insulin sensitivity, mg·kg⁻¹·min⁻¹·μU·ml⁻¹</td>
<td>0.025 ± 0.005</td>
<td>0.047 ± 0.005</td>
<td>&lt;0.001</td>
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<td>Basal fat oxidation, mg·kg⁻¹·min⁻¹</td>
<td>0.35 ± 0.05</td>
<td>0.56 ± 0.07</td>
<td>&lt;0.01</td>
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<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>22.4 ± 0.89</td>
<td>28.9 ± 1.68</td>
<td>&lt;0.001</td>
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Results


table 1. Primers

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<th>Forward</th>
<th>Reverse</th>
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<td>ATCAGCAAAGTCGGGGTGTTT</td>
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<td>FIS1</td>
<td>GTCCAAAGCAGGCGACGTTG</td>
<td>AGAAATGACGACCGATCGATT</td>
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<tr>
<td>PARK2</td>
<td>ATGAGCTGCTGGTGATGACAGT</td>
<td>AGACATGACGATCTAGGCCAACC</td>
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<tr>
<td>MFN1</td>
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<td>AGACATGACGATCTAGGCCAACC</td>
<td>NM_033540</td>
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<tr>
<td>MFN2</td>
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<td>TTGACGCAGCCCTCTAGGACAC</td>
<td>NM_00127660</td>
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<td>PINK1</td>
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<td>ATCTGTTGCTAAATTCAGATCACTTCAGG</td>
<td>NM_032409</td>
</tr>
<tr>
<td>OPA1</td>
<td>AGGCTGCGAATTTTTTG</td>
<td>AGGCGATCTCGATATGAGTACG</td>
<td>NM_130837</td>
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</table>

Primer pairs for genes involved in mitochondrial dynamics. MFN1, Mitofusin 1; MFN2, Mitofusin 2; OPA1, Optic atrophy 1; FIS1, Fission 1; DNMT1, Dnmt1-like protein; PINK1, Pten-induced putative kinase 1; PARK2, Parkin 2.

Statistical analyses. Values were tested for normality using the D’Agostino and Pearson omnibus normality test on GraphPad Prism 4.0 (Graphpad Software, San Diego CA). Pre- to postintervention changes were assessed using repeated-measures ANOVA for normally distributed samples. A Wilcoxon signed-rank test was used to determine changes in gene expression. Linear regression analysis was used to determine associations between normally distributed data. In addition, Spearman’s rank correlation analyses were used to identify relationships between variables that failed the normality test [Δinsulin sensitivity]. Statistical significance was accepted when P < 0.05. These analyses were carried out using StatView for Windows 5.0.1 (SAS Institute), and all data are expressed as means ± SE.
increased following the intervention ($P < 0.001$), as was basal fat oxidation ($P < 0.01$; Table 2).

**Protein expression.** Exercise training resulted in a significant reduction in Drp1 phosphorylation at Ser$^{616}$ (ratio of phosphorylated Drp1 to total Drp1; $P = 0.01$; Fig. 1). Total Drp1 (ratio of Drp1 to actin) protein expression was not increased by the exercise intervention (pre vs. post: $0.91 \pm 0.22$ vs. $1.02 \pm 0.13$, $P = 0.63$).

**Gene expression.** Dynamin1-like protein (DNM1L) is the gene that codes for Drp1, and its expression was also significantly upregulated ($P < 0.01$ following the intervention. Further, exercise training increased OPA1 gene expression ($P = 0.05$), while there was a trend towards an increase in expression of both MFN1 ($P = 0.08$) and MFN2 ($P = 0.07$) genes. The expression of FIS1 was unchanged and no change was observed in the expression of PINK1 or PARK2 genes (Fig. 2).

**Correlations.** There was a significant correlation between changes in Drp1 Ser$^{616}$ phosphorylation and fat oxidation ($r = -0.58; P < 0.05$; Fig. 3A), and also changes in Drp1 Ser$^{616}$ phosphorylation and insulin sensitivity ($\rho = -0.52; P < 0.05$; Fig. 3B).

**DISCUSSION**

Our data show that a 12-wk aerobic exercise intervention that induced significant weight loss in obese adults improved peripheral insulin sensitivity and fat oxidation in parallel with alterations in basal Drp1 activation. Since phosphorylation of Drp1 at Ser$^{616}$ increases mitochondrial fission activity (56), our data suggest that Drp1-mediated mitochondrial fission may be decreased following exercise training. Furthermore, this down-regulation of Drp1 activity was significantly correlated with improvements in fat oxidation and insulin sensitivity. These data are novel because they provide the first in vivo evidence in humans that supports the findings of recent research using cell and animal models, which suggest that Drp1-mediated mitochondrial fission may be an important determinant of skeletal muscle insulin resistance.

Much has been made in recent years of the potential role of mitochondria in the development of diabetes. Despite this, no mechanism has thus far emerged linking impairments in mitochondrial function to insulin resistance. The hypothesis that mitochondrial fission may result in mitochondrial dysfunction and insulin resistance is intriguing, as this mechanism would require no intrinsic mitochondrial respiratory chain defect and can also account for how mitochondrial dysfunction could be induced without reductions in mitochondrial mass or content.

The precise mechanism by which Drp1 activation may mediate improvements in insulin resistance and fat oxidation is unclear. However, multiple studies have reported that Drp1-mediated mitochondrial fission is associated with a transient loss of mitochondrial membrane potential in the excised mitochondrial fragment (3, 32, 33, 56). Disposal of mitochondrial membrane potential may result in accelerated respiration, but this necessarily requires suppression of ATP production (11). Consequently, higher basal rates of mitochondrial fission may result in increased uncoupling and a corresponding reduction in overall mitochondrial network efficiency. Interestingly, data from Conley et al. (7) suggest that sedentary individuals indeed exhibit higher mitochondrial respiration and uncoupling compared with active subjects, while Chavez et al. (5) demonstrated decreased mitochondrial membrane potential in the presence of decreased insulin sensitivity in healthy subjects following lipid infusion. Furthermore, increased uncoupling is evident in several obesity-related pathologies (6, 8). Therefore, it is conceivable that Drp1 activation may result in loss of membrane potential through sensitization of the mitochondrial permeability transition pore (mPTP) (25, 64). Importantly, activation of the mPTP in this case would potentially result in increased reactive oxygen species production despite a loss of membrane potential (40, 48, 64, 66) rather than membrane hyperpolarization as has previously been suggested (63). Furthermore, such an event would likely result in sufficient permeabilization of the mitochondrial membrane to factors such as fatty-acyl CoAs and acylcarnitines, which under normal circumstances would be sequestered in the mitochondria and have the ability to interfere with the insulin signaling pathway (26). This postulation is supported by recent work of Taddeo et al. (55), which suggests that opening of the mPTP may indeed lead to cell damage.

![Fig. 1. A: representative blots of phosphorylated-Drp1 at Ser$^{616}$, total Drp1, and actin. B: densitometric analysis of phosphorylated Drp1 (pDrp1), total Drp1 (Drp1), and actin. Exercise resulted in a reduction in Drp1 Ser$^{616}$ phosphorylation ($P = 0.01$). Data are means ± SE. *$P < 0.05$.

![Fig. 2. Expression of genes related to mitochondrial dynamics. Exercise resulted in increased expression of both OPA1 ($^*P < 0.05$) and DNM1L ($^*P < 0.01$) but no change in the expression of the other genes. MFN1, Mitofusin 1; MFN2, Mitofusin 2; OPA1, Optic atrophy 1; FIS1, Fission 1; DNM1L, Dynamin1-like protein; PINK1, PTEN-induced putative kinase 1; PARK2, Parkin 2.](http://jap.physiology.org/)}
be an important novel mediator of insulin resistance. In that study, inhibition of the mPTP restored glucose uptake in muscle cells, although the increase in GLUT-4 expression on the cell membrane appeared to be independent of the classical insulin signaling pathway. Although we did not directly measure membrane potential in vivo in the present study, it is reasonable to infer that the exercise training-mediated reductions in basal Drp1 activation are likely accompanied by increased mitochondrial membrane potential, decreased mitochondrial permeability within the mitochondrial population, and improved mitochondrial efficiency and reduced insulin resistance.

Further to this hypothesis, Jheng et al. (20) recently reported that in C2C12 cells preincubation with palmitic acid induced Drp1-mediated mitochondrial fragmentation, higher levels of oxidative stress, reduced ATP production, and reduced insulin-mediated glucose uptake. Knockdown of Drp1 prevented these palmitate-induced effects. Furthermore, ob/ob mice treated with Mdivi-1, a Drp1 inhibitor, recovered tubular mitochondrial percentage and membrane potential, decreased oxidative stress to baseline levels, and increased insulin-stimulated glucose uptake. The data presented herein indicate that exercise training may also reduce Drp1-mediated mitochondrial fission. Additionally, these alterations correlate with changes in fat oxidation and insulin sensitivity. Taken together, these data corroborate the hypothesis that mitochondrial fission may be a determinant of insulin resistance in obesity. However, further research is required to determine whether Drp1 activation and its downstream effects, either directly or indirectly, regulate insulin sensitivity in human skeletal muscle.

Our data are also consistent with previous research suggesting that energy balance may be a key determinant of mitochondrial dynamics activity (13, 20, 35, 47, 60, 61). The weight loss observed in the current study confirms that individuals were placed in energy deficit. The increased expression of fusion related genes combined with total Drp1 protein expression suggests that either the exercise training itself, or the energy deficit, initiated a profusion state within the skeletal muscle. However, this finding cannot be confirmed without imaging the mitochondrial network, something that was outside the scope of the current investigation.

Interestingly, DNML1, the gene that codes for Drp1, was significantly upregulated following the intervention. It is possible that the increase in DNML1 gene expression is related to the last exercise bout. It was previously reported that total Drp1 protein content was increased in skeletal muscle of lean healthy males 24 h after a single bout of high-intensity interval training and peaked 24 h after the third exercise session; however, total Drp1 protein expression trended downward thereafter for up to 2 wk of training (42). Nevertheless, we cannot discount the possibility that the effects observed in the current study are in part due to an acute rather than chronic effect of exercise on gene expression. Similarly, previous reports have demonstrated that both fat oxidation (14, 30) and insulin sensitivity (37) are increased following a single bout of exercise. Further research will be required to determine whether the exercise effects observed here are driven by short-term gains following a single exercise bout or chronic adaptations to exercise training. Interestingly, the increase in DNML1 gene expression in the current study was not reflected in total Drp1 protein expression, suggesting that Drp1 in skeletal muscle may undergo posttranscriptional regulation.

In summary, this study provides new in vivo evidence that altering energy balance through an aerobic exercise intervention may result in decreased Drp1 activity and a profusion environment in skeletal muscle of older obese individuals with insulin resistance and that these alterations are associated with improvements in fat oxidation and insulin sensitivity. These findings support the hypothesis that elevated mitochondrial fission may result in mitochondrial dysfunction and insulin resistance and that exercise training may cause improvements in insulin sensitivity through inhibition of this pathway. The data presented here also suggest that a more complete investigation into the role of Drp1 activation and its downstream effects on mitochondrial morphology, mitochondrial function, and insulin sensitivity is warranted and may result in novel approaches for therapeutic treatment of insulin resistance and type 2 diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

Author contributions: C.E.F. and J.P.K. conception and design of research; C.E.F. and A.M. performed experiments; C.E.F., A.M., and J.P.K. analyzed data; C.E.F., A.M., N.L., and J.P.K. interpreted results of experiments; C.E.F. prepared figures; C.E.F. drafted manuscript: C.E.F., A.M., N.L., and J.P.K. edited and revised manuscript; C.E.F., A.M., N.L., and J.P.K. approved final version of manuscript.

REFERENCES


