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Exercise Training Remodels Human Skeletal Muscle Mitochondrial Fission and Fusion Machinery Towards a Pro-Elongation Phenotype

Christopher L. Axelrod[1,2], **Ciarán E. Fealy**[1], **Anny Mulya**[1], and **John P. Kirwan**[1,2]

[1]Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195

[2]Integrated Physiology and Molecular Medicine Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, 70808

Abstract

Aims: Mitochondria exist as a morphologically plastic network driven by cellular bioenergetic demand. Induction of fusion and fission machinery allows the organelle to regulate quality control and substrate flux. Physiologic stressors promote fragmentation of the mitochondrial network, a process implicated in the onset of metabolic disease, including type 2 diabetes and obesity. It is well known that exercise training improves skeletal muscle mitochondrial volume, number, and density. However, the effect of exercise training on muscle mitochondrial dynamics remains unclear.

Methods: Ten sedentary adults (65.8 ± 4.6 years; 34.3 ± 2.4 kg/m^2) underwent 12 weeks of supervised aerobic exercise training (5 day/wk, 85% of HR_{MAX}). Body composition, cardio-metabolic testing, hyperinsulinemic-euglycemic clamps, and skeletal muscle biopsies were performed before and after training. MFN1, MFN2, OPA1, OMA1, FIS1, Parkin, PGC-1α, and HSC70 protein expression was assessed via Western blot.

Results: Exercise training led to improvements in insulin sensitivity, aerobic capacity, and fat oxidation (all P<0.01), as well as reductions in bodyweight, BMI, fat mass and fasting glucose (all P<0.001). When normalized for changes in mitochondrial content, exercise reduced skeletal muscle FIS1 and Parkin (P<0.05), while having no significant effect on MFN1, MFN2, OPA1, and OMA1 expression. Exercise also improved the ratio of fusion to fission proteins (P<0.05), which positively correlated with improvements in glucose disposal (r^2=0.59, P<0.05).

Conclusions: Exercise training alters the expression of mitochondrial fusion and fission proteins, promoting a more fused, tubular network. These changes may contribute to the

Corresponding Author: John P. Kirwan, Ph.D., Integrated Physiology and Molecular Medicine Laboratory, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA, 70808, Location: L-4030, Phone: (225) 763-3171, john.kirwan@pbrc.edu.

Author Contributions

C.L. Axelrod, C.E. Fealy, A. Mulya, and J.P. Kirwan designed the study, performed data collection, analysis, and interpretation. J.P. Kirwan supervised data collection, analysis, and interpretation. C.L. Axelrod drafted the manuscript and all authors contributed to editing and finalization of the manuscript. J.P. Kirwan is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Christopher L. Axelrod and John P. Kirwan are now affiliated with Pennington Biomedical Research Center, Baton Rouge, LA, 70808. C.E. Fealy is now affiliated with Maastricht University, Maastricht, NL.

CONFLICT OF INTEREST

None of the authors have a conflict of interest related to this work.

improvements in insulin sensitivity and substrate utilization that are observed after exercise training.

Keywords

mitochondrial dynamics; exercise training; insulin resistance; obesity; skeletal muscle

Introduction

Peripheral insulin resistance is implicated in numerous chronic diseases, including non-alcoholic fatty liver disease (NAFLD), polycystic ovarian syndrome (PCOS), central obesity, hypertension, dyslipidemia, and type 2 diabetes[1–4]. Yet, the mechanisms underlying the onset and development of insulin resistance remain unclear. A growing body of research has implicated mitochondrial dysfunction in the pathogenesis of insulin resistance[5–11]. However, the integrity, morphology, structure, and functionality of mitochondria in chronic disease states remains heavily disputed[12–14].

Recent advances in molecular biology have redefined mitochondria as a morphologically plastic network sensitive to the bioenergetic demands of the cell. Mitochondria continuously undergo cycles of fission and fusion in order to balance input from mitochondrial biogenesis with output from damaged or poorly functioning mitochondria[15]. This process, referred to as mitochondrial dynamics, plays an essential role in cellular homeostasis, the extent of which has been reviewed elsewhere[16]. Induction of the cellular fusion and fission machinery allows the organelle to regulate mitochondrial quality control[17,18], ion transport[19], ATP synthesis[20], membrane potential ($\Delta\psi m$)[21], metabolic byproducts[22], and substrate flux[23].

Mitochondrial fusion occurs in two discrete yet coordinated events. Fusion of the outer mitochondrial membrane (OMM) is mediated by mitofusins 1 and 2 (MFN1 and MFN2, respectively)[24]. The inner mitochondrial membrane (IMM) of partially fused mitochondria is then coordinated by optic atrophy 1 (OPA1). OPA1-mediated IMM fusion is regulated by the peptidase OMA1 and *i*-AAA protease YME1L[25]. Mitochondrial fission is primarily regulated by dynamin-related protein 1 (DRP1), a cytosolic GTPase. Upon activation, DRP1 is phosphorylated by multiple known serine kinases, and is translocated to the mitochondria[26]. DRP1 is recruited to the OMM by a number of adapter proteins, the most well studied being mitochondrial fission 1 (FIS1)[27]. Damaged organelles may then be cleared by Parkin, an E3 ubiquitin-protein ligase, which mediates mitochondrial autophagy (mitophagy)[28].

Bioenergetic stress arising from nutrient oversupply induces fragmentation of the mitochondrial network, resulting in uncontrolled fission and inhibition of fusion[29]. In addition, acute and chronic exposure to saturated fatty acids (SFA's) and hyperglycemia fragment the mitochondrial network[30], diminish $\Delta\psi m$ and total ATP content[30], enhance ROS generation[22], and impair glucose uptake in β-cells[31], adipocytes[32], and myocytes[30,33]. Furthermore, impairments in mitochondrial fusion have been observed in obese rats and humans[34]. In contrast, cells exposed to nutrient deprivation develop elongated tubules, and are protected against mitophagy[35]. Collectively, these findings implicate mitochondrial

dynamics as a potential regulatory mechanism linking excess nutrient intake to skeletal muscle insulin resistance.

It is well established that exercise training improves the volume, number, density, and function of skeletal muscle mitochondria[36]. However, little is known about the effect of exercise training on mitochondrial dynamics. We have previously reported that 12 weeks of exercise training reduces DRP1 activity at Serine 616 in human skeletal muscle, a known site of mitochondrial fission[37]. To our knowledge, exercise-induced adaptations to skeletal muscle mitochondrial dynamics in humans has been scarcely reported[38,39].

Thus, the purpose of this investigation was to examine changes in human skeletal muscle mitochondrial dynamics after 12 weeks of aerobic training. We hypothesized that exercise training would enhance expression of fusion proteins, while inhibiting fission and mitophagy activation. It was also hypothesized that changes in mitochondrial dynamics would be associated with improvements in insulin sensitivity.

Results

12 weeks of exercise training significantly reduced body weight ($p<0.001$), BMI ($P<0.001$), body fat % ($P=0.001$), VAT ($P<0.001$), FPG ($P=0.020$), FPI ($P=0.033$), triglycerides ($P=0.025$), cholesterol ($P=0.003$), VLDL ($P=0.019$), LDL ($P=0.006$), and HOMA-IR ($P=0.039$) (Table 1). Furthermore, the intervention significantly improved VO_{2MAX} ($P<0.001$), GDR ($P<0.001$), NOGD ($P<0.0001$), and M/I ($P<0.001$) (Table 1).

Next, we assessed differences in mitochondrial dynamics protein expression pre- and post-exercise training (Figure 1a). To assess the influence of biogenesis on mitochondrial dynamics, we additionally measured PGC-1α expression and citrate synthase activity. PGC-1α expression and citrate synthase activity significantly increased after exercise training (ALL $P<0.01$; Figure 1b). To account for these changes, we normalized protein expression data to citrate synthase activity, and observed significant reductions in Parkin ($P<0.0001$), and FIS1 ($P<0.001$), with no significant changes to MFN1, MFN2, OPA1, or OMA1 (Figure 1c). To further understand our findings in the context of the balance between fission and fusion, we generated ratios of OPA1/FIS1 and OPA1/Parkin pre- and post-training (Figure 2). Exercise training significantly increased the ratio of both OPA1/FIS1 ($P=0.001$; Figure 2a) and OPA1/Parkin ($P=0.007$; Figure 2b). Changes in the ratio of OPA1/FIS1 expression significantly correlated with improvements in glucose disposal rate ($P=0.01$, $r^2=0.59$) (Figure 3a). Also, changes in Parkin expression significantly correlated with FPG ($P=0.02$, $r^2=0.54$) (Figure 3b).

Discussion

We have previously reported that exercise reduces mitochondrial fission in insulin resistant skeletal muscle, which correlated with improvements in fatty acid oxidation, and insulin sensitivity[37]. Herein we extend those findings to demonstrate that 12 weeks of exercise training also reduces FIS1, a regulatory protein of the mitochondrial fission and mitophagy machinery. We also provide the first *in vivo* evidence that exercise reduces skeletal muscle Parkin activation, a protein directly implicated in the mitophagy pathway. These changes

were observed in concert with improvements in oxidative capacity, glycolytic activity, and whole-body insulin sensitivity, as well as reductions in body fat, peripheral lipid and glucose accumulation. Taken together, these data suggest that exercise can enhance skeletal muscle mitochondrial dynamics and quality control, leading to increased whole-body insulin sensitivity via reductions in mitochondrial insults due to excess nutrient intake.

Exercise Training and Mitochondrial Dynamics

Few studies have examined changes in mitochondrial dynamics after prolonged exercise training. Konopka et al. reported a similar increase in skeletal muscle expression of MFN1 and MFN2 in both young and older adults after 12 weeks of aerobic exercise training[38]. Zampieri et al. reported increased OPA1 expression after 12 weeks of skeletal muscle electrical stimulation, but not resistance training[39]. Interestingly, further mitochondrial analysis revealed greater network integration and elongation after electrical stimulation, suggestive of enhanced fusion activity. In a pre-clinical model, after 7 days of exercise Sprague-Dawley rats displayed a pro-fusion shift similar to the results described herein[40]. Furthermore, prolonged sedentarism or aging induced pro-fission phenotypes, suggesting a relationship between skeletal muscle oxidative capacity and mitochondrial dynamics. Activation of fission and inhibition of fusion proteins have additionally been reported in muscle denervation models, further strengthening this notion[41,42]. However, generalizability of these findings is limited to due lack of normalization to changes in mitochondrial content.

It was previously shown that aerobic exercise training leads to a 60–120% increase in mitochondrial protein content[43,44]. To account for the contributions of biogenesis to changes in mitochondrial dynamics, we measured PGC-1α expression, a known transcriptional regulator of mitochondrial biogenesis[45], and normalized protein expression to citrate synthase activity, a validated biomarker of mitochondrial content[46]. We found that citrate synthase activity accounted for exercise-induced changes in fusion activity (MFN1, MFN2, OPA1, OMA1). However, the reductions in fission and mitophagy (FIS1 and Parkin) appeared to be independent of changes in mitochondrial content. Pre-clinical models, in concert with data described herein, support the notion that exercise drives post-translational modifications in the mitochondria which contribute towards a pro-fusion phenotype.

The Mitophagic Response in Trained Skeletal Muscle

Data from Egan et al. suggest that an adequate mitophagic response is essential for balancing oxidative stress from nutrient metabolism, with mitochondrial network integrity, structure, and function[47]. This is further evidenced by studies demonstrating the onset of metabolic diseases with loss of autophagic function in the liver and pancreas[48,49]. For this reason, it has been postulated that enhancing mitochondrial quality control may be a critical adaptation to exercise[50]. In this regard, exercised muscle benefits from increased mitophagic rates, clearance and quality control as a result of increased skeletal muscle oxidation rates. This idea is largely supported by acute exercise studies in skeletal muscle, which have demonstrated upregulation in mitochondrial fission, with modality specific effects on fusion[51–53].

However, our intervention data support the notion that exercise training may result in fewer and/or less frequent mitophagic events - a product of enhanced metabolic function and improved mitochondrial architecture and supporting machinery. In this view, exercise increases the pool of skeletal muscle mitochondria that can be integrated into larger tabulated networks. Kim et al. found that mice with a skeletal muscle mitophagy specific knockout were protected against high fat diet-induced insulin resistance, had greater energy expenditure, and enhanced insulin sensitivity compared to wild type mice[54]. This observation is further supported by the increased abundance of antioxidants and free radical scavengers in trained skeletal muscle[55].

It appears therefore, that mitophagy in skeletal muscle is discrete and possibly more disposable than in other tissues such as the liver or pancreas. Thus, exercise may alter mitochondrial turnover via a biphasic mechanism. Acute exercise promotes enhanced mitochondrial turnover, priming the mitochondrial network for future bouts. Chronic exercise may then reduce mitochondrial turnover by improving the integrity of the mitochondrial network, or by increasing the size and abundance of intact mitochondrial networks. Clearly, further research is necessary to elucidate the effects of chronic exercise on skeletal muscle mitophagy.

Overall, these results highlight the role of exercise in the regulation of skeletal muscle mitochondrial dynamics with regards to the balance of fusion, fission, and quality control. Our data in concert with pre-clinical models demonstrate that chronic exercise alters expression of key mitochondrial proteins involved in the regulation of mitochondrial dynamics. Furthermore, we provide additional insight into the role of mitochondrial dynamics proteins in the regulation of skeletal muscle insulin sensitivity. Previous data from our group in conjunction with the findings described herein implicates altered mitochondrial fission and mitophagy in the pathogenesis of type 2 diabetes. Furthermore, exercise training carries the therapeutic potential to normalize or enhance mitochondrial dynamics, reducing fission and mitophagy and increasing fusion by input from mitochondrial biogenesis. The current investigation was limited by assessing proteins governing mitochondrial fission and fusion processes. More detailed studies to elucidate the mechanisms driving mitochondrial fission-induced insulin resistance and type 2 diabetes using imaging-based approaches are underway.

Materials and Methods

Subjects:

Ten obese, prediabetic adults completed a 12 week exercise training intervention. Medical screenings excluded individuals with heart, kidney, liver, thyroid, intestinal, and pulmonary diseases or 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 high intensity physical activity. All women were postmenopausal and not using hormone replacement therapy. Participants had also been weight stable for at least the previous 6 months. The study was approved by the Cleveland Clinic Institutional Review Board, and all subjects provided written informed consent in accordance with our guidelines for the protection of human subjects.

Exercise Intervention:

Subjects underwent 60 minutes of aerobic exercise 5 days/week for 12 weeks (treadmill walking and cycle ergometry) at ~85% (± 5 beats per minute) of their maximal heart rate (HR_{MAX}). HR_{MAX} was obtained at baseline, and at 4 week increments during the intervention and was recorded during an incremental maximal aerobic-exercise (VO_{2MAX}) test using a modified Bruce protocol. All sessions were supervised by an exercise physiologist. The test was deemed to be maximal if at least three of the following criteria were satisfied: a respiratory exchange ratio of ≥1.10, a leveling off in oxygen consumption with increasing workloads, volitional fatigue, or a heart rate greater than or equal to age-predicted maximum. Subjects were instructed to maintain eucaloric intake and dietary records were collected at baseline, and 4 week increments to ensure compliance. Dietary analysis was performed with Nutritionist Pro software (Axxya Systems, Stafford, TX).

Inpatient control period:

All measures collected pre- and post-intervention were obtained 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 measures 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:

After a 12-hour overnight fast, height and weight were measured using standard techniques in a hospital gown. Dual-energy X-ray absorptiometry (Lunar iDXA; Madison, WI) was then used to determine whole body fat and lean mass. Estimation of fat and lean tissue content was obtained from iDXA software according to the manufacturer's instructions. Computerized tomography scanning was additionally used to quantify visceral adipose tissue (VAT) with a SOMOTOM Sensation 16 Scanner (Siemens Medical Solutions, Malvern, PA), as previously described[56]. Non-contrast scans were performed locally in the lumbar region.

Insulin Sensitivity Testing:

Measurements of insulin sensitivity were obtained after an overnight fast using a four hour, euglycemic-hyperinsulinemic clamp (90 mg/dl, 40 $mU \cdot m^{-2} \cdot min^{-1}$), as described previously[57,58]. Briefly, a primed (3.28 mg/kg) continuous (0.036 $mg \cdot kg^{-1} \cdot min^{-1}$) infusion of D-[6,6-$^2H_2$]glucose began at −120 min and continued throughout the procedure to calculate endogenous glucose production (EGP). At 0 minutes, simultaneous infusion of insulin (constant) and 20% dextrose (variable) began. Arterialized heated-hand venous blood was sampled at 5 minute intervals (YSI 2300; STAT Plus, Yellow Springs, OH), and the glucose infusion rate (GIR) was adjusted according to the correction algorithm of DeFronzo et al.[57]. Insulin sensitivity was then calculated as insulin-stimulated glucose metabolism (M; $mg \cdot kg^{-1} \cdot min^{-1}$) during the last 30 minutes of the clamp. Plasma for assessing glucose kinetics was deproteinized, extracted and derivatized before analysis by gas

chromatography-mass spectrometry as previously described[37]. The isotopic enrichment (mole 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. Endogenous glucose production (EGP) and the rate of glucose disposal (GDR) was then derived using the Steele equation[59]. Hepatic insulin resistance was estimated by the suppression of EGP after insulin stimulation. Homeostatic model assessment of insulin resistance (HOMA-IR) {[fasting plasma glucose (FPG) × fasting plasma insulin (FPI)] ÷ 405} was also calculated as an additional measure of insulin resistance. Whole body respiratory exchange ratio (RER) and substrate metabolism was assessed basally and under insulin-stimulated conditions via indirect calorimetry as previously described (Vmax Encore; Viasys, Yorba Linda, CA)[60]. Non-oxidative glucose disposal was estimated by subtracting the glucose oxidation rate from the GDR.

Percutaneous Muscle Biopsy:

Skeletal muscle tissue was obtained from the medial vastus lateralis using a modified Bergström technique as previously described[61]. Samples were obtained approximately 36 hours after the final exercise bout. Upon collection, samples were dissected of fat and connective tissue, and immediately frozen in liquid nitrogen. All muscle samples were then stored at −140°C until the time of analysis.

Tissue Homogenization, Western Blot Analysis, and Quantification:

Muscle homogenates were prepared by grinding muscle tissue in ice-cold lysis buffer (Invitrogen) in the presence of protease inhibitor cocktail, 5 mM phenylmethylsulfonyl fluoride (Sigma), and Phos-STOP (Roche Applied Sciences, Indianapolis, IN). The homogenates were then centrifuged for 10 min at 14,000 x *g*. The resulting supernatant was decanted and the tissue lysates were stored at −80°c until the time of analysis. Protein concentrations were measured using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). 32 μg's (0.8 μg/mL) of muscle lysate were solubilized in Laemmli sample buffer containing 5% β-mercaptoethanol and boiled for 5 min. 40 μL's of sample was then loaded into 4–12% Tris Glycine gels (Novex) and separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis at 125 volts for 1.5 hours (SDS-PAGE: Invitrogen).

The gels were transferred to polyvinylidene fluoride membranes (Bio-Rad), and blocked with 5% bovine serum albumin (BSA) in phosphate-buffered saline with 0.1% Tween-20 (PBST) for 1 hour. Membranes were then incubated overnight with anti-MFN1 (R & D Systems; Minneapolis, MN, catalog no. AF7880), anti-MFN2 (Cell Signaling Technology; Danvers, MA, catalog no. 9482), and anti-OPA1 (Abnova; Walnut, CA, catalog no. 12083), anti-OMA1 (Abcam; Cambridge, MA, ab154949), anti-FIS1 (Thermo Fisher Scientific; Waltham, MA, PA1–41082), anti-Parkin (Cell Signaling Technology; 4211), anti-PGC1α (Novus Biologicals; NBP1–04676) and anti-HSC70 (Santa Cruz Biotechnology; Dallas, TX, SC-7298) antibodies diluted 1:1000 in 5% BSA solution. Membranes were washed with PBST and incubated with species-specific horseradish peroxidase-conjugated secondary antibodies (GE Healthcare, Piscataway, NJ; catalog no. NA931) diluted 1:10,000 in 5% BSA solution. Immunoreactive proteins were visualized by enhanced chemiluminescence reagent (ECL Prime; GE Healthcare) and quantified by densitometric analysis using ImageJ

software[62]. All antibodies were internally validated prior to use. All bands displayed were subject to quantification with the exception of MFN1 and OMA1. For MFN1, only the bottom band, which appears at the predicted molecular weight (90 kDa), was used. For OMA1, only the middle band, which appears at the predicted molecular weight (60 kDa), was used. Protein expression was normalized to changes in mitochondrial biogenesis by dividing the raw values by PGC-1α expression. Values were then expressed as fold induction relative to pre-intervention normalized to loading control. The fold induction ratios of OPA1/FIS and OPA1/Parkin were subsequently derived from the raw densitometric values.

Citrate Synthase Activity:

Citrate synthase activity was measured via commercially available kinetic assay (Sigma-Aldrich; CS0720). Briefly, ~5 mg of frozen muscle tissue were homogenized (FastPrep-24™; MP Biomedicals, LLC) in 500 μL's of lysis buffer (Sigma-Aldrich; C3228) containing protease inhibitor cocktail (Sigma-Aldrich; P8340). Protein concentrations were measured using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). 8 μg's of protein were then diluted in assay buffer, and assessed for endogenous net citrate synthase activity on a spectrophotometer.

Statistical Analysis:

Student's paired samples *t*-test was used to assess differences pre- to post-exercise intervention. Normality of distribution was verified using the Shapiro-Wilks test. Equality of variance was assessed visually using a Q-Q plot. Correlations between study variables were assessed using Pearson's *r*. If data sets were abnormally distributed, log transformation was used to approach normality, and reassessed using the Shapiro-Wilks test. Graph Pad Prism 5 was used for statistical analysis. R was used to generate the Q-Q plot. The level of significance was set at $P<0.05$. *,†,‡ Indicates a significant treatment effect at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

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New & Noteworthy:

This study provides novel insight into changes in muscle mitochondrial fusion, fission, and mitophagy in humans before and after exercise training. Importantly, our data utilize adequate normalization to decipher contributions from mitochondrial dynamics, not content, in exercise-related cardiometabolic improvements.

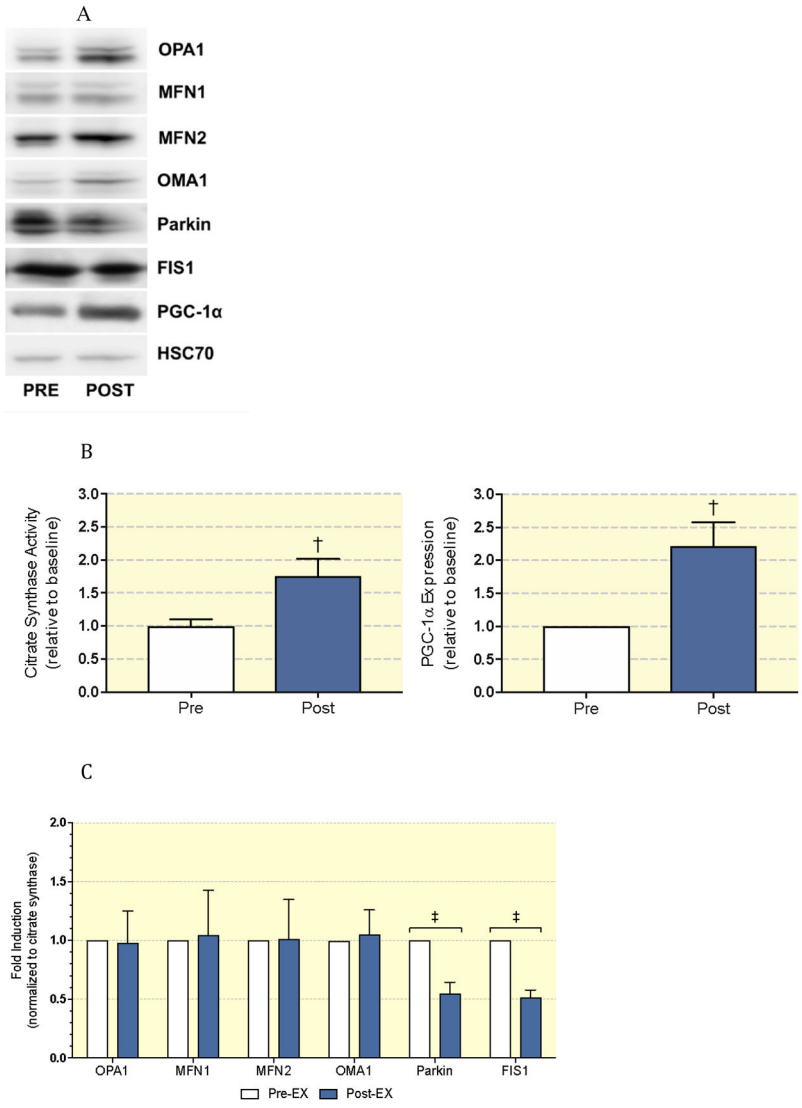

Figure 1:

(A) Representative immunoblots pre (−) to post (+) exercise training in human skeletal muscle. **(B)** Changes in citrate synthase activity and PGC-1α expression pre/post exercise training. **(C)** Densitometric analysis of mitochondrial dynamics proteins pre (white) to post (blue) exercise training.

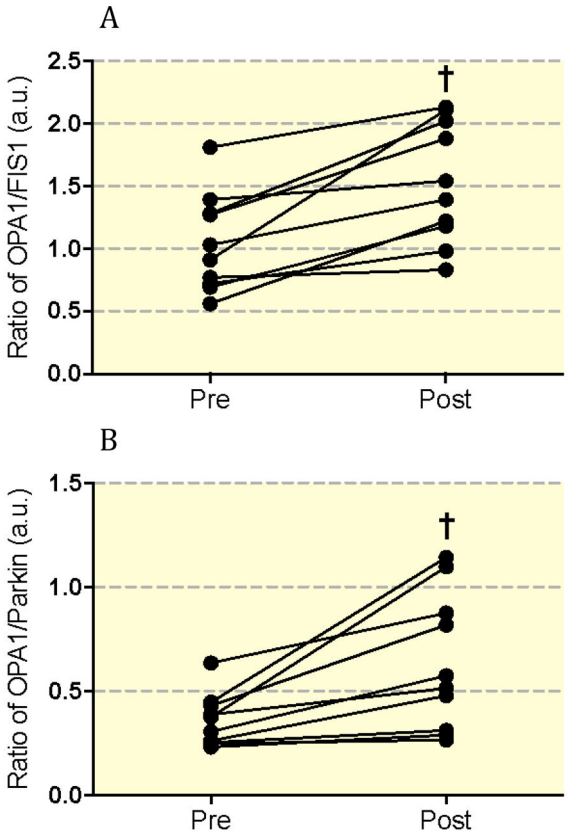

Figure 2:

(A) Effect of exercise training on OPA1/FIS and **(B)** OPA1/Parkin expression ratios.

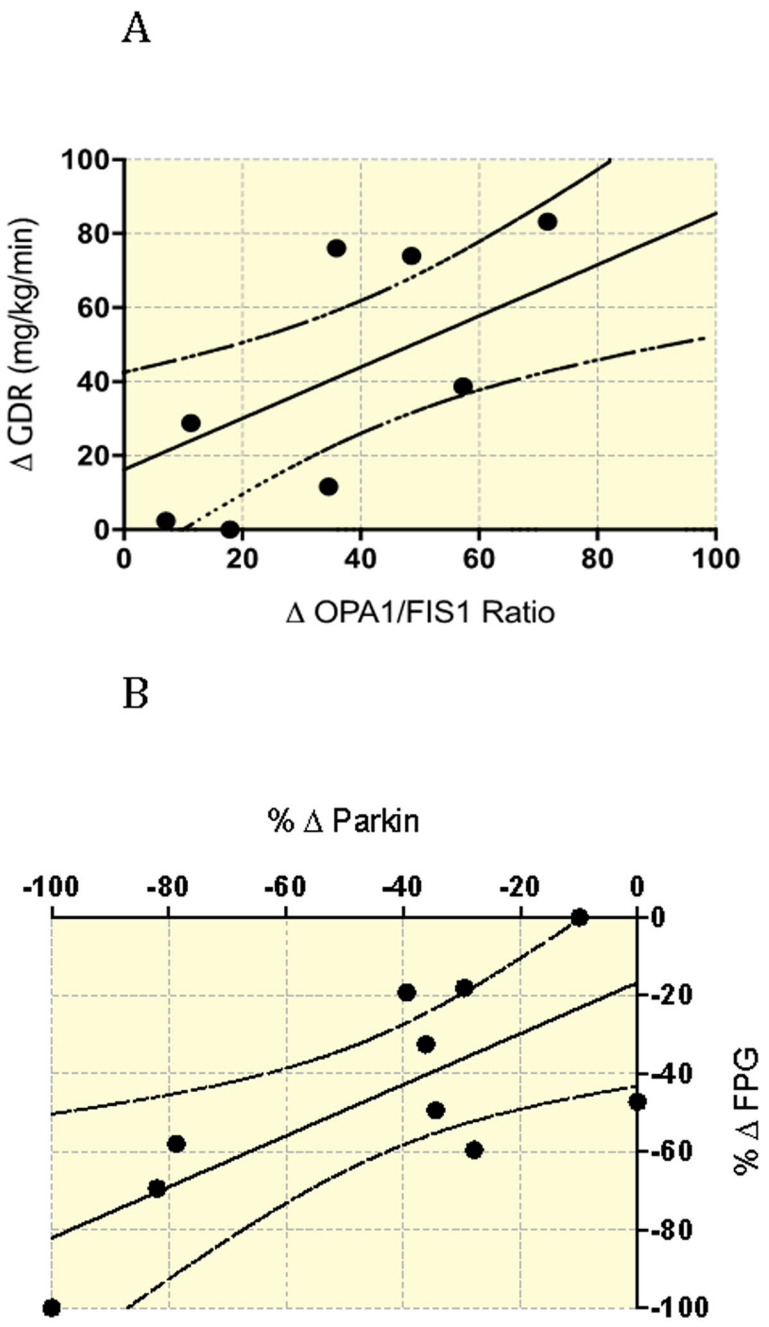

Figure 3:

(A) Significant correlation (P=0.01, R^2=0.59) between Δ OPA1/FIS1 ratio (pre to post intervention) and Δ GDR (pre to post intervention). **(B)** Significant correlation (P<0.02, R^2=0.54) between %Δ parkin (pre to post intervention) and %Δ FPG (pre to post intervention).

Table 1.

Changes in subject characteristics after 12 weeks of exercise training

Characteristic	PRE		POST		p-value (2-tailed)
	M	***SEM***	***M***	***SEM***	
n (m/f)	10	(8/2)			
Age (yrs)	66.3	1.6	-	-	-
Weight (kg)	106.0	4.2	92.8	3.3	<0.001
BMI (kg/m^2)	34.5	0.7	30.2	0.8	<0.001
Body Fat %	40.6	2.1	34.7	3.5	0.001
VAT (cm^2)	107.7	17.2	60.1	17.8	<0.001
VO_{2MAX} (ml/kg/min)	23.8	1.2	32.0	2.5	<0.001
Blood Chemistry					
FPG (mg/dL)	98.2	2.5	94.1	1.8	0.020
FPI (μU/mL)	14.1	1.5	10.9	1.2	0.033
HOMA-IR	3.1	0.5	2.3	0.4	0.019
Triglycerides (mg/dL)	154.0	24.6	96.2	15.6	0.025
Cholesterol (mg/dL)	209.5	12.2	174.2	9.5	0.003
HDL (mg/dL)	46.6	4.5	48.6	4.1	0.329
VLDL (mg/dL)	29.0	5.2	17.3	2.4	0.019
LDL (mg/dL)	133.9	9.2	108.3	8.8	0.006
Insulin Sensitivity					
$Glucose_{90–120}$ (mg/L)	89.9	0.8	88.2	0.3	0.087
$Insulin_{90–120}$ (μU/mL)	97.4	5.5	95.4	7.0	0.660
$M_{90–120}$ (mg/kg/min)	2.6	0.4	4.7	0.4	<0.0001
$GDR_{90–120}$ (mg/kg/min)	2.5	0.3	4.9	0.4	<0.001
EGP (mg/kg/min)	2.2	0.2	1.4	0.1	0.030
Suppression of EGP (%)	49.7	10.4	84.1	9.8	0.012
NOGD (mg/kg/min)	1.0	0.3	3.0	0.5	<0.0001