SUPPLEMENTARY DATA

**Mitochondrial Biochemistry.** NADH-oxidase, citrate synthase, and creatine kinase activities, and cardiolipin were measured as described in our previous publications[1-3]. To prepare soluble and particulate fractions, homogenates were centrifuged at 45,000*xg* for 20 min[1]. The soluble fraction was mixed (1:1) with buffer containing 50% glycerol[1] and saved for analysis of creatine kinase and citrate synthase released from mitochondria. The pellet (particulate fraction) containing mitochondria was suspended in storage medium containing 0.1 mg/ml BSA, 20μmol/l leupeptin, and 25% glycerol[1]. To release mitochondria trapped by the myofibrillar matrix, the particulate fraction was treated with the KCl/pyrophosphate medium method[1,2,4]. Samples were stored at -80°C before analyses. The activity of creatine kinase was measured in the soluble fraction at 30°C by HPLC monitoring of the generation of NADPH in a coupled enzymatic reaction (hexokinase/glucose-6P dehydrogenase)[3,5] The activity of citrate synthase was determined by high-performance liquid chromatography monitoring of the generation of CoA-SH after conversion to a fluorescent adduct in a reaction with ThioGlo-1[3,6]. NADH-oxidase activity and cardiolipin content in the particulate fraction were measured with high-performance liquid chromatography[3,7,8]. Cardiolipin peaks were identified from retention time and relative intensity as previously described[9,10]. Citrate synthase and NADH oxidase were expressed relative to creatine kinase activity to normalize for minor variations in muscle biopsy fiber content[1]. Succinate dehydrogenase activity was measured using histochemical methods as described previously[11].

**Fiber Bundle Preparation**

Fiber bundles were prepared under magnification, by gently teasing apart individual fibers with two sharp tweezers, submerged in ice-cold BIOPS media in a petri dish. The fiber bundles were permeabilized with saponin (2ml of 50 μg/ml) for 20 minutes and then washed twice in Buffer Z (105mM K-MES, 30 mM KCl, 10mM $KH_2PO_4$, 5mM $MgCl_2$-$6H_2O$, 5mg/ml BSA, 1mM EGTA, pH 7.4 with KOH) with blebbistatin (25μM) to inhibit contraction.

**Analysis of Sphingolipid and Diacylglycerol (DAG) Species.** Intramuscular sphingolipids and DAGs were quantified by high-pressure liquid chromatography (HPLC)-tandem mass spectrometry as described previously[12] and in the supplemental section. (Lipidomics Core, Medical University of South Carolina). Briefly, liquid nitrogen-frozen samples (~30mg) were homogenized in ice-cold buffer (250mM sucrose, 25 mM KCl, 50 mM Tris, and 0.5 mM EDTA, pH 7.4) and were fortified with internal standards and extracted into a one-phase neutral organic solvent system (ethyl acetate/isopropyl alcohol/water; 60:30:10 v/v/v), evaporated and reconstituted in methanol and quantified by a surveyor/TSQ 7000 liquid chromatography/mass spectrometry system (Thermo Finigan, Thermo Fisher Scientific Inc., Waltham, MA). Quantitative analysis was performed in a positive multiple-reaction monitoring mode, based on calibration curves generated by adding to an artificial matrix known amounts of target analytes, synthetic standards, and an equal amount of internal standard. Intramuscular DAG and ceramide content was normalized to tissue-wet weight (pmol/mg tissue).

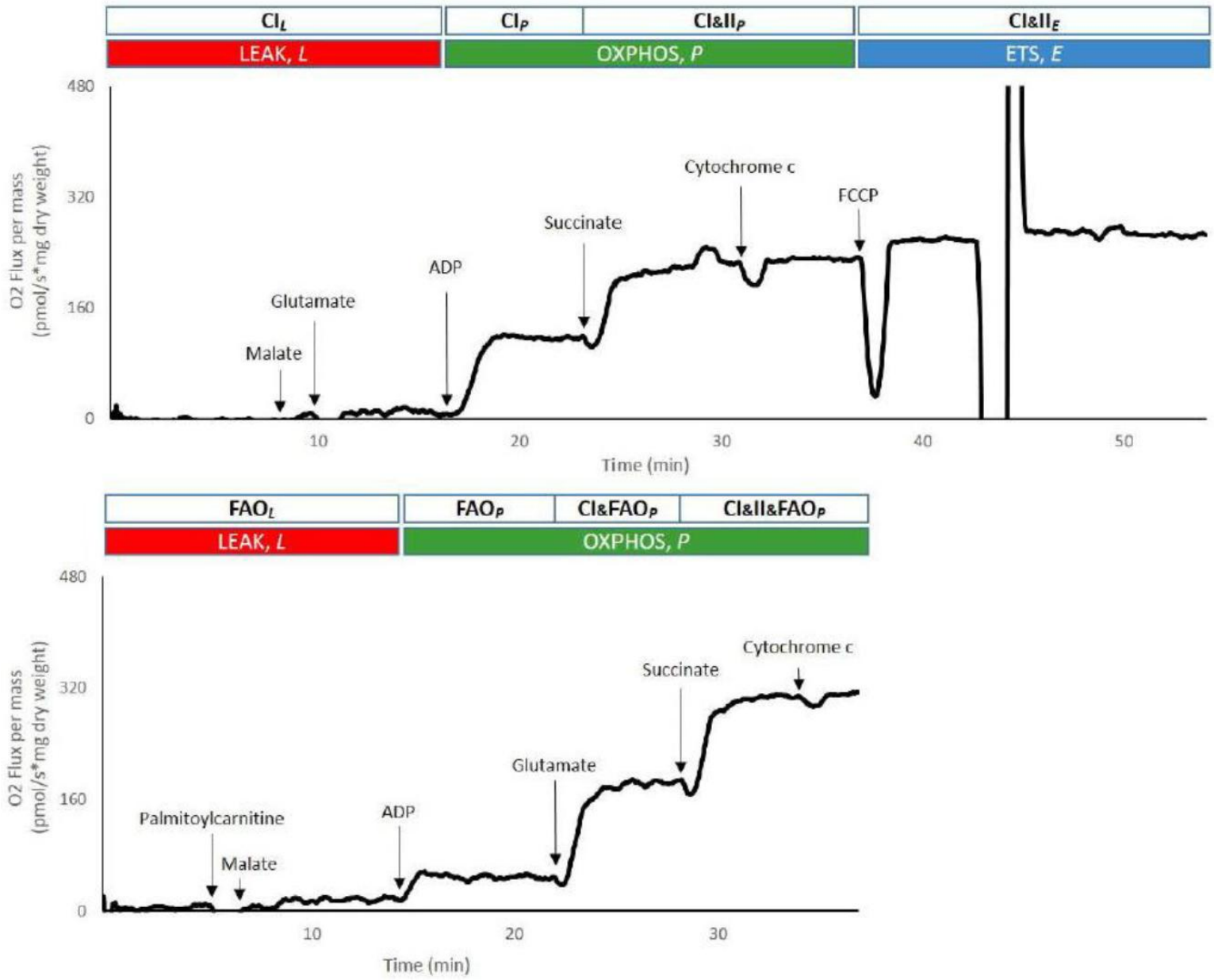
SUPPLEMENTARY DATA

**Fiber Type and Intramyocellular Triglyceride (IMTG) Content.** Determination of IMTG content was performed using a modified version of methods previously used in our laboratory[11]. Briefly, serial transverse sections (10 μm) of mounted biopsy samples were generated using a cryostat (Cryotome E; Thermo Shandon, Pittsburgh, PA) at -20 °C and placed on a cleaned glass slide (Fisherfinest, Fischer Scientific, Pittsburgh, PA). Sections were then stained in a filtered solution of Oil Red O (300 mg/ml in 36 % triethylphosphate) for 30 minutes at room temperature. Thereafter, sections were incubated with primary antibodies for anti-human myosin heavy chain (MYH)7 (type I myocytes) and MYH2 (type IIa myocytes) overnight at room temperature and subsequently incubated with fluorescein (FITC) (type IIa myocytes) and Rhodamine (type I myocytes) conjugated secondary antibodies (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). Type IIx fibers remained unstained. Images were visualized using a Leica microscope (Leica DM 4000B; Leica Microsystems, Bannockburn, IL), digitally captured (Retiga 2000R camera; Q Imaging, Surrey, Canada), and semi-quantitative image analysis was conducted using specialized software (Northern Eclipse, v6.0; Empix Imaging, Cheektowaga, NY). Oil red O staining intensity and cross sectional area was determined in type I and type II myocytes. Analysis is based on >200 fibers per section.

**Protein Content by Immunoblot.** A portion of frozen muscle (~30mg) was prepared for immunoblot for GLUT protein expression as described in the supplemental section. The frozen muscle sample was homogenized (T8 Ultra Turrax; IKA Inc., Wilmington, NC) in ice-cold cell lysis buffer (Cell signaling Technology, Danvers, MA) including a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). The homogenates were incubated with gentle rocking (1 hr, 4°C) and then centrifuged (10 min, 12000 xg, 4°C) and the supernatant removed. Protein content of the supernatant was determined using the bicinchoninic acid assay (BCA) (Thermo Scientific, Rockford, IL). Aliquots of supernatant were mixed with 5x Laemmli buffer (10 % SDS, 10 % glycerol, 10 mM beta-mercaptoethanol, 0.05 % bromophenol blue, 0.2 M Tris-HCl pH 6.8) and denatured by heating (5 min, 100 °C). Samples were separated on a 4-20% SDS-PAGE gel followed by transfer onto polyvinylidine difluoride membranes (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked in 5 % non-fat milk, and incubated with the following primary antibodies: Anti-GLUT4 (#2213, Cell Signaling, Danvers, MA), anti-GLUT1, anti-GLUT12 (#GT12-A and #GT122-A, Alpha Diagnostics International, San Antonio, TX), anti-OXPHOS antibody cocktail (#MS601, Mitosciences, Eugene, OR), and anti-α tubulin (Mouse #DM1A and Rabbit #11H10, Cell Signaling, Danvers, MA). Membranes were then incubated in appropriate species-specific secondary antibodies (IRDye 800CW anti-Rabbit #926-32211 and IRDye 680RD anti-Mouse #926-68070, Li-Cor Biosciences, Lincoln, NE). Protein bands were visualized using a Li-Cor Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE) and analyzed with Image Studio v2.1 software (Li-Cor Biosciences, Lincoln, NE). Gel-to-gel variation was controlled for by using a standardized sample on each gel. Protein loading was controlled by normalizing bands of interest to α-Tubulin.

SUPPLEMENTARY DATA

**Supplementary Figure 1**



SUPPLEMENTARY DATA

**Supplementary Table 1. Exercise Compliance Data for participants in the EX group.**

| | Sessions/wk | Min/Session | Min/week | HR/session | % max HR |
|---|---|---|---|---|---|
| **Mean** | 3.5 | 44.2 | 154.4 | 132.5 | 78.4 |
| **SD** | 1.2 | 14.6 | 87.6 | 15.6 | 7.2 |
| **SEM** | 0.2 | 2.1 | 17.0 | 2.2 | 1.0 |

Abbreviations: wk, week; Min, minutes; HR, heart rate

SUPPLEMENTARY DATA

## SUPPLEMENTARY REFFERENCES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