Electronic Supplemental Methods

Study Participant Screening

Body Composition

Each participant underwent a whole-body dual energy X-ray absorptiometry (DXA) scan (Hologic Discovery GDR 1000/W, software version 12.6.2 OD; Waltham, MA) to assess regional and total lean body and fat masses (1). This test was administered by a licensed physical therapist and a certified DXA technologist within the Washington University in St. Louis (WUSTL) School of Medicine.

Blood Chemistries

HbA1c, a lipid panel, a comprehensive metabolic panel, and a complete blood count were obtained from blood samples collected after an overnight fast on the morning of the screening visit in the WUSTL Clinical and Translational Research Unit (CTRU). All samples were analyzed by the CTRU Core Lab for Clinical Studies.

Oral glucose tolerance test (OGTT)

Each participant completed a two-hour, 75-gram OGTT after a 12-hour overnight fast during their screening visit in the WUSTL Clinical and Translational Research Unit (CTRU) (2). The test was administered by CTRU nursing staff and supervised by the study physician.

One-repetition Maximum (1RM) Strength Assessment

One repetition maximum strength was measured for leg press, chest press, seated row, pull down, knee extension, shoulder press, and biceps curl exercises on a Hoist (San Diego, CA, USA) single pod exercise machine according to guidelines established by the American College of Sports Medicine (3). The 1RM testing was administered by a licensed physical therapist within the WUSTL Clinical and Translational Research Unit (CTRU) at the end of the screening visit.

Daily Physical Activity Monitoring

Participants' daily physical activity was measured by using an ActiGraph (Pensacola, FL) GT3X+ accelerometer. The monitor was placed on the non-dominant wrist with a non-removable wristband by a licensed physical therapist after completion of all screening procedures and worn for 7 days prior to the lipid metabolism study.

Glucose Metabolism Study

Cross-Over Procedures

For the order of conditions (rest>exercise vs exercise>rest) participants selected from 10 pieces of paper from a bowl (5 stating "rest>exercise" and 5 stating "exercise>rest") to ensure an equal number of participants in each order. The paper was not returned to the bowl to ensure that 5 participants were assigned to each order.

Standardized Meals

The composition of the standardized meals was as follows: breakfast: 2.18 MJ, 19% protein, 31% fat, 50% carbohydrates; lunch: 3.02 MJ, 19% protein, 32% fat, 49% carbohydrates; afternoon snack: .75 MJ, 4% protein, 15% fat, 81% carbohydrates; and dinner: 3.26 MJ, 20% protein, 28% fat, 52% carbohydrates.

Liquid Test Meal

To ensure meal consistency for both the exercise and rest visits for each participant, a single meal was prepared and then divided in half. Participants consumed the liquid meal within 16 minutes (1/4 of the meal provided every 4 minutes). After each aliquot was consumed, the liquid meal containers were rinsed with 10 ml of water and the rinse was given to participants to ensure they ingested the entire study meal.

Exercise Protocol:

The exercise prescription used in this study (3 sets, 10-12 repetitions, 80% 1RM) was selected to be consistent with the recommendations of the American Diabetes Association, the American College of Sports Medicine, and the existing literature on resistance exercise for the treatment of type 2 diabetes (3,4). Since there is a positive correlation between energy expenditure and the reduction in postprandial TG, we selected a higher intensity to maximize the effects of the single exercise session (5).

Skeletal Muscle Mitochondrial Respiration

6-10 mg (wet weight) of tissue were transferred into BIOPS solution (50 mM K^+-MES, 20 mM taurine, 0.5 mM dithiothreitol, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM

phosphocreatine, 20 mM imidazole, pH 7.1, adjusted with 5N KOH at 0 °C, 10 mM Ca–EGTA buffer (2.77 mM CaK_2EGTA + 7.23 mM K_2EGTA; 0.1mM free calcium)) and then placed onto a glass plate where fibers were separated using forceps (6). To ensure complete permeabilization, the fibers were incubated by gentle agitation at 4°C in BIOPS solution containing 50 µg/ml saponin for 20 min. Fibers were washed for 10 min at 4°C in ice-cold (MiR05; 0.5 mM EGTA, 3mM $MgCl_2 \cdot 6H_2O$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 1g/L BSA, 60 mM potassium-lactobionate, 110 mM sucrose, pH 7.1) and the wet weight of the fibers was measured on a microbalance (Mettler Toledo, Greifensee, Switzerland) (6).

3 mg (wet weight) of permeabilized muscle fibers were used per respirometer chamber (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) containing 2.090 ml MiR05 at 37°C. Oxygen concentration (µM) and oxygen flux (pmol s^{-1} mg^{-1}; negative time derivative of oxygen concentration, divided by muscle mass per volume) were recorded using DatLab software (Oroboros Instruments). The oxygen concentration in the chamber was maintained between 150 and 400 µM to avoid oxygen limitation of fiber respiration.

The substrate-uncoupler-inhibitor titration protocol was as follows (final concentrations): palmitoylcarnitine (0.05 mM), L-carnitine (5 mM), and malate (0.5 mM) to support electron entry from fatty acid β-oxidation through electron-transferring flavoprotein (ETF) and Complex I (CI) to coenzyme Q. Prior to the addition of ADP, oxygen utilization occurs due to proton slip across the inner mitochondrial membrane (LEAK respiration). ADP (4 mM) was then added to stimulate fatty-acid supported oxidative phosphorylation $(ETF + CI)_{Lip}$. The subsequent addition of pyruvate (10 mM)

stimulates glycolytic oxidative phosphorylation. Glutamate (10 mM) was then added, followed by succinate (10 mM) to recapitulate the TCA cycle and stimulate maximal oxidative phosphorylation [$(ETF + CI + CII)_{Lip + Pyr}$] through both ETC complex I and complex II. Cytochrome *c* (10 μM) is added to test the integrity of the outer mitochondrial membrane. The increase of flux with cytochrome *c* was on average 3.3 ± 0.4%. Electron transfer system capacity ($CI+II_{E}$) was reached by stepwise (0.5 μM) addition of the uncoupler carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP). Finally, the addition of rotenone (0.5 μM), an inhibitor of complex I was used to measure succinate-supported ETS capacity (CII_{E}). Supplemental Table 2 outlines the flux control and substrate control ratios calculated in this study.

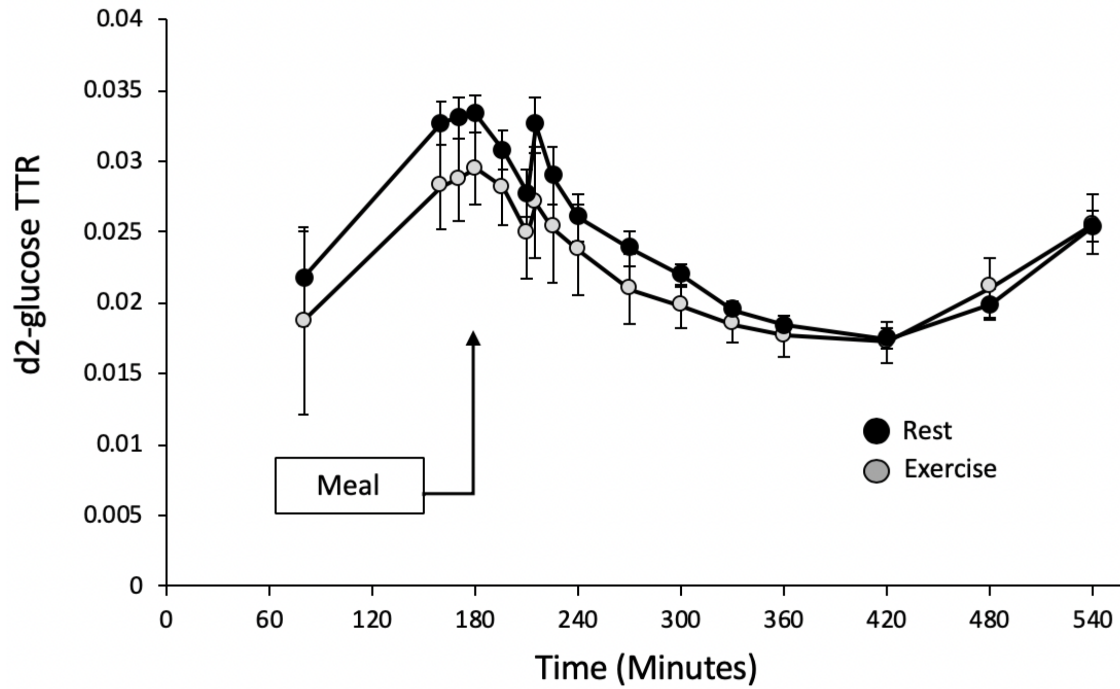
ESM Table 1. Substrate-uncoupler-inhibitor titration (SUIT) protocol (7)

Definition	Abbreviation	Flux State	Titration
Leak	$(ETF + CI)_{LEAK}$	L	PC + Mal
FFA Oxidation through CI	$(ETF + CI)_{Lip}$		PC + Mal + ADP
Combined FFA and Pyruvate Oxidation Through CI	$(ETF + CI)_{Lip+Pyr}$		PC + Mal + ADP + Pyr + Glut
Max OXPHOS	$(ETF + CI + CII)_{Lip+Pyr}$	P	PC + Mal + ADP + Pyr + Glut + Succ
Maximal ETS	$(ETF + CI + CII)_{E}$	E	PC + Mal + ADP + Pyr + Glut + Succ + FCCP
CII Supported Respiration	$(CII)_{E}$		PC + Mal + ADP + Pyr + Glut + Succ + FCCP + Rot

FFA = free fatty acids. P and OXPHOS = oxidative phosphorylation (coupled respiration). E and ETS = electron transport chain capacity/uncoupled respiration. ETF = electron transport flavoprotein. CI = electron transport chain complex I. L and LEAK = leak respiration. Lip = respiration in response to palmitoylcarnitine titration. CII = electron transport chain complex II. PC = palmitoylcarnitine, Mal = malate. Pyr = pyruvate. Glut = glutamate. Succ = succinate. FCCP = Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone. Rot = rotenone.

Electronic Supplemental Results

ESM Figure 1. [6,6-$^2H_2$]glucose Tracer:Tracee Ratio (TTR)



Time-course values are mean ± 95% CI. Black circles = rest condition. Gray circles = exercise condition. Meal denotes timing of test meal. Time point “0” corresponds to the start of the [6,6-$^2H_2$]glucose tracer.

Supplemental Works Cited

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