Adam R. Konopka,[1] Albert Asante,[1] Ian R. Lanza,[1] Matthew M. Robinson,[1] Matthew L. Johnson,[1] Chiara Dalla Man,[2] Claudio Cobelli,[2] Mark H. Amols,[1] Brian A. Irving,[1] and K.S. Nair[1]

Defects in Mitochondrial Efficiency and H_2O_2 Emissions in Obese Women Are Restored to a Lean Phenotype With Aerobic Exercise Training

Diabetes 2015;64:2104–2115 | DOI: 10.2337/db14-1701

OBESITY STUDIES

The notion that mitochondria contribute to obesity-induced insulin resistance is highly debated. Therefore, we determined whether obese (BMI 33 kg/m^2), insulin-resistant women with polycystic ovary syndrome had aberrant skeletal muscle mitochondrial physiology compared with lean, insulin-sensitive women (BMI 23 kg/m^2). Maximal whole-body and mitochondrial oxygen consumption were not different between obese and lean women. However, obese women exhibited lower mitochondrial coupling and phosphorylation efficiency and elevated mitochondrial H_2O_2 (mtH_2O_2) emissions compared with lean women. We further evaluated the impact of 12 weeks of aerobic exercise on obesity-related impairments in insulin sensitivity and mitochondrial energetics in the fasted state and after a high-fat mixed meal. Exercise training reversed obesity-related mitochondrial derangements as evidenced by enhanced mitochondrial bioenergetics efficiency and decreased mtH_2O_2 production. A concomitant increase in catalase antioxidant activity and decreased DNA oxidative damage indicate improved cellular redox status and a potential mechanism contributing to improved insulin sensitivity. mtH_2O_2 emissions were refractory to a high-fat meal at baseline, but after exercise, mtH_2O_2 emissions increased after the meal, which resembles previous findings in lean individuals. We demonstrate that obese women exhibit impaired mitochondrial bioenergetics in the form of decreased efficiency and impaired mtH_2O_2 emissions, while exercise effectively restores mitochondrial physiology toward that of lean, insulin-sensitive individuals.

Obesity is escalating at epidemic rates, with the majority of the U.S. population (69%) being overweight or obese. Obesity is accompanied with decreased insulin-stimulated skeletal muscle glucose uptake (i.e., insulin resistance), which often precedes overt type 2 diabetes and cardiometabolic disease. Insulin-resistant individuals also have a reduced ability to adjust substrate utilization to substrate availability (i.e., metabolic flexibility) (1). Altered mitochondrial physiology is implicated in the etiology of skeletal muscle insulin resistance and metabolic inflexibility owing to reports of reduced mitochondrial content and/or oxidative capacity in individuals who are insulin resistant or have type 2 diabetes (2–5). However, growing evidence suggests a disassociation between insulin sensitivity and mitochondrial oxidative capacity (6–14).

We previously showed that obese individuals are insulin resistant while having similar capacity for skeletal muscle mitochondrial ATP production compared with lean individuals, matched for age, sex, and activity levels (6). Mitochondrial energetics are primarily governed by the balance between supply and demand. Thus, energy restriction that is well-known to enhance insulin sensitivity also reduces redox burden on the mitochondria, resulting in lower reactive oxygen species emissions (15). In

[1]Division of Endocrinology, Diabetes, Metabolism, and Nutrition Research, Mayo Clinic College of Medicine, Rochester, MN
[2]Department of Information Engineering, University of Padova, Padova, Italy
Corresponding author: K.S. Nair, nair@mayo.edu.

Received 7 November 2014 and accepted 13 January 2015.
Clinical trial reg. nos. NCT02105428 and NCT01477164, clinicaltrials.gov.
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db14-1701/-/DC1.

contrast, low-energy demand of sedentary behavior coupled with a high-energy supply in the context of obesity or high-fat feeding results in an energy surplus that places a large redox burden on mitochondria. Emergent evidence suggests that a chronic positive energy balance increases by-products of mitochondrial metabolism that may trigger the pathogenesis of insulin resistance and metabolic inflexibility (16,17). A recent report (10) indicated that mitochondrial oxidative capacity was not different, but mitochondrial hydrogen peroxide (mtH_2O_2) emissions were elevated in obese, insulin resistant versus lean, insulin-sensitive individuals, thus positing mitochondrial oxidative stress rather than oxidative capacity as a contributor of obesity-induced insulin resistance.

In support of this finding, nutritional overload with acute high-fat feeding and short-term high-fat diet increased mtH_2O_2 emissions in lean humans to levels observed in obese, insulin-resistant states (18). Thus, a high-calorie, high-fat diet is deleterious to mitochondrial and metabolic health in lean individuals, but we know very little about whether exercise in obese individuals is an effective remedy to restore mitochondrial physiology to a lean profile. Although compelling evidence strongly links mitochondrial redox balance in the etiology of insulin resistance, it remains to be determined whether aerobic exercise training (AET) that is known to increase energy expenditure and insulin sensitivity alters skeletal muscle mtH_2O_2 production under different states (i.e., postprandial and postabsorptive). This knowledge is critical to understand not only how redox biology in skeletal muscle governs insulin sensitivity but also how exercise works as a therapeutic strategy to improve insulin action in individuals at high risk of developing cardiometabolic disease.

To address these gaps in knowledge, we chose to study women with polycystic ovary syndrome (PCOS) because these women present clinically with obesity and severe insulin resistance but preserved skeletal muscle mitochondrial respiration compared with BMI-matched control subjects (19,20), thus providing additional impetus to evaluate how mtH_2O_2 emission influences insulin action. Twenty-five obese, insulin-resistant women diagnosed with PCOS were studied before and after either 12 weeks of AET or sedentary control (SED). Insulin sensitivity and skeletal muscle mitochondrial energetics were assessed in the fasted state and 4 h after a high-fat mixed meal. We also studied mitochondrial energetics in lean, insulin-sensitive women in the fasted state to provide a healthy reference group to compare with obese women before and after exercise.

RESEARCH DESIGN AND METHODS

After giving written informed consent, as approved by the Mayo Foundation Institutional Review Board, all participants received a physical and medical history examination. Obese women (BMI 28–40 kg/m^2) were diagnosed with PCOS using the Rotterdam criteria (i.e., two of three criteria: excess androgens, ovulatory dysfunction, and polycystic ovaries). Obese women were included if insulin resistant as determined by an index of insulin sensitivity (IS_{OGTT} 7.5) after an oral glucose tolerance test but were excluded if they had type 2 diabetes. For lean women, no known family history of type 2 diabetes was recorded during their medical examination. Both groups were sedentary, defined as <30 min of exercise twice per week and monitored via an accelerometer for ~1 week. Participants were not using tobacco, chronic nonsteroidal anti-inflammatory drugs, nutritional supplements, antihyperglycemic/insulin sensitizer therapies, β-blockers, statins, or any other cardiovascular medication; did not have type 1 diabetes; and did not have any condition that may limit the ability to exercise. All lean women were taking oral contraceptives, while one obese woman ceased consumption of metformin >1 month prior to beginning the study.

Study Protocol

Obese women were randomized and studied before and after 12 weeks of AET (n = 12) or SED (n = 13). The supervised exercise program consisted of 60 min of stationary cycling at a heart rate corresponding to 65% of VO_2 peak 5 days per week. VO_2 peak, determined from expired gas analysis during a graded exercise test on a stationary cycle ergometer, was measured at 0, 4, 8, and 12 weeks. 65% of VO_2 peak was recalculated after each exercise test. Body composition was measured using DXA (Lunar DPX-L; Lunar Radiation, Madison, WI).

A standardized, weight-maintaining diet was provided for 3 days (50% carbohydrate, 30% fat, and 20% protein) prior to and during the study days for both lean and obese women. On the evening of day 3, participants reported to the clinical research unit and stayed overnight in the fasted state to complete the studies described below (Fig. 1).

Hyperinsulinemic-Euglycemic Pancreatic Clamp

Somatostatin (100 ng· kg FFM^{-1} · min^{-1}), insulin (1.5 mU · kg FFM^{-1} · min^{-1}), glucagon (1 ng · kg FFM^{-1} · min^{-1}), and growth hormone (4.7 ng · kg FFM^{-1} · min^{-1}), where FFM is fat-free mass, were infused constantly throughout the 3-h hyperinsulinemic-euglycemic pancreatic clamp (study day A). Glucose (40% dextrose) was infused to maintain plasma glucose at ~90 mg/dL. Plasma glucose was not different between groups or time. The average glucose infusion rate (GIR) (micromoles per kilogram of FFM per minute) during the final 30 min of the clamp was used to determine insulin sensitivity. The clamp was completed in a subset of obese women (AET = 8, SED = 7) and performed ~71 h after the last exercise bout.

Muscle Biopsies and Mixed-Meal Tolerance Test

A percutaneous skeletal muscle biopsy was obtained in the fasted state (Fig. 1) from both lean and obese women at 0700 h from the vastus lateralis under local anesthesia (2% lidocaine). After the muscle biopsy (~0745 h), obese women consumed a high-fat mixed meal (35% of daily caloric intake) (Supplementary Table 1). Net insulin action was determined from the insulin sensitivity index using the oral minimal model (23). One participant in

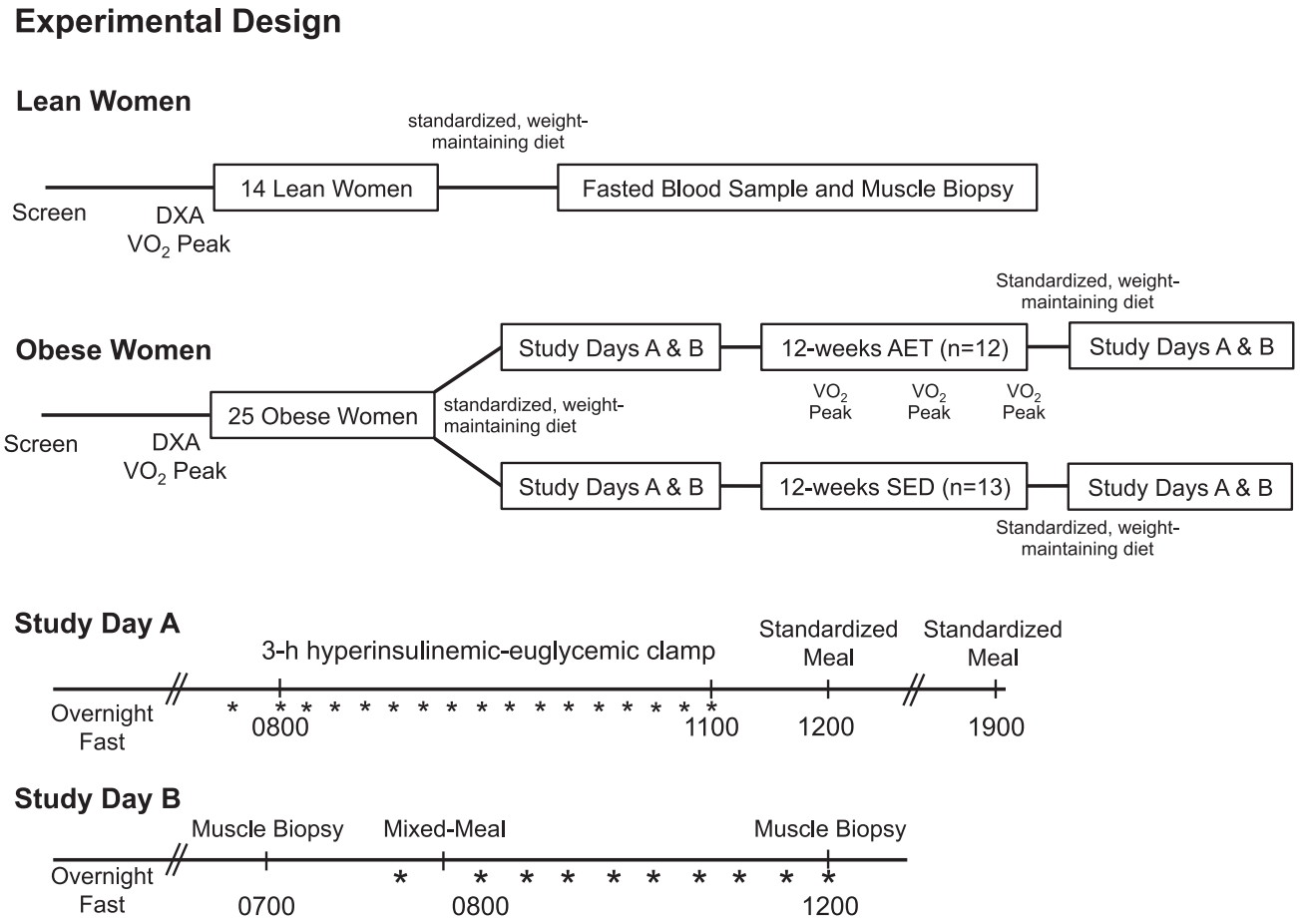

Figure 1—Experimental design. Blood and skeletal muscle biopsy samples were obtained in the fasted state from lean women (n = 14) after 3 days of a standardized weight-maintaining diet. Obese women were randomized to AET (n = 12) or SED (n = 13) and participated in study days A and B before and after the interventions. Obese women also consumed a standardized, weight-maintaining diet 3 days before and during their study days. Study day A consisted of a 3-h hyperinsulinemic-euglycemic pancreatic clamp. Blood samples were obtained every 10 min to adjust the GIR to maintain euglycemia (~90 mg/dL). After the clamp was completed, standardized meals were provided to keep participants weight stable. Study day B consisted of a fasted skeletal muscle biopsy, followed by consumption of a high-fat mixed meal. Blood samples were collected every 30 min for 4 h after the meal. A second muscle biopsy was obtained 4 h after the meal as previously described (18). *Blood sample.

the exercise group was excluded owing to failure to complete the entire meal. A second muscle biopsy was obtained from obese women 4 h after the completion of the meal. Therefore, muscle biopsies were obtained ~94 and 99 h after the last exercise bout (study day B).

Mitochondrial Energetics

Mitochondrial Respiration

Muscle samples (100 mg) were immediately placed in ice-cold BIOPS buffer and then transferred to a chilled glass, homogenized, and prepared for assessment of mitochondrial oxygen consumption using high-resolution respirometry (Oroboros Instruments, Innsbruk, Austria) (11). Respiration in isolated mitochondria was measured in the presence of 2.5 mmol/L ADP to stimulate state 3 respiration with electron flow through complex I (CI), complex I and II (CI+II), and complex II (CII) using carbohydrate substrates (malate, glutamate, and succinate) and inhibitors as previously described (11). Cytochrome c (cyto c) was added at 10 μmol/L to assess integrity of the outer mitochondrial membrane. State 4 respiration was measured in the presence of 2 μg/mL oligomycin to inhibit ATP synthase activity. Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) was added to determine the maximum uncoupled respiration of the electron transport chain. Last, 2.5 μmol/L antimycin A was added to inhibit mitochondrial oxygen consumption to confirm that oxygen consumption was of mitochondrial origin. Oxygen flux rates were expressed relative to mitochondrial protein content. Mitochondrial coupling efficiency was evaluated from the respiratory control ratio (RCR) as the quotient of state 3 (CI+II) to state 4 respiration rates. ADP:oxygen ratio (ADP:O) was determined by quantifying the amount of oxygen consumed in response to a nonsaturating pulse of ADP (15 μmol/L), providing an index for mitochondrial phosphorylation efficiency.

H_2O_2 Production

The reactive oxygen species–emitting potential of isolated mitochondria was evaluated under state 2 conditions as previously described (11). Briefly, a Fluorolog 3 (Horiba Jobin Yvon) spectrofluorometer with temperature control and continuous stirring was used to monitor Amplex Red (Invitrogen) oxidation in a freshly isolated mitochondrial suspension. Amplex Red oxidation was measured in the presence of glutamate (10 mmol/L), malate (2 mmol/L), and succinate (10 mmol/L). The fluorescent signal was corrected for background auto-oxidation and calibrated to a standard curve. H_2O_2 production rates were expressed relative to mitochondrial protein.

H_2O_2 emission was also measured in permeabilized muscle fibers as previously described (15,18). Duplicate sets of fiber bundles (~5 mg) were mechanically separated using sharp forceps and incubated on ice in saponin (50 μg/mL) for 30 min. Permeabilized fibers were then washed in buffer containing (in millimoles per liter) 110 K-MES, 35 KCl, 1 EGTA, 5 K_2HPO_4, 3 $MgCl_2$-$6H_2O$, 0.05 pyruvate, and 0.02 malate and 5 mg/mL BSA (pH 7.4, 295 mOsm). Fibers were preincubated in 10 mmol/L pyrophosphate to deplete endogenous adenylates. Fiber bundles were placed in a quartz cuvette with 2 mL buffer z containing 2 μg/mL oligomycin followed by a stepwise addition of glutamate (5 μmol/L), malate (2 μmol/L), and succinate (0.25–12 mmol/L) to stimulate H_2O_2 production under state 4 conditions. H_2O_2 emission rates were expressed per tissue wet weight.

Antioxidant Activity and mRNA Expression

Catalase activity was determined by measuring the removal of hydrogen peroxide in mixed-muscle homogenates (15,24). Superoxide dismutase isoform (SOD)1 and SOD2 antioxidant activity was measured as the consumption of xanthine oxidase–generated superoxide radical by SOD in a competitive reaction with a tetrazolium salt. Potassium cyanide was added to inhibit cytoplasmic SOD1, with the remaining activity deemed mitochondrial SOD2 (Cayman Chemical Company, Ann Arbor, MI). Total RNA was isolated from ~15 mg skeletal muscle tissue, and cDNA was prepared using the TaqMan reverse transcription kit per the manufacturer's instructions. Real-time PCR was performed on a Viia7 Real-Time PCR system as previously described (11). The probes utilized are outlined in Supplementary Table 2.

Oxidative Damage

DNA oxidative damage was determined as the adduct biomarker 8-oxo-2'deoxyguanosine (8-oxo-dG) in mixed-muscle homogenate as previously described (15). Protein oxidative damage was measured as the content of protein carbonyls in mixed-muscle homogenate using the Oxiselect Protein Carbonyl Immunoblot kit (Cell Biolabs, San Diego, CA) following the manufacturer's guidelines. Muscle was homogenized using radioimmunoprecipitation assay buffer with Halt protease and phosphatase inhibitors (Pierce, Rockford, IL) with EDTA and spun at 10,000*g* at 4°C. Samples were loaded equally (~30 μg), resolved using gel electrophoresis, and transferred to a polyvinylidene fluoride membrane. Digital images of protein carbonyl content were captured using a chemiluminsescent imaging system. Verification of equal protein loading was achieved by use of vinculin (cat. no. CP74; Merck-Millipore, Darmstadt, Germany) using a fluorescent imaging system on the same membrane (LiCOR Odyssey, Lincoln, NE).

Progesterone and Estradiol

Progesterone and estradiol were measured via ELISA in serum from blood samples obtained in the fasted state per the manufacturer's instructions (ALPCO, Salem, NH).

Statistical Analysis

Obese versus lean comparisons were performed using an unpaired Student *t* test. Group comparisons were also made after adjustment for difference in age between groups. For determination of the effects of the intervention and effects of acute, high-fat feeding, data were analyzed using a two-way ANOVA with repeated measures (group × time). When an interaction was present, post hoc testing was performed using the Holm-Šidák multiple comparisons test. Since all mitochondrial data were unaltered with the SED group, we proceeded to compare the data before and after AET with the lean reference group using a one-way ANOVA and Fisher least significant difference post hoc test. Significance was set at $P < 0.05$ for all parameters.

RESULTS

Participant Characteristics

Baseline characteristics of body weight, BMI, whole-body fat, FFM, age, and plasma concentrations of insulin and glucose as well as HOMA were not different between SED and AET obese women but were greater than in lean women (Table 1). BMI, weight, and whole body fat were increased after 12 weeks in the SED group but were maintained at baseline values after 12 weeks of AET (Table 2).

Table 1—Clinical characteristics of obese and lean women

	Obese	Lean
N	25	14
BMI (kg/m^2)	33 ± 1	24 ± 1#
Weight (kg)	91 ± 2	67 ± 3#
Fat mass (kg)	40 ± 2	23 ± 2#
FFM (kg)	47 ± 1	40 ± 2#
Age (years)	35 ± 1	25 ± 1#
Fasting plasma glucose (mg/dL)	104 ± 3	94 ± 5#
Fasting plasma insulin (μU/mL)	12.4 ± 1.3	5.6 ± 0.8#
HOMA	3.4 ± 0.5	1.3 ± 0.2#

Data are means ± SEM. #Difference for obese vs. lean ($P < 0.05$).

Table 2—Change in characteristics with AET or SED

	AET	SED
N	12	13
ΔBMI (kg/m^2)	−0.5 ± 0.4	1.1 ± 0.2*
ΔWeight (kg)	−1.4 ± 1.0	3.1 ± 0.8*
ΔFat mass (kg)	−1.4 ± 0.7	2.4 ± 0.7*
ΔFFM (kg)	0.4 ± 0.6	1.1 ± 0.7
ΔFasting plasma glucose (mg/dL)	−5.3 ± 2.6	2.6 ± 2.9^
ΔFasting plasma insulin (μU/mL)	−1.4 ± 0.5	1.0 ± 1.4
ΔFasting plasma C-peptide (nmol/L)	−0.09 ± 0.03*	0.03 ± 0.03
ΔHOMA	−0.6 ± 0.2	0.3 ± 0.4^
ΔProgesterone (ng/mL)	−0.3 ± 0.4	0.3 ± 1.0
ΔEstradiol (pg/mL)	−8.6 ± 7.2	12.4 ± 10.2

No characteristics were different between groups at baseline. *$P < 0.05$ vs. before intervention (PRE); ^$P \leq 0.06$, interaction.

Lower Mitochondrial Efficiency and Increased mtH_2O_2 Emissions in Obese Women

Absolute whole-body oxygen consumption at baseline was similar between lean and obese women (VO_2 peak) (liters per minute) (Fig. 2*A*) but lower in obese women when expressed relative to lean mass (milliliters per minute per kilogram FFM). Maximal activity of citrate synthase was not different between obese and lean women (Fig. 2*B*). Maximal mitochondrial oxygen flux during state 3 respiration (CI, CI+II, and CII) and uncoupled respiration (FCCP) were also not different between obese and lean (Fig. 2*C*). However, state 4 respiration was greater in obese individuals, indicating increased oxygen consumption not linked with phosphorylation, which is consistent with decreased ($P < 0.05$) mitochondrial coupling efficiency in the form of reduced RCR (Fig. 2*D*). Mitochondrial phosphorylation efficiency was also lower ($P < 0.05$) in obese women as measured by ADP:O (Fig. 2*E*). mtH_2O_2 emissions in isolated mitochondria were greater ($P < 0.05$) in obese versus lean women (Fig. 2*F*). Since there was a small age difference between groups, we correlated age with mitochondrial function and found no relationship between age and any mitochondrial parameter (state 4 respiration, R^2 = 0.05; RCR, R^2 = 0.11; ADP:O, R^2 = 0.08; and mtH_2O_2, R^2 = 0.03). Moreover, after adjustment for age, obese women still exhibit mitochondrial derangements evidenced from lower bioenergetics efficiency (decreased RCR and ADP:O) and elevated mtH_2O_2 emissions compared with lean, insulin-sensitive women.

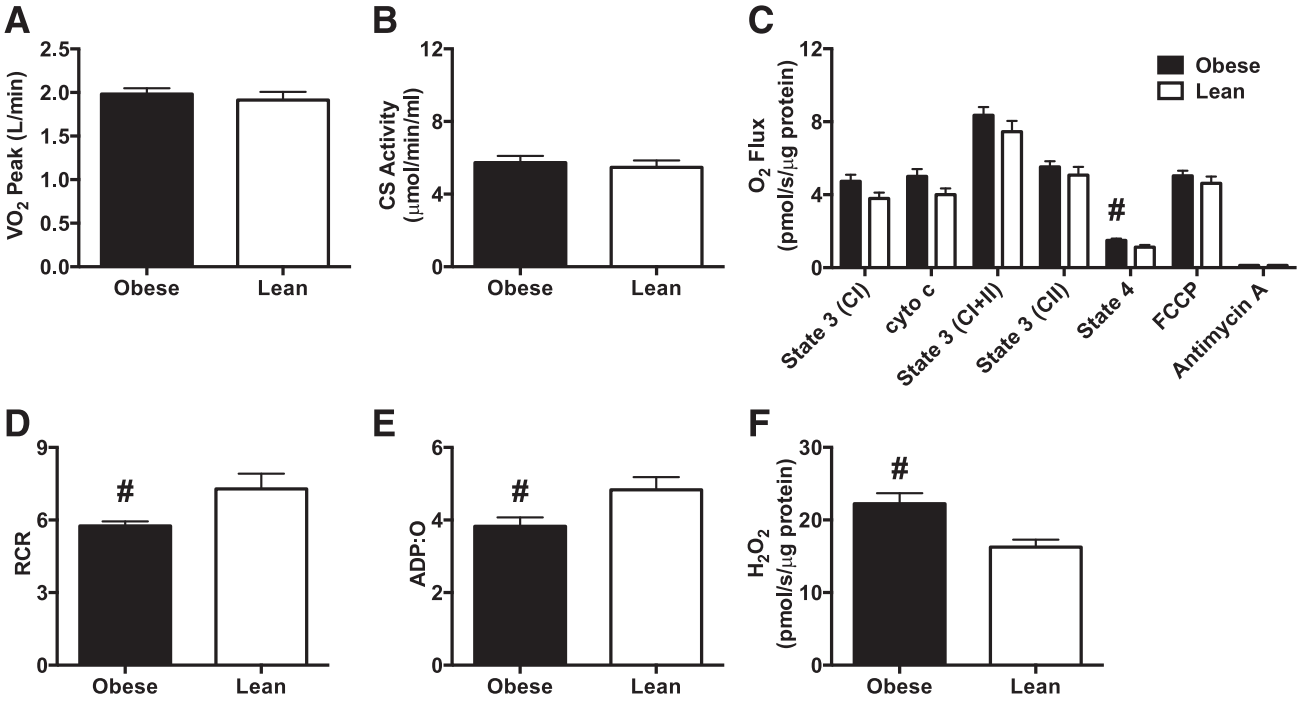

Figure 2—Obese women exhibited lower mitochondrial bioenergetics efficiency and increased mtH_2O_2 emissions. Whole-body oxygen consumption (*A*), maximal citrate synthase (CS) activity (*B*), and mitochondrial oxidative capacity (*C*) were not different between sedentary obese and lean women. Maximal mitochondrial oxidative capacity was determined during state 3 respiration with substrates targeting CI, CI+II, and CII. However, greater basal and state 4 respiration (*C*) and lower RCR (state 3/state 4) (*D*) indicate decreased mitochondrial coupling efficiency in obese women. Obese women also demonstrated reduced phosphorylation efficiency (ADP:O) (*E*). mtH_2O_2 emissions were increased in obese compared with lean women during state 2 conditions with glutamate (10 mmol/L), malate (2 mmol/L), and succinate (10 mmol/L). Mitochondrial energetics in obese and lean women were evaluated in isolated mitochondria and expressed relative to mitochondrial protein content (micrograms of protein). Data presented as mean ± SEM. #Statistical differences from lean ($P < 0.05$).

After Exercise, Mitochondrial Efficiency and mtH_2O_2 Emissions Are No Longer Different in Obese Versus Lean Women

Mitochondrial energetics in obese women were assessed in the fasted state before and after AET and SED. Obese women in AET were also compared with the lean women. AET increased ($P < 0.05$) VO_2 peak (Fig. 3*A*) and maximal citrate synthase activity (Fig. 3*B*). Maximal mitochondrial oxidative capacity during state 3 and FCCP-induced respiration was also elevated after AET (Fig. 3*D*). RCR and ADP:O increased ($P < 0.05$) after AET (Fig. 3*F* and *G*) and were no longer different from lean women. These data indicate that exercise increased the capacity and bioenergetics efficiency of mitochondria in skeletal muscle.

The H_2O_2-emitting potential of isolated mitochondria in the fasted condition was decreased ($P < 0.05$) after AET, achieving levels similar to those in lean women (Fig. 3*H*). These data are supported by lower ($P < 0.05$) mtH_2O_2 emission during a stepwise succinate titration in permeabilized myofibers (Fig. 3*I*). Decreased mtH_2O_2 emission after AET occurred concomitantly with increased ($P < 0.05$) catalase activity, a myocellular antioxidant, and decreased ($P < 0.05$) 8-oxo-dG, a marker of DNA oxidative damage (Fig. 4*A* and *B*). Moreover, the protein carbonyl content, an indicator of protein oxidative damage, was maintained after AET but increased ($P < 0.05$) with SED (Fig. 4*C*). SOD1 and SOD2 activities were unaltered after AET (Fig. 3*D*). Antioxidant activity and DNA oxidative damage were not different with SED. mRNA expression of antioxidant Sod1 was lower after AET, while catalase, Sod2, Txnrd2, Gpx4, and CoxIV were not different between groups.

Relationship Between Mitochondria and Acute High-Fat Feeding Is Altered With Exercise

Acute high-fat feeding did not change mitochondrial respiration (Fig. 5), RCR, or ADP:O before or after either intervention. Similarly, a high-fat mixed meal did not did not alter mtH_2O_2 emissions in sedentary obese women (Fig. 5). However, mtH_2O_2 emissions were lower in the fasted state after AET, while feeding increased ($P < 0.05$) mtH_2O_2 emission in the trained state (Fig. 5).

Exercise Improves Insulin Sensitivity

The GIR ($\mu mol \cdot kg\ FFM^{-1} \cdot min^{-1}$) required to maintain euglycemia during the clamp was 42% greater ($P < 0.05$) after AET and unchanged after SED (Fig. 6*A*). The high-fat mixed meal was an average of 874 kcal (Supplementary Table 1). Insulin sensitivity after the mixed-meal tolerance test increased with time ($P < 0.05$), which was driven mainly by improvements after AET ($P = 0.06$ interaction) (Fig. 5*B*). Glucose tolerance measured by the glucose area under the curve (AUC) in response to the meal was lower ($P < 0.05$) after AET and unaltered after SED (Fig. 6*C*). Insulin AUC was lower after AET ($P = 0.05$) and unchanged after SED (Fig. 6*D*). No change in C-peptide AUC was seen in either group (Fig. 6*E*).

DISCUSSION

The current study shows that obese, insulin-resistant women with PCOS exhibit mitochondrial derangements in the form of lower bioenergetics efficiency and increased mtH_2O_2 emissions and that exercise training reversed these derangements to resemble a lean phenotype. Furthermore, exercise training increased catalase antioxidant activity, which, in concert with lower mtH_2O_2 production, likely explains why exercise training decreased skeletal muscle DNA oxidative damage. Exercise training also increased maximal mitochondrial respiration and tricarboxylic acid (TCA) cycle enzyme activity. Compared with the results in SED subjects, insulin sensitivity was improved and mtH_2O_2 emissions were responsive to feeding after exercise. We find that obese women with PCOS exhibit distinct mitochondrial derangements involving increased mtH_2O_2 production and impaired coupling efficiency. We show that a realistic, short-term exercise program not only improves skeletal muscle mitochondrial oxidative capacity but also normalizes the impairments in mitochondrial efficiency and oxidant emission, increases activity of endogenous antioxidant systems, and decreases cellular oxidative damage. These physiological adaptations to exercise are likely key elements that contribute to the improvement of insulin sensitivity in these women.

In the current study, we show that insulin sensitivity is lower in obese women in spite of mitochondrial oxidative capacity similar to that in lean control subjects. The current study is consistent with emerging evidence from our laboratory and others that maximal mitochondrial oxidative capacity may not be a primary determinant of insulin sensitivity in obesity or PCOS (6,7,10,11,14,19). However, we found marked differences in oxidative stress in the form of elevated mtH_2O_2 emissions in obese insulin-resistant compared with lean insulin-sensitive women, which supports the relationship between mtH_2O_2 emissions and insulin resistance (9,10,25). Obesity or a high-calorie diet generates an energy surplus when paired with low energy demands of sedentary behavior. The nutrient burden creates a positive mitochondrial membrane potential, which increases mtH_2O_2 emissions (17), and chronic exposure to reactive oxygen species has been shown to inhibit insulin-mediated GLUT4 translocation (26). This lends merit to chronically elevated mtH_2O_2 production as a mechanism to diminish insulin sensitivity in obese women. Of interest is that while obese women displayed constitutively elevated mtH_2O_2 emissions in the fasting state, an acute, high-fat meal did not change mtH_2O_2 emissions. The observation that obese women did not have altered mtH_2O_2 production after high-fat feeding is consistent with obese, insulin-resistant animals and humans that have an impaired ability to alter mitochondrial-derived metabolites and substrate utilization to changes in substrate availability (27–29). Thus, our results are in line with the concept of impaired metabolic flexibility at the level of the mitochondrion. Collectively, these findings suggest that mtH_2O_2 emissions are

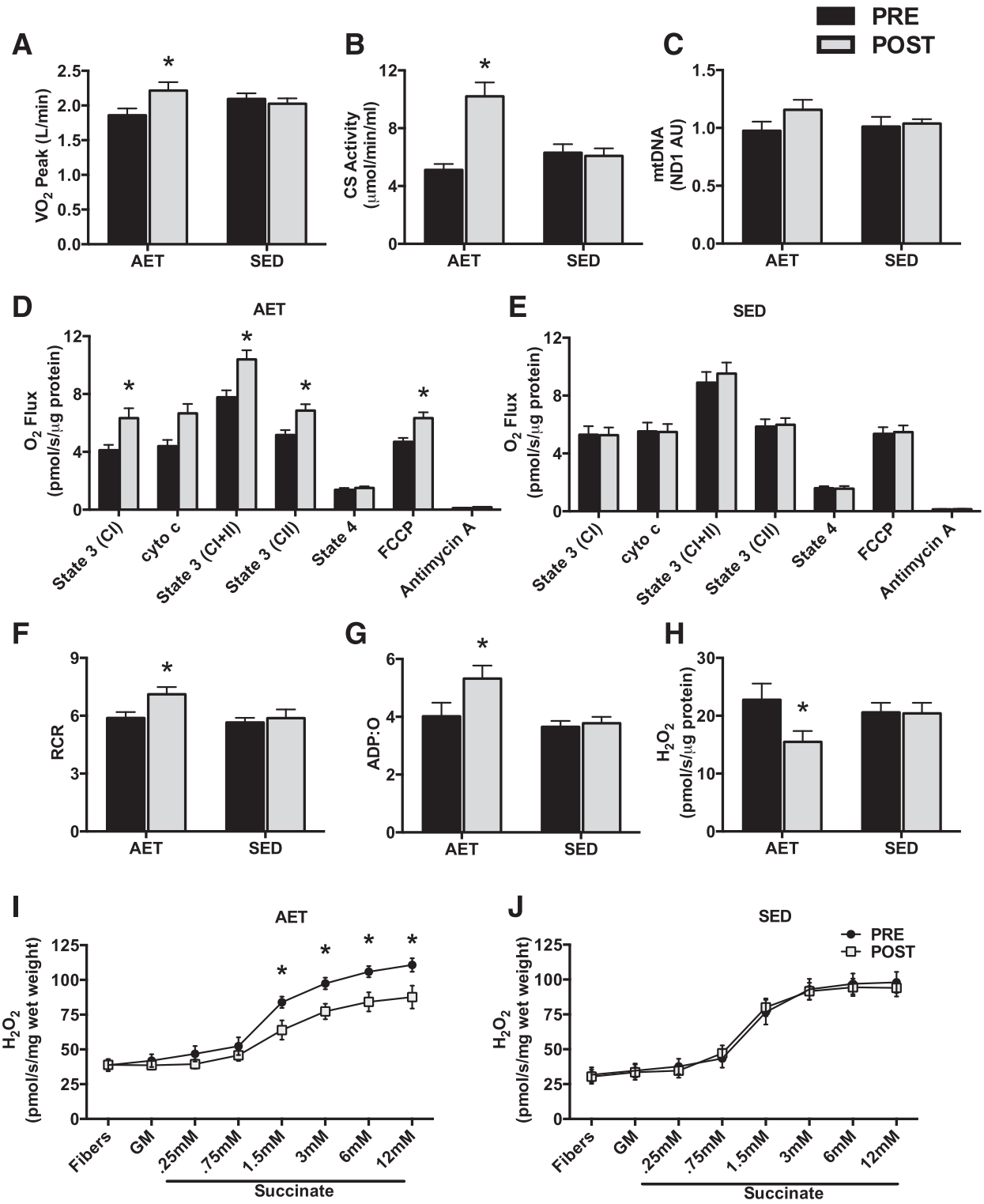

Figure 3—Mitochondrial efficiency and mtH_2O_2 emission are restored with exercise training. Whole-body oxygen consumption (*A*), maximal citrate synthase (CS) activity (*B*), and mitochondrial oxidative capacity (*D* and *E*) in obese women were increased after 12 weeks of AET and mtDNA was unaltered (*C*). Moreover, deficiencies in mitochondrial coupling (RCR) and phosphorylation efficiency (ADP:O) in obese women were improved after exercise and no longer different than in lean women (*F* and *G*). mtH_2O_2 emission in isolated mitochondria was reduced after exercise training, reaching values that were not different than lean women (*H*). The finding that exercise lowers mtH_2O_2 emission was recapitulated in permeabilized myofibers during a stepwise succinate titration protocol (0.25–12 mmol/L) during state 4 conditions (2 μg/μL oligomycin) (*I* and *J*). Baseline comparisons between groups (AET versus SED) via an unpaired Student *t* test revealed no statistical differences ($P = 0.17$). SED did not alter any mitochondrial parameters. Data from isolated mitochondria were expressed relative to mitochondrial protein content (micrograms of protein). mtH_2O_2 emission from permeabilized myofibers were expressed relative to fiber bundle wet weight (milligrams). Data are mean ± SEM. *Statistical differences from before intervention (PRE) ($P < 0.05$). AU, arbitrary units.

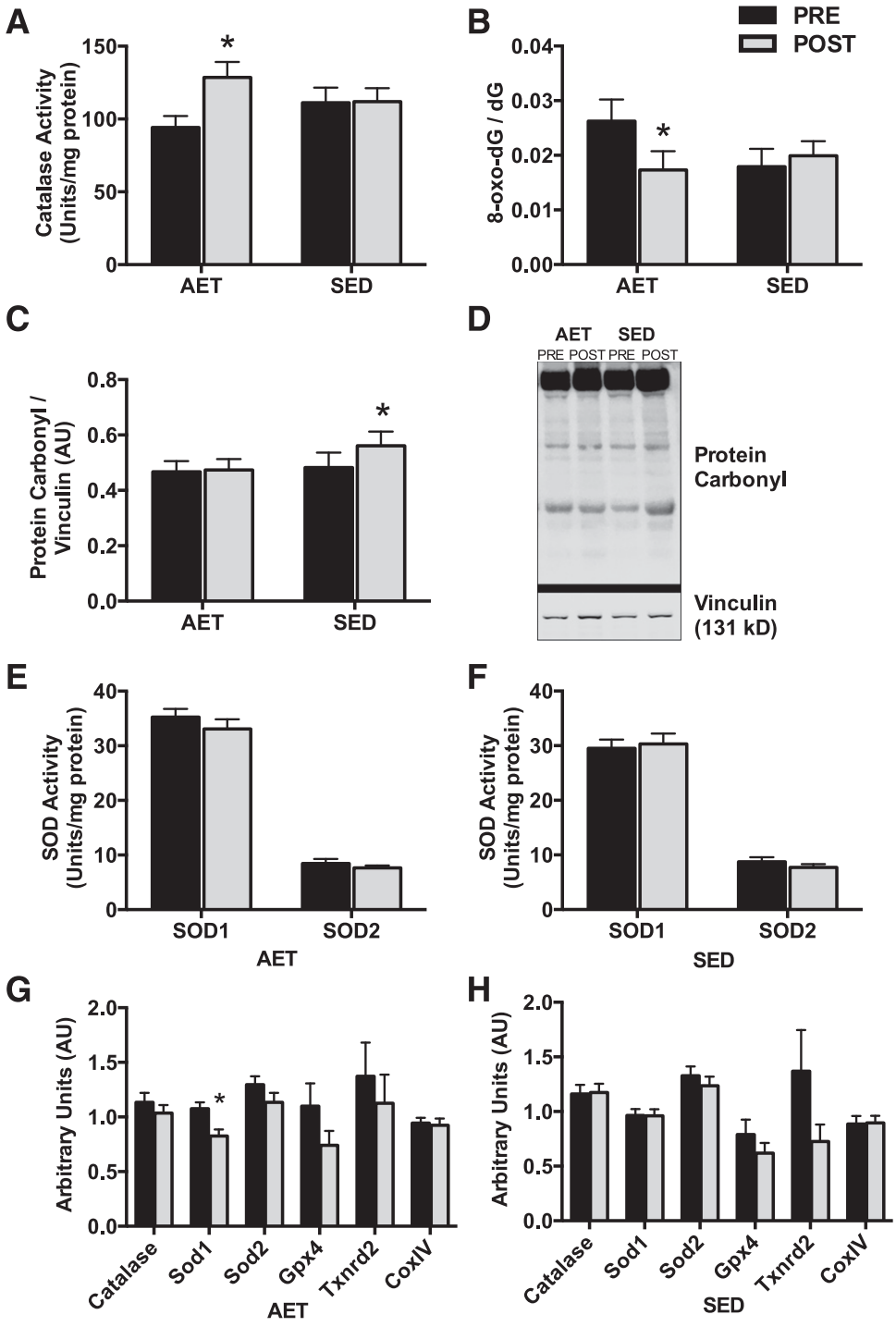

Figure 4—Catalase activity is increased and DNA oxidative damage is reduced after exercise training. Maximal catalase buffering capacity assessed in skeletal muscle homogenates was increased after AET (*A*). Skeletal muscle DNA oxidative damage was reduced when determined from 8-oxo-dG, an abundant adduct formed by DNA oxidation (*B*). Protein oxidative damage was preserved with exercise, while the SED group accumulated damaged proteins (*C*). Assessment of protein carbonyl provides a crude assessment of cellular protein oxidative damage as seen in the representative blot. Vinculin was used to confirm equal protein loading (*D*). Maximal activities of cytoplasmic (SOD1) and mitochondrial (SOD2) SOD were unaltered with exercise training or sedentary behavior (*E* and *F*). mRNA expression of antioxidants catalase, Sod2, Gpx4, and Txnrd2 and mitochondrial marker CoxIV was not different between groups (*G* and *H*). Sod1 mRNA expression decreased after AET. Antioxidant activity is expressed relative to muscle homogenate protein content (milligrams of protein). Data are mean ± SEM. *Statistical differences from before intervention (PRE) ($P < 0.05$).

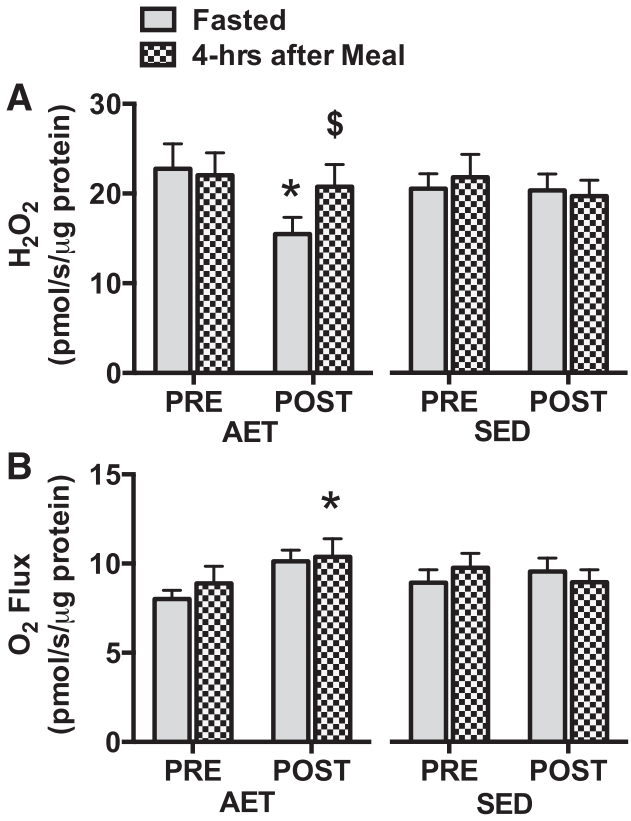

Figure 5—mtH_2O_2 emissions were increased after acute, high-fat feeding in the trained state. During SED conditions (before intervention [PRE] AET, PRE and POST SED), acute high-fat feeding did not alter mtH_2O_2 emissions. However, after exercise training (POST AET), feeding increased mtH_2O_2 production (*A*). mtH_2O_2 emissions were evaluated in isolated mitochondria during state 2 conditions. Acute, high-fat feeding did not alter mitochondrial respiration before or after either intervention (*B*). Mitochondrial oxygen consumption presented in Figure 5 is during state 3 respiration through CI+II supported by glutamate, malate, succinate, and ADP. Data are presented as mean ± SEM. $Effect of feeding ($P < 0.05$); *difference from PRE ($P < 0.05$). hrs, hours.

associated with insulin resistance and metabolic inflexibility. Identification of the precise mechanism(s) connecting these impairments requires future study.

Although maximal mitochondrial oxidative capacity was not different between obese and lean women, impaired mitochondrial bioenergetics efficiency was apparent in obese women. Obese women had greater oxygen consumption during oligomycin-supported state 4 respiration, suggesting that oxygen consumption was not well coupled to phosphorylation and may be related to proton leak across the inner mitochondrial membrane. This is consistent with a lower RCR, which represents greater mitochondrial uncoupled respiration. Moreover, obese women also exhibited a lower ADP:O ratio, indicating that more oxygen is needed to phosphorylate ADP to ATP. Even though the obese and lean women were both young (35 vs. 25 years old), there was a statistical difference in age. Our previous work suggests a 5% decrease in maximal oxidative capacity per decade of increasing age (30), but this was not apparent in the current study. Additionally, we found no correlation between age and any mitochondrial parameter, and when age was used as a covariate our findings did not change. Therefore, we are confident that our data demonstrate impaired mitochondrial bioenergetics efficiency in obese compared with lean women. The mechanisms underlying detriments in mitochondrial efficiency are not certain; however, lipid stress can evoke mitochondrial uncoupling potentially through uncoupling protein 3 (31). Increased mitochondrial uncoupling has been proposed to protect against mtH_2O_2 emissions, but it appears that the degree of mitochondrial redox burden in this cohort overrides any substantial protective effects. An alternative hypothesis may be that H_2O_2-induced post-translational modifications to complexes of the electron transport chain (32) and/or the inner mitochondrial membrane (33) alter proper function and promote uncoupling.

In the current study, 12 weeks of exercise training in obese women not only increased insulin sensitivity but also reduced mtH_2O_2 emission and increased mitochondrial bioenergetics efficiency. Therefore, exercise altered the obesity-related defects in mitochondrial energetics, correcting these functional parameters to values observed in lean, insulin-sensitive women. It is important to note that this enhancement of insulin sensitivity and mitochondrial remodeling occurred despite the fact that these women remained obese and lost only minimal (nonsignificant) weight after exercise training. Improvements in insulin sensitivity with exercise are induced by a myriad of alterations (34–37), while our data suggest that improvements in cellular redox status play a prominent role. There are several precedents within the literature that strongly connect cellular redox status with insulin sensitivity. Anderson et al. (18) convincingly established a link between energy balance, mitochondrial redox state, and insulin sensitivity. Consistent with this hypothesis, we found evidence that women with PCOS, who are inherently insulin resistant, exhibit marked improvements in mtH_2O_2 production, coupling efficiency, catalase activity, and DNA oxidative damage after exercise training. Moreover, lower mtH_2O_2 emissions may allow increased GLUT4 translocation (26), while overexpression of catalase activity can rescue insulin-stimulated glucose disposal by 65% during insulin resistance in vitro (38), which supports our 42% increase in GIR during the clamp in vivo. Together, these adaptations appear to improve the myocellular redox status through mechanisms similar to those that we previously reported preserved mitochondrial performance during senescence in lifelong calorie-restricted mice (15). The current study extends these earlier observations to suggest that interventions that modulate cellular redox status also improve insulin sensitivity.

After AET, there was a change in the relationship among a single high-fat mixed meal, mtH_2O_2 emissions, and insulin sensitivity. In the trained state, high-fat feeding increased mtH_2O_2 emissions in obese women, and this change in mtH_2O_2 production and plausibly

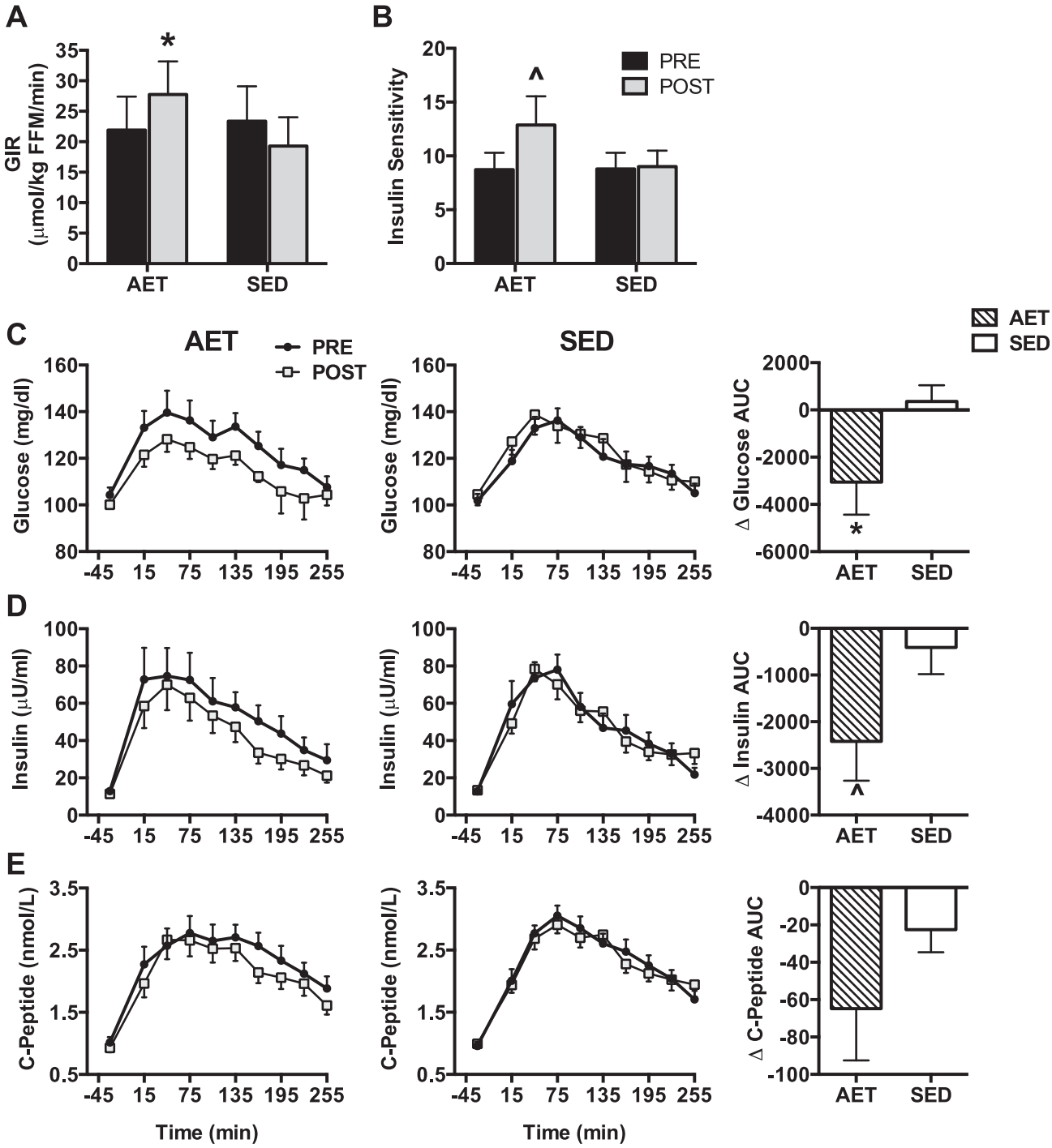

Figure 6—Exercise improves insulin sensitivity. The GIR required to maintain euglycemia during the hyperinsulinemic clamp was increased after exercise training and was not altered during SED (*A*). A similar trend ($P = 0.06$) was observed for postprandial insulin sensitivity using the oral minimal model (*B*). AET lowered the glucose AUC after a high-fat mixed meal (*C*). Insulin AUC after the meal was also lower ($P = 0.05$) (*D*), but C-peptide was not altered ($P = 0.17$) with exercise (*E*). ΔAbsolute change in the AUC. Data are means ± SEM. *Difference from before intervention (PRE) ($P < 0.05$); ^difference from PRE ($P \leq 0.06$).

metabolic flexibility occurred concurrently with improvements in postprandial insulin sensitivity and glucose tolerance. These findings disassociate an acute rise in mtH_2O_2 emissions with impaired insulin sensitivity and suggest that a brief elevation in mtH_2O_2 emissions after a nutritional stimulus may be a healthy response, as shown previously in lean, insulin-sensitive individuals (18). Indeed, an acute dose of H_2O_2 can increase insulin-stimulated glucose uptake (39), consistent with the findings in the current study. It is possible that with repeated consumption of high-calorie, high-fat meals, the repeated increase in mtH_2O_2 production may accumulate over

time, which strengthens the link that constitutively high rates of mtH_2O_2 emissions participate in the etiology of insulin resistance during obesity.

AET enhanced mitochondrial bioenergetics efficiency, correcting the impairment in obese women, and improved citrate synthase activity and oxidative capacity, which is consistent with classical mitochondrial adaptations (40–44). It is important to note that we observed increased maximal respiration with substrates targeting CII succinate dehydrogenase (SDH). SDH is an enzyme common to both the TCA cycle and electron transport chain and, accordingly, our study found that citrate synthase and SDH, both involved in the TCA cycle, were increased after training, indicating increased maximal capacity for TCA cycle flux in obese women. These data may have important implications, as previous models of obesity have indicated that β-oxidation outpaces TCA cycle, leading to accumulation of acetyl-CoA, acylcarnitine esters, and mtH_2O_2 (16,45,46). These metabolites can allosterically inhibit glucose oxidation and may induce posttranslational modifications such as oxidation and acetylation to mitochondrial proteins, potentially worsening mitochondrial uncoupling, incomplete β-oxidation, TCA cycle function, and insulin action. Indeed, we and others have shown that exercise increases electron transport coupling efficiency (current study), complete β-oxidation (28,46), and TCA cycle enzymes (47), including citrate synthase, SDH, and isocitrate dehydrogenase, which may collectively alleviate the metabolic bottleneck and metabolite accumulation during nutrient stress. Consistent with this notion, our current study demonstrates that exercise prevents the accumulation of global protein oxidation, which is in accord with our recent findings that 8 weeks of exercise training in healthy adults decreased oxidation of mitochondrial proteins, creating a cellular milieu conducive for improved metabolic flux (48).

In conclusion, we have demonstrated that derangements in mitochondrial bioenergetics efficiency and mtH_2O_2 emissions in obese women are improved with 12 weeks of AET and modulating myocellular redox status occurs concomitantly with improved insulin sensitivity.

Acknowledgments. The authors are greatly indebted to the skillful assistance of Katherine Klaus, Daniel Jakaitis, Jill Schimke, Dawn Morse, Roberta Soderberg, Deborah Sheldon, Lynne Johnson, and Melissa Aakre.

Funding. The authors are grateful for support from National Institutes of Health (NIH) grants T32DK007352 (to A.R.K. and M.M.R.) and DK41973 (to K.S.N.). Additional support was provided by the Mayo Foundation and funding for the Murdock-Dole Professorship (to K.S.N.). This publication was made possible by the Mayo Clinic Metabolomics Resource Core through grant U24DK100469 from the National Institute of Diabetes and Digestive and Kidney Diseases and the Mayo Clinic Center for Translational Science Activities grants UL1TR000135 and KL2TR000136 (to M.L.J.), and KL2RR024151 (to B.A.I.) from the National Center for Advancing Translational Sciences.

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official view of the NIH.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.R.K. contributed to data collection, data analysis, and data interpretation and wrote the manuscript. A.A. contributed to data collection and data analysis. I.R.L. contributed to conceptual design, data collection, data interpretation, and manuscript writing and editing. M.M.R. and M.L.J. contributed to data collection, data analysis, and manuscript editing. C.D.M. and C.C. contributed to data analysis. M.H.A. and B.A.I. contributed to conceptual design and data collection. K.S.N. contributed to conceptual design, data analysis, data interpretation, and manuscript writing and editing. K.S.N. 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.

Prior Presentation. Parts of this study were presented as an oral presentation at the 74th Annual American Diabetes Association Scientific Meeting, San Francisco, CA, 13–17 June 2014.

References

1. Kelley DE, Goodpaster B, Wing RR, Simoneau J-A. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 1999;277:E1130–E1141
2. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002;51:2944–2950
3. Ritov VB, Menshikova EV, Azuma K, et al. Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Endocrinol Metab 2010;298:E49–E58
4. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003;300:1140–1142
5. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 2004;350:664–671
6. Karakelides H, Irving BA, Short KR, O'Brien P, Nair KS. Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. Diabetes 2010;59:89–97
7. Nair KS, Bigelow ML, Asmann YW, et al. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. Diabetes 2008;57:1166–1175
8. Larsen S, Ara I, Rabøl R, et al. Are substrate use during exercise and mitochondrial respiratory capacity decreased in arm and leg muscle in type 2 diabetes? Diabetologia 2009;52:1400–1408
9. Lefort N, Glancy B, Bowen B, et al. Increased reactive oxygen species production and lower abundance of complex I subunits and carnitine palmitoyltransferase 1B protein despite normal mitochondrial respiration in insulin-resistant human skeletal muscle. Diabetes 2010;59:2444–2452
10. Fisher-Wellman KH, Weber TM, Cathey BL, et al. Mitochondrial respiratory capacity and content are normal in young insulin-resistant obese humans. Diabetes 2014;63:132–141
11. Lanza IR, Blachnio-Zabielska A, Johnson ML, et al. Influence of fish oil on skeletal muscle mitochondrial energetics and lipid metabolites during high-fat diet. Am J Physiol Endocrinol Metab 2013;304:E1391–E1403
12. Han D-H, Hancock CR, Jung SR, Higashida K, Kim SH, Holloszy JO. Deficiency of the mitochondrial electron transport chain in muscle does not cause insulin resistance. PLoS ONE 2011;6:e19739
13. Hancock CR, Han D-H, Chen M, et al. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci U S A 2008;105:7815–7820
14. Szendroedi J, Schmid AI, Chmelik M, et al. Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. PLoS Med 2007;4:e154
15. Lanza IR, Zabielski P, Klaus KA, et al. Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metab 2012;16:777–788
16. Muoio DM, Neufer PD. Lipid-induced mitochondrial stress and insulin action in muscle. Cell Metab 2012;15:595–605

17. Fisher-Wellman KH, Neufer PD. Linking mitochondrial bioenergetics to insulin resistance via redox biology. Trends Endocrinol Metab 2012;23:142–153
18. Anderson EJ, Lustig ME, Boyle KE, et al. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest 2009;119:573–581
19. Rabøl R, Svendsen PF, Skovbro M, et al. Skeletal muscle mitochondrial function in polycystic ovarian syndrome. Eur J Endocrinol 2011;165:631–637
20. Eriksen MB, Minet AD, Glintborg D, Gaster M. Intact primary mitochondrial function in myotubes established from women with PCOS. J Clin Endocrinol Metab 2011;96:E1298–E1302
21. Amato MC, Galluzzo A, Merlino S, et al. Lower insulin sensitivity differentiates hirsute from non-hirsute Sicilian women with polycystic ovary syndrome. Eur J Endocrinol 2006;155:859–865
22. Ciampelli M, Leoni F, Cucinelli F, et al. Assessment of insulin sensitivity from measurements in the fasting state and during an oral glucose tolerance test in polycystic ovary syndrome and menopausal patients. J Clin Endocrinol Metab 2005;90:1398–1406
23. Cobelli C, Dalla Man C, Toffolo G, Basu R, Vella A, Rizza R. The oral minimal model method. Diabetes 2014;63:1203–1213
24. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 2010;5:51–66
25. Abdul-Ghani MA, Jani R, Chavez A, Molina-Carrion M, Tripathy D, Defronzo RA. Mitochondrial reactive oxygen species generation in obese non-diabetic and type 2 diabetic participants. Diabetologia 2009;52:574–582
26. Rudich A, Tirosh A, Potashnik R, Hemi R, Kanety H, Bashan N. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. Diabetes 1998;47:1562–1569
27. Muoio DM, Noland RC, Kovalik J-P, et al. Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. Cell Metab 2012;15:764–777
28. Battaglia GM, Zheng D, Hickner RC, Houmard JA. Effect of exercise training on metabolic flexibility in response to a high-fat diet in obese individuals. Am J Physiol Endocrinol Metab 2012;303:E1440–E1445
29. Ukropcova B, Sereda O, de Jonge L, et al. Family history of diabetes links impaired substrate switching and reduced mitochondrial content in skeletal muscle. Diabetes 2007;56:720–727
30. Short KR, Bigelow ML, Kahl J, et al. Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 2005;102:5618–5623
31. Anderson EJ, Yamazaki H, Neufer PD. Induction of endogenous uncoupling protein 3 suppresses mitochondrial oxidant emission during fatty acid-supported respiration. J Biol Chem 2007;282:31257–31266
32. Choksi KB, Nuss JE, Deford JH, Papaconstantinou J. Age-related alterations in oxidatively damaged proteins of mouse skeletal muscle mitochondrial electron transport chain complexes. Free Radic Biol Med 2008;45:826–838
33. Paradies G, Petrosillo G, Pistolese M, Ruggiero FM. Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage. Gene 2002;286:135–141
34. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. J Appl Physiol Bethesda Md (1985) 2005;99:338–343
35. Perseghin G, Price TB, Petersen KF, et al. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. N Engl J Med 1996;335:1357–1362
36. Coen PM, Goodpaster BH. Role of intramyocelluar lipids in human health. Trends Endocrinol Metab 2012;23:391–398
37. Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest 2007;117:1690–1698
38. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944–948
39. Higaki Y, Mikami T, Fujii N, et al. Oxidative stress stimulates skeletal muscle glucose uptake through a phosphatidylinositol 3-kinase-dependent pathway. Am J Physiol Endocrinol Metab 2008;294:E889–E897
40. Konopka AR, Suer MK, Wolff CA, Harber MP. Markers of human skeletal muscle mitochondrial biogenesis and quality control: effects of age and aerobic exercise training. J Gerontol A Biol Sci Med Sci 2014;69:371–378
41. Short KR, Vittone JL, Bigelow ML, et al. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. Diabetes 2003;52:1888–1896
42. Ghosh S, Lertwattanarak R, Lefort N, et al. Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance. Diabetes 2011;60:2051–2060
43. Menshikova EV, Ritov VB, Ferrell RE, Azuma K, Goodpaster BH, Kelley DE. Characteristics of skeletal muscle mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity. J Appl Physiol (1985) 2007;103:21–27
44. Hutchison SK, Teede HJ, Rachoń D, Harrison CL, Strauss BJ, Stepto NK. Effect of exercise training on insulin sensitivity, mitochondria and computed tomography muscle attenuation in overweight women with and without polycystic ovary syndrome. Diabetologia 2012;55:1424–1434
45. Koves TR, Ussher JR, Noland RC, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 2008;7:45–56
46. Koves TR, Li P, An J, et al. Peroxisome proliferator-activated receptor-γ coactivator 1α-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. J Biol Chem 2005;280:33588–33598
47. Lanza IR, Short DK, Short KR, et al. Endurance exercise as a countermeasure for aging. Diabetes 2008;57:2933–2942
48. Johnson ML, Irving BA, Lanza IR, et al. Differential Effect of Endurance Training on Mitochondrial Protein Damage, Degradation, and Acetylation in the Context of Aging. J Gerontol A Biol Sci Med Sci. 10 December 2014 [Epub ahead of print]