The notion that mitochondria contribute to obesity-induced insulin resistance is highly debated. Therefore, we determined whether obese (BMI 33 kg/m²), insulin-resistant women with polycystic ovary syndrome had aberrant skeletal muscle mitochondrial physiology compared with lean, insulin-sensitive women (BMI 23 kg/m²). 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 H₂O₂ (mtH₂O₂) 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 mtH₂O₂ 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. mtH₂O₂ emissions were refractory to a high-fat meal at baseline, but after exercise, mtH₂O₂ 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 mtH₂O₂ 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
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 (mtH2O2) 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 mtH2O2 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 mtH2O2 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 mtH2O2 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/m²) 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 (ISOGTT 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, anti-hyperglycemic/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 VO2 peak 5 days per week. VO2 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 VO2 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 μU · 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
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$_2$O$_2$ 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 mM), malate (2 mM), and succinate (10 mM). The fluorescent signal was corrected for background auto-oxidation and calibrated to a standard curve. H$_2$O$_2$ production rates were expressed relative to mitochondrial protein.

H$_2$O$_2$ 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$_2$HPO$_4$, 3 MgCl$_2$-6H$_2$O, 0.05 pyruvate, and 0.02 malate and 5 mg/mL BSA (pH 7.4, 295 mOsm). Fibers were preincubated in 10 mM pyrophosphate to deplete endogenous adenylates. Fiber bundles were placed in a quartz cuvette with 2 mL buffer containing 2 μM oligomycin followed by a stepwise addition of glutamate (5 μmol/L), malate (2 μmol/L), and succinate (0.25–12 mM) to stimulate H$_2$O$_2$ production under state 4 conditions. H$_2$O$_2$ 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 Carbyl Immunoblot kit (Cell Biolabs, San Diego, CA) following the manufacturer’s guidelines.

**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 ($P < 0.05$).
Lower Mitochondrial Efficiency and Increased mtH2O2 Emissions in Obese Women

Absolute whole-body oxygen consumption at baseline was similar between lean and obese women (VO2 peak) (liters per minute) (Fig. 2A) 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. 2B). 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. 2C). 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. 2D). Mitochondrial phosphorylation efficiency was also lower (P < 0.05) in obese women as measured by ADP:O (Fig. 2E). mtH2O2 emissions in isolated mitochondria were greater (P < 0.05) in obese versus lean women (Fig. 2F). 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, R2 = 0.05; RCR, R2 = 0.11; ADP:O, R2 = 0.08; and mtH2O2, R2 = 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 mtH2O2 emissions compared with lean, insulin-sensitive women.

Figure 2—Obese women exhibited lower mitochondrial bioenergetics efficiency and increased mtH2O2 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). mtH2O2 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).

Table 2—Change in characteristics with AET or SED

|       | AET  | SED  |
|-------|------|------|
| N     | 12   | 13   |
| ΔBMI (kg/m²) | −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 ≤ 0.06, interaction.
After Exercise, Mitochondrial Efficiency and mtH2O2 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) VO2 peak (Fig. 3A) and maximal citrate synthase activity (Fig. 3B). Maximal mitochondrial oxidative capacity during state 3 and FCCP-induced respiration was also elevated after AET (Fig. 3D). RCR and ADP:O increased (P < 0.05) after AET (Fig. 3F 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 H2O2-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. 3H). These data are supported by lower (P < 0.05) mtH2O2 emission during a stepwise succinate titration in permeabilized myofibers (Fig. 3I). Decreased mtH2O2 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. 4A 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. 4C). SOD1 and SOD2 activities were unaltered after AET (Fig. 3D). Antioxidant activity and DNA oxidative damage were not different with SED. mRNA expression of antioxidant Sod1 was lower after AET, while catalase, Sod2, Txnrdr2, 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 alter mtH2O2 emissions in sedentary obese women (Fig. 5). However, mtH2O2 emissions were lower in the fasted state after AET, while feeding increased (P < 0.05) mtH2O2 emission in the trained state (Fig. 5).

Exercise Improves Insulin Sensitivity

The GIR (µmol·kg FFM⁻¹·min⁻¹) required to maintain euglycemia during the clamp was 42% greater (P < 0.05) after AET and unchanged after SED (Fig. 6A). 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. 5B). 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. 6C). Insulin AUC was lower after AET (P = 0.05) and unaltered after SED (Fig. 6D). No change in C-peptide AUC was seen in either group (Fig. 6E).

DISCUSSION

The current study shows that obese, insulin-resistant women with PCOS exhibit mitochondrial derangements in the form of lower bioenergetics efficiency and increased mtH2O2 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 mtH2O2 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 mtH2O2 emissions were responsive to feeding after exercise. We find that obese women with PCOS exhibit distinct mitochondrial derangements involving increased mtH2O2 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 mtH2O2 emissions in obese insulin-resistant compared with lean insulin-sensitive women, which supports the relationship between mtH2O2 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 mtH2O2 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 mtH2O2 production as a mechanism to diminish insulin sensitivity in obese women. Of interest is that while obese women displayed constitutively elevated mtH2O2 emissions in the fasting state, an acute, high-fat meal did not change mtH2O2 emissions. The observation that obese women did not have altered mtH2O2 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 mtH2O2 emissions are
Mitochondrial efficiency and mtH$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ emission was recapitulated in permeabilized myofibers during a stepwise succinate titration protocol (0.25–12 mmol/L) during state 4 conditions (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$_2$O$_2$ 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 (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 Txmd2 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).
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 detriment 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 mtH2O2 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 H2O2-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 mtH2O2 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 mtH2O2 production, coupling efficiency, catalase activity, and DNA oxidative damage after exercise training. Moreover, lower mtH2O2 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, mtH2O2 emissions, and insulin sensitivity. In the trained state, high-fat feeding increased mtH2O2 emissions in obese women, and this change in mtH2O2 production and plausibly...
metabolic flexibility occurred concurrently with improvements in postprandial insulin sensitivity and glucose tolerance. These findings disassociate an acute rise in mtH2O2 emissions with impaired insulin sensitivity and suggest that a brief elevation in mtH2O2 emissions after a nutritional stimulus may be a healthy response, as shown previously in lean, insulin-sensitive individuals (18). Indeed, an acute dose of H2O2 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 mtH2O2 production may accumulate over

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 ($P \leq 0.06$).
time, which strengthens the link that constitutively high rates of mtH$_2$O$_2$ 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 CI 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$_2$O$_2$ (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$_2$O$_2$ emissions in obese women are improved with 12 weeks of AET and modulating myocellular redox status occurs concomitantly with improved insulin sensitivity.

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

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