Skeletal muscle respiratory capacity is enhanced in rats consuming an obesogenic Western diet

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Skeletal muscle respiratory capacity is enhanced in rats consuming an obesogenic Western diet. Am J Physiol Endocrinol Metab 302: E1541–E1549, 2012. First published April 10, 2012; doi:10.1152/ajpendo.00590.2011.—Obesity-induced lipid over-supply promotes skeletal muscle mitochondrial biogenesis. Previous investigations have utilized extreme high-fat diets (HFD) to induce such mitochondrial perturbations despite their disparity from human obesogenic diets. Here, we evaluate the effects of Western diet (WD)-induced obesity on skeletal muscle mitochondrial function. Long-Evans rats were given ad libitum access to either a WD [40% energy (E) from fat, 17% protein, and 43% carbohydrate (30% sucrose); n = 12] or a control diet (CON; 16% of E from fat, 21% protein, and 63% carbohydrate; n = 12) for 12 wk. Rats fed the WD consumed 23% more E than CON (P = 0.0001), which was associated with greater increases in body mass (23%, P = 0.0002) and adiposity (17%, P = 0.03). There were no differences in fasting blood glucose concentration or glucose tolerance between diets, although fasting insulin was increased by 40% (P = 0.001). The maximal activities of several mitochondrial enzymes (citrate synthase, β-hydroxyacyl-CoA dehydrogenase, carnitine palmitoyltransferase) were also elevated in WD (86%, P = 0.001). The maximal capacity of the electron transfer system was greater following WD (37%, P = 0.02), as were the maximal activities of several mitochondrial enzymes (citrate synthase, β-hydroxyacyl-CoA dehydrogenase, carnitine palmitoyltransferase). Protein expression of citrate synthase, UCP3, and individual respiratory complexes was increased in response to WD (P = 0.0002). There were no differences in respiratory coactivator-1 mRNA or protein abundance. We conclude that the respiratory capacity of skeletal muscle is enhanced in response to the excess energy supplied by a WD. This is likely due to an increase in mitochondrial density, which, acting as ligands to peroxisome proliferator-activated receptors (PPARs) (18), are thought to increase mitochondrial biogenesis via the PPARγ-coactivator-1α (PGC-1α) pathway (18, 21, 26, 45). The results of these studies, as well as previous work from our laboratory (15), suggest that certain aspects of mitochondrial function (i.e., β-oxidation and oxidative phosphorylation) may be upregulated following a HFD.

The issue of whether mitochondrial “deficiency and/or dysfunction” contributes to the obesity sequelae has been questioned in light of recent reports of high-fat diet (HFD)-induced increases in mitochondrial enzyme activities and protein expression (13, 21, 25, 51, 52). Indeed, such diets cause an increase in circulating free fatty acids (FFA) (19–21, 53), which, acting as ligands to peroxisome proliferator-activated receptors (PPARs) (18), are thought to increase mitochondrial biogenesis via the PPARγ-coactivator-1α (PGC-1α) pathway (18, 21, 26, 45). The results of these studies, as well as previous work from our laboratory (15), suggest that certain aspects of mitochondrial function (i.e., β-oxidation and oxidative phosphorylation) may be upregulated following a HFD.

High-fat fed rodents rapidly gain weight while concomitantly developing insulin resistance (32, 33, 57). As such, this model is used frequently to investigate the underlying mechanisms of obesity and its associated conditions. Investigations are often undertaken based on the assumption that these pathologies develop similarly in rodents as they do in humans. However, the typical human obesogenic diet, i.e., a Western diet (WD), is moderately high in fat [~40–45% of total energy (E)] yet still comprises a substantial proportion of E from carbohydrate (particularly simple sugars) and protein (43). In contrast, a typical rodent HFD usually contains extreme levels of fat (≤80% of total available E) and very low carbohydrate (9, 43). Given the role diet composition plays in the mechanistic progression of obesity and insulin resistance, the disparity between the macronutrient breakdown of a rodent HFD and a typical human obesogenic diet is an important factor to consider when interpreting results (9, 56). Whereas diets high in fat and diets high in sugar may result in similar outcomes, such as rapid weight gain (9, 56), increased circulating lipids (9, 56), and insulin resistance (9), the mechanisms underlying these changes are likely to be markedly different (9).
In the current investigation, we fed rats a commercially available diet that closely mimics a human WD (a diet both high in fat and in sucrose) and measured the mitochondrial respiratory capacity of skeletal muscle using high-resolution respirometry. We hypothesized that 12 wk of a WD would increase the respiratory capacity of skeletal muscle mitochondria via the upregulation of PPARδ and PGC-1α.

**METHODS**

**Animals.** Eight-week-old male Long-Evans rats (*n* = 24) were obtained from Monash Animal Services (Monash, Australia) and housed under a controlled 12:12-h light-dark cycle at a constant temperature of 22°C. Animals had ad libitum access to food and water and were allowed to acclimate to the Royal Melbourne Institute of Technology (RMIT) Animal Facility for 1 wk prior to commencement of the study. All procedures were approved by the RMIT Animal Ethics Committee.

**Experimental groups.** Following the acclimation period rats were randomly assigned to one of two 12-wk dietary interventions: a control diet (CON; *n* = 12, 16% of E from fat, 21% from protein, and 63% from carbohydrate, 16.1 kJ/g; Specialty Feeds, Glen Forrest, Western Australia, Australia) or a commercially available WD (*n* = 12; 40% E from fat, 17% from protein, and 43% from carbohydrate, 19.4 kJ/g; Specialty Feeds). The macronutrient breakdown of each diet is presented in Table 1. Body mass and food intake were monitored twice weekly.

**Intraperitoneal glucose tolerance tests.** After 11 wk, all rats were subjected to a glucose challenge to assess glucose clearance. Following an overnight fast (14 h), ~15 μl of blood was collected via tail cut, and glucose concentration was measured on a handheld glucometer (Roche Diagnostics, Castle Hill, New South Wales, Australia). Rats then received a single glucose bolus (2 g/kg body wt) via intraperitoneal injection, and blood glucose concentration was monitored at 30-min intervals over 120 min postinjection.

**Tissue collection and analyses.** After 12 wk, animals were decapitated and hindlimb muscles immediately excised. The left soleus (~87% type I and ~13% type IIA fibers) was placed in ice-cold muscle preservation medium [BIOPS; 50 mM K+ -MES, 20 mM taurine, 0.5 mM dithiothreitol, 6.56 mM MgCl2, 5.77 mM ATP, 15.72 mM K2EGTA, 0.1 mM phosphocreatine, and 20 mM imidazole, pH 7.1, adjusted with 5 mM HEPES, 20 mM HEpes, adjusted to pH 7.1 with KOH at 37°C, and 1 g/l fatty acid-free BSA; ACROS Organics] (38). Duplicate tissue samples (2–3.5 mg) were transferred to the chambers of an O2X-Oxygraph high-resolution respirometer (Oroboros, Innsbruck, Austria) containing 2 ml of MiR06 (MiR05 + 280 IU/ml catalase) and calibrated to air saturation. Individual chambers were oxygenated to ~485 nmol/ml with pure O2 (BOC Australia).

**Substrate uncoupler inhibitor titration protocol.** Malate (2 mM) was added to the chamber and the mass specific oxygen flux (ΔO2; pmol·s−1·mg−1 wet wt) stabilized for 5–10 min. The tissue was then subjected to a substrate uncoupler inhibitor titration (SUIT) protocol that sequentially evaluates complex (C)I leak state (the addition of 5 mM pyruvate and 10 mM glutamate; state 2 respiration), oxidative phosphorylation with electron flux through CI by titration (the addition of 0.25, 0.75, 2.5, and 5 mM ADP; state 3 respiration), the integrity of the outer mitochondrial membrane (the addition of 10 μM cyclo-trime concrete c), maximal coupled respiration with electron flux through both CI and CII (the addition of 10 mM succinate; state 3 respiration), maximal capacity of the electron transport system (ETS) by uncoupling with a stepped titration (the addition of 0.5 and 1 μM carboxyl cyanide p-trifluoromethoxyphenylhydrazone), uncoupled oxidative phosphorylation with electron flux through both CI and CII (the addition of 0.5 μM of the CI inhibitor rotenone), and residual O2 consumption (ROX; with the addition of NaNO2, 200 mM). Chambers were maintained at 37°C, and O2 saturation was maintained between 300 and 450 nmol/ml via regular titrations of H2O2. Mass-specific O2 flux was determined using DatLab (Oroboros) from steady-state O2 normalized to tissue wet weight and adjusted for instrumental background. ROX was used in addition to flux control ratios (results normalized to the maximal capacity of the ETS) were calculated to determine mitochondrial function independent of mitochondrial density (38).

**Mitochondrial enzyme activities.** Muscle homogenates (*n* = 10/group) were prepared over ice from snap-frozen soleus or EDL (10–20 mg for each) in buffer (175 mM KCl and 2 mM EDTA, pH 7.4, 1.5% or 1:100 dilution) and then subjected to three freeze-thaw cycles. Citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (β-HAD), and total carnitine palmitoyltransferase (CPT) activities were determined using the methods of Sreer (49), Bergmeyer (5), and Bieber et al. (6), respectively, with the following modifications. Briefly, aliquots of homogenate were added to the appropriate wells of a 96-well microplate with a working solution, with final concentrations of either 72.5 mM Tris-HCl, 0.45 mM acetyl-CoA, 0.1 mM DTNB (for CS), 50 mM Tris-HCl, 2 mM EDTA, 250 μM NADH (for β-HAD), and 116 mM Tris-HCl or 1 mM EDTA, 1.1 mM L-carnitine, and 100 μM DTNB (for CPT). After the wells were monitored for background activity, reactions were initiated via the addition of 0.5 mM oxaloacetic acid (for CS) and 100 μM acetocetate-CoA (for β-HAD) or 1.9 mM palmitoyl-CoA (for CPT). Enzyme activities were determined by monitoring Δabsorbance at 412 nm (for CS and CPT) and 355 nm (for β-HAD) for 3 min at 25°C. Extinction coefficients of 13.6 (for CS and CPT) and 6.22 μmol/cm2 (for β-HAD) were used to calculate rates of enzyme activity. Rates are expressed as μmol·min−1·g−1·wt wt.

**Table 1. Daily energy intake and nutritional parameters for control rats and rats receiving the WD**

<table>
<thead>
<tr>
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<th>Control</th>
<th>WD</th>
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<tr>
<td>Daily energy intake, kJ</td>
<td>273.52 ± 3.94</td>
<td>335.41 ± 5.92*</td>
</tr>
<tr>
<td>Protein (casein)</td>
<td>21.00</td>
<td>17.00</td>
</tr>
<tr>
<td>g/day</td>
<td>3.39 ± 0.05</td>
<td>3.37 ± 0.06</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>63.00</td>
<td>43.00</td>
</tr>
<tr>
<td>g/day</td>
<td>1.69 ± 0.02</td>
<td>5.89 ± 0.10*</td>
</tr>
<tr>
<td>Starch</td>
<td>9.10 ± 0.13</td>
<td>2.66 ± 0.05*</td>
</tr>
<tr>
<td>Lipid</td>
<td>E%</td>
<td>16.00</td>
</tr>
<tr>
<td>E%</td>
<td>1.19 ± 0.02</td>
<td>3.63 ± 0.06*</td>
</tr>
<tr>
<td>Unsaturated, g/day</td>
<td>0.08 ± 0.001</td>
<td>2.37 ± 0.04*</td>
</tr>
<tr>
<td>Saturated, g/day</td>
<td>1.10 ± 0.02</td>
<td>1.24 ± 0.02</td>
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</tbody>
</table>

Values represent means ± SE, as determined over 12 wk; *n* = 12 animals/group. WD, Western diet; E%, energy percent. *P < 0.05.
were extracted in chloroform-methanol (13). The organic portion was then evaporated under a stream of N₂ and reconstituted in chloroform. Silicic acid was then added to the organic portion, and the phospholipids were removed by centrifugation. The resultant supernatant was then evaporated under a stream of N₂ and reconstituted in chloroform.  

**Reverse transcription and real-time PCR.** First-strand cDNA synthesis was performed for RNA extracts using Superscript VILO (Invitrogen). Relative mRNA expression was determined using commercially available Taqman primer/probe sets (Applied Biosystems, Mulgrave, Australia) for PGC-1α (Ppargc1a; cat. no. Rn00580241_m1) and PPARγ (Ppard; cat. no. Rn00565707_m1). Glyceraldehyde-3-phosphate dehydrogenase (Gapdh; cat. no. Rn01775763_g1) was included as a housekeeping gene to normalize threshold cycle (C_T) values. Mitochondrial DNA (mtDNA) copy number was determined as described previously (55, 58), using Taqman primer/probes sets for mitochondrially encoded NADH dehydrogenase-1 (MT-ND1; cat. no. Rn03296764_s1) and nuclear-encoded Gapdh. Quantification was performed in duplicate using a 72-well Rotor-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, Australia). PCR conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Data were calculated using the ΔΔC_T method.  

**Immunoblotting.** Approximately 50 mg of frozen soleus and EDL was homogenized in buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 50 mM NaF, 5 mM Na pyrophosphate, 10% glycerol, 1% Triton X-100, 10 µg/ml trypsin inhibitor, 2 µg/ml aprotonin, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride; 1:8 dilution) and centrifuged at 20,000 g for 30 min at 4°C. Protein concentration of the supernatant was determined using the bicinechonic method (Pierce). Muscle lysates containing either 5 or 20 µg of protein were prepared in 4× Laemmli buffer, subjected to SDS-PAGE, and then transferred to polyvinylidene difluoride membranes. Membranes were blocked (5% nonfat dry milk) for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies specific for signaling proteins [PPARα (no. PA1–823A; Thermo Scientific), PPARγ (no. ab24509; Abcam), and PGC-1 (no. ab3242; Chemicon) and their targets CPT-I (no. sc20670; Santa Cruz Biotechnology) and UCPI (no. PA1-055; Affinity BioReagents)], for mitochondrial proteins [complexes I, II, III, IV (subunit I), and V of the electron transfer system (no. MA604; MitoSciences), complex IV (subunit II, no. MS405; MitoSciences), complex IV (subunit IV, no. MS407; MitoSciences), CS (no. ab96600; Abcam), and as a protein loading control (α-tubulin; Sigma, St. Louis, MO)]. Protein was detected using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) and quantified by densitometry.  

**Statistical analyses.** All values expressed are means ± SE. An unpaired t-test was used to compare groups for all analyses except the intraperitoneal glucose tolerance test and the stepped titration of ADP, where a two-way analysis of variance was used. All statistical analyses were performed using Graph Pad Prism software. Significance is reported where P < 0.05.

**RESULTS**

**WDs are obesogenic.** Rats fed the WD maintained a greater energy intake than CON throughout the study (335 ± 6 and 274 ± 4 kJ/day, respectively, P < 0.0001; Table 1). From 6 wk, WD rats were heavier than CON (379.1 ± 7.1 and 356.6 ± 3.9 g, respectively, P = 0.01) and remained heavier throughout the intervention (Fig. 1A). As illustrated in Fig. 1B, epididymal fat pads were heavier in WD compared with CON (8.1 ± 0.4 and 6.9 ± 0.3 g, respectively, P = 0.03). IMTGs were elevated 37% in WD compared with CON in the soleus (42.8 ± 5.1 and 31.2 ± 4.3 µmol/g dry wt, respectively), although values did not attain statistical significance (P = 0.10; Table 2). In the EDL, IMTGs were not different between groups (P > 0.05).

**Table 2. Physiological parameters in blood and skeletal muscle of fasted animals**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>WD</th>
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<tbody>
<tr>
<td>Fasting blood glucose, mM</td>
<td>5.70 ± 0.24</td>
<td>5.10 ± 0.18</td>
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<tr>
<td>Fasting serum insulin, ng/ml</td>
<td>1.70 ± 0.12</td>
<td>2.39 ± 0.20*</td>
</tr>
<tr>
<td>Fasting serum cholesterol, mM</td>
<td>2.99 ± 0.18</td>
<td>3.53 ± 0.19</td>
</tr>
<tr>
<td>Fasting serum triglycerides, mM</td>
<td>1.66 ± 0.13</td>
<td>3.09 ± 0.36*</td>
</tr>
<tr>
<td>Fasting serum free fatty acids, mM</td>
<td>0.61 ± 0.02</td>
<td>0.73 ± 0.07</td>
</tr>
<tr>
<td>Intramyocellular triglycerides, µM/g dry wt</td>
<td>31.17 ± 4.27</td>
<td>42.78 ± 5.14</td>
</tr>
<tr>
<td>Soleus</td>
<td>31.17 ± 4.27</td>
<td>42.78 ± 5.14</td>
</tr>
<tr>
<td>EDL</td>
<td>5.93 ± 1.16</td>
<td>5.97 ± 1.26</td>
</tr>
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</table>

Values represent means ± SE, as determined after 12 wk; n = 8–12 animals/group. EDL, extensor digitorum longus. *P < 0.05.
In oxidative muscle the capacity of the ETS was greater following consumption of a WD. To evaluate the capacity of the ETS and thus determine maximal O2 flux through the mitochondria, we measured O2 flux in the soleus during a SUIT protocol with substrates for CI and CII (Fig. 2A). State II respiration with electron leak through CI was greater in WD compared with CON (WD 9.9 ± 0.6 pmol·s⁻¹·mg wet wt⁻¹ and CON 5.8 ± 0.6 pmol·s⁻¹·mg wet wt⁻¹, P < 0.001). A similar trend was observed for state III respiration with flux through CI, although this difference did not attain statistical significance (WD 89.7 ± 8.9 pmol·s⁻¹·mg wet wt⁻¹ and CON 70.6 ± 6.8 pmol·s⁻¹·mg wet wt⁻¹, P = 0.11). However, greater sensitivity to ADP was observed (P < 0.05; Fig. 2B).

State III respiration with flux through both CI and CII together (WD 137.2 ± 11.8 pmol·s⁻¹·mg wet wt⁻¹ and CON 101.7 ± 9.8 pmol·s⁻¹·mg wet wt⁻¹) and noncoupled respiration with flux through CII (WD 73.9 ± 5.3 pmol·s⁻¹·mg wet wt⁻¹ and CON 56.5 ± 4.5 pmol·s⁻¹·mg wet wt⁻¹) were greater in WD (P = 0.04 and P = 0.03, respectively). Similarly, uncoupled/maximal flux through the ETS was greater in WD compared with CON (152.0 ± 11.3 pmol·s⁻¹·mg wet wt⁻¹ and 110.8 ± 9.8 pmol·s⁻¹·mg wet wt⁻¹, respectively, P = 0.02).

To assess O2 flux independently of mitochondrial density, we calculated the flux control ratios for each substrate combination (Table 3). There were no differences between dietary groups.

Consumption of a WD increases maximal activity of mitochondrial enzymes. Both CS (WD 23.5 ± 1.5 μM·min⁻¹·g wet wt⁻¹ and CON 19.4 ± 1.0 μM·min⁻¹·g wet wt⁻¹, P = 0.03; Fig. 3A) and total CPT activity (WD 0.8 ± 0.1 μM·min⁻¹·g wet wt⁻¹ and CON 0.4 ± 0.1 μM·min⁻¹·g wet wt⁻¹, P = 0.01; Fig. 3B) were higher in the soleus after WD compared with CON. There were no differences between dietary groups for β-HAD activity in the soleus (WD 4.9 ± 0.2 μM·min⁻¹·g wet wt⁻¹ and CON 4.1 ± 0.3 μM·min⁻¹·g wet wt⁻¹, P = 0.11; Fig. 3B). In the EDL, CS (WD 10.1 ± 0.5 μM·min⁻¹·g wet wt⁻¹ and CON 8.3 ± 0.5 μM·min⁻¹·g wet wt⁻¹, P = 0.01; Fig. 3A), β-HAD (WD 2.6 ± 0.1 μM·min⁻¹·g wet wt⁻¹ and CON 2.2 ± 0.2 μM·min⁻¹·g wet wt⁻¹, P = 0.04; Fig. 3B), and CPT (WD 0.13 ± 0.01 μM·min⁻¹·g wet wt⁻¹ and CON 0.09 ± 0.01 μM·min⁻¹·g wet wt⁻¹, P = 0.02; Fig. 3C) activities were all greater following the WD.

Enhanced respiratory capacity was not associated with changes in PPARα or PGC-1 expression in the soleus. No differences were observed in the gene expression of PPARα or PGC-1α in either soleus or EDL muscles (Fig. 4). There were also no differences in the protein expression of PGC-1, PPARα, or CPT I between dietary treatments for the soleus. Small but significant increases were observed in the protein expression of UCP3 in both soleus (3%, P = 0.04; Fig. 5) and EDL muscle (6%, P = 0.002, Fig. 6). In the EDL, PGC-1

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**Table 3. Flux control ratios**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>WD</th>
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<tbody>
<tr>
<td>Complex I, leak</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Complex I, OXPHOS (state III)</td>
<td>0.62 ± 0.02</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>Complex I and II, OXPHOS (state III)</td>
<td>0.92 ± 0.02</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>Complex II, uncoupled</td>
<td>0.53 ± 0.03</td>
<td>0.53 ± 0.04</td>
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Values represent means ± SE; n = 6 animals/group. OXPHOS, oxidative phosphorylation. Flux control ratios are the respirometry data normalized to maximal O2 consumption in uncoupled states (electron transport system).

**WD caused hyperinsulinaemia but did not alter fasting blood glucose concentrations or the response to a glucose challenge.** No differences were observed in fasting blood glucose concentrations. However, fasting serum insulin was greater in WD compared with CON (P = 0.007; Table 2). During the intraperitoneal glucose tolerance test there was no diet effect or interaction, although there was a main effect for time in response to the glucose challenge (P < 0.0001). The areas under the blood glucose curves for WD and CON were not different (results not shown).

**WD increased fasting serum triglycerides.** Serum triglycerides were greater in WD compared with CON (P = 0.001; Table 2). Total cholesterol and serum FFAs were also greater in WD; however, neither of these values attained statistical significance (18%, P = 0.052, and 20%, P = 0.12, respectively; Table 2).
protein expression was 4% greater following the WD ($P = 0.005$; Fig. 6), and there was a trend toward increased expression of PPARδ; however, this did not attain statistical significance ($P = 0.07$; Fig. 6).

A WD induces muscle-specific changes to mitochondrial protein expression. In the soleus, protein expression of CS was increased in WD (27%; $P = 0.03$; Fig. 5); however, expression of individual electron transfer system complexes was similar between WD and CON for all complexes except for CI, which was greater in WD than in CON (11%; $P < 0.05$; Fig. 5). There was also a strong trend toward increased mtDNA copy number following the WD (70%; $P = 0.07$; Fig. 4A), although this did not attain statistical significance. In the EDL (Fig. 6), CS protein expression was greater in WD for CS (45%, $P = 0.02$), CI (14%, $P = 0.04$), CII (16%, $P = 0.009$), CIII (18%, $P = 0.04$), and CV (13%, $P = 0.02$), whereas there was a trend toward increased CIV expression (10%, $P = 0.08$). Similarly to the soleus, there was a trend toward increased mtDNA copy number with the WD (42%, $P = 0.08$; Fig. 4B), although this did not attain statistical significance.

**DISCUSSION**

The mitochondrion has long been implicated in the pathogenesis of obesity and metabolic dysfunction (30, 34). However, the extent of mitochondrial involvement and whether these changes are secondary to lifestyle factors (i.e., physical inactivity) are a matter of current debate. Previously, it has been shown that a mitochondrial deficiency exists in obese humans (30) and rodents (11, 48). However, the results of recent studies suggest that there is dissociation between mitochondrial dysfunction and the progression of obesity (4, 19, 21, 51). Indeed, accumulating evidence indicates that HFDs induce increases in the expression (13, 19, 21, 51) and activity (1, 51)
of key mitochondrial enzymes and that this process is likely to be driven by an increase in circulating FFAs (21). Our data support this hypothesis and demonstrate for the first time that skeletal muscle respiratory capacity is enhanced after 12 wk on an obesogenic (high-fat, high-sucrose) WD.

We employed a method of high-resolution respirometry that allows the direct monitoring of O2 consumption by the ETS in permeabilized muscle fibers (38) and reflects the integrated activity of the tricarboxylic acid cycle and electron transfer system, providing a dynamic measurement of the oxidative capacity of tissue ex vivo (38). We show that 12 wk of a WD induces an increase in the activity of the ETS in skeletal muscle with a high oxidative capacity. When the data was normalized to allow interpretation of function independent of mitochondrial volume, this increase was attenuated, suggesting that mitochondrial biogenesis, rather than increased activity, is likely to be responsible for the increase in respiratory capacity.

This finding is supported by the increased expression and activity of the mitochondrial enzyme CS, an enzyme long used as a marker enzyme for mitochondrial content. Additional mitochondrial enzyme activity and protein measurements in the EDL muscle (composed mainly of primarily glycolytic fibers) further support this notion.

Previously, it has been shown in WD- (56) and HFD-fed rodents (19, 21, 51) that skeletal muscle has an increased capacity for fat oxidation after as little as 4 wk. In the current study, we observed increased activity of total CPT (CPT I and CPT II), which was indicative of an increase in the delivery of long-chain fatty acids into the mitochondria. Although we did not measure fat oxidation directly, HFD-induced increases in the activity of CPT I have previously been associated with greater rates of fat oxidation (10, 51). Interestingly, in the soleus we did not observe any differences in the activity of β-HAD, the third enzyme of the β-oxidation pathway, imply-

![Fig. 5](http://ajpendo.physiology.org/)

**Fig. 5.** A: representative Western blots for transcriptional coactivators PPARγ, PPARα, and PGC-1, their targets CPT I and UCP3, and mitochondrial proteins in the soleus muscle. B: relative protein expression as determined by densitometry. Values represent means ± SE; n = 8–10 animals/group. *P < 0.05. CS, citrate synthase.

![Fig. 6](http://ajpendo.physiology.org/)

**Fig. 6.** A: representative Western blots for transcriptional coactivators PPARγ, PPARα, and PGC-1, their targets CPT I and UCP3, and mitochondrial proteins in the EDL muscle. B: relative protein expression as determined by densitometry. Values represent means ± SE; n = 8–10 animals/group. *P < 0.05.
ing that the rate of fatty acid delivery to the mitochondria may be discordant with the capacity for β-oxidation in muscle with high oxidative capacity. However, it is important to note that these data are of maximal enzyme activity ex vivo and are not indicative of in vivo demand.

Although we can only speculate whether or not this mismatch is likely to contribute to cellular lipid deposition (IMTGs were elevated 37% in the soleus but remained similar in the EDL, where β-HAD activity was elevated), the notion that the β-oxidation and tricarboxylic acid cycle activities are disproportionate and contribute to lipid deposition in diet-induced obesity is not a new concept. Previously, Hoehn et al. (24) demonstrated that simply driving metabolism toward increased fat oxidation is not sufficient to increase energy expenditure, whereas Koves et al. (31) suggested that incomplete fatty acid oxidation and accumulation of lipid species occur when the import of fatty acid into the mitochondria exceeds metabolic demand. Moreover, human studies have shown that, despite increases in IMTG storage, rates of fatty acid oxidation may be impaired only in cases of extreme obesity, where mobility is reduced severely (4, 27). This implies that impaired fatty acid oxidation per se is unlikely to be responsible for the accumulation of lipid species during obesity but rather the reduction in physical activity. Our findings suggest that chronic feeding of a WD increases the cellular machinery necessary to cope with increases in energy expenditure, and yet without the appropriate energy demand substrates will accumulate in the tissue, eventually being stored. Pharmacological agonism of PPARδ has been shown previously to increase the expression of several oxidative proteins, including UCP3 and CPT I (36). However, unless combined with an endurance training program, no functional improvements are seen, implying that there must be an appropriate stimulus for any functional enhancements to manifest. We propose that the increases in mitochondrial respiratory capacity observed in the current investigation are a compensatory response in anticipation of a potential increase in substrate flux to the mitochondria.

A second aim of this investigation was to characterize potential mechanisms responsible for the increase in mitochondrial function. The PPARs are a family of ligand-activated nuclear transcription factors that sense and respond to dietary lipids and their metabolites (16). Of the three isoforms, PPARα and PPARδ are the two expressed preferentially in skeletal muscle, with PPARδ being the predominant isoform in this tissue (8). Both PPARα and PPARδ play key roles in fatty acid metabolism (18), acting with the transcriptional coactivator and “master regulator” of mitochondrial biogenesis, PGC-1α (40, 50), to induce increases in both nuclear and mitochondrial target genes for fatty acid catabolism (50, 56). Previous studies have identified PPARδ and PGC-1α as important mediators of diet-induced increases in mitochondrial function (29). Furthermore, PPARδ is known to interact directly with CPT I (50), whereas HFDs or pharmaceutical PPARδ agonists increase UCP3 mRNA (50, 56) and protein expression (22, 51). Unlike previous studies that have investigated the effect of a HFD on mitochondrial signaling, we did not find a large increase in circulating FFAs following the WD. However, total serum triglycerides were almost twofold greater. Moreover, we were unable to demonstrate marked differences in the gene and/or protein expression of PPARδ or PGC-1α, the two signaling proteins that are thought to be responsible for HFD-induced alterations to mitochondrial function (51). Despite this, we cannot discount the possibility that posttranslational regulation of protein activity may be a potential mechanism by which mitochondrial function is enhanced. Indeed, it is well known that the acetylation status of PGC-1α is important in regulating its activity (14), whereas PPARδ itself may posttranslationally modify PGC-1α (15).

Of note was that the expression of PPARδ’s downstream target protein CPT I remained unchanged despite an increase in total CPT activity. Previous studies have reported increases in UCP3 mRNA following a WD (56), and in line with this we show increases in UCP3 protein expression. Taken together, increased CPT activity and UCP3 protein expression could be indicative of an increase in PPARδ activity (17) despite no increase in PPARδ gene or protein expression. Although UCP3 remains without an established function (2, 35), increases in UCP3 expression due to PPAR stimulation implicate this protein in an array of metabolic processes that involve substrate handling. Further investigations will need to be undertaken over prolonged intervention periods to determine whether PPARδ activity is indeed altered by a WD. However, it is also plausible that the reduced percentage of fat in a WD may have less agonist potential than that of a HFD, and as such, other mechanisms may be responsible for the observed increase in CPT activity and UCP3 expression.

In conclusion, the results of the present investigation demonstrate that an increase in mitochondrial respiratory capacity occurs in response to WD-induced obesity. This is likely a compensatory mechanism to cope with excess energy availability in the absence of a simultaneous increase in energy demand. Such a scenario would be expected to contribute to intracellular lipid deposition and overall adiposity when sustained for prolonged periods. Further investigations are required to identify the precise mechanisms that lead to this adaptation.

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DISCLOSURES
The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS
E.J.S., T.A.J., S.K., J.A.H., and N.K.S. contributed to the conception and design of the research; E.J.S., D.M.C., T.A.J., J.S.L., S.K., and N.K.S. performed the experiments; E.J.S., D.M.C., J.S.L., and N.K.S. analyzed the data; E.J.S. interpreted the results of the experiments; E.J.S. prepared the figures; E.J.S. drafted the manuscript; E.J.S., J.A.H., and N.K.S. edited and revised the manuscript; E.J.S., D.M.C., J.A.H., and N.K.S. approved the final version of the manuscript.

REFERENCES


