Resveratrol improves exercise performance and skeletal muscle oxidative capacity in heart failure

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EXERCISE INTOLERANCE IS recognized as one of the major signs in patients with chronic heart failure (HF), which is manifested clinically by dyspnea and fatigue with exercise (7). Originally, it was thought that impaired exercise performance resulted solely from derangements in central hemodynamics in response to exercise, which leads to skeletal muscle hypoperfusion and early onset of lactic acidosis (33). However, it is now recognized that a poor correlation exists between left ventricular ejection fraction (LVEF) at rest and exercise capacity, as measured by peak oxygen consumption (V\textsuperscript{O\textsubscript{2}}) (22). Indeed, patients with HF that have similar reductions in LVEF display a broad range of exercise capacity, suggesting that noncardiac factors mediate this impaired exercise capacity. Furthermore, pharmacological interventions that acutely increase cardiac output (i.e., inotropic agents) have a limited effect on peak V\textsuperscript{O\textsubscript{2}} and exercise performance (36, 40, 66). This has led to the emergence of the concept that intrinsic impairments in skeletal muscle structure, function, and metabolism are key factors in the development of exercise intolerance in HF patients (6).

Numerous studies in animals and humans with HF have shown several skeletal muscle abnormalities, including changes in fiber type composition, reduced capillary density, muscle atrophy, and mitochondrial dysfunction (70). Some studies have suggested that metabolic alterations in skeletal muscle are a critical underlying mechanism contributing to skeletal muscle myopathy and exercise limitations in HF and may potentially play a bigger role than changes in intrinsic myofibrillar contractility (9, 19, 61). However, not all studies have produced similar conclusions (43, 65), and thus the precise impact and contribution of these skeletal muscle abnormalities on exercise intolerance in HF remains incompletely understood.

Resveratrol (Resv) is a naturally occurring polyphenol that has been shown to have a multitude of beneficial effects in cardiovascular and metabolic disease (55, 72). We have recently shown that Resv, when administered as a treatment for established pressure overload-induced HF, was able to nearly
double lifespan in these mice (54). Furthermore, Resv treatment significantly improved cardiac remodeling, diastolic cardiac function, and cardiac glucose metabolism in mice with HF (54). Studies have shown that Resv can increase skeletal muscle biogenesis (27), insulin sensitivity (54), and exercise performance in rodents (4, 27, 45). In addition, we have shown that Resv may act as an exercise mimetic by improving isometric force of isolated muscles and whole body oxidative metabolism in healthy rodents (15, 16). Based on these previous findings, we investigated whether Resv treatment would be able to improve exercise tolerance and physical activity in mice with pressure overload-induced HF and attempted to identify how the skeletal muscle may directly contribute to these effects.

Herein, we show that HF leads to significant impairments in systemic metabolic rate and an increased reliance on fatty acid utilization over glucose. As well, mice with HF display signs of severe exercise intolerance and impaired skeletal muscle oxidative capacity that was significantly improved with Resv treatment. Therefore, our data suggest that Resv may be an effective adjunct therapy that can act on peripheral tissues, such as the skeletal muscle, to help alleviate a major functional limitation of HF and may have the potential to dramatically improve quality of life in patients with HF.

MATERIALS AND METHODS

Materials. All chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO). All primary antibodies were purchased from Cell Signaling Technology (Danvers, MA), EMD Millipore (Billerica, MA), or Thermo Fisher Scientific (Waltham, MA), and secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Immunoblots were developed using Western Lightning Plus-ECL enhanced chemiluminescence substrate (Perkin Elmer, Waltham, MA). Trans-Resv was purchased from Lailabs (Durham, NC), and AIN-93G rodent diet was purchased from Dyets (Bethlehem, PA).

Experimental animals. All protocols involving mice were approved by the University of Alberta Institutional Animal Care and Use Committee and conform to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (8th edition; revised 2011). They adhere to the principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences and comply with the Canadian Council on Animal Care guidelines. Male C57Bl/6N mice (7 wk of age) were obtained from Charles River Laboratories and maintained on a 12:12-h light-dark cycle (0600–1800 light) with free ad libitum access to food and water for a 1-wk acclimatization period. At 8 wk of age, mice were randomly assigned into groups and were subjected to sham (n = 15) or transverse aortic constriction (TAC, n = 29) surgery to induce pressure overload-induced HF. Post surgery (3 wk), mice with an ejection fraction (EF) <45% were considered to be in HF and were randomly allocated into two cohorts of mice where the treatment group was administered Resv (4 g Resv/kg AIN-93G diet, n = 14; Dyets) in their diet while the control group (n = 10) received regular diet without Resv for an additional 2 wk. The dosage of Resv was equivalent to ~450 mg Resv·kg⁻¹·day⁻¹ in mice as described previously (16), which equates to plasma levels of 10–20 μmol/l in rodents (18).

TAC surgery. TAC surgery was performed as described previously (54). In brief, male 8-wk-old mice were anesthetized by an intraperitoneal injection of a cocktail of ketamine (100 mg/kg) and xylazine (10 mg/kg), intubated, and connected to a mouse ventilator (MiniVent; Harvard Apparatus, Holliston, MA). Following midline sternotomy, a double-blunted 27-gauge needle was tied to the aorta between the innominate and left common carotid arteries using 6/0 silk suture. The needle was then removed, and chest and skin were sutured closed. Sham mice underwent the same open-chest procedure as the TAC mice but without aortic banding.

Metabolic analysis in vivo. Indirect calorimetry was performed using the Comprehensive Laboratory Animal Monitoring System (Oxymax/CLAMS; Columbus Instruments, Columbus, OH) as previously described (25). Following a 24-h acclimatization period, mice were monitored for a full 12:12-h dark (active)-light (inactive) cycle. The respiratory exchange ratio [RER; RER = carbon dioxide production (VCO₂)/VO₂] was used to estimate the percent contribution of fat and carbohydrates to whole body energy metabolism in mice in vivo. Physical activity was monitored by dual-axis detection (x,z) using infrared phototell technology. Total activity was calculated by adding z-counts (rearing or jumping) to total counts associated with ambulatory movement and stereotypical behavior (grooming and scratching). Lipid and glucose oxidation rates (ml·kg⁻¹·h⁻¹) were calculated based on validated equations (21).

Endurance treadmill test. A two-lane motorized rodent treadmill attachment for the Oxymax/CLAMS Metabolic Cage Analysis System (Columbus Instruments) was used to determine exercise performance in shams, TAC and TAC + Resv mice. All mice were familiarized to treadmill running before sham or TAC surgery. Exercise performance was measured by an endurance treadmill running test to exhaustion performed at the following two time points: 1) 3 wk post-TAC (before treatment) and 2) after 2 wk of treatment with either vehicle or Resv (posttreatment). Immediately before the exercise protocol mice were acclimated for 30 min in the treadmill chamber at a speed of 0 m/min. At a 10° incline, the belt speed was programmed to increase by 1 m/min every minute for the first 5 min and then remained at 15 m/min for a total of 60 min or until the mouse reached a state of exhaustion, whichever occurred first. A mouse was deemed to be in exhaustion when it was no longer able to continue running on the treadmill as judged by the mouse spending more than five consecutive seconds at the back of the belt despite repeated prodding. Exercise capacity was determined by measuring total distance run (m) and time to exhaustion (min) on the treadmill by each mouse.

Mitochondrial respiration in isolated permeabilized skeletal muscle fibers. Mitochondrial respiration was assessed in saponin-permeabilized soleus and extensor digitorum longus (EDL) muscle fibers as previously described (26). Briefly, mice were euthanized with an intraperitoneal injection of pentobarbital sodium (0.5 mg/kg body wt), and freshly excised soleus and EDL muscles were dissected in an ice-cold sterile-filtered (0.45 μm) isolation solution containing: 2.77 mM CaK₂EGTA, 7.23 mM K₂EGTA, 20 mM imidazole, 20 mM taurine, 49 K-MES, 3 mM KH₂PO₄, 9.5 mM MgCl₂, 5.7 mM ATP, 15 mM phosphocreatine, and 1 μM leupeptin at pH 7.1. Fiber strips (2–5 mm long × 1 mm wide) were dissected into small bundles of six to eight muscle fibers. Following dissection, the fibers were transferred to 2 ml of isolation solution supplemented with 50 μg/ml saponin and permeabilized under gentle mixing at 4°C for 20 min. The fiber bundles were washed three times at 4°C in 4 ml of sterile-filtered (0.45 μm) respiration medium consisting of: 0.5 mM K₂EGTA, 3 mM MgCl₂, 20 mM taurine, 10 mM KH₂PO₄, 20 mM HEPES, 60 mM potassium lactobionate, 110 mM mannitol, 0.3 mM DTT, and 1 g/l bovine serum albumin BSA; fatty acid free at pH 7.1. Respiration was measured using the Oxygraph Plus System (Hansatech Instruments, Norfolk, UK) at 30°C. Basal state respiration was measured in the presence of 10 mM glutamate and 5 mM malate, and state 3 respiration was measured in the presence of 2 mM ADP. After each respiration measurement, the fibers were removed from the oxygraph chamber and subjected to drying at 60°C overnight. Respiration measurements were done in duplicate for each animal, and the rates were normalized per milligram of dry tissue. The final results were expressed as nanomoles O₂ per minute per milligram dry weight.

In vivo echocardiography. Mice were mildly anesthetized using 1.5% isoflurane, and transthoracic echocardiography was performed using a Vevo 770 high-resolution imaging system equipped with a
In situ isometric muscle force measurements. Mice were anesthetized with an intraperitoneal injection (100 mg/ml ketamine, 10 mg/ml acepromazine, and 0.9% sodium chloride; dose 17.5 ml/kg body mass) with additional doses given to maintain a surgical plane of anesthesia for the duration of measurements. Incisions were made along the dorsum of the right hindlimbs, and the soles and tibialis anterior (TA) muscles were exposed. The distal tendons of each muscle were isolated and individually secured with 2.0 silk to a Kulite strain gauge (model KH-102). A purpose-made nerve cuff (AS 632 Cooner Wire, Chatsworth, CA) was placed around the sciatic nerve for electrical stimulation. Skin incisions were then closed before functional measurements began. Core body temperature was maintained at 37°C with a heating lamp. Animals were placed in a prone position and secured with clamps at the knee and ankle joints. Before each series of recordings, the optimal resting length required to generate maximum isometric force was determined for each muscle. Maximum twitch and tetanic forces (in mN) were sequentially recorded in the right soleus and TA muscle. Twitch force was determined as the average of peak forces generated by five individual twitches elicited at 1 Hz. Maximum tetanic force was measured following stimulation of the nerve at 100 Hz (0.2-ms pulse width) for 500 ms.

Gut microbial profiling. Cecum from all three groups of mice was collected after a 5- to 6-h fast in sterile autoclaved DNase/RNase-free Eppendorf tubes. Genomic DNA was extracted from the cecum, and the variable 3 region of the 16S rDNA gene was subsequently amplified by the Illumina compatible multiplex PCR. Following this, the samples were sequenced using the MiSeq platform. As described previously (10), a custom in-house pipeline was used to process the FASTQ files (McMaster Genome Center, McMaster University). With the use of Cutadapt and PANDASEq, sequences were trimmed, aligned (38, 39), and then grouped into Operational Taxonomic Units (OTUs) based on 97% similarity with AbundantOTU+ (39, 68). OTUs were assigned against the 2011 version of the Greengenes reference database (12) using Quantitative Insights Into Microbial Ecology (5). α- and β-Diversity was calculated as previously described (5, 10). The closest root of the phylogenetic tree was used to assign OTUs, which can result in different OTUs being assigned to the same classification. Prediction of metagenome functional content from 16S rDNA library was developed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) software, and PICRUSt predictions were categorized as level 1 to 3 into KEGG pathways (29). The Linear Discriminant Analysis (LDA) Effect Size (LEfSe) algorithm was used to identify pathways with differentiating abundance in the different groups, as we have described previously (56).

Immunoblot analysis. Denatured samples of soleus muscle homogenates were subjected to SDS-PAGE, and proteins were transferred to a nylon membrane. Subsequent immunoblotting to determine expression of target proteins was employed. Primary antibodies used for immunoblotting were anti-phosphorylated AMPK (Thr172) (13, 58, 71), anti-Akt and anti-phosphorylated Akt (Ser473) (24, 54, 69), and α-tubulin (13, 17, 58) from Cell Signaling Technology used at a dilution of 1:1,000. Anti-phosphorylated insulin receptor substrate (IRS)-1 (Tyr612) antibody (53) from Thermo Fisher Scientific was used at a dilution of 1:500, and anti-sirtuin (SIRT)-1 antibody (54) from FMTU Millipore was used at a dilution of 1:500. Immunoblots were developed using the Western Lightning Plus-ECL enhanced chemiluminescence substrate (Perkin Elmer). Densitometric analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD). Densitometric data were corrected against tubulin or respective total protein levels as a loading control.

Statistical analysis. Results are expressed as means ± SE or whisker box plots. Statistical analyses were performed using GraphPad Prism software. Comparisons were performed by one- or two-way ANOVA and appropriate post hoc test or Student’s unpaired t-test when appropriate. For the microbiota sequencing data, the Benjamini-Hochberg multiple-testing adjustment procedure was conducted in R to account for the false discovery rate (FDR), where FDR-corrected P values were estimated for all taxonomic data. Results from PICRUSt analysis were evaluated for significance using the LEfSe tool with P values set at 0.05 and a LDA cutoff score of 2.0 (52).

RESULTS

Resv increases exercise capacity in mice with HF. Following TAC surgery to induce HF (3 wk), mice with an EF <45% (Table 1) were administered diet with vehicle or Resv (~450 mg Resv/kg body wt⁻¹·day⁻¹); calculated plasma levels from this dose in rodents is equivalent to humans receiving 150 mg/day) (18). Similar to our previous study (54), 2 wk of Resv treatment in mice with pressure overload-induced HF did not result in an improvement in resting LVEF compared with vehicle-treated HF mice (Fig. 1A and Table 2). Because exer-
tional fatigue and exercise intolerance are hallmark functional symptoms of HF (63), we measured physical activity and exercise capacity in these mice at 5 wk following TAC surgery. Using metabolic cage analysis, we observed that TAC mice had significantly reduced total basal physical activity levels compared with sham mice and that there is a trend toward increased physical activity levels in Resv-treated TAC mice (Fig. 1B). In addition, we subjected all groups of mice to endurance treadmill stress tests and showed that mice with HF displayed significantly reduced treadmill-running distance (Fig. 1C) and time to exhaustion (Fig. 1D) compared with

<table>
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<tr>
<th>Table 1. Physical characteristics and cardiac morphology and function from TAC mice at 3 wk post-TAC (pretreatment)</th>
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<tr>
<td><strong>TAC Control</strong></td>
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<td>Body weight, g</td>
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<td>Wall measurements</td>
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<td>Corrected LV mass, mg</td>
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<td>IVS-diastole, mm</td>
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<td>IVS-systole, mm</td>
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<td>LVID-diastole, mm</td>
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<td>LVID-systole, mm</td>
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<td>Systolic function</td>
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<td>EF, %</td>
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<td>FS, %</td>
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<td>LVEDV, μl</td>
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<td>CO, ml/min</td>
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<td>SV, μl</td>
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<td>Diastolic function</td>
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<td>IVRT, ms</td>
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<td>IVCT, ms</td>
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<td>ET, ms</td>
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Values are means ± SE; n = 6–7 mice/group. TAC, transverse aortic constriction; Resv, resveratrol; LV, left ventricle; HR, heart rate; IVS, interventricular septal wall thickness; LVFW, left ventricular posterior wall thickness; EF, ejection fraction; FS, fractional shortening; LVID, left ventricular internal diameter; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; CO, cardiac output; SV, stroke volume; IVRT, isovolumic relaxation time; IVCT, isovolumic contraction time; ET, ejection time. Groups were compared by unpaired Student’s t-test.
sham mice. Interestingly, 2 wk Resv treatment dramatically improved overall exercise capacity in HF mice, nearly doubling treadmill-running duration and distance ran compared with vehicle-treated TAC mice (Fig. 1, C and D). Whereas exercise capacity steeply declined by ~60% from 3 to 5 wk following TAC surgery in vehicle-treated HF mice, Resv treatment was able to blunt the decline in treadmill running duration by nearly 30% over this same period of time (Fig. 1 E). Together these data suggest that Resv improves exercise capacity and potentially physical activity levels in mice with severe HF, two major symptoms associated with HF.

Resv treatment increases metabolic rate and whole body glucose utilization in mice with pressure overload-induced HF. Because HF is known to involve alterations in cardiac and peripheral metabolism, we analyzed sham and HF mice treated with or without Resv using indirect calorimetry at 5 wk following surgery. As expected, sham mice displayed a normal circadian rhythm in their respiratory exchange ratio (RER), showing metabolic flexibility to switch between fatty acid and glucose as energy substrates during a 24-h cycle; however, TAC mice had a significantly reduced RER during the dark phase (active; 1800 – 0600), indicating a greater proportional use of fatty acids (Fig. 2, A and B). In contrast, HF mice treated with Resv for 2 wk displayed elevated RER levels, demonstrating that these mice used more carbohydrates (Fig. 2, A and B). In addition, significant reductions in \( V\dot{O}_2 \) and \( V\dot{CO}_2 \) were observed during the dark phase (active) in TAC mice compared with sham mice (Fig. 2, C and D), suggesting that mice in HF have a reduced overall metabolic rate. However, there was a strong trend toward increased rates of \( V\dot{O}_2 \) (Fig. 2 C) and significantly elevated rates of \( V\dot{CO}_2 \) (Fig. 2 D) during the dark phase in Resv-treated mice with HF compared with vehicle-treated HF mice. Consistent with this, there was a decline in heat production observed in TAC mice compared with sham mice, and there was a trend toward increased heat production by Resv treatment (Fig. 2 E). Furthermore, calculated fatty acid oxidation rates during the dark phase trended toward being increased in TAC mice and were subsequently lower in HF mice treated with Resv (Fig. 2F). In accordance with this, calculated glucose oxidation rates were significantly reduced in mice with HF, and this was increased with Resv treatment (Fig. 2 G). Taken together, our data indicate that mice in HF with reduced LVEF may have elevated rates of systemic fatty acid utilization. However, Resv treatment restores metabolic rate and shifts substrate utilization toward greater glucose use in mice with HF.

Figure 1. Resveratrol (Resv) increases exercise capacity in mice with heart failure (HF). Ejection fraction (%EF, A) \((n = 6–11)\), total physical activity (B) \((n = 6–7)\), total distance ran (C), and time to exhaustion (D) during an endurance treadmill test at 5 wk following transverse aortic constriction (TAC) surgery. E: %change in treadmill running duration following 2 wk of vehicle or Resv treatment in mice with HF \((n = 8–11)\). \(*P < 0.05\) by 1-way ANOVA and Sidak’s post hoc test.
Resveratrol and Exercise Capacity in Heart Failure

Table 2. Physical characteristics and cardiac morphology and function from sham and TAC mice at 2 wk posttreatment

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<th></th>
<th>Sham</th>
<th>TAC Control</th>
<th>TAC + Resv</th>
<th>Sham vs. Control</th>
<th>Sham vs. Resv</th>
<th>Control vs. Resv</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>26.33 ± 1.03</td>
<td>28.29 ± 1.02</td>
<td>26.5 ± 0.36</td>
<td>0.299</td>
<td>0.988</td>
<td>0.314</td>
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<td>HR, beats/min</td>
<td>410.7 ± 23.2</td>
<td>475.5 ± 16.0</td>
<td>478.4 ± 17.4</td>
<td>0.09</td>
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Wall measurements

<table>
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<th>Sham vs. Control</th>
<th>Sham vs. Resv</th>
<th>Control vs. Resv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected LV mass, mg</td>
<td>101.98 ± 8.24</td>
<td>175.38 ± 9.20</td>
<td>165.25 ± 7.13</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;1.00</td>
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<tr>
<td>IVS-diastole, mm</td>
<td>0.78 ± 0.04</td>
<td>1.00 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;1.00</td>
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<tr>
<td>IVS-systole, mm</td>
<td>1.03 ± 0.03</td>
<td>1.16 ± 0.02</td>
<td>1.15 ± 0.03</td>
<td>0.016</td>
<td>0.024</td>
<td>&gt;1.00</td>
</tr>
<tr>
<td>LV PW-diastole, mm</td>
<td>0.78 ± 0.04</td>
<td>1.00 ± 0.03</td>
<td>0.97 ± 0.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;1.00</td>
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<tr>
<td>LV PW-systole, mm</td>
<td>1.03 ± 0.03</td>
<td>1.16 ± 0.02</td>
<td>1.15 ± 0.03</td>
<td>0.018</td>
<td>0.034</td>
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<td>LVID-diastole, mm</td>
<td>4.28 ± 0.15</td>
<td>4.83 ± 0.09</td>
<td>4.79 ± 0.09</td>
<td>0.007</td>
<td>0.01</td>
<td>&gt;1.00</td>
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<td>LVID-systole, mm</td>
<td>3.20 ± 0.24</td>
<td>4.25 ± 0.14</td>
<td>4.11 ± 0.12</td>
<td>0.001</td>
<td>0.002</td>
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Systolic function

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<th>Sham vs. Resv</th>
<th>Control vs. Resv</th>
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<tbody>
<tr>
<td>EF, %</td>
<td>50.08 ± 5.01</td>
<td>26.00 ± 3.47</td>
<td>30.06 ± 2.69</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>FS, %</td>
<td>25.73 ± 3.25</td>
<td>12.21 ± 1.78</td>
<td>14.24 ± 1.38</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;1.00</td>
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<tr>
<td>LVEDV, μL</td>
<td>83.00 ± 6.91</td>
<td>109.65 ± 5.06</td>
<td>107.44 ± 4.88</td>
<td>0.011</td>
<td>0.015</td>
<td>&gt;1.00</td>
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<tr>
<td>LVESV, μL</td>
<td>43.19 ± 7.39</td>
<td>81.79 ± 6.98</td>
<td>75.68 ± 5.37</td>
<td>0.002</td>
<td>0.006</td>
<td>&gt;1.00</td>
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<tr>
<td>CO, ml/min</td>
<td>18.53 ± 0.87</td>
<td>12.80 ± 1.01</td>
<td>13.58 ± 1.41</td>
<td>0.015</td>
<td>0.031</td>
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<tr>
<td>SV, μL</td>
<td>41.16 ± 1.80</td>
<td>27.08 ± 2.86</td>
<td>29.52 ± 3.10</td>
<td>0.01</td>
<td>0.03</td>
<td>&gt;1.00</td>
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Diastolic function

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<th>Sham</th>
<th>TAC Control</th>
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<th>Sham vs. Resv</th>
<th>Control vs. Resv</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVRT, ms</td>
<td>20.30 ± 1.97</td>
<td>18.33 ± 1.47</td>
<td>18.62 ± 1.37</td>
<td>&gt;1.00</td>
<td>&gt;1.00</td>
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<tr>
<td>IVCT, ms</td>
<td>17.86 ± 1.71</td>
<td>16.02 ± 1.78</td>
<td>14.93 ± 2.21</td>
<td>&gt;1.00</td>
<td>0.941</td>
<td>&gt;1.00</td>
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<tr>
<td>ET, ms</td>
<td>51.73 ± 2.50</td>
<td>50.21 ± 2.27</td>
<td>50.68 ± 2.50</td>
<td>&gt;1.00</td>
<td>&gt;1.00</td>
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Values are means ± SE; n = 6–11 mice/group. Groups were compared by 1-way ANOVA and Bonferroni post hoc test.
may induce its beneficial effects on improved insulin signaling and/or glucose homeostasis.

Resv does not alter skeletal muscle strength but restores skeletal muscle oxidative capacity. Skeletal muscle dysfunction plays an important role in limiting peak functional exercise capacity and performance in patients with HF (70). It has been suggested that this results from a combination of muscle atrophy, decreased muscle strength, and metabolic dysfunction (19, 37, 44). To investigate the direct effects on skeletal muscle, we measured skeletal muscle mass of soleus and TA and found that there were no differences across the three groups of mice (Fig. 5A). Furthermore, we observed that maximum tetanic force generation was not reduced in mice with HF (Fig. 5B), suggesting that neither muscle atrophy/wasting and nor alterations in muscle function are the primary factors contributing to exercise intolerance in these mice. Given that skeletal muscle mitochondrial function has been proposed to be impaired in the setting of HF (9), we isolated EDL and soleus fibers and measured ex vivo VO2. Under basal conditions, we show that there is a HF-induced trend toward decreased VO2 in the more glycolytic EDL muscle fibers (Fig. 5C) and in the oxidative soleus muscle (Fig. 5D). Moreover, upon stimulation with ADP, state III respiration was significantly reduced in both EDL and soleus muscle fibers from TAC mice compared with sham mice (Fig. 5C and D). In contrast, levels of ADP-stimulated VO2 were increased in skeletal muscle fibers from HF mice treated with Resv compared with HF mice treated without Resv (Fig. 5C and D). Interestingly, despite the increase in oxidative capacity in skeletal muscle

Figure 2. Resv treatment increases metabolic rate and whole body glucose utilization in mice with HF. Respiratory exchange ratio (RER, A), peak RER (B), peak oxygen consumption (VO2, C), peak carbon dioxide production (VCO2, D), and heat production (E) during a 24-h cycle (light and dark phases) in sham, TAC, and TAC + Resv mice (n = 6–7). Calculated whole body fatty acid oxidation (F) and glucose oxidation (G) rates during the dark phase (active; n = 6–7). A: *P < 0.05, TAC + Resv vs. TAC (*), TAC vs. sham (#), and sham vs. TAC + Resv (§) by 2-way ANOVA and Sidak’s post hoc test. B-F: *P < 0.05 by 1-way ANOVA and Sidak’s post hoc test.
from TAC mice treated with Resv, we found that protein levels of mitochondrial electron transport chain complexes and peroxisome proliferator-activated receptor-γ coactivator-1α were unchanged between groups (data not shown). Furthermore, levels of LC3B, as a marker of autophagy, were also not different between sham and TAC mice treated with or without Resv, suggesting that neither mitochondrial biogenesis nor mitophagy is responsible for the beneficial effects of Resv on skeletal muscle oxidative capacity. Taken together, these findings suggest that Resv treatment may either improve or prevent the decline in skeletal muscle mitochondrial oxidative capacity, and this likely contributes to the ability of the exercising muscle to use O2 for contraction during exercise.

DISCUSSION

We have previously shown that Resv treatment of mice with established HF increases lifespan and lessens the severity of the HF phenotype by reducing cardiac fibrosis, improving cardiac energy metabolism, and enhancing diastolic cardiac function (54). Surprisingly, despite this, Resv did not increase resting cardiac systolic function in mice with HF (54). We add to our previous findings by showing that Resv treatment is able to treat one of the major symptoms of HF, namely exercise intolerance. Indeed, exercise intolerance is inextricably linked to HF diagnosis with the New York Heart Association (NYHA) classification of HF based on the degree of functional limitation imposed by HF. However, there exists a very poor correlation between resting left ventricular systolic function and exercise intolerance (22). Consistent with this, we show that endurance running capacity is reduced by >70% in HF mice with EF ranging from 10 to 40% compared with healthy age-matched sham control mice. However, 2 wk of Resv treatment significantly increased treadmill running duration and distance in mice with pressure overload-induced HF compared with vehicle-treated TAC mice. Interestingly, we have previously shown that Resv is able to increase exercise perf-
mance in healthy rodents (16). Taken together, these data support the idea that Resv may act as an exercise mimetic in both healthy and diseased settings. Although an earlier study by Rimbaud et al. (51) showed that Resv was able to improve skeletal muscle mitochondrial function in the Dahl salt-sensitive rat as a model of HF, we show that this translates into measureable functional improvements in exercise capacity. A majority of studies administer Resv before disease development, whereas we show that Resv is effective as a treatment after the onset of cardiac dysfunction and exercise intolerance. Because our previous study showed that Resv improved cardiac diastolic function in mice with HF, it is possible that heart rate was increased to a greater extent during exercise in Resv-treated mice, which may also contribute to the increase in exercise treadmill running capacity.

To investigate the potential physiological and molecular mechanisms that could be responsible for this significant exercise benefit of Resv, we first investigated the effects of HF and Resv on whole body energy metabolism. Although commonly measured during peak exercise, few studies have measured basal RER and metabolic rate in mice with HF to assess whether there are alterations in systemic whole body metabolism. Interestingly, mice with HF displayed lower RER levels (closer to 0.8) during the dark phase (active), suggestive of a loss in metabolic flexibility with a greater reliance on fatty acid utilization in TAC mice. We speculate that this may be due to

Figure 4. Resv treatment alters the gut microbiome of mice with HF. A: representative commensal gut microbial community in mice with HF receiving vehicle (TAC; n = 6) and Resv (TAC + Resv; n = 5). B: ratio of Bacteroidetes to Firmicutes in the cecum of TAC (n = 5) or TAC + Resv mice (n = 6). C: relative abundance of Lachnospiraceae (f) 2, Bacteroides, Parabacteroides, Bilophila, and Akkermansia in the cecum of TAC (n = 5) or TAC + Resv (n = 6) mice. D: metagenomic predictions using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) and Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis of the cecal microbial functional profiles between TAC (n = 5) or TAC + Resv (log LDA >2.0; n = 6) mice. Values in A are shown as means ± SE. *P < 0.05, analyzed by 1-way ANOVA with Tukey’s post hoc test in A. Data in C were analyzed vs. TAC + Resv determined by unpaired t-test. Only functional categories meeting a log LDA significant threshold value of >2.0 are shown in D.
the fact that high sympathetic activation and circulating nor-
epinephrine levels commonly observed in the setting of HF
may lead to increased lipolysis in adipose tissue and that this,
in combination with HF-induced insulin resistance, may pro-
mote greater fatty acid use (32). In agreement with this, studies
have shown that Resv is able to reduce norepinephrine levels in
rats following myocardial infarction (67), and thus we specu-
late that this Resv-mediated decrease in norepinephrine levels
may also occur in our HF model. In contrast, we found that
Resv-treated mice preferentially use glucose as an energy
substrate, as evidenced by a marked increase in RER during
both the light and dark phases compared with vehicle-treated
TAC mice. Interestingly, mice with HF had a reduced whole
body metabolic rate as shown by decreases in \( V_{\text{O}_2} \), \( V_{\text{CO}_2} \), and
heat production levels, whereas Resv partially restored rates in
mice with HF to the level of sham mice. Taken together, these
data suggest that mice with HF have a lower basal metabolic
rate, and Resv treatment was able to improve whole body
metabolism and shift substrate selection toward glucose. Inter-
estingly, Resv does not restore the normal circadian rhythm
of substrate use, and it remains unclear as to the reason behind
this.

Contrary to the results presented herein, some studies in
humans show that resting energy expenditure is actually in-
creased in patients with chronic HF, and this is associated with
increased fatty acid oxidation rates (32, 48, 50). Although the
reasons behind this discrepancy are not clear, it may be due to
the fact that most HF patients in clinical studies are on standard
medical therapy, including angiotensin-converting enzyme in-
hibitors and/or angiotensin receptor blockers, \( \beta \)-blockers, long-
acting nitrates, digoxin, diuretics, and aldosterone antagonists.
As well, indirect calorimetry was typically performed in pa-
patients after an overnight fast, whereas the mice in our study
were given ad libitum access to food for 24 h, and mice are
known to eat sporadically even throughout the inactive light
phase. Another potential confounding factor is the degree of
muscle wasting and cachexia found in patients with HF in the
aforementioned studies compared with our animal model,
which may influence metabolic rate (62). Indeed, we did not
observe significant muscle wasting in our animal model of
pressure overload-induced HF, at least at this early time point.

It is well known that diabetic patients have an increased risk
of developing HF; however, insulin resistance is also highly
prevalent in nondiabetic HF patients (1, 47). The degree of
insulin resistance increases significantly with worsening
NYHA class and is associated with impaired exercise perfor-
mance and peak \( V_{\text{O}_2} \) (1). Consistent with this, we showed that
phosphorylation status of IRS-1 and Akt under 6-h-fasted
conditions in soleus muscle were reduced in TAC mice com-
pared with sham and that Resv treatment increased phosphor-
ylation levels of these proteins, suggesting that Resv improves
peripheral insulin sensitivity in mice with HF. These data are
consistent with our findings using indirect calorimetry showing
the TAC mice use less glucose as an energy substrate, whereas
Resv promotes glucose utilization.

Given the fact that Resv has low bioavailability when
administered orally and largely arrives unmetabolized in the
colon (34), recent evidence has shown Resv’s ability to alter
the gut microbiota (8, 20, 56). This is relevant since changes in
the gut microbiota have been associated with obesity and the
development of type 2 diabetes (59, 60), such as decreased
Bacteroidetes-to-Firmicutes ratio (30). In addition, more recent
evidence has suggested that repeated exercise training can
oppose changes in the distal gut microbiome during diet-
duced obesity, without altering obesity in mice (10). Consis-
tent with these findings, our results show that Resv supplemen-
tation in mice during HF is able to decrease the Bacteroidetes-
to-Firmicutes ratio of the cecal microbiota. Although the
molecular mechanism associated with how alterations in the
gut microbial community improves insulin resistance in HF is
not clear, previous work has indicated the role of the gut
microbiome in modulating energy expenditure and storage
through regulation of host gene expression (46). This can also result in production of short-chain fatty acids (SCFA), which are bacterial waste products, via colonic fermentation. Of interest are acetate, which has been shown to improve glucose tolerance, and propionate and butyrate, which have been implicated in the protection against diet-induced obesity (3). In addition, the gut microbiome has been implicated in the regulation of metabolic pathways, including skeletal muscle AMPK phosphorylation (2), which may also be attributed to SCFA. Interestingly, previous analyses of fecal samples in mice orally administered Resv did not show an increase in SCFA (56), which may necessitate alterations in gut microbiome composition to precede the observed beneficial effects of Resv treatment. We found that Resv increased whole body carbohydrate metabolism in mice with HF, and this change was also mirrored by an increase in the predicted metagenomic capacity of the ecel microbe for carbohydrate metabolism. It is intriguing that the effects of Resv on carbohydrate metabolism are shared between the host and microbiota. Furthermore, many of the changes in the microbiota caused by Resv are in agreement with those changes induced by exercise (11). This is of particular interest, since Resv has been proposed to function as an exercise mimetic (16), and the similar changes in the gut microbiome induced by both Resv and exercise are intriguing. Together, these findings provide additional physiological mechanistic insight into how Resv may improve glucose utilization and mitochondrial oxidative capacity in HF.

Given that exercise intolerance in HF has been strongly linked to mitochondrial and oxidative impairment in skeletal muscle (70) and that Resv has been shown to increase mitochondrial number and/or function (4, 27), we isolated skeletal muscle fibers from all three groups of mice and measured rates of ex vivo VO₂. As expected, both EDL and soleus muscle fibers isolated from TAC mice had significantly reduced VO₂ rates compared with sham mice. Interestingly, Resv treatment prevented the decline or restored rates of VO₂ in skeletal muscle, and this occurred in the absence of changes in muscle mass and/or strength, as well as mitochondrial biogenesis or mitophagy. Although Resv is known to be an AMPK activator, we failed to see skeletal muscle AMPK activation in our study. It is possible that AMPK was activated earlier on and was normalized by the 5-wk time point or that the reduction in phosphorylated AMPK reflects improved skeletal muscle energetic status following Resv treatment.

SIRT1 has been linked to the beneficial effects of Resv on glucose metabolism and mitochondrial oxidative capacity (27, 49). Resv has been shown to act through SIRT1 and SIRT3 to increase mitochondrial cardiolipin synthesis, which may improve mitochondrial dysfunction (14). Although we have not fully investigated the role of sirtuins in this study, this may be a potential mechanism of action by which Resv acts to increase oxidative capacity in skeletal muscle from mice with HF and is worthy of future investigation.

Our data are largely consistent with studies in patients with HF where abnormal skeletal muscle metabolism is characterized by reduced oxidative metabolism with an earlier shift to glycolytic metabolism, utilization of high-energy phosphocreatine (PCr), early intracellular acidification, and delayed PCr recovery after exercise (6, 35). Interestingly, these metabolic abnormalities appear to be independent of changes in total limb perfusion (31, 41, 64) or muscle mass (28) observed in HF patients. Indeed, our data suggest that Resv is able to improve mitochondrial oxidative metabolism and substrate utilization in skeletal muscle. Our data are consistent with a study by Rimbaud et al. (51) showing that Resv preserves skeletal muscle oxidative capacity in the hypertensive Dahl salt-sensitive rat model. In addition, we and others have shown that Resv is able to improve endothelium-dependent vasodilatation in the femoral artery of mice with HF (51, 54). Impaired vascular function and vasodilatory response to exercise can limit blood flow to the exercising muscle and further limit exercise performance (7). Therefore, it is possible that improved vascular function may contribute to the beneficial effects of Resv on exercise capacity in HF mice by improving substrate and O₂ supply to skeletal muscle.

Overall, we conclude that HF leads to significant alterations in whole body metabolism and that Resv is able to improve systemic metabolic rate and alter substrate utilization in mice with established HF. Interestingly, these whole body metabolic changes were associated with distinct alterations in the composition and predicted function of the gut microflora, and this may be one of the mechanisms by which Resv improves energy metabolism. Most importantly, Resv significantly increased exercise tolerance in mice with HF. Therefore, Resv may be an effective adjunct therapy given to HF patients to reduce fatigue and exercise intolerance and may improve outcomes and quality of life in this patient population.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

MMS, NB, IR, VS, CLS, DF, NT, ED and KEJ performed the experiments; MMS, NB, IR, VS, NT, KEJ, TTK, ED, JMS and JRB contributed to experimental planning, data analysis and interpretation; MMS, TTK and JRB wrote the manuscript; all authors contributed to editing and reviewing the manuscript.

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