Aerobic exercise training reduces cardiac function in adult male offspring exposed to prenatal hypoxia

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¹Department of Physiology, University of Alberta, Edmonton, Alberta, Canada; ²Department of Obstetrics and Gynecology, University of Alberta, Edmonton, Alberta, Canada; and ³Women and Children’s Health Research Institute, University of Alberta, Edmonton, Alberta, Canada

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Reyes LM, Kirschenman R, Quon A, Morton JS, Shah A, Davidge ST. Aerobic exercise training reduces cardiac function in adult male offspring exposed to prenatal hypoxia. Am J Physiol Regul Integr Comp Physiol 309: R489–R498, 2015. First published July 8, 2015; doi:10.1152/ajpregu.00201.2015.—Intrauterine growth restriction (IUGR) has been associated with increased susceptibility to myocardial ischemia-reperfusion (I/R) injury. Exercise is an effective preventive intervention for cardiovascular diseases; however, it may be detrimental in conditions of compromised health. The aim of this study was to determine whether exercise training can improve cardiac performance after I/R injury in IUGR offspring. We used a hypoxia-induced IUGR model by exposing pregnant Sprague-Dawley rats to 21% oxygen (control) or hypoxic (11% oxygen; IUGR) conditions from gestational day 15 to 21. At 10 wk of age, offspring were randomized to a sedentary group or to a 6-wk exercise protocol. Transthoracic echocardiography assessments were performed after 6 wk. Twenty-four hours after the last bout of exercise, ex vivo cardiac function was determined using a working heart preparation. With exercise training, there was improved baseline cardiac performance in male control offspring but a reduced baseline cardiac performance in male IUGR exercised offspring (P < 0.05). In male offspring, exercise decreased superoxide generation in control offspring, while in IUGR offspring, it had the polar opposite effect (interaction P ≤ 0.05). There was no effect of IUGR or exercise on cardiac function in female offspring. In conclusion, in male IUGR offspring, exercise may be a secondary stressor on cardiac function. A reduction in cardiac performance along with an increase in superoxide production in response to exercise was observed in this susceptible group.

intrauterine growth restriction; aerobic exercise training; intervention; heart function

In 2008, sixty-three percent of all deaths worldwide were attributed to noncommunicable diseases and, from these, cardiovascular diseases (CVDs) were the leading cause of mortality (1). Every year, billions of dollars are expended on CVDs through both direct and indirect costs (16), and thus they have a great impact on public health care resources. Within the last three decades, an increasing body of evidence has demonstrated that suboptimal conditions in utero, which lead to intrauterine growth restriction (IUGR), can cause long-term consequences for the cardiovascular system. In fact, a low birth weight has been associated with the development of diabetes (34), hypertension (8), and increased susceptibility of ischemic heart disease (17). Together, these findings have raised important considerations for addressing IUGR as a risk factor to develop CVD later in life, especially in countries where pregnancy-related complications are common (12).

IUGR following a hypoxic insult has been associated with specific cardiac structural changes from early stages in development. It has been established that chronic exposure to hypoxia during pregnancy is associated with a decrease in fetal and neonatal cardiomyocyte proliferation and an increase in cardiomyocyte apoptosis (2, 32). Furthermore, an increase in collagen deposition soon after birth (32) and later in life (36) has been found in offspring exposed to hypoxia in utero. Moreover, cardiovascular functional changes in offspring born from hypoxic pregnancies have also been reported. Kane et al. (18) found that chronic exposure to hypoxia during pregnancy leads to an increased sympathetic output and increase in baroreflex gain in adult offspring. In addition, hypoxia-induced IUGR has been associated with an increased oxidized/reduced glutathione ratio in the myocardium, demonstrating an increase in oxidative stress (30). Moreover, adult offspring born from hypoxic pregnancies have an increased susceptibility to myocardial ischemia-reperfusion (I/R) injury (22, 29, 36, 37).

Given that the CVD burden is vast, an impressive amount of research has been conducted to determine the role interventions may have to prevent the development of these diseases. From these intervention studies, aerobic exercise training has been shown to be cardioprotective and, more importantly, evidence has shown that aerobic exercise training is associated with a reduction in mortality due to any cause (7). There are several mechanisms by which aerobic exercise training could potentially improve cardiac outcomes; however, a reduction in reactive oxygen species (ROS) is one of the main mechanisms by which aerobic exercise training may confer cardioprotection. Aerobic exercise training has been shown to improve the antioxidant capacity of cardiomyocytes; several studies have shown that superoxide dismutase isoforms 1 (SOD-1; Cu-ZnSOD) and 2 (SOD-2; MnSOD), catalase, and glutathione peroxidase protein expression and/or activity are upregulated following aerobic exercise training (reviewed in Ref. 2).

It is important to note that aerobic exercise training in healthy populations has been associated with cardioprotection (reviewed in Ref. 11). Moreover, exercise training has also been associated with an improvement of the redox status in CVD such as heart failure, hypertension, and myocardial infarction (reviewed in Ref. 5). Exercise, nonetheless, has also been shown to cause transient myocardial damage (19) and atrial fibrillation (6). Likewise, Laher et al. (20) found that in 8-mo-old obesegenic/diabetic db/db mice, aerobic exercise training increased myocardial oxidative stress, did not upregulate any SOD isoforms or catalase, and negatively altered
glutathione homeostasis. Thus whether cardioprotection can be achieved through exercise in disease populations is debatable, and further investigation is required to determine the effects of aerobic exercise training in a susceptible population such as IUGR offspring.

It has been shown that exercise training in IUGR animals improved the metabolic phenotype associated with being born growth restricted by increasing insulin sensitivity in female Sprague-Dawley rats (14) and by increasing relative islet surface area and β-cell mass in male Wistar-Kyoto rats (21). Furthermore, we found that aerobic exercise training had beneficial vascular effects in enhancing endothelium-derived hyperpolarization-mediated vasodilation in gastrocnemius muscle arteries from male IUGR offspring (26). The impact of aerobic exercise training on cardiac performance in IUGR offspring, however, remains unknown. Thus the aim of this study was to determine whether aerobic exercise training can improve cardiac performance after I/R injury in IUGR offspring.

**METHODS**

Animal model/aerobic exercise intervention. The present set of experiments comprises part of a study designed to determine the cardiovascular effects of aerobic exercise training in IUGR offspring. Thus a subset of offspring generated from each dam was used to assess vascular function, which has been previously published, and the details of the animal model and the aerobic exercise intervention used are described therein (26). The second subset of offspring was used for this study. Briefly, 3-mo-old Sprague-Dawley (n = 26) rats (Charles River, Wilmington, MA) were mated overnight. Upon confirmation of pregnancy, rats were exposed to control (room air, n = 13) or hypoxic (11% oxygen, n = 13) conditions from gestational day (GD) 15 to 21 to create an IUGR model. At the time of birth (GD 22), anthropometric parameters such as body weight, crown-to-rump length, and abdominal girth were measured in the pups. Litters were...
randomly reduced to eight pups (4 males and 4 females) to normalize access to maternal nutrition. Offspring were weaned at 3 wk, and the exercise intervention started at 10 wk of age. From each litter, two males and two females were randomly allocated to the exercise training group, and two males and two females were assigned to a sedentary group. One male and one female from each group were used in a separate series of vascular function experiments and were not included in the present article (26). Thus only one pup/sex/litter was used in the experiments for the current study. Offspring were progressively habituated to motor-driven treadmill running during 5 consecutive days and then exercised for 30 min at 20 m/min; 5° grade; 5 days/wk; for 6 wk. Rats were encouraged to run with a jet of air applied to the hindlimb. We have previously reported that male and female, control, and IUGR offspring have a similar exercise capacity (26). Offspring in the sedentary group were exposed to the same room environment for the same period of time as the training group. All procedures in this study were approved by the University of Alberta Animal Welfare Committee, were in accordance with the guidelines of the Canadian Council on Animal Care and confirmed to the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

**Echocardiography.** At 15 wk of age, rats (control and IUGR, sedentary and exercised, male and female) were anesthetized with inhaled isoflurane (2%) mixed with 1.5 L/min 19.5% O₂. Rats were immobilized on a heating platform in a supine position, and their extremities were fixed to electrodes on the heating platform surface. Electrocardiogram electrodes continuously monitored heart rate and respiratory rate. Body temperature was monitored using a rectal probe. After the chest area was depilated, a transthoracic echocardiography was performed using the Vevo 2100 digital imaging platform (VisualSonics) with a 13- to 23-MHz transducer. M-mode images from the parasternal short and long axis views, as well as pulse-wave Doppler images, were taken. Animals from the exercise groups did not perform the exercise protocol that day. The following formulas were used to calculate echocardiogram parameters: left ventricular internal diameter in diastole (LVIDdias); left ventricular internal diameter in systole (LVIDsys); left ventricular volume in diastole (LV-Vdias); and left ventricular volume in systole (LV-Vsys):

\[
\text{Left ventricular volume; diastole} = \left[\frac{7.0}{(2.4 + \text{LVID}_{\text{dias}})}\right] \times \text{LVID}_{\text{dias}}
\]

**Table 3. Echocardiographic data obtained from female control and IUGR, sedentary and exercised offspring**

<table>
<thead>
<tr>
<th>Morphologic parameters</th>
<th>Control Sedentary</th>
<th>Control Exercised</th>
<th>IUGR Sedentary</th>
<th>IUGR Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS; diastole, mm</td>
<td>0.95 ± 0.08</td>
<td>0.97 ± 0.05</td>
<td>1.10 ± 0.12</td>
<td>1.08 ± 0.10</td>
</tr>
<tr>
<td>IVS; systole, mm</td>
<td>1.58 ± 0.05</td>
<td>1.43 ± 0.12</td>
<td>1.88 ± 0.33</td>
<td>1.74 ± 0.15</td>
</tr>
<tr>
<td>LVVAW; diastole, mm</td>
<td>7.13 ± 0.19</td>
<td>6.95 ± 0.36</td>
<td>6.75 ± 0.28</td>
<td>7.22 ± 0.15</td>
</tr>
<tr>
<td>LVVAW; systole, mm</td>
<td>3.26 ± 0.17</td>
<td>3.43 ± 0.33</td>
<td>2.75 ± 0.32</td>
<td>3.23 ± 0.19</td>
</tr>
<tr>
<td>LVID; diastole, mm</td>
<td>1.68 ± 0.08</td>
<td>1.78 ± 0.11</td>
<td>1.70 ± 0.14</td>
<td>2.03 ± 0.13</td>
</tr>
<tr>
<td>LVID; systole, mm</td>
<td>3.19 ± 0.08</td>
<td>2.95 ± 0.13</td>
<td>3.12 ± 0.30</td>
<td>3.47 ± 0.14</td>
</tr>
<tr>
<td>LVVPW; diastole, mm</td>
<td>7.20 ± 0.22</td>
<td>7.09 ± 0.39</td>
<td>6.90 ± 0.26</td>
<td>7.18 ± 0.14</td>
</tr>
<tr>
<td>LVVPW; systole, mm</td>
<td>3.63 ± 0.21</td>
<td>3.77 ± 0.33</td>
<td>3.05 ± 0.36</td>
<td>3.59 ± 0.21</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>1.88 ± 0.08</td>
<td>1.86 ± 0.19</td>
<td>1.85 ± 0.10</td>
<td>1.83 ± 0.08</td>
</tr>
<tr>
<td>LV mass/body weight, mg/g</td>
<td>3.01 ± 0.10</td>
<td>3.09 ± 0.22</td>
<td>3.23 ± 0.15</td>
<td>3.04 ± 0.06</td>
</tr>
</tbody>
</table>

**Volume parameters**

| LV volume; diastole, μL                  | 703.04 ± 29.53    | 656.44 ± 68.82    | 673.80 ± 63.43 | 696.42 ± 53.64 |
| LV volume; systole, μL                  | 2.04 ± 0.14       | 1.99 ± 0.27       | 2.09 ± 0.14    | 1.94 ± 0.14    |

**Systolic function**

| Stroke volume, μL                       | 275.14 ± 18.21    | 267.77 ± 33.27    | 249.96 ± 20.27 | 270.90 ± 12.02 |
| Ejection fraction, %                    | 57.92 ± 6.82      | 64.43 ± 13.42     | 41.48 ± 10.61  | 55.70 ± 7.81   |
| Fractional shortening, %               | 224.40 ± 13.14    | 204.12 ± 20.28    | 206.45 ± 14.41 | 231.42 ± 9.95  |
| Cardiac output, ml/min                  | 83.56 ± 1.67      | 80.73 ± 2.40      | 87.54 ± 2.37   | 84.40 ± 1.71   |

Data are presented as means ± SE.

**Table 4. Pulse-wave Doppler data obtained from female control and IUGR, sedentary and exercised offspring**

<table>
<thead>
<tr>
<th>Aortic valve flow</th>
<th>Control Sedentary</th>
<th>Control Exercised</th>
<th>IUGR Sedentary</th>
<th>IUGR Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection time, ms</td>
<td>69.85 ± 2.74</td>
<td>74.32 ± 3.70</td>
<td>70.80 ± 4.52</td>
<td>69.98 ± 3.91</td>
</tr>
<tr>
<td>Outflow maximum ejection velocity, mm/s</td>
<td>972.52 ± 49.29</td>
<td>795.91 ± 38</td>
<td>908.58 ± 44.41</td>
<td>930.93 ± 61.80</td>
</tr>
<tr>
<td>Peak pressure gradient, mmHg</td>
<td>3.85 ± 0.38</td>
<td>2.56 ± 0.24</td>
<td>3.35 ± 0.33</td>
<td>3.56 ± 0.48</td>
</tr>
</tbody>
</table>

| Pulmonary valve flow                     |                   |                   |                |                |
| Acceleration time (PAT), ms              | 31.93 ± 2.49      | 36.41 ± 4.41      | 32.29 ± 2.57   | 27.75 ± 3.30   |
| Ejection time (PET), ms                  | 80.16 ± 2.51      | 77.32 ± 6.58      | 82.92 ± 2.82   | 77.20 ± 5.59   |
| Valve peak velocity, ms                  | 910.26 ± 49.47    | 809.86 ± 42.15    | 1,028.34 ± 74.82* | 904.50 ± 87.32 |
| PAT/PET                                  | 0.40 ± 0.03       | 0.47 ± 0.03       | 0.39 ± 0.03    | 0.37 ± 0.04    |
| Peak pressure gradient, mmHg             | 3.59 ± 0.40       | 2.66 ± 0.29       | 4.36 ± 0.63    | 3.46 ± 0.69    |

| Mitral valve                             |                   |                   |                |                |
| Ejection time, ms                        | 51.26 ± 3.08      | 54.15 ± 5.36      | 56.01 ± 3.76   | 55.97 ± 3.76   |
| IVCT, ms                                 | 25.78 ± 2.30      | 27.24 ± 2.88      | 21.97 ± 2.92   | 23.75 ± 4.49   |
| IVRT, ms                                 | 24.57 ± 1.90      | 26.01 ± 2.51      | 23.47 ± 0.44   | 24.40 ± 1.36   |
| E velocity, mm/s                         | 798.09 ± 29.84    | 719.75 ± 53.43    | 744.31 ± 47.03 | 771.69 ± 48.54 |
| A velocity, mm/s                         | 533.35 ± 27.13    | 542.25 ± 27.43    | 523.06 ± 47.29 | 602.02 ± 79.17 |
| E/A ratio                                | 1.52 ± 0.09       | 1.33 ± 0.10       | 1.46 ± 0.12    | 1.35 ± 0.12    |
| Tei index                                | 1.03 ± 0.10       | 1.03 ± 0.11       | 0.86 ± 0.14    | 0.92 ± 0.17    |

Data are presented as means ± SE. *Two-way ANOVA revealed an IUGR effect.
Fig. 1. Male cardiac performance during ischemia/reperfusion protocol. A: baseline cardiac performance of 30 min was obtained, followed by 10 min of global, no-flow ischemia and 40 min of reperfusion. Groups include control sedentary offspring (solid line, ■, n = 6); control exercised offspring (dashed line, □, n = 6); intrauterine growth restriction (IUGR) sedentary offspring (solid line, ▲, n = 9); and IUGR exercised offspring (dashed line, △, n = 8). B: summary data of cardiac performance during preischemia. C: summary data of cardiac power during reperfusion. D: summary data of percentage of recovery after the ischemic event. Data are summarized and presented as means ± SE. Sedentary male offspring (closed bars); exercised male offspring (checkered bars). Data were analyzed by two-way ANOVA; #P < 0.03, †P < 0.04, group effect control vs. IUGR; *P < 0.05 Bonferroni post hoc test control exercised vs. IUGR exercised.

Fig. 2. Female cardiac performance during ischemia/reperfusion protocol. A: baseline cardiac performance of 30 min was obtained, followed by 10 min of global, no-flow ischemia and 40 min of reperfusion. Groups include: control sedentary offspring (solid line, ■, n = 6); control exercised offspring (dashed line, □, n = 8); IUGR sedentary offspring (solid line, ▲, n = 7); and IUGR exercised offspring (dashed line, △, n = 8). B: summary data of cardiac performance during preischemia. C: summary data of cardiac power during reperfusion. D: summary data of percent recovery of cardiac power after the ischemic event. Data are summarized and presented as means ± SE and were analyzed by two-way ANOVA. Closed bars: sedentary female offspring; checkered bars: exercised female offspring.
Left ventricular mass; systole = \[
\frac{7.0}{(2.4 + LVV_{sys})} \times LVV_{sys}^3
\]

%Ejection fraction = \[
100 \times \left(\frac{LVV_{dias} - LVV_{sys}}{LVV_{dias}}\right)
\]

%Fractional shortening = \[
100 \times \left(\frac{LVID_{dias} - LVID_{sys}}{LVID_{dias}}\right)
\]

Left ventricular mass = \[
1.053 \times \left(\frac{LVID_{dias} + LVPW_{dias} + IVS_{dias}}{LVID_{dias}}\right)
\]

I/R protocol. At 16 wk of age (young adult), and 24 h after the last bout of exercise, offspring were anesthetized with a single dose (1.5 ml) of inhaled isoflurane. When the pedal reflex was absent, hearts were rapidly excised and the aortas were fixed to a cannula and perfused in a retrograde Langendorff mode with Krebs-Henseleit solution [in mmol/l: 120 NaCl, 25 NaHCO3, 5.5 glucose, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, and 2.5 CaCl2 (pH 7.4 gassed with 95% O2-5% CO2)]. The left atrium was cannulated and hearts were subsequently perfused in an anterograde working heart mode. Hearts were paced at 300 beats/min. The working heart protocol included 30 min of baseline (preischemia), followed by 10 min of global, no-flow ischemia and 40 min of reperfusion. This protocol was based on previous data where isolated heart experiments were carried out in young and aged offspring (27, 29). Measurements of cardiac function were obtained every 10 min. Signals from all sensors (flow, pressure, temperature, and ECG) were acquired using an interface and recorded using the Isoheart Software (Harvard Apparatus). Cardiac performance during the I/R protocol was determined as previously described (29, 35) by calculating cardiac power (peak systolic pressure (mmHg) − maximal preload (mmHg) × cardiac output (ml/min) × 0.13/heart dry weight (g)).

Western blot analyses in nonperfused hearts. Nonperfused heart tissue was homogenized in a lysis buffer containing [in mmol/l: 20 Tris (pH 7.4), 5 EDTA, 10 sodium pyrophosphate tetrabasic, 100 sodium fluoride, 1% NP-40, and Protease Inhibitor Cocktail (1:1 Halt Protease Inhibitor, Thermo Scientific)]. Protein concentrations were determined by bicinchoninic acid assay (Piefer). A total of 100 μg of protein was loaded, and subsequent sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed. Membranes were blocked with blocking buffer for fluorescent Western blotting (Rockland Immunochemicals), and they were incubated with anti-SOD-1 C-17 (1:1,000; Santa Cruz Biotechnology), anti-SOD-2 FL (1:2,000; Santa Cruz Biotechnology), anti-catalase (1:1,000; Abcam), or anti-glutathione peroxidase (1:5,000; Santa Cruz Biotechnology) antibodies preincubated in 3% nonfat milk for 1 h at room temperature, followed by secondary antibodies (1:10,000; IRDye 800 donkey and IRDye 680 donkey). Imaging was performed on a Li-Cor Odyssey system. Protein bands were quantified with corresponding software (Li-Cor Biosciences). Results were normalized to α-tubulin.

Superoxide detection in cardiac tissue. A piece of nonperfused left ventricle was embedded in optimal cutting medium (OCT) and snap-frozen in liquid nitrogen and stored at −80°C until use. Sections were cut at 20 μm using a cryostat. Superoxide generation was measured by staining the tissues with dihydroethidium (DHE). DHE is a cell-permeable compound that reacts with intracellular and extracellular superoxide to produce ethidium. Fluorescence is generated when ethidium then binds to nuclear DNA. Four slides with four sections (1 from each group: control sedentary, control exercised, IUGR sedentary, and IUGR exercised) were prepared for male and female offspring.

Fig. 3. Male control and IUGR, sedentary and exercised offspring: cardiac protein expression of antioxidant enzymes. Protein expression in nonperfused cardiac tissue of antioxidant enzymes in male sedentary offspring (closed bars, n = 9) and male exercised offspring (checkered bars, n = 7). All data are presented as a ratio of the protein of interest to α-tubulin. The sedentary control group was normalized to 100% and then the percent change of the other groups (control exercised, IUGR sedentary, and IUGR exercised) was assessed. A: representative image of a Western blot membrane probed for superoxide dismutase isoform 1 (SOD-1) and α-tubulin and summary data of SOD-1 protein expression. B: representative image of a Western blot membrane probed for SOD-2 and α-tubulin and summary data of SOD-2 protein expression. C: representative image of a Western blot membrane probed for catalase and α-tubulin and summary data of catalase protein expression. Data are summarized and presented as means ± SE and were analyzed by two-way ANOVA.
spring. This allowed for each tissue to be replicated four times. Slides were thawed, washed three times with Hank’s balanced salt solution (HBSS), and incubated for 10 min at 37°C. After removal of HBSS, fresh DHE (200 μmol/l) was added and the slides were incubated for 30 min at 37°C. After removal of DHE and washes with HBSS, slides were immediately viewed using a fluorescence microscope (IX81 Olympus), and images were obtained with cellSense (Olympus). All samples were analyzed within 1 h of staining. Images are presented at ×20 magnification.

Statistical analyses. The data are presented as means ± SE. This study had a two-way ANOVA design where the effect of being born growth restricted and the effect of aerobic exercise training were determined. Female and male offspring data were analyzed separately due to differences in their phenotype. All the variables were tested using a two-way ANOVA followed by a Bonferroni post hoc test. To determine cardiac performance after ischemia, a mean of the baseline cardiac power in each group was estimated and then the percentage recovery of cardiac power was calculated. For Western blot analyses, comparisons between the groups were performed by normalizing to the sedentary control group and then assessing the percentage change in the other groups (control exercised, IUGR sedentary, and IUGR exercised). For DHE analyses, the mean intensity of the staining was normalized to the number of nuclei/picture using ImageJ (NIH), and an average of the four pictures was calculated. Comparisons between the groups were performed by normalizing to the sedentary control group and then assessing the percentage change in the other groups (control exercised, IUGR sedentary, and IUGR exercised). Statistical significance was defined as P ≤ 0.05. All data were analyzed using GraphPad Prism 6 statistical software (GraphPad Software).

RESULTS

Animal model. Anthropometric parameters of the cohort were the same for both sets of offspring generated for these studies. Thus we demonstrated that exposure to hypoxia in utero decreased offspring body weight (13.8% in male offspring, P < 0.0001; and 11.4% in female offspring, P < 0.0001) and abdominal girth (6.1% in male offspring, P < 0.01; and 4.7% in female offspring, P < 0.05), whereas crown-rump length remained the same (26).

Echocardiography. In male offspring, neither being born from a hypoxic environment nor aerobic exercise training affected left ventricular wall dimensions or heart function variables such as ejection fraction, fractional shortening, or stroke volume (Table 1). No changes in the aortic or pulmonary valve flow were found and mitral valve function was preserved in all groups (Table 2).

In female offspring, there were no differences among the groups regarding left ventricular wall dimensions and systolic function (ejection fraction, fractional shortening, and stroke volume; Table 3). Moreover, we found that compared with control sedentary female offspring, pulmonary valve peak velocity was increased in IUGR sedentary female offspring (control 910.3 ± 49.5 ms vs. IUGR 1,028.3 ± 74.8 ms, P < 0.05; Table 4). There were no differences among the groups regarding diastolic function (mitral valve function; Table 4).

I/R protocol. In male offspring, cardiac performance was similar in control and IUGR sedentary groups during the preischemia period (P > 0.05; Fig. 1, A and B). A Bonferroni post hoc test revealed that aerobic exercise training increased baseline cardiac performance in control offspring, while cardiac performance in male IUGR offspring was reduced (Fig. 1, A and B). During reperfusion, being born growth restricted was associated with a decrease in cardiac power (P = 0.03; Fig. 4, Female offspring).
Moreover, being born growth restricted was associated with a decrease in the percentage of recovery following the ischemic insult ($P = 0.04$; Fig. 1D).

In female offspring, there were no differences in cardiac performance between control and IUGR sedentary offspring during the preischemia period (Fig. 2, A and B). Interestingly, and contrary to our observations in male offspring, aerobic exercise training did not affect pre- or postischemic cardiac performance in either control or IUGR female offspring (Fig. 2, A–C). Consequently, the percent recovery of cardiac power following the ischemic event was also similar in all groups (Fig. 2D).

Protein expression of antioxidant enzymes in nonperfused hearts. In male offspring, cardiac protein expression of SOD-1 (Fig. 3A), SOD-2 (Fig. 3B), catalase (Fig. 3C), and glutathione peroxidase (control sedentary 100 ± 24.0% vs. exercised 112.4 ± 15.3% and IUGR sedentary 131.4 ± 38.9% vs. exercised 103.6 ± 25.5%) was not different among the groups.

Conversely in female offspring, aerobic exercise training increased cardiac protein expression of SOD-1 in both control offspring (sedentary 100 ± 15.3% vs. exercised 272.7 ± 86.4%) and IUGR offspring (sedentary 163.8 ± 33.6% vs. exercised 231.2 ± 65.0%, $P < 0.05$; Fig. 4A). Cardiac protein expression of SOD-2 (Fig. 4B), catalase (Fig. 4C), and glutathione peroxidase was not different among the groups (control sedentary 100 ± 13.9% vs. exercised 82.2 ± 9% and IUGR sedentary offspring 129.9 ± 24.2% vs. exercised 109.6 ± 22.4%).

Fig. 5. Dihydroethidium (DHE) staining in nonperfused hearts from male control and IUGR, sedentary and exercised offspring. Superoxide production as assessed by DHE staining in nonperfused hearts. The sedentary control group was normalized to 100% and then the percent change of the other groups (control exercised, IUGR sedentary, and IUGR exercised) was assessed. Representative images of male control sedentary (A), male control exercised (B), male IUGR sedentary (C), male IUGR exercised (D), and negative control for DHE staining (E). F: summary data of male sedentary offspring (closed bars, $n = 4–5$) and male exercised offspring (checkered bars, $n = 4–5$). Data are summarized and presented as means ± SE and were analyzed by two-way ANOVA.

**DISCUSSION**

The impact of being born growth restricted on the cardiovascular system later in life has been shown to be far-reaching (reviewed in Ref. 15). Since CVDs are the primary cause of mortality worldwide, finding strategies to prevent or ameliorate CVDs is a priority for public health. Aerobic exercise training has been shown to be cardioprotective and to decrease mortality from all causes (7). Thus we tested aerobic exercise as an intervention to prevent susceptibility to myocardial I/R injury in an IUGR population using a rat model. To the best of our
knowledge, this is the first study designed to determine the impact of aerobic exercise training on cardiac function in offspring born from hypoxic pregnancies. As expected, in male control offspring, aerobic exercise training improved basal cardiac performance and decreased cardiac superoxide generation. In male IUGR offspring, however, the opposite was observed, whereby aerobic exercise reduced cardiac performance and increased cardiac superoxide generation. Recovery of cardiac performance during the reperfusion period was not affected by aerobic exercise training in either control or IUGR offspring. The interaction of growth restriction and exercise, therefore, had a detrimental effect on cardiac function in male offspring. In females, there was no effect of IUGR or exercise on basal cardiac performance nor did exercise training have an effect on the recovery of cardiac performance during the reperfusion period. We further observed that aerobic exercise training increased cardiac SOD-1 expression in both control and IUGR female offspring, which was not observed in male offspring. The female sex, therefore, appeared to be protected against the detrimental effects of a compromised in utero environment on cardiac function, and this may have been due to an improved antioxidant status. In males, exercise was found to have a differential effect on the levels of oxidative stress, as assessed by superoxide levels, dependent on the in utero condition experienced by the offspring. Since the levels of antioxidants included in this study were found to be unaltered, this might be due to differential expression of prooxidants or differential activity levels of either pro- or antioxidants. Further investigation is required to fully elucidate the effect of exercise on the oxidant status in male offspring born from either a normal or compromised pregnancy.

Our findings in male IUGR exercised offspring imply a basal cardiac maladaptation to aerobic exercise training in this group. Altered cardiac function in male IUGR exercised offspring could be secondary to an increase in sympathetic tone following exercise. Being born small for a given gestational age at term has been associated with reduced heart rate variability (13) and an increase in plasma norepinephrine (9), which suggests that IUGR is associated with an imbalance in autonomic tone. In a swine model of myocardial infarction, Duncker et al. (10) found that during exercise, swine had an exaggerated withdrawal of cardiac parasympathetic tone, increased norepinephrine and epinephrine plasma levels, and a blunted β-adrenergic cardiac response while β-adrenergic vasodilation in the coronary vasculature was maintained, suggesting cardiac specific β-adrenergic desensitization. Thus, in our animal model, a preexisting autonomic imbalance could lead to a state of β-adrenergic desensitization, which would be detrimental only under stress conditions such as aerobic exercise and would impair the normal cardiac adaptations to aerobic

Fig. 6. DHE staining in nonperfused hearts from female control and IUGR, sedentary and exercised offspring. Superoxide production as assessed by DHE staining in nonperfused hearts. The sedentary control group was normalized to 100% and then the percent change of the other groups (control exercised, IUGR sedentary and IUGR exercised) was assessed. Representative images of female control sedentary (A), female control exercised (B), female IUGR sedentary (C), female IUGR exercised (D), and negative control for DHE staining (E). F: summary data of female sedentary offspring (closed bars, n = 3–6) and female exercised offspring (checkered bars, n = 5). Data are summarized and presented as means ± SE and were analyzed by two-way ANOVA.
exercise training. Moreover, we also found that exercise in male offspring decreased cardiac superoxide generation in controls, while the opposite effect occurred in IUGR offspring, with no changes in the protein expression of any of the antioxidant enzymes studied. Since it has been previously shown that vagal nerve stimulation modulates cardiac redox status and adrenergic drive in mice with chronic heart failure (33), an association between an increased sympathetic tone and the increase in ROS in IUGR exercised offspring could be made.

In female offspring, despite preserved ex vivo cardiac function, we demonstrated that IUGR offspring had an increase in pulmonary valve peak velocity in vivo. It has been previously described that female IUGR offspring exhibited signs of pulmonary hypertension only with aging (28). Changes in the pulmonary valve peak velocity in our young female offspring suggest that cardiac remodeling could be occurring in the absence of overt signs of cardiac dysfunction either ex vivo or in vivo; aerobic exercise training did not alter any other echocardiogram functional or morphological parameters in either control or IUGR offspring. Morphological adaptations of the heart, however, may be modest unless comparing well trained and untrained populations (31), or severe pathological states (3).

Regarding the antioxidant balance in female offspring, aerobic exercise training increased SOD-1 while it had no effect either on the protein expression of the other antioxidant enzymes measured or superoxide production. These results are in accordance with McDonald et al. (23), who found that in streptozotocin-diabetic rats, SOD-1 but not SOD-2 was upregulated after 6 wk of aerobic exercise training on a treadmill. An increased antioxidant capacity might go some way to explaining the preserved cardiac function observed in this sex.

In conclusion, a decrease in cardiac performance before ischemia in association with an increase in ROS demonstrated that the impact of aerobic exercise training on cardiac function in male IUGR offspring was greater than in the female IUGR offspring. Interestingly, our previous findings in littermates demonstrated a more pronounced vascular dysfunction associated with aerobic exercise training in female compared with male offspring (24, 26). Since it has been previously shown that being born with IUGR secondary to a hypoxic pregnancy was associated with an increased cardiovascular and metabolic susceptibility to secondary stressors such as aging and a high-fat diet (4, 24, 27–29), we could interpret that aerobic exercise training may represent a secondary stressor to cardiac function that is not well tolerated in male offspring born from a hypoxic in utero environment. Our data support the fact that the impact of any insult during fetal development is crucial to cardiovascular development. Moreover, well-established preventive strategies to decrease the risk of cardiovascular diseases later in life may be detrimental in this susceptible population by further increasing risk. Thus more research is needed to counteract the effect of being born IUGR.

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REFERENCES


