CALL FOR PAPERS | Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

Effects of voluntary wheel running on cardiac function and myosin heavy chain in chemically gonadectomized rats

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Hydock DS, Lien C-Y, Schneider CM, Hayward R. Effects of voluntary wheel running on cardiac function and myosin heavy chain in chemically gonadectomized rats. Am J Physiol Heart Circ Physiol 293: H3254–H3264, 2007. First published September 28, 2007; doi:10.1152/ajpheart.00801.2007.—Reducing testosterone and estrogen levels with a luteinizing hormone-releasing hormone agonist such as Zoladex is a common treatment for many prostate and breast cancer patients, respectively. There are reports of surgical gonadectomy inducing cardiac dysfunction, and exercise has been shown to be cardioprotective under these circumstances. Minimal research has been done investigating the effects of chemical gonadectomy and physical activity on cardiac function. The purpose of this investigation was to examine the effects of chemical gonadectomy and physical activity on cardiac function. Male (M) and female (F) Sprague-Dawley rats received either Zoladex treatment (Zol) that suppressed gonadal function for 8 wk or control implants (Con) and either were allowed unlimited access to voluntary running wheels (WR) or remained sedentary (Sed) throughout the treatment period. In vivo and ex vivo left ventricle (LV) function were then assessed, and myosin heavy chain (MHC) expression and voluntary wheel running protected against this cardiac dysfunction.

Echocardiography; myosin heavy chain; orchiectomy; ovariectomy; sex hormones

DURING MATURATION, prostate development is highly dependent on male sex hormone (i.e., androgen) availability (18, 32, 77). Once fully developed, the prostate remains sensitive to androgens, which are vital to ensuring proper prostate cell maintenance and function (34, 73). However, because prostate epithelial cells express highly sensitive androgen receptors, androgens can also promote malignant cell growth and proliferation in the prostate (2, 24, 80). Similarly, mammary gland development and growth are dependent on the female sex hormone estrogen, because mammary epithelial cells express a variety of estrogen receptors (76). Because of this, endocrine-responsive breast tumor cell growth and proliferation is stimulated by estrogens (12, 17).

Because of the sex hormone-sensitive nature of endocrine-responsive prostate and breast cancer cells, treatments have been developed to withdraw sex hormones from these malignant cells in order to inhibit growth and proliferation. Historically, orchiectomy and ovariectomy (i.e., surgical gonadectomy) have been used to battle endocrine-responsive prostate and breast cancers, respectively. Chemical forms of sex hormone ablation, however, are currently being administered as effective alternatives to surgical gonadectomy. One such form of androgen and estrogen withdrawal in prostate and breast cancer patients, respectively, is the use of the luteinizing hormone (LH)-releasing hormone (LH-RH) agonist Zoladex (goserelin acetate). Zoladex reduces sex hormone concentrations to castrate levels by desensitizing the pituitary gland to LH-RH (16). Zoladex occupies LH-RH receptors on the pituitary, which causes a downregulation of LH-RH receptors. This downregulation eventually reduces LH release from the pituitary. Testicular and ovarian synthesis of testosterone and estrogen, respectively, are stimulated by LH, and inhibited production of LH leads to castrate levels of testosterone and estrogen.

Aside from the most commonly recognized side effects of LH-RH agonist therapy (i.e., decreased quality of life, fatigue, depression, hot flashes, loss of libido, sexual dysfunction, musculoskeletal issues), chemical gonadectomy may also be associated with impaired cardiac function because the myocardium expresses androgen and estrogen receptors (21, 45). Surgical gonadectomy has been shown to significantly impair cardiac function in male and female rats at the whole heart (60) and isolated cardiocyte (19) levels. In addition, surgical gonadectomy-induced cardiac dysfunction has been shown to be associated with a shift in cardiac myosin heavy chain (MHC) isoform distribution from the fast ATPase activity α-isoform to the slow ATPase activity β-isoform (4, 49, 60). With the exception of one report (29), minimal work has been done...
investigating the effects of chemical gonadectomy on cardiac function and cardiac MHC in the male or female heart. Therefore, the first purpose of this investigation was to determine the effects of chemical gonadectomy with Zoladex on cardiac function in adult male and female rats. It was hypothesized that Zoladex treatment would impair cardiac function and that this dysfunction would be associated with an increased expression of the slow ATPase activity β-MHC isoform.

Because of the negative conditions that hypogonadism poses for the myocardium, interventions to combat these deleterious cardiac side effects could be beneficial. Exercise training has been suggested as a possible means of minimizing the deleterious effects of hypogonadism in prostate cancer (28, 68) and breast cancer (56, 70) patients, but few studies have investigated the effects of exercise training on hypogonadism-induced cardiac dysfunction. Thus far, studies have shown a cardioprotective effect of exercise training during hypogonadal conditions (4, 44). However, these studies used surgical castration to induce hypogonadism in male and female rats, and, to our knowledge, only one study has explored the effects of motorized treadmill running on cardiac function during chemical gonadectomy in male rats (29) and no studies have examined the effects of exercise on the female heart during chemical gonadectomy. Therefore, the second purpose of this investigation was to investigate the effects of voluntary wheel running activity on cardiac function in adult male and female rats receiving Zoladex. It was hypothesized that increasing physical activity during Zoladex treatment would protect against cardiac dysfunction and that this cardioprotective effect would be associated with an attenuated β-MHC isoform upregulation.

METHODS

Animals and animal care. All protocols were approved by our Institutional Animal Care and Use Committee and were in compliance with Animal Welfare Act guidelines. Ten-week-old male and female Sprague-Dawley rats obtained from Harlan (Indianapolis, IN) were used in this study. Animals were housed in an environmentally controlled facility on a 12:12-h light-dark cycle and provided chow and water ad libitum throughout the duration of this study.

Chemical gonadectomy. Male (M, n = 40) and female (F, n = 40) rats were randomly assigned to receive either the LH-RH agonist Zoladex (M Zol, n = 20; F Zol, n = 20) or a control implant (M Con, n = 20; F Con, n = 20). Animals in the Zol group received a total of two 3.6-mg Zoladex depot formulations (gooserelin acetate housed in a 5-mm-long × 1-mm-wide biodegradable cylinder) implanted subcutaneously at the neck. One 3.6-mg depot formulation of Zoladex has been shown to effectively depress testosterone and estrogen levels in male and female rats, respectively, to castrate levels for 28 days (16). Animals received implants on day 1 and day 29, thereby blocking testosterone and estrogen production for a total of 56 days (8 wk). Animals in the Con group received subcutaneous control implants at the neck on days 1 and 29. Each control implant consisted of a 5-mm-long × 1-mm-wide section of biodegradable Silastic tubing (Dow Corning, Midland, MI).

Voluntary wheel running. Immediately after administration of the initial implants, male Con and Zol animals were randomly assigned to either the sedentary (Sed) or the voluntary wheel running (WR) group (M Sed + Con, n = 10; M Sed + Zol, n = 10; M WR + Con, n = 10; M WR + Zol, n = 10). Likewise, female Con and Zol animals were assigned to either the Sed or the WR group (F Sed + Con, n = 10; F Sed + Zol, n = 10; F WR + Con, n = 10; F WR + Zol, n = 10) immediately after the initial implant administration. Animals in the WR groups were housed individually in cages with commercially available running wheels (MiniMitter, Bend, OR) and given 24 h/day access to voluntary running wheels throughout the 56-day treatment period. Running distances were recorded with a VitalView data acquisition system (Mini Mitter). Animals in the Sed groups remained in standard rat cages throughout the 56-day treatment period. Cardiac function (in vivo and ex vivo) was then assessed on day 57.

Echocardiography. On completion of noninvasive blood pressure analysis, transthoracic echocardiography was conducted on sedated rats with a commercially available echocardiographic system (Toshiba Nemio 30, Tustin, CA; 10-MHz pediatric transducer). Animals were sedated with ketamine (40 mg/kg ip), and echocardiography was completed within 10–15 min after the administration of the sedative. Once the animals were sedated, the anterior and left lateral thoracic regions were shaved and animals were placed in the left lateral decubitus position. The probe was positioned to obtain short- and long-axis as well as four-chamber views. From the short-axis view, an M-mode tracing of the left ventricle (LV) was obtained for measures of septal wall thickness during systole (SWs) and diastole (SWd), posterior wall thickness during systole (PWs) and diastole (PWd), LV end-systolic diameter (LVEsd) and LV end-diastolic diameter (LVEDd). For all cardiac dimensions, we used a leading edge-to-leading edge technique as described by the American Society of Echocardiography (41).

Aortic and mitral valve blood flow profiles were obtained from an apical view with pulsed-wave Doppler, with the smallest possible sample volume placed at the aortic annulus and the tips of the mitral valve, respectively. LV mass was calculated as 1.04[(LVEDd + PWd + SWd)² – LVEDd²], relative wall thickness (RWT) was calculated as (PWd + SWd)/LVEDd, and fractional shortening (FS) was calculated as [(LVEDd – LVLd)/LVEDd]. The velocity of circumferential shortening (Vc) was calculated as FS/ET, where ET is ejection time. ET was obtained from Doppler measures of aortic flow and measured as the time between aortic valve opening and aortic valve closure. Vc was corrected to account for heart rate (HR) variability (VcHR) and was calculated as Vc/√(HR). From pulsed Doppler mitral and aortic flow images, the time–velocity integral (TVI), maximal flow velocity (Vmax), and mean flow velocity (Vmean) were measured. For measurements of cardiac dimensions, time intervals, and flow velocities, data from three consecutive cardiac cycles, when possible, were obtained and averaged for each rat.

Isolated working heart. After attainment of in vivo cardiac function measures, ex vivo cardiac function was assessed with an isolated working heart preparation. Each animal was anesthetized with an intraperitoneal injection of heparinized (500 U) pentobarbital sodium (50 mg/kg), and when a tail pinch reflex was absent the heart was rapidly excised and the aorta was cannulated. The heart was perfused with Krebs-Henseleit buffer (mM: 120 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 25 NaHCO₃, 17 glucose, and 0.5 EDTA, pH 7.4). Perfusion buffer was aerated with 95% O₂-5% CO₂, and temperature was maintained at 37°C. During retrograde perfusion, hearts were trimmed free of noncardiac tissue, and the left atrium was cannulated via the pulmonary vein. The flow of perfusate was then redirected through the pulmonary vein at a preload of 10 cmH₂O, and the aortic cannula was opened to allow for contraction against an afterload chamber set at 100 cmH₂O. After a 1.5-min equilibration period, hearts were paced at 300 beats/min with a stimulus isolator in conjunction with a PowerLab/8e data acquisition system (ADInstruments, Colorado Springs, CO). A microtip catheter pressure transducer (SPR-671, 1.4 F; Millar Instruments, Houston, TX) inserted at the aortic annulus and the tips of the mitral valve closure.

Myosin heavy chain isoform expression. LV homogenates were analyzed for MHC isoform expression according to the techniques described by Talmadge and Roy (71) and described previously by our

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RESULTS

Running wheel distances. Figure 1 illustrates running wheel distances for male and female animals. Zoladex-implanted male animals ran significantly less than control-implanted male animals during weeks 1 and 2 after the initial implant (P < 0.05, Fig. 1A). During weeks 3–8, however, no significant running distance differences were observed (P > 0.05). Female animals receiving Zoladex ran significantly less than female animals receiving control implants beginning 5 wk after initial implant, and this trend continued throughout the observation period (P < 0.05; Fig. 1B).

General observations. Body and heart mass data for male and female animals are presented in Table 1. No between-group differences were observed in male body masses at the times when the initial and second implants were administered (P > 0.05), and no differences were observed in body masses at the time of death (P > 0.05). Both absolute and relative heart mass (heart mass/body mass), however, were found to be significantly lower in M WR+Zol than M Sed+Con (P < 0.01).

No body mass differences were observed at the time of the initial implant for female animals (P > 0.05). Compared with F Sed+Con, however, F Sed+Zol and F WR+Zol had significantly greater body masses at the second implant and death time points (P < 0.01), which is consistent with reports of surgical ovariotomy promoting increased body mass in rodents (20). No absolute heart mass differences were observed at the time of death (P > 0.05); however, F WR+Zol had a significantly lower relative heart mass than F Sed+Con (P < 0.05), and F WR+Con had a significantly greater relative heart mass than F Sed+Con (P < 0.05).

In vivo cardiac function. Table 2 contains cardiac geometry measurements for male and female animals. In male animals, Zoladex treatment had a minimal effect on overall LV morphology, with only SWs differing between M Sed+Con and M Sed+Zol (P < 0.01). Zoladex treatment in female animals, on the other hand, had more profound effects on LV morphology. F Sed+Zol possessed significantly lower SWs (P < 0.05), SWd, PWs, PWd, LV mass, and RWT (P < 0.01) and significantly greater LVDs (P < 0.05) than F Sed+Con. No differences in cardiac geometry were noted between F WR+Zol and F Sed+Con (P > 0.05).
Table 1. Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sed+Con</th>
<th>Sed+Zol</th>
<th>WR+Con</th>
<th>WR+Zol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Implant BM, g</td>
<td>313±7</td>
<td>320±7</td>
<td>314±8</td>
<td>315±3</td>
</tr>
<tr>
<td>2nd Implant BM, g</td>
<td>377±6</td>
<td>372±9</td>
<td>348±8</td>
<td>359±3</td>
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<tr>
<td>Death BM (g)</td>
<td>413±10</td>
<td>407±13</td>
<td>382±8</td>
<td>397±3</td>
</tr>
<tr>
<td>Heart mass, g</td>
<td>1.52±0.03</td>
<td>1.45±0.06</td>
<td>1.43±0.05</td>
<td>1.32±0.01†</td>
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<td>Relative heart mass, g/kg</td>
<td>3.69±0.06</td>
<td>3.55±0.12</td>
<td>3.74±0.07</td>
<td>3.32±0.02†</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Implant BM, g</td>
<td>227±3</td>
<td>219±4</td>
<td>223±6</td>
<td>216±3</td>
</tr>
<tr>
<td>2nd Implant BM, g</td>
<td>255±5</td>
<td>292±4†</td>
<td>247±7</td>
<td>282±3†</td>
</tr>
<tr>
<td>Death BM, g</td>
<td>275±6</td>
<td>311±6†</td>
<td>261±6</td>
<td>313±4†</td>
</tr>
<tr>
<td>Heart mass, g</td>
<td>1.14±0.03</td>
<td>1.18±0.02</td>
<td>1.20±0.04</td>
<td>1.18±0.03</td>
</tr>
<tr>
<td>Relative heart mass, g/kg</td>
<td>4.15±0.14</td>
<td>3.81±0.07</td>
<td>4.60±0.14*</td>
<td>3.76±0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sed+Con, sedentary control; Sed+Zol, sedentary Zoladex; WR+Con, wheel running control; WR+Zol, wheel running Zoladex; BM, body mass; relative heart mass, heart mass per kilogram of body mass at death. *P < 0.05 vs. sedentary Sed+Con.

LV FS for male and female animals is illustrated in Fig. 2. No significant FS differences were observed between male groups (P > 0.05; Fig. 2A). Sex hormone suppression in sedentary female animals, however, promoted a 13% decline in FS compared with F Sed+Con (P < 0.05), whereas FS was preserved in F WR+Zol (P > 0.05; Fig. 2B).

Zoladex treatment had no effect on Vcf and Vfc in male animals (P > 0.05; Table 3). LVs from sedentary female animals receiving Zoladex, on the other hand, had significantly slower Vcf and Vfc than LVs from female sedentary controls (P < 0.01; Table 3), and this Vcf and Vfc decrement was not observed in F WR+Zol (P > 0.05; Table 3). Heats from M Sed+Zol performed with significantly slower aortic Vmax and Vmean than M Sed+Con hearts (P < 0.01 and 0.05, respectively), and this reduced aortic blood flow velocity was not observed in hearts from M WR+Zol (P > 0.05; Table 3). Analysis of mitral valve Doppler measures revealed that M Sed+Zol mitral TVI was significantly lower than that in M Sed+Con (P < 0.01; Table 3). In hearts from female animals, no significant between-group aortic or mitral Doppler measures were detected (P > 0.05; Table 3).

Ex vivo cardiac function. LVDPs acquired from an isolated working heart model are illustrated in Fig. 3. Compared with M Sed+Con, a 14% decline in LVDP was observed in M Sed+Zol (P < 0.01; Fig. 3A). Conversely, this LVDP decrement was not observed in M WR+Zol (P > 0.05). Likewise, compared with F Sed+Con, a 19% decline in LVDP was observed in F Sed+Zol (P < 0.05), but this decline was attenuated in F WR+Zol (P > 0.05 vs. F Sed+Con; Fig. 3B).

A 14% decline in +dP/dt was observed in M Sed+Con (P < 0.05), and this Zoladex-induced reduction in +dP/dt was not observed in M WR+Zol (P > 0.05; Fig. 4A). Although ventricles from F Sed+Zol

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Table 2. Cardiac geometry

<table>
<thead>
<tr>
<th></th>
<th>Sed+Con</th>
<th>Sed+Zol</th>
<th>WR+Con</th>
<th>WR+Zol</th>
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<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWs, mm</td>
<td>2.78±0.10</td>
<td>3.16±0.09†</td>
<td>2.96±0.10</td>
<td>2.98±0.07</td>
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<td>SWD, mm</td>
<td>1.65±0.05</td>
<td>1.79±0.05</td>
<td>1.67±0.09</td>
<td>1.64±0.06</td>
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<td>PWs, mm</td>
<td>2.96±0.09</td>
<td>3.14±0.10</td>
<td>3.14±0.14</td>
<td>3.04±0.10</td>
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<tr>
<td>PWd, mm</td>
<td>1.68±0.07</td>
<td>1.79±0.08</td>
<td>1.83±0.09</td>
<td>1.81±0.11</td>
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<tr>
<td>LVDs, mm</td>
<td>3.35±0.08</td>
<td>3.18±0.12</td>
<td>3.29±0.28</td>
<td>3.16±0.20</td>
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<tr>
<td>LVDd, mm</td>
<td>6.70±0.14</td>
<td>6.33±0.11</td>
<td>6.49±0.23</td>
<td>6.44±0.21</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>740±37</td>
<td>749±29</td>
<td>750±31</td>
<td>726±27</td>
</tr>
<tr>
<td>RWT, mm</td>
<td>0.50±0.01</td>
<td>0.57±0.02</td>
<td>0.55±0.04</td>
<td>0.55±0.04</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWs, mm</td>
<td>2.79±0.11</td>
<td>2.40±0.07*</td>
<td>2.77±0.07</td>
<td>2.76±0.11</td>
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<tr>
<td>SSWd, mm</td>
<td>1.50±0.05</td>
<td>1.19±0.06†</td>
<td>1.42±0.10</td>
<td>1.41±0.05</td>
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<tr>
<td>PWs, mm</td>
<td>2.93±0.10</td>
<td>2.43±0.08†</td>
<td>2.90±0.09</td>
<td>2.89±0.10</td>
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<td>PWd, mm</td>
<td>1.66±0.08</td>
<td>1.27±0.05</td>
<td>1.54±0.08</td>
<td>1.58±0.08</td>
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<tr>
<td>LVDs, mm</td>
<td>6.29±0.13</td>
<td>3.34±0.17*</td>
<td>2.92±0.17</td>
<td>2.72±0.14</td>
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<tr>
<td>LVDd, mm</td>
<td>5.94±0.13</td>
<td>6.37±0.16</td>
<td>6.12±0.21</td>
<td>6.02±0.11</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>566±26</td>
<td>404±48†</td>
<td>544±29</td>
<td>534±24</td>
</tr>
<tr>
<td>RWT, mm</td>
<td>0.53±0.02</td>
<td>0.39±0.02†</td>
<td>0.49±0.02</td>
<td>0.50±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. SWs, end systole septal wall thickness; SSWd, end diastole septal wall thickness; PWs, end systole posterior wall thickness; PWd, end diastole posterior wall thickness; LVDs, end systole left ventricular (LV) dimension; LVDd, end diastole LV dimension; RWT, relative wall thickness. *P < 0.05 vs. sex-matched Sed+Con.

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Fig. 2. Echocardiography-derived left ventricular fractional shortening of male and female Zoladex and control animals allowed access to running wheels or remaining sedentary. A: data obtained from male animals; B: data obtained from female animals. M Sed+Con, male sedentary control; M Sed+Zol, male sedentary Zoladex; F Sed+Con, female sedentary control; F Sed+Zol, female sedentary Zoladex. *Significantly lower than F Sed+Con (P < 0.05).
displayed a 13% decline in +dP/dt (compared with F Sed+Con), no between-group +dP/dt difference was observed in female animals (P > 0.05; Fig. 4B). A significant between-group −dP/dt difference was observed in male animals, and although −dP/dt in M Sed+Zol was 10% slower than in M Sed+Con, post hoc testing revealed that only M WR+Con had a significantly faster rate of pressure decline than M Sed+Con (P < 0.05; Fig. 5A). Although F Sed+Zol demonstrated a 13% decline in −dP/dt (compared with F Sed+Con), no significant between-group −dP/dt difference was detected in female animals (P > 0.05; Fig. 5B).

**Myosin heavy chain analysis.** To help explain decrements in cardiac function associated with chemical gonadectomy and the protective effect of increased physical activity, MHC isoform expression was analyzed. Figure 6 illustrates α- and β-MHC isoforms expressed in LVs from male and female animals. Ventricles from M Sed+Zol expressed a 13% lower level of the fast ATPase activity α-MHC isoform with a correspondingly greater expression of the slow ATPase activity β-MHC isoform than M Sed+Con (P < 0.01; Fig. 6A). Wheel running during treatment attenuated this isoform shift, because MHC distribution in M WR+Zol ventricles did not differ from MHC distribution in M Sed+Con ventricles (P > 0.05). Similarly, ventricles from F Sed+Zol expressed a 28% lower level of the α-isoform with a correspondingly greater expression of the β-isoform than F Sed+Con (P < 0.01), and F WR+Zol did not have this altered ventricular MHC distribution (P > 0.05; Fig. 6B).

**DISCUSSION**

There were definitive sex differences in the response of cardiac function and morphology following chemical gonadectomy in sedentary animals as assessed by echocardiography. Whereas hearts from sedentary castrated female animals exhibited alterations in seven of the eight measured LV geometric parameters, hearts from castrated male animals exhibited alterations in only one of the measured parameters. Similarly, FS in chemically castrated sedentary female animals was significantly lower than in sedentary controls, while there were no differences in FS between chemically castrated sedentary males and their sedentary controls. In contrast, hearts from chemically orchietomized male rats exhibited significant alterations in echocardiographic blood flow characteristics, whereas chemically ovarioctomized female rats did not exhibit such alterations.

When cardiac dysfunction was analyzed ex vivo, however, we observed a great deal of similarity between male and female hearts from sedentary animals undergoing LH-RH agonist treatment. Sedentary chemically gonadectomized animals, whether male or female, exhibited lower LVDP values than controls, and this LV dysfunction was associated with an upregulation in the slow ATPase activity β-MHC isoform. Alterations in the cardiac MHC profile have been shown to profoundly affect cardiac function. Specifically, there is an inverse linear relationship between β-MHC isoform expression and power output in isolated rat cardiac myocytes (26, 40), with some studies reporting as much as a twofold greater peak power output in cells exclusively expressing α-MHC versus cells exclusively expressing β-MHC (25). This relationship appears to be consistent with data obtained from isolated rodent heart experiments that demonstrate a decline in cardiac performance as the percentage of cardiac β-MHC increases (40, 74).

**Chemical gonadectomy and the male heart.** Studies on the effects of male sex hormones on the myocardium have had mixed results. Available testosterone has been shown to be instrumental in both cardioprotection and cardiac dysfunction. Testosterone is necessary for heat shock protein 70 upregulation induced by metabolic inhibition (43) and exercise preconditioning (47), but testosterone availability has been shown to exacerbate ischemia-reperfusion injury (7, 79) and cardiac dysfunction associated with trauma-hemorrhage (59). Testosterone, however, does promote cardiac myocyte hypertrophy (45, 78), and surgical orchectomy has been shown to depress

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**Table 3. Echocardiography-derived and Doppler measures**

<table>
<thead>
<tr>
<th></th>
<th>Sed+Con</th>
<th>Sed+Zol</th>
<th>WR+Con</th>
<th>WR+Zol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>382±12</td>
<td>401±8</td>
<td>405±10</td>
<td>407±12</td>
</tr>
<tr>
<td>V_cat, cm/s</td>
<td>0.95±0.03</td>
<td>0.89±0.05</td>
<td>0.94±0.06</td>
<td>0.93±0.06</td>
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<tr>
<td>V_mean, cm/s</td>
<td>0.076±0.003</td>
<td>0.073±0.004</td>
<td>0.078±0.005</td>
<td>0.077±0.006</td>
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<tr>
<td>Aortic TVI, cm</td>
<td>3.42±0.13</td>
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<td>3.56±0.10</td>
<td>3.72±0.22</td>
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<tr>
<td>Aortic V_max, cm/s</td>
<td>105±4</td>
<td>84±6*</td>
<td>103±2</td>
<td>111±5</td>
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<tr>
<td>Aortic V_mean, cm/s</td>
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<td>54±4*</td>
<td>65±2</td>
<td>67±4</td>
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<td>Mitral TVI, cm</td>
<td>3.91±0.16</td>
<td>3.18±0.19†</td>
<td>3.63±0.15</td>
<td>3.68±0.10</td>
</tr>
<tr>
<td>Mitral V_max, cm/s</td>
<td>98±4</td>
<td>90±4</td>
<td>107±4</td>
<td>103±4</td>
</tr>
<tr>
<td>Mitral V_mean, cm/s</td>
<td>72±3</td>
<td>64±3</td>
<td>77±3</td>
<td>75±3</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>441±6</td>
<td>406±17</td>
<td>405±8</td>
<td>416±29</td>
</tr>
<tr>
<td>V_cat, cm/s</td>
<td>1.07±0.03</td>
<td>0.75±0.09†</td>
<td>0.94±0.05</td>
<td>1.02±0.04</td>
</tr>
<tr>
<td>V_mean, cm/s</td>
<td>0.091±0.003</td>
<td>0.063±0.008†</td>
<td>0.078±0.005</td>
<td>0.085±0.004</td>
</tr>
<tr>
<td>Aortic TVI, cm</td>
<td>3.24±0.15</td>
<td>3.63±0.30</td>
<td>3.34±0.14</td>
<td>3.53±0.18</td>
</tr>
<tr>
<td>Aortic V_max, cm/s</td>
<td>96±3</td>
<td>89±6</td>
<td>95±5</td>
<td>98±5</td>
</tr>
<tr>
<td>Aortic V_mean, cm/s</td>
<td>63±2</td>
<td>61±4</td>
<td>60±2</td>
<td>65±3</td>
</tr>
<tr>
<td>Mitral TVI, cm</td>
<td>4.19±0.10</td>
<td>3.87±0.18</td>
<td>4.05±0.11</td>
<td>4.05±0.11</td>
</tr>
<tr>
<td>Mitral V_max, cm/s</td>
<td>108±2</td>
<td>103±5</td>
<td>107±3</td>
<td>108±2</td>
</tr>
<tr>
<td>Mitral V_mean, cm/s</td>
<td>79±2</td>
<td>72±5</td>
<td>78±2</td>
<td>78±2</td>
</tr>
</tbody>
</table>

Values are means ± SE. V_cat, velocity of circumferential fiber shortening; V_mean, heart rate-corrected velocity of circumferential fiber shortening; TVI, time-velocity integral; V_max, maximum velocity; V_mean, mean velocity. *P < 0.05, †P < 0.01 vs. sex-matched Sed+Con.
LV function (19, 60) and alter cardiac myosin isozyme distribution (44, 60). Therefore, testosterone appears to play a beneficial role in maintaining function and morphology in the healthy heart. Additionally, it is well known that thyroid hormones are instrumental in maintaining β-MHC expression (22), and there is evidence that surgical orchiectomy reduces thyroid hormone concentrations (11). Exercise, however, has been shown to enhance thyroid hormone metabolism (36) and increase myocardial thyroid hormone receptor expression (30). This exercise-induced thyroid hormone receptor upregulation is associated with an increased expression of β-MHC (38), which provides a possible mechanism for the exercise-induced MHC preservation in chemically castrated male rats observed in the present study.

Chemical gonadectomy and the female heart. Hypogonadism has a profound effect on the female myocardium. Menopause is associated with cardiovascular dysfunction (83, 84), but the nature of menopausal estrogen withdrawal differs from that of chemical ovariectomy employed in the present study (i.e., high estrogen levels rapidly reduced to near-castrate levels). Immediate female sex hormone depletion has been shown to be detrimental to the cardiovascular system, as evidenced by surgical ovariectomy models that promote cardiac dysfunction associated with disrupted MHC isoform expression (60). There is also evidence of surgical ovariectomy having deleterious effects on myocardial Ca^{2+} activation (4, 81), and although myofilament Ca^{2+} sensitivity may (46) or may not (14, 63) be affected by MHC isoform expression, Ca^{2+} sensitivity may be an alternative mechanism explaining LH-RH agonist-induced cardiac dysfunction.

It is well known that estrogen availability is considered cardioprotective (6, 13, 66), and the positive effects of estrogen on the female myocardium may very well be linked to 17β-estradiol’s antioxidant effect on the cardiovascular system (15, 31, 48). Surgical ovariectomy has been shown to exacerbate myocardial oxidative stress-induced damage caused by anthracycline administration (50), and estrogen administration protects against ischemia-reperfusion injury (6, 57). This suggests that the cardiac dysfunction observed in hearts from chemically ovariectomized rats in the present study may be attributed to compromised antioxidant status. In fact, the MHC isoform disruption observed in the present study may be a result of an impaired ability of the chemically ovariectomized rat heart to quench free radical production, because increased oxidative stress has been shown to promote β-MHC isoform upregu-

**Fig. 3.** Left ventricular developed pressures obtained with an isolated working heart model. A: data obtained from male animals. B: data obtained from female animals. *Significantly lower than F Sed+Con (P < 0.05); †significantly lower than M Sed+Con (P < 0.01).

**Fig. 4.** Left ventricular rates of pressure development obtained with an isolated working heart model. *Significantly lower than M Sed+Con (P < 0.05).
related to MHC expression. However, as to whether or not changes in wall thickness are in the contractile state of the ventricular wall. We are unsure, related to increased wall thickness suggestive of improvements alterations. Improvements in cardiac performance were indeed only molecular alterations (i.e., MHC) but also morphological tile dysfunction in the female heart is partially a result of not increase in LVDs. This suggests that Zoladex-induced contrac-
creases in SWs, SWd, PWs, and PWd, respectively, and a 24% Zoladex treatment promoted 14%, 21%, 17%, and 23% de-

heart demonstrated a much different response. Eight weeks of to be depressed in M Sed

prived sedentary male hearts exhibited a 14% greater SWs, but
dimensional. Compared with sedentary controls, androgen-de-
flows. This phenomenon, however, was not detected when isolated working heart preparation, because both M Sed+Zol and F Sed+Zol hearts possessed significantly lower LVDP than their respective controls. The in vivo and ex vivo cardiac function differences may be due to the influence of neurohumoral factors present in vivo that were negated during ex vivo analysis.

Testosterone has been shown to be a potent vasodilator of the aorta and large arteries (10, 82, 85), and androgen depriv-
tation therapy promotes large artery stiffness (67). In the present study, therefore, M Sed+Zol may have possessed decreased aortic and large artery compliance, in turn affecting aortic blood flow velocity without a FS decrement. In fact, in vivo FS may have been maintained during androgen depriv-
ivation in an attempt to maintain close to normal systemic blood flow. This phenomenon, however, was not detected when hearts were analyzed ex vivo since the perfusate and systemic loads used were identical for hearts from each experimental group.

In vivo cardiac function analysis revealed that hearts from F Sed+Zol possessed a 7% decline in aortic \( V_{\text{max}} \), but this change was not found to be statistically significant. Tatchum-Talom et al. (75) reported that aortas from surgically ovariec-
tomized rats possessed wall stiffness similar to that of sham-operated controls. In addition, the authors reported that the aortas from ovariectomized animals had significantly greater lumen cross-sectional area than controls. These findings help explain the lack of significantly altered aortic blood flow in F Sed+Zol, because normal aortic stiffness with increased lumen size could account for close to normal aortic blood flow despite FS decrements.

There also appears to be a differential MHC effect of Zoladex on male and female rat hearts. Sedentary males receiving Zoladex had a 13% reduction in the fast ATPase activity \( \alpha \)-MHC isoform, whereas sedentary females receiving

![A](https://example.com/image1.png)  
![B](https://example.com/image2.png)

Fig. 5. Left ventricular rates of pressure decline obtained with an isolated working heart model. *Significantly greater than M Sed+Con \((P < 0.05)\).
Zoladex had a 28% reduction in the fast ATPase activity α-MHC isoform. The difference in MHC alteration was reflected in functional data, because M Sed+Zol FS was not significantly different from that in M Sed+Con but F Sed+Zol FS was found to be significantly different from that in F Sed+Con. Additionally, ex vivo LVDP was 14% lower in M Sed+Zol and 19% lower in F Sed+Zol compared with their sex-matched sedentary controls. The greater magnitude of cardiac dysfunction observed in the estrogen-deprived female heart is partially explained by the greater magnitude of MHC shifting.

Wheel running protects against chemical gonadectomy cardiac dysfunction. Animals allowed free access to running wheels during chemical gonadectomy treatment did not exhibit the cardiac dysfunction and β-MHC upregulation observed in animals remaining sedentary during chemical gonadectomy. Increased physical activity with voluntary wheel running has been shown to improve cardiovascular performance in healthy rats (51, 52), hypertensive rats (23, 37), and post-myocardial infarction rats (58). To our knowledge, there have been no previous investigations analyzing the effects of voluntary wheel running on cardiac function in sex hormone ablation models. The effects of exercise on the myocardium after surgical castration have been investigated with swimming (44) and motorized treadmill models (4), and we reported previously (29) that motorized treadmill running protected against androgen deprivation therapy-induced cardiac dysfunction. Swim training and forced treadmill training, however, may not translate well to the intensity of exercise typically prescribed to cancer survivors, since low-to-moderate intensity aerobic exercise training has been shown to be advantageous to cancer patients (5, 61, 62) and voluntary wheel running provides a training stimulus that is less stressful than the training stimulus provided by motorized treadmill running (53).

Sex, sex hormones, and voluntary wheel running. The large disparity in voluntary running distances observed between control male and female animals is consistent with the study of Jones et al. (35), who reported females running at higher rates than males. On average, control females ran 44% further than control males throughout the 8-wk study period (244 vs. 129 km). Nonetheless, 8 wk of wheel running did not promote improvements in cardiac function in control males or females (WR+Con). Previously, our laboratory reported (9) that 8 wk of voluntary wheel running did not improve ex vivo cardiac function in healthy female rats, and although Konhilas et al. (39) reported increased heart mass following 7 wk of nonresisted wheel running in male C57BL/6 mice, they reported no significant upregulation in β-MHC mRNA. Likewise Schultz et al. (64) recently reported that 16 mo of voluntary wheel running did not induce echocardiographic or hemodynamic alterations in control rats. Additionally, 9 wk of motorized treadmill running resulted in no significant increase in maximum cardiac ATPase activity and α-MHC protein expression in control animals (4). However, exercise mode may play a role in promoting advantageous cardiac adaptations, as evidenced by Malhotra et al. (44), who reported that swim training performed for 90 min two times per day for 8 wk promoted a significant myosin V1 isoform upregulation. It has also been shown that 8 wk of progressive treadmill training significantly upregulates cardiac stress proteins whereas 8 wk of voluntary wheel running does not (53). Thus beneficial adaptation in the healthy rat heart may be stimulated by longer-duration and/or
high-intensity exercise not present in the present study’s voluntary wheel running model.

Although wheel running provided protection against Zoladex-induced cardiac dysfunction, the effects of sex hormone ablation on running wheel activity also warrant discussion. Reduced running wheel activity observed in chemically gonadectomized male and female rats is consistent with other reports of surgical castration reducing running wheel activity in female (20) and male (33) rodents. Although F WR+Zol ran significantly less than F WR+Con during weeks 5–8, these distances were similar to that of M WR+Con. Since control males ran distances similar to those of chemically castrated females, it is possible that estrogens contribute to the increased running activity typically observed in female rodents (27, 55).

Regardless of the effects of sex hormone ablation on running wheel activity, Zoladex treatment may in fact lower the baseline of cardiac function to a point where voluntary wheel running promotes adaptation. Therefore, the present study demonstrates that hypogonadal cardiac dysfunction can be attenuated by substantially lower exercise loads than that employed to control animals.

Voluntary wheel running may more closely mimic the conditions experienced by recovering prostate and breast cancer patients receiving chemical gonadectomy. Even though chemically castrated animals ran less than their noncastrated controls, the reduced wheel running volumes were still cardioprotective. Previous studies reporting exercise-induced cardioprotection during surgical castration have employed identical exercise workloads for animals in castrated and sham-operated groups (4, 44). The present study suggests that a sex hormone-deprived heart can maintain its morphology, function, and MHC profile through low-intensity exercise. The novel observation of lower exercise volumes preserving cardiac function in the present study holds promise for prostate and breast cancer patients receiving LH-RH agonist treatment because this therapy is typically associated with increased fatigue levels (54, 65, 69). Thus fatigue during therapy often translates to an inability to participate in high-volume or high-intensity exercise during LH-RH agonist treatment. Low-volume exercise training, as demonstrated in this study, may be adequate for cancer survivors to address LH-RH agonist-induced cardiac dysfunction.

Summary and conclusions. Chemical gonadectomy with the LH-RH agonist Zoladex promoted in vivo and ex vivo cardiac dysfunction in both male and female sedentary rats. This dysfunction was associated with an upregulation in the slow ATPase activity β-MHC isoform. Voluntary wheel running during chemical gonadectomy provided protection from this form of hypogonadism-induced cardiac dysfunction, possibly by preserving MHC distribution. The findings of the present investigation provide insight into factors affecting LH-RH agonist-treated prostate and breast cancer patients’ decreased quality of life. The cardioprotective effect afforded by voluntary wheel running in the present study also provides insight as to how to manage LH-RH agonist-induced side effects. Even though LH-RH agonist-treated cancer patients may lack the motivation to exercise at high-volume workloads (because of increased fatigue), it is promising that the lower running workloads performed by hypogonadal animals afforded preservation of cardiac function.

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**REFERENCES**


