Effect of ovariectomy in heart failure-prone SHHF/Mcc-fa<sup>op</sup> rats

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Departments of 1Veterinary Biosciences, 2Medical Biochemistry, and 3Food Science and Technology, The Ohio State University, Columbus, Ohio 43210; and 4Department of Anatomy, Indiana University School of Medicine, Fort Wayne, Indiana 46800

Sharkey, Leslie C., Bethany J. Holycross, Sonhee Park, Sylvia A. McCune, Roger Hoversland, and M. Judith Radin. Effect of ovariectomy in heart failure-prone SHHF/Mcc-fa<sup>op</sup> rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1968–R1976, 1998.—The importance of the loss of ovarian function to the progression of hypertension and heart disease in women is controversial. We investigated whether ovariectomy would accelerate development of hypertension, congestive heart failure, and neurohumoral activation in adult spontaneous hypertension heart failure (SHHF) rats, a genetic model of heart failure. Six months after ovariectomy, no significant differences between control and ovariectomized rats were seen in systolic or diastolic blood pressure, left ventricular fractional shortening by echocardiography, or heart weight. Percent V<sub>1</sub> myosin isozyme was significantly lower in ovariectomized rats. Northern blot analysis failed to show significant differences between groups in expression of hepatic angiotensinogen, renal renin, or left ventricular atrial or brain natriuretic peptide mRNA. In a second experiment, serial measures of systolic pressure and left ventricular shortening fractions failed to document a significant difference between control and ovariectomized rats as they developed heart failure, although there was a significant decline in shortening fraction in both groups at the age when regular estrous cycling naturally ceases. Survival time was similar between groups. In summary, ovariectomy of adult SHHF rats does not appear to affect the progression of genetically programmed hypertension and heart failure in this model.

hypothesis; shortening fraction; atrial natriuretic peptide; renin-angiotensin system; heart failure

CONTROVERSY PERSISTS regarding the significance of menopause to cardiovascular health in women and the rationale for estrogen replacement therapy. Data demonstrating that women with functional ovaries have lower rates of heart disease than age-matched men or postmenopausal women suggest that ovarian hormones, primarily estrogen, may have a protective effect on the heart (28). Women with heart failure due to nonischemic causes have significantly better survival than men, and women at an early phase of menopause have superior myocardial contractility compared with women of a similar age whose menopause is of longer duration (1, 23). Postmenopausal women have more pronounced cardiovascular responses to mental stress and higher ambulatory diastolic blood pressures compared with premenopausal women (22).

Studies have been undertaken in rats to clarify the effects of the loss of ovarian function on the cardiovascular system. Compared with intact rats, ovariectomized Wistar rats have impaired contractile function as well as decreased myosin ATPase activity and reduced V<sub>1</sub> myosin isoenzyme, a marker of hypertrophic adaptation in rats (25, 26). Unfortunately, work in rats has shown inconsistent effects of ovariectomy on the development of hypertension in various models; the age at ovariectomy may also be a factor. Decreased blood pressure (3), increased blood pressure (21), and no difference in blood pressure (7) have all been reported in ovariectomized animals compared with controls. Inconsistencies in previous work may reflect differences in the populations studied. Individuals genetically predisposed to hypertension and heart disease may show enhanced responses to changes in sex steroids, including the loss of ovarian hormones. The purpose of this study is to examine the effect of ovariectomy on the progression of heart disease in spontaneous hypertension heart failure rats (SHHF/Mcc-fa<sup>op</sup>, abbreviated as SHHF), in which males die of heart failure six or more months earlier than females. All rats in this strain develop genetically determined hypertension, cardiac hypertrophy, activation of neurohumoral systems, and terminal congestive heart failure as they age; this occurs in the absence of dietary or surgical interventions, making them a superior model for human heart disease in a “high-risk” population (20). As in people, SHHF females progress to heart failure later than SHHF males.

METHODS

Experiment 1

Animal model. Sixteen 8-mo-old lean female SHHF rats were obtained from the breeding colony of Dr. Sylvia McCune at The Ohio State University. The facility is American Association for Accreditation of Laboratory Animal Care accredited, the protocols were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee, and animals were cared for according to National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were paired by initial tail cuff blood pressures and randomly assigned to ovariectomy (OVX1, n = 8), nonsurgical control (n = 4), or sham-operated control (n = 4) groups. Because no differences were observed between control groups for any parameters, these were combined for data analysis (Con1, n = 8). Seven milligrams of ketamine (Ketaset; Fort Dodge Laboratories, Fort Dodge, IA) and 0.7 mg xylazine/
100 g body wt (Rompun; Bayer, Shawnee Mission, KS) were administered intraperitoneally. For the ovariectomy group, the ovaries were exteriorized, ligated, and removed via bilateral paralumbar incisions, which were closed with wound clips. The sham procedures consisted of anesthesia, visualization of the ovaries through incisions into the abdominal cavity, and closure of the wounds. Adequacy of ovariectomy was verified by vaginal cytology, plasma estrogen levels, and examination of the ovaries and uterus at necropsy. All animals were housed in pairs in a light-controlled room (12 h light, 12 h dark) and provided with ad libitum access to tap water and food. Conscious resting tail cuff blood pressures were taken once a month for 6 mo. After blood pressure measurement, rats were placed in stainless steel metabolic cages for 24-h urine collection, after which fasted plasma samples were collected by tail bleeding.

M-mode echocardiographic evaluation was conducted at 13 mo of age (5 mo after ovariectomy). At 14 mo of age, four randomly selected OVX2 rats and their paired Con1 rats had indwelling carotid catheters placed for the direct measurement of conscious resting blood pressure and for collection of samples for plasma renin activity (PRA) analysis. Rats were pretreated with 6 mg/100 g body wt carbenicillin in the drinking water to control infection and anesthetized with ketamine-xylazine as described above. A single polyethylene catheter (PE-50) was inserted into the left carotid artery of each rat, tunneled subcutaneously to the dorsal interscapular region, and secured by jacketing. Forty-eight hours after surgery, cannulas were connected to a pressure transducer (model 1280; Hewlett-Packard, Palo Alto, CA) and conscious resting diastolic and systolic arterial blood pressures were recorded on a physiological recorder (model 7758A; Hewlett-Packard). Blood samples were collected through the catheters into chilled tubes containing EDTA.

All rats were killed at 14 mo of age with 100 mg/kg pentobarbital sodium administered intraperitoneally. Heart, kidneys, liver, retroperitoneal and mesenteric fat pads, and the brain were weighed. Tissue samples were quick frozen on dry ice and stored at −70°C. Only retroperitoneal fat was frozen for assays. Organ-to-body weight ratios were calculated. The presence and follicular activity of the ovaries were noted.

Experiment 2

Thirteen 10-mo-old lean female SHHF rats were obtained from Dr. Sylvia McCune’s breeding colony at The Ohio State University. The rats were documented by vaginal cytology to have normal estrous cycles. Rats were paired according to initial blood pressure measurements and then randomly assigned to ovariectomy (OVX2, n = 7) or control groups (Con2, n = 6). Surgery was performed as described in Experiment 1. Every 8 wk, vaginal cytology was performed to assess the estrous cycling status of the Con2 rats and to determine when cycling stopped. Cytology was also performed on OVX2 rats after ovariectomy to confirm successful termination of estrous cycles. Estrogen levels were determined at 11, 12, 13, 16, and 20 mo of age. Systolic tail cuff blood pressures were taken before ovariectomy at 10 mo of age and at approximately every 4–8 wk thereafter. Blood pressures were also determined at the time of euthanasia. The readings for 10, 11, 12, and 13 mo were conscious measurements, whereas the remainder were collected under ketamine-xylazine sedation just before echocardiography. To assess functional changes in the heart after surgical removal of the ovaries, we performed echocardiographic analysis every 1–2 mo beginning 4 mo after ovariectomy. In the case of Con2 rats, functional data were collected for interpretation in relation to age and the natural cessation of estrous cycles. A final recording was made just before euthanasia.

The rats were allowed to progress into terminal heart failure and were euthanized by an intraperitoneal overdose of pentobarbital sodium (100 mg/kg) when they appeared to be in distress. Some rats developed other life-threatening medical conditions and had to be euthanized before the development of terminal heart failure, and they were categorized as either in early failure or not in heart failure at the time of death or euthanasia. Terminal heart failure was characterized by dyspnea, edema, moderate to marked thoracic or abdominal effusions at necropsy, systolic blood pressure in the normo- or hypotensive range (i.e., <110 mmHg), and left ventricular fractional shortening (LVFS) below 50%. Rats were characterized as being in early congestive heart failure if mild thoracic or abdominal effusion was present at necropsy, systolic blood pressure became normo- or hypotensive (i.e. <110 mmHg), and LVFS was below 50%. If rats had no effusion, were still hypertensive (tail cuff systolic pressure >140 mmHg), and had LVFS above 50%, they were categorized as not being in heart failure. Full necropsies were done for tissue collection and to determine the cause of death in all rats.

Blood collection and analysis. Blood samples were collected after a 24-h fast. Tubes for plasma collection contained lithium heparin and aprotinin, with the exception of samples for renin analysis, which were collected in tubes containing 20 µl of 20% EDTA. Plasma cholesterol in experiment 1 was measured enzymatically using a commercially available kit (Stanbio Laboratories, San Antonio, TX), and plasma glucose was measured using the hexokinase method of Bergmeyer et al. (4). Plasma insulin concentrations were measured at 9 and 14 mo of age using a commercially available double antibody RIA kit (EquateRIA; Binax, Portland, ME), and plasma atrial natriuretic peptide (ANP) concentrations were determined at 9 and 13 mo of age using an RIA kit (Peninsula Laboratories, Belmont, CA) modified by the method of Radin et al. (24). Plasma estradiol was determined using a commercially available RIA kit (Coat-a-Count estradiol kit; Diagnostic Products, Los Angeles, CA), and PRA was measured in samples obtained from conscious, cannulated rats using a commerically available kit (GammaCoat plasma renin activity RIA; Incstar, Stillwater, MN).

Myosin isoform. Isolation and separation of myosin isoforms from the left ventricle in experiment 1 was done following the method of Hoh et al. (15). Myosin extracts were prepared and stored at −70°C until analysis by 3.5% polyacrylamide gel electrophoresis using nondissociating conditions. Gels were stained with Coomassie blue and quantitated by a laser densitometer (LKB Gelscan XL; LKB Probbker, Bromma, Sweden).

RNA isolation and Northern blot analysis. Total RNA was extracted from fresh frozen left ventricle, kidney, liver, and retroperitoneal adipose tissue samples by the method of Chirgwin et al. (9) and stored at −70°C. Northern blot analysis was done to identify and quantitate mRNA for renal renin, hepatic and abdominal adipose angiotensinogen, left ventricular proANP, and brain natriuretic peptide (BNP). Full-length cDNA probes for rat renin (1.4 kb) and rat angiotensinogen (1.7 kb) were generously provided by Dr. Kevin Lynch of the University of Virginia, Charlottesville, VA (6). Dr. Christopher Glombotski of San Diego State University, San Diego, CA, provided the full-length cDNA probe for ANP (13), and a nonlinearized full-length cDNA probe for BNP was obtained from A. J. de Bold of the University of Ottawa Heart Institute, Ottawa, Ontario, Canada (13). Expression of ANP and BNP mRNA in the left ventricle was
normalized to a constitutively expressed mRNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A 905-bp cDNA probe for GAPDH was acquired from Ambion (Austin, TX) and is reported to have a high degree of binding for rat mRNA (13). The probes were labeled by random priming with [α-32P]dCTP using Prime-a-Gene reagents (Promega, Madison, WI) to a specific activity of 10⁶ to 10⁷ counts·min⁻¹·µg⁻¹ DNA. Percent incorporation was calculated and was >50% for all reactions; therefore unincorporated nucleotides were not separated from the labeled probes.

For analysis, 10–20 µg of total RNA per sample was denatured in a formamide and formaldehyde solution and loaded onto a 1.2% agarose, 6% formaldehyde gel. RNA was fractionated by size and blotted onto an MSI nylon membrane (0.45 µm; MagnaGraph, Westborough, MA) according to the method of Thomas (27). Blots were examined under ultraviolet (UV) light for degradation of RNA, location of the 28S and 18S ribosomal RNA bands, and estimation of uniformity of loading. The RNA was fixed to the blot by UV transillumination (254 nm, 500 µW/cm²) for 1.5 min and by baking under vacuum at 80°C for 2 h. Blots were prehybridized using the method of Church and Gilbert (10) for renin and a modification of the method of Chen et al. (8) that does not include yeast transfer RNA for angiotensigen and ANP. Blots for BNP were prehybridized in 6X saline sodium citrate (SSC), 5X Denhardt's solution, 0.5% SDS, 0.5 M phosphate buffer, and 200 µg herring sperm DNA. The renin blots were hybridized and washed as previously described (10). Hybridization for the other probes was accomplished by adding the labeled probes to the prehybridization solution and incubating them at 42°C overnight. Blots were washed two times for 10 min at room temperature using 1X SSC and 0.1% SDS, two times for 10 min at room temperature using 0.2X SSC and 0.2% SDS, and one time for 10 min using 0.2X SSC and 0.2% SDS at 50°C. BNP blots were washed twice for 20 min at 65°C in 2X SSC and 0.1% SDS and then two times for 20 min at the same temperature in 0.2X SSC and 0.1% SDS. Blots were rinsed in 0.05 M phosphate buffer for 10 s at room temperature and air dried. Dried blots were wrapped in plastic wrap and placed on a Molecular Dynamics PhosphorImager, and ImageQuant Software was used for densitometric analysis. After initial analysis, blots were stripped by boiling for 10–15 min in 0.1X SSPE. Successful stripping was confirmed by placing the blots on a phosphorimager for 24 h, resulting in no image on scanning. The blots were then considered ready for constitutive mRNA probing.

**Echocardiogram**

M-mode echocardiograms were performed on a Sonos 1000 echocardiograph machine (Hewlett-Packard) using a 7.5 MHz pediatric transducer. Images were recorded on a digital storage optical disc for later analysis with the software package of the echocardiograph machine. Restraint was provided by intraperitoneal injection of 5.0 mg ketamine and 0.5 mg xylazine per 100 g body wt. LVSF was calculated using the following formula: ((left ventricular internal diameter in diastole – left ventricular internal diameter in systole)/left ventricular internal dimension in diastole) × 100.

**Blood Pressure Measurement**

Tail cuff blood pressures were measured using a Gilson Duograph (model ICT-2H; Middleton, WI). Three acceptable tracings were obtained for each animal, and the values were averaged.

Heart, kidneys, liver, lungs, uterus, and the brain were weighed. Once the whole heart weight was measured, the atria were separated by a single cut with a razor blade and weighed together. The right ventricle was removed by cutting along its attachment to the left ventricle and septum and was weighed separately. The left ventricle and septum were weighed as a single unit. All animals were examined for the presence or absence of ovaries.

Statistical analysis. Results are means ± SE. Data were analyzed using the statistical software package Instat. Student's t-tests were performed on data sets with comparable standard deviations. For sets with significant differences in the variation between the ovariectomized and control groups, a nonparametric Mann-Whitney's rank sum test was done. Differences were reported as significant if P < 0.05. Correlations were linear regressions performed using Instat. The Kaplan-Meier curves were constructed, and the log rank test was used to compare the survival of OVX2 and Con2 rats that were in terminal or early congestive heart failure at the time of death.

**RESULTS**

**Experiment 1**

All Con1 rats were cycling normally, whereas OVX1 rats were persistently in diestrus or proestrus according to vaginal cytology (data not shown). The success of the ovariectomy procedure was further confirmed by measuring plasma estradiol levels, which were significantly lower in OVX1 than Con1 rats at 13 mo of age (9.0 ± 2.5 and 30.5 ± 7.8 pg/ml, respectively, Mann-Whitney's test). Figure 1A shows the effect of ovariectomy on body weight in lean female SHHF rats. Rats had similar fasted body weights before ovariectomy, but from 4 wk to the end of the study, OVX1 rats weighed significantly more than Con1 rats. Table 1 contains comparisons of the brain, heart, liver, and kidney weights and the ratios of these organ weights to total body weight. OVX1 rats had significantly higher kidney weights than Con1 rats. OVX1 rats had lower brain-to-body weight ratios because of the postovariectomy weight gain. OVX1 rats also had smaller livers relative to body size, although the gross weights were not different. This is also probably related to the smaller body size of the Con1 compared with OVX1 rats.

Table 1 shows fasting plasma glucose and insulin concentrations in OVX1 and Con1 rats. Insulin levels increased significantly in OVX1 rats from 9 to 14 mo of age, with no significant change over time in Con1 rats. Fasting plasma glucose concentrations did not change over time. Plasma cholesterol levels are compared in Fig. 2. Ovariectomy resulted in significantly higher total cholesterol levels from 1 mo after ovariectomy until the termination of the study.

In this study, ovariectomy at 8 mo of age had minor effects on cardiovascular function. There were no significant differences in tail cuff blood pressures between the groups at any time (data not shown). As with the tail cuff data, there were no differences between the groups for blood pressure obtained via arterial catheter (systolic blood pressure Con1 144 ± 6.2 vs. OVX1 161 ± 10
mmHg); however, there was a trend for the OVX1 rats to have higher diastolic pressures (Con1 109 ± 5 vs. OVX1 128 ± 8 mmHg; P = 0.10). M-mode echocardiographic measurements showed no difference in left ventricular shortening fraction at 13 mo of age, corresponding to 5 mo after ovariectomy (Con1 79.1 ± 5.4 vs. 66.8 ± 4.3%).

Although no gross evidence of hypertrophy was noted in OVX1 rats at necropsy, myosin isoenzyme distribution was significantly altered by ovariectomy (Fig. 3). OVX1 rats had lower %V1 isoenzyme than Con1, but we found no correlation between %V1 isoenzyme and heart weight (r = 0.03, P = 0.92), heart-to-body weight ratio (r = 0.39, P = 0.15), or M-mode fractional shortening (r = 0.46, P = 0.09).

PRA was similar in both groups (OVX1 7.96 ± 2.0, Con1 7.49 ± 1.4 ng ANG I generated·ml⁻¹·h⁻¹), as expected for groups with similar blood pressures and heart sizes. OVX1 rats had 103 and 98% of control angiotensinogen mRNA expression in hepatic and retroperitoneal adipose tissue, respectively. There were no significant differences in renal renin mRNA expression, and all blots showed single transcripts of the expected sizes.

Plasma ANP concentrations did not differ between OVX1 and Con1 rats at 13 mo of age (Table 2). There were no significant differences in ANP or BNP expression between the groups when expressed as a ratio to constitutive GAPDH expression (Table 2). No statistically significant differences were observed in mRNA expression between rats that underwent carotid cannulation and those that did not (data not shown). Plasma ANP concentration correlated with heart weight (r = 0.77, P = 0.005, n = 16) and was inversely correlated with tail cuff blood pressure (r = −0.61, P = 0.016, n = 16). Additionally, plasma ANP concentration was correlated with left ventricular ANP mRNA expression normalized to GAPDH (r = 0.61, P = 0.0159, n = 15). There was no correlation between ANP mRNA expression in the left ventricle and fractional shortening (n = 15, r = 0.016, P = 0.57).

Experiment 2

Ovariectomy performed at 10 mo of age successfully terminated estrous cycles. On average, spontaneous cessation of estrous cycling occurred in the Con2 rats at 17.4 ± 0.7 mo of age. Serum estradiol concentration was significantly greater in the Con2 compared with OVX2 rats at the beginning of the study (Fig. 4). Estradiol levels decreased with age in the Con2 rats and were similar to OVX2 rats by 16 mo of age, just before the spontaneous cessation of estrous cycling in the Con2 rats. As was seen in the previous experiment, ovariectomy resulted in significantly increased body weights in OVX2 compared with Con2 rats (Fig. 1). By 25 mo, this difference was no longer statistically significant due to body weight loss in OVX2 rats. As heart function deteriorated terminally, the OVX2 rats began to lose weight and no longer weighed more than the Con2 rats by the end of the study. Weight loss is characteristic of SHHF rats and humans in congestive heart failure. The mean survival time for OVX2 rats was 25.00 mo (range 19–26 mo, confidence interval 24.20–25.80) and for Con2 rats was 25.75 (range 18–27 mo, confidence interval 24.81–26.29). The difference was not significant by the log rank analysis, indicating that ovariectomy did not significantly shorten the life span of lean female SHHF rats.

As in the first experiment, ovariectomy did not result in significant differences in systolic tail cuff blood pressure between OVX2 and Con2 rats (Fig. 5). Left ventricular function did not appear to be affected by ovariectomy. OVX2 rats had comparable fractional shortening to age-matched Con2 rats at all times measured (Fig. 6). A significant decline in LVSF in both groups occurred between 18 and 20 mo of age (P < 0.001), which corresponded to the time of spontaneous cessation of estrous cycling in Con2 rats and was preceded by the fall in serum estradiol concentration (Fig. 4). This decline was seen simultaneously in the OVX2 rats that had stopped cycling much earlier and...
Table 1. Organ weights, insulin, and glucose in ovariectomized and control SHHF in experiments 1 and 2

<table>
<thead>
<tr>
<th></th>
<th>Con1 (n=8)</th>
<th>OVX1 (n=8)</th>
<th>Con2 (n=6)</th>
<th>OVX2 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain, g</td>
<td>1.80 ± 0.05</td>
<td>1.84 ± 0.03</td>
<td>1.81 ± 0.06</td>
<td>1.80 ± 0.08</td>
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<tr>
<td>Heart, g</td>
<td>1.46 ± 0.45</td>
<td>1.59 ± 0.44</td>
<td>1.92 ± 0.24</td>
<td>1.89 ± 0.17</td>
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<tr>
<td>Liver, g</td>
<td>8.96 ± 0.36</td>
<td>9.70 ± 0.19</td>
<td>9.53 ± 1.30</td>
<td>9.67 ± 8.82</td>
</tr>
<tr>
<td>Kidneys, g</td>
<td>1.54 ± 0.04*</td>
<td>1.81 ± 0.10</td>
<td>1.68 ± 0.08</td>
<td>1.63 ± 0.04</td>
</tr>
<tr>
<td>Abdominal fat, g</td>
<td>8.63 ± 1.25</td>
<td>11.03 ± 1.01</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>Brain:body weight</td>
<td>0.77 ± 0.02*</td>
<td>0.65 ± 0.01</td>
<td>0.89 ± 0.05*</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>Heart:body weight</td>
<td>0.61 ± 0.05</td>
<td>0.54 ± 0.05</td>
<td>0.92 ± 0.09</td>
<td>0.90 ± 0.15</td>
</tr>
<tr>
<td>Liver:body weight</td>
<td>3.81 ± 0.09*</td>
<td>3.35 ± 0.06</td>
<td>4.21 ± 0.25</td>
<td>3.93 ± 0.25</td>
</tr>
<tr>
<td>Kidneys:body weight</td>
<td>0.63 ± 0.03</td>
<td>0.63 ± 0.03</td>
<td>0.84 ± 0.06</td>
<td>0.69 ± 0.04</td>
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<tr>
<td>Abdominal fat:body weight</td>
<td>3.96 ± 0.42</td>
<td>3.81 ± 0.32</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>Fasted insulin, µU/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 mo of age</td>
<td>4.8 ± 1.4</td>
<td>3.1 ± 1.1</td>
<td></td>
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<tr>
<td>14 mo of age</td>
<td>6.6 ± 1.9t</td>
<td>8.8 ± 1.9</td>
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<tr>
<td>Fasted glucose, mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 mo of age</td>
<td>82.1 ± 6.2</td>
<td>79.3 ± 3.3</td>
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<tr>
<td>14 mo of age</td>
<td>89.8 ± 3.4</td>
<td>83.4 ± 2.3</td>
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</table>

Data are means ± SE. SHHF, spontaneous hypertension heart failure. *P < 0.05 for ovariectomized (OVX) 1 vs. control (Con) 1 or OVX2 vs. Con2, Student’s t-test. Fasting plasma insulin and glucose concentrations are from OVX1 and Con1 rats at 9 and 14 mo of age, corresponding to 1 and 6 mo after ovariectomy, †P < 0.05 for OVX1 at 9 mo vs. OVX1 at 14 mo.

therefore appears unlikely to be due to changes in ovarian function.

Because there were no statistically significant differences between OVX2 and Con2 rats in blood pressure or fractional shortening, the groups were combined for the following analysis. All rats were categorized as dying from terminal congestive heart failure, dying in early failure with complications from other causes, or having no signs of heart failure at the time of death from an unrelated disease. Other conditions that contributed to removal before the development of terminal congestive heart failure included pituitary tumors (1 OVX2 and 1 Con2 rat), lymphoma (1 OVX2 and 1 Con2 rat), and periportal masses (1 OVX2 and 1 Con2 rat). Rats exhibiting any clinical signs of failure had lower systolic pressures than rats with no signs of heart failure (104.7 ± 7.1 vs. 151.4 ± 19.7 mmHg, P = 0.04). Rats with no signs of failure had significantly higher left ventricular shortening fractions compared with rats in early or terminal failure (Fig. 7).

Significant decreases in left ventricular shortening fraction were accompanied by increased total heart weight (Table 3), with rats in terminal heart failure having significantly heavier hearts compared with early-failure and no-failure rats. The left ventricle and septum weights were similar for all the groups. The difference in total heart weight was due to significant differences in the weights of the right ventricle and atria. Consequently, the left ventricle comprised a lower percentage of the total heart weight in the terminal-failure rats compared with the early- or no-failure rats.

Organ weights at necropsy are given in Table 1. All organ weights were similar in the two groups except for the uterus. Uteri weighed significantly less and were grossly atrophic in the OVX2 compared with Con2 rats (data not shown). Ovaries were present in all Con2 rats, and no ovaries were seen in OVX2 rats.

DISCUSSION

Ovariectomy in the SHHF rat produced physical and metabolic changes consistent with previous studies in other rat strains. Ovariectomized SHHF rats weighed 20–25% more than controls, which is similar to the 13–25% weight gains reported for ovariectomized...
Wistar and spontaneously hypertensive rats (SHR) (7, 17, 25, 26). Fasting plasma cholesterol was significantly increased in OVX1 compared with Con1 rats, similar to findings in ovariectomized SHR (17). Insulin levels also increased over the study period in OVX1 but not Con1 rats. Increases in cholesterol and insulin are associated with a greater risk of cardiovascular disease in humans, and there is evidence that hyperinsulinemia may contribute to the development of hypertension in humans and SHR (5). However, these changes in the SHHF rat did not coincide with significant progression of hypertension or the induction of heart failure.

Hypertension is a common, powerful, and yet modifiable risk factor for cardiovascular disease. Significant gender differences exist, beginning at puberty, in the development of hypertension in humans, and, as young and middle-aged adults, men have higher blood pressure than women. With advancing age and decreasing ovarian function, the gap narrows between men and women (5). Several studies have examined the effects of gonadal steroids on the development of hypertension in other rat models with conflicting results. Various investigators have found that ovariectomy decreased blood pressure (3), had no effect (7), or increased blood pressure (11). In addition to strain differences, it appears that the loss of ovarian function may have more pronounced effects on blood pressure if performed at younger ages. It is possible that our study failed to document differences in blood pressure between intact and ovariectomized SHHF rats because of the older age of the rats at the time of ovariectomy or because ovarian hormones do not protect against hypertension in this strain. A significant decrease in blood pressure did occur as SHHF rats functionally decompensated and progressed into congestive heart failure (Fig. 5).

Despite the physical and metabolic changes induced by ovariectomy, there were no significant differences in cardiovascular function, progression to congestive heart failure, or survival compared with controls. Antemortem measurement of heart function using echocardiography failed to detect differences in LVSF between OVX and Con rats in either study. If differences in heart function between OVX and Con were present in SHHF rats, they may have been too subtle to detect using this

Table 2. ANP and BNP gene expression in left ventricle and plasma ANP concentrations in Con1 and OVX1 SHHF rats in experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Plasma ANP, pg/ml</th>
<th>ANP/GAPDH*</th>
<th>BNP/GAPDH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con1</td>
<td>7</td>
<td>318.1 ± 70.6</td>
<td>0.67 ± 0.03</td>
<td>0.49 ± 0.25</td>
</tr>
<tr>
<td>OVX1</td>
<td>8</td>
<td>289.9 ± 110.5</td>
<td>1.06 ± 0.22</td>
<td>0.41 ± 0.13</td>
</tr>
</tbody>
</table>

Data are means ± SE. No significant differences were seen between Con1 and OVX1 spontaneous hypertension heart failure (SHHF) rats for plasma atrial natriuretic peptide (ANP) levels at 13 mo of age (5 mo after ovariectomy) or for ANP and brain natriuretic peptide (BNP) mRNA expression at 14 mo of age by Northern blot analysis. *Data are expressed as a ratio to expression of constitutively present enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Fig. 5. Ovariectomized (OVX2) and control (Con2) lean female SHHF/Mcc-fa<sup>p</sup> rats had similar tail cuff systolic blood pressures from 10 to 25 mo of age (ovariectomy was performed at 10 mo of age; 10-mo value expressed here was determined before surgery). Each point of data is mean ± SE of surviving rats (Con2: n = 6 for 10–18 mo, n = 5 for 20–21 mo, n = 4 for 22 and 24 mo, and n = 3 for 25 mo; OVX2: n = 7 for 10–20 mo, n = 6 for 21 mo, n = 4 for 22 and 24 mo, and n = 2 for 25 mo), Student’s t-test.

Fig. 6. Ovariectomized (OVX2) and control (Con2) lean female SHHF/Mcc-fa<sup>p</sup> rats had similar left ventricular fractional shortening percentage by M-mode echocardiography from 14 to 25 mo of age. Each point is mean ± SE of surviving rats (Con2: n = 6 for 10–18 mo, n = 5 for 20–21 mo, n = 4 for 22 and 24 mo, n = 3 for 25 mo; OVX2: n = 7 for 10–20 mo, n = 6 for 21 mo, n = 4 for 22 and 24 mo, and n = 2 for 25 mo), Student’s t-test.
Early failure, n = 5; terminal failure, n = 6. Total heart weight. *P < 0.05, Student's t-test. No failure, n = 5; early failure, n = 3; terminal failure, n = 5.

A significant decline in LVSF did occur between 18 and 20 mo of age in OVX2 and Con2 rats, after the fall in serum estradiol concentration in Con2 rats and corresponding to the time when the last of the control rats stopped having regular estrous cycles. However, OVX2 rats showed a simultaneous decline in function with Con2 rats even though they had stopped cycling at 10 mo of age. The cause of this decline in cardiac function in both OVX2 and Con2 rats appears to be more complex than a simple loss of ovarian function and may be the result of some other senescent or preprogrammed change. The LVSF does correlate with the stage of failure in SHHF rats, because rats in early failure had fractional shortenings intermediate between terminal- and no-failure rats (Fig. 7).

Table 3. Heart weights for lean female SHHF rats in early and terminal congestive heart failure compared with rats showing no signs of failure

<table>
<thead>
<tr>
<th></th>
<th>No Failure (n = 5)</th>
<th>Early Failure (n = 2)</th>
<th>Terminal Failure (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total heart, g</td>
<td>1.46 ± 0.10</td>
<td>1.65 ± 0.00</td>
<td>2.36 ± 0.11*</td>
</tr>
<tr>
<td>Left ventricle + septum, g</td>
<td>1.03 ± 0.10</td>
<td>1.17 ± 0.03</td>
<td>1.23 ± 0.05</td>
</tr>
<tr>
<td>Right ventricle, g</td>
<td>0.19 ± 0.07</td>
<td>0.20 ± 0.02</td>
<td>0.39 ± 0.02*</td>
</tr>
<tr>
<td>Atria, g</td>
<td>0.21 ± 0.03</td>
<td>0.18 ± 0.01</td>
<td>0.52 ± 0.06*</td>
</tr>
<tr>
<td>Left ventricle-to-total heart ratio, %</td>
<td>68.8 ± 1.3</td>
<td>70.6 ± 1.5</td>
<td>52.0 ± 0.9*</td>
</tr>
</tbody>
</table>

Data are means ± SE. Total heart weight for lean female SHHF/Mcc-fa<sup>®</sup> rats in terminal congestive heart failure is significantly greater compared with rats in early congestive heart failure or with no signs of failure. This is due primarily to increased size of right ventricle and atria. There were no differences between groups in weights of left ventricle and septum, resulting in the left ventricle of terminal-failure rats contributing a significantly lower percentage of total heart weight. *P < 0.05 for terminal vs. early and no failure, ANOVA with Tukey-Kramer multiple comparisons post test.

\[ \beta \]-Myosin heavy chain protein and ANP have been called "stable late markers" of cardiac hypertrophy, because their gene expression in the adult rodent has been strongly linked to the development of pathological left ventricular hypertrophy in numerous experimental models of hypertension and hypertrophy. Although rats normally show some myosin isozyme shifts with aging, the changes seen in SHHF rats are greater than those observed in other strains of the same age. Also, marked shifts to V<sub>3</sub> are associated with the progression to congestive heart failure in SHHF rats, regardless of the age of the rat (19). Several studies have shown that ovariectomy of female rats results in a reduction of V<sub>1</sub> isoenzyme and impairment of heart function in Wistar rats (25, 26). A similar, but less marked, change in myosin distribution occurred in OVX1 rats, but this change did not result in a measurable depression of contractile function when echocardiography was used.

Plasma ANP concentrations are elevated in humans with left ventricular dysfunction and are even higher when patients develop overt heart failure (12). ANP mRNA and protein levels in the left ventricle increase with the development of hypertension and myocardial hypertrophy in SHR (2), and renovascular hypertensive rats show increases in ANP and BNP expression in relation to hypertrophy (18). In SHHF rats, plasma ANP concentrations increase with hypertension and are positively correlated with the severity of failure (20). Our study failed to show any significant difference between OVX1 and Con1 rats in left ventricular mRNA expression or plasma ANP levels, which does not support an effect of ovarian function on ANP expression or secretion in SHHF rats. This finding is consistent with the similar heart size, left ventricular function, and blood pressure seen in the two groups.

Female SHHF rats have delayed elevations in PRA compared with male rats, suggesting that female hormones may influence the renin-angiotensin system (RAS) and progression of heart disease in this strain (16). In fact, elevated PRA is not seen until 3–6 mo after the cessation of normal estrous cycling in the lean female SHHF rats. This led us to hypothesize that ovariectomy would lead to earlier RAS activation and progression of disease, but our data did not support this hypothesis. Divergent results have been reported for the effect of ovariectomy on the RAS in other hypertensive rat strains. In stroke-prone SHR rats, PRA and ANG II fell after ovariectomy, but total renin and plasma angiotensinogen did not differ (3). As in our study, ovariectomy was not associated with changes in renal renin or hepatic angiotensinogen mRNA (8). It may be that significant strain differences in the etiopathology of hypertension, protocol differences such as age of ovariectomy, or differences in methods for evaluation of RAS activation lead to these conflicting results.

Fig. 7. Lean female SHHF/Mcc-fa® rats with no signs of congestive heart failure at time of death had significantly higher left ventricular fractional shortening percentage by M-mode echocardiography compared with rats in early or terminal congestive heart failure. Data are expressed as means ± SE. *P < 0.05, Student's t-test. No failure, n = 5; early failure, n = 3; terminal failure, n = 5.
In summary, despite the presence of clear physical and metabolic changes consistent with ovarioctomy, there was no evidence of accelerated hypertrophy, nor was congestive heart failure prematurely initiated by ovarioctomy in SHHF rats. Additionally, there was no difference in survival between OVX2 and Con2 rats. Both OVX and Con SHHF rats showed a similar pattern of functional and morphological changes at the time of decompensation and failure. Heart weights were significantly greater in terminal-failure compared with early- and no-failure rats because of significantly heavier atria and right ventricles. Left ventricular systolic failure resulted in accelerated hypertrophy of the other chambers in the terminal phase of failure.

Perspectives

There is a great deal of controversy regarding the role of ovarian hormones and menopause in the development of cardiovascular disease in women. One of the important goals of this study was to determine if rats that underwent premature surgical loss of ovarian function would have an accelerated development of congestive heart failure and a shorter life span compared with rats that underwent natural cessation of cycling. The SHHF rat model is particularly pertinent in that this strain is genetically preprogrammed to develop congestive heart failure and there is a marked sexual dimorphism in the development of disease that resembles the human condition. OVX2 rats did not have any change in average life span compared with Con2 rats. With more rats, the small difference in age at death from cardiac failure between OVX2 and Con2 rats may have achieved statistical significance; however, this difference does not compare with the large gender difference in life span observed in this strain. The surgically induced loss of ovarian function may not be detrimental in genetically programmed heart failure. However, this study does not assess the benefit of estrogen replacement therapy, which may still have value. This study does suggest that the effects of ovarioctomy may not parallel sex differences seen within the SHHF rat strain.

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