Unveiling gender differences in demand ischemia: a study in a rat model of genetic hypertension

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Abstract

Objective: Female gender is associated with reduced tolerance against acute ischemic events and a higher degree of left ventricular hypertrophy under chronic pressure overload. We tested whether female and male rats with left ventricular hypertrophy present the same susceptibility to demand ischemia. Methods: Hearts from hypertrophied female and male salt-resistant and salt-sensitive Dahl rats (n = 8 per group) underwent 30 min of demand ischemia induced by rapid pacing (7 Hz) and an 85% reduction of basal coronary blood flow, followed by 30 min of reperfusion on an isovolumic red cell perfused Langendorff model. Results: In female hearts, high-salt diet induced a pronounced hypotrophy of the septum (2.38 ± 0.09 vs 2.17 ± 0.08 mm; p < 0.01), whereas male hearts showed the greatest increase in the anterior/posterior wall of the left ventricle (LV) (3.19 ± 0.22 vs 2.01 ± 0.16 mm; p < 0.05) compared with salt-resistant controls. At baseline, LV-developed pressure/g LV was significantly higher in female than male hearts (200 ± 13 and 196 ± 14 vs 161 ± 10 and 152 ± 15 mmHg g⁻¹; p < 0.01), independent of hypertrophy, indicating greater contractility in females. During ischemia, LV-developed pressure decreased in all groups; at the end of reperfusion, hypertrophied female and male hearts showed higher developed pressures independent of gender (148 ± 3 and 130 ± 8 vs 100 ± 7 and 85 ± 6 mmHg; p < 0.01). In contrast, diastolic pressure was more pronounced in female than in male hypertrophied hearts during ischemia and reperfusion (24 ± 3 vs 12 ± 2 mmHg; p < 0.01). Conclusions: In the pressure overload model of the Dahl salt-sensitive rat, female gender is associated with a more pronounced concentric hypertrophy, whereas male hearts develop a more eccentric type of remodeling. Although present at baseline, after ischemia/reperfusion systolic function is gender-independent but more determined by hypertrophy. In contrast, diastolic function is gender-dependent and aggravated by hypertrophy, leading to pronounced diastolic dysfunction. We can conclude that in the malignant setting of demand ischemia/reperfusion gender differences in hypertrophied hearts are unmasked: female hypertrophied hearts are more susceptible to ischemia/reperfusion than males. To determine whether in female hypertensive patients with acute coronary syndromes, diastolic dysfunction could contribute to the worse clinical course, further experimental and clinical studies are needed.

Keywords: Gender; Hypertrophy; Ischemia; Reperfusion; Diastolic function

1. Introduction

Cardiac hypertrophy and, in particular, left ventricular hypertrophy (LVH) is an adaptive response to hypertension. By increasing ventricular wall thickness, hypertrophy distributes a pressure overload over a greater myocardial cross-sectional area such that systolic wall stress, fiber shortening, and stroke volume can remain normal despite the increased load [1].

In experimental studies, female gender is associated with more concentric hypertrophy under pressure overload [2, 3], after myocardial infarction (MI) [4] or post-MI with hypertension [5] compared with males. In clinical studies, hypertension in premenopausal women is associated with increased concentric hypertrophy and contractile performance compared with men [6]. Similarly, in postmenopausal women with systolic hypertension and aortic stenosis [7], the pattern of hypertrophy is eccentric, too. This concentric pattern designates both an increase in left ventricular mass index and an increase in relative wall thickness, and results in a normalization of wall stress. In contrast, the pattern seen in men with equivalent disease is more consistent with a cardiomyopathic eccentric response [7].

Female gender is also associated with greater susceptibility to acute ischemic syndromes [8]. Data from the US National Registry of Myocardial Infarction clearly indicate that there is a gender-based difference in mortality: among
patients with less than 50 years of age, mortality rate for women is twice that for men [9]. Similarly, in the GUSTO IIb study women had more complications than men during hospitalization and a higher mortality rate [10]. These differences seem unrelated to the presence of estrogen, since estrogen has been found to be protective of ischemia–reperfusion (I/R) injury [11].

In pressure overload hypertrophy, I/R injury is increased, whereby diastolic dysfunction is predominant [12,13]. The extent of ischemic diastolic and systolic dysfunction is dependent on the type of hypertrophy [14]. To this day, it is unclear whether hypertension-induced hypertrophy leads to similar gender differences as other models of pressure overload with respect to development of LVH. In addition, nothing is known about the susceptibility of hypertrophied female and male hearts toward I/R injury. We hypothesized that in hypertension-induced hypertrophy, females would have increased concentric hypertrophy that increases the susceptibility to ischemia–reperfusion injury. Using a model of genetic hypertension we therefore investigated the influence of gender (1) on the development and extent of compensated LVH and (2) on the vulnerability to I/R injury.

2. Methods

2.1. Animal model

The Dahl salt-sensitive (DS) rat is an established experimental model of hypertrophy [5,14]. DS rats develop hypertension with low renin and aldosterone levels and compensated LVH. The Dahl salt-resistant (DR) rat develops neither hypertension nor hypertrophy and serves as age- and sex-matched control group.

Inbred DS (male n = 8, female n = 8) and DR (male n = 8, female n = 8) rats obtained from Harlan Sprague Dawley were housed, one rat per cage, in the Animal Facility of Boston University Medical Center as per the Guide for the Care and Use of Laboratory Animals (NIH Publication No.85-23, revised 1996). As described previously [5,14], rats arrived at 8—9 weeks of age and were fed low-salt diet (0.12% NaCl) for 1 week for acclimatization. The rats were then switched to high-salt diet (7.8% NaCl) and water ad libidum for 4 weeks. Systemic blood pressure was measured by tail-cuff method at 30 °C.

2.2. Isolated heart preparation

After 4 weeks of high-salt diet hemodynamic changes, left ventricle (LV) remodeling, and tolerance to ischemia–reperfusion injury were studied in the isolated isovolumically beating (balloon in LV) heart preparation perfused with red blood cells as previously described [12]. Briefly, rats were injected intraperitoneally with sodium pentobarbital (1 ml, 15 mg ml⁻¹) and heparinized (300 IU) via the right iliacal vein. The chest was opened rapidly, the heart excised, placed in iced saline, weighed, the aortic cannula inserted and perfusion started within 10 s retrogradely via the aortic root. All coronary venous effluents were collected by a cannula in the ligated pulmonary artery and measured by timed collection. Two pacing electrodes were placed on the epicardial surface and the hearts were paced according to the study protocol (Grass Instruments, Model 59, Quincy, MA). A fluid-filled latex balloon connected, to a Statham P23Db pressure transducer (Statham Instruments, Hato Rey, Puerto Rico) by a short, stiff polyethylene tubing was then placed into the left ventricle through the mitral valve and fixed. Coronary perfusion pressure was measured via a Statham P23Db pressure transducer connected to the aortic cannula. Coronary pressures, LV pressure, and its first derivative were recorded on a multichannel recorder (Gould Cleveland, Ohio).

2.3. Perfusion system and perfusate

The perfusion system has been described in detail [12]. Briefly, it consisted of a venous reservoir, a variable flow pump, an oxygenator, a water-jacketed arterial reservoir, and a filter of 20 μm pore size. The red blood cell perfusate consisted of bovine red blood cells at a final hematocrit of 40% in Krebs–Henseleit buffer (in mM): NaCl 118, KCl 4.7, CaCl₂ 2.0, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 26.6, glucose 5.5, lactate 1.0, palmitic acid 0.4 (as a source for free fatty acid metabolism), and 40 g l⁻¹ bovine serum albumin. Gentamicin (0.02 g l⁻¹) was added to retard bacterial growths. The perfusate was equilibrated with 20% O₂, 3% CO₂, and 77% N₂ at 37 °C and pH 7.4.

2.4. Perfusion protocol

During baseline hearts were initially perfused at 80 mmHg for the nonhypertrophied and at 100 mmHg for the hypertrophied hearts. This ensured similar coronary blood flow per LV mass (Table 2). Before the onset of ischemia (see below), the perfusion mode was switched to constant flow such that changes in perfusion pressure now indicated changes in vascular resistance. At the end of baseline perfusion, LV balloon volume was increased until a LVEDP of 10 mmHg. Thereafter, LV balloon volume was held constant. Under these conditions, changes in LVEDP reflect changes in diastolic chamber stiffness. After the demand ischemia protocol, hearts were switched back to preischemic baseline conditions (5 Hz) and reperfused for 30 min under these stable conditions.

2.4.1. Demand ischemia protocol

Hearts were subjected to 30 min of low-flow ischemia at 15% of their initial coronary blood flow. To simulate demand ischemia, pacing rate was increased from 5 Hz to 7 Hz after 5 min of ischemia and reduced to 5 Hz for the last 5 min of ischemia. Dahl salt-sensitive rats are characterized by an isoform-specific modulation of the Na⁺/K⁺-ATPase that impairs sodium excretion from the myocytes [15]. We hypothesized that tachycardia, therefore, would further impair ischemic diastolic function secondary to a sodium overload of the myocytes.

Hemodynamic measurements were performed according to a fixed schedule and were paralleled by sampling of arterial and venous (coronary sinus) perfusate to calculate cumulative lactate production (μmol min⁻¹ g⁻¹ LV mass), as described recently [12].
2.4.2. Pressure—volume curves

Prior to ischemia and at the end of reperfusion, diastolic pressure—volume relationship was determined over a range of balloon filling volumes. Using an airtight Hamilton syringe containing saline, left ventricular balloon volume was increased by 0.02 ml increments up to an LVEDP of 40 mmHg. The diastolic pressures generated for given volumes were used to describe a pressure—volume relationship by an exponential curve and equation ($p = \frac{b}{c^2} e^{kv}$) according to a model of Fletcher et al. [16]. Individuals with an $R^2$ value greater than 0.95 were included. The formula was solved for given pressures and a final exponential pressure—volume relationship for each individual was determined.

2.5. Fixation and histologic preparation

Following the postischemic pressure—volume experiments, hearts were arrested in diastole through an infusion of 1 ml high concentration KCl, flushed with 2 ml of saline, perfused at 50 mmHg with 200 ml of 10% buffered formalin acetate (Fisher Scientific), and fixed at a LVEDP of 5 mmHg. After fixation, hearts were processed for histologic examination, cut into five sections of equal thickness, and stained with hematoxylin and eosin. Morphometry was performed on the mid-papillary muscle section of the heart using a digital imaging and computer-aided quantification (Sigma Scan Pro 4.0).

2.6. Statistical analysis

Results were given as mean ± SEM. Comparison of animal characteristics were analyzed with a one-factor analysis of variance and post-HOC tests (Tukey HSD and Fisher PLSD). Comparison of pressure—volume relationship between experimental groups was performed by two-factor analysis of variance, and comparison of repeated hemodynamic measurements was performed by two-factor analysis of variance for repeated measurements. If overall analysis of variance indicated a significant difference of groups or interaction, values at specific time points were examined by the method of least significant differences (Fisher PLSD). A probability of $p < 0.05$ was considered to be significant. Two symbols were used to identify gender-specific (*) and hypertrophy-specific (#) differences.

3. Results

3.1. Animal characteristics

Fig. 1 shows body weight (Fig. 1A) and systolic blood pressures (Fig. 1B) before 2 weeks and 4 weeks after the onset of high-salt diet. Body weight was smaller in females compared with males. After 2 weeks of high-salt diet, female and male DS rats had developed hypertension compared with DR rats ($p < 0.05$) that became even more pronounced after 4 weeks of high-salt diet ($p < 0.01$, Fig. 1B). Hypertension-induced LV hypertrophy, as assessed by an increased LV/body weight ratio, was more pronounced in female than male hearts ($4.2 ± 0.2$ vs $3.7 ± 0.1$; $p < 0.05$; Table 1).

In addition, the high-salt diet in female hearts induced a pronounced hypertrophy of the septum (Table 1 and Fig. 2A), whereas male hearts showed the greatest increase in the anterior, posterior, and free wall of the LV (Table 1 and Fig. 2A). Compensated LVH was also observed by a leftward shift of the diastolic pressure/volume curve in the DS groups. However, female hearts were positioned significantly left to the respective male hearts, indicating smaller LV cavities and, therefore, a more concentric hypertrophy ($p < 0.05$; Fig. 3A).

3.2. Coronary blood flow

At baseline, coronary blood flow/g LV was similar in all groups (Table 2). Accordingly, coronary blood flow/LV mass
was identical during demand ischemia and reperfusion. Coronary perfusion pressure was significantly increased in hypertrophied hearts, indicating an increased coronary resistance during ischemia \((p < 0.01)\). During reperfusion coronary perfusion pressure and resistance were normalized in all groups except in female hypertrophied hearts, where it increased slightly but not significantly (Table 2).

### 3.3. Systolic function

LV-developed pressure (systolic—diastolic LV pressure) was similar in nonhypertrophied female and male hearts and similarly increased in hypertrophied male and female hearts (Fig. 4). When developed pressure was normalized for LV mass (mmHg g\(^{-1}\)), female hearts developed higher pressures than male hearts, independent of hypertrophy, suggesting a higher contractility in female hearts \((200 \pm 13 \text{ and } 196 \pm 14 \text{ vs } 161 \pm 10 \text{ and } 152 \pm 15 \text{ mmHg g}^{-1}, \text{ female DS and DR vs male DS and DR}; p < 0.01)\). During demand ischemia, LV-developed pressure decreased to the same extent in all groups and remained stable throughout ischemia. During reperfusion, hypertrophied male and female hearts had higher LV-developed pressures than nonhypertrophied hearts \((148 \pm 3 \text{ and } 130 \pm 8 \text{ vs } 100 \pm 7 \text{ and } 85 \pm 6 \text{ mmHg}; p < 0.01)\), independent of gender. Maximal positive \(dp/dt\) (mmHg\(^{-1}\)) paralleled LV-developed pressure (data not shown).

![Fig. 2. Representative prints of female (panel A) and male (panel B) DS and DR rats at mid-papillary level, stained with hematoxylin-eosin. Whereas in females the hypertrophic response has its maximum in the septum (panel A), male hearts show the most pronounced increase in the anterior, posterior, and free wall of the LV (panel B).](https://academic.oup.com/ejcts/article-abstract/31/2/298/454547/31239454547)

![Fig. 3. Before ischemia (panel A) end-diastolic pressure-volume relationship of female hearts are positioned left of male hearts \((p < 0.05)\), indicating smaller LV cavity size and more concentric hypertrophy in hypertensive hearts. After ischemia (panel B) all curves have shifted to the left, indicating greater myocardial stiffness. Female hearts are still positioned left of male hearts \((p < 0.05)\).](https://academic.oup.com/ejcts/article-abstract/31/2/298/454547/31239454547)
During ischemia, LV diastolic pressure increased in all groups (Fig. 5A). This increase in diastolic pressures was higher in female hypertrophied than nonhypertrophied hearts ($p < 0.05$ and $p < 0.01$, respectively). In addition, female hypertrophied hearts also had higher diastolic pressures than male hypertrophied and nonhypertrophied hearts ($p < 0.05$ and $p < 0.01$, respectively). During reperfusion, diastolic function improved in all groups. However, it remained significantly elevated in female hypertrophied hearts compared with male hearts ($24 \pm 3$ vs $11 \pm 2$ mmHg; $p < 0.01$) and nonhypertrophied female hearts ($13 \pm 3$ mmHg; $p < 0.05$). When diastolic dysfunction was expressed as diastolic pressure/g LV, differences between hypertrophied and nonhypertrophied hearts were no longer present. However, female hearts again showed persistent and increased diastolic dysfunction as compared with hypertrophied and nonhypertrophied male hearts during ischemia and reperfusion ($p < 0.05$ and $p < 0.01$, respectively, Fig. 5B). Maximal negative dp/dt (mmHg$^{-1}$) paralleled diastolic pressure in all groups (data not shown).

### 3.4. Diastolic function

During ischemia, LV diastolic pressure increased in all groups (Fig. 5A). This increase in diastolic pressures was higher in female hypertrophied than nonhypertrophied hearts ($p < 0.05$ and $p < 0.01$, respectively). In addition, female hypertrophied hearts also had higher diastolic pressures than male hypertrophied and nonhypertrophied hearts ($p < 0.05$ and $p < 0.01$, respectively). During reperfusion, diastolic function improved in all groups. However, it remained significantly elevated in female hypertrophied hearts compared with male hearts ($24 \pm 3$ vs $11 \pm 2$ mmHg; $p < 0.01$) and nonhypertrophied female hearts ($13 \pm 3$ mmHg; $p < 0.05$). When diastolic dysfunction was expressed as diastolic pressure/g LV, differences between hypertrophied and nonhypertrophied hearts were no longer present. However, female hearts again showed persistent and increased diastolic dysfunction as compared with hypertrophied and nonhypertrophied male hearts during ischemia and reperfusion ($p < 0.05$ and $p < 0.01$, respectively, Fig. 5B). Maximal negative dp/dt (mmHg$^{-1}$) paralleled diastolic pressure in all groups (data not shown).

### 3.5. Pressure—volume relationship

After ischemia and reperfusion the diastolic pressure—volume curve shifted leftward in all hearts, indicating increased myocardial stiffness. Female hearts remained left of male hearts ($p < 0.05$; Fig. 3B). Although female hypertrophied hearts shifted more than hypertrophied male hearts, this difference did not reach statistical significance.

### 3.6. Lactate production

Cumulative lactate production per LV mass ($\mu$mol min$^{-1}$ g$^{-1}$) was significantly higher in female hypertrophied than in nonhypertrophied hearts (650.8 ± 160.1 vs 144.9 ± 32.2; $p < 0.002$) and tended to be higher in male hypertrophied than in nonhypertrophied hearts (428.5 ± 70.9 vs 276 ± 38.6; ns).

### 4. Discussion

The major findings of the present study are: (1) in a model of pressure overload (compensated state), female hearts show...
more concentric hypertrophy and a higher contractility than male hearts. (2) During low flow ischemia in females, this more pronounced concentric hypertrophy results in increased susceptibility to ischemia–reperfusion injury.

### 4.1. Gender differences in LVH

Our study is in accordance with other experimental studies in hypertensive hearts and different models of pressure overload hypertrophy that showed increased concentric hypertrophy with a predominant hypertrophy of the septum in females compared with males after aortic banding [2,17] and post-MI [4]. Similarly, clinical studies in pre- and postmenopausal women showed that hypertension and aortic stenosis were associated with increased concentric hypertrophy [7]. The small concentric hypertrophied chambers result in an increased contractile function during baseline and conversely might be responsible for the increased diastolic dysfunction during ischemia in female hearts (see below). The mechanisms that contribute to this unique remodeling of the LV in pressure- and hypertension-induced LVH in females are not entirely known. Differences in hormonal status, i.e. an increased estrogen level or a lack of testosterone might conceivably contribute. Estrogen has been shown to influence cardiac hypertrophy via its vasodilative NO-mediated properties [18]. With respect to direct influences of estrogen on the myocardium, it has been shown that estrogen regulates myocardial hypertrophy via influences on MAP-kinase-phosphatase-1 (MAPK), atrial natriuretic peptide (ANP) [6] or a modulation of the AT-1 receptor [19]. In a model of aortic constriction in ovarectomized mice, 17β-estradiol significantly decreased hypertrophy relative to the placebo-treated mice. No effect of 17β-estradiol was detected in sham-operated animals nor did 17β-estradiol reduce cardiac fibrosis in the hypertrophied hearts in pressure-overloaded mice. Therefore, estrogen may prevent cardiac hypertrophy and a lack of estrogen may play a role in the development of LV hypertrophy late in life [20]. Similarly, estrogen and its regulatory effects on MAP-kinase-phosphatase-1 could also be the reason for delayed mortality and hypertrophy in female mice [21].

### 4.2. Gender difference in I/R injury in normotensive rats

We found no difference in the susceptibility to I/R in normotensive male and female rats. This is in agreement with studies in wild-type mice where no gender difference was found in isolated hearts undergoing an I/R protocol [17,22]. Therefore, physiological levels of estrogen in normotensive animals seem not to exert any protective or deleterious effect. In humans, female gender is associated with worse prognosis in acute coronary syndromes [8,9]. Our data suggest that differences in co-morbidity and other factors (e.g. more left ventricular hypertrophy, smaller coronary size etc.) might contribute greatly to this difference.

### 4.3. Gender differences in I/R injury in hypertrophied hearts

We and others have repeatedly shown that pressure overload hypertrophy increases susceptibility to I/R injury, whereby diastolic dysfunction is predominant [12,23,24]. In the present experiments we chose a demand ischemia model to increase the sodium load via the Na/Ca-exchanger in the myocytes through tachycardia. Since the Dahl rat has a mutation of the alpa1-Na, K-ATPase sodium excretion from the myocytes could be impaired.

Surprisingly, no increase in diastolic dysfunction was found in the male hypertrophied versus nonhypertrophied hearts in this study. This is in agreement with previous studies in this model, where we did not find an increased susceptibility in male hypertensive animals after 4 weeks of high-salt diet [14]. Left ventricular hypertrophy per se seems not in all cases deleterious, rather the increased susceptibility of hypertrophied hearts is dependent on the stimulus for hypertrophy and the stage of compensation of the hypertrophied heart [14].

In contrast, hypertrophied females showed increased diastolic dysfunction compared with nonhypertrophied females and compared with male hypertrophied hearts. Differences in blood flow could not contribute to this finding, since coronary blood flow before and after demand ischemia was similar in all groups. During reperfusion increased coronary perfusion pressure in female hypertensive hearts, however, indicates an increased vasoconstriction in these animals.

Female hypertensive hearts also had the highest lactate production. In this model of demand ischemia with residual flow, a mixed aerobic and anaerobic metabolism exists. The increased lactate production indicates greater areas of severe underperfusion where metabolism is mainly anaerobic. The heterogeneous metabolism is a known phenomenon of global ischemia in the rat heart. Areas of aerobic conditions with near normal pH exist next to areas of severe underperfusion and acidosis [14]. It is reasonable to speculate that in female hypertrophied hearts with increased concentric hypertrophy increased diastolic dysfunction developed during demand ischemia mainly because of this geometric difference. The increased diastolic pressure resulted in (subendocardial) areas of severe underperfusion with increased glycolysis and accelerated depletion of high-energy phosphates that resulted in the poorer recovery of these hearts.

It is important to note that in our experiments, the perfusate contained physiological concentrations of glucose, lactate and free fatty acids, the metabolites usually consumed by the heart. This was deemed important since the response to ischemia is highly dependent on the concentrations of the metabolic substrates [25], which might be even more important in female hearts.

In summary, the present study demonstrates that hypertrophy is significantly higher in female than male hypertensive rats. Furthermore, female hearts show significantly higher LV contractile performance per LV mass. In addition to these findings, the present study for the first time documents that during demand ischemia this more pronounced concentric hypertrophy results in no difference in systolic function but an increase in diastolic dysfunction and lactate production in female hypertrophied hearts compared with female nonhypertrophied and male hearts. To determine whether in female hypertensive patients with acute coronary syndromes diastolic dysfunction could contribute to
the worse clinical course, further experimental and clinical studies are needed.

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