Sex and β-adrenergic-induced cardiac remodelling

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Heart failure (HF) = complex clinical syndrome

manifestations of clinical HF are:
  reduced blood flow
  elevated cardiac filling pressure
At Risk for Heart Failure

STAGE A
At high risk for HF but without structural heart disease or symptoms of HF.
- e.g., Patients with:
  - hypertension
  - atherosclerotic disease
  - diabetes
  - obesity
  - metabolic syndrome
  - Patients using cardiotoxins
  - with FHx CM

THERAPY
GOALS
- Treat hypertension
- Encourage smoking cessation
- Treat lipid disorders
- Encourage regular exercise
- Discourage alcohol intake, illicit drug use
- Control metabolic syndrome

DRUGS
- ACEI or ARB in appropriate patients
  - (see text)
- Beta-blockers in appropriate patients
  - (see text)

DEVICES IN SELECTED PATIENTS
- Implantable defibrillators

STAGE B
Structural heart disease but without signs or symptoms of HF.
- e.g., Patients with:
  - preexisting MI
  - LV heart modeling including LVH and LVEF
  - asymptomatic valvular disease

THERAPY
GOALS
- All measures under Stage A
- Dietary salt restriction

DRUGS
- ACEI or ARB in appropriate patients (see text)
- Beta-blockers in appropriate patients (see text)

DEVICES IN SELECTED PATIENTS
- Implantable defibrillators

STAGE C
Structural heart disease with prior or current symptoms of HF.
- e.g., Patients with:
  - known structural heart disease
  - and shortness of breath and fatigue, reduced exercise tolerance

THERAPY
GOALS
- All measures under Stages A and B
- Dietary salt restriction

DRUGS FOR ROUTINE USE
- Diuretics for fluid retention
- ACEI
- Beta-blockers

DEVICES IN SELECTED PATIENTS
- Aldosterone antagonist
- ARBs
- Digitalis
- Hydralazine/nitrates

STAGE D
Refractory HF requiring specialized interventions.
- e.g., Patients who have marked symptoms at rest despite maximal medical therapy (e.g., those who are recurrently hospitalized or cannot be safely discharged from the hospital without specialized interventions)

THERAPY
GOALS
- Appropriate measures under Stages A, B, C
- Decision re: appropriate level of care

OPTIONS
- Compassionate end-of-life care/hospice
- Extraordinary measures
  - heart transplant
  - chronic inotropes
  - permanent mechanical support
  - experimental surgery or drugs

Hunt et al, 2005
Adverse cardiac remodelling (dilatation)

Normal Heart

Dilatation

Increased wall stress neurohormonal activation

$r$ (ventricular radius)

$h$ (wall thickness)
Proposed mechanism of dilatation:

- Apoptotic or necrosis
- Lengthening
- Collagen disruption

(Norton 2003)
Neurohormonal activation

Adrenergic

RAAS

Endothelin

Vasopressin

Inflammatory mediators

Oxidative stress...
Another classification:

- HF with preserved ejection fraction (EF)
- HF with reduced EF

→ 2 distinct disease processes
HF with **reduced** EF

↑

Cardiac Dilatation

Male gender

HF with **preserved** EF

↑

older, more often hypertensive, less likely to have coronary disease

Female gender
In pressure overload,
Sympathetic Nervous System (SNS) activation

Left ventricle

Lengthening
Cell death
Collagen disruption
Reactive fibrosis

Cardiac Dilatation

Sympathetic Nervous System (SNS) activation

? Gender
To determine the effect of sex in β-adrenergic-induced cardiac remodelling in the presence or the absence of pressure overload.
Rodent models of dilatation:

- Post myocardial infarction
- Toxic models (doxorubicin or high dose of isoproterenol)

Other models:
- Transgenic mice
- Volume overload (aortocaval fistula model)
- Pressure overload (Spontaneously Hypertensive rats)
- Chronic low level of β-adrenergic receptor stimulation
**2-D directed M-Mode Echocardiography**

In vivo,

**Left ventricular dimensions:**
- A: End diastolic diameter (EDD)
- B: End systolic diameter (ESD)
- Posterior Wall Thickness (PWT ED and PWT ES)

**LV systolic function:**
- Endocardial fractional shortening (FSend)
  
  \[
  F_{\text{Send}} = \frac{(EDD - ESD)}{EDD} \times 100
  \]

- Midwall fractional shortening (FSmid)
  
  \[
  F_{\text{Smid}} = \left[\frac{(EDD + PWT ED) - (ESD + PWT ES)}{(EDD + PWT ED)}\right] \times 100
  \]
Isolated Perfused Heart System

Ex vivo,

load-independent measures of cardiac function and dimensions in paced hearts
LV diastolic and systolic pressure-volume relations

Diastolic pressure (mm Hg)

Increasing volume of the balloon

Developed pressure (mm Hg)

Diastolic pressure

\[ V_0 \] Volume

Developed pressure

Volume
Objective 1

To determine whether sex affect β-adrenergic-induced cardiac remodelling in normotensive SD rats (honours projects – unpublished data)
Materials & Methods : Groups

Start of experiment
\( t=0 \)

Daily injection of Isoproterenol or vehicle

\( t=+3 \) months

4 groups (n=12 per groups):

3-month-old normotensive (SD) rats

Male
\[ \text{Vehicle (saline solution)} \]
\[ \text{Isoproterenol (0.01 mg.kg}^{-1}.\text{day}^{-1}) \]

Female
\[ \text{Vehicle (saline solution)} \]
\[ \text{Isoproterenol (0.01 mg.kg}^{-1}.\text{day}^{-1}) \]

Echocardiography
Objective 2

To determine whether castration affect β-adrenergic-induced cardiac remodelling in male normotensive SD rats

(Hodson et al., J. Cardiovasc Pharmacol 2014)
Materials & Methods : Groups

Start of experiment
\[ t=0 \]

Daily injection of Isoproterenol or vehicle
\[ t=+6 \text{ months} \]

4 groups (n=8-10):

2-month-old male normotensive (SD) rats

- SHAM-operated
  - Vehicle (saline solution)
  - Isoproterenol (0.015 mg.kg\(^{-1}\).day\(^{-1}\))

- Castrated
  - Vehicle (saline solution)
  - Isoproterenol (0.015 mg.kg\(^{-1}\).day\(^{-1}\))

Terminal procedures

- Echocardiography
- Isolated Perfused Heart
- Tissue collection
LV dimensions and systolic function

Greater LV end diastolic diameter with injection of ISO
Similar in SHAM-operated and in Castrated rats.

<table>
<thead>
<tr>
<th></th>
<th>Sham Operated</th>
<th>Castrated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline Vehicle</td>
<td>ISO n = 10</td>
</tr>
<tr>
<td>LV EDD (mm)</td>
<td>7.91 ± 0.24</td>
<td>*<em>9.05 ± 0.16</em></td>
</tr>
<tr>
<td>LV ESD (mm)</td>
<td>4.38 ± 0.24</td>
<td>5.34 ± 0.24*</td>
</tr>
<tr>
<td>LV FS_{end} (%)</td>
<td>44.90 ± 1.68</td>
<td>41.18 ± 1.86</td>
</tr>
<tr>
<td>LV FS_{mid} (%)</td>
<td>27.13 ± 1.03</td>
<td>26.85 ± 2.50</td>
</tr>
</tbody>
</table>

*P < 0.05 versus saline-treated groups.
Load-independent measures of cardiac dimensions

- Greater LV $V_0$ with injection of ISO
- Similar in SHAM-operated and Castrated rats.
No difference in LV function
Conclusions

In normotensive male SD rats,

Chronic low level of β-adrenergic receptor stimulation (for 3 months) produces LV chamber dilatation to an equal extent in male and female

The removal of male steroids by castration does not affect the β-adrenergic-induced cardiac dilatation.

(Estrogen-deficiency does not affect the β-adrenergic-induced cardiac dilatation.)
In SHR,

Left Ventricular Hypertrophy (LVH)

Left ventricle

Cardiac Dilatation

Lengthening
Cell death
Collagen disruption
Reactive fibrosis

Sympathetic Nervous System (SNS) activation

Gender

?
To determine whether female less susceptible to β-adrenergic-induced cardiac remodelling than male in the context of chronic hypertension.

(Michel et al., J. Cardiac Failure 2017)
Materials & Methods: Groups

Start of experiment $t=0$

Daily injection of Isoproterenol or vehicle

$t=+5$ months

4 groups (n=12/13):

- SHR males 9-month-old
  - Vehicle (saline solution)
  - Isoproterenol (0.04 mg.kg$^{-1}$.day$^{-1}$)

- SHR females 9-month-old
  - Vehicle
  - Isoproterenol (0.04 mg.kg$^{-1}$.day$^{-1}$)

Terminal procedures
- Echocardiography
- Isolated Perfused Heart
- Tissue collection
Characteristics of the rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male SHRs</th>
<th>Female SHRs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 12)</td>
<td>ISO (n = 13)</td>
</tr>
<tr>
<td>Body weight (BW; g)</td>
<td>378.3 ± 5.4†</td>
<td>382.9 ± 4.8†</td>
</tr>
<tr>
<td>Tibial length (TL; mm)</td>
<td>42.0 ± 0.5†</td>
<td>42.1 ± 0.6†</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.46 ± 0.03†</td>
<td>1.64 ± 0.04†</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>1.15 ± 0.02†</td>
<td>1.32 ± 0.04†</td>
</tr>
<tr>
<td>LV weight/BW × 100</td>
<td>0.31 ± 0.01†</td>
<td>0.35 ± 0.01†</td>
</tr>
<tr>
<td>LV weight/TL × 10</td>
<td>0.27 ± 0.005†</td>
<td>0.32 ± 0.01†</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>3.26 ± 0.18</td>
<td>4.32 ± 0.24*</td>
</tr>
<tr>
<td>ED PWT (mm)</td>
<td>2.31 ± 0.12</td>
<td>2.31 ± 0.12</td>
</tr>
<tr>
<td>ES PWT (mm)</td>
<td>3.08 ± 0.08†</td>
<td>2.93 ± 0.06†</td>
</tr>
<tr>
<td>FS_end (%)</td>
<td>50.5 ± 1.6</td>
<td>45.5 ± 2.1</td>
</tr>
<tr>
<td>FS_mid (%)</td>
<td>28.5 ± 1.1†</td>
<td>26.7 ± 1.1†</td>
</tr>
</tbody>
</table>

- Greater LV weight in male with injection of ISO compared to other groups
- Greater LV $V_0$ and LV EDD in male with injection of ISO compared to other groups
- Smaller RWT in male with injection of ISO compared to other groups
No major difference in function
Acute hemodynamic response to ISO

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male SHRs (n = 6)</th>
<th>Female SHRs (n = 6)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline FS_{end} (%)</td>
<td>45.0 ± 2.4</td>
<td>45.1 ± 2.7</td>
<td>.98</td>
</tr>
<tr>
<td>Baseline FS_{mid} (%)</td>
<td>21.1 ± 3.5</td>
<td>23.5 ± 1.0</td>
<td>.49</td>
</tr>
<tr>
<td>Baseline heart rate (beats/min)</td>
<td>219 ± 9</td>
<td>232 ± 2</td>
<td>.12</td>
</tr>
<tr>
<td>EC_{50} for FS_{end} (µg/kg)</td>
<td>7.7 ± 1.6</td>
<td>5.7 ± 1.9</td>
<td>.47</td>
</tr>
<tr>
<td>EC_{50} for FS_{mid} (µg/kg)</td>
<td>6.8 ± 2.2</td>
<td>3.7 ± 0.9</td>
<td>.19</td>
</tr>
<tr>
<td>EC_{50} for heart rate (µg/kg)</td>
<td>10.5 ± 2.7</td>
<td>9.2 ± 2.2</td>
<td>.72</td>
</tr>
<tr>
<td>E_{max} for FS_{end} (%)</td>
<td>78.7 ± 2.3</td>
<td>74.7 ± 2.1</td>
<td>.22</td>
</tr>
<tr>
<td>E_{max} for FS_{mid} (%)</td>
<td>39.2 ± 2.6</td>
<td>35.1 ± 0.9</td>
<td>.15</td>
</tr>
<tr>
<td>E_{max} for heart rate (beats/min)</td>
<td>414 ± 16</td>
<td>401 ± 8</td>
<td>.44</td>
</tr>
</tbody>
</table>

- No difference in acute hemodynamic response to ISO
Greater collagen area in male with injection of ISO compared to other groups
Conclusions

- Male SHR, but not female, develops β-adrenergic-induced cardiac dilatation

  Male more susceptible than female to adverse cardiac remodelling induced by β-adrenergic stimulation in the context of hypertension

- Development of reactive myocardial fibrosis in male, but not in female SHR
Sympathetic Nervous System (SNS) activation

LVH

Cardiac Dilatation

↑ +

Male gender
Absence of estrogen (protective effect of estrogen)
OR
Testosterone ?
Objective 4

To determine whether ovariectomy or castration affect susceptibility to β-adrenergic-induced cardiac remodelling in the context of chronic hypertension

(Magubane et al., J. Cardiovasc Pharmacol 2017)
Start of experiment
\(t=0\)  

Daily injection of Isoproterenol or vehicle

\(t=+5\) months

8 groups:

9-month-old SHR

SHAM-operated
Male
Or
Female

Vehicle (saline solution)

Isoproterenol (0.04 mg.kg\(^{-1}\).day\(^{-1}\))

Terminal procedures
Echocardiography
Isolated Perfused Heart
Tissue collection

Vehicle (saline solution)

Isoproterenol (0.04 mg.kg\(^{-1}\).day\(^{-1}\))

Gonadectomy
Male
Or
Female
- increased LVEDD with ISO but no change in LV $V_0$ and RWT
- Similar in sham-operated and ovariectomised rats
No difference in LV function
No difference in function
Deficiency in testicular hormones prevents β-adrenergic-induced adverse ventricular remodelling in male SHR.

However, a deficiency of ovarian hormones may not account for the lack of effect of chronic β-adrenergic stimulation on cardiac remodelling in female SHR.

- Testosterone may be responsible for the chronic β-adrenergic-induced LV dilatation and eccentric remodelling observed in male but not female SHR.
With normal blood pressure,

**Cardiac Dilatation**

**Sympathetic Nervous System (SNS) activation**
With hypertension,

LVH

Sympathetic Nervous System (SNS) activation

Male gender
Testosterone

Cardiac Dilatation
Acknowledgements

Colleagues:
Gavin Norton
Angela Woodiwiss
Mhlengi Magubane
Bryan Hodson
WITS Central Animal Service

Fundings:
NRF, FRC, CPGRU


Results

### Sham-operated SHR

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ISO</th>
<th>Ovariectomised SHR</th>
<th>Saline</th>
<th>ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>225.3 ± 3.9</td>
<td>226.3 ± 2.4</td>
<td>274.9 ± 3.8†</td>
<td>270.7 ± 3.3†</td>
<td></td>
</tr>
<tr>
<td>Tibial length (mm)</td>
<td>37.1 ± 0.4</td>
<td>36.9 ± 0.4</td>
<td>39.2 ± 0.5†</td>
<td>39.4 ± 0.7†</td>
<td></td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.14 ± 0.07</td>
<td>1.07 ± 0.03</td>
<td>1.17 ± 0.05†</td>
<td>1.31 ± 0.07†</td>
<td></td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>0.90 ± 0.02</td>
<td>0.86 ± 0.02</td>
<td>0.95 ± 0.05†</td>
<td>1.06 ± 0.05†</td>
<td></td>
</tr>
<tr>
<td>LV weight / BW x100</td>
<td>0.38 [0.36-0.40]</td>
<td>0.37 [0.35-0.40]</td>
<td>0.34 [0.30-0.38]</td>
<td>0.41 [0.37-0.42]</td>
<td></td>
</tr>
<tr>
<td>LV weight / TL x100</td>
<td>0.23 [0.22-0.25]</td>
<td>0.23 [0.22-0.25]</td>
<td>0.23 [0.22-0.25]</td>
<td>0.28 [0.24-0.30]</td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>148.8 ± 6.1</td>
<td>130.0 ± 5.3</td>
<td>133.1 ± 8.0</td>
<td>135.1 ± 10.6</td>
<td></td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>98.7 ± 4.0</td>
<td>85.7 ± 3.5</td>
<td>89.6 ± 6.6</td>
<td>90.5 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>197.8 ± 6.1</td>
<td>175.1 ± 6.5#</td>
<td>206.0 ± 7.1</td>
<td>166.8 ± 8.4#</td>
<td></td>
</tr>
<tr>
<td>LV ESD (mm)</td>
<td>2.96 ± 0.19</td>
<td>2.97 ± 0.13</td>
<td>3.09 ± 0.08</td>
<td>3.09 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>PWED (mm)</td>
<td>2.00 [1.93-2.30]</td>
<td>2.01 [1.90-2.15]</td>
<td>2.13 [1.87-2.25]</td>
<td>2.20 [1.90-2.30]</td>
<td></td>
</tr>
<tr>
<td>PWES (mm)</td>
<td>2.74 ± 0.04</td>
<td>2.73 ± 0.05</td>
<td>2.65 ± 0.06</td>
<td>2.66 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Fbpeak (%)</td>
<td>51.6 ± 2.2</td>
<td>53.3 ± 1.1</td>
<td>49.3 ± 1.0</td>
<td>53.4 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Fbend (%)</td>
<td>29.6 ± 1.5</td>
<td>31.5 ± 1.1#</td>
<td>30.0 ± 0.7</td>
<td>34.1 ± 1.8#</td>
<td></td>
</tr>
</tbody>
</table>

### Castrated SHR

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>374.9 ± 4.9†</td>
<td>384.9 ± 6.0†</td>
</tr>
<tr>
<td>Tibial length (mm)</td>
<td>42.1 ± 0.5</td>
<td>41.9 ± 0.5</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.43 ± 0.03</td>
<td>1.65 ± 0.07#</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>1.15 ± 0.02</td>
<td>1.32 ± 0.06#</td>
</tr>
<tr>
<td>LV weight / BW x100</td>
<td>0.30 [0.29-0.32]</td>
<td>0.34 [0.30-0.38]#</td>
</tr>
<tr>
<td>LV weight / TL x100</td>
<td>0.27 [0.26-0.29]</td>
<td>0.32 [0.29-0.33]#</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>141.0 ± 9.1</td>
<td>138.5 ± 7.3</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>98.7 ± 4.8</td>
<td>92.9 ± 4.8</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>208.1 ± 6.1</td>
<td>186.8 ± 7.5#</td>
</tr>
<tr>
<td>LV ESD (mm)</td>
<td>3.29 ± 0.16</td>
<td>4.49 ± 0.17#</td>
</tr>
<tr>
<td>PWED (mm)</td>
<td>2.14 [2.02-2.37]</td>
<td>2.14 [1.95-2.22]</td>
</tr>
<tr>
<td>PWES (mm)</td>
<td>2.96 ± 0.09</td>
<td>2.92 ± 0.06</td>
</tr>
<tr>
<td>Fbpeak (%)</td>
<td>50.9 ± 1.4</td>
<td>45.5 ± 1.4</td>
</tr>
<tr>
<td>Fbend (%)</td>
<td>30.3 ± 1.0</td>
<td>27.5 ± 1.0#</td>
</tr>
</tbody>
</table>

➢ No difference in BP in male and female gonadectomised or not with or without Iso
Chronic Hypertension

High prevalence of HT in black South Africans

LV Hypertrophy 2 to 3 fold more common in black population than in white population (Drazner et al. 2005)
Alteration in ventricular performance:

– Deranged systolic and diastolic function at rest

– Decreased contractile reserve on stress

– Persistent and progressive increased in end systolic wall stress
Effect of gender

- More severe phenotype of LVH, dilated cardiomyopathy or HF in male
- Earlier HF or more severe LVH (Du XJ Cardiovasc Res 2004)
- Gao In B2 overexpressed mice

- Toxic model doxorubicin
- Toxic model ISO very high dose
- Volume overload
- Pressure overload
Effect of gender

- Gao In B2 overexpressed mice
- Toxic model doxorubicin
- Toxic model ISO very high dose
- Volume overload
- Pressure overload