Female rats are less prone to clinical heart failure than male rats in a juvenile rat model of right ventricular pressure load

Bossers, Guido P. L.; Hagdorn, Quint A. J.; Koop, Anne-Marie C.; van der Feen, Diederik E.; Bartelds, B.; van Leusden, Tom; De Boer, Rudolf A.; Sillje, Herman H. W.; Berger, Rolf M. F.

Published in: AMERICAN JOURNAL OF PHYSIOLOGY-HEART AND CIRCULATORY PHYSIOLOGY

DOI: 10.1152/ajpheart.00071.2022

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version Publisher's PDF, also known as Version of record

Publication date: 2022

Link to publication in University of Groningen/UMCG research database


Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Sex is increasingly emerging as determinant of right ventricular (RV) adaptation to abnormal loading conditions. It is unknown, however, whether sex-related differences already occur in childhood. Therefore, this study aimed to assess sex differences in a juvenile model of early RV pressure load by pulmonary artery banding (PAB) during transition from pre to postpuberty. Rat pups (*n* = 57, 3 wk old, 30–45 g) were subjected to PAB or sham surgery. Animals were euthanized either before or after puberty (4 and 8 wk postsurgery, respectively). Male PAB rats demonstrated failure to thrive already after 4 wk, whereas females did not. After 8 wk, female PAB rats showed less clinical symptoms of RV failure than male PAB rats. RV pressure-volume analysis demonstrated increased end-systolic elastance after 4 wk in females only, and a trend toward preserved end-diastolic elastance in female PAB rats compared with males (*P* = 0.055). Histology showed significantly less RV myocardial fibrosis in female compared with male PAB rats 8 wk after surgery. Myosin heavy chain 7-to-6 ratio switch and calcineurin signaling were less pronounced in female PAB rats compared with males. In this juvenile rat model of RV pressure load, female rats appeared to be less prone to clinical heart failure compared with males. This was driven by increased RV contractility before puberty, and better preservation of diastolic function with less RV myocardial fibrosis after puberty. These findings show that RV adaptation to increased loading differs between sexes already before the introduction of pubertal hormones.

In this study, we describe sex differences in our unique weanling rat model of increased RV pressure load by pulmonary artery banding. We are the first to assess temporal sex-related differences in RV adaptation during pubertal development. Female rats show superior RV function and less diastolic dysfunction and fibrosis compared with male rats. These differences are already present before puberty, indicating that the differences in RV adaptation are not only determined by sex hormones.

## INTRODUCTION

Right ventricular (RV) dysfunction is an important determinant of outcome in various cardiovascular diseases, such as congenital heart disease (CHD), pulmonary arterial hypertension (PAH), and heart failure with preserved ejection fraction (HFpEF) (1, 2). Incidence of RV failure becomes higher with increasing age, but in patient that develop heart failure before reaching adulthood, morbidity, and mortality is high (3). In the past decade, increasing evidence has emerged indicating that in patients with these conditions substantial differences between sexes exist with regard to disease severity, clinical course of the disease, and mortality (4, 5). The prevalence of CHD, in general, is higher in females, whereas the prevalence of severe CHD, as well as mortality, is higher in males (2, 6). This also applies to patients with PAH, as well as in patients with HFpEF: female patients are more likely to develop these conditions, whereas men show worse RV function and clinical outcomes (5, 7–9). These observations raise the question of which factors underlie these observed sex differences. Data from large studies in cardiovascular disease-free subjects have demonstrated higher levels of estradiol and estradiol metabolism to be associated with better RV function, suggesting a mechanistic role for sex hormones (10, 11). Although an

---

*G. P. L. Bossers and Q. A. J. Hagdorn contributed equally to this work.*

Correspondence: G. P. L. Bossers (g.p.l.bossers@umcg.nl).

Submitted 9 February 2022 / Revised 22 March 2022 / Accepted 22 March 2022
important role of sex hormones has been consistently supported by clinical and experimental studies, further clinical studies to dissect the effects of sex chromosomes from sex hormones are scarce, but the suggested role of sex hormones is consistently supported by experimental studies (12, 13). Various protective properties of estrogen have been demonstrated in both general cardiac dysfunction and specifically pressure-loaded RV dysfunction (14–19). In contrast, male sex hormones are associated with worse RV function in healthy humans, and experimental RV pressure load (10, 20).

Altogether, it seems that sex substantially affects RV adaptation to abnormal ventricular loading conditions. It has not been yet elucidated whether these sex differences are solely driven by sex hormones or whether genetic constitution also plays a role. Therefore, it is important to know whether sex differences also occur in childhood before sex hormones become active. Unfortunately, data on sex-specific patterns of RV adaptation in pediatric, prepubertal heart diseases are scarce. Both clinical and experimental studies have all been conducted in adult humans, or animals. Therefore, the present study aimed to assess sex-related differences in RV adaptation during transition from pre to postpuberty in a rat pup model of early RV pressure load by pulmonary artery banding (PAB).

**MATERIALS AND METHODS**

Animal care and experiments were conducted according to the Dutch Animal Experimental Act and conform to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and the Declaration of Helsinki. The Animal Experiments Committee of the University of Groningen, in The Netherlands approved the experimental protocol (Permit No. AVDI05002015IS134).

**Animal Model**

To induce RV pressure overload in rat pups, we adapted the previously described procedure for PAB used in our laboratory (21). Wistar rat pups (Charles River, France) were as soon as possible after weaning (3rd to 4th wk of life, 30–40 g) randomized into two groups: sham (male \(n = 13\)/female \(n = 10\)) or pulmonary artery banding (PAB, male \(n = 22\)/female \(n = 14\)). PAB was applied under general anesthesia with ventilation (isoflurane/oxygen mixture, 5% induction; 2 to 3% maintenance) and warmed at 37°C. RV pressure load was defined as pressure gradient over the pulmonary artery (PA), measured by maximal flow velocity in the PA. Two female PAB rats showed a PAB gradient <20 mmHg, which was regarded as a nonsignificant pressure load, and these were excluded due to failure of adequate PAB surgical procedure, leaving 11 female PAB rats. Tricuspid annular plane systolic excursion (TAPSE) was measured, representing longitudinal systolic RV function. LV cardiac output (CO) was calculated using aortic flow and diameter as follows: (aortic diameter)\(^2\) \times 3.14 \times velocity time integral \times heart rate. Beat-to-beat variation was accounted for by averaging measurement from 6 to 12 consecutive beats. TAPSE was indexed by dividing by tibia length (TL), and LV CO was indexed by dividing by TL to the power of three (TL\(^3\)), according to the previously published methods for indexing (24).

**Invasive RV Pressure-Volume Analyses**

Before termination (either at 4 wk after PAB or at 8 wk after PAB), open-chest pressure-volume measurements were performed in anesthetized and intubated rats using a pressure-admittance catheter (1.9-Fr, 6-mm spacing, Transonic, Ithaca, NY). After thoracotomy and pericardiotomy, the catheter was introduced in the apex of the RV toward the outflow tract. The pressure signal was recorded in Chartlab 5 with an ADVantage PV system (ADV500) processor (Transonic, Ithaca, NY). Analyses of the obtained signal were performed with Circlab 2015 (developed by P. Steendijk, Leiden University Medical Center, Leiden, The Netherlands). LV stroke volume as measured by echocardiography (in mL) was used to calibrate the RV conductance-derived stroke volume. Steady-state pressure-volume loops were used for calculation of all parameters. End-systolic and end-diastolic elastance (\(E_{es}\) and \(E_{ed}\), respectively) were determined using the single-beat method (25).

**Organ Weights and Histological Analyses**

After catheterization, the rats were euthanized by blood withdrawal. After flushing the heart with NaCl 0.9%, the heart and organs were removed. Tibia’s were collected to measure TL. RV, ventricular septum, left ventricle, and both atria were dissected and weighed. Cardiac weights were indexed according to previously published formulas (24). After fixation of the RV (formalin), cardiomyocyte cross-sectional area (CCSA, on wheat germ agglutinin staining), fibrosis (Masson Trichrome) were measured. Capillary density (on Lectin staining) was assessed in the RV-free wall by counting the number of capillaries on five views of \(\times 100, 100 \mu m\), and \(\times 20\) magnification. Capillary-to-myocyte ratio was measured by dividing the...
capillary density by the mean number of cardiomyocytes (calculated as area of analysis divided by mean CCSA).

**Gene Expression in RV Tissue**

RT-qPCR of specific genes (26–29) representing cardiomyocyte stress [natriuretic peptide B (NPPB)], fetal gene switch [myosin heavy chain 7 (MYH7) to myosin heavy chain 6 (MYH6) ratio], profibrotic signaling [TIMP metalloproteinase inhibitor 1 (TIMP1)] and pathological hypertrophy [regulator of calcineurin (RCAN1)] were measured as described previously (30). Expression data were corrected for the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by means of the ∆ΔCT method. PAB groups were compared with its respective sex and time point control group.

**Statistical Analyses**

Quantitative data are expressed as means ± SE. Only for body weight SD was used to allow assessment of the 95% confidence interval (1.96 SD). ANOVA with Holm–Sidak correction for multiple tests was performed to test differences between sham and PAB animals at the different time points for continuous variables (GraphPad Prism 5.04 for Windows), χ² test for categorical variables (SPSS, v. 23, 2015). Kaplan–Meier survival curves were plotted, and log-rank test was performed to compare survival between sexes. Rats were censored when planned euthanasia took place, mortality was defined as death or unplanned euthanasia when a humane endpoint was reached. α < 0.05 was considered significant.

**RESULTS**

### Clinical Status

At the 4-wk time point, three out of 21 male PAB rats had died, whereas there was no mortality among female rats and none of the remaining PAB rats showed clinical signs of RV failure. Eight weeks after PAB surgery, one additional male PAB rat had died, as well as one female PAB rat. Furthermore, 70% of the remaining male PAB rats had developed at least one clinical sign of RV failure (5/10 ascites, 7/10 tachypnea, 7/10 pleural effusion, Fig. 1A), whereas 40% of the remaining female PAB rats had developed at least one clinical sign of RV failure (2/5 ascites, 1/5 tachypnea, 0/5 pleural effusion). The occurrence of pleural effusion was significantly lower in female PAB rats compared with male PAB rats (P = 0.003). The occurrence of ascites and tachypnea, as well as the occurrence of at least one clinical sign and the total number of clinical signs did not differ significantly between sexes. Also, the survival difference between

---

**Figure 1.** Symptoms, survival, and growth. **A:** graphical representation of the proportion of rats that have one of the following symptoms: ascites, tachypnea, or pleural effusion. Black represents the proportion with the symptom present, gray represents the proportion without the symptom present. **B:** Kaplan–Meier survival curve of PAB rats. Red line represents females, blue line represents males. Rats are censored when planned euthanasia at 4 wk took place. **C:** graph representing body weight at various time points of male PAB rats in blue and female PAB rats in red, displayed as means and standard deviation (SD), superimposed on 95% confidence interval (1.96 SD) of control rats. **D:** graph representing tibia length at various time points of male PAB rats in blue and female PAB rats in red, displayed as means and SD, superimposed on 95% confidence interval (1.96 SD) of control rats. *P < 0.05 compared with control (graph A, χ² test, graph C and D ANOVA). PAB: pulmonary artery banding.
sexes did not reach statistical significance \( (P = 0.33, \text{Fig. 1B}) \). From the 4-wk time point, male PAB rats showed failure to thrive, defined as decreased body weight, when compared with control male rats \( (P < 0.001 \text{ for both 4 and 8 wk}) \). Also, tibia length, a measure of body size independent of body composition, was significantly lower in male PAB rats compared with male controls at the 8-wk time point \( (P < 0.001, \text{Fig. 1, C and D}) \). Female PAB rats did not show decreased body weight nor tibia length compared with female control rats at any time point.

Right Ventricular Function

Echocardiography showed that the pressure load induced by PAB was equal between groups \( (\text{Fig. 2A}) \). Cardiac index appeared to be lower in PAB rats compared with controls, similarly in both male and female rats, although this decrease did not reach statistical significance \( (P = 0.459; \text{Fig. 2C}) \). TAPSE, a measure of longitudinal RV function, decreased over time in PAB rats compared with control groups \( (4 \text{ wk: females } P = 0.003, \text{ males } P = 0.186, 8 \text{ wk: females } P = 0.007, \text{ males } P = 0.018) \), without significant differences between males and females \( (\text{Fig. 2B}) \).

PV-loop analysis showed an increase in RV \( E_{es} \), a load-independent measure of RV contractility, in female PAB rats compared with female controls at both 4 and 8 wk \( (P < 0.001 \text{ and } P = 0.011, \text{ Fig. 2D}) \), whereas in male PAB rats, RV \( E_{es} \) did not increase compared with male controls. RV \( E_{es} \)-to-\( E_{s} \) ratio did not significantly differ between PAB rats and controls in either sex. RV \( E_{ed} \), a load-independent measure of RV stiffness, increased over time in both male and female PAB rats, when compared with control groups, reaching statistical significance at 8 wk \( (\text{males } P < 0.001 \text{ and females } P = 0.010, \text{ Fig. 2E}) \). RV \( E_{ed} \) increased more pronouncedly in male PAB rats compared with female PAB rats \( (P = 0.055) \).

Right Ventricular Remodeling

Both male and female PAB rats demonstrated a marked increase in RV and RA weight, both at the 4- and 8-wk time point compared with control groups. In addition, immunohistochemistry demonstrated increased CCSA also already at both time points compared with control animals \( (\text{Fig. 3, A–D}) \). No differences in RV hypertrophy, defined by these variables, could be demonstrated between sexes. At 8 wk after PAB, capillary/myocyte ratio was increased similarly in both sexes \( (\text{male } P = 0.002 \text{ and female } P = 0.002, \text{ Fig. 3F}) \). Already at 4 wk after PAB, increased RV fibrosis was present in both male and female rats when compared with control animals \( (P = 0.002 \text{ and } P = 0.048, \text{ respectively}) \). However, at 8 wk after PAB, the extent of RV fibrosis was significantly higher in male PAB rats compared with female PAB rats \( (P = 0.004, \text{ Fig. 3E}) \).

Increased gene expression in the RV of stress marker NPPB was demonstrated by RT-qPCR at both 4 and 8 wk after PAB, similarly in male and female PAB rats compared with respective controls \( (\text{Fig. 4A}) \). Profibrotic signaling marker TIMP1 showed a similar gene expression pattern. Gene expression of RCAN1, activator of pathological hypertrophy, increased similarly in the RV of both male and female PAB rats after 4 wk compared with control \( (P = 0.005 \text{ and } P = 0.044, \text{ respectively, Fig. 4C}) \), whereas after 8 wk, this

Figure 2. Echocardiography and pressure-volume analyses. A–C: echocardiographic measures: pulmonary artery (PA) gradient, tricuspid annular plane systolic excursion (TAPSE), and cardiac index, respectively. D and E: pressure-volume derived measures: RV end-systolic elastance \( (E_{es}) \) and RV end-diastolic elastance \( (E_{ed}) \), respectively. Data are presented as means ± SE. * \( P < 0.05 \) compared with control, # \( P < 0.05 \) between sexes (ANOVA). PAB, pulmonary artery banding; RV, right ventricular.
increased RCAN1 expression persisted only in male PAB rats, which differed significantly from the expression in female PAB rats ($P < 0.001$). Finally, different from female PAB rats, male PAB rats showed a marked increase of MYH7-to-MYH6 ratio, marker of a switch to the fetal gene program due to cardiac injury compared with controls already at 4 wk after PAB ($P = 0.016$). At 8 wk after PAB, this increase was even more pronounced, as was the difference with female PAB rats (Fig. 4, D–F). The differences in MYH7/6 ratio between male and female PAB rats were mainly driven by a strong increase in MYH7 in male PAB rats, whereas no such increase was observed in female PAB rats. The key findings and differences of the present study are displayed in Table 1.

**DISCUSSION**

The present study in a rat pup model of increased RV pressure load is the first to describe temporal sex-related differences in RV adaptation during pubertal development. In this model, female sex appeared to be protective for clinical signs of heart failure, including failure to thrive, and was associated with increased adaptive systolic RV function and less diastolic RV dysfunction compared with male sex. These sex-related differences in functional adaptation of the RV to increased pressure load were accompanied by differences in structural, molecular RV adaptation. Early pressure load of the RV induced less RV fibrosis in female rats, less pronounced fetal gene switch, and RCAN1 signaling compared
with male rats. The first signs indicating differences in RV adaptation between male and female rats started already at prepubertal age, although differences between sexes became more predominant after the onset of puberty and longer duration of the pressure overload.

We chose to adhere to previous studies demonstrating that puberty in rats starts at around 7.5 wk of age (22, 23). In the current study design, this means that puberty starts predominantly in the 5th wk after PAB. Therefore, 4-wk PAB groups were considered prepubertal and the 8-wk PAB groups were considered postpubertal.

Male PAB rats demonstrated reduced body growth compared with male control rats, whereas body growth was not affected in female PAB rats. In male PAB rats, not only body weight, but also tibia length was reduced, indicating that the reduced body weight is not solely caused by altered body composition, but reflects failure to maintain growth: failure to thrive. This, together with the more frequent occurrence of clinical symptoms of RV failure in male PAB rats, demonstrates that male rats compared with female rats, were more prone to develop clinical heart failure at an equal degree of RV pressure overload.

**Figure 4.** Gene expression levels of NPPB (A), TIMP1 (B), RCAN1 (C), MYH7-to-MYH6 ratio (D), MYH7 (E), and MYH6 (F). Data are presented as means ± SE. *P < 0.05 compared with control, #P < 0.05 between sexes (ANOVA). PAB, pulmonary artery banding.
The more favorable clinical profile of female PAB rats was associated with increased RV contractility and less diastolic impairment when compared with male PAB rats. Cardiac index, however, was equally reduced in males and females. The assessment of RV function revealed seemingly contradictory results between echocardiographic measurement of longitudinal systolic function (TAPSE) or invasive pressure-volume analysis. Where TAPSE decreased after PAB, most prominent in female rats, RV contractility reflected by $E_{ed}$ appeared to be increased exclusively in female PAB rats. We propose that female PAB rats were, better than male PAB rats, able to adapt their RV contraction pattern more efficiently from longitudinal toward more circumferential contraction, a known and effective RV compensatory mechanism to increased afterload (31). This apparent beneficial systolic adaptation in female rats occurred already at prepubertal age. Diastolic dysfunction has been previously shown to be closely related to clinical RV failure, both in patients and animal studies,(25, 32) which is in line with the current observation that preservation of diastolic function in female PAB rats was accompanied by less clinical RV failure. In contrast to the difference in systolic RV function, this better preservation of RV diastolic function occurred not before 8 wk after PAB, so at postpubertal age of the rats, and coincides with a lower degree of RV fibrosis in females compared with males, a difference that also became apparent not before postpubertal age. This difference between sexes in postpubertal structural adaptation was further associated with a more pronounced signal of pathological hypertrophy (RCAN1) at 8 wk in male compared with female rats. Altogether, juvenile, female PAB rats demonstrate a favorable RV adaptation pattern, characterized by maintained growth and better systolic adaptation before the onset of puberty, and less fibrosis, less diastolic dysfunction, and less signs of clinical heart failure after puberty compared with male PAB rats under equal hemodynamic loading conditions.

The unfavorable profile of RV function in male PAB rats may have originated from a more pronounced maladaptive response to stress, in terms of calcineurin signaling (RCAN1) and myosin heavy chain switch, in comparison to female PAB rats. A switch in myosin heavy chains is regarded as a stress-related marker of a switch to the fetal gene program in response to cardiac injury,(27) and the calcineurin pathway is described as an activator of pathological hypertrophy, associated with impaired cardiac function (29). In the LV, pathological hypertrophy negatively influences contractility but does not induce fibrosis (28). Therefore, the sex differences in levels of fibrosis were presumably not induced by alternate hypertrophic responses. RV fibrosis, however, is a direct target of estrogens, both in vivo and in vitro (12, 16). This, combined with the fact that sex differences in fibrosis arose not before puberty, suggests that the lower degree of fibrosis in female PAB rats compared with male PAB rats, results from hormonal influences rather than from myocardial stress. However, the present study also provides evidence that RV adaptation to increased pressure load differed between sexes already in juvenile, prepubertal rats. These phenomena have not been shown nor investigated previously, neither in animal studies, nor in human studies. It is important to keep in mind that in the current study, sex differences that only became apparent after puberty may be attributed to the effect of sex hormones but could also result from the longer duration of pressure load. Nevertheless, the results of the current study suggest that sex-related differences in RV function and adaptation to increased pressure load are not only due to sex hormones, but may also be affected by differences in genetic constitution between sexes. These insights are relevant for a variety of heart diseases, since understanding the differences in RV adaptation to pressure overload between males and females may lead to the identification of new treatment targets for patients with premature RV failure due to abnormal loading conditions, as is the case in CHD, PAH, or HFpEF.

### LIMITATIONS

In the current study design, RV adaptation to pressure load at prepubertal and postpubertal stages is compared. Pubertal stage was defined by age of the rats, based on previous studies. However, sexual maturation of the individual rats was not assessed by serum levels of sex hormones in female rats or testicular studies in male rats. The latter would have allowed for more confident classification in pre or postpubertal groups. Nevertheless, the picture provided by the current study is clear: clinically relevant sex differences occur already in prepuberty in juvenile RV pressure load. Furthermore, the duration of pressure loading and the growth of the rats, potentially leading to progressive pulmonary artery constriction due to the fixed banding could have played a role as confounding factors. However, the increase of right ventricular afterload, measured by peak-systolic pressure gradient over the banding, was similar in male and female rats. The current study did not
intervene in hormonal pathways, prohibiting to demonstrate causal effects of sex hormones or to clarify the underlying mechanisms of the observations. Further mechanistic exploration of sex differences in juvenile RV dysfunction will require early interventions in hormonal pathways, including ovarioectomy, castration or hormonal replacement, or inhibition therapies but should also go beyond such hormonal effects.

Conclusions
The present study in a pup rat model of RV pressure load showed that female rats were less prone to clinical heart failure compared with male rats. This was driven by sex-related differences in RV adaptation, expressed by increased RV contractility in prepuberty and followed by improved preservation of diastolic function with less RV myocardial fibrosis in postpuberty in female PAB rats compared with males. These findings indicate that sex differences in RV adaptation are not solely determined by sex hormones but occur already in prepuberty and may thus be related also to sex chromosomes.

GRANTS
This study was funded by the Sebald fund, Stichting Hartekind, and The Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centres, The Netherlands Organization for Health Research and Development, and Royal Netherlands Academy of Sciences (CVON-Phaedra) Grant 2012-08 and Dutch Heart Foundation Grant NKS2013-T091, Cobra3.

DISCLOSURES
R. A. de Boer declares speaker fees from Abbott, AstraZeneca, Novartis, and Roche. The University Medical Center Groningen (UMCG), which employs R. A. de Boer, has received research grants and/or fees from AstraZeneca, Abbott, Bristol-Myers Squibb, Novartis, Novo Nordisk, and Roche. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

REFERENCES
SEX DIFFERENCES IN RIGHT VENTRICULAR PRESSURE LOAD


