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The effects of prenatal androgen exposure on cardiac function and tolerance to ischemia/reperfusion injury in male and female rats during adulthood

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ABSTRACT

Aims: Cardiovascular diseases may originate from suboptimal intrauterine environments. We aimed to examine the effects of prenatal androgen exposure (PAE) on heart basal hemodynamic parameters and tolerance to ischemia/reperfusion (I/R) injury, in PAE adult females and males.

Main methods: Pregnant Wistar rats in the experimental group ($n = 8$) received 5 mg of testosterone (s.c. injection) on the 20th day of pregnancy, while controls received solvent. The hearts of adult female and male offspring were isolated and perfused in a Langendorff apparatus, values of left ventricular systolic pressure (LVSP), left ventricular developed pressure(LVDP), rate pressure product(RPP) and peak rates of positive and negative changes in left ventricular pressure(± dp/dt) were recorded using a power lab system.

Key findings: At baseline, PAE adult males demonstrated significant higher values of LVSP, LVDP, RPP and ± dp/dt, compared to controls and PAE adult females ($p < 0.05$), while PAE adult females showed no significant differences compared to controls.

In PAE adult males, LVSP, LVDP, RPP and ± dp/dt had significant decreasing trends per phases after I/R, compared to their controls and PAE females, while these decreasing trends were not statistically significant in PAE adult female rats *vs.* their controls.

Significance: The impact of prenatal androgen exposure on adulthood cardiac function and tolerance to I/R is gender dependent, which may be partly explained by different cardiac effects of hyperandrogenism in males *versus* females. After prenatal androgen exposure, the baseline hemodynamic parameters of the hearts of adult males are increased; although they had less tolerance to I/R, findings however not observed in females.

1. Introduction

Developmental programming is a complex process whereby the hormonal, nutritional, and metabolic disturbances occurring during the critical period of intrauterine development lead to alterations in the developmental trajectory of the growing fetus [1]. These changes can affect molecular and gene expression levels, structure and function of the developing organs, eventually leading to both adaptive and beneficial or to maladaptive consequences later in life.

It has been reported that the origin of cardiovascular diseases may be a result of suboptimal intrauterine environments such as disturbed hormonal milieu [2]. Previous studies report some adverse consequences of prenatal testosterone exposure in occurrence of cardiovascular diseases (CVDs) such as hypertension, cardiac hypertrophy, altered cardiac function, focal myocardial disarray in left ventricle, and adverse left ventricular remodeling [3–5]; however data documented remains unclarified and further studies are needed.

Recently, attention has focused on maternal androgen exposure because the number of pregnant women with elevated circulating testosterone levels and followed by the adverse consequences in their offspring during adult life is rapidly increasing; elevated androgens levels (hyperandrogenism) are reported in some obstetric pathological conditions such as preeclampsia, polycystic ovary syndrome (PCOS), obesity, stress and smoking [6–11]; it has been proposed that in

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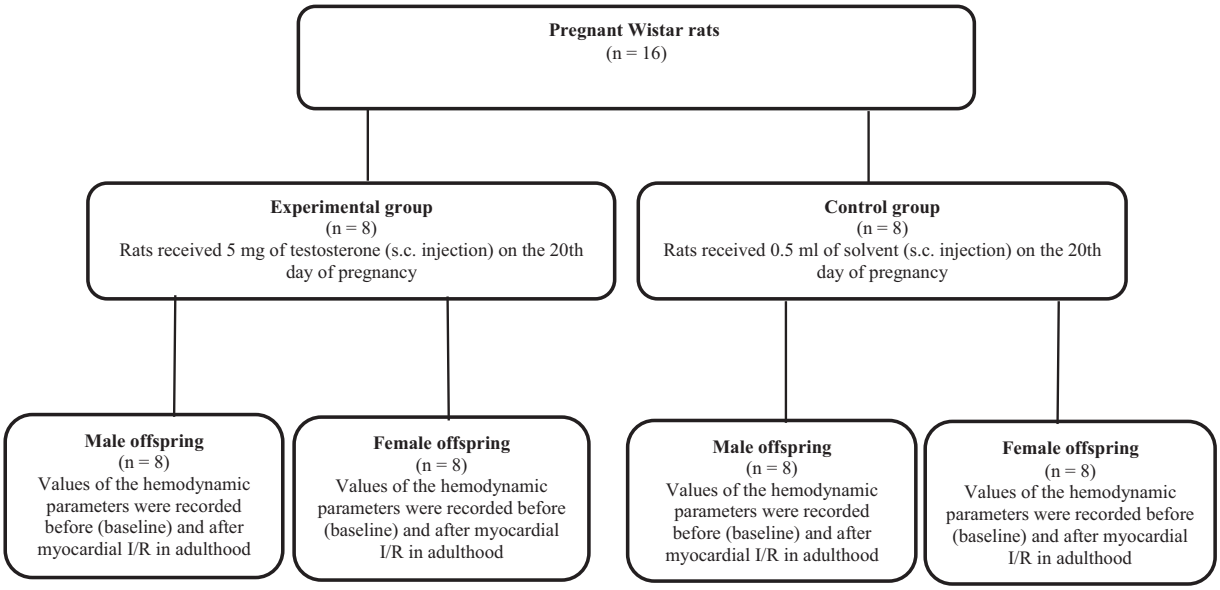

Fig. 1. Study Flowchart. s.c.: Subcutaneous. Hemodynamic parameters: LVEDP, LVSP, LVDP, ± dp/dt, HR and RPP. I/R: Ischemia/reperfusion.

pregnant women with PCOS the fetus is exposed to androgens during prenatal life [7,12,13], causing reproductive and endocrine disturbances *e.g.*, hyperandrogenism, in both male and female offspring later in life. Filippou and Homburg reported that a hyperandrogenic intrauterine environment programs the genes related to ovarian steroidogenesis and gonadotropin secretion, resulting in higher risk of the occurrence of diseases, accompanied with hormonal disorders *e.g.*, PCOS in adult life [14].

It is well known that numerous health problems are affected by gender. Based on basic and clinical research, gender-related differences in cardiac function, myocardial mechanical properties, cardioprotection, structural and functional adaptation to differential stresses (exercise-induced cardiac hypertrophy, non-ischemic myocardial remodeling, cardiac ischemia/reperfusion (I/R) injury) and risk of CVD have been reported [15–18]; these sex discrepancies are believed to be due to the actions of sex steroids, especially estrogens and androgens [15]. It is well documented that under both physiological and pathological conditions, the male and female heart differs significantly in many parameters. In addition, gender differences in the myocardial ischemic tolerance in adulthood can be affected by interventions (*e.g.* hypoxia) imposed during the early phases of ontogenetic development [15].

Most studies have focused on the effects of estrogens on heart while limited attention has been paid to the effects of testosterone on cardiovascular disease, myocardial injury or infarction and in the I/R heart (cardiac I/R injury, induced by the occlusion of coronary arteries followed by reperfusion). One of the target organs for androgens is the heart, so they may play an important role in cardiac injury [19].

Based on our knowledge, there is limited data available on cardiac function and tolerance to I/R injury, in adults (*e.g.*, adult rats) exposed to androgens during the critical period of their fetal life.

Accordingly, in the present study we aimed to examine and compare the effects of prenatal androgen exposure (PAE) on heart basal hemodynamic parameters and tolerance to I/R injury in male and female rats during adulthood.

2. Materials and methods

2.1. Ethics approval

In the present study, animal care and handling were in accordance with the principles of laboratory animal care. Local approval granted by the institutional (Research Institute for Endocrine Sciences) ethics review board and include the approval reference number (*RIES; 10ECRIES93/10/23*).

2.2. Animals and care

Female Wistar rats (n = 16, age 75–95 days, weight 170–190 g) were obtained from animal facility of the Research Institute for Endocrine Sciences (RIES), Shahid Beheshti University of Medical Sciences (SBMU) (Tehran, Iran).

One pair of male and female rats were kept separately in a polypropylene cage overnight in standard animal housing conditions (12 h/12 h light/dark cycles and controlled temperature of 22 ± 3 °C, relative humidity 45–55%). Observation of the vaginal plug after mating was considered as the first day of pregnancy. Pregnant rats were randomly divided into two groups, the experimental and vehicle (controls) groups (n = 8 in each group).

2.3. Hormonal treatment

For hormonal treatment, we used the same method for inducing a rat model of PCOS, details published previously [20]; in brief, in the experimental group, pregnant rats on the 20th day of pregnancy received a subcutaneous (s.c.) injection of 5 mg of free testosterone (T1500; Sigma, Germany) dissolved in a 500 μl cocktail containing sesame oil (S3547; Sigma, Steinheim, Germany) and benzyl benzoate (B6630; Sigma, Germany) at a ratio of 4:1; pregnant rats in the vehicle group received only 500 μl of solvent. After weaning, both male and female offspring were separately housed in groups of four per cage with free access to food and water. Both PAE male and female rats (n = 16, 8 in each group) and their controls (n = 16, 8 in each group) between 110 and 120 days of age (adulthood) were assessed for measurement of heart hemodynamic parameters and I/R experiments (Fig. 1).

2.4. Blood collection and measurement of testosterone levels

Body weights (BWs) of PAE adult male and female rats and their controls were measured (weight 180–200 g for females and 290–310 g for males). Rats were anaesthetized by intraperitoneal (i.p.) injection of pentobarbital sodium (P3761; 5 g; Sigma, St Louis, MO, USA) dissolved in normal saline 0.9% [60 mg (kg BW) − 1], heparin sodium (500 IU/kg) was i.p. injected 15 min before anaesthesia. Blood samples for

testosterone (T) measurement were collected from the abdominal aorta. Blood samples were centrifuged at 6000*g* for 5 min, at 4 °C and sera were immediately stored at −80 °C till the time of assay.

Serum total testosterone levels were measured using the enzyme-linked immunosorbent assay (ELISA) kit (Testosterone, ELISA, Diagnostics Biochem Canada Inc., Ontario, Canada, Sensitivity: 0.022 ng/ml). Intra-assay coefficient of variation for T was < 10%.

2.5. Isolated heart preparation and measurement of hemodynamic parameters

Following blood collection, the hearts were rapidly removed and placed in an ice-cold Krebs–Henseleit (KH) buffer, as quickly as possible to avoid any damage; each heart was then suspended from the Langendorff apparatus (ADInstruments, Australia) and perfused in retrograde *via* the aorta at a constant-pressure mode (75 mmHg) with KH buffer. Perfusion solution (KH) was gassed with a mixture of 95% O2 and 5% CO2 with a pH level of 7.4 at 37 °C. A fluid-filled latex balloon connected to a pressure transducer was inserted into the left ventricle through the left atria *via* a small incision in the left atrium to give a preload of 5–10 mmHg to measure pressure in the left ventricle (LVP), left ventricular systolic pressure (LVSP), heart rate (HR), the maximal rate of rise in the left ventricular pressure during systole (+dp/dt), the maximum rate of reduction in pressure during diastole (−dp/dt) and to calculate development of pressure in the left ventricular (LVDP) (difference between left ventricular peak systolic pressure and left ventricular end diastolic pressure) and rate pressure product (RPP) (HR × LVDP) [21].

After stabilization, baseline levels of these hemodynamic parameters (LVEDP, LVSP, LVDP, ± dp/dt, HR and RPP) were recorded, and the perfusion solution was cut off for 30 min in order to induce global ischemia, followed by reperfusion started and continued for 60 min; during reperfusion all of the hemodynamic parameters were again recorded every 15 min by a data acquisition system (Power Lab, AD instrument, Australia).

- Temperature (37 ± 0.5 °C) and humidity of the environment surrounding the heart were kept constant by a glass chamber.
- (KHS in mM contained: 115 NaCl; 4.6 KCl; 2.5 CaCl2; 1.6 MgSO4; 1.2 KH2PO4; 25 NaHCO3; 11.1 glucose (all from Merck, Darmstadt, Germany), equilibrated with 95% O2: 5% CO2 (pH = 7.4)) [21].
- At the time of surgery (blood collection and isolation of the heart), all female rats were in estrus phase, determined by vaginal smear. Collection of vaginal samples was done by inserting a cotton-tipped sterile swab into the vagina; the swab was rotated two or three times against the vaginal wall and then withdrawn and rolled on a clean glass slide and vaginal smears were fixed at the air, then stained with Giemsa and examined by light microscopy (×100 magnification). Estrous cycle phases were identified based on the proportion among three types of cells (epithelial cells, cornified cells and leukocytes) observed in the vaginal smear, using the method described by Marcondes et al. [22].

2.6. Cardiomyocyte injury

In order to assess cardiomyocyte injury, the coronary effluent accumulated before (perfusion period) and after (first 10 min of reperfusion phase) induction of ischemia and the levels of LDH were measured by the spectrophotometric method, using LDH assay kit (Pars Azmun Co., Tehran, Iran).

2.7. Statistical analysis

Normality assumption was examined by the Kolmogorov-Smirnov test; in case of significant results appropriate transformation was applied. To depict trend in means of variable changes graphically, mean plots (95% CI for mean) are presented according to their prenatal androgen exposure status (PAE), gender and phase of study (before ischemia, 15, 30, 45 and 60 min after ischemia). Firstly, to evaluate the effect of gender and PAE on the indices of heart damage before ischemia, a Generalized Linear Regression Model (GLM) was applied. Then, since there were four repeated measures of cardiac indices after ischemia (15, 30, 45 and 60 min), to assess the effect of PAE in subgroups of gender before and after induction of ischemia, a Generalized Estimating Equation Model (GEE) was used; in fact the, GEE approach provides advanced method of estimating regression parameters, which overcomes classical methods' weaknesses such as repeated measures Multivariate Analysis of Variance (MANOVA); it is also able to provide accurate effect size estimation in case of incomplete data which is very common in repeated measures and also modified parameter estimations to deal with confounding variables and also possible interaction effect [23]. Although this approach does not require normality assumption, we transformed the outcome in case of homogenous results with the first part. Interaction effects of PAE and gender were used to identify the influential effect of gender on changes in indices of heart damage after PAE. This regression model is defined as follows;

$$Mean(LVSP \mid PAE, Gender) = \beta_0 + \beta_1 PAE + \beta_2 Gender + \beta_3 PAE * Gender$$

The variable “phase” was added to this model for assessment of I/R injury and defined as phase 1 (before ischemia) and phases 2–5 (15, 30, 45 and 60 min after ischemia). This model was stratified for various statuses of PAE and gender, resulting in the following equations:

$$\begin{aligned} &Mean(LVSP \mid PAE, Phase2, Phase3, Phase4, Phase5) \\ &= \beta_0 + \beta_1 PAE + \beta_2 Phase2 + \beta_3 Phase3 + \beta_4 Phase4 + \beta_5 Phase5 + \beta_6 Phase2 * PAE + \beta_7 Phase3 * PAE + \beta_8 Phase4 * PAE + \beta_9 Phase5 * PAE \end{aligned}$$

$$\begin{aligned} &Mean(LVSP \mid Gender, Phase2, Phase3, Phase4, Phase5) \\ &= \beta_0 + \beta_1 Gender + \beta_2 Phase2 + \beta_3 Phase3 + \beta_4 Phase4 + \beta_5 Phase5 + \beta_6 Phase2 * Gender + \beta_7 Phase3 * Gender + \beta_8 Phase4 * Gender + \beta_9 Phase5 * Gender \end{aligned}$$

Here, phase 1 (before ischemia) has been considered as the reference group, and other phases were added as four dummy variables to the model.

In addition, t-student unpaired test was used to compare results of T and LDH levels, between the control and PAE rats.

3. Results

3.1. Testosterone (T) levels

T levels were significantly increased in PAE adult males, compared to their controls (6.78 ± 0.87 *vs.* 4.08 ± 0.66, respectively; $p < 0.05$). T levels were higher in PAE adult females than controls (1.26 ± 0.08 *vs.* 1.02 ± 0.09); however no significant differences were observed.

3.2. Heart indices

Figs. 2–4 present the trend of means and 95% CIs for heart indices in male and female rats according to the phases of study and PAE.

3.2.1. Heart indices at baseline and after I/R for androgen-unexposed rats (control groups)

Results from GLM analysis showed that, at baseline (before I/R) there were significant differences between androgen-unexposed males and females in terms of HR, which showed higher level of the HR by 52

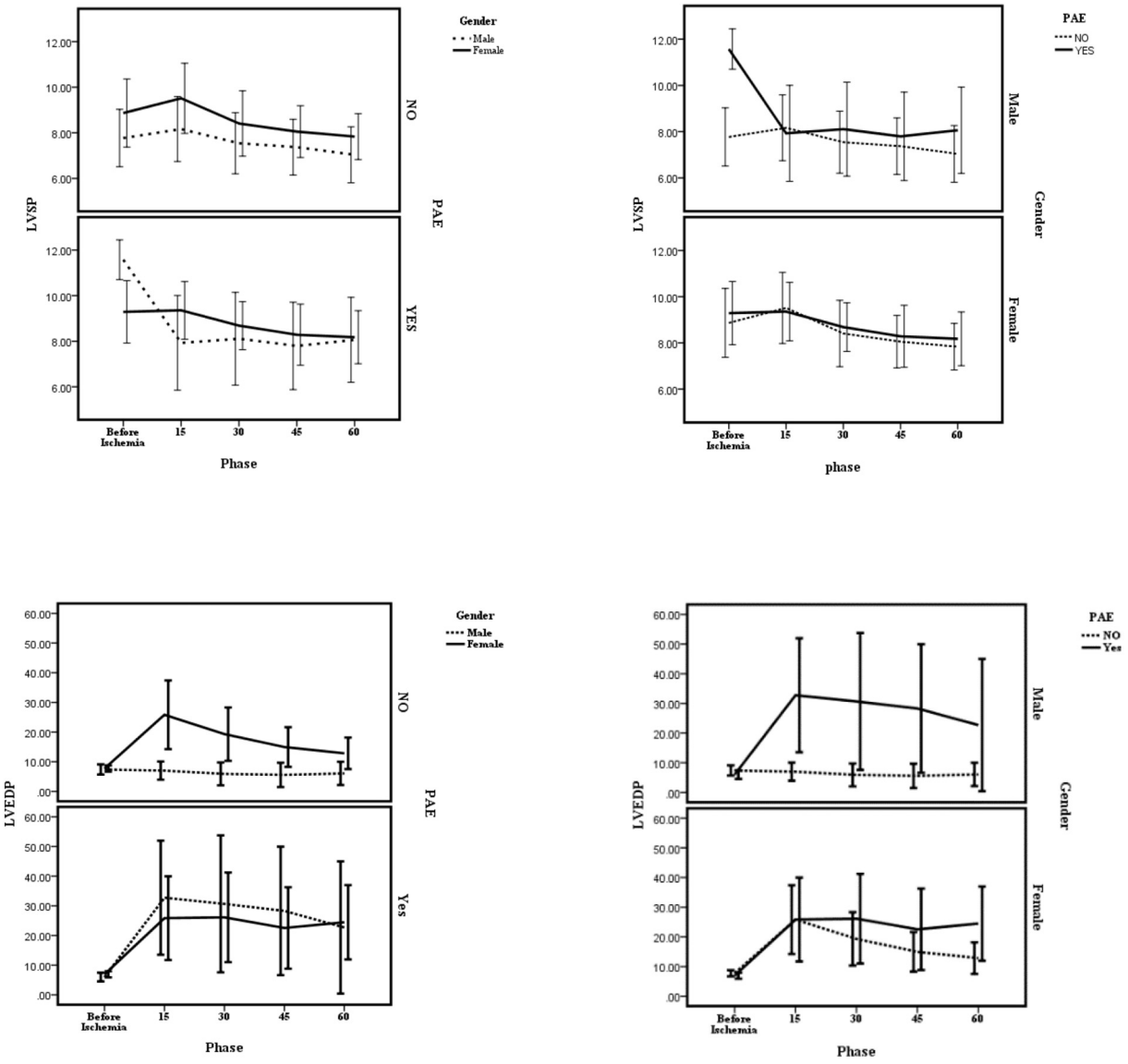

Fig. 2. Left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) in different phases of the study according to their prenatal androgen exposure (PAE) status in gender subgroups. (n = 8 in each group). Generalized Estimating Equation Model (GEE) analysis was applied to test the differences between gender (male *vs.* female), PAE (yes *vs* no), and phases (before ischemia and 15, 30, 45 and 60 min after ischemia).

(95% CI: 20, 85) in males. There were no statistically significant differences in other heart indices at initiation of the study between androgen-unexposed male and female rats (Table 1). In addition, GEE analysis showed that, LVEDP had a significant decreasing trend at 15 to 60 min after I/R, compared to before I/R in males *vs,* female androgen-unexposed rats (Table 2).

Heart indices at baseline (before I/R) for PAE adult rats.

3.2.2. Heart indices at baseline (before I/R) for PAE adult rats

Table 1 shows the results of before I/R obtained from GLM analysis. PAE adult male rats *vs.* controls and also PAE adult male rats *vs.* PAE adult females had significantly higher levels of LVSP, LVDP, RPP and ± dp/dt by 4 (95% CI: 2, 5), 37(95% CI: 14, 61), 31(95% CI: 9, 53), 22(95% CI: 14, 30), 16(95% CI: 7, 26) and 2(95% CI: 0.8, 4), 48 (95% CI: 18, 78), 44 (95% CI: 12, 75), 15(95% CI: 5, 25), and 11 (95% CI: 3, 19), respectively. No significant differences were observed in heart indices for PAE adult females *vs.* their controls.

3.2.3. Heart indices after I/R for PAE adult rats

Table 2 demonstrates the results of after I/R in 4 phases of 15, 30, 45 and 60 min compared to before I/R, obtained from GEE analysis. PAE adult male rats *vs.* controls and also PAE adult male rats *vs.* PAE adult females, showed significant decreasing trend in heart indices including LVSP, LVDP, RPP and ± dp/dt. Although these decreasing trends were observed in PAE adult female rats *vs.* their controls, it was not statistically significant in any of the phases. Moreover, LVEDP showed a significant increasing trend in PAE adult male rats *vs.* controls.

3.3. Cardiomyocyte injury

In order to examine cardiomyocyte injury after ischemia, LDH levels were measured in coronary effluent. As presented in Table 3, LDH levels increased in males and females after ischemia compared to before ischemia; although in PAE adult males the levels of LDH were significantly higher than their controls, after ischemia ($p < 0.05$), in females, no significant differences were observed between PAE adult rats and controls after ischemia (Table 3).

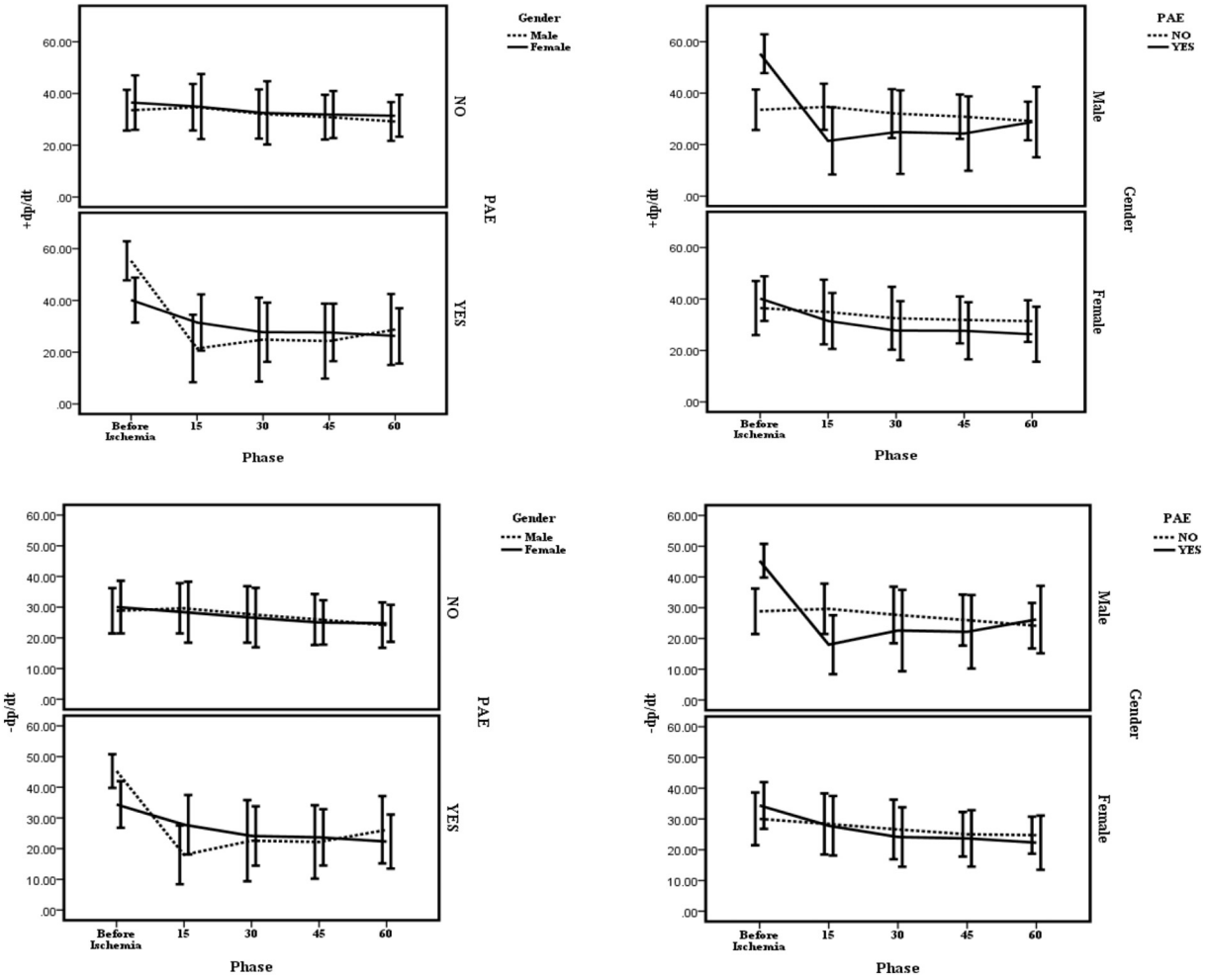

Fig. 3. Maximal rate of rise in the left ventricular pressure during systole (+dp/dt) and maximum rate of reduction in pressure during diastole (−dp/dt), in different phases of the study according to their prenatal androgen exposure (PAE) status in gender subgroups. (n = 8 in each group). Generalized Estimating Equation Model (GEE) analysis was applied to test the differences between gender (male *vs.* female), PAE (yes *vs* no), and phases (before ischemia and 15, 30, 45 and 60 min after ischemia).

4. Discussion

Fetal programming studies have shown gender-related differences in the occurrence of postnatal diseases, with a more pronounced effect in males than in females [24–26]. Given that testosterone is an important regulator of growth and differentiation during fetal development [27,28], in the current study we presented the impact of prenatal testosterone exposure on cardiac function and resistance to I/R injury in male and female rats later in life.

To the best of our knowledge, our study for the first time, revealed that, despite significant improvement in baseline cardiac function of PAE adult male rats, their cardiac condition worsens after I/R and they have lower tolerance to I/R injury in terms of reduced recovery of hemodynamic parameters, compared to their controls and PAE adult female rats. In PAE adult females, we observed no significant changes at baseline or even after induction of ischemia in comparison to their controls.

It has been reported that, prenatal testosterone exposure can lead to increased levels of androgens in both male and female offspring later in life, as we observed in our study rats (PAE adult rats). Androgens play a critical role in the cardiovascular system and are considered to be cardioregulatory hormones, which exert their biological effects on cardiomyocytes through androgen receptors [29,30]. Despite some of the detrimental effects of androgens on the cardiovascular system [31], some other studies have revealed favorable effects of androgens on this system [32]. Androgens affect expression of the L-type calcium channel, the Na^+/Ca^{2+} exchanger, cardiomyocyte Ca^{2+} handling, and contractile properties in rat ventricular myocytes and thus play an important role in cardiac performance in males [30,33–36], contributing thereby to gender differences in cardiac function. Based on a previous study conducted on mice, androgens had positive effects on cardiac circadian rhythms, contractile gene expression, and myocardial functional reserve [37]. Another study of isolated rat hearts showed that testosterone supplementation can restore a reduction in cardiac contractile functions, including ejection fraction, peak systolic pressure, and cardiac output to normal values [38,39]. In a study of rats with heart failure after infarction and low testosterone levels, testosterone treatment favorably affected immunomodulatory cytokine levels, improved cardiac function, and reduced maladaptive cardiac remodeling [40]. In addition, testosterone therapy has been associated with significant increases in cardiac output, improved cardiac function capacity, and reduced symptoms in men with heart failure [41,42]. Our results on the improvement of baseline cardiac function of PAE adult male rats, are in agreement with those of other studies reporting favorable cardiovascular effects of androgens [32,37]. Conversely after induction of ischemia, these beneficiary effects are reversed and these rats (PAE adult males) had lower tolerance to I/R injury compared to their controls and also PAE adult females; this paradox may partly be explained by the adverse effects of testosterone on cardiac function in the I/R hearts [43]. However there are some reports regarding the

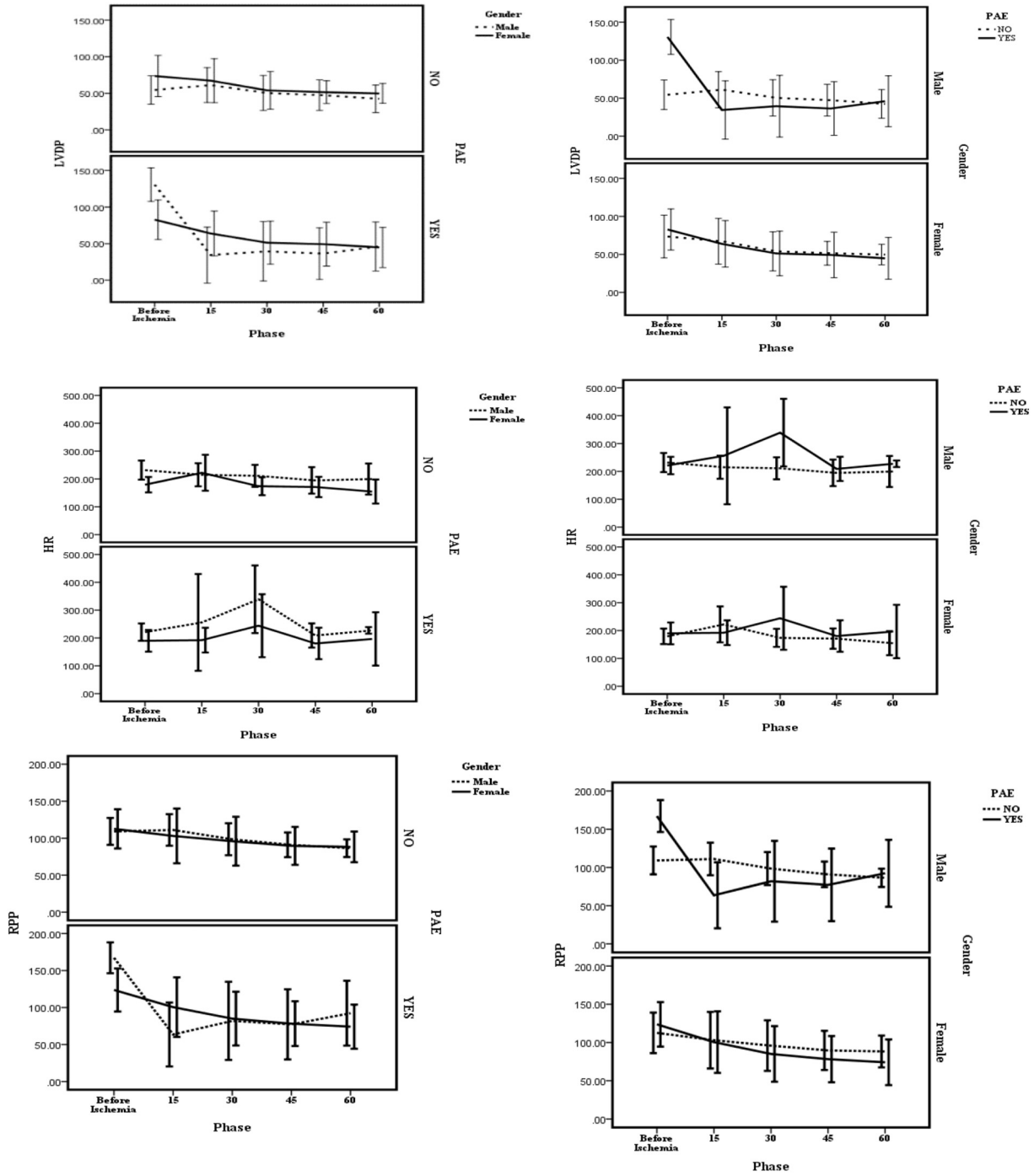

Fig. 4. Left ventricular developed pressure (LVDP), heart rate (HR), and rate pressure product (RPP) in different phases of the study according to their prenatal androgen exposure (PAE) status in gender subgroups. (n = 8 in each group). Generalized Estimating Equation Model (GEE) analysis was applied to test the differences between gender (male *vs.* female), PAE (yes *vs* no), and phases (before ischemia and 15, 30, 45 and 60 min after ischemia).

positive effects of physiological or low doses of testosterone on heart ischemic tolerance and its cardioprotective role against I/R injury [43]; conversely, some other studies indicate that testosterone can negatively affect cardiac function in the myocardial infarction model or in the I/R heart [43]. Testosterone may play an important role in the contractile dysfunction induced by I/R injury for several reasons, some of which report that worsened effects of testosterone on myocardial function after I/R may be due to "reducing cardioprotective signaling proteins such as STAT-3 (signal transducers and activators of transcription 3) and SOCS-3 (suppression of the cytokine signaling 3), and also decreasing myocardial Akt activation, leading to reduced Bad phosphorylation and decreased Bcl-2 levels" [43]. Based on evidence, cytokines have an effect on injury-induced cardiovascular dysfunction [44]. After acute I/R, the myocardium produces inflammatory cytokines (TNF-α and IL − 1), resulting in myocardial dysfunction and cardiomyocyte apoptosis [44]. Previous studies conducted on male rats have shown that testosterone increases TNF-α, IL-1, IL-6, activated p38 MAPK, caspase-1, caspase-3, and caspase-11; while decreasing Bcl-2 expression, consequently worsening myocardial function after I/R [44,45].

In animal models, testosterone induces an exaggerated inflammatory response after myocardial infarction that been associated

Table 1
Comparison of measurement of heart hemodynamic parameters before I/R (at baseline) phase in groups of gender and PAE *vs* controls: results from GLM model.

Parameters	[a]PAE adult male rats *vs.* Controls	[b]PAE adult male rats *vs.* PAE adult females	[a]PAE adult female rats *vs.* Controls	[b]Control males *vs.* females
Sqrt (LVSP)	4(2, 5)***	2(0.8, 4)**	0.4 (−1, 2)	-1(−2.6, 0.4)
LVEDP	−1.4(3, 0.1)	−0.9 (−2, 0.5)	−0.8 (−2, 0.5)	−0.3(−2, 1)
LVDP	37(14, 61)**	48(18, 78)**	9 (−22, 41)	−19(47, 9)
HR	−11(−53, 31)	31(−12, 74)	11(−30, 51)	52(20, 85)*
Sqrt (RPP)	31(9, 53)**	44(12, 75)**	11 (−21, 44)	−3(−30, 23)
Sqrt (+ dp/dt)	22(14, 30)***	15(5, 25)**	4(−6, 13)	−3(−14, 8)
Sqrt (− dp/dt)	16 (7, 26)***	11(3, 19)**	4(−4, 12)	−1(−10, 8)

PAE: Prenatal androgen exposed.
I/R: Ischemia/reperfusion.
LVSP: Left ventricular systolic pressure.
LVEDP: Left ventricular end-diastolic pressure.
LVDP: Left ventricular developed pressure.
HR: Heart rate.
RPP: Rate pressure product.
+ dp/dt: Maximal rate of rise in the left ventricular pressure during systole.
- dp/dt: Maximum rate of reduction in the left ventricular pressure during diastole.
n = 8 in each group.
Significant levels:
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
[a] β_1(95 % *CI*):(PAE), $\beta_1+\beta_3$(95 % *CI*):(PAE + PAE *Gender effect), parameter estimated *via* main effect (Mean difference) term in Generalized Linear Regression Model (GLM), respectively.
[b] β_2(95 % *CI*):(Gender effect), $\beta_2 + \beta_3$(95 % *CI*):(Gender + PAE*Gender effect) Subgroup analysis: Parameter estimated *via* interaction effect term in GLM model.

with an increased risk of cardiac rupture [46]. Wang et al., have reported that hearts from male mice demonstrate poorer recovery of contractile function (+dP/dt) after I/R in comparison to females [47]. However the dose dependent detonating effect of testosterone in male has not yet been reported.

In our study, despite more cardiac I/R injury in PAE adult female rats compared to their controls, this difference was not statistically significant. It seems that while increased androgens in these PAE female rats may had negative effects on their heart, they were more resistant to ischemia and they demonstrated less cardiac I/R injury, compared to PAE adult males; the gender-related differences observed on the tolerance to I/R injury can be due to the following reasons:

i. The protective effects of estrogen (given that in the current study, the hearts of female rats isolated in the estrus phase of the reproductive cycle, which has high levels of estrogens), which exert their effects through nuclear estrogen receptors in the cardiomyocytes [48]. Estrogen can reduce calcium loading during I/R, which results in less injury after ischemia, which at least in part, may be due to increased expression of nitric oxide synthase (NOS) [48]. Nitric oxide (NO) produced from NOS has been shown to regulate a number of calcium transporters and decrease Ca^{2+} entry by the L-type Ca^{2+} channel, resulting in less calcium loading during ischemia [48]. Based on other studies, reducing calcium concentration during ischemia is cardioprotective [49,50]. In addition, estrogens have been shown to be cardioprotective by activation of the phosphatidylinositol 3-kinase (PI3-kinase/AKT) pathway and many cardioprotective genes such as the heat shock proteins [31,51].

Previous studies have reported that administration of exogenous estradiol (E2) in adult males and females, gonad-intact and gonadectomized animals leads to reduced I/R injury. [52] Furthermore it has been shown that a higher percentage (23%) of women aged ≥40 years, with a first myocardial infarction (MI) will die during one year, compared to men (18%) [53]. These findings can be due to reduction in the levels of endogenous E2, followed by reduced ischemic tolerance in postmenopausal women.

ii. Another possible explanation as to why female myocardium suffers less injury with I/R, is the lower Ca^{2+} uptake rate by mitochondria and lower cardiac mitochondria contents which lead to production of less free radicals, subsequently lowering cardiac oxidative damage in these animals [15,54].
iii. Differences in levels of anti-apoptotic protein (Bcl2) and pro-apoptotic proteins (Bax and phosphor-p38) between two genders; levels of Bcl2 were significantly decreased after I/R in male rats compared to females. Additionally, higher levels of Bax and phosphor-p38 were observed after I/R in male rats compared to females [55].
iv. Autophagy plays a key role in survival of cellular homeostasis, differentiation, and tissue remodeling. It has also been reported that autophagy increases tolerance to myocardial I/R injury [55]. According to previous reports female rat hearts show increased autophagy protein (LC3B) expression and higher autophagy activities compared to male rats in response to I/R.

Wang et al., using global heart ischemia in mouse found improved recovery of ± dP/dt in females compared to males [47,48]. Increased tolerance of female hearts to I/R injury has been reported in dogs, rats, mice and rabbits [15]. On the other hand, some other experiments conducted on animals have demonstrated lack of male–female differences in I/R injury, recovery of LVDP and infarct size [56–58]. These inconsistencies may be explained by differences in the time or the type of ischemia, animal strain and the phase of reproductive cycle in females.

4.1. Strengths and limitations

While this is one of the first investigations exploring the impact of PAE on cardiac function and tolerance to I/R injury of both female and male adult rats, our findings need to be carefully interpreted, considering the limitations, which included lack of measuring levels of some inflammatory factors, antioxidants and estradiol; also we did not examine the relationship between testosterone levels and cardiac function before and after I/R in adult male rats, separately.

Further comprehensive studies are needed to identify underlying mechanisms of our observations considering comprehensive hormonal

Table 2
Comparison of measurement of heart hemodynamic parameters after I/R with before I/R (at baseline) in subgroups of study phases by gender and PAE: results from GEE analysis.

Parameters	Study phase	[a]PAE adult male rats *vs.* Controls	[b]PAE adult male rats *vs.* PAE adult females	[a]PAE adult female rats *vs.* Controls	[b]Control Males *vs.* Females
Sqrt (LVSP)	15 min after Ischemia	−4(−6, −2) ***	−4(−6, −1) **	−0.6(−2.5,1.4)	−0.2(−0.9,0.5)
	30 min after Ischemia	−3(−5, −1) ***	−3(−5, −0.7) *	−0.1(−1.6,1.3)	0.2(−0.4,0.9)
	45 min after Ischemia	−3(−5, −2) ***	−3(−5, −0.7) *	−0.2(−1.8,1.5)	0.4(−0.6,1.4)
	60 min after Ischemia	−3(−5, −1) ***	−2(−4, −0.4) *	−0.1(−1.6,1.4)	0.3(−0.9,1.5)
	Before Ischemia	Reference	Reference	Reference	Reference
LVDP	15 min after Ischemia	−103(−139, −66) ***	−77(−131, −22) **	−13(−57, 32)	13(−6, 32)
	30 min after Ischemia	−87(−125, −49) ***	−60(−112, −7) *	−12(−52, 28)	15(0.2, 31) *
	45 min after Ischemia	−87(−121, −54) ***	−61(−109, −12) *	−11(−51, 28)	15(−4, 34)
	60 min after Ischemia	−73(−107, −37) ***	−47(−94, 0.2)	−14(−52, 24)	12(−10, 34)
	Before Ischemia	Reference	Reference	Reference	Reference
Sqrt (RPP)	15 min after Ischemia	−105(−141, −70) ***	−81(−135, −26) **	−14(−63, 36)	11(−16, 39)
	30 min after Ischemia	−75(−116, −32) ***	−47(−103, 10)	−22(−65, 21)	6(−14, 26)
	45 min after Ischemia	−72(−109, −34) ***	−45(−94, 5)	−23(−59, 14)	5(−13, 22)
	60 min after Ischemia	−52(−83, −2) ***	−25(−69, 19)	−25(−61, 10)	2(−16, 20)
	Before Ischemia	Reference	Reference	Reference	Reference
Sqrt (+dp/dt)	15 min after Ischemia	−35(−48, −22) ***	−25(−43, −8) **	−7(−22, 8)	3(−5, 10)
	30 min after Ischemia	−29(−41, −15) ***	−18(−37, −1) **	−9(−23, 6)	3(−4, 9)
	45 min after Ischemia	−28(−41, −15) ***	−19(−36, −11) *	−8(−21, 5)	1(−4, 8)
	60 min after Ischemia	−22(−35, −9) ***	−13(−29, 4)	−9(−22, 4)	0.7(−6, 7)
	Before Ischemia	Reference	Reference	Reference	Reference
Sqrt (−dp/dt)	15 min after Ischemia	−28(−37, −19) ***	−21(−34, −7) **	−5(−18, 8)	3(−4, 9)
	30 min after Ischemia	−22(−32, −10) ***	−12(−28, 3)	−7(−18, 5)	2(−3, 7)
	45 min after Ischemia	−20(−30, −10) ***	−12(−26, 1)	−6(−16, 5)	2(−3, 7)
	60 min after Ischemia	−14(−24, −4) ***	−7(−19, 16)	−7(−17, 4)	0.6(−5, 6)
	Before Ischemia	Reference	Reference	Reference	Reference
HR	15 min after Ischemia	52(−94, 197)	33(−113, 178)	−41(−108, 27)	−60(−126, 7)
	30 min after Ischemia	139(40, 239) *	63(−64, 192)	60(−31, 150)	−15(−51, 21)
	45 min after Ischemia	25(−24, 74)	−3(−68, 63)	−1(−56, 54)	−29(−62, 4)
	60 min after Ischemia	38(7, 70) *	−0.4(−78, 77)	31(−47, 110)	−7(−41, 26)
	Before Ischemia	Reference	Reference	Reference	Reference
LVEDP	15 min after Ischemia	27(13, 41) ***	8(−10, 26)	1(−14, 16)	−19(−28, −9) ***
	30 min after Ischemia	26(9, 34) ***	5(−16, 26)	8(−7, 22)	−13(−20, −6) ***
	45 min after Ischemia	24(8, 34) ***	6(−13, 26)	8(−4, 21)	−9(−15, 3) **
	60 min after Ischemia	18(2, 34) ***	−0.9(−20, 18)	12(1, 24) *	−6(−11, −2) **
	Before Ischemia	Reference	Reference	Reference	Reference

PAE: Prenatal androgen exposed. I/R: Ischemia/reperfusion. LVSP: Left ventricular systolic pressure. LVEDP: Left ventricular end-diastolic pressure. LVDP: Left ventricular developed pressure. HR: Heart rate. RPP: Rate pressure product. + dp/dt: Maximal rate of rise in the left ventricular pressure during systole. - dp/dt: Maximum rate of reduction in the left ventricular pressure during diastole. n = 8 in each group.
Significant levels:
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
[a] Main effect (Mean difference) of PAE and Gender, (95%CI).
[b] Interaction effect of Gender*Phase in subgroup of PAE, (95%CI).

Table 3
Coronary effluents LDH levels in control and PAE rats before (baseline) and after I/R.

Variable	Male				Female			
	Controls (n = 8)		PAE (n = 8)		Controls (n = 8)		PAE (n = 8)	
	Before	After	Before	After	Before	After	Before	After
LDH (U/L)	137.44 ± 22.22	579.18 ± 27.33	130.78 ± 16.93	680.78 ± 27.14*	115.54 ± 13.50	279.4813 ± 26.42	118.02 ± 12.87	334.73 ± 25.57

Data are presented as mean ± SEM. PAE rats: Prenatal androgen exposed rats, I/R: Ischemia/reperfusion, LDH: lactate dehydrogenase. n = 8 in each group.
* Significance level $p < 0.05$, p value was obtained from t-student test in comparison between PAE adult and controls after ischemia.

and inflammatory profiles. Comprehensive understanding may allow therapeutic manipulation of sex hormone signaling mechanisms in the treatment of I/R injury. These findings may also have important clinical implications in gender-dependent consultations in later life of those exposed to androgen during prenatal life.

5. Conclusions

In conclusion, the impact of prenatal androgen exposure on adulthood cardiac function and tolerance to I/R is gender dependent, which may be partly explained by different cardiac effects of hyperandrogenism in males *versus* females. After exposure to testosterone during the critical period of fetal life, the baseline hemodynamic parameters of the hearts of androgen-exposed adult males are increased, although they had less tolerance to I/R, findings however not observed in females.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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References

[1] D. Barker, The developmental origins of adult disease, J. Am. Coll. Nutr. 23 (2004) 588S–595S, https://doi.org/10.1080/07315724.2004.10719428.
[2] S.B. Sherman, N. Sarsour, J. Hill, Prenatal androgen exposure of single-injection of testosterone induces cardiovascular and metabolic dysfunction in adult female Wistar rats, FASEB J. 31 (2017) 885–889 (Abstract).
[3] A.J. King, N.B. Olivier, P.S. Mohankumar, J.S. Lee, V. Padmanabhan, G.D. Fink, Hypertension caused by prenatal testosterone excess in female sheep, Am. J. Physiol. Endocrinol. Metab. 292 (2007) E1837–E1841, https://doi.org/10.1152/ajpendo.00668.2006.
[4] V. Chinnathambi, M. Balakrishnan, C. Yallampalli, K. Sathishkumar, Prenatal testosterone exposure leads to hypertension that is gonadal hormone-dependent in adult rat male and female offspring, Biol. Reprod. 86 (2012), https://doi.org/10.1095/biolreprod.111.097550 ARTN 137.
[5] A.K. Vyas, V. Hoang, V. Padmanabhan, E. Gilbreath, K.A. Mietelka, Prenatal programming: adverse cardiac programming by gestational testosterone excess, Sci. Rep. 6 (2016) 28335, , https://doi.org/10.1038/srep28335.
[6] E. Salamalekis, P. Bakas, N. Vitoratos, M. Eleptheriadis, G. Creatsas, Androgen levels in the third trimester of pregnancy in patients with preeclampsia, Eur. J. Obstet. Gynecol. Reprod. Biol. 126 (2006) 16–19, https://doi.org/10.1016/j.ejogrb.2005.07.007.
[7] T. Sir-Petermann, M. Maliqueo, B. Angel, H.E. Lara, F. Perez-Bravo, S.E. Recabarren, Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization, Hum. Reprod. 17 (2002) 2573–2579, https://doi.org/10.1093/humrep/17.10.2573.
[8] D.J. Evans, R.G. Hoffmann, R.K. Kalkhoff, A.H. Kissebah, Relationship of androgenic activity to body fat topography, fat cell morphology, and metabolic aberrations in premenopausal women, J. Clin. Endocrinol. Metab. 57 (1983) 304–310, https://doi.org/10.1210/jcem-57-2-304.
[9] D.B. Kandel, J.R. Udry, Prenatal effects of maternal smoking on daughters' smoking: nicotine or testosterone exposure? Am. J. Public Health 89 (1999) 1377–1383, https://doi.org/10.2105/AJPH.89.9.1377.
[10] C.H. Ramlau-Hansen, A.M. Thulstrup, J. Olsen, E. Ernst, C.Y. Andersen, J.P. Bonde, Maternal smoking in pregnancy and reproductive hormones in adult sons, Int. J. Androl. 31 (2008) 565–572, https://doi.org/10.1111/j.1365-2605.2007.00807.x.
[11] S. Rizwan, J.T. Manning, B.J. Brabin, Maternal smoking during pregnancy and possible effects of in utero testosterone: evidence from the 2D:4D finger length ratio, Early Hum. Dev. 83 (2007) 87–90, https://doi.org/10.1016/j.earlhumdev.2006.05.005.
[12] J.A. Barry, A.R. Kay, R. Navaratnarajah, S. Iqbal, J.E. Bamfo, A.L. David, et al., Umbilical vein testosterone in female infants born to mothers with polycystic ovary syndrome is elevated to male levels, J. Obstet. Gynaecol. 30 (2010) 444–446, https://doi.org/10.3109/01443615.2010.485254.
[13] M. Maliqueo, H.E. Lara, F. Sanchez, B. Echiburu, N. Crisosto, T. Sir-Petermann, Placental steroidogenesis in pregnant women with polycystic ovary syndrome, Eur. J. Obstet. Gynecol. Reprod. Biol. 166 (2013) 151–155, https://doi.org/10.1016/j.ejogrb.2012.10.015.
[14] P. Filippou, R. Homburg, Is foetal hyperexposure to androgens a cause of PCOS? Hum. Reprod. Update 23 (2017) 421–432, https://doi.org/10.1093/humupd/dmx013.
[15] B. Ostadal, I. Netuka, J. Maly, J. Besik, I. Ostadalova, Gender differences in cardiac ischemic injury and protection—experimental aspects, Exp. Biol. Med. 234 (2009) 1011–1019, https://doi.org/10.3181/0812-MR-362.
[16] R.A. Brown, M.J. Anthony, P. Petrovski, J. Ren, The influence of gender, diabetes, and acetaldehyde on the intrinsic contractile properties of isolated rat myocardium, Cardiovasc. Toxicol. 1 (2001) 35–42, https://doi.org/10.1385/ct:1:1:35.
[17] A. Foryst-Ludwig, U. Kintscher, Sex differences in exercise-induced cardiac hypertrophy, Pflugers Arch. 465 (2013) 731–737, https://doi.org/10.1007/s00424-013-1225-0.
[18] J.S. Janicki, F.G. Spinale, S.P. Levick, Gender differences in non-ischemic myocardial remodeling: are they due to estrogen modulation of cardiac mast cells and/or membrane type 1 matrix metalloproteinase, Pflugers Arch. 465 (2013) 687–697, https://doi.org/10.1007/s00424-013-1229-9.
[19] M. Krieg, K. SMITH, W. BARTSCH, Demonstration of a specific androgen receptor in rat heart muscle: relationship between binding, metabolism, and tissue levels of androgens, Endocrinology 103 (1978) 1686–1694, https://doi.org/10.1210/endo-103-5-1686.
[20] F.R. Tehrani, M. Noroozzadeh, S. Zahediasl, A. Piryaei, F. Azizi, Introducing a rat model of prenatal androgen-induced polycystic ovary syndrome in adulthood, Exp. Physiol. 99 (2014) 792–801, https://doi.org/10.1113/expphysiol.2014.078055.
[21] M. Dianat, M. Esmaeilizadeh, M. Badavi, A.R. Samarbaf-Zadeh, B. Naghizadeh, Protective effects of Crocin on ischemia-reperfusion induced oxidative stress in comparison with vitamin E in isolated rat hearts, Jundishapur J. Nat. Pharm. Prod. 9 (2014) e17187, , https://doi.org/10.1055/s-0033-1360383.
[22] F. Marcondes, F. Bianchi, A. Tanno, Determination of the estrous cycle phases of rats: some helpful considerations, Braz. J. Biol. 62 (2002) 609–614, https://doi.org/10.1590/S1519-69842002000400008.
[23] K.Y. Liang, S.L. Zeger, Longitudinal data-analysis using generalized linear-models, Biometrika 73 (1986) 13–22, https://doi.org/10.1093/biomet/73.1.13.
[24] T. Ozaki, H. Nishina, M.A. Hanson, L. Poston, Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring, J. Physiol. 530 (2001) 141–152, https://doi.org/10.1111/j.1469-7793.2001.0141m.x.
[25] M.D. Franco, A.P.V. Dantas, E.H. Akamine, E.M. Kawamoto, Z.B. Fortes, C. Scavone, et al., Enhanced oxidative stress as a potential mechanism underlying the programming of hypertension in utero, J. Cardiovasc. Pharmacol. 40 (2002) 501–509, https://doi.org/10.1097/01.Fjc.0000019077.73742.35.
[26] L.L. Woods, J.R. Ingelfinger, R. Rasch, Modest maternal protein restriction fails to program adult hypertension in female rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 289 (2005) R1131–R1136, https://doi.org/10.1152/ajpregu.00037.2003.
[27] F. De Zegher, H. Devlieger, R. Eeckels, Fetal growth: boys before girls, Horm. Res. 51 (1999) 258–259, https://doi.org/10.1159/000023382.
[28] R.S. Viger, D.W. Silversides, J.J. Tremblay, New insights into the regulation of mammalian sex determination and male sex differentiation, Vitam. Horm. 70 (2005) 387–413, https://doi.org/10.1016/S0083-6729(05)70013-3.
[29] N. Witayavanitkul, W. Woranush, T. Bupha-Intr, J. Wattanapermpool, Testosterone regulates cardiac contractile activation by modulating SERCA but not NCX activity, Am. J. Physiol. Heart Circ. Physiol. 304 (2012) H465–H472, https://doi.org/10.1152/ajpheart.00555.2012.
[30] K.L. Golden, J.D. Marsh, Y. Jiang, J. Moulden, Acute actions of testosterone on contractile function of isolated rat ventricular myocytes, Eur. J. Endocrinol. 152

(2005) 479–483, https://doi.org/10.1530/eje.1.01845.
[31] L. Fazal, F. Azibani, N. Vodovar, A. Cohen Solal, C. Delcayre, J.L. Samuel, Effects of biological sex on the pathophysiology of the heart, Br. J. Pharmacol. 171 (2014) 555–566, https://doi.org/10.1111/bph.12279.
[32] Y. Ikeda, K. Aihara, S. Yoshida, M. Akaike, T. Matsumoto, Effects of androgens on cardiovascular remodeling, J. Endocrinol. 214 (2012) 1–10, https://doi.org/10.1530/JOE-12-0126.
[33] K.L. Golden, J.D. Marsh, Y. Jiang, T. Brown, J. Moulden, Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E449–E453, https://doi.org/10.1152/ajpendo.00054.2003.
[34] C.L. Curl, L.M. Delbridge, B.J. Canny, I.R. Wendt, Testosterone modulates cardiomyocyte ca(2+) handling and contractile function, Physiol. Res. 58 (2009) 293–297.
[35] K.L. Golden, Q.I. Fan, B. Chen, J. Ren, J. O'Connor, J.D. Marsh, Adrenergic stimulation regulates Na+/Ca2+ exchanger expression in rat cardiac myocytes, J. Mol. Cell. Cardiol. 32 (2000) 611–620, https://doi.org/10.1006/jmcc.2000.1104.
[36] J.-M. Lobaccaro, N. Poujol, B.a. Térouanne, V. Georget, S.p. Fabre, S. Lumbroso, et al., Transcriptional interferences between normal or mutant androgen receptors and the activator protein 1—dissection of the androgen receptor functional domains, Endocrinology 140 (1999) 350–357, https://doi.org/10.1210/endo.140.1.6418.
[37] S.D. Praktiknjo, S. Picard, C.F. Deschepper, Comparisons of chromosome Y-substituted mouse strains reveal that the male-specific chromosome modulates the effects of androgens on cardiac functions, Biol. Sex Differ. 7 (2016) 61. ARTN 61 https://doi.org/10.1186/s13293-016-0116-4.
[38] T.F. Schaible, J. Scheuer, Comparison of heart function in male and female rats, Basic Res. Cardiol. 79 (1984) 402–412, https://doi.org/10.1007/BF01908140.
[39] T.F. Schaible, A. Malhotra, G. Ciambrone, J. Scheuer, The effects of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats, Circ. Res. 54 (1984) 38–49, https://doi.org/10.1161/01.res.54.1.38.
[40] Y.Z. Zhang, X.W. Xing, B. He, L.X. Wang, Effects of testosterone on cytokines and left ventricular remodeling following heart failure, Cell. Physiol. Biochem. 20 (2007) 847–852, https://doi.org/10.1159/000110444.
[41] C.J. Malkin, P.J. Pugh, J.N. West, E.J. van Beek, T.H. Jones, K.S. Channer, Testosterone therapy in men with moderate severity heart failure: a double-blind randomized placebo controlled trial, Eur. Heart J. 27 (2006) 57–64, https://doi.org/10.1093/eurheartj/ehi443.
[42] P.J. Pugh, T.H. Jones, K.S. Channer, Acute haemodynamic effects of testosterone in men with chronic heart failure, Eur. Heart J. 24 (2003) 909–915, https://doi.org/10.1016/S0195-668X(03)00083-6.
[43] W. Pongkan, S.C. Chattipakorn, N. Chattipakorn, Roles of testosterone replacement in cardiac ischemia–reperfusion injury, J. Cardiovasc. Pharmacol. Ther. 21 (2016) 27–43, https://doi.org/10.1177/1074248415587977.
[44] M. Wang, B.M. Tsai, A. Kher, L.B. Baker, G.M. Wairiuko, D.R. Meldrum, Role of endogenous testosterone in myocardial proinflammatory and proapoptotic signaling after acute ischemia-reperfusion, Am. J. Physiol. Heart Circ. Physiol. 288 (2005) H221–H226, https://doi.org/10.1152/ajpheart.00784.2004.
[45] P.R. Crisostomo, M. Wang, G.M. Wairiuko, E.D. Morrell, D.R. Meldrum, Brief exposure to exogenous testosterone increases death signaling and adversely affects myocardial function after ischemia, Am. J. Physiol. Regul. Integr. Comp. Physiol. 290 (2006) R1168–R1174, https://doi.org/10.1152/ajpregu.00833.2005.
[46] M.A. Cavasin, Z.Y. Tao, A.L. Yu, X.P. Yang, Testosterone enhances early cardiac remodeling after myocardial infarction, causing rupture and degrading cardiac function, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H2043–H2050, https://doi.org/10.1152/ajpheart.01121.2005.
[47] M. Wang, P. Crisostomo, G.M. Wairiuko, D.R. Meldrum, Estrogen receptor-alpha mediates acute myocardial protection in females, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H2204–H2209, https://doi.org/10.1152/ajpheart.01219.2005.
[48] E. Murphy, C. Steenbergen, Gender-based differences in mechanisms of protection in myocardial ischemia–reperfusion injury, Cardiovasc. Res. 75 (2007) 478–486, https://doi.org/10.1016/j.cardiores.2007.03.025.
[49] C. Steenbergen, E. Murphy, L. Levy, R.E. London, Elevation in cytosolic free calcium concentration early in myocardial ischemia in perfused rat heart, Circ. Res. 60 (1987) 700–707, https://doi.org/10.1161/01.RES.60.5.700.
[50] H.M. Piper, K. Meuter, C. Schafer, Cellular mechanisms of ischemia-reperfusion injury, Ann. Thorac. Surg. 75 (2003) S644–S648, https://doi.org/10.1016/S0003-4975(02)04686-6.
[51] R.D. Patten, I. Pourati, M.J. Aronovitz, J. Baur, F. Celestin, X. Chen, et al., 17β-estradiol reduces cardiomyocyte apoptosis in vivo and in vitro via activation of phospho-inositide-3 kinase/Akt signaling, Circ. Res. 95 (2004) 692–699, https://doi.org/10.1161/01.RES.0000144126.57786.89.
[52] D.H. Korzick, T.S. Lancaster, Age-related differences in cardiac ischemia–reperfusion injury: effects of estrogen deficiency, Pflugers Arch. 465 (2013) 669–685, https://doi.org/10.1007/s00424-013-1255-7.
[53] V.L. Roger, A.S. Go, D.M. Lloyd-Jones, R.J. Adams, J.D. Berry, T.M. Brown, et al., Heart disease and stroke statistics—2011 update: a report from the American Heart Association, Circulation 123 (2011) e18–e209, https://doi.org/10.1161/CIR.0b013e3182009701.
[54] B. Colom, J. Oliver, P. Roca, F.J. Garcia-Palmer, Caloric restriction and gender modulate cardiac muscle mitochondrial H2O2 production and oxidative damage, Cardiovasc. Res. 74 (2007) 456–465, https://doi.org/10.1016/j.cardiores.2007.02.001.
[55] C. Chen, L.-X. Hu, T. Dong, G.-Q. Wang, L.-H. Wang, X.-P. Zhou, et al., Apoptosis and autophagy contribute to gender difference in cardiac ischemia–reperfusion induced injury in rats, Life Sci. 93 (2013) 265–270, https://doi.org/10.1016/j.lfs.2013.06.019.
[56] H.R. Cross, L. Lu, C. Steenbergen, K.D. Philipson, E. Murphy, Overexpression of the cardiac Na+/Ca2+ exchanger increases susceptibility to ischemia/reperfusion injury in male, but not female, transgenic mice, Circ. Res. 83 (1998) 1215–1223, https://doi.org/10.1161/01.res.83.12.1215.
[57] S.C. Schuit, F.H. de Jong, L. Stolk, W.N. Koek, J.B. van Meurs, M.W. Schoofs, et al., Estrogen receptor alpha gene polymorphisms are associated with estradiol levels in postmenopausal women, Eur. J. Endocrinol. 153 (2005) 327–334, https://doi.org/10.1530/eje.1.01973.
[58] S.A. Gabel, V.R. Walker, R.E. London, C. Steenbergen, K.S. Korach, E. Murphy, Estrogen receptor beta mediates gender differences in ischemia/reperfusion injury, J. Mol. Cell. Cardiol. 38 (2005) 289–297, https://doi.org/10.1016/j.yjmcc.2004.11.013.