Pregnancy mitigates cardiac pathology in a mouse model of left ventricular pressure overload

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Running Head: Pregnancy mitigates TAC-induced cardiac pathology

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ABSTRACT

In western countries heart disease is the leading cause of maternal death during pregnancy. The effect of pregnancy on the heart is difficult to study in patients with preexisting heart disease. Since experimental studies are scarce, we investigated the effect of pressure-overload, produced by transverse aortic constriction (TAC) in mice, on the ability to conceive, pregnancy outcome, and maternal cardiac structure and function. Four weeks of TAC produced left ventricular (LV) hypertrophy and dysfunction with marked interstitial fibrosis, decreased capillary density and induced pathological cardiac gene expression. Pregnancy increased relative LV and right ventricular weight without affecting the deterioration of LV function following TAC. Surprisingly, the TAC-induced increase in relative heart and lung weight was mitigated by pregnancy, which was accompanied by a partial normalization of capillary density and natriuretic peptide type A expression. Additionally, the combination of pregnancy and TAC increased the cardiac phosphorylation of c-Jun, and STAT1, but reduced PI3K phosphorylation. Finally, TAC did not significantly affect conception rate, pregnancy duration, uterus size, litter size and pup weight. In conclusion, we found that, rather than exacerbating the changes associated with cardiac pressure-overload, pregnancy actually attenuated pathological LV remodeling and mitigated pulmonary congestion, capillary rarefaction and pathological gene expression produced by TAC, suggesting a positive effect of pregnancy on the pressure-overloaded heart.

NEW & NOTEWORTHY

Little is known about the effect of pregnancy on heart disease. We found that aortic stenosis did not affected fetal outcome and that pregnancy, rather than exacerbating LV dysfunction, attenuated
cardiac remodeling and mitigated pulmonary congestion and pathological gene expression, suggesting positive effects of pregnancy on the pressure-overloaded heart.

**KEYWORDS:** Pregnancy; Aorta Stenosis; Cardiac remodeling; Cardiac dysfunction; Fertility
INTRODUCTION

Cardiac disease in pregnancy is a growing and challenging health care problem and a leading cause of maternal mortality (6). Yet, little is known about the effect of pregnancy on cardiac dimensions and function, especially in the case of preexisting heart disease (6). Circulating blood volume and cardiac output rise by 30-50% during pregnancy (13, 31), which may cause major problems in the context of left ventricular outflow tract (LVOT) obstruction. Both pregnancy and LVOT obstruction (e.g. aortic stenosis) produce left ventricular hypertrophy (LVH) to compensate for the increased loading conditions. However, whereas pregnancy induces physiological LVH, which is not associated with dysfunction (12), aortic constriction induces pathological LVH, associated with myocardial fibrosis and left ventricular (LV) dysfunction (35). However, data on the impact of pregnancy on cardiac outcome in the presence of aortic stenosis remains inconclusive. Although in some studies pregnancy was associated with clinical status deterioration and detrimental effects on the newborn in women with aortic stenosis (3, 24, 34), others report that pregnancy, whether or not related to stenosis severity, is generally well tolerated (33, 38). Additionally, very little is known about the effect of pregnancy on ventricular remodeling and function in aortic stenosis as only one study, reports that LV ejection fraction was maintained during pregnancy (24).

In view of the lack of clinical and experimental studies on myocardial structure and function and on fertility and pregnancy outcome in woman with aortic stenosis, we set out to investigate the impact of pregnancy on these variables in the well-established murine model of transverse aortic constriction (TAC) (35). Specifically, we assessed whether TAC mice with impaired cardiac function could conceive and evaluated the impact of LVOT obstruction on pregnancy outcome and, conversely, the impact of pregnancy on cardiac function, structure and the expression of molecular makers of hypertrophy.
METHODS

A total of 46 female C57BL6 mice (10-12 weeks) entered the study and were randomly assigned to one of the experimental groups (TAC or sham) and after 28 days again randomly assigned to mated versus non-mated. All experiments were performed in accordance with the “Guiding Principles in the Care and Use of Animals” as approved by the Council of the American Physiological Society and with prior approval of the Animal Care Committee of the Erasmus MC Rotterdam.

Experimental procedure. All mice were weighed, sedated with 4% isoflurane, intubated and connected to a pressure-controlled ventilator (SAR-830/P; CWE), set at 90 breaths per minute with a peak inspiratory pressure of 18 cm H₂O and a positive end expiratory pressure of 4 cm H₂O. A gas mixture of O₂/N₂ (1:2 vol/vol) containing 2.5% isoflurane was used to maintain anesthesia. Body temperature was kept at 37°C and buprenorphine (50 µg/kg) was injected s.c. for postsurgical analgesia. TAC was produced in 27 mice by constricting the aorta between the truncus brachiocephalicus and the arteria carotis communis sinistra using a 27G needle as previously described (35). Age-matched sham operated mice (n=19) underwent an identical surgical procedures except for constriction of the aorta.

Twenty-eight days after surgery, mice were again anesthetized with isoflurane and ventilated to perform echocardiography. LV diameter was measured with an Aloka SSD 4000 echo device (Aloka; Tokyo, Japan) using a 12-MHz probe. Twelve sham and 16 TAC mice were mated with weight- and age-matched C57BL6 male mice for 1 week. Vaginal plugs were checked carefully twice a day during the mating period. The first day of plug presence was considered to be day 0.5 of pregnancy (17). The other 7 sham and 7 TAC mice were housed solitary as non-pregnant, non-mated controls.
Eighteen days after the start of pregnancy, or after being assigned to the non-mated group, all mice were weighed and re-anesthetized to perform echocardiography. Subsequently, mice were sacrificed and pups collected by caesarean section and weighed. The LV was excised for histological and molecular biological analysis. The LV, right ventricle (RV), lung, liver, and uterus were dissected and weighed. Subsequently, part of the LV was rapidly snap-frozen in N2 for molecular analysis and the other LV part was stored in paraformaldehyde for histological processing.

**Echocardiography.** LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD) and LV wall thickness were measured from the M-Mode images (35). Fractional shortening was calculated from short axes M-Mode images as 100x(LVEDD-LVESD)/LVEDD.

**Histomorphometry.** Paraffin embedded LV tissue was serially sectioned into 4-µm slices. Cross sectional area and aspect ratio (long axis/short axis) were determined in transverse sections of Hematoxylin and Eosin (HE) stained cardiomyocytes. Cardiomyocytes with an aspect ratio above 1.5 or without a visible nucleus were excluded from the analysis to ensure that only transversally cut myocytes measured in the central part of the cell were included. Interstitial fibrosis content was measured using Picrosirius Red staining and Lectin staining was used to measure capillary density as the number of capillaries per area (35). LV sections of 6 mice per group, were analyzed with a quantitative image analysis system (Clemex Technologies).

**Quantitative real-time PCR.** Total RNA was isolated from the LV of each mouse, using the miRNeasy Mini Kit (Qiagen Ltd, Crawley, UK) and reverse transcribed to cDNA. Quantitative PCR was performed with Power SYBR Green PCR master mix (Applied Biosystems). Expression levels of natriuretic peptide type A (Nppa), natriuretic peptide type B (Nppb), Myosin Heavy Chain 6 and 7 (MYH6 and MYH7), and Sarco-Endoplasmic Reticulum Ca$^{2+}$-ATPase (Atp2a2) were measured.
mRNA data were expressed relative to the amount of the constitutively expressed housekeeping gene, GAPDH.

**Cell Signaling Assay.** Levels of phosphorylated cell signaling proteins were analyzed using a Bio-Plex Pro cell signaling multiplex assay (Bio-Rad). Heart tissue was homogenized using a Precellys bead homogenizer in the supplied lysis buffers, according to the manufacturer’s instructions. 10ug protein lysate was incubated for 16 hours with magnetic antibody-tagged beads, against phosphorylated c-Jun N-terminal kinase (JNK) (Thr$^{183}$/Tyr$^{185}$), phosphorylated c-Jun (Ser$^{63}$), phosphorylated mitogen-activated protein kinase kinase 1 (MEK-1) (Ser$^{217}$/Ser$^{221}$), phosphorylated extracellular signal-regulated kinase (ERK)1/2 (Thr$^{202}$/Tyr$^{204}$, Thr$^{185}$/Tyr$^{187}$), phosphorylated p38 (Thr$^{180}$/Tyr$^{182}$), phosphorylated vascular endothelial growth factor receptor 2 (VEGFR2) (Tyr$^{1175}$), phosphorylated phosphoinositide 3-kinase (PI3K) p85 (Tyr$^{458}$), phosphorylated Akt (Ser$^{473}$), phosphorylated signal transducers and activators of transcription (Stat) 1 (Tyr$^{701}$), phosphorylated Stat3 (Ser$^{727}$), phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) (Ser$^{32}$/Ser$^{36}$) and phosphorylated nuclear factor-κB (NFκB) p65 (Ser$^{536}$). The positive control was supplied by the manufacturer for each of the analytes. Plates were analyzed using Bio-Plex Magpix multiplex reader and Bio-Plex Manager6.1 software (Bio-Rad). Data is expressed as a ratio of mean fluorescence intensity of a sample to mean fluorescence intensity of the positive control. One animal was excluded in its entirety from the TAC pregnant group as it proved to be an extreme outlier for all analytes measured.

**Statistical analysis.** The echocardiographic parameters 28 days after TAC (Table 1), organ weight, histological and molecular analysis as well as protein phosphorylation status were tested using GraphPad Prism software using a 2-way (pressure-overload x pregnancy) ANOVA followed by a Bonferroni post-test. Two separate interactions were studied in the echocardiographic
comparison of pregnant and non-pregnant sham and TAC mice at day 28 (4 wks after TAC before the onset of pregnancy) and day 46 (sacrifice) (Table 2). Within each surgery group (sham and TAC) a repeated measures ANOVA, followed by a Bonferroni post-test, was performed to test whether the differences in means of the echographic parameters between pregnant and non-pregnant mice in a surgery group changed with time after surgery. Additionally, for both the 28 days and 46 days data sets separately, 2-way ANOVAs, followed by a Bonferroni post-test, were performed to test whether the effect of TAC compared to sham on echocardiographic parameters differed between pregnant and non-pregnant mice. Fetal outcomes were compared using an unpaired t-test. A value of $P \leq 0.05$ was considered statistically significant and data are presented as mean $\pm$ SEM.

RESULTS

The distribution of the 46 mice across the various subgroups is shown in Fig. 1.

Maternal outcome

Mortality. In the TAC group, 4 out of 27 mice died within 24 hours after the operation. Two additional TAC mice died after mating (one after at least 10 days of confirmed pregnancy and one 14 days after mating, without pregnancy), which could be attributed to cardiac dysfunction as suggested by a significant increase of lung weight postmortem. These 6 mice were excluded from further analysis. No deaths occurred in the 21 sham-operated mice.

Fertility. In the sham group, 2 out of the 12 mated mice failed to become pregnant (17%), whereas in 6 of the 16 mated TAC animals (38%) mating did not result in pregnancy ($p=0.2$).

Effect pregnancy on TAC-induced alterations in cardiac geometry and function. The impact of TAC was analyzed using echocardiography performed at 28 days postoperatively (Table 1). No
significant difference in heart rate was detected. LVEDD and particularly LVESD were significantly increased with TAC compared to sham animals, resulting in a reduction of LVFS in TAC mice.

Repeated measures ANOVA of the echocardiographic data revealed that for none of the LV dimension and functional parameters, or any of the surgery groups (sham and TAC), a significant interaction was found between time after surgery (28 and 46 days) and pregnancy.

Similar to the combined groups in Table 1, 28 days of TAC enlarged LVEDD and LVESD and decreased LVFS (P<0.001 for all groups) but these TAC-induced alterations were not different between the non-pregnant and pregnant mice.

In contrast, 46 days after surgery, the TAC-induced increase in LVEDD was significantly mitigated by pregnancy (interaction term of surgery by pregnancy: P=0.05). Accordingly, LVEDD was smaller in the pregnant TAC mice compared to the non-pregnant TAC animals (P=0.05) and the LVEDD of pregnant TAC mice was not significantly different from the LVEDD of pregnant sham animals at day 46 (P=0.11) (Table 2). Additionally at day 46, LVESD was increased in TAC mice (P<0.001) and reduced by pregnancy (P=0.02). However, there was no significant interaction between pregnancy and surgery on LVESD (interaction term of surgery by pregnancy: P= 0.28), meaning that pregnancy reduced LVESD equally in sham and TAC mice. Similarly, 46 days of TAC decreased LVFS (P<0.001) and pregnancy enhanced LVFS (P<0.001), but pregnancy did not significantly affect the TAC-induced decrease of LVFS (interaction term of surgery by pregnancy P=0.20) (Table 2).

Effect pregnancy and TAC on organ weight. As TAC and pregnancy both likely influence body weight, relative organ weights were corrected for initial body weight at day 0 before induction of TAC and pregnancy. As expected, 46 days of TAC increased relative LV, RV and lung weights in non-pregnant as well as pregnant mice (Fig. 2). Pregnancy increased relative LV and RV weights
but did not significantly affect relative lung weight in sham-operated mice (Fig. 2 A, B and C). Relative LV and RV weight were not significantly affected by pregnancy in TAC mice, but lung weight (a marker for pulmonary congestion) was lower in pregnant TAC mice compared to non-pregnant TAC mice (interaction term 0.026). TAC increased liver weight similarly in both pregnant and non-pregnant animals and did not affect corrected uterus weight (Fig. 2 F and G) that was, as expected, markedly increased in the pregnant animals.

Histomorphometry. In line with relative LV weights, mean cardiomyocyte cross sectional area seemed higher in pregnant than in non-pregnant sham mice but this failed to reach statistical significance (Fig. 3 A and B). Pregnancy did not induce interstitial fibrosis or affect capillary density in pregnant sham mice (Fig. 3 A, C and D). As expected, TAC increased cardiomyocyte cross-sectional area, which was accompanied by an increase in LV interstitial collagen content and a reduction in capillary density. Pregnancy did not significantly affect cross sectional area, fibrosis content or capillary density in TAC mice (interaction terms 0.56, 0.72, 0.19 respectively) (Fig. 3).

Molecular Markers for Cardiac Pathology. TAC increased mRNA expression of Nppa and Nppb (Fig. 4 A and B) as well as the MYH6/MYH7 ratio (Fig. 4 C) and reduced Atp2a2 mRNA expression (Fig. 4 D) irrespective of pregnancy. Interestingly, the increase of Nppa expression was less pronounced in pregnant TAC mice than in non-pregnant TAC mice and a similar trend was observed for the MYH6/MYH7 ratio (interaction term = 0.1) (Fig. 4 A and C).

Next, we assessed the activity of several pathways that have been shown to have important effects on cardiac function. Pregnancy did not affect the phosphorylation of any of our studied proteins in sham-operated mice. Similarly TAC by itself did not affect the activity of these pathways in non-pregnant animals. However in TAC pregnant compared to TAC non-pregnant mice JNK phosphorylation was reduced (Fig. 5 A), the phosphorylation of c-Jun was increased (Fig. 5 B) and
both MEK1 \((p=0.052)\) (Fig. 5C) and ERK1/2 phosphorylation \((p=0.068)\) (Fig. 5D) tending to be increased while p38 phosphorylation was unchanged (Fig. 5E). Additionally in TAC mice pregnancy reduced the phosphorylation of PI3K (Fig. 5F), yet left VEGFR2 and Akt phosphorylation unaltered (Fig. 5 G and H), while the phosphorylation of STAT1, but not STAT3, was increased by pregnancy in TAC mice (Fig. 5 I and J) and the NFκB axis was not affected (Fig. 5 K and L).

**Fetal outcome**

In none of the TAC mice was pregnancy terminated before the planned sacrifice on day 18, suggesting that TAC did not affect pregnancy duration. There was no significant difference in litter size between TAC and sham-operated mice \((6.5±0.6 \text{ vs. } 6.6±0.5)\). Pup weight was ~15% lower in TAC vs sham operated mice \((1.23±0.04 \text{ g vs. } 1.06±0.09 \text{ g})\), although this did not reach significance \((P=0.11)\).

**DISCUSSION**

The present study is the first to investigate the impact of LVOT obstruction induced LVH and LV dysfunction on maternal fertility and pregnancy outcome and assess the impact of pregnancy on LVOT obstruction-induced changes in myocardial structure and function. The main findings were that (i) aortic stenosis did not significantly affect fertility, litter size, pregnancy duration or pup weight. (ii) Pregnancy tended to reduce TAC-induced LVH and blunted TAC-induced LV dilation as well as pulmonary congestion, coinciding with a higher capillary density and reduced expression of the LVH-associated gene Nppa and the MYH6/MYH7 ratio. The implications of these findings are discussed.

*Effect of Aortic Stenosis on Fertility, Pregnancy, Delivery and Fetal Outcome.* TAC has been...
validated as a reproducible method to study the cardiac response to LVOT obstruction in rodents (32, 35). In the present study, we focused on the development of LV hypertrophy, the type of hypertrophy (physiological or pathological) and the severity of LV dysfunction observed after TAC with and without pregnancy. We found that 28 days of severe transverse aortic constriction caused LV hypertrophy with progressive LV dilation and dysfunction. However, even in this compromised state, TAC mice were still able to conceive normal sized litters with normal pup weight, and carry pregnancies to full term. However, while litter size was well maintained, birth weight tended to be somewhat (~15%) lower in TAC than in sham mice (p=0.11). The trend towards lower body weight was not due to differences in uterus weights between sham and TAC mice, suggesting that although there was severe outflow obstruction, these tissues had developed normally. Several clinical studies have suggested that a reduced cardiac output results in uterine under-perfusion and is associated with a shorter pregnancy duration and lower birth weight (4, 16, 30, 38). Our study does not support these observations, as pregnancy was of a normal duration and birth weights were not significantly lower in the pups of TAC mice.

Impact of Pregnancy on Maternal Outcome and on Cardiac structure and Function. Interestingly, pregnancy did not adversely affect maternal outcome or cardiac function. In fact, pregnancy appeared to ameliorate pulmonary congestion and LV remodeling, which was confirmed at the molecular level by lower expression of molecular markers of pathological hypertrophy, such as Nppa and MYH6/MYH7 ratio. Likely as a consequence of the reduced LV remodeling, capillary rarefaction in TAC mice was blunted by pregnancy. Taken together, these findings indicate that, despite adding to the hemodynamic LV overload by TAC, pregnancy has a beneficial effect on pathological LV remodeling.

The reduction in TAC-induced LV dilation by pregnancy was associated with the absence of
pulmonary congestion. The prevention pulmonary congestion cannot be ascribed to a better LV function, as the progressive deterioration of LV fractional shortening between 28 and 46 days of TAC was not affected by pregnancy. Hence, it appears that an improvement in LV diastolic properties was responsible for the alleviation of pulmonary congestion. Important determinants of LV compliance are LV wall thickness, collagen content and passive force of cardiomyocytes. Since the severe myocardial interstitial fibrosis produced by TAC was not influenced by pregnancy, it follows that pregnancy may have blunted the TAC-induced increase in passive force of cardiomyocytes in TAC mice (35), or that pregnancy improved LV diastolic properties via the reduction in LV mass, translating into a more compliant chamber. Determining the exact mechanism by which pregnancy affects LV diastolic properties should be the subject of future studies.

The mechanism by which pregnancy blunted LV remodeling in TAC mice is unclear, but it is possible that higher circulating levels of estrogen play a pivotal role (15, 17). High estrogen levels in adult females (particularly during pregnancy) are known to exert cardioprotective effects (11, 25) and protect against pressure overload induced cardiac hypertrophy (10, 36). Activation of the endothelial nitric oxide synthase pathway by estrogen, enhances endothelial function, arterial dilation and angiogenesis that combined reduces cardiac afterload and improves LV function in the setting of pathological LV remodeling (21). Finally, estrogen has been shown to exert anti-hypertrophic effects on isolated myocytes (22) and positively influences the cardiac response to ischemic heart disease (7, 20).

The pregnancy-induced improvement in capillary density in TAC mice may have been, at least in part, responsible for the attenuation of LV remodeling and may be related to the pregnancy-associated increase in placental growth factor, a member of the vascular endothelial growth factor (VEGF) family (1, 27). However, we found no increased activation (phosphorylation)
of the VEGF receptor 2 in either TAC treated groups compared to sham treated animals, suggesting
that the activity of this axis was not enhanced. Interestingly, it has been shown that a switch in
cardiomyocyte VEGF signaling from VEGR2 (also known as KDR) to VEGFR1 (Flt-1) can reduce
the effects of pressure overload and the hypertrophic phenotype (26, 40). PI3K has two isoforms:
p110α, thought to be involved in adaptive hypertrophy i.e. in response to exercise and p110γ, which
signals through G protein-coupled receptors and is thought to be involved in pathological
hypertrophy (2, 28). Pregnancy did not alter PI3K phosphorylation levels in sham animals, but did
show a statistically significant decrease in pregnant compared to non-pregnant TAC animals.
Unfortunately our assay cannot determine which isoform of PI3K is being phosphorylated, but it
could be argued that we are seeing a decrease in the p110γ forms activity as we see no significant
increases in MEK1 and ERK1/2 and the pregnant TAC animals displayed a reduced hypertrophic
phenotype.

There is some controversy over the role of MAPK terminal effectors in hypertrophy (29). We
saw no change in phosphorylation levels of p38 in any of the four in vivo groups and a decrease in
JNK phosphorylation in pregnant TAC compared to non-pregnant TAC animals. A similar effect was
seen in an elegant in vivo experiment by Bueno et al (5). Differential dual-specificity phosphatase
MKP-1 limits the cardiac hypertrophic response in vitro and in vivo and may explain why c-Jun is
elevated, but JNK is reduced. Bueno et al showed that constitutive expression of MKP-1 led to
dephosphorylation of JNK and p38, and to a lesser extent, ERK1/2 (with no change in total protein
levels), resulting in a significant attenuation of load-induced hypertrophy in the mice themselves.
We did find a significant increase in c-Jun phosphorylation, which has been associated with a
reduced hypertrophic phenotype by Windak et al. (37), who used striated-muscle specific c-Jun−/−
knockout mice, to show that c-Jun is necessary to correct cytoskeleton and sarcomere organization
and to prevent cardiomyocyte apoptosis. Thus, its absence inhibits adaptive remodeling in hearts subjected to pressure overload and leads to pathological hypertrophy and cardiomyopathy (37).

The reduced hypertrophy phenotype seen in our pregnant TAC mice compared to non-pregnant TAC mice, alongside this reduced expression of molecular markers of pathological hypertrophy, is supported by Haq et al (19) who showed that increased JNK, p38 and ERK1/2 phosphorylation was associated with failing hearts rather than hypertrophy, supporting our view that pregnancy is improving the phenotype in TAC animals. It has also been postulated that MEK1 and ERK1/2 are activated by progesterone during pregnancy, promoting a ‘prosurvival pathway’ to counteract the normally harmful prolonged volume overload, which is a normal feature of the cardiac system in pregnancy (9). Additionally, increased MAPK activity has been associated with an E2-induced reversal of LVH (22) and therefore may have mediated the protective effects in our model through this mechanism too.

We saw no significant changes between the four experimental groups in either IKBα nor NFκB-phosphorylation levels, although NFκB is postulated to have a role in cardiac hypertrophy (18). Zelarayan et al (39) showed that this role is sex-specific, being adaptive in males only, so we do not believe it is playing a role in our model. STAT1 phosphorylation was increased, but STAT3 phosphorylation was unaffected. Most data described the effect of the JAK/STAT3 pathway in cardiac hypertrophy, where STAT3 is predominantly thought to be protective (14). In contrast, in some situations, STAT1 was found to be pro-apoptotic (23). Thus, our finding of increased STAT1 phosphorylation is unlikely to contribute to the protective effect of pregnancy.

It should be noted that we studied our animals at the end of pregnancy, when the physiological hypertrophic effects are decreasing (8), so the exact mechanisms responsible for the beneficial effect on cardiac function may have occurred earlier and might be obscured at the time of the study.
In conclusion, the present study shows that cardiac dysfunction associated with LVOT obstruction does not impair fertility, pregnancy outcome and that pregnancy does not deteriorate LV dysfunction but actually exerted a beneficial effect on pathological cardiac remodeling as well as pulmonary congestion and pathological gene expression produced by aortic stenosis.

GRANTS

HX received funding from Shanghai Jiao Tong University for one-year research fellowship in Rotterdam, the Netherlands. Funding for MRJ, BRH and SRS was provided by Borne, the Chelsea & Westminster Health Charity, UK.

DISCLOSURES

None

REFERENCES

8. Chung E, Heimiller J, and Leinwand LA. Distinct cardiac transcriptional profiles defining pregnancy and


**Figure Legends.**

**Figure 1**
The distribution of mice across the various subgroups of the study.

**Figure 2**
The effects of TAC and pregnancy on animal body and relative organ weight. Sham non-preg, n= 9; sham preg, n=10; TAC non-preg, n=12; TAC preg, n=9. TAC, transverse aortic constriction; BWd0, bodyweight at day 0; preg, pregnant; LV, left ventricular; RV, right ventricular; d46, at day 46. *P<0.05 vs. corresponding sham; †P<0.05 vs. corresponding non-pregnant.

**Figure 3**
Histological analyses of the effects of TAC and pregnancy on cardiomyocyte cross sectional area (B), fibrosis (C) and capillary density (D) with representative images of Hematoxylin and Eosin (HE), Picrosirius Red (PSR) and Lectin staining (A). 6-8 histology images of 6 mice per group were analyzed. *P<0.05 vs. corresponding sham; †P<0.05 vs. corresponding non-pregnant.

**Figure 4**
Molecular analysis of the influence of TAC and pregnancy on hypertrophy related biomarkers. TAC, transverse aortic constriction; Nppa, natriuretic peptide type A; Nppb, natriuretic peptide type B; MYH, Myosin heavy chain; Atp2a2, sarcoplasmic reticulum Ca^{2+} ATPase; preg, pregnant. (Sham non-preg, n= 9; sham preg, n=9; TAC non-preg, n=12; TAC preg, n=9). *P<0.05 vs. corresponding sham; †P<0.05 vs. corresponding non-pregnant.
Figure 5

Phosphorylation status of proteins involved in cardiac pathology. JNK, c-Jun N-terminal kinase; MEK-1, mitogen-activated protein kinase kinase 1; ERK1/2, extracellular signal-regulated kinase 1/2; PI3K, phosphoinositide 3-kinase; VEGFR2, vascular endothelial growth factor receptor 2; Stat, signal transducers and activators of transcription; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; NFκB, nuclear factor-κB. (Sham non-preg, n=9; sham preg, n=10; TAC non-preg, n=12; TAC preg, n=8). †P<0.05 vs. corresponding non-pregnant.

The data is represented as median (dotted line) and interquartile range (error bars). Graphs A, G and K have been plotted on a log10 scale whilst all others are on a linear scale.
Table 1: Echocardiographic changes in LV parameters in mice with TAC versus sham from baseline to 28 days post-surgery

<table>
<thead>
<tr>
<th></th>
<th>group</th>
<th>sham</th>
<th>TAC</th>
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<tbody>
<tr>
<td><strong>Mice (n)</strong></td>
<td>0 days</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>0 days</td>
<td>500 ± 11</td>
<td>524 ± 9</td>
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<tr>
<td></td>
<td>28 days</td>
<td>508 ± 6</td>
<td>502 ± 8</td>
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<tr>
<td><strong>LVEDD (mm)</strong></td>
<td>0 days</td>
<td>3.3 ± 0.04</td>
<td>3.3 ± 0.14</td>
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<tr>
<td></td>
<td>28 days</td>
<td>3.3 ± 0.05</td>
<td>3.8 ± 0.10 *†</td>
</tr>
<tr>
<td><strong>LVESD (mm)</strong></td>
<td>0 days</td>
<td>1.8 ± 0.05</td>
<td>1.9 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>1.8 ± 0.06</td>
<td>3.0 ± 0.10 *†</td>
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<tr>
<td><strong>LVFS (%)</strong></td>
<td>0 days</td>
<td>45 ± 1</td>
<td>44 ± 3</td>
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<tr>
<td></td>
<td>28 days</td>
<td>44 ± 1</td>
<td>22 ± 1 *†</td>
</tr>
</tbody>
</table>

HR, heart rate; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVFS, left ventricular fractional shortening. *P<0.05 vs. corresponding sham; †P<0.05 vs. corresponding baseline (0 days).
Table 2: Influence of pregnancy on TAC-induced alterations of echocardiographic LV parameters 28 days (potential onset of pregnancy) and 46 days post surgery (potential late pregnancy).

<table>
<thead>
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<th>Group</th>
<th>28 days post surgery (onset of pregnancy)</th>
<th>46 days post surgery (late pregnancy)</th>
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</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sham</td>
<td>non-pregnant 511 ± 8 506 ± 10</td>
<td>529 ± 11 518 ± 6</td>
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<tr>
<td>TAC</td>
<td>non-pregnant 490 ± 11 522 ± 10</td>
<td>487 ± 16 514 ± 12</td>
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<td>LVEDD (mm)</td>
<td>non-pregnant 3.32 ± 0.06 3.14 ± 0.07</td>
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<tr>
<td>TAC</td>
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<td>4.41 ± 0.15* 3.95 ± 0.12†</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>non-pregnant 1.92 ± 0.05 1.80 ± 0.05</td>
<td>2.12 ± 0.05 1.92 ± 0.03</td>
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<td>sham</td>
<td>non-pregnant 3.17 ± 0.16 2.83 ± 0.13</td>
<td>3.73 ± 0.20 3.21 ± 0.14</td>
</tr>
<tr>
<td>TAC</td>
<td>non-pregnant 3.17 ± 0.16 2.83 ± 0.13</td>
<td>3.73 ± 0.20 3.21 ± 0.14</td>
</tr>
<tr>
<td>LVFS (%)</td>
<td>non-pregnant 44 ± 2 46 ± 1</td>
<td>39 ± 1 16 ± 1</td>
</tr>
<tr>
<td>sham</td>
<td>non-pregnant 43 ± 1 20 ± 2</td>
<td>39 ± 1 16 ± 1</td>
</tr>
<tr>
<td>TAC</td>
<td>non-pregnant 43 ± 1 20 ± 2</td>
<td>39 ± 1 16 ± 1</td>
</tr>
</tbody>
</table>

HR, heart rate; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVFS, left ventricular fractional shortening. *P≤0.05 vs. corresponding sham; †P≤0.05 vs. corresponding non-pregnant; #P<0.001 TAC (combined non-pregnant & pregnant) vs sham (combined non-pregnant & pregnant); $P≤0.05 pregnant (combined sham & TAC) vs. non-pregnant (combined sham & TAC).
Figure 2
Figure 3
Figure 4
Figure 5