Sex differences in exercise-induced physiological myocardial hypertrophy are modulated by oestrogen receptor beta

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Aims

Oestrogen receptor alpha (ERα) and beta (ERβ) are involved in the regulation of pathological myocardial hypertrophy (MH). We hypothesize that both ER are also involved in physiological MH. Therefore, we investigated the role of ER in exercise-induced physiological MH in loss-of-function models and studied potential mechanisms of action.

Methods and results

We performed 1 and 8 weeks of voluntary cage wheel running (VCR) with male and female C57BL/6J wild-type (WT), ERα- and ERβ-deleted mice. In line with other studies, female WT mice ran more than males (P ≤ 0.001). After 8 weeks of VCR, both sexes showed an increase in left ventricular mass (females: P ≤ 0.01 and males: P ≤ 0.05) with more pronounced MH in females (P < 0.05). As previously shown, female ERα-deleted mice run less than female WT mice (P < 0.001). ERβ-deleted mice showed similar running performance as WT mice (females vs. males: P ≤ 0.001), but did not develop MH. Only female WT mice showed an increase in phosphorylation of serine/threonine kinase (AKT), ERK1/2, p38-mitogen-activated protein kinase (MAPK), and ribosomal protein s6, as well as an increase in the expression of key regulators of mitochondrial function and mitochondrial respiratory chain proteins (complexes I, III, and V) after VCR. However, ERβ deletion abolished all observed sex differences. Mitochondrial remodelling occurred in female WT-VCR mice, but not in female ERβ-deleted mice.

Conclusion

The sex-specific response of the heart to exercise is modulated by ERβ. The greater increase in physiological MH in females is mediated by induction of AKT signalling, MAPK pathways, protein synthesis, and mitochondrial adaptation via ERβ.

Keywords

Exercise-induced physiological MH • Sex • Oestrogen receptor • Hypertrophy associated • Signalling pathway • Mitochondrial adaptation

1. Introduction

Exercise-induced physiological myocardial hypertrophy (MH) is characterized by an increase in cardiac mass and cardiomyocyte dimension.1 In contrast to pathological MH, physiological MH does not lead to fibrosis and is fully reversible.1 Physiological MH-associated signalling pathways include the phosphoinositide 3-kinase (PI3-K)-serine/threonine kinase (AKT) and mitogen-activated protein kinase (MAPK) pathways, glycogen synthase kinase-3β (GSK-3β), AMP-dependent protein kinase (AMPK), and p70 s6 kinase (S6K) and its down-stream...
target s6 ribosomal protein (s6), which is involved in protein synthesis.\textsuperscript{2,3} Specifically, activation of AKT plays a pivotal role in the development of exercise-induced physiological MH.\textsuperscript{3} Stimulation of physiological MH-associated signalling pathways in pathological settings has been shown to be cardioprotective.\textsuperscript{4,5} For instance, increased PI3-K–AKT activity led to improved survival in a mouse model of dilated cardiomyopathy and to favourable effects on cardiac function and fibrosis under pressure overload.\textsuperscript{5,6} Therefore, understanding the mechanisms and signalling pathways leading to physiological MH may lead to new strategies to prevent and treat pathological MH.

Sex differences exist in exercise-induced physiological MH. Female mice showed a higher increase in cardiac mass compared with males, independent of forced or voluntary exercise character.\textsuperscript{7–9} The greater capacity of female hearts to develop physiological MH could contribute to the more favourable remodelling in women under pathological pressure overload.\textsuperscript{10,11} This sexually dimorphic cardiac response may be due to sex hormones or sex differences in gene expression.\textsuperscript{12}

We and others showed that 17β-oestradiol (E2) and its receptors, oestrogen receptor alpha (ERα), and beta (ERβ) impact the heart by modulating cardiac morphology, function, and gene expression in both sexes.\textsuperscript{13–21} Recently, we showed that ERα agonist (16α-LE2) inhibited MH, fibrosis and supported maintenance of cardiac function after transverse aortic constriction (TAC) in female ovariectomized mice.\textsuperscript{18} Furthermore, ERβ deletion increased TAC-induced cardiomyocyte hypertrophy in both sexes. However, ERβ deletion increased cardiac fibrosis only in female mice, whereas it decreased fibrosis in male mice.\textsuperscript{17} Thus, ERα and ERβ contribute in a sex-specific manner to the development of pathological MH. Therefore, we hypothesized that ER are also involved in physiological MH development. Dissecting the related pathways may offer new therapeutic opportunities to shift pathological MH to a more physiological form.

During exercise-induced physiological MH, mitochondrial adaptations are needed to cope with the heart’s increased energy demand, which can be achieved by increased mitochondrial biogenesis\textsuperscript{22,23} or mitochondrial remodelling.\textsuperscript{24} A number of studies indicate that E2/ER affect the mitochondria directly by transcriptional regulation of mitochondrial proteins and regulate electron transport chain activity and oxygen consumption in different cell types and tissues.\textsuperscript{25}

The aim of our present study was to determine whether ERα and ERβ modulate the development of exercise-induced physiological MH and associated signalling pathways in a sex-specific manner using loss-of-function models. We also explored sex differences in mitochondrial adaptation that could contribute to a sexual dimorphism in physiological MH in an ER-dependent manner.

2. Methods

2.1 Animals

Experiments were performed in accordance with the guidelines of Charité Universitätsmedizin, were approved by the Landesamt für Gesundheit und Soziales (LaGeSo, Berlin, Germany; G0148/06, G0370/08), and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of health (NIH Publication No. 85-23, revised 1996). Twelve-week-old male and female C57Bl/6J- (WT mice; Harlan Winkelmann), ERβ-deleted (ERβ−/−),\textsuperscript{26} ERα-deleted mice (ERα−/−),\textsuperscript{27} and their WT littermates, were kept on a 12:12 h light–dark cycle in temperature-controlled rooms, fed with commercial standard chow (ssniff-Spezialdiäten GmbH, Soest, Germany) and water ad libitum. WT mice were randomly assigned to 1 or 8 weeks of voluntary cage wheel running (VCR; 8 weeks: n = 11, 1 week: n = 8 for each sex) or sedentary (sed) group (8 weeks: n = 8, 1 week: n = 6 for each sex). ERβ−/− and ERα−/− mice and WT littermates were also randomly assigned to both the VCR and sed groups (n = 12 each group and sex; details on the assessment of daily running distance see Supplementary material online). Since WT mice and WT littermates of ER-deleted mice showed similar exercise performance and cardiac adaptation, animals were taken together and compared with ERβ−/− and ERα−/− mice. To assess the oestrous cycle stage in females, vaginal smears were examined.

2.2 Echocardiography

Echocardiography was performed at week 0, 1, and 8 as previously described (for details see Supplementary material online).\textsuperscript{17}

2.3 Tissue sampling

After VCR, mice were anaesthetized with isoflurane (1.5%) and killed by cervical dislocation. Hearts were harvested, left ventricle (LV) isolated, immediately snap frozen in liquid nitrogen, and stored at −80°C or fixed in 4% formaldehyde for at least 24 h; until use for gene and protein or histological analyses.

2.4 Histology

Cardiac histology was performed as described earlier.\textsuperscript{17} Haematoxylin/eosin staining was carried out to determine cardiomyocyte diameter and picrosirius red staining to obtain collagen content (for details see Supplementary material online).

2.5 Quantitative real-time polymerase chain reaction

Total RNA isolation and quantitative real-time PCR were carried out as previously described.\textsuperscript{17,21} mRNA content of target genes was normalized to expression of hypoxanthine phosphoribosyltransferase. Primer sequences are listed in Supplementary material online, Table S1.

2.6 Western blot

Whole cell extracts were isolated using modified RIPA buffer and separated by SDS–polyacrylamide gel electrophoresis as recently described.\textsuperscript{21} Antibodies used for western blotting are listed in Supplementary material online.

2.7 Transmission electron microscopy

LV samples were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) over night, post-fixed with 2% Osmiumtetroxid in 0.1 M cacodylate buffer for 2 h, dehydrated with graded ethanol solutions, and embedded in Epon (SERVA). Ultrathin sections were stained with uranyl acetate and lead citrate.\textsuperscript{18} Electron micrographs were captured using a transmission electron microscope (EM 906, Zeiss), and mitochondrial area and number were evaluated (for details see Supplementary material online).

2.8 Cell culture

AC16 cells, a human cardiomyocyte-like cell line,\textsuperscript{29} were cultured as recently described.\textsuperscript{21} Prior to E2- and ERβ-agonist treatment, cells were starved with 2.5% charcoal-stripped FBS in phenol red-free media, and subsequently incubated with E2 (10−8 M; Sigma), ERβ agonist (10−7 M; Karo Bio, Sweden), or ethanol as vehicle for 12 h at 37°C in 5% CO2.

2.9 Statistical analysis

Data are shown as mean ± standard error of the mean (SEM). Data were analysed for sex and treatment separately in the genotypes by two-way ANOVA followed by Bonferroni post hoc test, unpaired t-test was used for comparisons between two groups, and one-way ANOVA followed by Dunnett’s post hoc test was used to analyse E2 and ERβ-agonist effects in AC16 cells using GraphPad Prism 5.01. A P-values ≤0.05 were considered statistically significant.
3. Results

3.1 Effect of sex on the development of physiological MH

After 8 weeks of VCR, female WT mice ran longer distances (Figure 1) with higher speed and for a longer time period than males (see Supplementary material online, Figure S1A and B). A significant increase in left ventricular mass related to tibia length (LVM/TL) was induced by VCR in both sexes compared with sed controls (Table 1). However, the increase in LVM/TL was more pronounced in female VCR mice than in male VCR mice (see Supplementary material online, Figure S1C). Cardiomyocyte diameter increased only in female VCR mice (Table 1 and see Supplementary material online, Figure S1D). Cardiac stress markers, such as atrial natriuretic peptide type A (Nppa) and brain natriuretic peptide type B (Nppb) and collagen content in LV tissue, were not induced by VCR (see Supplementary material online, Figure S2). These data indicate the development of physiological MH in WT mice with a clear sexual dimorphism.

3.2 Effect of ER on running behaviour and development of physiological MH

To dissect the role of ER on exercise-induced physiological MH, we subjected female and male ERα−/− and ERβ−/− mice to 8 weeks of VCR. Female ERα−/− mice ran less than female WT mice (Figure 1A) based on lower running time and speed (see Supplementary material online, Figure S1A and B). However, male ERα−/− mice performed similar to male WT mice (Figure 1A and see Supplementary material online, Figure S1A and B), indicating that ERα is required for exercise behaviour in females, but not in males. In line with their limited running performance, female ERα−/− mice did not increase LVM/TL after VCR (see Supplementary material online, Table S2). In contrast, ERβ deletion did not modify running behaviour. Female and male ERβ−/− mice had similar running performances compared with WT mice (Figure 1A and see Supplementary material online, Figure S1A and B). However, in contrast to WT mice, LVM/TL did not increase in ERβ−/− mice compared with sed controls (Table 1). Thus, exercise-induced physiological MH development depends on ERβ in both sexes.

3.3 Effect of sex and ERβ on phosphorylation of AKT and p38-MAPK

We next tested whether hypertrophic-associated signalling pathways display sex differences and are modulated by ERβ. Phosphorylation of AKT (pAKT) and p38-MAPK (p-p38-MAPK) were significantly increased in females after VCR, but not in males (Figure 2A and B). ERβ deletion precluded AKT and p38-MAPK activation in female mice after VCR.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Running performance of female and male WT, ERβ−/−, and ERα−/− mice. Female WT and ERβ−/− mice display significantly higher average daily running distance compared with males. Female ERα−/− mice show significantly less exercise performance compared with female WT mice. WT mice: n = 19–21 per group; ERβ−/−: n = 11–12 per group; ERα−/−: n = 9–10 per group. Two-way ANOVA: ***p < 0.001. WT, wild-type mice; ERβ−/−/ERα−/−, oestrogen receptor beta/beta-alpha deletion.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Echocardiographic parameters and cardiomyocyte diameter of male and female WT and ERβ−/− mice after 8 weeks of VCR</th>
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<tbody>
<tr>
<td><strong>Sex</strong></td>
<td><strong>WT-sed</strong></td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>19</td>
</tr>
<tr>
<td>LVM (mg)</td>
<td>110.4±3.0</td>
</tr>
<tr>
<td>LVM/TL (mg/mm)</td>
<td>6.3±0.15</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.64±0.01</td>
</tr>
<tr>
<td>PWd (mm)</td>
<td>0.65±0.01</td>
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<tr>
<td>LVIDd (mm)</td>
<td>4.54±0.06</td>
</tr>
<tr>
<td>EF (%)</td>
<td>45.8±1.8</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>434.5±17.7</td>
</tr>
<tr>
<td>Cardiomyocyte diameter (μm)</td>
<td>11.53±0.23</td>
</tr>
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</table>

Values show means ± SEM. All measurements were performed after 8 weeks of VCR. N, number of mice in each group; *N for cardiomyocyte diameter, 5–11; WT, wild-type mice; ERβ−/−, oestrogen receptor beta-deleted mice; sed, sedentary; VCR, voluntary cage wheel running; TL, tibia length; LVM, left ventricular mass; IVSd, septum thickness during diastole; PWd, posterior wall thickness during diastole; LVIDd, left ventricular internal diameter during diastole; HR, heart rate; EF, ejection fraction.

Two-way ANOVA: (*) intervention (sed vs. VCR) *p < 0.05, **p < 0.01, ***p < 0.001; (§) sex (female vs. male) WMP ≤ 0.001, HMP ≤ 0.01 and MP ≤ 0.05.
Figure 2  AKT, p38-MAPK, ERK1/2, and s6 are phosphorylated in female WT mice after VCR, abolished by ERβ deletion. (A) pAKT and (B) p38-MAPK are significantly induced only in female WT mice compared with sed after VCR, (C and D) which is abolished in ERβ⁻/⁻ mice. (E) Phosphorylation of ERK1/2 and (F) s6 are significantly induced in female WT mice, but not in males, after 1 week of VCR. (G) No changes in p-AKT and (H) p38-MAPK could be observed after 1 week of VCR. WT mice: n = 6–11 per group and sex (8 weeks); n = 4–8 per group and sex (1 week); ERβ⁻/⁻: 6–11 per group and sex. Data of 8 weeks of VCR are expressed as fold of male sed WT-/ERβ⁺/+ mice expression. Two-way ANOVA: *P < 0.05, **P < 0.01, ***P < 0.001; unpaired t-test: $P < 0.01$. WT, wild-type mice; ERβ⁻/⁻, ERβ-deleted mice; VCR, voluntary cage wheel running; sed, sedentary.
Furthermore, phosphorylation of ERK1/2, GSK-3β, AMPK, and S6k and its down-stream target s6 were not induced after 8 weeks of VCR (see Supplementary material online, Figure S3A—E). Since hypertrophy-associated signalling pathways can be activated at different time points, as shown by others,8 we examined pathways involved in protein synthesis at an earlier time point and subjected male and female WT mice to 1 week of VCR. Exercise behaviour of both sexes at 1 week of VCR was comparable to 8 weeks (see Supplementary material online, Table S3). Female mice exhibited a significant increase in phosphorylation of ERK1/2 and s6 (Figure 2E and F) without induction of p-AKT and p-p38-MAPK (Figure 2G and H). These data suggest that exercise-induced MH in female mice is accompanied by activation of AKT, p38-MAPK, ERK1/2 signalling, and protein synthesis in a time-dependent manner, mediated by ERβ.

3.4 Effect of sex and ERβ on mitochondrial gene and protein expression

Since hypertrophic growth is associated with changes in mitochondrial biogenesis,23 we tested whether mitochondrial key regulators are regulated in a sex-specific manner and by ERβ in VCR. The transcription factors nuclear respiratory factor (NRF)-1 and -2 were induced after VCR only in female WT mice (Figure 3A and B). This was abolished by ERβ deletion, suggesting the regulation of NRF-1 and -2 in exercising female mice is mediated by ERβ. MEF2C and ATP5K expression were significantly increased only in female WT mice after VCR (Figure 3E and F). This was abolished by ERβ deletion, suggesting the regulation of MEF2C and ATP5K in exercising female mice is mediated by ERβ. The data suggest that exercise-induced MH in female mice is accompanied by activation of AKT, p38-MAPK, ERK1/2 signalling, and protein synthesis in a time-dependent manner, mediated by ERβ.

**Figure 3** Mitochondrial key modulators are regulated in a sex-specific manner after VCR. (A) NRF-1 and (B) NRF-2 protein levels are higher in female WT mice compared with males after VCR. (C and D) Abolished in ERβ−/− mice. (E) Mef2a and (F) Atp5k expression are significantly increased only in female WT mice after VCR. (G and H) Abolished by ERβ deletion. WT mice: n = 7−11 per group and sex. ERβ−/−: 7−11 per group and sex. Data are expressed as fold of male sed WT-/ERβ2/2-mice expression. Two-way ANOVA: *P ≤ 0.05. WT, wild-type mice; ERβ−/−, ERβ-deleted mice; VCR, voluntary cage wheel running; sed, sedentary.
females by ERβ (Figure 3C and D). Myocyte enhancer factor 2A (Mef2a) and Atp5k, coding for a subunit of mitochondrial F1F0-ATPase, were up-regulated by VCR in female WT mice, but not in males (Figure 3E and F). Increased expression of both genes after VCR in females was abolished by ERβ deletion (Figure 3G and H), suggesting a regulating role of ERβ.

3.5 Effects of sex and ERβ on oxidative phosphorylation complex I, III, and V proteins

Protein level from complex I (NDUFB8) of oxidative phosphorylation (OXPHOS) chain was significantly increased in female, but not in male running WT mice (Figure 4A). Protein from complex III (UQCRC2; A) and complex V (ATPSa; B) were significantly higher in female VCR WT mice compared with VCR males. (C–F) Differences were abolished in ERβ−/− mice. WT mice: n = 5–11 and ERβ−/− mice: n = 9–11 per group and sex. GAPDH was used as internal standard. Data are expressed as fold of male sed WT-/ERβ−/−-mice expression. Two-way ANOVA: **P ≤ 0.01. WT, wild-type mice; ERβ−/−, ERβ-deleted mice; VCR, voluntary cage wheel running; sed, sedentary.
Figure 4B) and protein from complex V (ATPSa; Figure 4C) were higher in VCR female WT mice compared with males. All these differences were abolished by ERβ deletion (Figure 4D–F), suggesting that increased expression in female hearts of OXPHOS proteins is modified by ERβ.

3.6 Effects of sex and ERβ on mitochondrial remodelling

Exercise is also associated with mitochondrial remodelling. Therefore, we measured mitochondrial number and size distribution in the mouse heart. Mitochondrial number was increased after VCR in WT mice of both sexes (Figure 5A), abolished by ERβ deletion (Figure 5B). Particularly, the number of smaller mitochondria in both sexes increased (≤0.5 μm²; Figure 5C). In contrast, the number of large mitochondria (≥1 μm²) decreased significantly only in female WT mice after VCR (Figure 5D and E). Furthermore, we found a significant reduction in Mito-fusin (Mfn)-2 protein only in females (Figure 5F). These data indicate that exercise increased mitochondrial number and mitochondrial remodelling in both sexes, with a shift from large towards smaller mitochondria only in female VCR WT mice, modulated by ERβ.

3.7 Effects of 17β-oestradiol and ERβ on mitochondrial key regulators in a human cardiomyocyte-like cell line

To confirm that E2 and ERβ regulate MEF2A, NRF-1, and -2 in cardiomyocytes, we tested the effects of E2 and specific ERβ agonist in a cardiomyocyte-like cell line (AC16 cells). Supplementary material

![Figure 5](https://academic.oup.com/cardiovascres/article-abstract/102/3/418/2931074)
online, Figure S4). E2 treatment and selective ERβ activation significantly increased MEF2A, NRF-1, and -2 gene expression, indicating that E2 and ERβ agonists can increase the expression of mitochondrial key modulators in cardiomyocytes.

4. Discussion

The present study brings novel insights into the sexual dimorphic and ER-dependent development of exercise-induced MH. The development of exercise-induced MH depends on ERβ in both sexes. In females, but not in males, ERβ supports the activation of pro-hypertrophic signalling pathways, the regulation of mitochondrial regulators, OXPHOS chain proteins, and mitochondrial size distribution. Regulation of these pathways may explain the greater degree of exercise-induced physiological MH in females compared with males.

4.1 ERα is involved in running behaviour in female mice

The observed impaired running behaviour in female ERα-deleted mice is in agreement with other studies, showing that E2 and ERα are involved in running behaviour and locomotor activity in females, probably mediated by ERα in the brain. However, running behaviour in males was not sensitive to ERα deletion, possibly due to different oestrogenic sensitivity and control mechanisms as shown by others. Male ERα-/- mice showed an increase in LVM/TL, similar to male WT mice, indicating that ERα does not modulate the cardiac response due to exercise in males.

4.2 ERβ is involved in physiological MH in both sexes

ERβ deletion abolished an exercise-induced increase in LVM/TL in both sexes. In females, ERβ modulates pro-hypertrophic signalling pathways and mitochondrial adaptation (Figure 6). Male mice have circulating E2 levels and synthesize E2 from testosterone by aromatases in the heart locally and can therefore activate cardiac ERβ. Thus, we suggest that ERβ plays a role in the development of physiological MH also in male mice. Since expression levels of ERβ in the mouse heart are not different between sexes (see Supplementary material online, Figure S5A and B), it is conceivable that post-translational modification of ERβ may contribute to the observed sex differences in the cardiac response to exercise, which will be clarified in future studies.

4.3 Sex differences in intracellular signalling in physiological MH

In agreement with other studies, we observed sex differences in exercise-induced MH with a greater increase in cardiac mass and AKT activation in female WT mice. Going beyond these findings, we now show the activation of p38-MAPK, MAPK-ERK1/2, and s6 in female, but not in male, hearts after VCR. Thus, we describe novel pathways, which may explain greater exercise-induced MH in females.

After 1 week of VCR, female hearts exhibited a significant increase in ERK1/2 and s6 phosphorylation. This is in agreement with findings in MAPK-1 transgenic mice, indicating a prominent role for MAPK-ERK1/2 signalling in stimulating physiological MH. Furthermore, MAPK-ERK1/2 phosphorylates s6 in cardiomyocytes, leading to increased protein synthesis in females.
synthesis and cardiomyocyte growth. Thus, we suggest that hypertrophic growth in female hearts is induced after 1 week of VCR mainly via induction of both signalling pathways. After 8 weeks of VCR, female hearts showed significantly increased pAKT and p38-MAPK compared with males. Both are involved in the development of physiological MH and cardiomyocyte hypertrophy. In agreement with our recent study, showing that sex differences in gene and protein expression precede sex differences in cardiac morphology and function at a later time point, we assume that sexual dimorphism in MAPK-ERK1/2 and protein synthesis after 1 week causes sex differences in cardiac morphology at 8 weeks after VCR. We suggest that the time-dependent activation of MAPK-ERK1/2, p38-MAPK, AKT, and protein synthesis may be due to cyclic activation of the respective pathways upon exercise, leading to MH.

### 4.4 Role of ERβ in hypertrophy-associated signalling pathways

In agreement with other studies, showing that ERβ was necessary for the activation of PI3K/ AKT pathway in female rat hearts, in skeletal muscle, and rat heart myoblasts, exercise-induced activation of AKT in female hearts was abolished by ERβ deletion in our study. This may be partially due to a direct interaction between ERβ and AKT. Furthermore, it has been shown that the E2/ERβ complex had a stimulatory effect on p-p38-MAPK in cancer cells. Based on that, we suggest that ERβ impacts exercise-induced physiological MH in female hearts by modulating AKT and p38-MAPK phosphorylation. Although ERβ deletion abolished the development of physiological MH in male mice, we could not identify underlying mechanisms in the present study, which remain to be elucidated.

### 4.5 Sex differences in mitochondrial adaptation and the role of ERβ

We showed that physiological MH in females was accompanied with increased expression of mitochondrial-associated proteins and genes; e.g. NRF-1, -2, Mef2a, Atp5k, and OXPHOS proteins from complexes I, III, and V. NRF-1 and -2 are primarily involved in the regulation of nuclear- and mitochondrial-encoded genes. It has been also reported that regulatory effects of E2/ER and ERβ agonist diapropionitrite on mitochondrial proteins in a variety of cell types and tissues are mediated by NRF-1 and -2. This corroborates our findings that the increase in NRF-1, -2, Mef2a, Atp5k, and proteins of the OXPHOS complexes in female WT VCR mice is mediated by ERβ and is further supported by increased expression of these genes in AC16 cells upon ERβ activation.

Adaptation in mitochondrial function during exercise-induced MH can also be achieved by mitochondrial remodelling, regulated by ERβ. In agreement with our recent study, supported by increased expression of these genes in AC16 cells upon exercise, leading to MH.

### 5. Clinical relevance

Activation of physiological MH-associated signalling pathways in the pathological setting is suggested to be cardioprotective. Sex differences in the development of pathological MH in humans have been reported. Recently, we and others showed in animal models that in both sexes the development of pathological MH and cardiac remodelling is affected by ERβ. Therefore, we now propose that targeting ERβ with selective agonists to stimulate signalling pathways of physiological cardiac adaptation may be rewarding in both sexes and could provide novel therapeutic options for the diseased heart. Furthermore, since E2 has been shown to up-regulate SUR2A levels, a regulatory subunit of the KATP channel, which confers cardioprotection under ischaemic conditions in both sexes induced by exercise, future studies considering the role of ERβ and SUR2A may also reveal additional mechanisms of clinical relevance.

### 6. Study limitations

Our study is limited to 1 and 8 weeks of VCR, since we wished to analyse initial and steady-state effects in exercise-induced MH. Thereby, we may have missed intermediary effects since different pathways can be activated at different time points. Using global ER-knockout mouse models, we cannot exclude systemic effects on non-cardiac tissues, such as observed for ERα in the mouse brain, leading to impaired running performance in female mice. Furthermore, we cannot determine which cardiac cell types are involved in the observed effects. Future studies with cardiac cell-specific ER deletion mice could help to overcome these limitations.

### 7. Conclusion

We offer a novel explanation for the greater ability for cardiac adaptation to VCR in female than in male mice. Physiological MH is characterized by greater activation of AKT, MAPKs, and protein synthesis as well as mitochondrial adaptation in females, and requires ERβ in both sexes.

### Supplemental material

Supplementary Material is available at Cardiovascular Research online.

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### Conflict of interest

M.M.D. is the patent holder on AC16 cells. Other authors declared no conflict of interest.

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### References


