Molecular and Functional Signature of Heart Hypertrophy During Pregnancy

Mansoureh Eghbali, Rupal Deva, Abderrahmane Alioua, Tamara Y. Minosyan, Hongmei Ruan, Yibin Wang, Ligia Toro, Enrico Stefani

Abstract—During pregnancy, the heart develops a reversible physiological hypertrophic growth in response to mechanical stress and increased cardiac output; however, underlying molecular mechanisms remain unknown. Here, we investigated pregnancy-related changes in heart structure, function, and gene expression of known markers of pathological hypertrophy and cell stretching in mice hearts. In late pregnancy, hearts show eccentric hypertrophy, as expected for a response to volume overload, with normal left ventricular diastolic function and a moderate reduction in systolic function. Pregnancy-related physiological heart hypertrophy does not induce expression changes of known markers of pathological hypertrophy like: α- and β-myosin heavy chain, atrial natriuretic factor, phospholamban, and sarcoplasmic reticulum Ca$^{2+}$-ATPase. Instead, it induces the remodeling of Kv4.3 channel and increased c-Src tyrosine kinase activity, a stretch-responsive kinase. Cardiac Kv4.3 channel gene expression was downregulated by ≈3- to 5-fold, both at the mRNA and protein levels, and was paralleled by a reduction in transient outward K$^+$ currents, a longer action potential and by prolongation of the QT interval. Downregulation of cardiac Kv4.3 transcripts was mimicked by estrogen treatment in ovariectomized mice, and was prevented by the estrogen receptor antagonist ICI 182,780. c-Src activity increased by ≈2-fold in late pregnancy and after estrogen treatment. We propose that, in addition to mechanical stress, the rise of estrogen toward the end of pregnancy contributes to pregnancy-related heart hypertrophy by increased c-Src activity and that the rise of estrogen is one factor that down regulates cardiac Kv4.3 gene expression providing a molecular correlate for a longer QT interval in pregnancy. (Circ. Res. 2005;96:1208-1216.)

Key Words: heart hypertrophy ■ pregnancy ■ estrogen ■ $I_{so}$ ■ Kv4.3 channel

During pregnancy, the heart undergoes hypertrophic growth to compensate for the increased cardiac output. Cardiac hypertrophy has been defined as an increase in cardiomyocyte size that can be beneficial and adaptive (physiological) or a maladaptive (pathophysiological) phenomenon to compensate for the hemodynamic stress resulting from pressure or volume overloads. Pressure overload induces concentric hypertrophy characterized by wall thickening without significant chamber enlargement. Volume overload, as in pregnancy, induces eccentric hypertrophy characterized by chamber enlargement with a proportional change in wall thickness.1 Physiological hypertrophy is reversible and occurs during maturation, pregnancy, and exercise without morbid effects on cardiac function.2-4 Despite the growing knowledge of the molecular changes that can occur during pathological heart hypertrophy,1 the underlying molecular mechanisms of pregnancy-related heart hypertrophy are unknown.

In pathological heart hypertrophy, expression of a set of genes has been reported to be altered and, therefore, can be used as markers for this class of hypertrophy. Examples are, α-, β-myosin heavy chain (MHC), atrial natriuretic factor (ANF), phospholamban, sarcoplasmic reticulum Ca$^{2+}$-ATPase (SERCA),1,5 and the fast transient outward potassium current molecular constituents, contributing to QT interval, Kv4.3 and Kv4.2 transcripts.6-10

As sex hormone levels change during pregnancy, it is conceivable that genes that change during pregnancy-related cardiac hypertrophy are under hormonal control. In support of this view, Kv4.3 gene activity is downregulated by estrogen in the myometrium.11 In addition, reduction of Kv4.3 and Kv4.2 reduction after myocardial infarction can be reversed by the administration of thyroid hormone.12 Aside from the potential role of hormonal changes, increased stretching of cardiac myocytes is critical in inducing hypertrophy and is followed by activation of many signaling molecules within minutes of mechanical stimulation.13 Mechanical stretch of cardiomyocytes activate second messengers such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and Src tyrosine kinase.14,15 Interestingly, c-Src activity can

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From the Department of Anesthesiology (M.E., R.D., A.A., T.Y.M., H.R., Y.W., L.T., E.S.), Division of Molecular Medicine, Department of Molecular and Medical Pharmacology (L.T.), Department of Physiology (E.S.), Department of Medicine (Y.W.) and Brain Research Institute (L.T., E.S.), David Geffen School of Medicine at the University of California Los Angeles.

Correspondence to Dr Enrico Stefani, UCLA School of Medicine, Department of Anesthesiology, BH-520A CHS, Box 957115, Los Angeles, CA 90095-7115. E-mail estefani@ucla.edu

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be upregulated by estrogen.\textsuperscript{16} Because during pregnancy there is an increased risk of arrhythmias\textsuperscript{17} and a longer QT interval,\textsuperscript{18} we hypothesized that hormone and stretch responsive genes such as Kv4.3 and c-Src, respectively, could be modified in pregnancy-related heart hypertrophy. Here, we examined the molecular mechanisms leading to pregnancy-related hypertrophy in mice; we compared heart structure, function, and gene expression of known hypertrophic markers including the molecular constituents of the transient outward current ($I_{\text{to}}$), and c-Src activity in hearts of nonpregnant (NP) versus late pregnant (LP) mice. Our data reveal that pregnancy-related heart hypertrophy is associated with increased c-Src activity and Kv4.3 remodeling controlled by estrogen.

## Materials and Methods

### Animals and Hormonal Treatment

Nonpregnant (NP; diestrus), late-pregnant (LP, 19 to 20 days), and ovariectomized mice (C57/B6) were used. Protocols received institutional approval. Ovariectomized mice were injected subcutaneously with 3.5 $\mu$g/kg/d $17\beta$-estradiol (estrogen) for 6 days (twice a day) or 21 days (once a day), with estrogen (3.5 $\mu$g/kg/d) or ICI 182,780 (25 $\mu$g/kg) (6 days) or with vehicle (control). As a model of heart failure by pressure overload, male mice were subjected to

### Reagents

Antibodies were from: Anti-Kv4.3, Alomone (Jerusalem, Israel); anti-Src monoclonal, Upstate (Lake Placid, NJ), polyclonal (SRC 2), Santa Cruz; and antivinculin, Sigma (St. Louis, Mo). Monoclonal anti-Src monoclonal, Upstate (Lake Placid, NJ), polyclonal (SRC 2), and anti-Src recognizes c-Src and does not cross-react with Fyn, Yes or Lyn recombinant proteins (unpublished observations) and is referred to as monoclonal anti-c-Src.

### Cardiac Function

The echocardiography was performed under anesthesia (Avertin 2% IP) using a VisualSonics Vevo 660 equipped with a 35-MHz linear transducer.\textsuperscript{20} Electrocardiograms (ECGs) were obtained using built-in ECG electrode-contact pads (THM100, Indus Instruments) under constant isoflurane/O2 face mask anesthesia. Body temperature was maintained at 37°C. Standard Lead II ECGs were acquired to as monoclonal anti-c-Src.

### Myocyte Isolation

Isolated hearts were perfused through the aorta for three 10 to 30 minute sequential periods with: (1) Ca\textsuperscript{2+}-free Tyrode (mmol/L): 130 NaCl, 5.4 KCl, 1 MgCl\textsubscript{2}, 0.6 NaH\textsubscript{2}PO\textsubscript{4}, 10 HEPES, 5 Glucose, pH 7.4; (2) Ca\textsuperscript{2+}-free Tyrode plus 0.03 mmol/L CaCl\textsubscript{2}, 20 mmol/L taurine, 0.1% bovine serum albumin (BSA) and 52 U/mL Collagenase (GibcoBRL) with or without 0.4 mg/L protease Type XIV (Sigma); (3) KB solution (mmol/L): 25 KCl, 10 KH\textsubscript{2}PO\textsubscript{4}, 2 MgSO\textsubscript{4}, 5 HEPES, 0.5 EGTA, 20 taurine, 100 K-glutamate, 5 creatine, 20 glucose, 10 aspartic acid and 1% BSA, pH 7.4 or Ca\textsuperscript{2+}-free Tyrode followed by progressive addition of CaCl\textsubscript{2} up to 1 mmol/L.

### Electrophysiology

Whole-cell, voltage-, and current-clamp recordings were obtained at room temperature from right ventricular myocytes.\textsuperscript{22} Patch electrodes had resistances of 1.5 to 2.5 M\textOmega. Voltage clamp bath solution was (mmol/L): 136 NaCl, 4 KCl, 2 MgCl\textsubscript{2}, 1 CaCl\textsubscript{2}, 5 CoCl\textsubscript{2}, 0.02 tetrodotoxin (TTX), 10 HEPES, and 10 glucose, pH 7.4; action potential recordings were without Co\textsuperscript{2+} and TTX. The pipette solution contained (mmol/L): 135 KCl, 10 EGTA, 10 HEPES, and 5 glucose, pH 7.2. Analog signals were filtered at one-fifth of the sampling frequency (1 to 10 kHz). Series resistances were electronically compensated (\textapprox 85%); voltage errors resulting from the uncompensated series resistance were \textless 10 mV. Current amplitudes were normalized to the cell capacitance. Action potentials were recorded in response to 1 ms depolarizing current pulses.

### Cell and Tissue Staining

Cells were plated on laminin (10 $\mu$g/mL)-precoated coverslips, labeled with Texas Red-conjugated wheat germ agglutinin, and fixed with 4% paraformaldehyde. Myocytes were labeled with Texas Red-conjugated wheat germ agglutinin, and fixed with 4% paraformaldehyde.

### Cardiac Function in NP and LP mice

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>LP</th>
</tr>
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<tbody>
<tr>
<td>LV Volume (mm$^3$)</td>
<td>43.68±2.58</td>
<td>67.30±4.32*</td>
</tr>
<tr>
<td>LV %EF</td>
<td>73.28±4.49</td>
<td>56.95±1.27*</td>
</tr>
<tr>
<td>LV %SF</td>
<td>42.29±3.88</td>
<td>29.42±0.81*</td>
</tr>
<tr>
<td>E (mm/s)</td>
<td>897.2±57</td>
<td>1035.8±74.4</td>
</tr>
<tr>
<td>A (mm/s)</td>
<td>423.1±17.7</td>
<td>446.4±63.9</td>
</tr>
<tr>
<td>E/A</td>
<td>2.17±0.22</td>
<td>2.69±0.4</td>
</tr>
<tr>
<td>Electrocardiography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{\text{to}}$ ($\beta$A/PF)</td>
<td>48.9±6.7</td>
<td>29.5±2.7**</td>
</tr>
<tr>
<td>$I_{\text{f1}}$ ($\beta$A/PF)</td>
<td>24.8±4.1</td>
<td>12.2±1.7**</td>
</tr>
<tr>
<td>$\tau_{\text{decay,IVS}}$ (s)</td>
<td>0.11±0.01</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>$I_{\text{f2}}$ ($\beta$A/PF)</td>
<td>19.0±2.3</td>
<td>13.2±1.4*</td>
</tr>
<tr>
<td>$\tau_{\text{decay,IVS}}$ (s)</td>
<td>1.16±0.05</td>
<td>1.52±0.13*</td>
</tr>
<tr>
<td>$I_{\text{f3}}$</td>
<td>3.8±0.7</td>
<td>4.9±0.4</td>
</tr>
<tr>
<td>Em (mV)</td>
<td>−72.8±1.0</td>
<td>−73.9±0.6</td>
</tr>
<tr>
<td>APD (0 mV) (ms)</td>
<td>2.4±0.3</td>
<td>5.2±0.6*</td>
</tr>
<tr>
<td>APD (−60 mV) (ms)</td>
<td>16.6±0.9</td>
<td>34.5±3.5*</td>
</tr>
</tbody>
</table>

Values, mean±SEM. NP indicates non-pregnant; LP, late-pregnant; IVS, intra-ventricular septum; LV PW, left ventricular posterior wall; EF, ejection fraction; SF, shortening fraction; LVM, left ventricular mass; E, peak velocity of early diastolic filling; A, the peak velocity of late filling associated with atrial contraction; Echocardiogram, n=7 LP, n=8 NP; Iodic currents at +40 mV, n=13 NP, n=20 LP; Action potential, n=7 NP, n=12 LP; Echocardiogram, n=9 NP, n=11 LP. *P<0.05, **P<0.001, ***P<0.0005.
(20 minutes) in: 4% paraformaldehyde, 2% picric acid in 0.1 mol/L Na2HPO4, pH 7.4. Stacks of confocal images were every 0.25 μm at 0.115 μm/pixel. Cryostat ventricular transversal sections (14 μm) were stained with hematoxylin-eosin after heart fixation (2 hours). Measurements were performed with MetaMorph (Universal Imaging Corporation).

Biochemistry
Membrane fractions and immunobLOTS were described previously.23 c-Src activity was assessed using immunoprecipitated c-Src from cardiac lysates (500 μg protein) and measuring phosphorylation of Src peptide (KVEKIGEGTYGVVYK) (Upstate) with [γ-32P]ATP. Phosphorylated and mock samples were spotted onto phosphocellulose binding paper, washed, air-dried, and counted.

Real-Time PCR
Total RNA was isolated using Trizol (Invitrogen) and reverse transcribed with gene specific primers for real-time PCR.23 Controls were: (1) mock reverse transcription product tested in regular 30 cycle PCR; and (2) H2O instead of cDNA tested in parallel to real-time PCR reactions. For primers see online Table (http://circres.ahajournals.org).

Statistics
Student’s t-test was used. Probability values ≤0.05 were considered statistically significant. Values are mean±SE.

Results
Heart Develops Functional Eccentric Hypertrophy With Pregnancy
Figure 1A shows that the size of the heart increases enormously in late-pregnancy. The heart weight in LP mice (178±2 mg, n=20) was significantly higher than in NP animals (126±2 mg; n=18) (Figure 1B) and reversed within days (not shown). The larger body weight in LP mice (NP=20±0.4 g, n=18 versus LP=36±0.68 g, n=20, Figure 1C) makes the heart/body weight ratio lower in LP ((4.93±0.09)*10^-3) when compared with NP ((6.27±0.09)*10^-3) (Figure 1D). This phenotype is in sharp contrast to that of pathological heart hypertrophy where the heart/body weight ratio increases.24

Hematoxylin-eosin staining of transversal ventricular sections near the middle of the heart demonstrated a larger left ventricle (LV) chamber in LP versus NP animals (Figure 1E and 1F) with a modest but significant decrease in wall thickness (Figure 1E and 1G). The LV chamber area increased from 5.8±0.96 mm² in NP (n=6), to 21±1 mm² in LP (n=6) mice. The right ventricle (RV) chamber area was also increased from 2±0.19 mm² in NP to 7.7±2.1 mm² in LP mice (Figure 1F). Because of the dilation of both LV and RV chambers in LP mice, the total surface area was larger in LP (70±4.0 mm², n=6) than in NP mice (37±3 mm², n=6). The interventricular septum (IVS) and posterior wall (PW) thickness were slightly decreased in LP mice from 1.8±0.06 mm (n=6) to 1.4±0.1 mm (n=6), and from 2.0±0.1 mm (n=6) to 1.5±0.1 mm (n=6), respectively (Figure 1E and 1G). These morphometric data indicate that the heart develops eccentric hypertrophy during pregnancy as predicted for a response to volume overload.

Assessment of Cardiac Structure and Function in Late-Pregnancy With Echocardiography
Cardiac Structure
LV chamber dimensions, IVS and PW thicknesses, and LV mass index (LV mass/body weight ratio) were obtained from M-mode images as those in Figure 2A. Both LV end-diastolic (ED) and LV end-systolic (ES) diameters are larger in LP versus NP mice (Figure 2B, Table) confirming a bigger LV chamber in late-pregnancy as revealed in the histological sections (Figure 1E). The LV chamber dilation in late-pregnancy is evident when measuring the LV volume that increased ∼2 to 3 fold at the end of diastole (ED) and systole (ES), respectively (Figure 2C, Table). The LV chamber dilation in LP mice was associated with a reduction in the thickness of the LV, PW, and the IVS both at the end of systole and diastole (Table) consistent with the hematoxylin-eosin staining (Figure 1G).

Cardiac Function
LV systolic function was assessed by measuring the shortening fraction (SF) and ejection fraction (EF) from M-mode measurements.25 Both parameters were reduced in LP mice (Figure 2D, Table) showing a moderate decrease in cardiac function likely due to the increased chamber dimensions and the higher blood volume to be pumped in late-pregnancy. LV diastolic function was evaluated from the ratio E/A of Doppler tracings (blood flow across the aortic and mitral valves), where E is the...
peak velocity of early diastolic filling (E wave), and A is the peak velocity of late filling associated with atrial contraction (A wave). Figure 2E shows early diastolic (E) and late diastolic (A) waves in NP and LP mice with faster E than A waves in both conditions. Mean E and A wave peak amplitudes and E/A ratios were not significantly different with pregnancy (Figure 2F, Table). The lack of alteration in the diastolic function at the end of pregnancy supports a functional hypertrophic pattern in LP mice inasmuch patients with pathological LV hypertrophy show early diastolic velocities lower than normal. In summary, during late pregnancy there is a moderate decrease of systolic function with normal diastolic function.

Pregnancy-Related Heart Hypertrophy Results From Increased Myocyte Size

Myocytes from 3 NP (n=30 cells) and 3 LP (n=30 cells) preparations were chosen at random and cell volume, length, width, and thickness were measured after wheat germ agglutinin staining. Cardiomyocyte volume increased in LP mice from 13,610±700 μm³ to 22,960±1400 μm³. The larger myocyte volume in LP was because of an increase in cell length but not of width or thickness. Myocytes from NP mice were 101.4±3.6 μm in length which increased to 119.9±5.4 μm in LP mice; whereas cell thickness and width were practically the same, 7.6±0.28 versus 8.0±0.23 μm and 25.3±1.09 versus 29.7±1.3 μm (NP versus LP), respectively. Thus, the increase in ventricular size in late-pregnancy results from an increase in cell and myofibril length by adding new sarcomeres in series and leading to eccentric hypertrophy.

Pregnancy-Induced Cardiac Hypertrophy Is Unrelated to Classical Markers of Pathological Hypertrophy but Is Associated With Decreased Expression of Kv4.3 Channel

We quantified in hearts of NP and LP mice transcript levels of classical markers of pathological hypertrophy, α-myosin heavy chain (α-MHC), β-myosin heavy chain (β-MHC), atrial natriuretic factor (ANF), phospholamban, and sarcoplasmic reticulum Ca²⁺-ATPase (SERCA). The real-time PCR curves (fluorescence versus cycle number) were practically identical for each set of genes, indicating that their transcripts do not change with pregnancy (Figure 3A through 3E). GAPDH transcripts (control) were also unchanged in hearts form NP and LP mice (Figure 3F). The melting curves (dT/ΔT versus T) show a single sharp peak for all genes, indicating the presence of a single PCR product (Figure 3A through 3F, insets). Relative transcript expression was quantified by measuring fluorescence intensities in the linear region of the fluorescence versus cycle number curves. LP values were normalized to NP fluorescence values (Figure 3A through 3F, lower panels) demonstrating no change for the tested genes. As a positive control of pathological hypertrophy (PH), Figure 3G and H shows an increase of 26-fold in β-MHC and 18-fold in ANF transcripts of mice hearts after aortic banding. GAPDH transcripts were unchanged (not shown).
Pregnancy Induces a Reduction of Outward Transient K⁺ Currents, a Prolongation of the Action Potential and a Longer QT Interval

Besides the classical “hypertrophy gene program” cardiomyocytes from hypertrophic and failing hearts are known to display decreased transcript expression of Iₖs molecular constituents like Kv4.3, Kv4.2, and Kv1.4. However, we investigated if pregnancy resulted in transcript remodeling of these genes and a regulatory subunit of Kv4.x channel, KChIP2 (Figure 4A through 4D). Real-time PCR curves were practically superimposed for Kv1.4, Kv4.2, and KChIP2 (Figure 4A through 4C) indicating that hearts from NP and LP animals have similar quantities of these transcripts. In contrast, transcript levels of Kv4.3 were much lower in LP than in NP mice (Figure 4D) with a mean ∼5-fold decrease at the end of pregnancy. The presence of a single PCR product was confirmed by a single sharp peak in the melting curves (Figure 4A through 4D, insets) and by agarose-gel electrophoresis of the final product (not shown). Consistent with real-time PCR experiments, cardiac Kv4.3 total protein measured in crude membrane preparations was also downregulated ∼3-fold at the end of pregnancy (n=5 LP, n=5 NP) (Figure 4E). Thus, heart hypertrophy during pregnancy can be distinguished from hypertrophy in pathological states by the lack of change in α-, β-MHC, ANF, phospholamban, SERCA, Kv4.2, and Kv1.4 but has in common a reduction in Kv4.3 transcripts.

Pregnancy Induces a Reduction of Outward Transient K⁺ Currents, a Prolongation of the Action Potential and a Longer QT Interval

Whole-cell patch-clamp recordings revealed that outward peak K⁺ current density at +40 mV is larger in hearts of NP (48.9±6.7 pA/pF, n=13) than in LP (29.5±2.7 pA/pF, n=20) mice (Figure 5A and 5B) (Table). The macroscopic components of outward K⁺ currents, Iₒ, Iₖslow, and Iₖs, were analyzed according to previous work. The amplitudes and decay of the outward K⁺ currents in right ventricular cells from NP mice were well described by the sum of fast (∼100 ms) and slow (∼1 s) exponential components corresponding to Iₒ, Iₖslow, and a steady-state current, Iₛ (Table). For pulses to +40 mV, the time constant of the fast component (τₒ = dIₒ/dt) did not change with pregnancy (NP=0.11±0.01 s versus LP=0.14±0.03 s), while the time constant of the slow component (τslow = dIₖslow/dt) was somewhat increased from 1.16±0.05 s in NP to 1.52±0.13 s in LP mice (Table). Iₒ, Iₖslow density was reduced to a higher degree (∼2-fold) than Iₖslow density (∼1.5 fold) in LP mice, while Iₛ remains unmodified (Table).

Concomitant with the reduction in outward K⁺ current density in LP mice, the action potential duration and QT interval were also prolonged with pregnancy (Figure 5C and 5D, Table). Action potential duration measured at 0 and -60 mV increased from 2.4±0.3 ms and 5.2±0.6 ms in NP mice to 16.6±0.9 ms and 34.5±3.5 ms in LP mice, respectively. The corrected QT interval (QTc) was increased from 40.5±2.3 ms to 48.2±1.5 ms.

Estrogen-Mediated Downregulation of Cardiac Kv4.3 Transcripts

Biochemical and functional data indicate that the change in Kv4.3 expression is one of the factors that defines heart excitability during pregnancy. Because estrogen levels are greatly increased toward the end of pregnancy, we explored the possibility that estrogen could trigger the downregulation
of Kv4.3 channel transcripts in heart. The dose used produced estrogren plasma concentrations of 56±6 pg/mL (n=3, 6 day treatment) and 44±6 pg/mL (n=3, 21 day treatment), which are fairly close to the levels in late pregnancy (39±2 pg/mL, n=3). Real-time PCR experiments showed that fluorescence versus cycle curves were right-shifted in estrogen- with respect to sham-treated animals indicating downregulation of Kv4.3 transcripts; while cotreatment with the estrogen receptor antagonist ICI 182,780 was able to reverse the hormone effect (Figure 6A). Mean values in Figure 6B demonstrate that Kv4.3 transcript levels are reduced ~50% by estrogen treatment for 6 days and by 75% by treatment for 21 days, and completely reversed with ICI 182,780. The latter provides a strong argument for direct estrogen-receptor mediated regulation of transcriptional activity of the Kv4.3 gene. GAPDH (control) transcript levels were similar in oil, estrogen and estrogen+ICI 182,780 treated mice (not shown).

Discussion

We show for the first time the molecular and functional signature of pregnancy-related physiological heart hypertrophy and define the phenotypic alterations that occur in the mouse heart. The heart-to-body weight ratio is the most commonly used index for hypertrophy, which can increase by 10% to 15% in mild hypertrophy and up to 50% in severe heart failure. During pregnancy, the heart-to-body weight ratio decreases because of the higher body weight at the end of pregnancy. Therefore, pregnancy-related heart hypertrophy can be seen as a normal physiological and compensatory response that enables the heart to enhance its pumping capacity in response to increased demand.

The volume overload during pregnancy induces eccentric hypertrophy resembling that developed in dynamic exercise. Athletes involved in high dynamic exercise like running, develop eccentric hypertrophy caused by volume overload in response to the high cardiac output. On the other hand, athletes involved in static exercise like weightlifting develop...
concentric hypertrophy caused by pressure overload in response to high systemic arterial pressure.3,31

When the ventricle is subjected to hemodynamic stress, the ventricular myocytes experience morphological and biochemical changes that mark the initiation of hypertrophy. It is generally accepted that cardiac growth during hypertrophy results primarily from an increase in the size of the individual cells rather than an increase in the number of cells. This idea is based on the view that myocardial cells are terminally differentiated and cannot divide after birth.32 However, it has been reported that under certain circumstances adult ventricular myocytes can proliferate, and therefore, cell division may contribute to the increase in the size of the heart.33,34 Nevertheless, our results show that eccentric heart hypertrophy in pregnancy can be explained by an increased myocyte size because of an increase in cardiomyocyte length.

Cardiac Kv4.3 transcripts are downregulated by estrogen in ovariectomized mice. (A) Real-time PCR fluorescence intensity versus cycle number plots for Kv4.3 in ovariectomized mice treated with oil (sham) (●), estrogen (E2), 6 days (○), and E2+ICI 182,780 (ICI), 6 days (■). (B) Bar graph shows mean relative expression normalized to sham, values were: sham, 1±0.15; E2, 0.53±0.09 (6 day treatment) and 0.30±0.06 (21 day treatment); and E2+ICI, 1±0.07 (6 days of injection), n=3 independent RNA isolations in each group. Doses are in Materials and Methods.

Figure 5. Smaller outward transient K⁺ currents, longer action potential duration and longer QT intervals in hearts of LP versus NP mice. Currents were elicited with pulses from −70 mV to +40 mV in 10 mV steps from −70 mV holding potential in myocytes of NP (A) and LP (B) mice. Action potentials from NP (C) and LP (D) mice elicited by 1 ms pulses. Dashed lines indicate the 0 and −60 mV levels. ECG recordings in NP (E) and LP (F) mice at a similar heart rate (~550/ min). Mean values are in Table.

Figure 6. Cardiac Kv4.3 transcripts are downregulated by estrogen in ovariectomized mice. (A) Real-time PCR fluorescence intensity versus cycle number plots for Kv4.3 in ovariectomized mice treated with oil (sham) (●), estrogen (E2), 6 days (○), and E2+ICI 182,780 (ICI), 6 days (■). (B) Bar graph shows mean relative expression normalized to sham, values were: sham, 1±0.15; E2, 0.53±0.09 (6 day treatment) and 0.30±0.06 (21 day treatment); and E2+ICI, 1±0.07 (6 days of injection), n=3 independent RNA isolations in each group. Doses are in Materials and Methods.
Our data demonstrate that Kv4.3 but not transcripts of Kv4.2, Kv1.4, KChiP2, or other known markers of pathological hypertrophy like α- and β-MHC, ANF, phospholamban, and SERCA\(^{1,5}\) changed during pregnancy (Figures 3 and 4). The loss of Kv4.3 transcripts and protein in late pregnancy coincided with reduced \(I_{\text{Ks}}\) density (Figure 5), which seems to be underlined by Kv4.3 and Kv4.2 heteromultimeric channels.\(^7\) Although our experiments show that Kv4.2 transcripts do not change with pregnancy, its potential change at the protein level needs to be addressed in the future. Similarly, whether changes in Kv2.1 and/or Kv1.5 (typical molecular correlates of murine \(I_{\text{Ks,slow}}\))\(^{35}\) explain the modest \(I_{\text{Ks,slow}}\) decrease during pregnancy remain an open question. Nevertheless, our results point to Kv4.3 as a common gene marker of pregnancy-related and pathological hypertrophies. The facts that, in estrogen primed mice cardiac Kv4.3 transcripts were downregulated, and that inhibition of estrogen receptor activity reversed the effect (Figure 6) suggest that the rise of estrogen at the end of pregnancy is one mechanism responsible for cardiac Kv4.3 remodeling in pregnancy.

The contribution of Kv4.3 channel changes to myocardial disease has been highlighted.\(^6,27,36\) Direct evidence for Kv4.3 role in pathological hypertrophy was given by demonstrating that its downregulation and cardiac hypertrophy after aortic stenosis could be restored by in vivo gene transfer of Kv4.3.\(^10\) In vivo Kv4.3/Kv4.2 functional ablation (with dominant-negative nonconducting constructs) cause decreased \(I_{\text{Ks}}\) and prolongation of both the action potential and the QT interval.\(^5,9\) Our studies demonstrate that Kv4.3 protein downregulation in late pregnancy is associated with a decreased \(I_{\text{Ks,p}}\), a prolonged action potential, and an increased QT interval (Figure 5) providing a molecular explanation for the increase in QT interval and cardiac arrhythmogenesis in human pregnancy.\(^17,18\)

Recent evidence shows that the tyrosine kinase c-Src upstream the Ras/MAPK(ERK, extracellular signal-regulated kinase) pathway is activated at the onset of pressure overload- or Endothelin-1-induced hypertrophy; and that estrogen is able to activate this c-Src/Ras/MAPK(ERK) pathway in brain.\(^15,37\) Here we found that c-Src activity increased by \(\approx 2\)-fold during late pregnancy and that this increase was mimicked by estrogen treatment (Figure 7). The increase in c-Src activity in late-pregnancy and by estrogen is somehow close to what was reported (2- to 3-fold) for reversible induced hypertrophy in guinea pig hearts but more than 3 times lower than c-Src activity measured in irreversible hypertrophy.\(^37,38\) We propose that the degree of c-Src activity associated with an increase of estrogen levels is one mechanism regulating pregnancy-related functional hypertrophy.

In conclusion, pregnancy-related heart hypertrophy is associated with activation of c-Src pathway and Kv4.3 gene remodeling, which are triggered by estrogen hormone.

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**References**


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<th>Mouse Gene</th>
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<th>Forward primer</th>
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Gene specific primers for real-time PCR

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