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Gender Differences in Non-Ischemic Myocardial Remodeling: Are They Due to Estrogen Modulation of Cardiac Mast Cells and/or Membrane Type 1 Matrix Metalloproteinase

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SUMMARY

This review is focused on gender differences in cardiac remodeling secondary to sustained increases in cardiac volume (VO) and generated pressure (PO). Estrogen has been shown to favorably alter the course of VO-induced remodeling. That is, the VO-induced increased extracellular matrix proteolytic activity and mast cell degranulation responsible for the adverse cardiac remodeling in males and ovariectomized rodents do not occur in intact premenopausal females. While less is known regarding the mechanisms responsible for female cardioprotection in PO-induced stress, gender differences in remodeling have been reported indicating the ability of premenopausal females to adequately compensate. In view of the fact that, in male mice with PO, mast cells have been shown to play a role in the adverse remodeling suggests favorable estrogen modification of mast cell phenotype may also be responsible for cardioprotection in females with PO. Thus, while evidence is accumulating regarding premenopausal females being cardioprotected; there remains the need for in-depth studies to identify critical downstream molecular targets that are under the regulation of estrogen and relevant to cardiac remodeling. Such studies would result in the development of therapy which provides cardioprotection while avoiding the adverse effects of systemic estrogen delivery.

I: INTRODUCTION

A significant cause of morbidity and mortality in post-menopausal women is heart failure (HF) [40]. While the mechanisms which contribute to the increased incidence and acceleration of HF in this patient population remain unresolved, a common structural event is adverse left ventricular (LV) remodeling. LV remodeling in response to sustained elevations in LV pressure or volume and/or injury entails structural changes to the

cardiomyocytes and extracellular matrix (ECM). The remodeling is considered to be compensatory if it results in the normalization of the myocardial stress and the maintenance of LV function. If, on the other hand, the limits to the compensatory remodeling mechanism are attained without achieving normalization, then the remodeling process becomes maladaptive with the end result being a thin walled, dilated, failing ventricle. While it is likely that decreased estrogen levels and thereby reduced local stimulation of estrogen receptors contribute to the HF process in post-menopausal women, the results from large clinical trials of hormone replacement therapy have been disappointing and remain controversial [34;66;81]. However, of particular interest to this review are recent findings indicating that use of hormone therapy in younger women has been associated with a lower risk of coronary heart disease and reduced overall mortality with minimal to no side effects [68;69;72]. Also, there is accumulating experimental evidence to indicate that premenopausal female animals, when faced with increased cardiac stress, have a greater ability to attain and maintain cardiac compensation than males. Nevertheless, this systemic estrogen treatment remains problematic in women who start hormone therapy many years after menopause [68;69;72], suggesting that more specific targeting downstream of the estrogen receptors in the context of LV remodeling and HF would be a relevant therapeutic target thereby avoiding the adverse effects of systemic estrogen delivery in all age groups. To this end, this review will be focused on gender differences in cardiac remodeling secondary to sustained increases in cardiac volume (VO) and ventricular pressure (PO) and in particular highlight the ability of estrogen to modulate cardiac mast cells and/or membrane type-1 matrix metalloproteinases as novel possible therapeutic targets.

II: STRUCTURAL GENDER REMODELING DIFFERENCES

a) Global Geometric Remodeling

As described by Stauffer and Leinwand [75], left ventricular mass is similar for males and females until puberty. At this time, relative to females, male hearts undergo an increase in wall thickness and chamber size likely due to cardiomyocyte hypertrophy. This difference persists throughout adulthood. Rate of relaxation decreases with ageing in both sexes, however, males tend to also have impairments in systolic function not present in females [30]. With ageing, there is a loss of cardiomyocytes and a corresponding increase in volume of the remaining cardiomyocytes in male, but not female hearts [75]. This describes the gender related changes in structure and function of the heart during the normal ageing process. However, there are stark differences in how the male and female heart remodels under pathological conditions. Global remodeling in response to a sustained elevation in myocardial wall stress can result in wall thinning or thickening and either no change or an increase or decrease in chamber size. If the ratio of LV wall thickness to chamber diameter or LV mass to chamber volume remains unchanged or increases the remodeling is referred to as concentric hypertrophy. If on the other hand wall thinning and LV dilatation occurs, the remodeling process is referred to as eccentric hypertrophy.

Volume Overload—Using an abdominal aorta to vena cava fistula model of VO, significant gender remodeling differences have been reported as depicted in figure 1 [10;26]. After 8 wks of VO, there is significant ventricular dilation (172%) and wall thinning or eccentric hypertrophy in male hearts. In contrast, female hearts subjected to VO are only slightly enlarged and the wall thickness is appropriately increased (concentric hypertrophy). Mortality secondary to congestive HF is significant in males at 8 wks of VO and is near 100% by 20 wks, while intact females with VO exhibit no signs or symptoms of HF throughout the 20 wk period. That estrogen is responsible for the cardioprotection has been verified by the fact that male and ovariectomized female rats with VO adversely remodel and develop HF [10;26], while supplementary estrogen to male and ovariectomized rats with

VO markedly attenuates or prevents the adverse remodeling and the development of HF [25;27].

Pressure Overload—Gender differences in cardiac remodeling in response to PO have been extensively studied. Recently, Chan et al. [13] provided an extensive characterization of LV remodeling in male and female spontaneously hypertensive rats (SHR) from 3 to 24 months of age. Both male and female SHR develop an initial concentric hypertrophy in response to PO. However, by 9 to 15 months of age, LV weight indexed to body weight tended to be greater in female SHR, although this only reached statistical significance at 12 months of age. This trend occurred despite the blood pressure level in the female SHR not reaching that of males during this time span. Despite the greater mass to body weight ratio, LV wall thickening was delayed in the female SHR and did not reach the levels of male SHR until 18 months of age. This coincided with the beginning of LV wall thinning in the males as they began to decompensate and transition to HF. Conversely, female SHR maintained the increased LV wall thickness throughout the remaining study period. In the male SHR, chamber size was decreased in comparison to normotensive controls over the first 15 months of age, consistent with concentric hypertrophy. Thereafter the male SHR LV dilated continuously. As a consequence, ejection fraction dramatically decreased after 18 months of age, indicative of HF. Conversely, the chamber of the female SHR LV was maintained at normal size throughout their lifespan and systolic function, as measured by ejection fraction, remained within normal limits.

Male and female SHR both experienced right ventricular hypertrophy, although it was delayed in the females (21 versus 18 months of age). One could speculate that, as a result of the right ventricular hypertrophy and the fact that the female SHR LV did not dilate, the female SHR would be prone to developing diastolic HF with preserved ejection fraction. However, while the mitral valve passive early (E) filling velocity wave and the atrial systolic (A) velocity wave ratio (E/A), as assessed with pulsed wave Doppler, dramatically increased in the male SHR at 21 months, E/A remained normal in the female SHR suggesting that that diastolic function was maintained in the female SHR. There are, however, examples in mouse models where 9 weeks of transaortic constriction (TAC) induced greater hypertrophy in male hearts than in female hearts, and both genders had similar declines in ejection fraction [24]. Interestingly though, end diastolic pressure and lung weights were increased in males in this study, but not in females. Skavdahl et al. [73] found that LV hypertrophy was less in female mice than in males at two weeks post-TAC again indicating that the hypertrophic response is delayed in females.

Studies using ovariectomy and estrogen replacement suggest that estrogen is likely responsible for the cardioprotection in PO. PO in female rats due to abdominal aortic banding also leads to LV hypertrophy and increased end diastolic pressure; both of which were further increased in ovariectomized rats [4]. In ovariectomized mice, replacement estrogen significantly reduced LV hypertrophy in response to TAC-induced PO [80].

In Table 1, the long-term (i.e, the time associated with decompensation in males) changes in LV wall thickness, chamber size and function in response to VO and PO are summarized for both genders.

b) Cardiomyocyte Remodeling

Depending on the nature of the stress, cardiomyocyte remodeling involves the parallel and/or in-series addition of sarcomeres, resulting in cardiomyocyte thickening and/or lengthening, respectively. In general, concentric hypertrophy is the result of cardiomyocyte thickening or adding sarcomeres in parallel and eccentric hypertrophy results from cardiomyocyte thinning and elongating secondary to an in-series addition of sarcomeres.

Volume Overload—Despite the gender differences in geometrical remodeling, both sexes were reported to have significant increases in LV weight relative to controls at 8 wks post VO (77% increase for female & 114% for male rats). Accompanying increases in right ventricular weight were also observed (134% for female & 161% for male rats) [26]. While most of these increases were attributable to increases in myocyte size, significant gender differences in the cardiomyocyte temporal remodeling response and geometry to VO have been reported. In response to an aortocaval fistula induced VO, Liu et al. [45;46] reported progressive, temporally proportional increases in cardiomyocyte cross-sectional area (parallel sarcomeres) and length (in-series sarcomeres) at 5 days post-fistula and beyond in female rats. Contrastingly in male rats, cardiomyocyte length and width did not increase significantly during the first 35 days of VO [21]. Thereafter, the increase in cardiomyocyte size was primarily due to increases in myocyte length. The mechanisms underlying these remarkable gender differences in cardiomyocyte remodeling secondary to VO remain to be identified. However, they may be related to gender differences in extracellular matrix (ECM) responses or cardiomyocyte integrin function (see sections IIc and IIId).

Pressure Overload—Cardiomyocyte remodeling in response to PO consists initially of an increase in cross-sectional area of the cell [1;83]. Over time, these thicker cardiomyocytes begin to decrease in cross-sectional area and become elongated; coinciding with ventricular dilatation and an increase in wall stress in the male heart. Surprisingly, little has been done to study gender differences in cardiomyocytes at the structural level in PO. Tamura et al. [78] studied the spontaneously hypertensive heart failure rat (SHHF) and found that male SHHF cardiomyocytes had greater cross-sectional areas at 2, 4, and 6 months of age than females. Cardiomyocytes were slightly longer in males at 2 months of age, but thereafter there were no gender differences in length. What is interesting though is that there appears to be a greater relative increase in size of the female SHHF cardiomyocytes (~50% increase), compared to the males (~20% increase). This greater relative increase may be important in providing cardioprotection since 24 month old SHHF females maintained a far greater fractional shortening than 18 month old males (42.1 versus 28.6%). Re-enforcing the role of estrogen, replacement estrogen decreased cardiomyocyte cross-sectional area in ovariectomized mice undergoing TAC-induced PO [80].

In Table 1, the short-term as well as the long-term (i.e., the time associated with decompensation in males) changes in cardiomyocyte width and length in response to VO and PO are summarized for both genders.

c) Extracellular Matrix Remodeling

Interspersed between cardiomyocytes, nerves and blood vessels, the extracellular matrix (ECM) is comprised of ground substance and connective tissue which is predominantly collagen with relatively small amounts of fibronectin, laminin and elastin. Because fibrillar collagen is a relatively stiff material that is in intimate contact with all other components of the myocardium, it plays a crucial role in the maintenance of ventricular shape, size and function [3;36]. Collagen is synthesized within fibroblasts and myofibroblasts. Once deposited in mature form and subsequently cross-linked, interstitial fibrillar collagen is extremely stable and resistant to degradation. Nevertheless the concentration of interstitial collagen in the myocardium is dependent on the balance between its synthesis and degradation and an imbalance will result in adverse remodeling of the collagen network and myocardium. To the best of our knowledge there are no studies in VO or PO that specifically investigate the role of gender on cardiac fibroblast function in vivo. Interestingly though, isolated male and female cardiac fibroblasts respond quite differently to estrogen. Cardiac fibroblasts from male rats increased expression of collagen I and III in response to estrogen, whereas expression of these genes was decreased in female cells incubated with

estrogen [60]. However, relevant to PO, estrogen inhibited proliferation and collagen synthesis by cardiac fibroblasts [22]. Interestingly, estrogen metabolites were even more potent in inhibiting these responses. Further, estrogen has also been shown to prevent cardiac fibroblasts from increasing levels of both $\alpha 1$ and $\beta 1$ integrins in response to angiotensin II [76]. Relevant to VO, estrogen treatment of isolated cardiac fibroblasts also from adult rats significantly decreased MMP-2 mRNA and protein in both male and female cells [48]. As discussed below, matrix metalloproteinase (MMP) initiated degradation of myocardial collagen typically results in ventricular dilatation and a decrease in ventricular stiffness, while an abnormal increase in interstitial collagen concentration and/or cross-linking results in a stiffer myocardium and ventricular diastolic dysfunction.

Volume Overload—Following the creation of a fistula-induced VO in male rats, a significant increase in MMP activity occurs within 12 hrs that is then sustained for approximately the first week. As a consequence, there is significant fibrillar collagen degradation by the third day [7] and the onset of progressive ventricular dilatation and hypertrophy becomes apparent after one week of VO [8]. This myocardial ECM disruption in the initial response to VO is clearly deleterious in that it subsequently results in a significant depression in chamber contractility and the LV is clearly more compliant. As further evidence of a cause and effect relation between collagen degradation and ventricular dilatation, MMP inhibitors have been shown to attenuate ventricular dilatation using the fistula model of VO and other experimental models of HF in male rats [14;59] and male pigs [74]. From these findings, one could conclude that elevations in MMP activity during the early stages of injury or elevated wall stress and the consequent degradation of fibrillar collagen are responsible for the initiation of a progressive remodeling process that eventually leads to a thinned wall and dilated failing LV. Once initiated, the adverse remodeling process continues despite a subsequent restoration and maintenance of near normal fibrillar collagen concentration after two weeks of VO.

Recently, it has been reported that this degradation of ECM collagen during the first 5 days of VO in male rats does not occur in intact premenopausal female rats with VO [47]. On the other hand, ovariectomized rats have a response to VO similar to males in that a significant decrease in myocardial collagen concentration occurs shortly after the initiation of VO. Accordingly, the cardioprotection mediated by the presence of estrogen appears to be largely due to the preservation of the ECM. Furthermore, the fact that, following VO in intact females, the ECM remains intact may explain the gender-related marked differences in cardiomyocyte remodeling discussed above. This possibility clearly requires additional research.

Pressure Overload—Under conditions of PO, there is an increased accumulation of ECM proteins, particularly fibrillar collagen, resulting in myocardial fibrosis [35;84;85]. This fibrosis continues to accumulate over time [13] and has the detrimental effect of causing an increase in LV stiffness and diastolic dysfunction [35;84;85]. Both male and female SHR continually accumulated similar levels of excess LV collagen over time with the exception of 24 months of age where males had greater amounts. Interestingly though, Chan et al. [13] found that the similar levels of increasing fibrosis in female hearts did not translate to the increases in diastolic stiffness that were observed in males. It remains to be determined if this was due to differences in types of collagen or the degree of collagen cross-linking between males and females [2;16;55]. Similarly, estrogen replacement failed to reduce cardiac fibrosis in ovariectomized mice exposed to TAC-induced PO [80]. This study did not include non-ovariectomized female mice so the effect PO has on fibrosis in the intact female remains unknown. In contrast to these studies, Fliegner et al. [24] found fibrosis to be increased in male mice hearts following 9 weeks of TAC, but not in females. The reason for these discrepant findings is not clear.

In Table 1, the short-term and long-term changes in the ECM in response to VO and PO are summarized for both genders.

III: Estrogen Modulation of Remodeling Mechanisms

a) Role of Mast Cells

Cardiac mast cells produce and store a wide variety of cytokines, growth factors, vasoactive agents and other biologically active mediators that are capable of mediating tissue remodeling. Importantly, several of these mediators are capable of activating MMPs, which in turn degrade the collagen matrix of the heart. Alternatively, cardiac mast cells have also been implicated in the fibrotic remodeling of the heart secondary to PO. However, little is known regarding the mechanisms by which cardiac mast cells are activated in VO and PO. Furthermore, as discussed below, there are gender differences in mast cell phenotype and function, which contribute to the cardioprotection afforded to pre-menopausal females.

Volume Overload—In male rats, LV cardiac mast cell density rapidly increases following the creation of VO. This event correlates with a significant increase in myocardial MMP activity and a 50 and 60% reduction in collagen volume fraction by 3 and 5 days post VO [7]. Both the VO-induced MMP activation and subsequent collagen degradation have been shown to be prevented with a mast cell membrane stabilization compound and in mast cell deficient rats [7;43], indicating a direct causal relationship between mast cell degranulation and increased MMP activity. Furthermore, adverse long-term VO-induced remodeling was significantly attenuated in mast cell deficient rats [43] and when mast cell degranulation was pharmacologically prevented [9]. Another outcome of the long-term prevention study was a significant decrease in mortality secondary to congestive HF, thus providing direct evidence that the early mast cell-related alterations of the ECM are critical events that culminate in a dilated, highly compliant failing heart (figure 1).

Evidence exists to indicate that there are gender differences in mast cell function, which contribute to the cardioprotection afforded to premenopausal females. As stated earlier, there is no reduction in collagen volume fraction following the creation of VO in intact females [47]. In addition the greater mast cell density that was noted in male [7] and ovariectomized rats [47] did not occur in the intact female [47]. The fact that the VO-induced increase in mast cell density did not occur in males when mast cell degranulation was prevented [7] indicates that activation of mast cells is necessary for the greater density. This was verified by the recent findings of Li et al. [44] whereby the increase in mast cell density post the creation of VO was reported to be due to the release of chymase causing a rapid increase in the level of stem cell factor which in turn stimulated the maturation of resident immature mast cells. Thus, it appears that there is a gender difference in the endogenous secretagogue responsible for VO-related mast cell activation. One possible candidate is endothelin-1, which is known to activate mast cells [52] and which is transiently elevated in male [53] and ovariectomized rats with VO and also in intact female rats but to a much lesser extent [47]. Other possibilities such as substance P [50] and oxidative stress [17;51] need to be investigated further.

Another possible mechanism responsible for the cardioprotection in intact females with VO is estrogen phenotypic modulation of cardiac mast cell contents. This has been documented in a study by Chancey et al. as follows [15]. Following stimulation with the mast cell secretagogue, compound 48/80, isolated hearts from ovariectomized rats experienced a marked increase in MMP activity, a decrease in collagen volume fraction and ventricular dilatation when compared with hearts from intact females and from ovariectomized rats treated with supplementary estrogen. The fact that, in all three groups compound 48/80

resulted in 97% of the mast cells being degranulated, clearly indicates differences in their secretory products secondary to the presence of estrogen.

In male rats cardiac mast cells have been shown to be an important source of tumor necrosis factor alpha (TNF- α) [29]. TNF- α has been reported to induce MMP-2 activity and membrane type 1-MMP (MT1-MMP or MMP-14) expression in a model of nucleus pulposus tissue degeneration and MMP-2 and -9 in human corneal epithelial cells [70;87]. Jobe et al. [37] have reported that progression of adverse myocardial remodeling secondary to VO was markedly attenuated by inhibition of TNF- α . For example, the myocardial collagen degradation seen in male rats at 3 days post VO was prevented by TNF- α inhibition indicating that mast cell derived TNF- α is responsible for the initial MMP activation. In hearts from males [50] and intact and ovariectomized rats, [47] LV myocardial TNF- α levels were determined after the creation of VO. While there was no increase in TNF- α levels in the sham and intact female groups, there was a sustained twofold increase in ovariectomized [47] and male [50] rats at 3 and 5 days of VO relative to their respective sham groups. Furthermore, the mast cell stabilizer nedocromil prevented these increases [47] and no increase in TNF- α occurred in male mast cell deficient rats with VO [43], thus providing additional support to the observation that, in the absence of estrogen, activated mast cells contribute significantly to myocardial TNF- α synthesis. It is also of interest to note that ovariectomy alone resulted in a doubling of myocardial TNF- α compared to that in the intact sham female rats [47].

Currently, it is not known if there are gender differences in other mast cell secretory products which may affect VO remodeling. In general, connective tissue type MCs contain the proteases chymase and tryptase in addition to the cytokine TNF- α which are known to influence the remodeling process associated with VO. For example as discussed above, mast cell derived chymase is responsible for the VO-induced increase in mast cell density [44]. Chymase is also capable of generating angiotensin II and converting precursors of TGF- β and MMP-2 and -9 to their active forms [12;79]. Tryptase appears to activate MMP-2 and possibly MMP-9 via its ability to activate proMMP-3 [31;41].

There is also the possibility that the activated mast cell could interact with MT1-MMP which directly causes ECM degradation, activates other pro-MMPs, and processes bioactive signaling molecules such as TNF- α [18]. Preliminary results from our laboratory identified estrogen response elements on the MT1-MMP promoter. In addition, LV MT1-MMP activity was determined in intact female and ovariectomized rats following 5 days of VO. The preliminary results demonstrated a significant increase in myocardial MT1-MMP activity in both the ovariectomized sham and VO rats, whereas the activity in the intact female after 5 days of VO was similar to that in the normal control. The >3-fold increase in MT1-MMP activity following ovariectomy is of particular interest as it clearly establishes the suppressive regulatory ability that estrogen exerts on MT1-MMP activity which may contribute to gender remodeling differences secondary to VO.

Pressure Overload—Similar to VO, mast cell density increases in the hypertensive/PO heart [56;71]. Hara et al. [32] used the mast cell-deficient mouse to determine if mast cells were important in developing HF following PO induced by aortic banding. They found that an absence of mast cells protected the PO hearts from dilatation and the loss of systolic function. We investigated the role of mast cells in the early development of myocardial remodeling and reported that inhibition of mast cell function with the mast cell stabilizing compound nedocromil prevented the development of fibrosis in the SHR heart, independent of blood pressure [42]. The mechanisms of this prevention appeared in part to be related to the mast cell specific protease tryptase, since it was elevated in the hypertensive heart. We further demonstrated that tryptase could induce cardiac fibroblast conversion to a

myofibroblast phenotype and increased collagen synthesis by activation of protease activated receptor-2 and subsequent induction of ERK1/2 signaling, but not p38 or JNK [42;49]. Additionally, we found that recruitment of macrophages into the hypertensive heart and altered cytokine levels were both normalized following mast cell stabilization, suggesting that these are also likely mechanisms by which mast cells induce fibrosis in the hypertensive heart. We also reported that mast cells regulated the levels of interferon- γ and IL-4 in the hypertensive heart. IL-4 has subsequently been shown to regulate fibrosis in PO [38]. Shiota et al. [71] reported that infusion of the mast cell secretagogue, compound 48/80, into isolated hearts led to increased levels of IL-6. They also identified the presence of TNF- α and TGF- β 1 in cardiac mast cells.

As was mentioned previously in VO, there is a possibility that cardiac mast cells may interact with MT1-MMP to induce remodeling. An interesting finding in the TAC model of PO is that MT1-MMP mRNA was up-regulated 148% in male mice following 2 weeks of TAC, but only 42% in female mice [73]. MT1-MMP expression is sensitive to changes in mechanical load, whereby increased wall tension accelerated MT1-MMP promoter activity in-vitro [67]. Increased myocardial MT1-MMP expression has been identified in patients and animals with sustained LV PO [33;88;90]. This could have an impact on the development of fibrosis through MT1-MMPs ability to activate pro-fibrotic pathways. For example MT1-MMP processes latent TGF binding protein-1 (LTBP-1) resulting in the release of active TGF β [5;19;23;28;39;54;62]. This may be relevant to mast cell contribution of TGF β 1 in PO hearts. Similarly, the ability of MT1-MMP to activate TNF- α may be an important factor in releasing mast cell-derived TNF- α . MT1-MMP could also be important later in the remodeling process via its ability to activate other MMPs that degrade the matrix and initiate ventricular dilatation. Given the important role of MT1-MMP, estrogen modulation of this protease could be an important mechanism of cardioprotection in the female heart.

While there is strong evidence for a role for mast cells in fibrosis and ultimately HF in PO, we do not know if estrogen-induced changes in mast cell phenotype have any role in the cardioprotection observed in the female heart in response to PO. Given the evidence in VO studies presented above, it is reasonable to assume that this could also be the case in PO. However, this remains to be investigated.

In Table 1, the short-term changes in TNF- α and mast cell density and the long-term changes in mast cell density in response to VO and PO are summarized for both genders.

b) Role of Integrins

Integrins serve to maintain the three-dimensional spatial relationship of the myocardial components by mediating the attachment of the cardiac myocyte to the ECM, and thereby contributing to the transmission of cardiomyocyte contractile forces to the ventricular chamber [64;65]. Accordingly, changes in the interaction between cardiomyocyte integrins and the ECM represent a potential mechanism that may contribute to the adverse functional and structural alterations that result from chronic elevations in ventricular wall stress.

Volume Overload—We are in the process of completing studies designed to determine whether alterations in cardiomyocyte integrin function (i.e., isolated male myocyte adhesion to ECM substrate components) correlate with the adverse structural myocardial remodeling induced by VO. A significant decrease in adhesion of cardiomyocytes to laminin, fibronectin and collagen types I and IV were found to coincide with the ECM degradation occurring during the first 5 days of VO. Also, the subsequent development of symptomatic congestive HF was associated with significant increases in ventricular dimensions [11], which correlated with striking reductions in integrin-mediated cardiomyocyte adhesion to

the ECM substrates. While not conclusive, these preliminary findings implicate alterations in integrin-mediated adult cardiomyocyte adhesion as an underlying mechanism responsible for the temporal progression of myocardial remodeling secondary to VO. However, it remains to be determined if the decreased adhesion was due to ECM degradation [7] or increased TNF- α levels [61]. In either case, intact female cardiomyocyte adhesion more than likely would remain normal with VO since ECM degradation and increased TNF- α do not occur [47]. This, however, remains to be determined.

Pressure Overload— $\alpha 1$ integrin was found to be increased in isolated cardiac fibroblasts from rat hearts exposed to three days of aortic banding [77]. This preceded the increase in collagen volume fraction in the LV of the banded animals. $\alpha 2$ integrin levels were elevated in these isolated fibroblasts from banded rats at 7 and 14 days. This corresponded with a period of increasing fibrosis in the hearts of the banded animals. Thus, up-regulation of integrin function may precede the development of fibrosis representing an important step in fibroblast migration to specific areas of the heart, at which point they continue to be up-regulated. Similarly, Bouzeghrane et al. [6] reported that the $\alpha 8\beta 1$ integrin unit is expressed on cardiac fibroblasts and that expression was up-regulated in the PO heart following infusion of angiotensin II. Noteworthy is that areas of myocardial scarring were heavily infiltrated by $\alpha 8\beta 1$ myofibroblasts. From the point of view of the cardiomyocyte, $\beta 1$ and $\beta 3$ integrins are required for compensatory hypertrophic growth in response to PO of the heart by interacting with other proteins essential for normal or hypertrophic cardiac function. Further, Ding et al. [20] identified abnormal $\beta 1$ integrin deposition in hearts in the early stages of failure from mice with TAC-induced PO. In these hearts, $\beta 1$ integrin was found in the ECM surrounding cardiomyocytes as well as on the cardiomyocytes themselves. Thus, it seems that integrins play an important role in fibroblast function and cardiomyocyte growth. Cardiac fibroblasts pretreated with estrogen did not increase production of $\alpha 1$ or $\beta 1$ integrins following activation with angiotensin II [76]. Functionally, this was manifest as a decrease in collagen gel contraction in response to angiotensin II. However, whether gender differences exist in integrin function in the whole heart and the overall role this might play in cardioprotection in PO is currently unknown.

In Table 1, the short-term changes in integrins in response to VO and PO are summarized for both genders.

b) Role of Estrogen Receptor

The biological actions of estrogen are mediated by its binding to specific estrogen receptors, ER- α and ER- β . Estrogen is known to mediate its effects on cells of the cardiovascular system via genomic and non-genomic mechanisms. Both male and female cardiomyocytes and fibroblasts possess estrogen receptor (ER) mRNA [48;57;86] and, thus, presumably are susceptible to the influence of estrogen under conditions of VO and PO. In addition, there is evidence that stimulation of these receptors by estrogen activates multiple intracellular events [48;63]. For example in neonatal rat LV myocytes, estrogen inhibits NF- κ B activity by its binding to nuclear receptors [58]. Finally, while no one has investigated whether ERs are present in cardiac mast cells, Zaitzu et al. reported that a human mast cell line and rat bone marrow derived mast cells expressed mRNA for ER- α but not ER- β [89].

Volume Overload

Eight weeks after the initiation of VO in young intact females, a 2-fold increase in the myocardial ER- β protein level and no change in ER- α protein level has been reported compared to sham-operated intact females. In contrast, ovariectomy prevented the VO induced increase in ER- β protein and ER- α was significantly reduced following 8 weeks of VO. Estrogen supplementation to the ovariectomized rats increased the protein levels of

both receptors [82]. Thus, while this study indicates that ER levels are affected by VO and ovariectomy, it does not indicate which ER is responsible for cardioprotection. Clearly, additional studies are required to determine the relative roles of the ERs in mediating the cardioprotective effects of estrogen whereby adverse remodeling in response to VO is prevented.

Pressure Overload

In mice subjected to 9 weeks of TAC-induced PO, deletion of ER- β led to greater wall thickening in the female hearts and consequently an increased relative wall thickness indicative of concentric hypertrophy [24] when compared to ER- $\beta^{-/-}$ males. Deletion of ER- β led to greater increases in cardiomyocyte cross-sectional area in both sexes in response to TAC. Thus, ER- β may serve to limit cardiomyocyte hypertrophy in both sexes. While fibrosis occurred in male, but not female hearts following TAC, of great interest is the finding that deletion of ER- β decreased the amount of fibrosis in the male hearts in response to TAC, but dramatically increased the amount of fibrosis in the female hearts. Thus, in female hearts ER- β appears to be important in limiting both cardiomyocyte hypertrophy and fibrosis. Interestingly though, while not statistically significant, ER- $\beta^{-/-}$ females tended to have a slightly improved ejection fraction, compared to ER- $\beta^{-/-}$ males. Skavdahl et al. [73] used female ER- α and ER- β knockout mice to elucidate the role of each receptor over the shorter time period of two weeks post-TAC. They found that deletion of ER- α had no effect on hypertrophy, however, deletion of ER- β resulted in an increased hypertrophic response in the female hearts. This may be due to a down-regulation of lipoprotein lipase in the ER- β knockout mice which is the rate limiting enzyme for delivery of fatty acids to muscle [73]. This is similar to the findings of Fliegner et al. [24] described above, albeit at an earlier time-point in the remodeling process. Somewhat in contrast with Fliegner et al. though, they did not find any effect of ER- β knockout on male hearts. Overall though, protection from PO may be conferred via ER- β .

Other than the observation that both ERs are affected by VO and ovariectomy nothing is known regarding the relative roles of the ERs in VO cardioprotection via estrogen. In contrast, the current results in PO using ER- α and ER- β knockout mice indicate that ER- β is responsible for the estrogen-mediated cardioprotection.

IV: Summary

A summary of the gender remodeling differences in response to VO and PO are presented in Table 1. While there remains the need for additional research regarding gender differences in myocardial remodeling secondary to a sustained increase in stress, evidence exists indicating that premenopausal female hearts have the ability to compensate. In the case of VO-induced stress, estrogen alters the course of remodeling, primarily through reduced extracellular matrix degradation, whereby the interstitial cardiac mast cell plays a mechanistic role in this process. Directional alterations in extracellular matrix proteolytic activity, primarily through the matrix metalloproteinases, including MT1-MMP, and mast cell degranulation were associated with the LV remodeling in VO in male and ovariectomized (OVX) rodents but not in intact pre-menopausal females. While less is known regarding the mechanisms responsible for estrogen-related cardioprotection in PO-induced stress, gender differences in remodeling have been reported again indicating the ability of premenopausal females to adequately compensate. In view of the fact that, in male mice, mast cells have been shown to play a role in the adverse remodeling secondary to PO suggests that estrogen modification of mast cell phenotype for cardioprotection occurs in females with PO. However, this remains to be determined.

The fact that, a significant cause of morbidity and mortality in post-menopausal women is HF, emphasizes the need for further research into gender remodeling differences. While the mechanisms which contribute to the increased incidence and acceleration of HF in this patient population remain unresolved, a common structural event is adverse LV remodeling. In addition, cardiac remodeling in response to a sustained elevation in myocardial injury and/or stress is a progressive process. Therefore, in order to understand the mechanisms underlying gender remodeling differences, temporal information regarding LV shape and size, myocyte dimensions and integrin function, and ECM status need to be determined. While such information is accumulating as discussed in the sections above and summarized in Table 1, there remain specific questions which need to be addressed. While there is convincing evidence that mast cells are playing a major role in remodeling in response to VO and PO, the endogenous secretagogues responsible for mast cell activation need to be identified as well as whether estrogen prevents their activation ability. Further, what factors dictate whether activated mast cells cause ECM degradation in VO or fibrosis in PO is still unknown, and how estrogen interferes with these outcomes is of critical importance. Similarly, the factors responsible for determining MT1-MMP's ability to cause ECM degradation and to cause fibrosis depending on the type of stress and the preventative role of estrogen in this process are not fully understood. Such information would be invaluable in identifying critical downstream molecular triggers that are under the regulation of estrogen and relevant to LV remodeling to which more targeted therapy could be developed thereby providing cardioprotection possibly to both genders while avoiding the adverse effects of systemic estrogen delivery.

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Reference List

1. Anversa P, Melissari M, Beghi C, Olivetti G. Structural compensatory mechanisms in rat heart in early spontaneous hypertension. *Am J Physiol.* 1984; 246:H739–H746. [PubMed: 6742139]
2. Badenhorst D, Maseko M, Tsotetsi OJ, Naidoo A, Brooksbank R, Norton GR, Woodiwiss AJ. Cross-linking influences the impact of quantitative changes in myocardial collagen on cardiac stiffness and remodelling in hypertension in rats. *Cardiovasc Res.* 2003; 57:632–641. [PubMed: 12618225]
3. Baicu CF, Stroud JD, Livesay VA, Hapke E, Holder J, Spinale FG, Zile MR. Changes in extracellular collagen matrix alter myocardial systolic performance. *Am J Physiol Heart Circ Physiol.* 2003; 284:H122–H132. [PubMed: 12485818]
4. Bhuiyan MS, Shioda N, Fukunaga K. Ovariectomy augments pressure overload-induced hypertrophy associated with changes in Akt and nitric oxide synthase signaling pathways in female rats. *Am J Physiol Endocrinol Metab.* 2007; 293:E1606–E1614. [PubMed: 17878225]
5. Bobik A. Transforming growth factor-betas and vascular disorders. *Arterioscler Thromb Vasc Biol.* 2006; 26:1712–1720. [PubMed: 16675726]
6. Bouzeghrane F, Mercure C, Reudelhuber TL, Thibault G. Alpha8beta1 integrin is upregulated in myofibroblasts of fibrotic and scarring myocardium. *J Mol Cell Cardiol.* 2004; 36:343–353. [PubMed: 15010273]
7. Brower GL, Chancey AL, Thanigaraj S, Matsubara BB, Janicki JS. Cause and effect relationship between myocardial mast cell number and matrix metalloproteinase activity. *Am J Physiol.* 2002; 283:H518–H525.
8. Brower GL, Henegar JR, Janicki JS. Temporal evaluation of left ventricular remodeling and function in rats with chronic volume overload. *Am J Physiol.* 1996; 271:H2071–H2078. [PubMed: 8945927]

9. Brower GL, Janicki JS. Pharmacologic inhibition of mast cell degranulation prevents left ventricular remodeling induced by chronic volume overload in rats. *J Cardiac Fail.* 2005; 11:548–556.
10. Brower GL, Gardner JD, Janicki JS. Gender mediated cardiac protection from adverse ventricular remodeling is abolished by ovariectomy. *Mol Cell Biochem.* 2003; 251:89–95. [PubMed: 14575309]
11. Brower GL, Janicki JS. Contribution of ventricular remodeling to pathogenesis of heart failure in rats. *Am J Physiol Heart Circ Physiol.* 2001; 280:H674–H683. [PubMed: 11158966]
12. Caughey GH, Raymond WW, Wolters PJ. Angiotensin II generation by mast cell α - and β -chymases. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology.* 2000; 1480:245–257.
13. Chan V, Fenning A, Levick SP, Loch D, Chunduri P, Iyer A, Teo YL, Hoey A, Wilson K, Burstow D, Brown L. Cardiovascular changes during maturation and ageing in male and female spontaneously hypertensive rats. *J Cardiovasc Pharmacol.* 2011; 57:469–478. [PubMed: 21283019]
14. Chancey AL, Brower GL, Peterson JT, Janicki JS. Effects of matrix metalloproteinase inhibition on ventricular remodeling due to volume overload. *Circ.* 2002; 105:1983–1988.
15. Chancey AL, Gardner JD, Murray DB, Brower GL, Janicki JS. Modulation of cardiac mast cell-mediated extracellular matrix degradation by estrogen. *Am J Physiol Heart Circ Physiol.* 2005; 289:H316–H321. [PubMed: 15722408]
16. Collier P, Watson CJ, van Es MH, Phelan D, McGorrian C, Tolan M, Ledwidge MT, McDonald KM, Baugh JA. Getting to the heart of cardiac remodeling; how collagen subtypes may contribute to phenotype. *J Mol Cell Cardiol.* 2012; 52:148–153. [PubMed: 22008391]
17. Combs AB, Acosta D. Toxic mechanisms of the heart: a review. *Toxicol Pathol.* 1990; 18:583–596. [PubMed: 2091237]
18. d'Ortho MP, Will H, Atkinson S, Butler G, Messent A, Gavrilovic J, Smith B, Timpl R, Zardi L, Murphy G. Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. *Eur J Biochem.* 1997; 250:751–757. [PubMed: 9461298]
19. Dallas SL, Sivakumar P, Jones CJ, Chen Q, Peters DM, Mosher DF, Humphries MJ, Kielty CM. Fibronectin regulates latent transforming growth factor-beta (TGF beta) by controlling matrix assembly of latent TGF beta-binding protein-1. *J Biol Chem.* 2005; 280:18871–18880. [PubMed: 15677465]
20. Ding B, Price RL, Goldsmith EC, Borg TK, Yan X, Douglas PS, Weinberg EO, Bartunek J, Thielen T, Didenko VV, Lorell BH. Left ventricular hypertrophy in ascending aortic stenosis mice: anoikis and the progression to early failure. *Circulation.* 2000; 101:2854–2862. [PubMed: 10859293]
21. Du Y, Plante E, Janicki JS, Brower GL. Temporal evaluation of cardiac myocyte hypertrophy and hyperplasia in male rats secondary to chronic volume overload. *Am J Pathol.* 2010; 177:1155–1163. [PubMed: 20651227]
22. Dubey RK, Gillespie DG, Jackson EK, Keller PJ. 17Beta-estradiol, its metabolites, and progesterone inhibit cardiac fibroblast growth. *Hypertension.* 1998; 31:522–528. [PubMed: 9453356]
23. Feng XH, Derynck R. Specificity and versatility in tgf-beta signaling through Smads. *Annu Rev Cell Dev Biol.* 2005; 21:659–693. [PubMed: 16212511]
24. Fliegner D, Schubert C, Penkalla A, Witt H, Kararigas G, Dworatzek E, Staub E, Martus P, Ruiz NP, Kintscher U, Gustafsson JA, Regitz-Zagrosek V. Female sex and estrogen receptor-beta attenuate cardiac remodeling and apoptosis in pressure overload. *Am J Physiol Regul Integr Comp Physiol.* 2010; 298:R1597–R1606. [PubMed: 20375266]
25. Gardner JD, Murray DB, Voloshenyuk TG, Brower GL, Bradley JM, Janicki JS. Estrogen attenuates chronic volume overload induced structural and functional remodeling in male rat hearts. *Am J Physiol Heart Circ Physiol.* 2010; 298:H497–H504. [PubMed: 19933421]
26. Gardner JD, Brower GL, Janicki JS. Gender differences in cardiac remodeling secondary to chronic volume overload. *Journal of Cardiac Failure.* 2002; 8:101–107. [PubMed: 12016634]

27. Gardner JD, Brower GL, Voloshenyuk TG, Janicki JS. Cardioprotection in female rats subjected to chronic volume overload: synergistic interaction of estrogen and phytoestrogens. *Am J Physiol Heart Circ Physiol*. 2008; 294:H198–H204. [PubMed: 17965290]
28. Ghosh AK. Factors involved in the regulation of type I collagen gene expression: implication in fibrosis. *Exp Biol Med* (Maywood). 2002; 227:301–314. [PubMed: 11976400]
29. Gilles S, Zahler S, Welsch U, Sommerhoff CP, Becker BF. Release of TNF- α during myocardial reperfusion depends on oxidative stress and is prevented by mast cell stabilizers. *Cardiovascular Research*. 2003; 60:608–616. [PubMed: 14659806]
30. Grandi AM, Venco A, Barzizza F, Scalise F, Pantaleo P, Finardi G. Influence of age and sex on left ventricular anatomy and function in normals. *Cardiology*. 1992; 81:8–13. [PubMed: 1477859]
31. Gruber BL, Marchese MJ, Suzuki K, Schwartz LB, Okada Y, Nagase H, Ramamurthy NS. Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J Clin Invest*. 1989; 84:1657–1662. [PubMed: 2553780]
32. Hara M, Ono K, Hwang MW, Iwasaki A, Okada M, Nakatani K, Sasayama S, Matsumori A. Evidence for a Role of Mast Cells in the Evolution to Congestive Heart Failure. *J Exp Med*. 2002; 195:375–381. [PubMed: 11828013]
33. Heymans S, Schroen B, Vermeersch P, Milting H, Gao F, Kassner A, Gillijns H, Herijgers P, Flameng W, Carmeliet P, Van de WF, Pinto YM, Janssens S. Increased cardiac expression of tissue inhibitor of metalloproteinase-1 and tissue inhibitor of metalloproteinase-2 is related to cardiac fibrosis and dysfunction in the chronic pressure-overloaded human heart. *Circulation*. 2005; 112:1136–1144. [PubMed: 16103240]
34. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *Jama*. 1998; 280:605–613. [PubMed: 9718051]
35. Janicki, JS.; Matsubara, BB. Myocardial collagen and left ventricular diastolic dysfunction. In: Gaasch, WH.; LeWinter, MM., editors. *Left Ventricular Diastolic Dysfunction*. Lea & Febiger; Philadelphia: 1994. p. 125-140.
36. Janicki JS, Brower GL, Gardner JD, Forman MF, Stewart J, Murray DB, Chancey AL. Cardiac mast cell regulation of matrix metalloproteinase-related ventricular remodeling in chronic pressure or volume overload. *Cardiovascular Research*. 2006; 69:657–665. [PubMed: 16376324]
37. Jobe LJ, Melendez GC, Levick SP, Du Y, Brower GL, Janicki JS. TNF- α inhibition attenuates adverse myocardial remodeling in a rat model of volume overload. *Am J Physiol Heart Circ Physiol*. 2009; 297:H1462–H1468. [PubMed: 19666842]
38. Kanellakis P, Ditiatkovski M, Kostolias G, Bobik A. A pro-fibrotic role for interleukin-4 in cardiac pressure overload. *Cardiovasc Res*. 2012; 95:77–85. [PubMed: 22492684]
39. Koli K, Saharinen J, Hyytiainen M, Penttinen C, Keski-Oja J. Latency, activation, and binding proteins of TGF- β . *Microsc Res Tech*. 2001; 52:354–362. [PubMed: 11170294]
40. Lam CS, Carson PE, Anand IS, Rector TS, Kuskowski M, Komajda M, McKelvie RS, McMurray JJ, Zile MR, Massie BM, Kitzman DW. Sex differences in clinical characteristics and outcomes in elderly patients with heart failure and preserved ejection fraction: the Irbesartan in Heart Failure with Preserved Ejection Fraction (I-PRESERVE) trial. *Circ Heart Fail*. 2012; 5:571–578. [PubMed: 22887722]
41. Lees M, Taylor DJ, Woolley DE. Mast cell proteinases activate precursor forms of collagenase and stromelysin, but not of gelatinases A and B. *Eur J Biochem*. 1994; 223:171–177. [PubMed: 8033891]
42. Levick SP, McLarty JL, Murray DB, Freeman RM, Carver WE, Brower GL. Cardiac mast cells mediate left ventricular fibrosis in the hypertensive rat heart. *Hypertension*. 2009; 53:1041–1047. [PubMed: 19398662]
43. Levick SP, Gardner JD, Holland M, Hauer-Jensen M, Janicki JS, Brower GL. Protection from adverse myocardial remodeling secondary to chronic volume overload in mast cell deficient rats. *Journal of Molecular and Cellular Cardiology*. 2008; 45:56–61. [PubMed: 18538342]

44. Li J, Lu H, Plante E, Melendez GC, Levick SP, Janicki JS. Stem cell factor is responsible for the rapid response in mature mast cell density in the acutely stressed heart. *J Mol Cell Cardiol.* 2012; 53:469–474. [PubMed: 22850284]
45. Liu Z, Hilbelink DR, Crockett WB, Gerdes AM. Regional changes in hemodynamics and cardiac myocyte size in rats with aortocaval fistulas. 1. Developing and established hypertrophy. *Circ Res.* 1991; 69:52–58. [PubMed: 1829028]
46. Liu Z, Hilbelink DR, Gerdes AM. Regional changes in hemodynamics and cardiac myocyte size in rats with aortocaval fistulas. 2. Long-term effects. *Circ Res.* 1991; 69:59–65. [PubMed: 2054942]
47. Lu H, Melendez GC, Levick SP, Janicki JS. Prevention of adverse cardiac remodeling to volume overload in female rats is the result of an estrogen-altered mast cell phenotype. *Am J Physiol Heart Circ Physiol.* 2012; 302:H811–H817. [PubMed: 22160000]
48. Mahmoodzadeh S, Dworatzek E, Fritschka S, Pham TH, Regitz-Zagrosek V. 17beta- Estradiol inhibits matrix metalloproteinase-2 transcription via MAP kinase in fibroblasts. *Cardiovasc Res.* 2010; 85:719–728. [PubMed: 19861308]
49. McLarty JL, Melendez GC, Brower GL, Janicki JS, Levick SP. Tryptase/Protease-activated receptor 2 interactions induce selective mitogen-activated protein kinase signaling and collagen synthesis by cardiac fibroblasts. *Hypertension.* 2011; 58:264–270. [PubMed: 21730297]
50. Melendez GC, Li J, Law BA, Janicki JS, Supowit SC, Levick SP. Substance P Induces Adverse Myocardial Remodeling via a Mechanism Involving Cardiac Mast Cells. *Cardiovasc Res.* 2011; 92:420–429. [PubMed: 21908647]
51. Melendez GC, Voloshenyuk TG, McLarty JL, Levick SP, Brower GL. Oxidative stress mediated cardiac mast cell degranulation. *Toxicological and Environmental Chemistry.* 2010; 92:1293–1301.
52. Murray DB, Gardner JD, Brower GL, Janicki JS. Endothelin-1 mediates cardiac mast cell degranulation, matrix metalloproteinase activation, and myocardial remodeling in rats. *Am J Physiol Heart Circ Physiol.* 2004; 287:H2295–H2299. [PubMed: 15231495]
53. Murray DB, Gardner JD, Brower GL, Janicki JS. Effects of nonselective endothelin-1 receptor antagonism on cardiac mast cell-mediated ventricular remodeling in rats. *Am J Physiol Heart Circ Physiol.* 2008; 294:H1251–H1257. [PubMed: 18178727]
54. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, Dietz HC. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet.* 2003; 33:407–411. [PubMed: 12598898]
55. Norton GR, Tsotetsi J, Trifunovic B, Hartford C, Candy GP, Woodiwiss AJ. Myocardial stiffness is attributed to alterations in cross-linked collagen rather than total collagen or phenotypes in spontaneously hypertensive rats. *Circulation.* 1997; 96:1991–1998. [PubMed: 9323091]
56. Panizo A, Mindan FJ, Galindo MF, Cenarruzabeitia E, Hernandez M, Diez J. Are mast cells involved in hypertensive heart disease? *J Hypertens.* 1995; 13:1201–1208. [PubMed: 8586812]
57. Pedram A, Razandi M, O'Mahony F, Lubahn D, Levin ER. Estrogen receptor-beta prevents cardiac fibrosis. *Mol Endocrinol.* 2010; 24:2152–2165. [PubMed: 20810711]
58. Pelzer T, Neumann M, de JT, Jazbutyte V, Neyses L. Estrogen effects in the myocardium: inhibition of NF-kappaB DNA binding by estrogen receptor-alpha and -beta. *Biochem Biophys Res Commun.* 2001; 286:1153–1157. [PubMed: 11527420]
59. Peterson JT. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. *Circ.* 2001; 103:2303–2309.
60. Petrov G, Regitz-Zagrosek V, Lehmkuhl E, Krabatsch T, Dunkel A, Dandel M, Dworatzek E, Mahmoodzadeh S, Schubert C, Becher E, Hampl H, Hetzer R. Regression of myocardial hypertrophy after aortic valve replacement: faster in women? *Circulation.* 2010; 122:S23–S28. [PubMed: 20837918]
61. Qiu Y, Liao R, Zhang X. Intervention of cardiomyocyte death based on the impedancesensing technique of monitoring cell adhesion. *Conf Proc IEEE Eng Med Biol Soc.* 2009; 2009:4457–4460. [PubMed: 19964632]
62. Rifkin DB. Latent transforming growth factor-beta (TGF-beta) binding proteins: orchestrators of TGF-beta availability. *J Biol Chem.* 2005; 280:7409–7412. [PubMed: 15611103]

63. Ropero AB, Eghbali M, Minosyan TY, Tang G, Toro L, Stefani E. Heart estrogen receptor alpha: distinct membrane and nuclear distribution patterns and regulation by estrogen. *J Mol Cell Cardiol.* 2006; 41:496–510. [PubMed: 16876190]
64. Ross RS. The extracellular connections: the role of integrins in myocardial remodeling. *J Card Fail.* 2002; 8:S326–S331. [PubMed: 12555140]
65. Ross RS, Borg TK. Integrins and the Myocardium. *Circ Res.* 2001; 88:1112–1119. [PubMed: 11397776]
66. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *Jama.* 2002; 288:321–333. [PubMed: 12117397]
67. Ruddy JM, Jones JA, Stroud RE, Mukherjee R, Spinale FG, Ikonomidis JS. Differential effects of mechanical and biological stimuli on matrix metalloproteinase promoter activation in the thoracic aorta. *Circulation.* 2009; 120:S262–S268. [PubMed: 19752377]
68. Salpeter SR, Walsh JM, Greyber E, Ormiston TM, Salpeter EE. Mortality associated with hormone replacement therapy in younger and older women: a meta-analysis. *J Gen Intern Med.* 2004; 19:791–804. [PubMed: 15209595]
69. Schierbeck LL, Rejnmark L, Tofteng CL, Stilgren L, Eiken P, Mosekilde L, Kober L, Jensen JE. Effect of hormone replacement therapy on cardiovascular events in recently postmenopausal women: randomised trial. *BMJ.* 2012; 345:e6409. [PubMed: 23048011]
70. Seguin CA, Pilliar RM, Madri JA, Kandel RA. TNF-alpha induces MMP2 gelatinase activity and MT1-MMP expression in an in vitro model of nucleus pulposus tissue degeneration. *Spine.* 2008; 33:356–365. [PubMed: 18277865]
71. Shiota N, Rysa J, Kovanen PT, Ruskoaha H, Kokkonen JO, Lindstedt KA. A role for cardiac mast cells in the pathogenesis of hypertensive heart disease. *J Hypertens.* 2003; 21:1823–1825. [PubMed: 14508186]
72. Simon JA. What's new in hormone replacement therapy: focus on transdermal estradiol and micronized progesterone. *Climacteric.* 2012; 15(Suppl 1):3–10. [PubMed: 22432810]
73. Skavdahl M, Steenbergen C, Clark J, Myers P, Demianenko T, Mao L, Rockman HA, Korach KS, Murphy E. Estrogen receptor-beta mediates male-female differences in the development of pressure overload hypertrophy. *Am J Physiol Heart Circ Physiol.* 2005; 288:H469–H476. [PubMed: 15374829]
74. Spinale FG, Coker ML, Krombach SR, Mukherjee R, Hallak H, Houck WV, Clair MJ, Kribbs SB, Johnson LL, Peterson JT, Zile MR. Matrix metalloproteinase inhibition during the development of congestive heart failure : effects on left ventricular dimensions and function. *Circ Res.* 1999; 85:364–376. [PubMed: 10455065]
75. Staufer BL, Leinwand LA. Sex differences in cardiac muscle and remodeling. *Advance in Molecular and Cell Biology.* 2004; 34:131–145.
76. Stewart JA Jr, Cashatt DO, Borck AC, Brown JE, Carver WE. 17beta-estradiol modulation of angiotensin II-stimulated response in cardiac fibroblasts. *J Mol Cell Cardiol.* 2006; 41:97–107. [PubMed: 16765374]
77. Stewart JA Jr, Massey EP, Fix C, Zhu J, Goldsmith EC, Carver W. Temporal alterations in cardiac fibroblast function following induction of pressure overload. *Cell Tissue Res.* 2010; 340:117–126. [PubMed: 20217135]
78. Tamura T, Said S, Gerdes AM. Gender-related differences in myocyte remodeling in progression to heart failure. *Hypertension.* 1999; 33:676–680. [PubMed: 10024326]
79. Tchougounova E, Lundquist A, Fajardo I, Winberg JO, Abrink M, Pejler G. A Key Role for Mast Cell Chymase in the Activation of Pro-matrix Metalloprotease-9 and Pro-matrix Metalloprotease-2. *J Biol Chem.* 2005; 280:9291–9296. [PubMed: 15615702]
80. van Eickels M, Grohe C, Cleutjens JP, Janssen BJ, Wellens HJ, Doevendans PA. 17beta-estradiol attenuates the development of pressure-overload hypertrophy. *Circulation.* 2001; 104:1419–1423. [PubMed: 11560859]
81. Vickers MR, MacLennan AH, Lawton B, Ford D, Martin J, Meredith SK, DeStavola BL, Rose S, Dowell A, Wilkes HC, Darbyshire JH, Meade TW. Main morbidities recorded in the women's

- international study of long duration oestrogen after menopause (WISDOM): a randomised controlled trial of hormone replacement therapy in postmenopausal women. *BMJ*. 2007; 335:239. [PubMed: 17626056]
82. Voloshenyuk TG, Gardner JD. Estrogen improves TIMP-MMP balance and collagen distribution in volume-overloaded hearts of ovariectomized females. *Am J Physiol Regul Integr Comp Physiol*. 2010; 299:R683–R693. [PubMed: 20504902]
 83. Wang X, Li F, Gerdes AM. Chronic pressure overload cardiac hypertrophy and failure in guinea pigs: I. Regional hemodynamics and myocyte remodeling. *J Mol Cell Cardiol*. 1999; 31:307–317. [PubMed: 10093044]
 84. Weber KT, Janicki JS, Shroff SG, Pick R, Chen RM, Bashey RI. Collagen remodeling of the pressure overloaded, hypertrophied nonhuman primate myocardium. *Circ Res*. 1988; 62:757–765. [PubMed: 2964945]
 85. Weber KT, Brilla CG, Janicki JS. Myocardial fibrosis: functional significance and regulatory factors. *Cardiovasc Res*. 1993; 27:341–348. [PubMed: 8490934]
 86. Weinberg EO, Thienelt CD, Katz SE, Bartunek J, Tajima M, Rohrbach S, Douglas PS, Lorell BH. Gender differences in molecular remodeling in pressure overload hypertrophy. *J Am Coll Cardiol*. 1999; 34:264–273. [PubMed: 10400020]
 87. Yang YN, Wang F, Zhou W, Wu ZQ, Xing YQ. TNF-alpha Stimulates MMP-2 and MMP-9 Activities in Human Corneal Epithelial Cells via the Activation of FAK/ERK Signaling. *Ophthalmic Res*. 2012; 48:165–170. [PubMed: 22710949]
 88. Yarbrough WM, Mukherjee R, Stroud RE, Rivers WT, Oelsen JM, Dixon JA, Eckhouse SR, Ikonmidis JS, Zile MR, Spinale FG. Progressive induction of left ventricular pressure overload in a large animal model elicits myocardial remodeling and a unique matrix signature. *J Thorac Cardiovasc Surg*. 2012; 143:215–223. [PubMed: 22056365]
 89. Zaitsu M, Narita S, Lambert KC, Grady JJ, Estes DM, Curran EM, Brooks EG, Watson CS, Goldblum RM, Midoro-Horiuti T. Estradiol activates mast cells via a non-genomic estrogen receptor-alpha and calcium influx. *Mol Immunol*. 2007; 44:1977–1985. [PubMed: 17084457]
 90. Zile MR, Baicu CF, Stroud RE, Van Laer A, Arroyo J, Mukherjee R, Jones JA, Spinale FG. Pressure overload-dependent membrane type 1-matrix metalloproteinase induction: relationship to LV remodeling and fibrosis. *Am J Physiol Heart Circ Physiol*. 2012; 302:H1429–H1437. [PubMed: 22287584]

Figure 1. Cardiac cross-sections depicting gender remodeling differences after 8 weeks of sustained cardiac volume overload. While significant hypertrophy occurred in the male and female hearts, the male heart becomes markedly dilated with significant wall thinning while the female heart develops thicker walls with little chamber dilation.

Table 1

(A) Short-term* responses in the heart under conditions of volume and pressure overload.				
Parameter	VO-male	VO-female	PO-male	PO-female
LV Weight	++	+	++	+
LV Wall Thickness	/	+	+++	++
LV Chamber Size	++	+	–	–
Cardiomyocyte Width	/	+	++	+
Cardiomyocyte Length	/	+	+	/
ECM	---	/	+	+
MMP Activity	+++	/	++	?
MC Density	+++	/	++	?
TNF- α	+++	/	++	?
Integrins	--	?	+	?

(B) Decompensated responses in the heart under conditions of volume and pressure overload.				
Parameter	VO-male	VO-female	PO-male	PO-female
LV Weight	+++	++	+++	++
LV Wall Thickness	---	++	---	++
LV Chamber Size	+++	–	++	/
LV Function	---	/	---	/
LV Stiffness	---	/	+++	/
Cardiomyocyte Width	/	++	–	–
Cardiomyocyte Length	+++	++	++	/
ECM	– or /	/	+++	++
MC Density	/	/	++	?

VO=volume overload, PO=pressure overload, MC=mast cell, ECM=extracellular matrix, MMP=matrix metalloproteinase, LV=left ventricle.

+ = slight increase, ++ = moderate increase, +++ = large increase

– = slight decrease, -- = moderate decrease, --- = large decrease

/ = no change from normal, ? = unknown

* Short-term in Table A refers to the first week in volume overload. In pressure overload it is model specific and refers to the first 2 weeks in the transaortic constriction model and the first 12 months in the spontaneously hypertensive rat model.