

STATE-OF-THE-ART PAPER

Sex-Related Differences in Myocardial Remodeling

Maddalena Piro, MD, PhD,* Roberta Della Bona, MD,* Antonio Abbate, MD, PhD,*†
Luigi M. Biasucci, MD,* Filippo Crea, MD*

Rome, Italy; and Richmond, Virginia

Sex has a profound impact on myocardial remodeling, which is defined as the molecular and cellular events after an injury to the myocardium (i.e., necrosis, pressure overload, volume overload, and aging) leading to a change in shape, dimension, and function of cardiac chambers. Indeed, experimental studies and post-mortem and observational clinical studies suggest the presence of important differences in myocardial remodeling between females and males in response to different types of injuries including aging, pressure and volume overload, and myocardial infarction. Interestingly, the remodeling process appears to be more favorable in women versus men; women are more likely to present heart failure with preserved systolic function and are at greater risk for low output syndrome acutely. These differences between men and women are widely held to be related to sex hormones such as estrogen, although the molecular effects of estrogen on ventricular cardiomyocytes are incompletely understood. In this review, we summarize the evidence supporting these notions and discuss the underlying mechanisms and the clinical implications. (J Am Coll Cardiol 2010;55:1057-65) © 2010 by the American College of Cardiology Foundation

Myocardial remodeling is defined as the molecular and cellular events after an injury to the myocardium (i.e., necrosis, pressure overload, volume overload, and aging) leading to a change in shape, dimension, and function of cardiac chambers. There are 2 patterns of remodeling: concentric and eccentric hypertrophy. The former is associated with an increase in wall thickness and initial preservation of cavity size and ejection fraction (EF). The latter is associated with progressive chamber dilation and dysfunction but with initial preservation of the stroke volume (1-3).

Sex has a profound impact on remodeling. Indeed, observational clinical studies and post-mortem and experimental studies suggest the presence of important differences in cardiac remodeling between females and males. In the first part of this review, we compare these differences in specific pathophysiological settings including aging cardiomyopathy, pressure and volume overload, myocardial ischemia, cardiogenic shock, and diastolic heart failure. We have included post-mortem and clinical and experimental observations. In the second part of the review, we discuss the plausible biological mechanisms responsible for sex-related differences, including the effect of estrogen on vascular and cardiac cell.

Aging Cardiomyopathy

Myocyte loss is the common final pathway leading to heart failure due to multiple causes, including aging. Aging cardiomyopathy is a clinically recognized entity (4). In an autopsy study, Olivetti et al. (5) analyzed the changes in number and size of ventricular myocytes in the hearts of 53 women and 53 men (whose body weight was not >20% or <20% of the optimal weight for sex, height, and age), in the age interval from 17 to 95 years. Among autopsy criteria for exclusion, they considered heart weight >400 g and >450 g for women and men, respectively, severe atherosclerosis, and acute and chronic myocardial infarction (MI). They demonstrated that, while aging was associated with preservation of cardiac weight and myocyte number and volume in females, ≈ 1 g/year of myocardium was lost in males, in association with a loss of 64 millions cells/year. Furthermore, myocyte cell volume increased at rate of $158 \mu\text{m}^3/\text{year}$ in the left ventricle (LV) and $167 \text{m}^3/\text{year}$ in the right ventricle in men but not in women. Moreover, the fraction of mononucleated and binucleated myocytes remained constant in the female heart with aging. In contrast, in men, mononucleated myocytes decreased by 0.3% per year in the LV and 0.2% per year in the right ventricle, while the percent of binucleated myocytes increased by 0.3% per year in the LV and 0.2% per year in the right ventricle.

These findings support the notion that sex differences may play a significant role in the detrimental effect of aging on the heart. In men, the progressive increase in average myocyte cell volume probably represents a reactive hypertrophic response to myocyte loss, thus preserving left and

From the *Catholic University of the Sacred Heart, Rome, Italy; and the †VCU Pauley Heart Center, Virginia Commonwealth University, Richmond, Virginia. Dr. Biasucci is a consultant for Pfizer, Sanofi-Aventis, Roche Diagnostic, and Siemens diagnostic, and receives grants from Sanofi-Aventis and Boehringer-Ingelheim.

Manuscript received February 13, 2009; revised manuscript received July 27, 2009, accepted September 1, 2009.

**Abbreviations
and Acronyms**

ANF = atrial natriuretic factor
EF = ejection fraction
EPC = endothelial progenitor cell
ER = estrogen receptor
IGF = insulin growth factor
LV = left ventricle/ ventricular
LVH = left ventricular hypertrophy
MI = myocardial infarction
mRNA = messenger ribonucleic acid
MSC = mesenchymal stem cell
SHR = spontaneously hypertensive rat

right ventricular wall thickness with aging. The basis for the differential impact of aging on the heart of males and females is currently unknown. A potential explanation may be related to the higher cardiac work load of male hearts throughout life, leading to an attenuation of the growth reserve of the myocardium with age in men (6–8). Moreover, sex appears to be an important determinant of the occurrence of apoptosis (9). Apoptosis, or programmed cell death, is the principal form of chronic cell loss (10). In a post-mortem study on humans, Mallat et al. (11) found that the apoptotic index was 3-fold higher in men than in women free of any cardiovascular disease who died of either violent

or natural causes. The lack of an increase in apoptosis rate and of a decrease in LV mass in the aging female heart might help to explain the reduced incidence of aging cardiomyopathy in women as compared with men. This hypothesis is in line with the findings by an animal study by Zhang et al. (12) in monkeys (*Macaca fascicularis*). Old male monkeys had a 4-fold increase in frequency of apoptosis compared with old female monkeys without any increase in proliferation-capable myocyte as assessed by Ki-67 expression.

Myocardial Response to Pressure Overload

Pressure overload is a common cause of cardiac remodeling and heart failure. Villari et al. (13) demonstrated a positive myocardial remodeling and a decrease in muscle mass in 15 patients who underwent aortic valve replacement. Subsequently, Morris et al. (14) attempted to characterize sex differences in recovery of ventricular function and survival after aortic valve replacement. Women showed an earlier improvement than men in EF after aortic valve replacement suggesting that sex-related factors may influence the adaptive response of the LV to pressure overload.

In line with these studies, Kostkiewicz et al. (15) studied 195 patients with isolated severe aortic stenosis at echocardiography to investigate the influence of sex on LV remodeling and preservation of systolic function. When compared with men, women had similar transvalvular aortic gradient and estimated aortic area, but had a greater degree of left ventricular hypertrophy (LVH) documented as changes in LV geometry (increased LV mass, increased relative wall thickness, and smaller end-diastolic and -systolic dimensions) and had preserved LV function (greater LV fractional shortening and EF), in keeping with the results of other studies (16–20).

Whether these differences result from intrinsic differences in molecular adaptation to pressure overload between men and women or are related to other factors extrinsic to the myocardium is unknown. Differences in geometric remodeling and an earlier onset of impaired systolic pump performance in male versus female animals have also been reported by Pfeffer et al. (21) in a spontaneously hypertensive rat (SHR), a model of pressure overload cardiomyopathy. When compared with male SHR, female SHR had greater EF and cardiac index and smaller end-diastolic and -systolic volumes, despite similar systolic blood pressure values. Female SHR (ages 6 to 18 months) had normal heart dimensions and function whereas male SHR had LV dysfunction and heart failure by 12 months.

Using banding of the transverse aorta as another model of pressure overload in male and female Wistar rats, Weinberg et al. (22) examined LV contractile reserve in the isolated heart 6 weeks after banding. Higher pressures (contractile force) developed in females than in males. Despite a similar degree of LVH and systolic wall stress, female hearts had preserved contractile reserve, whereas male hearts had depressed contractile reserve. The expression of β -myosin heavy chain and messenger ribonucleic acid (mRNA) of atrial natriuretic factor (ANF) in the ventricular myocardium was greater in male than in female hearts. Conversely, sarcoplasmic reticulum Ca^{++} -adenosine triphosphatase mRNA levels were depressed in male rats but not in female rats, compared with control rats. Thus, this study for the first time demonstrated that there are striking sex-related differences in the expression of key genes known to play critical roles in cardiac calcium regeneration and contractile function at an early stage of chronic pressure overload before the development of overt failure. In this animal model, the reduction in contractile reserve observed in male LVH rats may be explained in part by the greater up-regulation of heavy β -myosin, normally expressed in animal fetal life and down-regulated in the post-natal LV (23), and by the lower expression of sarcoplasmic reticulum Ca^{++} -adenosine triphosphatase, closely related to excitation-contraction coupling (24). Moreover, the removal of sex hormones by gonadectomy in rats significantly reduces cardiac function and induces a shift in myosin heavy chain content to the slower isoform ($V3/\beta$), indicative of a pathological shift that can be reversed by hormonal supplementation (25). These phenomena might play an important role also in humans, but this hypothesis has not specifically been tested.

Myocardial Response to Volume Overload

Volume overload leads to eccentric hypertrophy and eventually to heart failure. A clinical study by Rohde et al. (26) demonstrated that, in comparison with men, women with isolated aortic regurgitation had smaller end-diastolic and -systolic volumes, despite a similar degree of regurgitation. Multivariate analysis showed that female sex was independently associated with a greater LV mass/volume ratio after adjusting for the

severity of the valvular lesion, age, LV function, and concomitant bypass graft surgery. The LV mass and volume were calculated based on the criteria defined by Devereux and Reichek (Penn convention) (26), and adjusted for body surface area.

Additional evidence supporting the presence of sex-related differences in LV adaptation to volume overload derives from an animal study by Gardner et al. (27). They demonstrated that female rats adapt more favorably to volume overload induced by an infrarenal aortocaval fistula than do male rats. Female hearts developed concentric hypertrophy with no impairment of cardiac function, minimal ventricular dilation, and no changes in myocardial compliance after 8 weeks of fistula creation. In contrast, male hearts had significant fistula-induced dilation and decreased ventricular compliance. Moreover, the mortality rate was 10-fold higher for males than for females (25% vs. 3%), despite a similar degree of volume overload. The main difference between male and female hearts was not in the degree of hypertrophy, but rather in the degree of dilation. These findings suggest that in the presence of volume overload, female hearts develop appropriate concentric hypertrophy sufficient to maintain a stable compensated state, preventing the development of ventricular dilation and heart failure.

Myocardial Response to Acute Myocardial Ischemia

After acute coronary occlusion, the ensuing myocardial ischemia leads to excitation-contraction uncoupling, cell edema, apoptosis, and necrosis. Cardiomyocyte ischemic necrosis peaks at 24 h. Apoptosis peaks earlier (6 h), while being present also late after the index event, contributing to adverse remodeling (10). Unfavorable myocardial remodeling is a common consequence of acute MI (1). Heart failure is more frequent and severe among men than among women. In the CHARM (Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity) trial, women presenting with heart failure symptoms had higher LVEF and, after adjustment for all baseline characteristics, had better overall survival versus men, independent of menopause status (28).

Sex-related differences in cardiac remodeling are probably among the explanations of such sex-related differences in clinical outcome (29). Post-mortem data suggest that males and females may have a different modulation of the apoptotic pathway in the peri-infarct region. Females appear to be, at least partially, protected from ischemia-induced activation of the apoptotic cascade. Biondi-Zoccai et al. (9) showed that, among patients who died late after acute MI, males had a 10-fold higher apoptotic rate than women in peri-infarct region and greater *bax* expression than women in the peri-infarct region. These findings may explain the more aggressive course of post-infarction heart failure in men and its relatively more benign course in women. Guerra

et al. (29) found that apoptotic and necrotic myocyte death rate differed in the failing hearts of women and men. The lower rate of cell death in women was associated with a longer duration of the cardiomyopathy, a later onset of cardiac decompensation, and a longer interval between heart failure and transplantation.

In an animal model of experimental MI by Cavasin et al. (30), male mice, compared with female, had delayed myocardial healing, higher infarct expansion index, and a greater incidence in cardiac rupture. During the chronic phase of post-MI (12 weeks after induction of MI), the male survivors had worse cardiac function and more pronounced maladaptive remodeling, including a significantly greater dilation and myocyte hypertrophy. In contrast, females had 3 times lower mortality despite similar infarct size and showed a better outcome during the development of heart failure. The sex-related differences in early mortality and in cardiac rupture occurring during the first week post-MI may be attributable to differing mechanisms of infarct healing, tissue repair, degradation of the extracellular matrix, and myocyte slippage. In fact, male mice compared with female mice had premature extracellular matrix degradation, because of the higher number of neutrophils and increased activity of metalloproteinases, and they had a delayed removal of necrotic tissue and scar formation, probably related to the lower number of macrophages (30).

Cardiogenic Shock and “Diastolic” Heart Failure

The reduced ventricular dilation during remodeling in women compared with men, especially in the acute setting, may be beneficial on the one hand yet detrimental on the other. Indeed, an initial mild ventricular dilation during an acute insult (i.e., acute MI, acute myocarditis, and so forth) may be protective because it helps maintain an adequate stroke volume. In a study on patients presenting with shock, women had a significantly lower cardiac index despite similar LVEF. Moreover, women with cardiogenic shock complicating acute MI have more frequent adverse clinical events and mechanical complications (31). Low cardiac output syndrome after cardiac surgery is also more common among women (32).

The reduced ventricular dilation during remodeling in women compared with men helps explain why approximately one-half of women presenting with heart failure symptoms have preserved LVEF versus one-third of men (33). When affected by heart failure, women are also more likely than men to present with congestive symptoms (34). In an elegant study, Regitz-Zagrosek et al. (35) showed that women with heart failure and preserved LVEF had significantly greater impairment in diastolic filling compared with men. Both men and women with diastolic dysfunction and preserved EF showed an increase in the ventricular end-diastolic pressure-volume relation. When compared with men, women with heart failure and preserved systolic

function had smaller LV end-diastolic volume and stroke volumes but higher LV end-diastolic pressure.

Role of Estrogens In Myocardial Remodeling

The observed sex differences in heart disease, and particularly in ventricular remodeling, have led to considerable speculation regarding the underlying etiology, in which the role of circulating sex hormones must be certainly considered. Although the specific mechanisms by which estrogens exert their cardioprotective effects are not completely understood, they include indirect and direct effects of estrogens on vascular and cardiac cells.

Estrogens and menopause. It is well established that the great majority of women present with heart failure after menopause, when circulating levels of estrogen are low. Thus, the question is how estrogens can be protective after the menopause. In this regard, it is worth noting that it is indeed important to distinguish between ovarian synthesis of estrogens, which is subject to dramatic changes during the course of life, in particular after menopause, and intramyocardial synthesis, which is less influenced by such variations (36). Moreover, functional estrogen receptors (ERs [α and β]) have been demonstrated in ventricular myocardium of both males and females, and estrogen binding has both genomic and nongenomic effects (37). Estrogen binding has genomic effects as ERs are ligand-activated transcription factors and can activate transcription of a number of genes whose promoter regions contain tandem estrogen response elements (38). Conversely, ERs mediate nongenomic effects, inducing intracellular-signaling cascades, such as activation of protein kinase C and extracellular signal-regulated kinase, and modulating signaling by growth factors such as insulin-like growth factor (IGF)-1, epidermal growth factor, or transforming growth factor (39). In an animal model, Novotny et al. (40) demonstrated for the first time a protective role for nongenomic ER α signaling in the aged female rat heart. In particular, acute ER α activation was effective in reducing ischemic-reperfusion injury and necrotic and/or apoptotic cell death in the aged heart, which could have clinical relevance for selective ER α modulators as potential therapeutic targets in the aging female (40).

Probably, in post-menopausal women an up-regulation of nongenomic ER α signaling might contribute to explain sex differences in myocardial remodeling. Moreover, it should be considered that in the absence of estrogens, ERs can be recruited to transactivate estrogen-responsive genes. For example, IGF-1-induced transcription is dependent on the recruitment of ER α to the activator protein 1 complex but does not require estrogen to be present (41).

Metabolic and vascular effects of estrogens. Estrogens are known to exert beneficial effect on lipid metabolism and other endocrine effects that indirectly can regulate myocardial adaptation. Moreover, their protective role on modula-

tion of proinflammatory cytokines (42) and cardiac fibroblast growth (43) is well known.

Estrogens have also been demonstrated to exert their protective action on vascular and myocardial cells, both directly and through receptor-mediated effects. It has been demonstrated that estrogens directly relax coronary arteries (44) and restore endothelial function of peripheral resistance arteries in normotensive and hypertensive post-menopausal women (45) through mechanisms involving release of nitric oxide. Cardiac myocytes and cardiac fibroblasts also express functional ER α and β , and this activation downstream targets genes that play a key role in LVH, myocyte survival, and apoptosis (46).

Estrogens and LVH. Van Eickels et al. (47) demonstrated that greater LVH developed in female ovariectomized mice in a model of pressure overload than ovariectomized mice with replacement of physiological levels of 17 β -estradiol. They also observed a more pronounced ventricular expression of ANF in the banded, estrogen-supplemented group than in the banded, vehicle-treated animals. In line with these findings, to elucidate the underlying molecular mechanisms, the same group studied the relationship between estrogen antagonism of hypertrophy, ANF expression, and guanylyl cyclase A receptor signaling in cultured cardiomyocytes (48). They demonstrated that estrogen exerts profound antihypertrophic effects on ventricular myocytes, by transactivation of the ANF gene. Estrogen-induced ANF accumulation in the ventricular myocyte most likely results in ANF receptor activation in an autocrine/paracrine manner, which in turn evokes cytoplasmic cyclic-guanosine monophosphate signaling downstream of the guanylyl cyclase A receptor. In summary, estrogen-mediated ANF induction in cardiac hypertrophy contributes to reduce LVH.

Estrogens and apoptosis. Another molecular mechanism proven to reduce cytopathic damage associated with myocardial injury involves the activation of the serine/threonine protein kinase called *Akt* (also known as protein kinase B) that regulates a broad range of physiological responses including metabolism, gene transcription, and cell survival (49).

The activation of *Akt* in a sex-dependent manner may help explain different susceptibility to cardiovascular disease and support the beneficial role of estrogen stimulation (Fig. 1). Camper-Kirby et al. (50) demonstrated that adult premenopausal women display a significantly greater frequency of staining of *Akt1/2* (phospho-Ser473/474) in the nuclei of their cardiac myocytes than do men or post-menopausal women. These differences have also been seen in transgenic mice that specifically overexpress IGF-1, a proven stimulus for *Akt* activation, in the heart. In fact, both localization of phospho-*Akt* in myocardial nuclei and *Akt* kinase activity are increased in nuclear extracts from sexually mature female mice versus males. Moreover, they demonstrated an increased cytosolic localization of phospho-forkhead, a downstream nuclear target of *Akt*, in myocytes of female mouse hearts compared with male mouse hearts (50). This finding is in agreement with a greater

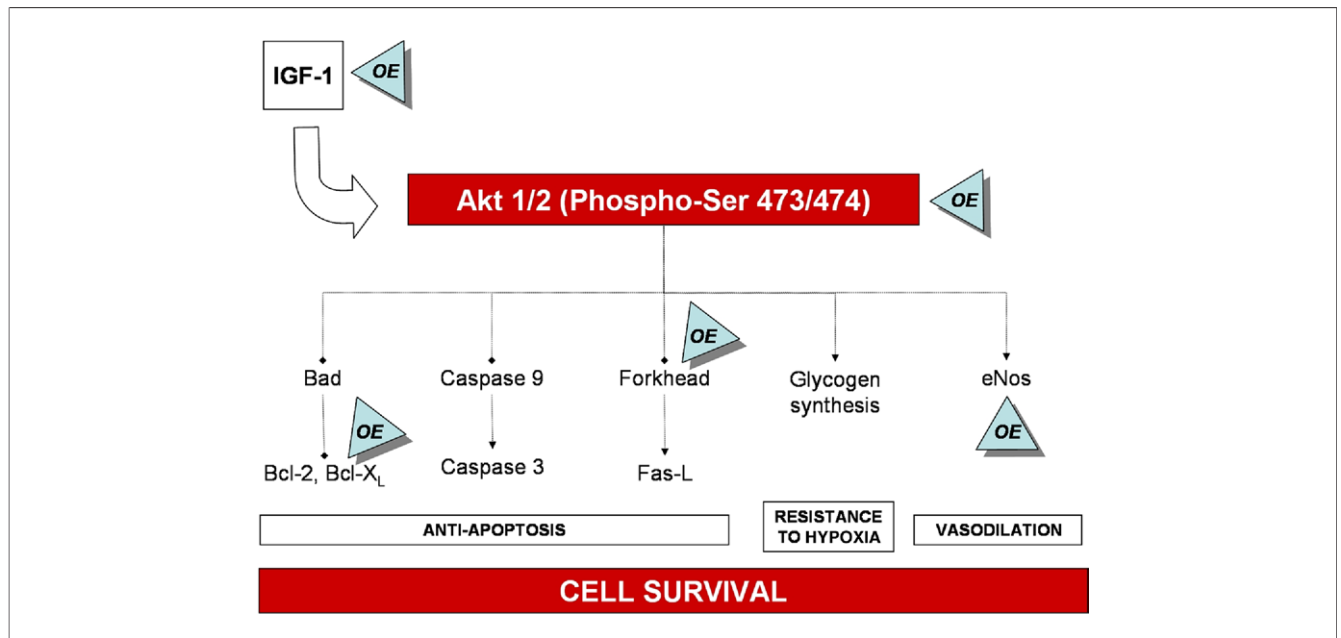


Figure 1 Activation of Akt in a Sex-Dependent Manner

The activation of Akt in a sex-dependent manner may help explain different susceptibility to cardiovascular disease and support the beneficial role of estrogen stimulation. Akt1/2 is overexpressed in the nuclei of the cardiac myocytes of adult pre-menopausal women. Akt prevents initiation of the mitochondrial pathway of apoptosis inducing Bad phosphorylation, reducing Bad inhibition of the antiapoptotic actions of Bcl-2 and Bcl-X; phosphorylates and inhibits caspase-9, responsible for activation of caspase-3 and for degradation of cellular macromolecules results; inhibits the receptor-mediated pathway of apoptosis by phosphorylating members of the forkhead family of transcription factors, inhibiting them to drive expression of Fas ligand (Fas L); and promotes glycogen synthesis that may increase the resistance to cellular hypoxia and to ischemia-reperfusion injury. In myocytes of female hearts, there is an increased cytosolic localization of phospho-forkhead, and an overexpression of the insulin growth factor (IGF)-1, a proven stimulus for Akt activation. Estrogen increases nuclear staining for Akt1/2 and cytoplasmatic staining for phospho-forkhead. Moreover, it induces the phosphorylation of the IGF-1 receptor on myocytes, improving cell survival, indirectly enhancing the expression of antiapoptotic gene products, such as Bcl-2 and Bcl-X, and decreasing the induction of proapoptotic proteins, such as Bax. Estrogen and IGF-1 stimulate nitric oxide, which promotes vasodilation and antithrombotic and inflammatory responses, increasing the cellular resistance to cytotoxic stimuli. eNOS = endothelial nitric oxide synthase; OE = estrogen.

degree of Akt phosphorylation and activation in female heart and illustrates a potential key sex-related difference in Akt-associated cardiac signaling.

In support of a role for estrogen in promotion of Akt signaling, exposure of rat cardiac myocyte cultures to 17β-estradiol or phytoestrogen genistein increased nuclear staining for Akt1/2 (phospho-Ser 473/474) and cytoplasmatic staining for phospho-forkhead (phospho-Ser 256) (51). Moreover, in line with the impact of phytoestrogen on Akt signaling, it has been reported that soy-based diets may affect the cardiovascular phenotypes in rats, playing a cardioprotective role in ischemia-reperfusion injury and in antihypertensive effects (52).

Estrogen-induced Akt activation could protect females against cardiovascular disease by increasing the resistance of their cardiomyocytes to cytotoxic stimuli.

Akt plays a central role in cell survival, modulating both the mitochondrial and the receptor-mediated pathway of apoptotic cascade (53). Akt-induced Bad phosphorylation promotes retention of Bad in the cytoplasm and prevents initiation of the mitochondrial pathway of apoptosis, reducing Bad inhibition of the antiapoptotic actions of Bcl-2 and Bcl-X (54). Moreover, Akt phosphorylates and inhibits caspase-9, which is responsible for activation of caspase-3 and for degradation of cellular macromolecules results (49).

Akt also inhibits the receptor-mediated pathway of apoptosis by phosphorylating members of the forkhead family of transcription factors, causing them to be retained in the cytoplasm and inhibiting them to drive expression of Fas ligand (55).

Increased phospho-forkhead levels, which have been linked to antiapoptotic effects, could account for decreased apoptosis in female failing heart relative to male hearts.

Furthermore, Akt promotes glycogen synthesis that may itself increase resistance to cellular hypoxia during ischemia, providing a greater pool of reserve for anaerobic glycolysis (53) and inducing cardioprotection from ischemia-reperfusion injury, as described in ovariectomized rats (56). Finally, the sex-related differences in Akt activity could be associated with a functional link among estrogen status, Akt activation, and cytoprotection at the level of the isolated cardiomyocytes. Convergent signaling of different pathways on Akt probably reflects important cross-talk between signal transduction mechanisms, as it has been described for estrogen-mediated stimulation of the IGF-1 receptor pathway and activation of the IGF-1 receptor by estrogen-mediated stimulation of phosphatidil-inositol-triphosphate-kinase/Akt (57). In fact, estrogen is able to phosphorylate IGF-1 receptor on myocytes, improving cell survival (58), indirectly enhancing the expression of antiapoptotic gene products, such as Bcl-2 and

Bcl-x, and decreasing the induction of proapoptotic proteins, such as *Bax* (59). Moreover, estrogen and IGF-1 stimulate nitric oxide, which promotes vasodilation and antithrombotic and inflammatory responses (60), increasing the cellular resistance to cytotoxic stimuli.

Estrogens and stem cells. As shown in the preceding text, female patients show relative cardiac protection from acute infarction and better outcome after MI compared with males (61). Such sex dimorphisms may also be related because of the facilitating effect of estrogens on mobilization of endothelial progenitor cells (EPCs), which appear to contribute to the preservation of cardiac function after acute MI (62). The ER α seems to play a more prominent role in this process (63). For the first time, Hamada et al. (62) demonstrated in a murine model that physiological levels of estradiol up-regulate ER α mRNA in EPCs, indicating that the ligand has potent effects on the expression of its own receptor (62).

Moreover, ER α contributes to up-regulation of vascular endothelial growth factor. It has been postulated that 17 β -estradiol may also mobilize EPCs through endothelial nitric oxide synthase-mediated activation of matrix metalloproteinase-9 (64). Up-regulation of matrix metalloproteinase-9 results in the release of soluble *Kit* ligand, which facilitates the transfer of endothelial cells from the quiescent to the proliferative pool (65). Interestingly, treatment with 17 β -estradiol significantly increases the number of EPCs in spontaneously hypertensive rats and delays senescence while augmenting telomerase activity in EPCs through the phosphatidil-inositol-triphosphate-kinase/*Akt* pathway (66,67). These data from an animal model are in concordance with the results deriving from a human study that demonstrated that women with higher plasma estrogen concentration showed a significantly higher level of circulating EPCs (68). The increase in the number of EPCs by 17 β -estradiol has been demonstrated to be mediated by a decreased rate of apoptosis through a caspase-8-dependent pathway (69). Finally, these data provide additional evidence of the importance of bone marrow-derived EPC phenotype in ischemic tissue repair.

Estrogens may exert an important influence on myocardial remodeling after ischemic injury, partially through paracrine growth hormone production by both EPC and bone marrow mesenchymal stem cells (MSC). In keeping with the hypothesis that sex differences exist in activated MSC function, Crisostomo et al. (70) demonstrated that lipopolysaccharide- and hypoxia-induced vascular endothelial growth factor production was significantly greater in female MSC compared with male MSC. Moreover, female MSC expressed significantly less pro-inflammatory cytokines, tumor necrosis factor- α and interleukin-6, compared with male MSC in response to acute lipopolysaccharide and hypoxia, suggesting their ability to limit inflammatory reactions (70). Moreover, stimulation of cardiac fibroblast with 17 β -estradiol induces nuclear translocation of ER protein, playing an important inhibitory role on renin-angiotensin system gene expression, and preventing fibro-

blast proliferation, synthesis of collagen types I and III, and expression of β 1 integrins (46,71).

Sex-related differences in signal transduction pathway may be associated with a greater resistance to hypoxia-induced stress in females versus males (72). Therefore, a better understanding of sex hormone regulation from a cell biology perspective will be critical in improving patient outcomes.

Role of Testosterone in Myocardial Remodeling

Whether estrogen alone is cardioprotective in humans needs to be clarified. Indeed, the observed sex differences and the increased incidence of cardiac events among women after menopause might not entirely be due to the depletion of estrogen, but might also be related to testosterone, as post-menopausal ovaries produce significant amount of androgens in the form of testosterone and androstenedione (73). A positive correlation has been demonstrated in post-menopausal women between increased testosterone levels and hypertension, decreased high-density lipoprotein levels, impaired vascular reactivity, cardiac hypertrophy, and coronary artery disease (74). For the first time in a mouse model of MI, Cavasin et al. (75) demonstrated that estrogen and testosterone play different and opposing roles in the development of heart failure and long-term remodeling after MI. In particular, estrogens (either endogenous or supplemental) prevent maladaptive chronic remodeling and further deterioration of cardiac performance, whereas testosterone (either endogenous or supplemental) adversely affects myocardial healing (as indicated by a higher rate of cardiac rupture), promotes cardiac dysfunction and remodeling, and exerts pronounced effects when estrogen levels are reduced. However, it should be observed that plasma estrogen and testosterone levels in males and females who received hormone replacement were much higher than physiological levels.

Conclusions

The incidence and severity of cardiovascular diseases among pre-menopausal women is lower than among men of comparable age, even after correction for various risk factors (Table 1). The causes of this difference are unclear. Cardiovascular factors strongly associated with sex include vascular function (endothelium-dependent flow-mediated dilation and aortic compliance are greater in females) and an LV mass index that is greater in males. After menopause, the rates of cardiovascular diseases converge, and once affected by ischemic heart disease, females may have a worse prognosis than their male counterparts. The differences of susceptibility are widely held to be related to sex hormones, such as estrogen, that exert potential benefit in inhibiting the progression of cardiac disease. Estrogen's ability to induce systemic vasodilation is in agreement with the hypothesis that sex differences in cardiac disease may depend on the properties of the vascular wall as well as on the

Table 1 Factors Influencing Cardiovascular Prognosis in Women and Men

Women	Men
Aging Cardiomyopathy	
Preservation of cardiac weight	Reduction in cardiac weight (1 g/yr)
Preservation of myocyte number	Reduction in myocyte number (64 million/yr)
Preservation of myocyte volume	Increase in myocyte cell volume
Constant mononucleate/binucleate myocytes ratio	Decreased mononucleate/binucleate myocytes ratio
Low apoptotic index	Apoptotic index 3-fold higher than women
<i>Increased apoptotic rate</i>	<i>Decreased apoptotic rate</i>
Myocardial Response to Pressure Overload	
Earlier improvement in EF after aortic valve replacement	Later improvement in EF after aortic valve replacement
Greater degree of LVH	Lower degree of LVH
Increased LV mass	
Increased relative wall thickness	
Smaller end-diastolic and -systolic dimensions	
Preserved LV function	Impaired LV function
<i>Later onset of impaired systolic pump performance</i>	<i>Earlier onset of impaired systolic pump performance</i>
Greater EF	
Greater cardiac index	
Smaller end-diastolic and -systolic volumes	
<i>Higher expression of β-myosin heavy chain</i>	<i>Lower expression of β-myosin heavy chain</i>
<i>Higher expression of ANF mRNA</i>	<i>Higher expression of ANF mRNA</i>
Myocardial Response to Volume Overload	
Smaller end-diastolic and -systolic volumes	Larger end-diastolic and -systolic volumes
Greater LV mass/volume ratio	Lower LV mass/volume ratio
<i>Concentric hypertrophy</i>	<i>No concentric hypertrophy</i>
<i>No impairment of cardiac function</i>	<i>Impairment of cardiac function</i>
<i>Minimal ventricular dilation</i>	<i>Significant ventricular dilation</i>
<i>No changes in myocardial compliance</i>	<i>Decreased ventricular compliance</i>
Myocardial Response to Acute Myocardial Ischemia	
Lower apoptotic rate in peri-infarct region	10-fold higher apoptotic rate in peri-infarct region
Lower <i>bax</i> expression in peri-infarct region	Greater <i>bax</i> expression in peri-infarct region
Longer duration of the cardiomyopathy	Shorter duration of the cardiomyopathy
Later onset of cardiac decompensation	Earlier onset of cardiac decompensation
Longer interval between heart failure and transplantation	Shorter interval between heart failure and transplantation
<i>Earlier myocardial healing</i>	<i>Delayed myocardial healing</i>
<i>Lower infarct expansion index</i>	<i>Higher infarct expansion index</i>
<i>Three times lower mortality</i>	<i>Greater incidence in cardiac rupture</i>
<i>Better cardiac function</i>	<i>Worse cardiac function</i>
<i>Better remodeling</i>	<i>Maladaptive remodeling</i>
	<i>Significantly greater dilatation</i>
	<i>Myocyte hypertrophy</i>
	<i>Premature extracellular matrix degradation</i>
	<i>Higher number of neutrophils</i>
	<i>Increased activity of metalloproteinases</i>
Cardiogenic Shock	
Significantly lower cardiac index	Higher cardiac index
More frequent adverse clinical events	Less frequent adverse clinical events
More frequent mechanical complications	Less frequent mechanical complications
More common low cardiac output syndrome	Less common low cardiac output syndrome
Heart Failure	
Preserved LV EF	Impaired LV EF
Smaller LV end-diastolic volume	Greater LV end-diastolic volume
Smaller stroke volumes	Greater stroke volumes
Higher LV end-diastolic pressure	Lower LV end-diastolic pressure
More frequent congestive symptoms	Less frequent congestive symptoms
Greater impairment in diastolic filling	Lower impairment in diastolic filling

Data from animal studies are reported in *italics*.

ANF = atrial natriuretic factor; EF = ejection fraction; LV = left ventricle/ventricular; LVH = left ventricular hypertrophy; mRNA = messenger ribonucleic acid.

cardiac myocytes, even if the molecular effects of estrogen on ventricular cardiomyocytes are less well understood. Considering the lower rates of cell loss associated with preservation of cardiac function and dimensions in females

versus males, it is not surprising that women presenting with congestive heart failure are likely to present with different clinical manifestations and significant differences in cardiac function.

Regardless of age and menopause, the remodeling process appears to be more favorable in women versus men. In contrast, women are more likely to present with “diastolic-only” dysfunction and are at greater risk for low output syndrome acutely. Lower awareness of heart disease among women is likely responsible for the worse outcome observed for women in some clinical series, and being a correctable issue, increased awareness of heart disease among women should still represent our number 1 priority.

Further, it is important to stress that being male or female is a variable that should be dealt with in both basic science and clinical research. It is clear that the response of humans and animals to various disease states can be profoundly affected by sex. These differences are unlikely to be due only to sex hormones. Indeed, some genes on the X chromosome are expressed at higher levels in females than in males, despite the process of X chromosome inactivation (76). Moreover, males express genes on the Y chromosome that are absent in females. Thus, a better understanding of the processes leading to differences in remodeling in women will most likely open the way to novel treatment modalities and ultimately benefit patients of both sexes.

Reprint requests and correspondence: Dr. Maddalena Piro, Catholic University of the Sacred Heart, Department of Cardiology, Largo A. Gemelli 8, Rome 00168, Italy. E-mail: pmarilena@tiscali.it.

REFERENCES

- Biondi-Zoccai GG, Baldi A, Biasucci LM, Abbate A. Female gender, myocardial remodeling and cardiac failure: are women protected from increased myocardiocyte apoptosis? *Ital Heart J* 2004;5:498–504.
- Mann DL. Mechanisms and models in heart failure: a combinatorial approach. *Circulation* 1999;100:999–1008.
- Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol* 2000;35:569–82.
- Wei JY. Age and the cardiovascular system. *N Engl J Med* 1992;237:1735–9.
- Olivetti G, Giordano G, Corradi D, et al. Gender differences and aging: effects on the human heart. *J Am Coll Cardiol* 1995;26:1068–79.
- Anversa P, Li P, Zhang X, Olivetti G, Capasso JM. Ischaemic myocardial injury and ventricular remodelling. *Cardiovasc Res* 1993;27:145–57.
- Fratlicelli A, Josephson R, Danziger R, Lakatta E, Spurgeon H. Morphological and contractile characteristics of rat cardiac myocytes from maturation to senescence. *Am J Physiol* 1989;257:H259–65.
- Capasso JM, Fitzpatrick D, Anversa P. Cellular mechanisms of ventricular failure: myocyte kinetics and geometry with age. *Am J Physiol* 1992;262:H1770–81.
- Biondi-Zoccai GG, Abbate A, Bussani R, et al. Reduced post-infarction myocardial apoptosis in women: a clue to their different clinical course? *Heart* 2005;91:99–101.
- Abbate A, Biondi-Zoccai GG, Baldi A. Pathophysiologic role of myocardial apoptosis in post-infarction left ventricular remodeling. *J Cell Physiol* 2002;193:145–53.
- Mallat Z, Fornes P, Costagliola R, et al. Age and gender effects on cardiomyocyte apoptosis in the normal human heart. *J Gerontol A Biol Sci Med Sci* 2001;56:M719–23.
- Zhang XP, Vatner SF, Shen YT, et al. Increased apoptosis and myocyte enlargement with decreased cardiac mass; distinctive features of the aging male, but not female, monkey heart. *J Mol Cell Cardiol* 2007;43:487–91.
- Villari B, Hess OM, Meier C, et al. Regression of coronary artery dimensions after successful aortic valve replacement. *Circulation* 1992;85:972–8.
- Morris JJ, Schaff HV, Mullany CJ, Morris PB, Frye RL, Orszulak TA. Gender differences in left ventricular functional response to aortic valve replacement. *Circulation* 1994;90:II183–9.
- Kostkiewicz M, Tracz W, Olszowska M, Podolec P, Drop D. Left ventricular geometry and function in patients with aortic stenosis: gender differences. *Int J Cardiol* 1999;71:57–61.
- Carroll JD, Carroll EP, Feldman T, et al. Sex-associated differences in left ventricular function in aortic stenosis of the elderly. *Circulation* 1992;86:1099–107.
- Buttrick P, Scheuer J. Sex-associated differences in left ventricular function in aortic stenosis of the elderly. *Circulation* 1992;86:1336–8.
- Aurigemma GP, Silver KH, McLaughlin M, Mauser J, Gaasch WH. Impact of chamber geometry and gender on left ventricular systolic function in patients >60 years of age with aortic stenosis. *Am J Cardiol* 1994;74:794–8.
- Aurigemma GP, Gaasch WH. Gender differences in older patients with pressure-overload hypertrophy of the left ventricle. *Cardiology* 1995;86:310–7.
- Douglas PS, Otto CM, Mickel MC, Labovitz A, Reid CL, Davis KB. Gender differences in left ventricle geometry and function in patients undergoing balloon dilatation of the aortic valve for isolated aortic stenosis. *NHLBI Balloon Valvuloplasty Registry. Br Heart J* 1995;73:548–54.
- Pfeffer JM, Pfeffer MA, Fletcher P, Fishbein MC, Braunwald E. Favorable effects of therapy on cardiac performance in spontaneously hypertensive rats. *Am J Physiol* 1982;242:H776–84.
- Weinberg EO, Thienelt CD, Katz SE, et al. Gender differences in molecular remodeling in pressure overload hypertrophy. *J Am Coll Cardiol* 1999;34:264–73.
- Izumo S, Lompré AM, Matsuoka R, et al. Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy. Interaction between hemodynamic and thyroid hormone-induced signals. *J Clin Invest* 1987;79:970–7.
- Kiss E, Ball NA, Kranias EG, Walsh RA. Differential changes in cardiac phospholamban and sarcoplasmic reticular Ca(2+)-ATPase protein levels. Effects on Ca2+ transport and mechanics in compensated pressure-overload hypertrophy and congestive heart failure. *Circ Res* 1995;77:759–64.
- Scheuer J, Malhotra A, Schaible TF, et al. Effects of gonadectomy and hormonal replacement on rat hearts. *Circ Res* 1987;61:12–9.
- Rohde LE, Zhi G, Aranki SF, Beckel NE, Lee RT, Reimold SC. Gender-associated differences in left ventricular geometry in patients with aortic valve disease and effect of distinct overload subsets. *Am J Cardiol* 1997;80:475–80.
- Gardner JD, Brower GL, Janicki JS. Gender differences in cardiac remodeling secondary to chronic volume overload. *J Card Fail* 2002;8:101–7.
- O’Meara E, Clayton T, McEntegart MB, et al. Sex differences in clinical characteristics and prognosis in a broad spectrum of patients with heart failure: results of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) program. *Circulation* 2007;115:3111–20.
- Guerra S, Leri A, Wang X, et al. Myocyte death in the failing human heart is gender dependent. *Circ Res* 1999;85:856–66.
- Cavasin MA, Tao Z, Menon S, Yang XP. Gender differences in cardiac function during early remodeling after acute myocardial infarction in mice. *Life Sci* 2004;75:2181–92.
- Wong SC, Sleeper LA, Monrad ES, et al. Absence of gender differences in clinical outcomes in patients with cardiogenic shock complicating acute myocardial infarction. A report from the SHOCK trial registry. *J Am Coll Cardiol* 2001;38:1395–401.
- Maganti MD, Rao V, Borger MA, et al. Predictors of low cardiac output syndrome after aortic valve surgery. *Circulation* 2005;112:1448–52.
- Cleland JG, Swedberg K, Follath F, et al. The EuroHeart Failure survey programme—a survey on the quality of care among patients with heart failure in Europe. Part 1: characteristics and diagnosis. *Eur Heart J* 2003;24:442–63.
- Dhar S, Koul D, D’Alonzo GE Jr. Current concepts in diastolic heart failure. *J Am Osteopath Assoc* 2008;108:203–9.

35. Regitz-Zagrosek V, Brokat S, Tschope C. Role of gender in heart failure with normal left ventricular ejection fraction. *Prog Cardiovasc Dis* 2007;49:241–51.
36. Grohe C, Kahlert S, Lobbart K, Vetter H. Expression of estrogen receptor α and β in rat heart: role of local oestrogen synthesis. *J Endocrinol* 1998;156:R1–7.
37. Mahmoodzadeh S, Eder S, Nordmeyer J, et al. Estrogen receptor alpha up-regulation and redistribution in human heart failure. *FASEB J* 2006;20:926–34.
38. Klein-Hitpass L, Schorpp M, Wagner U, Ryffel GU. An estrogen-responsive element derived from the 5' flanking region of the xenopus vitellogenin A2 gene functions in transfected human cells. *Cell* 1986;46:1053–61.
39. Levin ER. Cell localization, physiology, and nongenomic actions of estrogen receptors. *J Appl Physiol* 2001;91:1860–7.
40. Novotny JL, Simpson AM, Tomicek NJ, et al. Rapid estrogen receptor- α activation improves ischemic tolerance in aged female rats through a novel protein kinase C δ -dependent mechanism. *Endocrinology* 2009;150:889–96.
41. Baron S, Escanade A, Alberola G, et al. Estrogen receptor alpha and the activating protein-1 complex cooperate during insulin-like growth factor-1-induced transcriptional activation of the pS2/TFF1 gene. *J Biol Chem* 2007;282:11732–41.
42. An J, Ribeiro RC, Webb P, et al. Estradiol repression of tumor necrosis factor- α transcription requires estrogen receptor activation function-2 and is enhanced by coactivators. *Proc Natl Acad Sci U S A* 1999;96:15161–6.
43. Dubey RK, Gillespie DG, Jackson EK, Keller PJ. 17 β -Estradiol, its metabolites, and progesterone inhibit cardiac fibroblast growth. *Hypertension* 1998;31:522–8.
44. Williams JK, Adams MR, Herrington DM, Clarkson TB. Short-term administration of estrogen and vascular responses of atherosclerotic coronary arteries. *J Am Coll Cardiol* 1992;20:452–7.
45. Higashi Y, Sanada M, Sasaki S, et al. Effect of estrogen replacement therapy on endothelial function in peripheral resistance arteries in normotensive and hypertensive postmenopausal women. *Hypertension* 2001;37:651–7.
46. Grohe C, Kahlert S, Lobbart K, et al. Cardiac myocytes and fibroblasts contain functional estrogen receptors. *FEBS Lett* 1997;416:107–12.
47. Van Eickels M, Grohe C, Cleutjens JP, et al. 17 β -Estradiol attenuates the development of pressure-overload hypertrophy. *Circulation* 2001;104:1419–23.
48. Babiker FA, De Windt LJ, van Eickels M, et al. 17 β estradiol antagonizes cardiomyocytes hypertrophy by autocrine/paracrine stimulation of a guanylyl cyclase A receptor-cyclic guanosine monophosphate-dependent protein kinase pathway. *Circulation* 2004;109:269–76.
49. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to pkb. *Biochem J* 2000;346:561–76.
50. Camper-Kirby D, Welch S, Walker A, et al. Myocardial Akt activation and gender: increased nuclear activity in females versus males. *Circ Res* 2001;88:1020–7.
51. Patten RD, Pourati I, Aronovitz MJ, et al. 17 β -estradiol reduces cardiomyocyte apoptosis in vivo and in vitro via activation of phosphoinositide-3 kinase/Akt signaling. *Circ Res* 2004;95:692–9.
52. Konhilas JP, Leinwand LA. The effects of biological sex and diet on the development of heart failure. *Circulation* 2007;116:2747–59.
53. Sugden PH, Clerk A. Akt like a women: gender differences in susceptibility to cardiovascular disease. *Circ Res* 2001;88:975–7.
54. Zha J, Harada H, Yang E, et al. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X. *Cell* 1996;87:619–28.
55. Brunet A, Bonni A, Zigmond MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 1999;96:857–68.
56. Zhai P, Eurell TE, Cotthaus R, et al. Effect of estrogen on global myocardial ischemia-reperfusion injury in female rats. *Am J Physiol Heart Circ Physiol* 2000;279:H2766–75.
57. Kahlert S, Nuedling S, van Eickels M, et al. Estrogen receptor a rapidly activates the IGF-1 receptor pathway. *J Biol Chem* 2000;275:18447–53.
58. Richards RG, DiAugustine RP, Petrusz P, Clark GC, Sebastian J. Estradiol stimulates tyrosine phosphorylation of the insulin-like growth factor-1 receptor and insulin receptor substrate-1 in the uterus. *Proc Natl Acad Sci USA* 1996;93:12002–7.
59. Wang L, Ma W, Markovich R, Lee WL, Wang PH. Insulin-like growth factor I modulates induction of apoptotic signaling in H9C2 cardiac muscle cells. *Endocrinology* 1998;139:1354–60.
60. Huang A, Sun D, Koller A, Kaley G. Gender difference in myogenic tone of rat arterioles is due to estrogen-induced, enhanced release of NO. *Am J Physiol* 1997;272:H1804–9.
61. Wang M, Crisostomo P, Wairiuko GM, Meldrum DR. Estrogen receptor-alpha mediates acute myocardial protection in females. *Am J Physiol Heart Circ Physiol* 2006;290:H2204–9.
62. Hamada H, Kim MK, Iwakura A. Estrogen receptors a and b mediate contribution of bone marrow-derived endothelial progenitor cells to functional recovery after myocardial infarction. *Circulation* 2006;114:2261–70.
63. Pelzer T, Jazbutyte V, Hu K, et al. The estrogen receptor-alpha agonist 16 α -LE2 inhibits cardiac hypertrophy and improves hemodynamic function in estrogen-deficient spontaneously hypertensive rats. *Cardiovasc Res* 2005;67:604–12.
64. Iwakura A, Shastry S, Luedemann C, et al. Estradiol enhances recovery after myocardial infarction by augmenting incorporation of bone marrow-derived endothelial progenitor cells into sites of ischemia-induced neovascularization via endothelial nitric oxide synthase-mediated activation of matrix metalloproteinase-9. *Circulation* 2006;113:1605–14.
65. Heissig B, Hattori K, Dias S, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 2002;109:625–37.
66. Imanishi T, Kobayashi K, Hano T, Nishio I. Effect of estrogen on differentiation and senescence in endothelial progenitor cells derived from bone marrow in spontaneously hypertensive rats. *Hypertens Res* 2005;28:763–72.
67. Imanishi T, Hano T, Nishio I. Estrogen reduces endothelial progenitor cell senescence through augmentation of telomerase activity. *J Hypertens* 2005;23:1699–706.
68. Hoetzer GL, MacEneaney OJ, Irmiger HM, et al. Gender differences in circulating endothelial progenitor cell colony-forming capacity and migratory activity in middle-aged adults. *Am J Cardiol* 2007;99:46–8.
69. Strehlow K, Werner N, Berweiler J, et al. Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. *Circulation* 2003;107:3059–65.
70. Crisostomo PR, Wang M, Herring CM, et al. Sex dimorphisms in activated mesenchymal stem cell function. *Shock* 2006;26:571–4.
71. Zhou L, Shao Y, Huang Y, et al. 17 β -estradiol inhibits angiotensin II-induced collagen synthesis of cultured rat cardiac fibroblasts via modulating angiotensin II receptors. *Eur J Pharmacol* 2007;567:186–92.
72. Zhao X, Eghbali-Webb M. Gender-related differences in basal and hypoxia-induced activation of signal transduction pathways controlling cell cycle progression and apoptosis, in cardiac fibroblasts. *Endocrine* 2002;18:137–45.
73. Sluijmer AV, Heineman MJ, De Jong FH, et al. Endocrine activity of the postmenopausal ovary: the effects of pituitary down regulation and oophorectomy. *J Clin Endocrinol Metab* 1995;80:2163–7.
74. Philips GB, Pinkernell BH, Jing TY. Relationship between sex hormones and coronary artery disease in postmenopausal women. *Arterioscler Thromb Vasc Biol* 1997;17:695–701.
75. Cavasin MA, Sankey SS, Yu AL, et al. Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction. *Am J Physiol Heart Circ Physiol* 2003;284:H1560–9.
76. Dementyeva EV, Shevchenko AI, Zakian SM. X-chromosome up-regulation and inactivation: two sides of the dosage compensation mechanism in mammals. *Bioessays* 2009;31:21–8.

Key Words: sex ■ myocardial remodeling ■ estrogen ■ cardiomyopathy.