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REVIEW

Effects of biological sex on the pathophysiology of the heart

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Cardiovascular diseases are the leading causes of death in men and women in industrialized countries. While the effects of biological sex on cardiovascular pathophysiology have long been known, the sex-specific mechanisms mediating these processes have been further elucidated over recent years. This review aims at analysing the sex-based differences in cardiac structure and function in adult mammals, and the sex-based differences in the main molecular mechanisms involved in the response of the heart to pathological situations. It emerged from this review that the sex-based difference is a variable that should be dealt with, not only in basic science or clinical research, but also with regards to therapeutic approaches.

LINKED ARTICLES
This article is part of a themed section on Biological Sex and Cardiovascular Pharmacology. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-3

Abbreviations
AR, androgen receptor; CVD, cardiovascular disease; DOCA, deoxycorticosterone acetate; E-C, excitation-contraction; ET-1, endothelin-1; ER, oestrogen receptor; HRT, hormone replacement therapy; LV, left ventricular; MI, myocardial infarction; NP, natriuretic peptide; PCH, pathological cardiac hypertrophy; PR, progesterone receptor; RAAS, renin-angiotensin-aldosterone system; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; TAC, thoracic aorta constriction; VSMC, vascular smooth muscle cell

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death in men and women in industrialized countries. Over the last decade, while little change was noticed on the sex ratio of cohorts in the majority of CVD studies (Mosca *et al.*, 2011), several clinical trials provided evidence that sex is an important determinant of cardiovascular events in patients with vascular diseases or high-risk diabetes mellitus. Indeed, female diabetic patients had a higher risk for acute myocardial infarction compared to male diabetics (Kappert *et al.*, 2012). Women also exhibited a marked increase in the incidence of left ventricular (LV) hypertrophy after the menopause, when the prevalence of arterial hypertension increases (Lopez-Ruiz *et al.*, 2008). Furthermore, women generally display better cardiac function and survival in the face of CVD than men, although this advantage is lost when comparing postmenopausal women with age-matched men (Fujimoto *et al.*, 2013).

The effects of biological sex on cardiovascular physiology or pathology have long been known, but the biological mechanisms responsible for sex-related differences started to be unravelled over the last decades. Indeed, sex steroid hormones (oestrogens in female, testosterone in male) contribute significantly to the sex-based differences in the outcome of cardiac diseases, although the contribution of environmental oestrogen-like molecules, such as phytoestrogens, must not be neglected. Thus, hormonal therapy such as the hormone replacement therapy (HRT) after menopause using synthetic oestrogens and progesterone, while broadly debated, may help to understand the effects of oestrogen in cardiac pathophysiology. In addition, some elucidation of the interrelation between the sex steroid hormones and peptides and/or hormones directly involved in the cardiac physiopathology such as the components of the renin angiotensin-aldosterone system, and the natriuretic peptides (NPs) will be provided. At least, as adequate exercise and nutrition programmes were shown to improve the prevention and the treatment of CVD and metabolic disorders (Hagey and Warren, 2008), these lifestyle patterns are beginning to be taken into account in the treatment of postmenopausal women. The collection of such data will be of interest to analyse the relation between exercise, sex and CVD.

This review aimed at focusing on the effects of sexual hormones on the pathophysiology of the heart. The effect of adjuvant therapy such as hormonal replacement therapy and physical training on the cardiac adaptive response to pathological situations will also be discussed.

Sex-based differences in the adult heart in mammals

Sex-based differences in cardiomyocytes

With age, the number of cardiomyocytes significantly decreases in men through different processes including apoptosis and necrosis, whereas cardiomyocyte number and size are preserved in age-matched women (Swynghedauw, 1999; Kajstura *et al.*, 2010). At the cardiomyocyte level, various biochemical characteristics such as telomerase activity vary differently in male and female across the lifespan. Telomerase is an enzyme that repairs the telomeric repeat DNA lost during the cell cycle, thus restoring telomere length. Telomere maintenance is one mechanism through which cell viability is preserved, and telomere shortening occurs at the end stage of heart failure in humans (Oh *et al.*, 2003). Telomerase activity is detectable in the cardiomyocytes of young adults and decreases with aging in males, whereas it markedly increases in females. These data emphasize a gender difference in the cardiomyocyte viability and replication (Leri *et al.*, 2000; Torella *et al.*, 2004; Kajstura *et al.*, 2010). The enhanced telomerase activity in female cardiomyocytes provides a molecular basis for the preservation of cardiomyocyte population in women throughout their lifespan. Besides, oestrogen signalling prevents cardiac muscle mass loss through autophagy, in a context of cancer, resulting in lower cardiac atrophy in females than in males (Cosper and Leinwand, 2011). Altogether, these data suggest that oestrogens may be responsible for such differences in cardiomyocyte replication capacity/viability and may explain the greater ability of the female heart to resist the deleterious consequences associated with the aging process and/or the development of heart failure.

At the subcellular level of the cardiomyocyte, sex differences in excitation-contraction (E-C) coupling have been reported in adult rats. Ca^{2+} transients are smaller and the gain of E-C coupling is lower in the female cardiomyocytes than in the males. In addition, the aging-induced alterations of cardiac E-C coupling are more prominent in male, than in female hearts (Howlett, 2010). The sex-based differences in intracellular calcium handling also involved the phosphorylation state of phospholamban, L-type Ca^{2+} channel density and the K^+ currents. In mouse ventricle, Saito *et al.* (2009) proposed a gender-related differences in K^+ currents during ventricular repolarization, when examining fast transient outward K^+ current ($Ito_{,f}$) and ultrarapid delayed rectifier K^+ current ($IK_{,slow}$). They observed that under conditions where oestrogen levels were high, the induced K^+ current was reduced following a down-regulation of the $K_v4.3$ and $K_v1.5$ channels carrying the $Ito_{,f}$ and $IK_{,slow}$ respectively (channel and receptor nomenclature follows Alexander *et al.*, 2013). These changes provided a molecular correlation for the prolongation of the action potential duration and the corrected QT interval in female mice under conditions of high oestrogens. These findings suggest that knowledge of the hormonal status is important to set the appropriate timing of the treatment in women prone to arrhythmias (Saito *et al.*, 2009). In addition, female cardiomyocytes have a lower density of β-adrenoceptors and thus also a decreased inotropic response to stimulation of these receptors (Ostadal *et al.*, 2009).

Other sex-based differences are found at the level of mitochondria in the cardiomyocytes. The rate of Ca^{2+}uptake by cardiac mitochondria is lower in females than in males (see Ostadal *et al.*, 2009). In addition, female rats exhibit lower cardiac mitochondria contents, their lower number being compensated by a higher efficiency (Colom *et al.*, 2007). Therefore, the mitochondria from female hearts generate less free radicals, hence leading to lower cardiac oxidative damage in these animals. Such mitochondrial properties might be involved in the lower incidence of aging-related disorders and/or cardiac disease in women than in men.

Sex-based differences in the vascular cells of the coronary network

Another major cellular target for the sex-based differences in the CVDs is the endothelial cell, mainly through the modulation of the endogenous vasodilator NO by the endothelial NOS (NOS3). The oestrogens via the oestrogen receptors (ER)-α play a key role in the control of NOS3 activity (Mendelsohn and Karas, 1999; Chambliss and Shaul, 2002; Fleming and Busse, 2003). The microdomains, such as caveolae, are involved in the fine-tuned regulation of oestrogen-dependent NOS3 activity (Fleming and Busse, 2003; Loyer *et al.*, 2007a).

Sex differences have been also observed at the level of the vascular smooth muscle cell (VSMC) and affect the cell death and growth *in vivo* and *in vitro*. A 'gender memory' can be conserved in VMSC in primary culture (Straface *et al.*, 2009) and sex-based inhibition of VMSC proliferation by endothelin-1 (ET-1) was proposed to contribute, in part, to the cardioprotection noted in oestrogen-repleted states (Antoniucci *et al.*, 2001). Female VSMCs exhibit a resistance to anoikis, showing a more adhering phenotype that is characterized by a well-organized actin microfilament cytoskeleton and an increased level of phosphorylated kinases involved in focal adhesion, and more importantly, a higher propensity to undergo survival by autophagy (Straface *et al.*, 2009). The regulating effects of oestrogens on artery myogenic tone appear to involve regulation of calcium-activated potassium (BK_{Ca}) channels (Geary *et al.*, 1998; Rosenfeld *et al.*, 2000). BK_{Ca} channel expression and activity depend on a cohort of hormones and factors including those of the renin-angiotensin aldosterone system. Hence a down-regulation of BK_{Ca} channels in VSMC (Ambroisine *et al.*, 2007), is present only in males in presence of a cardiac hyperaldosteronism (Garnier *et al.*, 2004); the oestrogen levels in female counteracting the aldosterone effect on BK_{Ca} channel expression (C. Delcayre, pers. comm.).

Sex-based differences in the inflammatory cells and fibroblasts in cardiac pathophysiology

Inflammatory cells, mast cells and cardiac fibroblasts are known to have a detrimental role during cardiac disease and

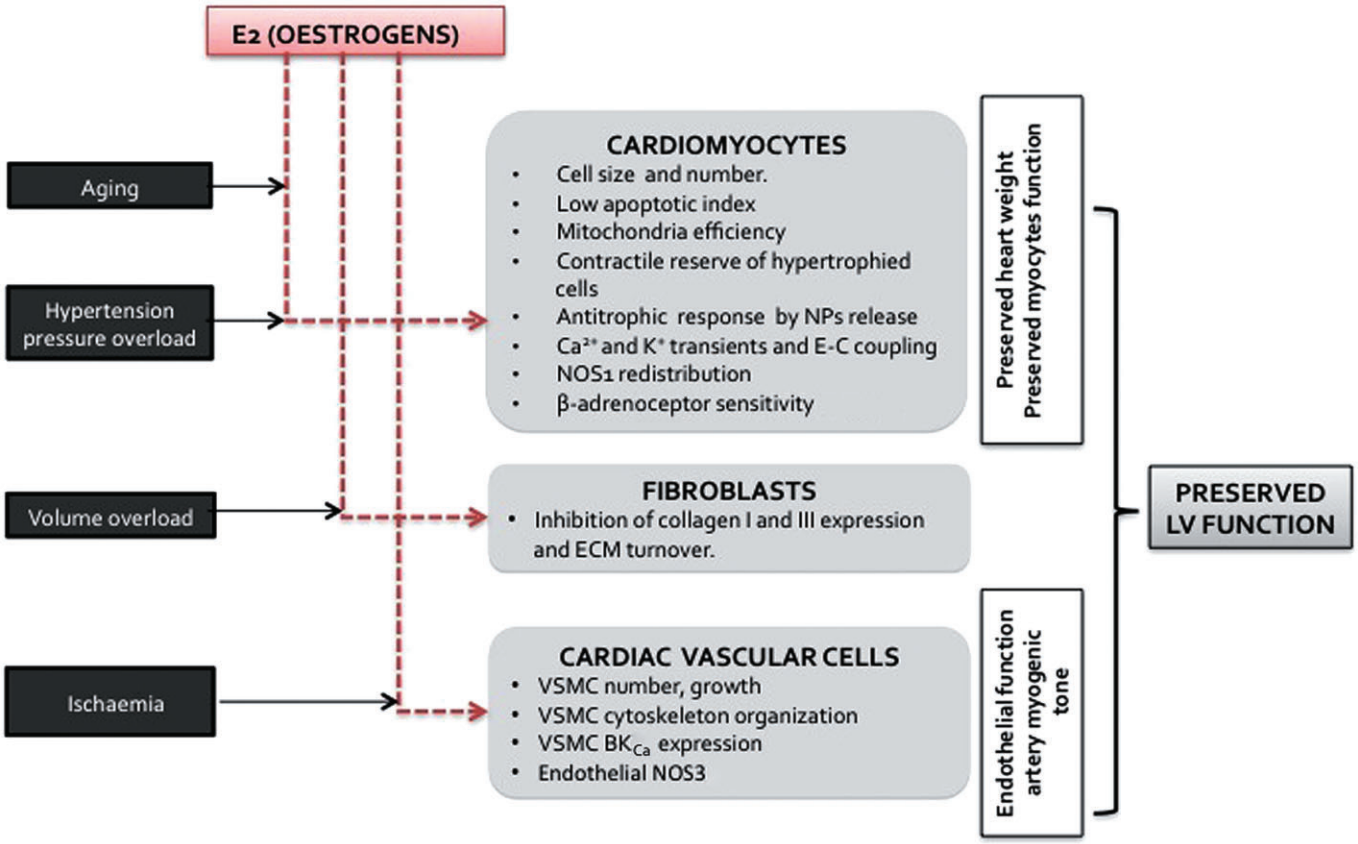

Figure 1

Summary of the effects of oestrogens, according to the cell types in the heart, which can be involved in cardioprotection induced by oestrogens.

these cell types might also be modulated by sex hormones. For example, oestrogens appeared to protect against the significant increases in mast cell density, collagen degradation, ET-1 and TNF-α, induced by volume overload (Lu *et al.*, 2012). The gender effects on the cardiac fibroblast, one of the key cells involved in the development of fibrosis, have been investigated recently (Montalvo *et al.*, 2012). By combining, gender analysis together with the effect of castration, it was demonstrated that circulating sex hormones contributed to the male sex-related increase in fibrosis and subsequent LV dysfunction after thoracic aorta constriction (TAC) through a mechanism involving TGF-β (Montalvo *et al.*, 2012). Based on these results, it was proposed that upon aging, the detrimental effects of the circulating androgens in males, rather than the protective actions of oestrogens in females, contributed to sex-related differences in myocardial remodelling.

Thus, oestrogens may trigger some of the major sex-based differences observed in cardiac pathophysiology, through unique effects in the different cell types present in the heart (Figure 1).

Sex hormones and receptors, effect on target organs

The sex-specific effects in the CVDs are mediated by the oestrogen, progesterone and androgen receptors (ERs, PRs and ARs respectively). The two known ERs, ERα and ERβ (ESR1 and ESR2), have been described in the human and rodent hearts, (see Fielitz *et al.*, 2007). All three receptor types, ERs, ARs and PRs, act by a number of genomic and non-genomic pathways. They act as transcription factors able to initiate the transcription of hormone-sensitive genes or to modulate the activity of other transcription factors. On binding the corresponding hormones, the ER, AR and PR can activate or interfere with many signalling pathways, including that of PI3K. Furthermore, an orphan GPCR, GPR30, has been proposed to mediate rapid actions of oestrogens (Revankar *et al.*, 2005), and was recently suggested to be a candidate receptor for non-genomic action of aldosterone in VSMC (see Wendler *et al.*, 2012). Signalling after activation of GPR30 may involve the stimulation of the adenyl cyclase and cAMP-dependent pathways.

Both ERs are expressed both in male and female myocardium. Apart from the production of oestrogens by the ovary, it has been suggested that both men and women synthesize oestrogens locally through the conversion of androgen to oestrogens by aromatase, particularly in adipose tissue (see Regitz-Zagrosek *et al.*, 2013). The increased oestrogen levels in older or obese men have been proposed to increase the risk for the development or the progression of CVD (Kararigas *et al.*, 2012). These elevated oestrogen levels in men are suggested to be responsible for the age-related changes in cardiac gene expression (see below). However, Banka (2012) pointed

out that longitudinal studies revealed a significant age-dependent decrease in estradiol bioavailability in men, whereas there was a high increase in estradiol levels associated with obesity. This adipose tissue-dependent increase in estradiol synthesis in men may account in part, for the increased risk of CVD associated with obesity and with the increase in cardiovascular events observed in a study on men treated for prostate cancer with high dose of diethylstilbestrol (Ferrini and Barrett-Connor, 1998) or of polyestradiol phosphate (Hedlund *et al.*, 2008).

Hence, the oestrogen actions differ between male and female with direct sex- and cardiomyocyte-specific effects in the heart (Kararigas *et al.*, 2012). The relative importance of ERα versus ERβ has not been addressed in normotensive models. However, genetic models of ERβ-deleted mice ($ER\beta^{-/-}$) showed that ERβ has a cardioprotective role in females, while having minor effects on fibrotic remodelling in heart of the $ER\beta^{-/-}$ male (Regitz-Zagrosek *et al.*, 2013). As described above, the stimulation of the ERα and ERβ by oestrogens activate kinases involved in different signalling pathways such as Akt, PI3K, ERK 1/2, the p38 MAPK, and regulate calcineurin expression (see Sussman *et al.*, 2011; Figure 2). Sussman *et al.* (2011) have clearly established sex differences in the basal levels of Akt, and demonstrated a nuclear accumulation of Akt in response to estradiol or a phytoestrogen treatment. The activation of Akt by oestrogen is known to influence events such as cell metabolism, cell cycle and cell survival. Studies from Sussman's group have also highlighted the role of the PI3K/Akt signalling cascade in the cardioprotective effects mediated by oestrogens and oestrogenic treatment. In addition, many cardioprotective genes such as the heat shock protein (Hsp) 72 or Hsp70 are up-regulated either directly or indirectly by oestrogens (Bhupathy *et al.*, 2010).

Numerous results indicate that oestrogens have favourable metabolic effects. Oestrogen deficiency increased heart and muscle lipid content and the atherogenic index (Picard *et al.*, 2000; Torto *et al.*, 2006) and was associated with metabolic disorders such as insulin resistance and altered glucose metabolism (Champion *et al.*, 2004; Bouwens and Rooman, 2005; Song *et al.*, 2005; Sitnick *et al.*, 2006).

Sex-based differences and neurohormonal disorders in CVD

An increasing body of evidence demonstrates sex differences in the renin-angiotensin-aldosterone system (RAAS), and their involvement in the development and progression of CVD and hypertension (see Bubb *et al.*, 2012). The greater activation of the RAAS leading to greater BP levels in males may be attributed in part to androgens, as castration leads to a normalization of BP and a down-regulation of the intrarenal RAAS. In addition, oestrogens have been shown to down-regulate the expression and activity of various components of the RAAS, potentially explaining the lower BP levels observed in females compared to males (see Maric-Bilkan and Manigrasso, 2012). Oestrogens decreased renin levels, ACE

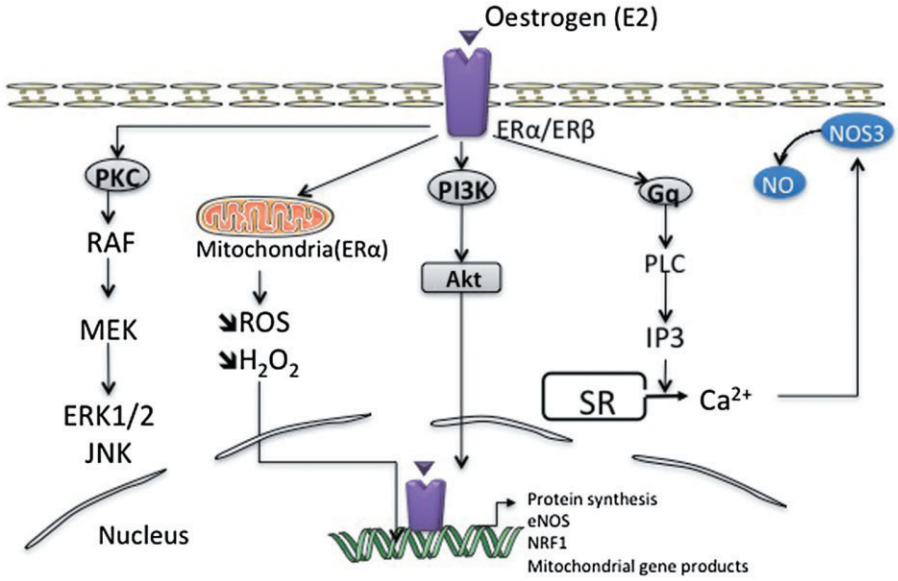

Figure 2

Molecular mechanisms of oestrogen action in cardiac cells, such as endothelial cells. Oestrogens can bind and activate the ERs, thereby inducing intracellular signalling cascades. Additionally, oestrogens influence other signalling pathways in the heart: oestrogens activate (i) the PI3K/Akt pathway, (ii) the Gq-coupled receptors, which results in the production of inositol trisphosphate (IP3) and the subsequent triggering of intracellular Ca^{2+} release and NO production, and (iii) the PKC/MAPK signal transduction pathways. All these signalling pathways are triggered at the level of the plasma membrane and activate intracellular cascades that converge to cytosolic targets and transcription factors and cofactors modulating gene expression. Finally, oestrogens can bind to mitochondria-specific receptors, through an increase in nuclear transcription of the nuclear respiratory factors (NRFs). MEK, mitogen-activated extracellular signal-regulated protein kinase; RAF, rapidly accelerated fibrosarcoma; ROS, reactive oxygen species.

activity, angiotensin AT_1 receptor density and aldosterone production (Bubb *et al.*, 2012). Besides, oestrogens increase the expression of NPs, AT_2 receptor density and angiotensin-(1–7) (Baiardi *et al.*, 2005; Bubb *et al.*, 2012; Gupte *et al.*, 2012; Maric-Bilkan and Manigrasso, 2012). The sex difference in the RAAS may also involve the GPR30 receptor, which has been proposed to trigger the non-genomic effects of oestrogens and aldosterone. Progesterone competes with aldosterone for the mineralocorticoid receptor. Little is known about androgens, but testosterone seems to increase renin levels and ACE activity (Komukai *et al.*, 2010). These effects of sex hormones on the RAAS can explain some of the sex differences in CVDs.

We previously described that cardiac hyperaldosteronism induced coronary artery defects in a sex-specific fashion (Garnier *et al.*, 2004). More recently, our data suggested that oestrogen may counteract the effect of hyperaldosteronism on the BK_{Ca} channel-mediated coronary relaxation in normotensive animals (Azibani *et al.*, 2013). In male mice, cardiac hyperaldosteronism was shown to worsen hypertension-induced fibrosis through two mineralocorticoid receptor-dependent mechanisms: the activation of inflammation/galectin-3-induced fibrosis and the inhibition of anti-fibrotic factor expression (NPs and bone morphogenetic protein-4; Azibani *et al.*, 2012). As proposed by Regitz-Zagrosek *et al.*, 2013, these features of adverse remodelling strongly suggested a role for ERβ in males. This suggestion was supported by the fact that in deoxycorticosterone acetate (DOCA)-salt mice, a model for low-renin salt-sensitive hypertension similar to aldosterone, a higher increase in cardiomyocyte diameter, pro-inflammatory and pro-fibrosis transcripts was observed in male when compared to female. In addition, female DOCA mice did not exhibit any signs of heart failure (Regitz-Zagrosek *et al.*, 2013). Interestingly, in diabetic animals, aldosterone plasma levels were shown to be increased in males, but not in females (Shimoni *et al.*, 2008). The sex-dependent elevation of aldosterone in plasma and in cardiac cells was proposed to contribute to oxidative stress in this metabolic disorder (Shimoni *et al.*, 2008).

Finally, the association of NPs, such as BNP and ANP, with gender has been examined in several studies. Despite disparity among results, higher BNP plasma levels were observed in females than in males. In basal conditions, NT-proBNP plasma levels, like those of BNP, tend to be higher in female patients and older individuals, through mechanisms involving either the clearance receptor for BNP or an increased NP expression (Redfield *et al.*, 2002; Costello-Boerrigter *et al.*, 2006). However, a population-based study indicated that in women, LV mass and NP concentrations increased to a lesser extent when compared to men and only upon severe LV dysfunction (Luchner *et al.*, 2002). Regarding postmenopausal women, HRT has been associated with higher BNP levels (Redfield *et al.*, 2002). In line with these findings, oestrogens exert anti-hypertrophic effects on cardiomyocytes *in vitro*, through the transactivation of the ANP gene (Horio *et al.*, 2000; Babiker *et al.*, 2004), hence preventing cardiomyocyte hypertrophy (Horio *et al.*, 2000). Taken together, it seems that tight regulation of NP expression is of importance for the sex-based differences involved in the development of cardiac hypertrophy.

From a pharmacological standpoint, the differences observed between males and females affected by neurohormonal disorders suggest that the potency of a number of cardiovascular drugs may vary with sex. A greater benefit for women or female animals was suggested by some studies for the aldosterone antagonist eplerenone (Kanashiro-Takeuchi *et al.*, 2009). Pharmacokinetic studies using eplerenone indicate that male rats metabolized the drug better than female rats due to an increased expression of cytochrome P-450 enzymes (Cook *et al.*, 2003). In addition, enhanced adrenergic responses have been described in females, in which direct sex hormone-dependent mechanisms may be involved. Indeed, women appear to have fewer α-adrenoceptors, resulting in a lower α-adrenoceptor response to noradrenaline, and an increased β-adrenoceptor sensitivity. The oestrogen-enhanced, β-adrenoceptor-mediated response partially involved NO mechanisms (Grossini *et al.*, 2008). Along these lines, the ability of the β-adrenoceptors to offset noradrenaline-mediated vasoconstriction that is seen in younger women disappears in postmenopausal women (Hart *et al.*, 2012). Altogether, these data highlight that efforts are still needed to take into account the sex of the patient when prescribing cardiovascular medication; efforts that should be undertaken from the initial pharmacokinetic and pharmacodynamic studies.

Effects of sex-based differences in the cardiac adaptability to haemodynamic overloads

Sex-based differences in the cardiac adaptability to pressure overload

Pathological cardiac hypertrophy (PCH) *per se* is an independent risk factor for heart failure (see Swynghedauw, 1999) and is frequently secondary to a mechanical pressure overload, due to arterial hypertension or aortic stenosis. The sex difference in the myocardial ability to adapt to mechanical overload has long been described (Douglas *et al.*, 1995; 1998), but received recently an increased interest (Loyer *et al.*, 2007a,b; Regitz-Zagrosek *et al.*, 2007; Luczak and Leinwand, 2009; Ostadal *et al.*, 2009; Bhupathy *et al.*, 2010; Petrov *et al.*, 2010). More interestingly, there are significant differences in the way male and female hearts respond to various challenges. In rodents, pressure overload increases left ventricular mass to the same extent in males and females, but cardiac function is better preserved in females (Weinberg *et al.*, 1999; 2003; Loyer *et al.*, 2007b). It is well known that premenopausal women have a better prognosis than men in response to hypertension and to aortic stenosis (Legget *et al.*, 1996). Based on clinical trials, heart failure with normal ejection fraction is much more common in women than in men and was related to sex-based differences in ventricular diastolic distensibility, in vascular stiffness and ventricular/vascular coupling and in skeletal muscle adaptation to heart failure (Regitz-Zagrosek *et al.*, 2007). When focusing on patients suffering from aortic stenosis (see Luczak and Leinwand, 2009), women, and specially the elderly, develop a more concentric form of hypertrophy than men. Interestingly, when analysing the regression of hypertrophy after aortic valve replacement, LV hypertrophy reversed more frequently in women than in men (Petrov *et al.*, 2010). Furthermore, women with

congestive heart failure survive better than men (Luczak and Leinwand, 2009), although recent epidemiological studies failed to demonstrate sex differences in death rate (Laribi *et al.*, 2012).

Experimental studies exposed sex-based differences in the development of PCH. Recently, Bubb *et al.* (2012) have highlighted the role of sex hormones in a genetic model of essential hypertension, the SHR. In response to mechanical triggers such as at the onset of a pressure overload secondary to a TAC, female rats developed more cardiac hypertrophy than male (Douglas *et al.*, 1998; Loyer *et al.*, 2007b) and did not exhibit any signs of acute heart failure (Loyer *et al.*, 2007b). In mice, similar sex-based differences were observed at later stages of cardiac hypertrophy (Witt *et al.*, 2008). Such sex differences in the adaptation of hearts to pressure overload draw attention to the underlying mechanistic pathways and induced gene expression profiles. Indeed, the sex-based differences in remodelling of the whole heart are mirrored by the differences in signalling pathways or gene expression profiles. These sex-based differences in cardiac response to TAC included a higher β-myosin heavy chain expression, lower levels of mRNA for the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and lower expression of several genes controlling mitochondrial function, including the transcription factor PGC-1α, in males displaying hypertrophied hearts than in females. These transcriptional changes were associated with a preserved contractile reserve in females with hypertrophied hearts (Weinberg *et al.*, 1999; Witt *et al.*, 2008).

Oestrogens can prevent PCH development indirectly by counteracting hypertension, through the direct triggering of ANP release (van Eickels *et al.*, 2001; Jankowski *et al.*, 2001; Zhu *et al.*, 2002), by blocking the p38 MAPK phosphorylation (van Eickels *et al.*, 2001) and by preventing Ca^{2+} deregulation (Xin *et al.*, 2002). Conversely, oestrogen deficiency enhanced adverse cardiac remodelling (capillary rarefaction, cardiomyocyte hypertrophy and loss) following TAC in rats (Marques *et al.*, 2006; Loyer *et al.*, 2007a). Recently, Kararigas *et al.* (2012) used elegant approaches including genome-wide expression profiling of oestrogen-treated human cardiac tissues and gene expression and functional analysis of mouse cardiomyocytes according to biological sex and oestrogen treatment in order to investigate the effects of oestrogens on gene regulation in the heart. Using this combined approach, they showed that the gene encoding the myosin regulatory light chain interacting protein was specifically induced in the cardiac tissues of men, in response to oestrogens. Conversely, the expression of the myosin regulatory light chain protein, a protein involved in cardiomyocyte contractility, was decreased only in male heart tissue. These changes in gene expression were related to impaired contractile function. All together, the data suggest a male-specific, oestrogen-regulated, effect resulting in an alteration of cardiac contractility.

Marked sex-based differences are also described in the development of cardiac fibrosis and include the expression of genes associated with the remodelling of the extracellular matrix (ECM), including those for collagen 3, MMP 2, TIMP2 and TGFβ2, which are lower in female heart after TAC in mice (Witt *et al.*, 2008; Fliegner *et al.*, 2010). Oestrogens reduced the turnover of the ECM, especially that of proteins involved in the collagen network (Xu *et al.*, 2003; Mahmoodzadeh *et al.*, 2010). Insights into the sex-specific regulation of fibrosis-related genes were provided by genetic heart failure models and *in vitro* approaches. In these models, β-estradiol significantly increased collagen-I and -III gene expressions in male fibroblasts, contrary to the effects observed in female cells (Petrov *et al.*, 2010); these effects being mediated by ERβ (Fliegner *et al.*, 2010). The sex-based differences observed in the regulation of genes encoding ECM proteins and MMPs may represent one of the major mechanisms slowing the progression of heart failure and the enhanced recovery of the heart in females.

Other lines of evidence of oestrogen-induced cardioprotection were provided by studies devoted to NO bioavailability or endothelial function. The reduction in the bioavailability of NO is a key feature of endothelial dysfunction during heart failure. In response to a severe TAC, sex differences changes in NOS3 activity were observed (Loyer *et al.*, 2007b). In the hypertrophied female rat heart, the NOS3 activity remained constant before the onset of signs of heart failure (Loyer *et al.*, 2007b) while after TAC, oestrogen deficiency blunted the increased NOS3 expression and exacerbated cardiac dysfunction (Loyer *et al.*, 2007a). Besides the putative role of NOS3-derived NO, the involvement of NOS1-derived NO has been demonstrated during the development of PCH and heart failure (Bendall *et al.*, 2004; Damy *et al.*, 2004; Loyer *et al.*, 2008). In male mice lacking both NOS isoforms, $NOS1/3^{-/-}$, a twofold increase in mortality was observed when compared to $NOS1/3^{-/-}$ females (Barouch *et al.*, 2003). The changes in NOS1 expression in hearts following TAC seemed to be triggered by mechanotransduction pathways, independently of oestrogen status (Loyer *et al.*, 2007a). However, in the failing heart, sex-based differences were reported regarding the sub-cellular localization of NOS1, as NOS1/caveolin-3 association was significantly higher in female mice in response to cardiac injury than in males (Sun *et al.*, 2006) or following TAC in rats (Loyer *et al.*, 2007b). According to Murphy and Steenbergen (2007), the increase in NOS1 co-localization with caveolin-3 in females under stress conditions (ischaemia/reperfusion) associated with increased Ca^{2+} (which activates NOS) resulted in an increase in S-nitrosylation of the L-type Ca^{2+} channel, lower Ca^{2+} entry and therefore lower Ca^{2+} load, altogether constituting a cardioprotective mechanism (Chakrabarti *et al.*, 2010).

Sex-based differences in the cardiac adaptability to volume overload

In rats, a volume overload secondary to aortocava fistula induces clear gender-specific differences in ventricular function, structural remodelling and mortality (Gardner *et al.*, 2002). The eccentric dilated hypertrophy was only observed in male Sprague-Dawley rats in response to volume overload, not in females (Gardner *et al.*, 2008). Using hormonal therapy in ovariectomized animal, it was proposed that oestrogens prevented adverse cardiac remodelling to a sustained volume overload through the direct or indirect inhibition of ET-1, the prevention of mast cell maturation and the inhibition of TNF-α synthesis by the mast cells (Gardner *et al.*, 2008; Lu *et al.*, 2012). Differences in the remodelling responses can also be seen after myocardial infarction (MI), as female rats developed less thickening of the non-infarcted

regions and a less pronounced diastolic dysfunction than their male counterparts. Also, post-MI rupture of the left ventricle was less frequent in female than in male mice (Deschepper and Llamas, 2007).

Sex-based differences in adjuvant therapy of CVD

Besides the classical therapeutic approach that includes ACE inhibitors, diuretics, β-blockers and that is prescribed to patients regardless of their gender, new therapeutic approaches taking into account the sex-based difference may profoundly affect the prognosis of the patient.

Sex-based differences in cardiac benefit following exercise training; effects during pathological conditions

Physical training is recognized as beneficial in the context of cardiac diseases. Sex-based difference during experimental physical training revealed better exercise capacity of female than male animals, and sex-specific difference in cardiac hypertrophic signalling have been identified, such as a relative higher cardiac increase in Ca^{2+}/calmodulin-dependent protein kinase in females than in males (Konhilas *et al.*, 2004). In human, physical exercise reveals clearly sex-related differences in both healthy subjects and patients with asymptomatic aortic stenosis (see Higginbotham *et al.*, 1984; Legget *et al.*, 1996; Regitz-Zagrosek *et al.*, 2007). Interaction between exercise training and female sex hormones on cardiac performance have been reported (Bupha-Intr and Wattanapermpool, 2004; Brown *et al.*, 2005; Coimbra *et al.*, 2008; Bupha-Intr *et al.*, 2009). Regular exercise cardioprotective in terms of cardiac sarcoplasmic reticulum Ca^{2+} uptake in oestrogen-deprived status through the regulation of SERCA expression and phospholamban B phosphorylation (Bupha-Intr *et al.*, 2009). In addition, exercising reduces inflammation and cell adhesion molecule expression in postmenopausal women (Wegge *et al.*, 2004). Such results support the idea that exercise training exerts much more benefit on cardiac function after menopause, albeit clinical studies revealed conflicting results (Zarins *et al.*, 2009; Swank *et al.*, 2010; Ryan *et al.*, 2012).

Exercise training in oestrogen-deficient rats improved resting haemodynamic status and arterial baroreflex sensitivity, most likely through the reduction of oxidative stress (Irigoyen *et al.*, 2005). Indeed, exercise training in oestrogen-deficient animals can restore cardiac reserve function, the normal levels of antioxidant molecules (Patten *et al.*, 2004; Rakpongsiri and Sawangkoon, 2008) and prevented pathology-related expression of β-myosin heavy chain (Bupha-Intr and Wattanapermpool, 2004). The metabolic syndrome and insulin resistance induced by oestrogen-deprivation were shown to be corrected by endurance exercise training alone or by oestrogen replacement alone (Saengsirisuwan *et al.*, 2009). In addition, Pighon *et al.* (2010) found that exercise training acts as oestrogen supplementation surrogate, by decreasing several genes of lipogenesis in liver, as well as decreasing several biomarkers of inflammation (IL-6, NFkB, TNF-α) in oestrogen-deprived rats. Growing evidence suggest that most of these effects are dependent on the AMP-activated protein kinase pathway (Park *et al.*, 2002; Lavoie and Gauthier, 2006).

During pathological situations, a prospective randomized controlled exercise trial indicated that exercise training had no major impact on the cardio-metabolic risk profile of overweight or obese postmenopausal women with moderate hypertension, despite considerable improvements in maximal oxygen consumption (Arsenault *et al.*, 2009). These results contrast with data obtained in aging men indicating that progressive resistance training can be used as antihypertensive therapy as well as for the control of metabolic diseases such as obesity or type 2 diabetes (Ibanez *et al.*, 2005). Furthermore, physical exercise was identified as a potent anti-senescent intervention to up-regulate telomere-stabilizing proteins and the telomerase activity in the diseased heart (Werner *et al.*, 2008).

The marked prevalence of hypertension observed in postmenopausal sedentary women (Staessen *et al.*, 1998) not only underlined the effects of oestrogen deficiency in the induction of vascular dysfunction, but also highlighted that exercise training may bring beneficial effects in this context. Postmenopausal women who engage in intermittent, moderate-intensity physical training experience demonstrated a significant reduction in systolic BP (Staffileno *et al.*, 2001). In rats, exercise training dramatically reduces systolic BP of both normo- and hypertensive oestrogen-deficient animals and prevents adverse cardiac remodelling (Irigoyen *et al.*, 2005; Marques *et al.*, 2006). Also in SHR rats, exercise training reduced BP only in males (Coimbra *et al.*, 2008). Among the molecular and biochemical mechanisms responsible for this cardioprotective effect of exercise, it has been postulated that these exercise-induced changes in the myocardium result from local increases in the oxidative stress detoxifying mechanisms or the levels of heat shock proteins (Powers *et al.*, 1998; Demirel *et al.*, 2001). Exercise training increased also antioxidant mechanisms through the expression of catalase and glutathione peroxidase (Adams *et al.*, 2005), and NO bioactivity through an enhanced NOS3 expression (Knowles and Moncada, 1994; Hagg *et al.*, 2004). Interestingly, Marques *et al.* (2006) demonstrated that a major effect of exercise training was the prevention of oestrogen deprivation-enhanced myocyte loss in the SHR. It is hypothesized that, as under normal conditions, regular exercise induces a protective effect on cardiac sarcoplasmic reticulum Ca^{2+} uptake in oestrogen-deficient animals (Bupha-Intr *et al.*, 2009). Additional benefit arose through lowering BP by increasing the capillary density in the heart and the muscles and physiological activation of cardiac hypertrophy (Akt pathway). Taken together, these data clearly demonstrate the sex difference on the effects of exercise on cardiac structure and function. Exercise training has beneficial effects by diminishing the PCH induced by pressure overload, mainly by reducing interstitial myocardial fibrosis, improving myocardial vascularization and preventing reduction in the number of cardiomyocytes.

Hormonal replacement therapy (HRT) and CVD

The change in relative CVD risk and incidence in women aged 50 years and older as a result of aging, loss of oestrogen

protection after menopause or the changing incidence of other cardiovascular risk factors is largely controversial (Valdiviezo *et al.*, 2013). The safety of HRT in postmenopausal women using synthetic oestrogens and progesterone was extensively debated after the report of increased risk of heart disease, stroke and venous thromboembolism (Rossouw *et al.*, 2002). Lam *et al.* indicated that the use of HRT in premenopausal women is associated with higher circulating NT-proBNP levels, compared with untreated age-matched women (Lam *et al.*, 2011). However, a recent randomized study (Schierbeck *et al.*, 2012) showed that women receiving HRT early after menopause had a significantly reduced risk of mortality, heart failure or MI, without any apparent increase in risk of cancer or stroke. In humans, HRT not only alleviated the metabolic consequences of menopause (Hassager and Christiansen, 1989; Arabi *et al.*, 2003; Green *et al.*, 2004) but maintained or improved cardiac performance (Alecrin *et al.*, 2004). Thus, the cardiac hypertrophy frequently observed after menopause can be significantly prevented by HRT (Bhupathy *et al.*, 2010). Consequently, the assessment of lifestyle patterns should be taken into account in the treatment of postmenopausal women, as adequate exercise and nutrition programmes were beneficial in the prevention and the treatment of obesity, diabetes and CVD in postmenopausal women (Hagey and Warren, 2008).

Hence, there is a need to encourage the implementation of well-proven interventions such as lifestyle changes of exercise, weight, BP and lipid control to prevent and reduce CVD risk, together with the inclusion of the sex-based differences in cardiac physiopathology in the therapeutic approaches adopted.

Conclusions

So far, the potentially important cardiovascular influences of endogenous oestrogens in men have received little attention. Recent evidence emphasizing the sexually dimorphic response of the heart to sex steroids according to pathophysiological status, suggests some novel therapeutic targets. For example, the negative responses to oestrogens in older men suggest the use of aromatase inhibitors as a potential pharmacological approach. In addition to the sex-based differences listed above, significant differences in the way the hearts of males and females respond to various challenges bring important insights into the mechanisms whereby female gender may influence favourably the remodelling and the adaptive response to myocardial insult. It is worth mentioning that, contrary to the apparently low improvement in treatment and outcome that has been suggested regarding women with MI over the past 25 years (Nauta *et al.*, 2012), a recent study demonstrated that the temporal mortality reductions between 1985 and 2008 were at least as high in women as in men with MI, in terms of both 30 day mortality and long-term mortality hazard (Nauta *et al.*, 2012). Recently, the epidemiological study of Laribi and co-workers also showed that over the last decade, the age-standardized death rate following heart failure was unrelated to sex differences, in seven European countries (Figure 3, Laribi *et al.*, 2012). Such data must not divert the efforts necessary to improve or develop therapeutic approaches to treatment of cardiac diseases that take into account gender-related differences.

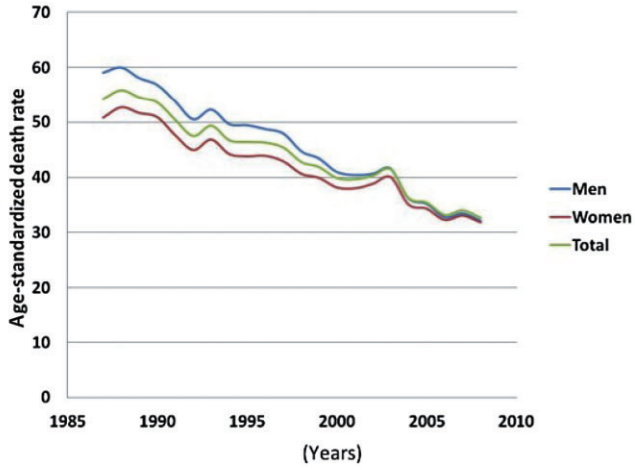

Figure 3
Heart failure as the underlying cause of death. The analysis clearly demonstrated that during the last decade, the age-standardized death rate per 100 000 inhabitants was unrelated to sex differences in seven European countries (reprinted from Laribi *et al.*, 2012 with permission).

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Conflict of interest

The authors state that there is no conflict of interest.

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