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## Book: Sex Differences in Cardiovascular Physiology and Pathophysiology

Ed B. Alexander

### Chapter “Sex differences in myocardial hypertrophy and heart failure”

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### Introduction

Heart failure (HF) is one of the major health threats of western societies. HF is a clinical syndrome arising from different pathophysiological conditions. HF affects up to 10 % of the elderly, in absolute numbers more women than men, as reviewed recently.<sup>1,2</sup> The likelihood for developing heart failure (HF) across the life course is determined by the interaction between genetic predisposition, a cumulative exposure to cardiovascular risk factors and age-related alterations in cardiac and vascular anatomy and physiology. In this process, sex differences come into play and determine the risk on developing clinically overt HF. In most studies and registries, women are more likely to survive than men and HF in women frequently occurs at an older age and with less ischemic etiology than men.<sup>3-5</sup>

While it remains subject of debate, most evidence points towards the existence of two HF phenotypes, each constituting approximately 50% of the total HF population and defined by differences in ejection fraction, preserved or reduced.<sup>6-8</sup> Recently, a third HF phenotype, named HF with a midrange ejection fraction (HFmrEF) has been acknowledged as a new entity within the HF syndrome.<sup>9</sup> HFmrEF patients comprise approximately 10-20% of the HF population.<sup>9</sup> As compared to heart failure with a preserved ejection fraction (HFpEF), patients with HFmrEF are younger and more frequently of the male sex.<sup>9</sup> A recent publication of the PROTECT-1 Study showed that all three HF entities differ from one another in both clinical features and biomarker profiles, with HFmrEF exhibiting the most heterogeneous profile.<sup>10</sup> Whereas biomarkers related to HFpEF were primarily of the inflammatory type and biomarkers related to heart failure with a reduced ejection fraction (HFrEF) were mostly associated with cardiac stretch, the biomarker profile of HFmrEF comprised both inflammatory and cardiac stretch biomarkers.<sup>10</sup> This implies that HFmrEF is indeed a mixture between HFpEF and HFrEF and that the prevalence of all three HF syndromes is different in men and women.

Few studies determined the true incidence of HFpEF and HFrEF in women and men, but the available evidence suggests that in western populations, HFpEF has a greater prevalence in women and HFrEF in men.<sup>2, 11-15</sup> Indeed, whereas women are more prone to develop HFpEF, men are more likely to develop HFrEF. In fact, the ratio of women to men in prevalence of HFpEF is an astounding 2:1.<sup>6-8</sup> It is not clear how frequent a transition from HFpEF to HFrEF occurs in the population and if this is sex-dependent. More studies are needed here. The evidence that HFrEF in men is mainly due to MI and

the evidence that women have a longer life expectancy and HFpEF has a greater prevalence in the older age groups may contribute to the difference in prevalence between these two HF entities.

The most important precursor of HF is myocardial hypertrophy (MH), as it occurs in hypertension and aortic stenosis, but diabetes, ischemic heart disease, genetic cardiomyopathies, arrhythmia also play an important role. Women and men with HF differ in their comorbidities. Aging, over- and under-nutrition, diabetes, hypertension, salt loading, as well as inflammatory or autoimmune diseases, are significant risk factors, which manifest differently between men and women and contribute to HF in a sex-dependent manner.<sup>16-18</sup> Furthermore, the relationship between sex, comorbidities and cardiovascular structure and function differs between women and men with HF.<sup>17</sup>

All triggers and comorbidities lead to increased wall stress, MH and/or relative myocardial ischemia and finally HF. This may combine with individual susceptibility, due to unfavorable gene variants, leading to more or less strong unfavorable responses of the heart. Sex specificity is introduced at the level of the triggers, but also of the pathophysiological substrate and the resulting response.

Hence, in the present review, we will briefly discuss sex differences in the trigger conditions/comorbidities before we approach the molecular mechanisms underlying the adaptive and maladaptive mechanisms leading to HF from these triggers.

## Sex differences in comorbidities of HF

### Sex differences in hypertension and obesity as risk factors for HF

One in three adults presents with arterial hypertension based on current guideline definitions in European countries and the United States.<sup>19,20</sup> Differences between women and men are well known as discussed.<sup>2</sup> In younger age and middle age groups hypertension has a significantly lower prevalence in women than in men.<sup>19</sup> Hypertension is less common in premenopausal women than in men of the same age<sup>21</sup> but its prevalence increases more steeply after the menopause. This has been linked to the loss of inhibition of the renin-angiotensin system by estrogens after menopause and to obesity. Postmenopausally sustained hypertension is frequently preceded by hypertension, eclampsia or preeclampsia during pregnancy.<sup>22</sup> In the elderly population, hypertension is more common in women than men.

Hypertension contributes to the risk for heart failure in women to a greater extent than in men<sup>23,24</sup> which is probably due to the greater prevalence in the older age groups and the fact that the women get older than men.

### Sex differences in pressure overload : aortic valve stenosis

Aortic stenosis (AS) is a common cause of MH in the elderly, and affects women and men, as reviewed previously.<sup>2</sup> In the European populations the prevalence is up to 5 % among subjects aged 70-79 years and up to 10 % among subjects > 80 years of age. AS due to a congenital bicuspid valve is 3 times more common among men than women<sup>25</sup>, while degenerative AS is more common among women than men with a women:men ratio of 1:0.76.<sup>26</sup> AS leads to pressure overload of the heart, similar to hypertension.

In recent studies, we observed that the phenotype of MH differs in women and men with AS. As a consequence of AS progression, leading to increasing pressure overload on the left ventricle, compensatory changes in left ventricular structure occur. Women develop more concentric MH with

well-preserved ejection fraction whereas men go more rapidly to ventricular dilatation and pump failure (Fig 1).<sup>27, 28</sup> Men also have more fibrosis in the pressure overloaded heart.<sup>28-32</sup> Phenotypic differences are associated with survival. If women exhibit the « male » dilated phenotype, their survival is significantly lower than those of women with the typical « female » concentric form of hypertrophy.<sup>29</sup> Lower myocardial function was also found in men compared to women with AS in the SEAS and in a smaller study.<sup>27, 33</sup> More longitudinal studies are needed to understand the sex differences in remodeling.

### Sex differences in obesity and diabetes

Obesity is a major risk factor for hypertension, diabetes, and coronary artery disease (CAD) in women and men (as reviewed in<sup>34</sup>). Obesity nowadays presents a major and gender-specific health problem due to its dramatic increase in younger women.<sup>35</sup> The HF risk introduced by obesity is better assessed by the waist-to-hip ratio than by body mass index (BMI)<sup>23</sup> due to the negative biological functions of visceral fat. Visceral obesity, which is more common in men and postmenopausal women, induces hypertension by different sex-dependent mechanisms, such as neurohormonal activation, an increase in intra-abdominal pressure, and glomerular and tubular effects. Premenopausal women in general develop peripheral adiposity, with predominantly gluteal fat accumulation, which is not associated with an equally poor cardiovascular prognosis.<sup>36</sup> After the menopause, concentrations of lipoproteins as well as body fat distribution shift to a more male pattern, to android obesity, which is linked to increased cardiovascular morbidity and risk for type 2 diabetes. Visceral fat and subcutaneous fat differ in the distribution of adrenergic and estrogen receptors and in the production of free fatty acids and inflammatory mediators which contribute to the development of hepatic insulin resistance.<sup>37</sup> A lower percentage of visceral fat may be one of the primary metabolic features that underlie the reduced risk for cardiovascular disease in premenopausal women.<sup>36</sup> Accumulation of visceral abdominal fat is accelerated by the menopause and is associated with the above mentioned increase in blood pressure, with insulin resistance and with increased cardiovascular risk.

Diabetes is a stronger risk factors for HF in women than in men.<sup>24, 38-40</sup> The interaction between diabetes and atherogenic and prohypertrophic mechanisms and cardiovascular risk in women has been related to sexual hormones but has not yet been completely understood. However, it has been shown that protective sex differences in endothelial function, in nitric oxide (NO) production and in the antithrombotic profile in women are abrogated by diabetes.<sup>41</sup> A major protective role for female sexual hormones in the interplay between insulin and atherosclerosis is suggested by the increased risk of young women with polycystic ovarian syndrome, which is characterized by low estrogen and relatively high testosterone serum levels, for diabetes and possibly also atherosclerosis.<sup>40, 42-44</sup>

### Sex differences in ischemic heart disease (IHD)

It has been pointed out frequently and also recently that in most western societies, CAD develops on average 7-10 years later in women compared to men.<sup>2</sup> However, manifestations of CAD in younger women are increasing.<sup>45, 46</sup> This may most likely be due to unfavorable life-style changes over the past decades. Acute coronary syndromes (ACS) occur 3-4 times more often in men than women below age 60, but after 75 yrs women represent the majority of patients. Recently, the number of ACS in younger women has significantly increased.<sup>45, 46</sup>

Whereas CAD classically focusses on the diseases of the epicardial coronary arteries, the recently defined entity IHD includes all damage due to ischemia in the myocardium, regardless whether the cause lies in the major coronary arteries, in the microcirculation or in a supply/demand disbalance.<sup>47</sup> IHD not due to atherosclerosis of major coronary arteries is more common in women than in men.

Accordingly, acute coronary syndromes (ACS), ST-elevation MI (STEMI) or Non-STEMI without epicardial CAD or structural heart disease occur more frequently in women than in men.<sup>47, 48</sup> In particular, younger women with ACS may present with open coronary arteries, with plaque erosions with distal embolization rather than plaque rupture with thrombus formation.<sup>49</sup> In Non-STEMI women it was recently demonstrated that coronary artery plaque area was associated with myocardial ischemia independent of presence of coronary stenosis.<sup>50</sup> Not unfrequently, angina or ACS in women may be due to coronary microvascular disease (CMD), also called microvascular angina.<sup>51, 52</sup> Women have more frequently components of pathological vasoreactivity, such as spasm and endothelial dysfunction (Figure 2).<sup>53-55</sup> IHD seems to lead more frequently to HF in women than in men.

### Sex differences in genetic cardiomyopathies

Genetic cardiomyopathies due to autosomal gene variations are expected to occur with the same prevalence between women and men.<sup>56</sup> However, dilated cardiomyopathy and hypertrophic cardiomyopathy (HCM) have a greater prevalence in men than in women.<sup>57-61</sup> Thus, compensation for the genetic defect in these syndromes appears to be more efficient in women than in men. A transition from a hypertrophic to a dilated, hypocontractile phenotype has been described in detail in a case study of a woman with HCM.<sup>62</sup> Sudden arrhythmic cardiac death is a frequent thread in HCM and sudden death in young athletes is frequently attributed to undiagnosed HCM. Noteworthy, sudden cardiac death in young and middle-aged athletes affects almost only men, also if unrelated to HCM.<sup>63</sup>

### Sex differences in arrhythmia

Women have a higher resting heart rate than men, a shorter sinus node recovery time and stronger modulation of heart rate variability, probably reflecting greater parasympathetic influences.<sup>56, 64-67</sup> Women also have longer rate-corrected QT intervals than men and manifest greater lengthening of the QT interval as heart rate slows.<sup>68</sup> At prolonged QT intervals, women are more susceptible to the development of “torsade des pointes”, a rare ventricular tachycardia, which is frequently self-limiting but may also degenerate into lethal ventricular fibrillation.<sup>67-72</sup> On the other hand, ventricular arrhythmias and sudden cardiac death are more common in men than in women in the short QT syndromes, e.g. the Brugada syndromes. Moreover, male sex is a strong predictor of risk for atrial fibrillation.<sup>73-77</sup> These phenotypes can be caused by pathological mechanisms affecting ion channels or factors related to regulatory pathways of ion channels in a sex-specific manner.

## Sex differences in major adaptive and maladaptive processes in HF

### Sex differences in energy metabolism

#### **Cardiac energy metabolism**

There is a strong link between myocardial metabolism and cardiac function. The heart is among the largest energy consumer organ in the body. Energy is stored in the form of adenosine triphosphate (ATP) and phosphocreatine (PCr) which is formed by the phosphorylation of creatine from ATP by the creatine kinase reaction (CK). The heart consumes around 1 mmole ATP/kg/sec. This means that all ATP and PCr content should be renewed every  $\approx 20$  sec. Heart muscle is a highly oxidative tissue as it produces more than 90% of its energy from mitochondrial respiration. Mitochondria are the site of

oxidative phosphorylations (OXPHOS). They are considered as the powerhouse of the cell because they produce the majority of energy rich bounds in the form of ATP. A unique feature of mitochondria is that they arose more than 1.45 billion years ago from endosymbiosis of ancient proteobacteria. Mitochondria contain their own DNA coding for 13 subunits of the respiratory chain and ribosomal and transfer RNAs. In mammals, the mitochondrial proteome contains more than 1000 proteins, not counting a wide array of splicing and post-translational variants.<sup>78</sup> Because most of the mitochondrial proteins are encoded by the nuclear genome, mitochondria have tissue-specific structure and function rendering them exquisitely well-adapted to the physiology of the host cell. Mitochondria produce more than 90% of energy needed for cardiac contraction, and occupy more than 30 % of the cardiac cell volume. They are densely packed, organized under the sarcolemma and in rows between myofilaments.

There is a strict correlation between cardiac work and oxygen consumption. Therefore, a strong energy signaling pathways should exist to ensure the close match between oxygen consumption and energy utilization. This is achieved in part by calcium as it regulates myosin and sarcoplasmic reticulum ATPases on one hand, and the major mitochondrial dehydrogenases and F<sub>0</sub>/F<sub>1</sub> ATPase on the other.<sup>79</sup> Mitochondrial respiration rate depends almost linearly upon the changes of localized ADP concentration in their physiological range. This mechanism of regulation relies on the existence of energetic microdomains at sites of energy production and utilization, interconnected by phosphotransfer kinases and cell architecture.<sup>80, 81</sup> The most important phosphotransfer system is the creatine kinase system which is free in the cytosol or bound to intracellular structures involved in energy production (mitochondria and glycolytic complexes) and energy utilization in close proximity to ATPases (sarcoplasmic reticulum, myofilaments, sarcolemma).

The heart is generally considered as a substrate omnivore with the capacity to oxidize fatty acids, carbohydrates, ketone bodies, lactate and even amino acids, the preferred substrate being fatty acids. The heart exhibits metabolic flexibility which is its ability to respond to changing workload, substrate availability, circulating hormones, coronary flow, fuel metabolism by choosing the right substrate at the right moment.<sup>82, 83</sup>

Adequate cellular energy supply and survival depend on mitochondrial life cycle, which involves mitochondrial biogenesis, dynamics, and recycling via mitophagy. Mitochondria proliferate by division of preexisting organelles, through a process called mitochondrial biogenesis. It includes mitochondrial protein expression and its molecular control, phospholipids synthesis, import of mitochondrial proteins and mitochondrial network dynamics. Mitochondrial biogenesis is under the control of the nucleus and necessitates the coordination of the two genomes, the nuclear and the mitochondrial ones.

The tuning of the mitochondrial protein expression depends on a highly interconnected network between coregulators and various DNA-binding transcription factors that regulate a large number of nuclear genes encoding mitochondrial proteins. The transcriptional coactivator peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) coactivator-1 (PGC-1 $\alpha$  and  $\beta$ ) are master regulators of energy metabolism at the level of gene transcription. Through their interaction with multiple transcription factors they enhance mitochondrial capacity for fatty acid oxidation and oxidative phosphorylation, the transcription and replication of mitochondrial DNA, mitochondrial dynamic proteins, and

angiogenesis. Among these transcription factors the PPARs and more specifically PPAR $\alpha$  plays a major role in regulation of proteins involved in lipid oxidation.

Aside from providing most of the energy to the host cell, mitochondria participate in multiple other cell functions like ionic homeostasis, production and regulation of ROS, pH regulation, steroid hormones synthesis, calcium homeostasis, thermogenesis, lipid and carbohydrate utilization, and cell death.

### **Sex differences in cardiac energy metabolism**

Cardiac energy metabolism is a place for sexual dimorphism. For example, women have lower myocardial glucose extraction fraction and utilization and higher myocardial oxygen consumption (MVO<sub>2</sub>) than men.<sup>84</sup> Female mice exhibit lower basal glycogen content<sup>85</sup> suggesting sex differences in the regulation of glycogen and glycophagy.<sup>85, 86</sup> However, cardiac triacylglycerol content does not differ between males and females.<sup>87</sup>

Mitochondria exhibit a strong tissue- and sex- specificity.<sup>88</sup> Estrogen and estrogen receptors have been implicated in the regulation of mitochondrial function and biogenesis (Fig 2) (for review see <sup>89, 90</sup>). A cross-talk between the PGC-1/PPAR axis regulating energy metabolism and substrate utilization and the estrogen/ERs pathway has important implications for the understanding of sex-differences in energy metabolism and pathologies. Different modes of interaction at the transcription level have been described (for review see <sup>89, 91</sup>). Estrogen responsive elements have been found on the PGC-1 $\alpha$  promoter.<sup>92</sup> Female sex hormones, estrogen and progesterone participate in the structural assembly of mitochondria and attenuate stress-induced mitochondrial ROS production while estrogens may also modulate mitochondrial ATP synthesis.<sup>93</sup> E2 deficiency induces mitochondrial compromise through miR-23a-mediated PGC-1 $\alpha$  downregulation, participating to the menopause-associated left ventricular concentric remodeling in females.<sup>94</sup>

A sexual dimorphism of mitochondrial oxidative capacities is evident in many tissues (Fig 3). However, in general the rate of respiration of cardiac mitochondria or homogenates as well as the amount of mtDNA/nDNA do not differ between males and females.<sup>95-100</sup> Cardiomyocytes from female rats exhibit lower mitochondrial content<sup>101</sup>, but a slightly higher mitochondrial content and mitochondrial size was found in mouse female heart.<sup>97</sup> Nevertheless, subtle differences have been described. When subsarcolemmal and intermyofibrillar mitochondria were studied, it was found that glutamate-malate-stimulated respiration was lower and ADP/O ratio higher in females.<sup>102</sup> A significant sexual dimorphism was shown in the expression of cardiac mitochondria genes with increased expression of genes of fatty acid metabolism in young females and higher expression levels of genes associated with oxidative phosphorylation in old female hearts compared to males.<sup>103</sup> This suggests that mitochondria from females have higher specific activity than the male ones, and preferentially use lipids.

In the heart, mitochondria are the essential source of production of reactive oxygen species (ROS) as well as the most direct target for their damaging effects. Interestingly, as in other tissues, mitochondria from female hearts produce less ROS and exhibit lower oxidative damage than males.<sup>97, 101, 104</sup> Part of these effects could be mediated by estrogens and estrogen receptors present in the cardiomyocytes. Indeed, estrogens are able to up-regulate genes involved in the protection against oxidative stress.<sup>105</sup> The enhanced mitochondrial function and lower oxidative stress in rat cardiac muscle could be mediated through G-protein coupled estrogen receptors.<sup>106</sup> Much less is known of the effects of male

hormones on mitochondrial structure and function. Testosterone has been shown to regulate myocardial cell growth and the activity of some inner mitochondrial membrane proteins in rodent hearts.<sup>107</sup>

Mitochondrial calcium regulation is an important player in cell fate and cell death. Together with ROS, calcium is an inducer of the permeability transition pore (PTP) leading to mitochondrial swelling, decreased ATP synthesis and ultimately cell death.<sup>108</sup> The capacity of mitochondria to control excess calcium is thus of crucial importance. This depends on the balance between calcium uptake along the electrochemical gradient and calcium extrusion mechanisms. Female cardiac mitochondria are more resistant both in the extent and in the rate of mitochondrial swelling at high calcium concentration<sup>109</sup> and have a greater mitochondrial calcium retention capacity than the male ones.<sup>102</sup> These properties of female cardiac mitochondria depends on gender modulation of the calcium uniporter.<sup>110</sup> Estrogens may be involved as it was shown that they protect heart mitochondria from high calcium-induced release of cytochrome c.<sup>111</sup>

### **Energy metabolism in hypertrophy and heart failure**

In response to stress, the heart hypertrophies to bear the extra load and normalize the pressure. Hypertrophy is accompanied by intracellular and extracellular remodeling that involves vascularization, excitation-contraction coupling cytoarchitecture and energy metabolism (for review see <sup>112</sup>). At first adaptive, some of these switches promotes a more favorable energetic economy, but when the stress is prolonged and strong, these adaptive mechanisms become insufficient to maintain oxidative metabolism and cardiac performances and participate in the cardiomyocyte failure. Metabolic alterations in heart failure encompass augmented energy demand due to the increased load, decreased energetic efficiency and diminished energy metabolism leading to energetic imbalance of the myocardium.<sup>81, 113</sup>

Complex changes in gene expression affect all cellular functions including energy metabolism. In response to stress, the genetic program is thought to reverse to a more fetal one. In general, a decrease in reliance on fatty acid is observed while glucose oxidation is first maintained and then also decreases in more severe states leading to loss of metabolic flexibility.<sup>82, 113, 114</sup> It is now appreciated that loss of metabolic flexibility and mitochondrial dysfunction precede cardiac dysfunction, indicating that metabolic remodeling is an early event in disease progression.<sup>114-116</sup> In response to physiological stimuli as well as during compensated hypertrophy, mitochondrial content increases in proportion to other cell components to maintain an optimal ratio between mitochondria and energy consuming organelles like myofilaments.<sup>117</sup> In uncompensated hypertrophy, mitochondrial dysfunction is associated with decreased energy production, decreased mitochondrial biogenesis, increased production of reactive oxygen species and increased mitochondrial permeability. Decreased mitochondrial biogenesis and mitochondrial dysfunction appear as a hallmark of the failing heart. Creatine kinase system is profoundly altered in heart failure. Content of cytosolic and more mitochondrial creatine kinases is decreased, affecting the coupling between energy production and energy transfer.<sup>81, 113</sup> Loss of metabolic flexibility, mitochondrial dysfunction, reduced mitochondrial content and altered energy transfer induce an energetic unbalance leading to altered calcium handling and contractile dysfunction.

### **Sex differences in energy metabolism in cardiac hypertrophy and heart failure**

The metabolic sexual dimorphism may be exacerbated following physiological or pathological stresses.



In response to exercise training, the heart hypertrophies and is remodeled in a sex-specific manner. In response to treadmill running, a preferential utilization of fatty acids and upregulation of cardiac genes involved in fatty acid metabolism is observed in female hearts compared to males, while myocardial glucose uptake is reduced, evidencing sex-specificity in cardiac substrate availability and utilization.<sup>118</sup>

Using loss-of-function animal models, it was shown that the sex-specific mitochondrial adaptation to exercise is modulated by the estrogen receptor ER $\beta$ .<sup>119</sup>

In the failing heart, sexual dimorphism has been identified in the expression of genes involved in energy metabolism. In patients with idiopathic dilated cardiomyopathy, the female-specific pattern of gene deregulation involves energy metabolism and regulation of transcription and translation while the male pattern involves genes related to muscular contraction.<sup>120</sup> In patients with aortic stenosis, women have more concentric hypertrophy with better systolic function, less upregulation of extracellular matrix genes and better reversibility after unloading while stressed female hearts maintain energy metabolism better than male hearts and are better protected against calcium overload.<sup>56</sup> These differences could contribute to the protection of females against heart failure.

Following transverse aortic constriction in mice, females have less hypertrophy and less signs of heart failure than males. This is accompanied by a stronger repression of genes involved in mitochondrial function and biogenesis as well as fatty acid oxidation and increased expression of matrix-related genes in males compared to females involving ER $\beta$ .<sup>92, 121</sup> Sex differences in energy metabolism and HF is underlined by the finding that deletion of PPAR $\alpha$  and simultaneous modulation of fatty acid utilization in mice leads to sex-specific phenotypes with HF-related death in male animals and survival in females.<sup>122</sup>

Anticancer therapies by anthracycline are limited by their cardiotoxicity which is considered as a complex multifactorial process involving oxidative stress and mitochondrial damages.<sup>123, 124</sup> A sexual dimorphism in doxorubicin (a widely used anthracycline) toxicity has been recently described. Female rats appear much less sensitive to its cardiotoxic effects than male rats.<sup>96, 125, 126</sup> The female protection involves energy metabolism, AMPK pathway, cardiolipins and mitochondria.<sup>96, 125</sup> The greater cardiotoxicity to doxorubicin of tumor-bearing spontaneously hypertensive male rats is attributed to the greater activation of oxidative stress and apoptosis without differences in tumor volume regression.<sup>127</sup>

IHD is the leading cause of morbidity and mortality in both men and women but women have lower risk before menopause.<sup>128, 129</sup> Ischemia and post-ischemic reperfusion cause mitochondrial functional and structural injuries due in part to excess production of ROS and calcium overload, triggering PTP opening, decrease in ATP supply and ultimately cell death.<sup>108</sup> Female hearts are more resistant to oxygen deprivation<sup>130, 131</sup>, and ischemic reperfusion injury induces lower infarct size in female than males rats.<sup>132</sup> Age and gender-specific modulation of mitochondrial oxygen consumption and mitochondrial free matrix calcium ([Ca<sup>2+</sup>]<sub>Mito</sub>) content occurs during early reperfusion.<sup>133</sup> Female protection from myocardial ischemia/reperfusion injury involves better mitochondrial calcium handling and maintained membrane potential.<sup>110</sup> Estrogen may be involved in the better preservation of mitochondrial function following cardiac ischemia/reperfusion.<sup>134</sup>

An interesting proteomic analysis in monkeys has shown that female hearts are protected against age-induced decrease in numerous enzymes of glycolysis, Krebs cycle and mitochondrial electron transport chain. The authors concluded that these differences may likely contribute to the cardiac contractile dysfunction of aging male hearts.<sup>135</sup>

Women show an increased prevalence of metabolic cardiomyopathy compared to males. Sex differences in metabolic cardiomyopathy has been recently reviewed.<sup>86</sup> Cardiovascular disease is the leading cause of death in the diabetic population. Metabolic cardiomyopathies like type 2 diabetes and metabolic syndrome are more common in women than men.<sup>86</sup> Diabetes increases the incidence of heart disease three to four times in women and two to three times in men.<sup>136</sup> Energy metabolism is among the possible targets for these sex differences. Baseline sex differences in energy metabolism may be involved. For example, PPAR $\alpha$ , the transcription factor involved in the regulation of lipid metabolism in heart and other organs show sex differences.<sup>100</sup> A role for estrogens in regulating insulin sensitivity has been suggested. E2/ER $\alpha$  elicits the metabolic effects of estrogens by genomic, non-genomic, and mitochondrial mechanisms that regulate insulin signaling, substrate oxidation, and energetics.<sup>137</sup> In rats, streptozotocin-induced type I diabetes affects female cardiac mitochondrial respiration more than the male one but insulin treatment restores mitochondrial function more effectively in the female heart<sup>138</sup> Female hearts exhibit increased resilience to ischemia/reperfusion injury and cell death as compared to males (for review see <sup>88</sup>). This advantage is however lost in diabetes as in the murine female heart, the metabolic gene switch from fatty acids to glucose and enhanced mitochondrial respiratory capacity is lost with the onset of obesity/type 2 diabetes.<sup>100</sup>

Pulmonary arterial hypertension (PAH) is a fast progressive fatal disease characterized by elevated pulmonary arterial pressure, pulmonary vascular remodeling and occlusive pulmonary vascular lesions. Evidence from recent epidemiological studies show an approximate female to male ratio of 4:1, depending on the underlying disease pathology showing that female sex is a risk factor for PAH (for review see <sup>139</sup>). However, the basal mechanisms of these sex differences are still largely unknown. Sex hormones have been involved in the development of PAH. Although generally protective, estrogens and its metabolites may in some instances be deleterious and more studies are needed to clearly decipher the potential therapeutic effects of estrogens in PAH.<sup>140, 141</sup> Mitochondrial dynamics and biogenesis have been implicated in the hyperproliferation of pulmonary artery smooth muscle cells and remodeling of the right ventricle in PAH<sup>142</sup>, but male and female potential differences have been poorly investigated so far. The major cause of death in PAH is right ventricle heart failure. Interestingly, the protective effect of estrogen on right ventricular function has been attributed to preservation of mitochondrial function and biogenesis.<sup>143</sup>

**In conclusion**, part of the sexual dimorphism of cardiovascular diseases is linked to energy metabolism and mitochondrial function. Better preservation of mitochondrial function and content in female heart and better ability to handle calcium and to decrease ROS production participate in the lesser sensitivity of females to heart failure. However, our understanding of sex differences is still incomplete and highlights the importance of developing comparative studies between males and females in basic and clinical sciences.

### Sex differences in cardiac inflammation

Sex differences in the development and outcome of cardiovascular diseases have been recently reviewed and the pathomechanisms of cardiac inflammation, leading to myocarditis or coronary artery disease appear to differ between men and women.<sup>56</sup> Thus, some differences in the development and outcome of cardiovascular diseases might be linked with a stronger infiltration of immune cells in male cardiac tissue.<sup>144</sup>

Myocarditis is characterized by an acute inflammation and is more prevalent in men than in women<sup>145-148</sup>, though the underlying cause is unknown.<sup>149</sup> Viral infections, e.g., coxsackievirus B (CVB3), are the major cause of myocarditis involving Th1, Th2 and Th17 cell-mediated immunity. Aside from lymphocytes, macrophages are also involved in myocardial inflammation.<sup>150</sup> Inflammatory macrophages can be divided in M1 macrophages, which have a pro-inflammatory signature, or in M2 macrophages, which are involved in anti-inflammatory actions, e.g., wound healing and fibrosis.<sup>151, 152</sup> The aberrant expression of cytokines during inflammation leads to the switch of the macrophage's phenotype over time, which under certain conditions may promote the perpetuation of the inflammation.<sup>153</sup> Therefore, M2 macrophages may play a pathogenic role, e.g., in chronic inflammatory diseases.<sup>154</sup>

Polarization of macrophages, i.e., differentiation in M1 or M2 phenotype, in CVB3 induced myocarditis is sex-specific: higher expression of activated macrophages M1 in male and M2 phenotype in female mice (Fig 4).<sup>155</sup> However, only male mice develop Th2-related fibrosis and DCM after CVB3 infection<sup>145, 156</sup>, indeed the activation of Th2 cells in male mice is essential for the perpetuation of the myocarditis related inflammation and development of heart failure.<sup>157</sup> Male mice infected with CVB3 virus present more infiltration of  $\gamma\delta$ T cells than female mice, which are involved in the activation of M1 macrophages during the acute inflammatory stage.<sup>158</sup> In contrast it is described that, more Th2 anti-inflammatory cells and T regulatory (Treg) cells infiltrate and therefore more M2 macrophage activation in the cardiac tissue of female mice after CVB3 infection.<sup>149, 158, 159</sup> Interestingly, female mice with myocarditis in the estrus phase showed significantly lower amounts of inflammatory cells. IFN- $\gamma$ -positive CD4 cells and activated T-cells were decreased in the estrus phase, while the amount of Treg cells was increased<sup>158</sup>, suggesting the pivotal role of sexual hormones in the development and chronicity of myocarditis.

In CAD also sex differences in the inflammatory processes are reported, which contribute to the development and maintenance of this disease.<sup>160</sup> Men suffer more frequently from occlusive CAD, while women exhibit more frequently microvascular dysfunction or spasm located in the epicardial coronary arteries due to pathological vasoreactivity based on endothelial dysfunction in younger ages.<sup>53, 161</sup> Moreover, spontaneous coronary artery dissection occurs >90% in women <60 years of age. It is frequently associated with strong changes in the sex hormone levels and occurs more frequently in immunologic and connective tissue diseases.<sup>162</sup> Chronic inflammation seems to play a crucial role in atherogenesis and in the maintenance of atherosclerotic lesions.<sup>163</sup> Particularly, monocytes and macrophages significantly contribute to the inflammatory processes in CAD.<sup>164-166</sup> Sexual hormones modulate the immune system via hormone receptors under physiological and pathological conditions.<sup>167</sup> Sexual hormone receptors are expressed in macrophages, mast cells, natural killer cells, dendritic cells, B cells and T cells.<sup>168</sup> Estrogens and androgens regulate inflammation through genomic and non-genomic pathways.<sup>169</sup> This modulation promotes sex differences in the activation of immune cells.<sup>169</sup>

While androgens enhance the innate immune response and have immunosuppressive actions, activating the pro-inflammatory Th1/Th17 immune response, E2 activates the regulatory IL-4 mediated Th2 response, which protects women and female animals from acute inflammation.<sup>170</sup> Furthermore, E2 activates the humoral immune response, leading to an increased antibody response and autoantibody production.<sup>149, 170</sup> In addition, sex differences in the balance between Th1 and Th2 immunity in humans are documented<sup>171</sup>, men develop a Th1/Th17 mediated acute immune reaction,

while women develop a prominent Th2 immune response in cardiac tissue. The sex hormone mediated imbalance of the Th1/Th2 immunity profoundly modulates the acute inflammatory stage in the heart.<sup>170</sup> Furthermore, Treg, which are prominent in the immune reaction in women, decrease Th1, Th2 and Th17 immune response and inhibit acute inflammation.<sup>172, 173</sup> Although both pro-and anti-inflammatory actions of estrogens in male and female animals and cells, have been described, which seems to play a dual role in chronic inflammation<sup>162</sup>, the majority of studies argue for anti-inflammatory effects of estrogen receptors (ER) activation in cardiac tissue. Particularly, E2 exhibits anti-inflammatory actions on endothelial cells and immune cells in vitro.<sup>165, 166</sup> and promotes cardioprotective effects in premenopausal women.<sup>174</sup>

E2 seems to inhibit directly the NF- $\kappa$ B dependent Th1 immune reaction via activation of the ER- $\alpha$ .<sup>175</sup> In addition, E2 loss leads to expression of pro-inflammatory cytokines in humans<sup>53</sup> and induces the premature development of atherosclerosis<sup>176</sup>, an effect that can be prevented in both species by estrogen-containing oral contraceptives.<sup>177, 178</sup> In accordance with this, E2 treatment of postmenopausal women attenuated progression of atherosclerosis.<sup>179</sup> However, the beneficial effects of a HRT seems to be strongly dependent of the timing of the hormonal substitution after menopause.<sup>178</sup> While the initiation of an E2 administration at the time of the menopause significantly decreased the risk of coronary heart disease in postmenopausal women, the late initiation of HRT has no positive effects in the prevention or improvement of atherosclerosis.<sup>177, 180-182</sup> In accordance with this, animal studies demonstrated that HRT prevents the formation of arterial lesions, but it did not improve the regression of established arterial lesions.<sup>177, 180</sup> The protective effects of E2 in atherosclerosis are related among other to the actions of E2 on chemokines and cytokines that are involved in the early inflammatory phase in atherosclerosis<sup>183, 184</sup>, which has positive effects in the stabilization of endothelium.<sup>185</sup> Likewise, E2 reduces myocardial phosphorylation of p38 and myocardial expression of pro-inflammatory mediators, e.g., TNF- $\alpha$ , IL-1 $\beta$  and IL-6, which are lower in female rodents after ischemia and reperfusion.<sup>186</sup>

Chronic inflammation has been shown to directly or indirectly disturb the endothelial integrity by changing the expression profile of vasoactive molecules or altering the cell-to-cell junctions. Likewise, chronic inflammation also activates monocytes to transmigrate endothelium and release a repertoire of cytokines leading to the loss of endothelial integrity and dysfunction.<sup>187</sup> Clinical and experimental studies suggest the importance of E2 in the inflammatory response-induced disturbance of the endothelial function. Indeed, an estrogen replacement therapy decreases the risk of cardiovascular disease in postmenopausal women<sup>188</sup>, which can be, at least partly, attributed to the reduced expression of vascular inflammatory markers, like E-selectin and ICAM-1, observed both in patients<sup>189, 190</sup> and endothelial cell culture models in vitro.<sup>191</sup> In vascular endothelium, E2 enhances eNOS expression and NO production<sup>192</sup> and improved cerebral artery dilation.<sup>193</sup> Furthermore, E2 suppresses TNF $\alpha$  -induced ICAM-1 and MCP-1 expression in endothelial cells<sup>191</sup> and improves the expression of tight junction protein occluding in endothelial cells.<sup>194</sup> In addition, E2 reduces leukocyte extravasation in different inflammation models.<sup>195, 175, 196</sup>

Monocytes also contribute in the endothelial destabilization, which plays an important role in the development of diastolic dysfunction in HFpEF, which is more common in postmenopausal women than in men. The development of HFpEF has been suggested to be related to microvascular endothelial cell inflammation and subsequent concentric cardiac remodeling and dysfunction.<sup>197</sup> Cardiac

inflammation plays a crucial role in the initiation of cardiac fibrosis and diastolic stiffness in HFpEF patients<sup>198</sup>, which further confirms the link between inflammatory cells and fibrosis. Monocyte infiltration and differentiation as well as activation of macrophages in the inflamed area play a prominent role in the activation and differentiation of fibroblasts into myofibroblasts, leading to collagen deposition and fibrotic tissue<sup>199</sup>. Macrophage and mast cells modulate degradation and synthesis of extracellular matrix and contribute directly in tissue remodeling processes in the inflamed heart.<sup>170, 200</sup> Pro- and anti-inflammatory cytokines like IL-1 $\beta$ , IL-4, IL-13, IL-17, TNF- $\alpha$  and TGF- $\beta$  promote fibroblast proliferation and activation, leading to an excessive collagen deposition.<sup>199</sup> Moreover, MCP-1 is more elevated in male heart during cardiac inflammation in comparison to female heart.<sup>201</sup> MCP-1 overexpression plays a fundamental role in the immune cell infiltration in cardiac tissue, promoting heart failure.<sup>202</sup> Testosterone seems to play a crucial role in these processes, leading to adverse cardiac remodeling in the male heart.<sup>145, 203</sup>

Studies on the transcriptomic response of the heart to pressure overload in female mice lacking ER $\beta$  showed an increase in natural killer cell-mediated cytotoxicity and leukocyte transendothelial migration pathways.<sup>204</sup> In human activated peripheral blood mononuclear cells (PBMCs) E2 inhibits the expression of pro-inflammatory cytokines<sup>205</sup> and decreases the ROS-mediated NF- $\kappa$ B activity.<sup>206</sup> In accordance with this, our previous studies showed that the expression of genes related with cytochrome P450 pathways were restored under the effects on the E2/ER $\beta$  axis<sup>204</sup>, contributing to the positive effects of E2 in cardiovascular diseases.

In addition, a loss of the endothelial barrier function results in increased vascular permeability and leakage of blood components, which may finally result in organ dysfunction.<sup>205</sup> In addition, cellular stress, like hypoxia evokes endothelial dysfunction e.g. through a decrease in the eNOS expression and an increased reactive oxygen species (ROS) production in endothelial cells, following by increased endothelial instability.<sup>207</sup> Likewise, chronic inflammation also activates monocytes to transmigrate endothelium and release a repertoire of cytokines inducing endothelial barrier failure.<sup>208</sup>

Recent reports suggest the role of mitochondrial function and biogenesis in inflammatory response, either in immune cells or in targeted cells, e.g. fibroblasts or endothelium.<sup>209</sup> There is a growing evidence that E2 supports mitochondrial function by promoting expression of tricarboxylic acid cycle and OXPHOS proteins and, therefore, may affect inflammatory response.<sup>210</sup> While NO seems to have anti-inflammatory effects, ROS promotes pro-inflammatory actions, leading to cell and organ damage. Recent reports suggest that NO/ROS balance is essential for mitochondrial function and resolution of inflammation.<sup>211</sup> The potential cellular mechanisms of the anti-inflammatory effect of E2 may be the improvement of mitochondrial function and reduction of ROS formation in cardiac cells. Certainly, inflammatory response is usually accompanied by excessive ROS formation resulting in mitochondrial stress.<sup>212</sup>

### Sex differences in cardiac fibrosis

Cardiac fibrosis is strongly and in a sex specific manner associated with the development of myocardial hypertrophy (MH) and progression to heart failure. Clinical studies have documented sex differences in cardiac fibrosis with higher collagen deposition and more frequently observed cross-hatching in men compared to women with aortic stenosis (AS), coronary artery disease and atherosclerosis.<sup>29, 56, 213-215</sup> Accordingly, TGF $\beta$ -1 and SMAD signaling was found to be more pronounced in male hearts compared with women with AS.<sup>29, 216</sup> Furthermore, men with AS showed a significant higher activation of pro-

fibrotic markers, i.e. collagen I and III, periostin, matrix metalloproteinase-2 (MMP-2) and -9, compared with women.<sup>29, 32, 216</sup> ER $\alpha$ , ER $\beta$  and AR control fibrotic pathways, collagen, and matrix-metalloproteinase synthesis in a sex-specific manner (Fig 6). In addition, estrogen attenuates the development of cardiac fibrosis differently in women and men. Estrogen directly inhibits collagen synthesis in women and supports it in men.<sup>32, 217-219</sup> Estrogen also increases matrix metalloproteinase expression via activation of the ER $\alpha$  and MAPK-ERK1/2 signaling pathway.<sup>220</sup> Furthermore, the E2 regulates a network of miRNAs mainly via ER  $\beta$ , that controls fibrosis.<sup>221</sup> Cardiomyocyte-specific ER $\alpha$  expression reduces the development of fibrosis following myocardial infarction (MI).<sup>222</sup> Androgens also interact with pro- and antifibrotic mechanisms. The genetic deletion of the AR leads to the exacerbation of angiotensin II-induced cardiac dysfunction and fibrosis.<sup>223</sup>

### Sex differences in calcium handling and contractility

Sex differences in cardiac contractility and calcium (Ca) handling have been recently reviewed in detail by us and others.<sup>56, 224</sup> Female rodents exhibit less SR Ca<sup>2+</sup> loading compared with males in response to  $\beta$ -adrenergic stimulation and smaller Ca<sup>2+</sup> transients.<sup>225, 226</sup> These sex differences are partly mediated by the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway.<sup>227</sup> Ovariectomy increases SR Ca<sup>2+</sup> content and peak Ca<sup>2+</sup> transient amplitudes. These effects are reversed by E2 treatment and regulated during the estrous cycle.<sup>228-232</sup>

The sensitivity of myofilament response to Ca<sup>2+</sup> is regulated by estrogen administration.<sup>233-235</sup> In particular, myofilament Ca<sup>2+</sup> sensitivity is increased in hearts of ovariectomized (OVX) rats compared with sham-operated rats, while this effect is reversed by E2 treatment.<sup>234, 236, 237</sup> Similarly, Ca<sub>v</sub>1.2 protein levels and the gain of excitation–contraction coupling are higher in hearts from OVX rats compared with those of sham-operated rats.<sup>230, 233</sup>

Female sex and E2 is associated with changes in the activity of the sarcolemmal and mitochondrial ATP-sensitive potassium (KATP)<sup>132, 238</sup> and sarcolemmal KATP channel and to the protection of cardiac cells against hypoxia/re-oxygenation injury.<sup>132, 238-240</sup> Furthermore, estrogen attenuates the occurrence of ischemia/reperfusion-induced arrhythmias through the modulation of NO and Ca<sup>2+</sup>-activated potassium channels, which is more pronounced in females than in males.<sup>241-243</sup>

Testosterone also affects cardiac Ca handling and contractility.<sup>244-246</sup> L- and T-type Ca<sup>2+</sup> currents are induced in neonatal rat cardiomyocytes by testosterone.<sup>247, 248</sup> Testosterone shortens the QT interval by activating potassium channels, giving a molecular explanation for the Brugada syndromes.<sup>249, 250</sup>

### Sex differences in relaxation

The molecular basis for diastolic function are manifold as discussed previously.<sup>251, 252</sup> Sequestration of calcium and cross bridge uncoupling after the end of systole in the cardiomyocyte are responsible for the active process of relaxation.<sup>253, 254</sup> The phosphorylation state of phospholamban is different in male and female hearts and leads to differences in the affinity of the sarcoplasmic reticulum for calcium re-uptake.<sup>254</sup> In addition, E2 protects single myocytes against calcium loading induced by hypoxia.<sup>255</sup> In female rat hearts, sex related differences in calcium signalling can also be partially explained by greater myofibrillar calcium sensitivity.<sup>256</sup> Testosterone regulates the L-type calcium channel and other calcium regulatory proteins.<sup>257</sup> Thus, differences in myocardial calcium handling due to estrogens or androgens may contribute to sex differences in diastolic function.

Increased availability of NO increases diastolic function in animal models and the human heart.<sup>258</sup> NO leads to an earlier onset of relaxation and increases diastolic distensibility via effects on MMPs and cGMP.<sup>258</sup> The NO-system exhibits sex specific regulations. Estrogens affect the expression and activity

of NO-synthases at different levels - at the level of gene transcription and cytoplasmic control of activity.<sup>34</sup> This leads probably to higher levels of NO in women compared with men and contributes to better endothelial dependent vasodilation in women. Polymorphisms in the NO system have greater effects in women than in men.<sup>259</sup> Thus, differences in the NO synthases represent a candidate to understand sex differences in relaxation. The cardiac endothelium is one of the main producers of NO and modulators of calcium signaling. It should also have a major role in the control of relaxation and sex differences.<sup>260</sup>

### Sex differences in vascular adaptation and maladaptation

Although the pathophysiology of the HF syndrome is multifactorial and complex, vascular dysfunction is known to be a major contributor to the development, progression and treatment of HF.<sup>197, 261-265</sup> The vascular wall is a biological, dynamic organ that, in addition to releasing vasoactive substances, reacts to various hemodynamic and metabolic stimuli with structural and functional adaptations.<sup>263</sup> Moreover, various physiological (i.e. age) and pathological (i.e. hypertension, diabetes) factors affect the vascular bed, thereby impairing its morphology and function.<sup>266</sup> Hence, vascular dysfunction is a result of a cumulative exposure of the vascular bed to the effects of numerous pathophysiological elements, with sex being a key determinant in this process.

Sex differences in vascular dysfunction are due to inherent sex differences in vascular biology as well as to sex differences in vascular ageing patterns and susceptibility to risk factors.<sup>197, 261-265, 267</sup> In HFrEF, the leading predisposing factors for its development are clinical syndromes of macrovascular disease, namely obstructive CAD and MI.<sup>261, 268-270</sup> Contrarily, in HFpEF, coronary microvascular impairment is thought to be the main driver.<sup>197, 261-265, 271, 272</sup> In coronary microvascular impairment, endothelial dysfunction yields microvascular rarefaction, autonomic imbalance and a reduced coronary flow reserve.<sup>273, 274</sup> Autopsy studies have indeed confirmed the process of microvascular rarefaction in hearts of patients diagnosed with HFpEF. Genetic studies indicate that genes involved in myocyte proliferation, transforming growth factor-beta (TGF- $\beta$ )/erbB signaling, and extracellular matrix formation may be causal to HFpEF, although no evidence was provided regarding differences between men and women.<sup>275</sup> TGF- $\beta$  can induce an endothelial-to-mesenchymal transition (EndMT), a mechanism in endothelial cells that may contribute to vascular disease. It may be causal to the microvascular endothelial dysfunction and coronary microvascular ischaemia seen in HFpEF.<sup>276-278</sup> Also a reduced coronary flow reserve is an important pathophysiological feature in HFpEF. Due to deficient functioning of the coronary microvasculature, cardiomyocytes stay intact, yet respond with a reduction in relaxation capacity. As such, the myocardium stiffens.<sup>262</sup> Microvascular impairment in HF is suggested to be preceded by abnormalities in the microcirculation such as damage to the arterial intima, swelling and degeneration of endothelial cells (yielding narrowing of the lumen of capillaries), the occurrence of degenerative foci and lipofuscin deposits in myofibers and an increase in adrenergic function and abnormal handling of norepinephrine in cardiac sympathetic nerve endings.<sup>276-278</sup>

It has been postulated that microvascular disease primarily affects women due to sex differences in vascular alterations related to hormonal exposure as well as to accumulation of risk factors and to intrinsic biological sex differences.<sup>47, 261</sup> An excellent example of the latter is provided by The Women's Ischemia Syndrome Evaluation (WISE) study.<sup>279, 280</sup> This study demonstrated that in 50% of all women who presented with chest pain without signs of significant obstructive CAD on coronary angiography, coronary microvascular dysfunction, as demonstrated by coronary flow velocity reserve testing, was in fact present and could not be predicted by risk factors for atherosclerosis or hormone levels.<sup>279, 280</sup> More importantly, during follow up, a considerable number of these women developed HFpEF,

implying that HFpEF as well as non-obstructive CAD go hand in hand with coronary microvascular dysfunction.<sup>280</sup> In addition to microvascular dysfunction, non-obstructive CAD appears to be accompanied by microvascular spasm, arterial stiffening, arterial wall thickening and myocardial bridging, all of which may result in myocardial ischemia.<sup>261, 281</sup> Moreover, thrombus on top of an intact eroded plaque, more often described in women than in men, is accompanied by microembolisations downstream in the heart.<sup>282</sup> This may potentially link female atherosclerotic disease to coronary microvascular disease.<sup>283</sup> Although a detailed description of sex differences in atherosclerotic plaque morphology goes beyond the scope of this chapter, it has been excellently covered in previous chapters.

### **Sex differences in vascular biology**

With macro- and microvascular dysfunction underlying a myriad of cardiovascular diseases, which in turn show great disparities in prevalence between the sexes, looking at sex differences in components of the vascular wall may provide insight into why these sex differences arise. (Fig 6) Already at a morphological level, the coronary arteries differ between men and women. Women have a smaller diameter of all coronary arteries as compared to men, and the same holds true for the smaller vessels in the heart.<sup>284, 285</sup> Macroscopic differences in disease are present as well. In early atherosclerosis of the coronary artery, men show more diffuse macrovascular endothelial dysfunction and larger atheroma burden than women in the coronary arteries.<sup>270</sup> On a genetic level, sex-specific genetic variants that associate with coronary endothelial dysfunction in the large vessels have been identified.<sup>286</sup> Variants in males are located in genes such as NFKB1 and KIF6, while for females they are present in LPA and ADORA3. In children already, high levels of plasma LPA are associated with dysfunctional endothelium.<sup>287</sup> ADORA1, adenosine receptor A1, is a gene with genetic variants in both sexes, but with different single-nucleotide polymorphisms. Adenosine-related biology in the coronary vasculature is subject to sex-differences.<sup>288, 289</sup> In pigs it has been shown that a sexual dimorphic response to adenosine is present in the coronary vasculature, depending as well on the type of vessel, be it venule or arteriole.<sup>289</sup> The regulation of vasorelaxation by NADPH oxidases in the endothelium also displays sexual dimorphism in coronary arteries of pigs. Interestingly, NADPH oxidase 5 (NOX5) is significantly higher expressed in males in human coronary arteries (GTex Portal). The role of NOX5 in vascular disease has just been reviewed, but only recently discovered, as rodents lack NOX5.<sup>290</sup> Dealing with oxidative stress is prone to sex differences, and might also partly explain differences in longevity between men and women.<sup>291</sup> In a mouse-model of hypertension, inhibiting mTOR signaling in females, but not in males, led to diminished expression of CD31 in the coronary microvascular endothelium, indicating that mTOR signaling is essential for cardioprotection in women but may not be essential in men.

An important contributor to microvascular impairment appears to be estrogen deficiency.<sup>261, 273, 292</sup> Sex hormones are a large cause of differential responses between men and women.<sup>2, 56</sup> It is known that in women, the age-related cardiovascular risk is more manifest in post-menopausal life than in pre-menopausal life.<sup>261</sup> This phenomenon is generally attributed to the menopausal transition, the withdrawal of endogenous sex steroids in midlife.<sup>261</sup> The degree to which biological ageing and menopause each add to the age related increment in cardiovascular risk in women persists to be a subject of ongoing debate. Due to the concurrent occurrence and interrelatedness of both processes, this debate is not easy.<sup>293</sup> Additionally, elucidating the role of estrogen in cardiovascular disease risk is difficult due to the recognition that around menopause, next to estrogen, other cardiovascular risk factors in women alter as well, which is likely related to a combination of ageing and the menopausal



transition.<sup>261, 294-296</sup> Hence, estrogen might independently add to cardiovascular risk, spuriously due to confounding or by serving as a mediator through which other cardiovascular risk factors act. However, evidence does support the existence of an association between endogenous estrogen and cardiovascular disease, including HF.<sup>292</sup> Estrogen receptors are present in numerous vascular and cardiac cells and may thereby affect microvascular disease and HF.<sup>297-299</sup> Moreover, in postmenopausal women, an inverse relation between years since menopause and brachial artery flow mediated dilation (FMD) has been reported.<sup>300</sup> Hence, estrogen appears important in maintaining proper vascular health before menopause, as loss of ovarian hormones leads to activation of the renin angiotensin aldosterone system, causing endothelial dysfunction in the end.<sup>301</sup> Also, studies have shown that whereas estrogen mediates vasodilation of the epicardial coronary arteries in pigs, testosterone might be beneficial to the vessels because of its conversion to estrogen by aromatase.<sup>302-304</sup> It has been reported that testosterone treatment in elderly women with heart failure is indeed beneficial.<sup>305</sup> Nonetheless, other lines of evidence did not observe any associations between menopausal status and cardiovascular outcome.<sup>306</sup> In addition, various studies have pointed out that already at pre-pubertal age, sexual dimorphism in (sub) clinical cardiovascular phenotypes is present.<sup>307-309</sup> This implies that, intrinsic, non-hormonal factors that act throughout life probably contribute more to sex differences in cardiovascular risk than midlife hormonal shifts do.

Indeed, sex chromosomes are beginning to be recognized as important to health and disease, independent of sex hormones. Aberrations in sex chromosome number and genetic variation within sex chromosomes have showed to be associated with several cardiovascular diseases.<sup>310</sup> Multiple genes located on the sex chromosomes are hypothesized to have tissue-specific roles, and are differentially expressed between men and women in, for example, the coronary artery (GTex Portal), and as such, most likely as well in the microvasculature. A study in a gonadal-free environment in ischemia/reperfusion injury showed that one X chromosome is better than two, as XX mice are more susceptible to damage and have lower functional heart recovery, underlining the importance of sex chromosomes.<sup>311</sup> Moreover, age-related loss of the Y chromosome in men was associated with incident cardiovascular disease (among which incident HF) in men with severe atherosclerotic disease.<sup>312</sup> Changes in X chromosome inactivation may regulate gene expression between sexes, individuals and tissues. The X chromosomes contain information involved in immunity, inflammation and endothelial function, potentially explaining why most autoimmune diseases predominantly affect women.<sup>313, 314</sup> These women with autoimmune diseases, in turn, have an increased risk for cardiovascular disease, suggesting an intriguing role for the female X chromosomes. However, data on sex chromosome alterations in cardiovascular biology is lacking, while the sex chromosomes harbor genes important in (epigenetically) regulating the autosome. As such, whether or not sex chromosome regulation is involved in the syndrome of HF warrants further investigation.

### **Sex differences in vascular ageing**

Age-related alterations in vascular function largely encompass progression of endothelial dysfunction, arterial dilatation and arterial stiffening, which usually goes hand in hand with an increase in systolic blood pressure.<sup>261, 315</sup> Up to the sixth decade of life, both the increment in arterial stiffening and endothelial dysfunction progress at a greater rate in men as compared to women.<sup>261</sup> However, from the sixth decade of life onwards, age-related vascular dysfunction progresses more rapidly in women than in men.<sup>261</sup> Before menopause, the general vascular function as well as the baroreceptor response and autonomic tone perform better in women when compared to their male counterparts of

comparable age.<sup>316, 317</sup> Yet, after menopause, arterial stiffness becomes more pronounced in women than in men, even after adjustments for confounding factors such as arterial diameter and body size.<sup>318, 319</sup> Clinically, this sex-specific change in vascular structure is reflected in hypertension prevalence patterns across the life span: whereas below the age of 45 years, hypertension is more prevalent in men than in women and between the ages of 45 and 64 years, its prevalence is comparable between the sexes, from the age of 65 years onwards, the prevalence of hypertension is higher in women than in men.<sup>320</sup>

The sex-specific alterations in biomechanical properties of the arteries with ageing have deleterious sex-specific effects on left ventricular diastolic function. Age-related arterial stiffening induces an increase in arterial pressure during systole and a decrease in arterial pressure during diastole.<sup>262, 321-323</sup> The increase in arterial pressure during systole enhances left ventricular load, thereby inducing left ventricular hypertrophy and, as such, prompts left ventricular dysfunction. The hypertrophied heart has a longer contraction and relaxation period, which prolongs the duration of systole and shortens the duration of diastole. A longer systole results in augmented transmission of antegrade and retrograde pressure waves.<sup>322, 323</sup> With arterial stiffening, antegrade pressure waves add to retrograde pressure waves, returning to the heart not at early diastole but at end systole, thereby increasing late systolic load and thus extending ventricular relaxation time even more.<sup>263, 315, 324, 325</sup> These changes prompt an increase in myocardial oxygen demand, which requires an increase in coronary blood flow.<sup>324</sup> Unfortunately, this requirement is not met. The ability to increase coronary blood flow is impaired as a result of a reduction in arterial pressure during diastole and a reduction in diastole duration, the latter being due to a prolonged systolic ejection period and ventricular relaxation time.<sup>263, 315, 326</sup> The mismatch of a decreased ability for coronary perfusion while there is a need for an increase in coronary perfusion induces ischemia. Ischemia prompts an additional increase in ejection time as well as a decrease in ventricular relaxation, which further impairs ventricular perfusion throughout diastole, thereby generating a vicious circle and giving rise to the development of left ventricular diastolic dysfunction.<sup>263, 315, 327</sup>

Due to the larger increment in arterial stiffening in later life, women are more prone to develop an increase in aortic pressure and a longer duration of ventricular relaxation than men.<sup>322, 328, 329</sup> This results in a larger burden of pulsatile arterial loading and pulsatile afterload in women, which deteriorates diastolic function and, as such, possibly adds to the development of HFpEF in women.<sup>322, 325, 330</sup> This theory is confirmed by *in vivo* studies that showed that only in women arterial stiffness is inversely related to diastolic relaxation.<sup>331</sup> Hence, in later life, arterial stiffening may not only progress at a higher rate in women, they may also be more susceptible to its deleterious effects on left ventricular diastolic function than their male counterparts.<sup>262</sup>

### **Sex differences in vascular risk factors**

Advancing age and comorbidities synergistically result in abnormalities in vascular and cardiac morphology and function. As such, comorbidities predispose for the development of HF, although it requires mentioning that also in the absence of cardiovascular disease, female sex as well as ageing have been related to an increment in vascular and left ventricular diastolic stiffness. Comorbidities related to vascular dysfunction contribute to the development and progression of HF, in a sex-specific manner.

Comorbidities are alleged to contribute more to HFpEF than to HFrEF since patients with HFpEF are more likely to die from non-cardiac causes and less likely to die from HF than patients with HFrEF.<sup>197,</sup>

<sup>265, 332-335</sup> Several lines of evidence showed that whereas HFrEF patients have a higher prevalence of CAD and MI, HFpEF patients have a higher prevalence of non-cardiac comorbidities such as hypertension, diabetes, obesity, anemia, chronic kidney disease (CKD), atrial fibrillation (AF) and COPD.<sup>271, 272, 336, 337</sup> For HFmrEF patients, studies showed that they more frequently had hypertension than HFrEF patients, more commonly had ischemic heart disease than HFpEF patients and that a significant proportion had diabetes.<sup>9</sup> Interestingly, although CAD is more common in HFrEF, it is also prevalent in HFpEF.<sup>264, 338</sup> Because of improvements in therapeutic strategies for acute coronary syndromes, survival rates for patients with CAD have increased dramatically over the past decades.<sup>339</sup> For example, enhanced salvage of the myocardium owing to revascularization enables the perseverance of ejection fraction.<sup>264</sup> Despite this therapeutic progress, due to chronic multi-vessel CAD, the majority of patients have persistent ischemia, that induces an increase in left ventricular filling pressure, a decrease in ventricular compliance and a deterioration in ventricular relaxation, which may all ultimately lead to HFpEF.<sup>264, 338, 340</sup>

A hypothesis postulated by Paulus et al. suggests that in HFpEF, comorbidities induce a systemic, pro-inflammatory state that affects the coronary microvascular endothelium, which, in turn, is causal to the development of HFpEF.<sup>197, 265</sup> Paulus et al. state that inflamed endothelial cells react to the pro-inflammatory state by producing reactive oxygen species that reduce nitric oxide (NO) bioavailability for neighboring cardiomyocytes. Decreased NO bioavailability prompts a decline in protein kinase G (PKG) activity in cardiomyocytes. Consequently, low PKG activity induces cardiomyocyte hypertrophy thereby favoring stiffening of the cardiomyocytes and concentric left ventricular remodeling due to hypophosphorylation of titin, a large and important cytoskeletal protein. Finally, the stiffened cardiomyocytes as well as interstitial fibrosis, the latter being due to an increase in collagen deposition by myofibroblasts, add to the development of left ventricular diastolic dysfunction, the key functional deficit in HFpEF.<sup>197</sup> According to the abovementioned the hypothesis proposed by Paulus et al. numerous comorbidities are capable of inducing a systemic pro-inflammatory state.<sup>197</sup> This pro-inflammatory state appears to predict the occurrence of HFpEF, yet not of HFrEF.<sup>197, 341</sup> For example, COPD, an important contributor to HFpEF development, morbidity and mortality, obviously is a chronic, systemic inflammatory disease.<sup>197, 265, 335</sup> Also for obesity, it has been proposed that release of pro-inflammatory cytokines in adipose tissue infiltrated by macrophages may yield a systemic inflammatory response.<sup>197, 342, 343</sup> Furthermore, in salt-sensitive hypertension, high salt intake prompts renal production of pro-inflammatory cytokines thereby provoking systemic oxidative stress.<sup>197, 344, 345</sup> Moreover, both diabetes and the metabolic syndrome are known to be preceded by among others oxidative stress and mitochondrial and endothelial derangements.<sup>264, 346, 347</sup>

In addition to functional vascular impairment, comorbidities also contribute to structural vascular deficits. For example, two major predisposing factors for microvascular damage (HFpEF) are diabetes and hypertension. Their cardiometabolic effects appear to affect endothelium dependent vasodilatation thereby inducing disturbances in the microvasculature.<sup>346, 347</sup> Whereas hypertension has been described in depth in the previous paragraph, diabetes is also a well-known risk factor for both HFpEF and HFrEF.<sup>264, 348</sup> Evidence points out that the presence of diabetes is related to a larger age-related increment in left ventricular wall thickness and a smaller age-related decrease in left ventricular diastolic dimensions, signifying the risk of volume overload.<sup>347, 349</sup> Additionally, in HFpEF, diabetes has been related to increased filling pressure and arterial stiffness as well as to left ventricular remodeling, the latter specifically occurring in women.<sup>340</sup> Markers of insulin resistance have also been related to both concentric remodeling and a greater mass of the left ventricle.<sup>346, 350</sup>

Besides diabetes, obesity prompts alterations in vascular structure and induces an increment in total and central blood volumes and simultaneously, a decrease in systemic arterial resistance.<sup>351, 352</sup> The combination of these hemodynamic changes and increase in oxygen consumption yield an increment in cardiac output.<sup>351, 352</sup> Perseverance of these hemodynamic alterations may pave the way for the development of subclinical left ventricular hypertrophy as well as for augmented pressures in the mean pulmonary artery and during left ventricular diastole, which can ultimately result in HFpEF.<sup>351, 352</sup> Also in CKD, anemia and chronic liver disease this condition of abnormal vascular and cardiac structure and function combined with a high-output state and extra-cardiac volume overload can arise, thereby possibly provoking HFpEF.<sup>264</sup> In metabolic syndrome, an enhanced tendency for CAD as well as left ventricular hypertrophy, myocardial stiffening and decreased energy availability have been described as major abnormalities that may induce HFpEF.<sup>346</sup> As for physical activity, a study observed a relation between physical inactivity and deterioration in ventricular function that appeared to closely mimic the ventricular impairments seen in HFpEF. With advancing age, women less frequently engage in strenuous physical activity than men. In fact, the extent of physical activity in women decreases from 54% at the ages of 18-44 years to 13% at the age of 75+ years.<sup>262, 353, 354</sup>

In conclusion, sex differences in vascular dysfunction play a multifactorial and crucial role in the pathophysiology of the HF syndrome. Nonetheless, more interdisciplinary research is warranted to untie all the knots in sex-stratified cardiovascular biology, including the role of sex in all stages and aspects of the HF syndrome.

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## Legends

Fig 1 . Sex specific development of myocardial hypertrophy. From <sup>56</sup>

Figure 2. Sex differences in energy metabolism and in response to stress involves mitochondrial capacity, production of reactive oxygen species, calcium handling leading to decreased opening probability of the PTP and better cell survival in females compared to males.

Fig 3 Estrogen effects in immune cell function, adapted from <sup>56</sup>

Fig 4 Sex differences in fibrosis Adapted from VRZ, <sup>56</sup>

Figure 5: Schematic overview of sex-specific determinants of vascular dysfunction. In women, vascular dysfunction of the microvessels may lead to HFpEF, In men, vascular dysfunction in the macrovessels is more common and may lead to HFrEF.

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Fig 1

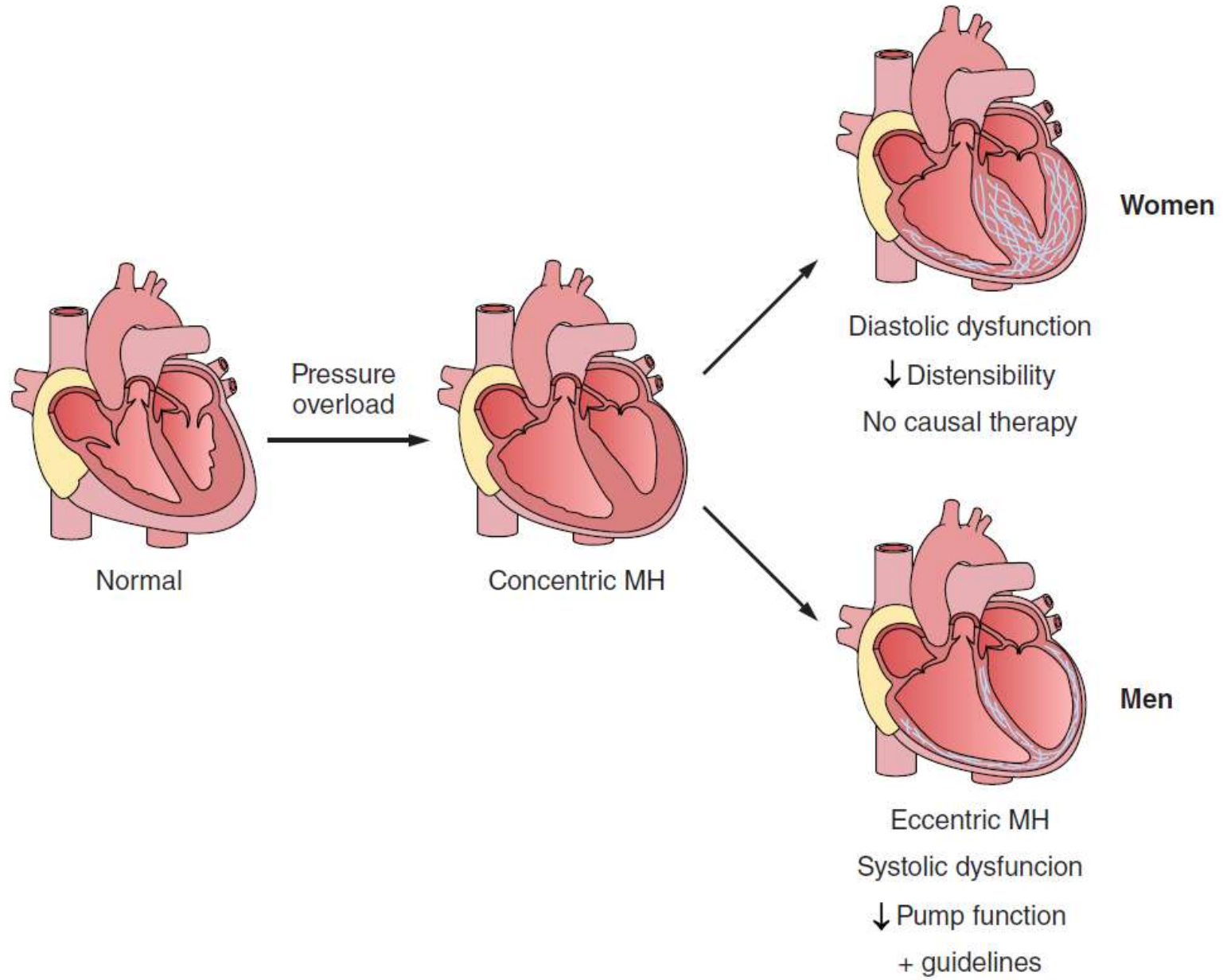


Fig 2

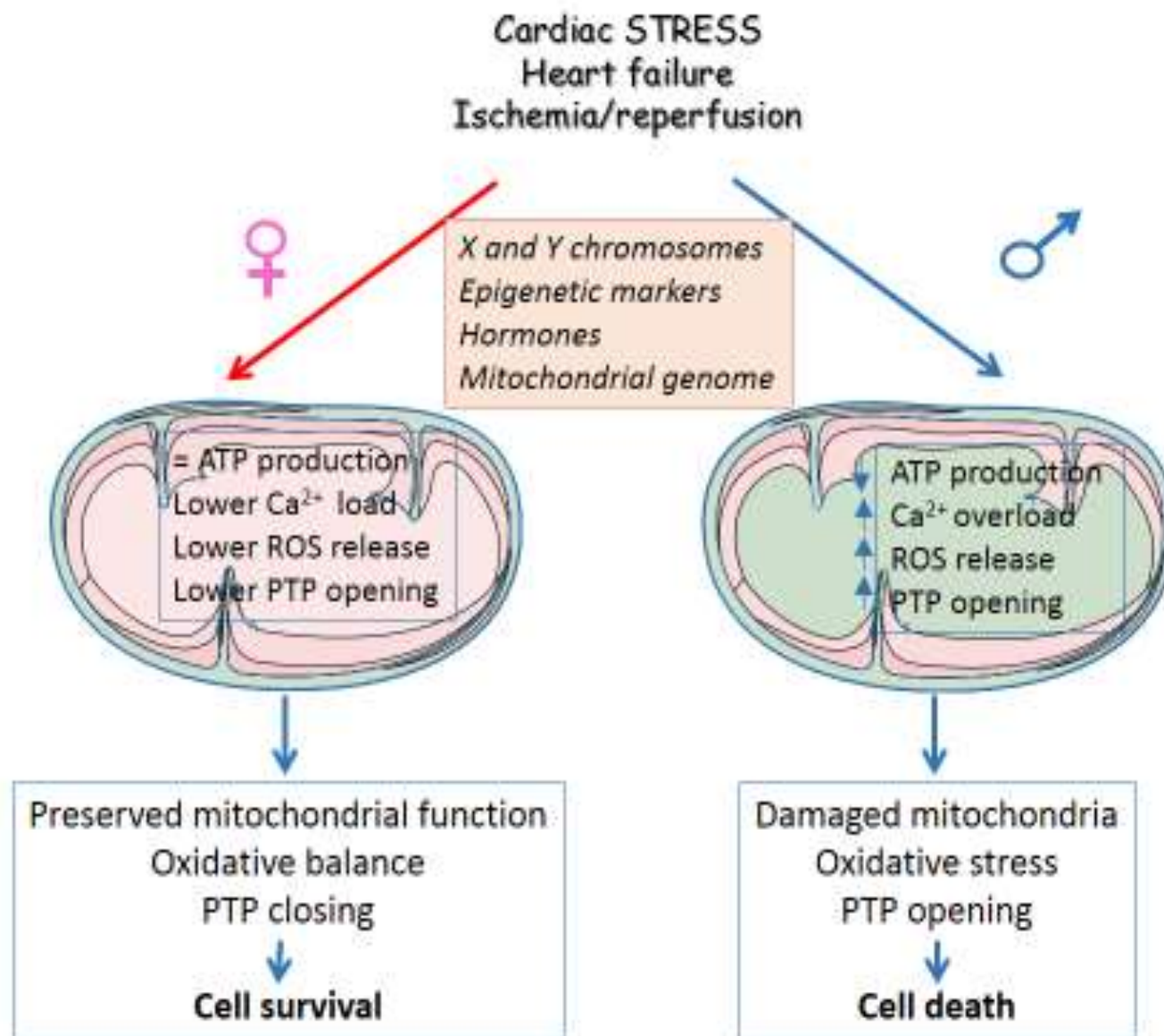


Fig 3

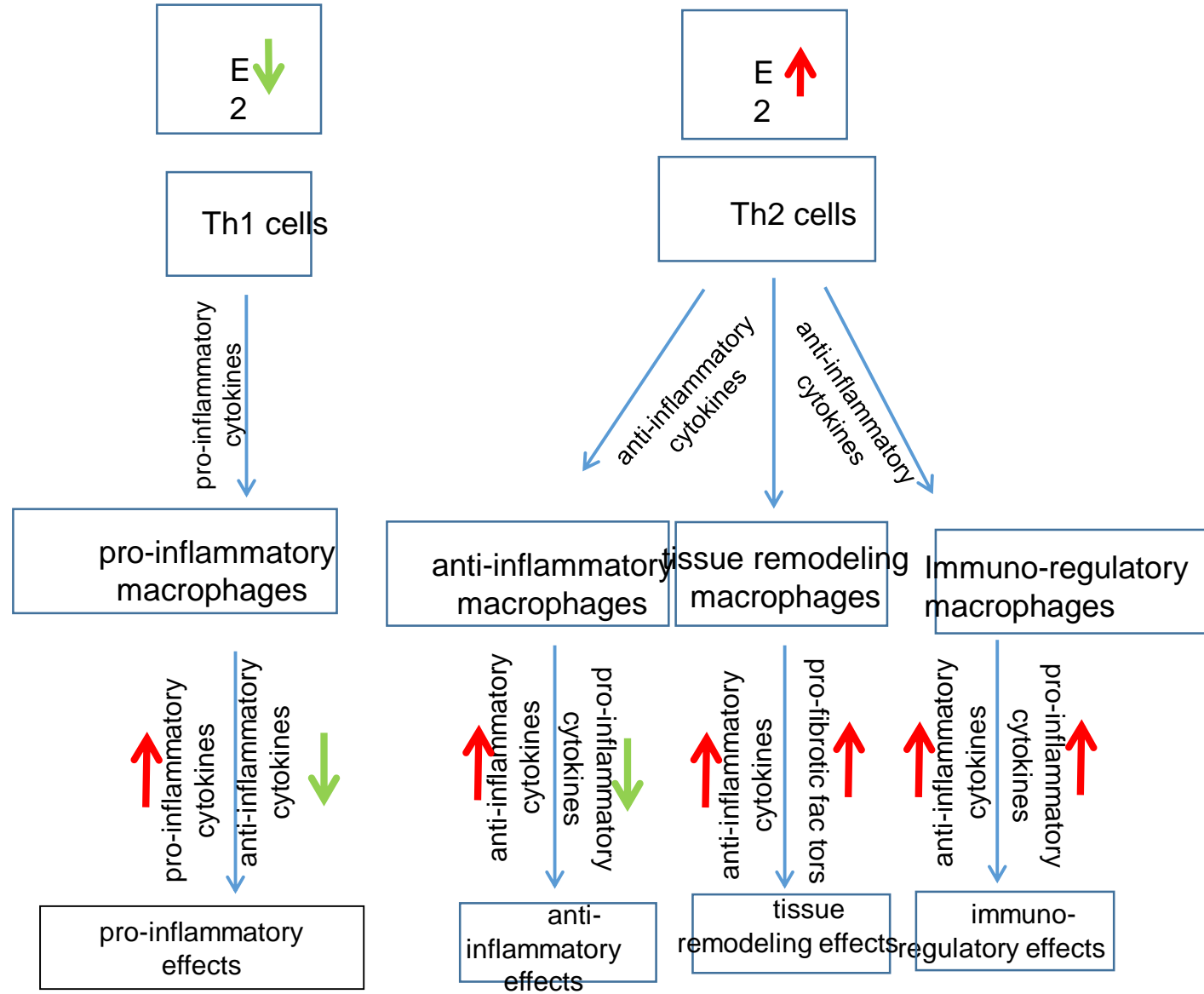




Fig 4

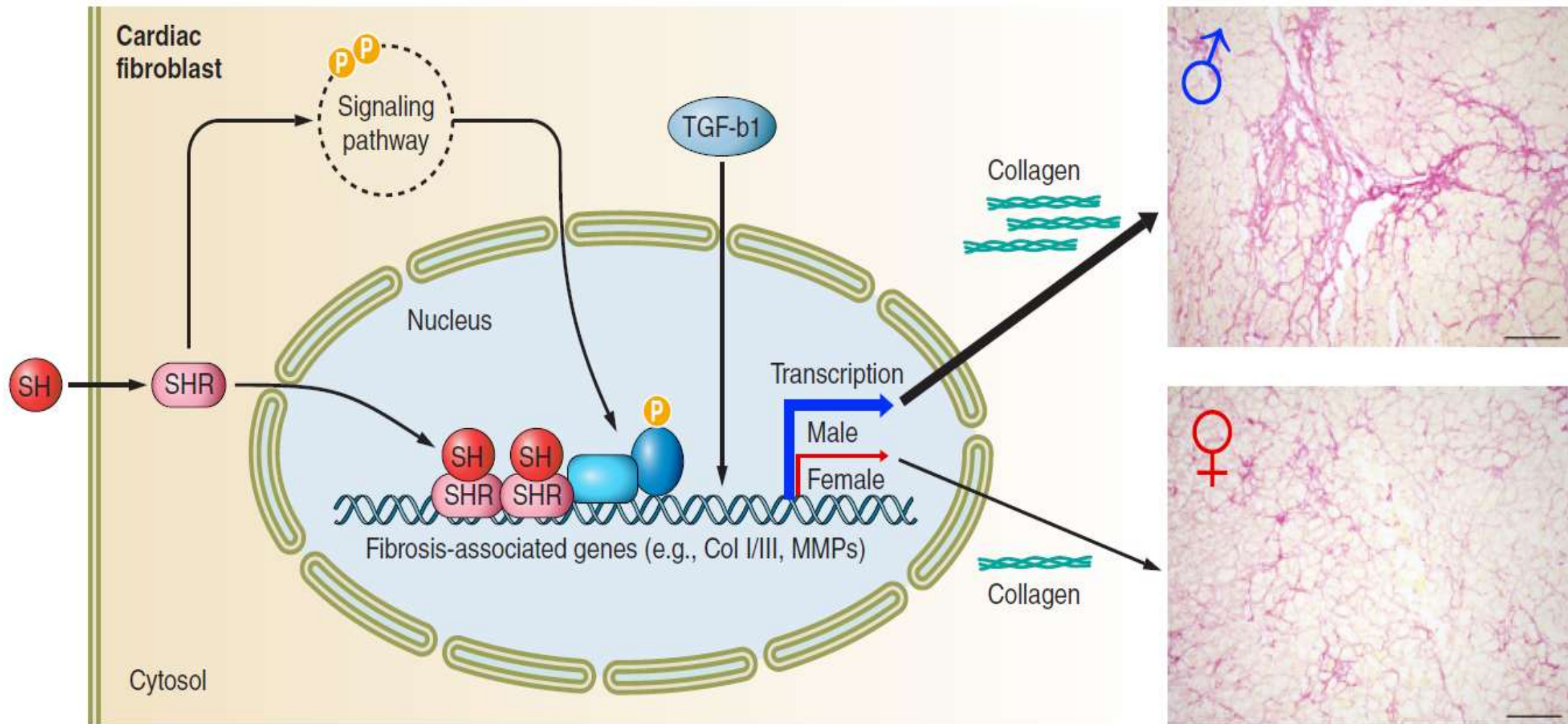


Fig 5

