Mechanisms of sex differences in atrial fibrillation - role of hormones and differences in electrophysiology, structure, function, and remodeling

Katja E. Odening[1,2,3], Sebastian Deiß[4], Dagmara Dilling-Boer[5], Maxim Didenko[6], Urs Eriksson[7,8], Sotirios Nedios[9,10], Fu Siong Ng[11], Ivo Roca Luque[12], Pepa Sanchez Borque[13], Kevin Vernooy[3,14], Adrianus Wijnmaalen[15], Hikmet Yorgun[16];
on the behalf of the DAS-CAM participants 2017-2018

[1]Department of Cardiology and Angiology I, Heart Center, University of Freiburg, Freiburg, Germany; [2]Faculty of Medicine, University of Freiburg, Freiburg, Germany; [3]Department of Cardiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center, Maastricht, The Netherlands; [4]Asklepios Medical Center Altona, Department of Cardiology, Arrhythmia unit, Hamburg, Germany; [5]Hartcentrum Hasselt, Jessa Ziekenhuis Hasselt, Belgium; [6]Department of surgical and interventional arrhythmology, Kuprianov's cardiovascular surgery clinic, Military Medical Academy, St. Petersburg, Russia; [7]Rhythmology Division, Department of Medicine, GZO Regional Health Center, Wetzikon, Switzerland; [8]Cardioimmunology, Center for Molecular Cardiology, University of Zurich, Zurich-Schlieren, Switzerland; [9]Heart Center, University of Leipzig, Germany; [10]Massachusetts General Hospital, Harvard University, Boston, MA, USA; [11]National Heart & Lung Institute, Imperial College London, UK; [12]Arrhythmia Unit, Cardiology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain; [13]Fundacion Jimenez Diaz - Quiron Salud, Madrid, Spain; [14]Department of Cardiology, Radboud University Medical Center, Nijmegen, The Netherlands; [15]Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands; [16]Department of Cardiology, Electrophysiology Unit, Hacettepe University, Ankara, Turkey

Corresponding author:
Katja E. Odening, MD
Heart Center University of Freiburg
Department of Cardiology and Angiology I
Hugstetter Str. 55
79106 Freiburg
Germany
katja.odening@uniklinik-freiburg.de

Abstract

Atrial fibrillation (AF) is the clinically most prevalent rhythm disorder with large impact on quality of life and increased risk for hospitalizations and mortality in both men and women. In recent years, knowledge regarding epidemiology, risk factors and patho-physiological mechanisms of AF has greatly increased. Sex differences have been identified in the prevalence, clinical presentation, associated comorbidities and therapy outcomes of AF. Although it is known that age-related prevalence of AF is lower in women than in men, women have worse and often atypical symptoms and worse quality of life as well as a higher risk for adverse events such as stroke and death associated with AF.

In this review, we evaluate what is known about sex differences in AF mechanisms - covering structural, electrophysiological, and hormonal factors - and underscore areas of knowledge gaps for future studies. Increasing our understanding of mechanisms accounting for these sex differences in AF is important both for prognostic purposes and the optimization of (targeted, mechanism-based, and sex-specific) therapeutic approaches.

Keywords

Atrial fibrillation; sex differences; mechanisms; electrical parameters; structural remodeling; hormones

Word count

4649 (text without references, tables, figure legends)

147 references

1. Introduction

Atrial fibrillation (AF) is the clinically most prevalent rhythm disorder with large impact on quality of life and increased risk for hospitalizations and mortality in both men and women.[1, 2] In recent years, knowledge regarding epidemiology, risk factors and patho-physiological mechanisms of atrial fibrillation has greatly increased.[3] Sex differences have been identified in the prevalence, clinical presentation, associated comorbidities and therapy outcomes of AF.[4-7] Although it is known that age-related prevalence of AF is lower in women than in men[8], women have worse and often atypical symptoms and worse quality of life as well as a higher risk for adverse events such as stroke and death associated with AF.[9, 10] This is also reflected in the current AF risk algorithms such as the CHA_2DS_2VASc score.[3] Despite the advances in the treatment of AF, women are more prone to AF recurrences compared to men.[6, 10] While the exact mechanism for these sex differences remains to be elucidated, the importance of structural, electrophysiological and hormonal factors have been proposed.[6] Data regarding sex differences in the mechanisms of AF is scarce and a better understanding is important both for prognostic purposes and the optimization of therapeutic approaches.

Based on a thorough electronic literature search conducted using PubMed, this review evaluates what is known about sex differences in AF mechanisms and underscores areas of knowledge gaps for future studies.

2. Epidemiology: Sex differences in AF prevalence, age of onset, clinical presentation and co-morbidities

Atrial fibrillation (AF) is more common in men (0.06%) than in women (0.04%).[11] In observational studies in Western countries, women have 30-50% lower age-adjusted incidence and prevalence of AF, indicating that the substrate for AF develops less readily in women. In East/Asian countries, overall AF prevalence is lower than in Western countries for which reports regarding sex distribution have been less consistent varying from equal to lower prevalence in

women than in men.[12] In general, AF incidence increases with age for both sexes. Given the higher life expectancy of women, the absolute numbers and lifetime risk (~23%) for AF are similar in both sexes.[13] The prevailing mechanisms predisposing to AF, however, differ between men and women (Table 1), as highlighted in the following chapters.

Women are more likely to present with persistent AF and atypical symptoms (weakness and fatigue) and to report a worse quality of life than men.[5] Although asymptomatic AF is less common among women,[14] atypical symptoms may delay diagnosis and therapy and may contribute to the worse outcomes seen in women.[15]

2.1 Sex differences in AF subtypes

2.1.1 Idiopathic AF and genetic AF

Genetic predisposition has been attributed to individuals with familial aggregation of early-onset idiopathic AF, but data on sex-specific differences in idiopathic AF have been controversial.[16] Mothers of patients with AF or atrial flutter and women with ≥2 affected siblings have higher AF risk than their male counterparts.[17] Similarly, a small study observed that more women had familial idiopathic AF.[18] This is in contrast to a large registry, which showed that men with a 1st degree relative with idiopathic AF have 37% higher AF risk than women.[19] The genetic basis of these differences is not known, but X-linked variants of the protective *KCNE5* gene and a deletion in the *EMD* gene have been proposed as contributing factors.[20, 21]

2.1.2 Exercise-associated AF and autonomic AF

Pronounced sex differences exist in the connection between exercise and AF: Moderate exercise reduces the risk of AF in women (-8.6%) and intense exercise is able to reduce AF risk in women even more by 30%. In male patients, moderate exercise also reduces AF risk. Strong exercise, however, increases the risk for AF in men, pointing towards a U-shape relation in men.[22-26] Excessive endurance sports (e.g., more than 1500 hours of sports/year) increases the

risk of AF three times in men. These patients also have higher recurrence rates after pulmonary vein isolation.[27-29]

Male athletes show more marked concentric ventricular and atrial remodeling with altered diastolic function, which associates with a higher blood pressure during exercise as well as a higher sympathetic tone compared to female athletes.[30] This atrial remodeling might be a reason for the higher rate of AF in sportsmen.[30] In addition, studies in rat models revealed that long-term endurance exercise causes a combination of structural remodeling and (central and end-organ) vagal enhancement,[31] which may further promote reentry by causing spatially heterogeneous shortening of atrial refractoriness.[32] Indeed, despite the observation of an overall higher sympathetic tone during exercise,[30] in (male) athletes, paroxysmal AF occurs three times more often in situations of high vagal tone (such as rest, sleep or postprandial) than in non-athletes.[33] In line with these observations, a low heart rate has generally been associated with an increased risk for AF.[34]

While in young male athletes, these vagal triggers predominate; in post-menopausal women an increased predominant sympathetic tone is observed.[35, 36] This increased sympathetic/parasympathetic balance has generally been associated with a high risk of AF[34] and is particularly often associated with AF in organic heart disease and post-cardiac surgeries.[37]

2.1.3 Co-morbidity-associated AF

Women often develop AF at an older age than men.[38] In the Framingham Heart Study, for example, 74% of women with AF were aged ≥70 years compared to only 58% of men, strongly suggesting that more comorbidity may be present and possibly causative in women with AF[1] (Table 1).

All classic cardiovascular risk factors, except for diabetes, are predictive of AF in both sexes. High body mass index has been shown to be the strongest predictor for AF with a higher hazard

ratio in men than in women. Moderate alcohol intake increases the risk for AF only in men, while high alcohol intake is associated with a heightened AF risk in both sexes [39, 40]. No sex specific differences have been consistently demonstrated for other risk factors such as hyperthyroidism.[24, 38, 40-42]

Several comorbidities, however, are differently associated with AF in men and women: Women with heart failure have a 14-fold risk and men a 8.5-fold risk of developing AF.[43] In addition, AF is an independent risk factor for new onset heart failure with preserved ejection fraction in women but not in men.[44] Although over the past 50 years the prevalence of valvular heart disease has generally decreased in women with AF in high income countries, there is still a higher prevalence of valvular heart disease and hypertension and lower prevalence of coronary heart disease in women than in men with AF.[1, 8, 45]

2.2 Sex hormone effects on AF

2.2.1 AF in menopausal and postmenopausal women

The incidence of AF in premenopausal women is low but the incidence increases after menopause particularly at ages over 50 years, suggesting a beneficial effect of estrogen and/or a harmful effect of postmenopausal hormonal changes - such as the pronounced decrease in estrogen - with regards to the development of AF.[46] With the decrease of estrogen levels during menopause, blood pressure, LDL cholesterol, metabolic syndrome and body mass index increase. All these effects are well established risk factors for developing AF[47] and their increase after menopause may explain the partial catch up in the incidence of AF in post-menopausal women (Table 1).

In line with these observations, there is evidence for a higher incidence of AF in patients undergoing anti-estrogen treatment and for a lower risk of AF with (estrogen-based) hormonal replacement therapy, albeit published data are conflicting.[48, 49 50] While estradiol was shown to reduce the risk for AF,[51] conjugated estrogens alone have been reported to increase AF risk

(two-fold compared to estradiol)[51, 52] and combined estrogen-progesterone based hormonal replacement therapy had either no effect[52] or decreased AF incidence,[49] suggesting a complex interaction and an impact of the hormonal preparation and its estrogen-receptor specificity.

2.2.2 AF during pregnancy and postpartum

Observational studies suggest that pregnancy may exacerbate supraventricular tachycardias in general.[53] Prevalence of AF in pregnant women, however, is very low (0.05%)[54] and is generally limited to patients with structural heart disease, in which AF incidence is a little higher (1.3%).[55] Nevertheless, several electrical and electro-mechanical changes have been observed during pregnancy such as an increase in P wave duration and dispersion and in atrial electro-mechanical coupling interval measured by tissue doppler[56] that are well known markers for increased AF incidence in normal hearts. As these observations were made in patients with preeclampsia, however, it is unclear whether these are alterations normally occurring during pregnancy or being simply associated with the vascular/atrial susceptibility of an abnormal pregnancy. AF during the peripartum period may occur mainly due to drug therapy such as terbutaline during tocolysis[57, 58] and also as an expression of peripartum cardiomyopathy.[59]

2.2.3 AF and testosterone

The epidemiological data on the link between AF and testosterone is conflicting. Data from the Framingham study show an association between AF incidence and reduced total testosterone levels in men aged 55 years and above, with the strongest association seen in men ≥ 80 years of age, with a 3.5-fold increase in AF risk for every standard deviation reduction in testosterone levels.[60] Similarly, a smaller cross-sectional study demonstrated a similar association between reduced testosterone levels and lone AF.[61] By contrast, the Multi-Ethnic Study of Atherosclerosis study showed that higher levels of endogenous bioavailable testosterone

seemed to contribute to AF development.[62] The differences in findings may in part be due to methodological differences in measuring testosterone (total vs. bioavailable testosterone) but may also relate to competing mechanisms of direct and indirect testosterone effects.

The data on the effects of testosterone replacement are equally conflicting. In a recent study on >76,000 individuals, normalization of testosterone levels with replacement therapy was associated with a decreased incidence of AF.[63] However, preclinical studies have shown the opposite effect, with testosterone replacement increasing arrhythmogenesis in pulmonary veins and the left atrium, probably by enhancing adrenergic activity.[64]

3. Mechanisms underlying sex differences in AF

3.1 Sex differences in electrophysiological properties of atria

3.1.1 Sex differences in electrophysiology and calcium handling

Experimental models show sex differences in the electrophysiology of the left atrium. In male mice, pulmonary veins (PV) have a higher spontaneous beating rate, increased burst firing and more delayed afterdepolarisations (57 vs. 16%).[65] Additionally, male mice have slower sino-atrial impulse-generation activity. Thus, spontaneously higher PV beating rates can compete with sino-atrial activity leading to arrhythmias. Sex differences in arrhythmogenesis can also be explained by differences in calcium and sodium channel regulation.[66] Mouse studies have shown that late sodium current, calcium transients and sarcoplasmic reticulum calcium contents of the posterior wall of the left atrium were greater in male cardiomyocytes than in females, which may contribute to increased ectopic activity.[66] Interestingly, no differences were found in the right atrium.

In contrast, a clinical study in women undergoing AVNRT ablation showed shorter atrial effective refractory period (AERP) in women rather than longer.[67] In a small cohort of patients that underwent AF ablation[68] these sex differences in AERP were not confirmed, likely due to

some electrical (and structural) remodelling present in these patients with paroxysmal AF. This small study suggested that the prevalence of non-PVI triggers was significantly more frequent in women than in men (16 vs. 8.4%).

3.1.2 Sex hormone effects on electrophysiology and calcium handling

Cardiac myocytes express estrogen and androgene receptors, strongly suggesting that sex hormones may directly affect ion channels and their expression.[69, 70]

Most research has focused on sex hormone effects on ion channels/currents expressed in atria and ventricles. Testosterone increases repolarizing I_{Kr}, I_{K1}, and I_{Ks},[71, 72] and acutely reduces $I_{Ca,L}$.[73] Estrogen exerts complex effects on I_{Kr}:[74] it blocks I_{Kr} directly[75] but may also increase I_{Kr} by promoting HERG trafficking.[76] In addition, estrogen reduces I_{Ks} by reducing its beta-subunit KCNE1,[77] reduces I_{to},[78] and increases $I_{Ca,L}$.[79] These alterations of ion currents result in a net estrogen-induced prolongation of action potential duration (APD) and QT interval and a net testosterone-induced shortening of APD/QT (Figure 1).[80] The shortened APD in male atria may be pro-arrhythmic by facilitating reentry, while the longer APD in female atria may exert anti-arrhythmic effects relating to AF (contrasting with its pro-arrhythmic effects in the ventricles). As most studies on hormone effects on ion currents were performed in ventricular cardiomyocytes, the transferability to the atrial electrical phenotype needs confirmation.

Only few data are available on sex hormone effects on "atrial" ion channels/currents and most were derived from non-cardiac tissue also expressing these channels. During pregnancy (with high estrogen levels), I_f current densities and automaticity are increased in mice.[81] Estrogen upregulates Ca^{2+}-activated small conductance potassium channels (SK3) in colonic smooth muscle cells[82] and downregulates two-pore domain K^+ channels (TASK-1) in neural cells (Figure 1).[83] A likewise estrogen-induced reduction of TASK-1 in the atria would prolong the atrial APD and exert pro-arrhythmic effects, as demonstrated in patients with lone AF harboring loss-of-function mutations in KCNK3/TASK-1.[84] As SK expression has been demonstrated to be

particularly high in the pulmonary veins (as compared to the rest of the atria),[85] estrogen-induced changes in SK expression might modulate triggered activity from the pulmonary veins. Whether similar estrogen-effects are indeed observed in the atria, however, needs to be assessed.

Sex hormone effects on calcium handling properties can also contribute to atrial arrhythmogenesis: Estrogen increases the propensity for triggered activity by increasing $I_{Ca,L}$,[79] NCX activity,[86] and RyR2 leakiness.[87] Testosterone in contrast reduces triggered activity by increasing SERCA activity,[79, 88] and decreasing $I_{Ca,L}$ (Figure 1).[73] In line with these findings, testosterone deficiency facilitates atrial arrhythmia by reducing binding of FKBP12.6 to RyR2 resulting in increased calcium leakage.[89]

3.1.3 Sex differences in electrical remodelling

There is little direct evidence on whether sex differences in atrial electrical remodelling play a role in sex-differences in AF risk. Biochemical and histological analysis of atrial tissue obtained during cardiac surgery showed that remodelling-induced changes of connexins and collagen in AF are broadly similar between men and women, though women exhibited somewhat stronger AF-induced increase in Cx40.[90] Indirect evidence indicating that sex may be an important determinant of the degree of electrical remodelling in the left atrium comes from a study on heart failure patients. Analysis of mRNA expression of genes encoding for ion channel subunits important in cardiac conduction and arrhythmogenesis in left atria of explanted human hearts showed differential remodelling between sexes, with lower expression levels in transcripts encoding for K(v)4.3, KChIP2, K(v)1.5, and K(ir)3.1 in the failing female left atrium as compared with the male left atrium.[91] Differential electrical remodelling between sexes was also seen in a study in rabbits on left ventricular hypertrophy with less pronounced APD prolongation in left ventricles in females than in males leading to longer APD in males than females - thus reversing the sex differences observed at baseline.[92] Whether similar sex differences in remodelling also

occur in the atria remains to be investigated.

3.2 Sex differences in structure and function of atria

3.2.1 Sex differences in atrial anatomy, structure and function

Morphological, structural and functional sex differences in the atria have been described in association with AF. Healthy women have smaller maximal left atrial volumes (89±21 ml vs. 103±30 ml), smaller left atrial anteroposterior diameter, and lower left atrial stroke volumes (48±15 ml vs. 58±23 ml).[93, 94] In addition, MRI analyses revealed sex differences in atrial mechanical function in healthy subjects, e.g. in atrial conduit and booster pump function.[95, 96] In contrast to the observations in healthy subjects, women referred for AF ablation usually have larger atria than men,[97] which may be partly due to the facts that these women were older, had a longer AF history, more hypertension and more valve disease than the men in the study.

Tissue fibrosis plays a major role in the development of AF and its progression to a persistent/permanent status. Sex differences regarding the degree of fibrosis in the different clinical variants of AF were demonstrated by histopathological studies and with MRI.[98] It is not clear, whether these sex differences in the extent of fibrosis with more pronounced fibrosis in women are mainly due to inherent differential expression of fibrosis-related genes and proteins or due to the age of men and women with AF (younger age in men with AF).[90]

A study looking at pulmonary vein sleeves from patients with and without longstanding persistent AF demonstrated increased fibrosis in females with AF.[90] These sex differences in fibrosis remodelling in longstanding persistent AF were mainly due to the inherent differential expression of fibrosis-related genes and proteins, with those related to the TGFβ/Smad3 pathway being up-regulated in females, suggesting sex-specific aggravation of fibrosis remodelling. There is also evidence to suggest that this more extensive left atrial structural remodelling leads to greater deterioration in left atrial appendage function in women with high calculated risk of stroke in AF compared with men.[99]

3.2.2 Sex hormone effects on atrial mechanical function

Data on sex hormone effects on atrial function were obtained in animal studies. In rats, androgens produce acute vasodilation, increase contractility and increase the sino-atrial recovery time by depressing spontaneous depolarization involved in atrial pace-making.[100] Androgens cause a larger response to inotropes in male atria via a postsynaptic increase in intracellular cAMP and independently of beta1-adrenoceptors.[101] In female mice though, estrogens are those that drive the sensitivity to catecholamine by down-regulating the beta1-receptors, depending on the estrous cycle.[102] In female sheep, in contrast, estrogen causes LV enlargement and increased stroke volume.[103]

Sex hormones also modify the response to volume overload and the secretion of atrial natriuretic peptide (ANP) as demonstrated in studies in rats. Estrogen increases the basal secretion, but does not influence the stretch-induced ANP secretion. By contrast, although testosterone does not affect basal secretion, it completely abolishes the stretch-induced increase in ANP secretion.[104]

3.2.3 Sex differences in structural remodelling of the heart the atria

Animal data suggests sex-specific differences in pathological remodelling. In mouse models of myocarditis as well as in models of isoproterenol-induced heart failure, fibrotic responses are generally more prominent in male mice, showing higher numbers of $TLR4^+$ $CD11b^+$ monocytes, neutrophils, mast cells and dendritic cells, and increased Th1 helper cell responses compared to females.[105] By contrast, protective Th2 responses, increased B cells, more inhibitory $Tim\text{-}3^+$ $CD4^+$ T cells, and more T regulatory cells dominate the picture in female animals.[105, 106] Consistent with these findings, intracardiac macrophages from male mice preferentially expressed iNOS, IL-12, TNFα, and CD16/32, markers associated with M1 activation,[107, 108] while

heart-infiltrating macrophages in females showed a M2 activation pattern including arginase 1, IL-10, and Mϕ-MR expression.

Fibrosis is an event that affects the atria as well as the ventricles, and several lines of evidence suggest that estrogens indeed play an important role in attenuating this process of adverse remodelling. Thus, it is not surprising that expression of fibrosis-related genes, mainly those related to the TGFβ/Smad3 pathway, is up-regulated in postmenopausal women with AF (Figure 2).[109] Nevertheless, the exact mechanisms through which sex modulates structural atrial remodelling still remain to be identified.[110]

3.3 Sex differences in cardiac autonomic modulation and neuro-humoral responses

3.3.1 Sex differences in cardiac autonomic activity

The autonomic nervous system (ANS) including the sympathetic and parasympathetic system and the intrinsic neuronal network - and its alterations - plays an important role in the pathogenesis of AF.[111-113] Both parts of the ANS are involved in the initiation and maintenance of AF. The role of the parasympathetic system in AF is mainly attributed to the shortening in APD and increased dispersion of refractoriness in the atrial myocardium facilitating initiation and maintenance of AF.[32] Vagal activation exerts these effects mostly via acetylcholine activated K^+ channels.[114] Sympathetic stimulation can also promote AF by increasing Ca^{2+} release; thereby causing afterdepolarisation formation as a trigger for AF (Figure 3).

When compared to men of the same age, women seem to have more dominant vagal tone indexed by measures of heart rate variability,[115, 116] although this sex difference disappears with aging (and consecutive changes in hormones).[117]

Sex hormones influence the autonomic tone. As the adrenergic tone influences conduction properties and refractoriness of cardiac tissue,[118] these hormone effects on the autonomic system may contribute to sex differences in the electrophysiological properties of the heart.[119] Low levels of estrogen and elevated levels of progesterone increase catecholamine levels and

there is higher sympathetic activity in the luteal phase of the menstrual cycle (which is characterized by low estrogen and high progesterone levels).[120, 121] Similarly, in post-menopausal women an increased predominant sympathetic tone is associated with reduced estrogen levels.[35, 36]

3.3.2 Sex differences in neuro-humoral responses

A complex nervous system controls the cardiovascular system, and affects the arterial pressure, heart rate and cardiac contractility. This neural control of the heart and vascular system is related to the sympatho-vagal balance and is regulated by vascular feedback. The renin-angiotensin system and natriuretic peptides, for example, contribute to the regulation of the cardiovascular system, partially acting as cardiac hormones.

Physiologically, women have a lower responsiveness of the mechanisms regulating arterial pressure.[122] In addition, women have higher values of natriuretic peptides.[123]

Studies evaluating sex differences in neuro-humoral control of the cardiovascular system in pathological conditions are scarce. Electrical and structural remodelling in atrial fibrillation does not seem to be mediated by changes in autonomic tone.[124] But neuro-humoral activation could be involved in differences of atrial fibrosis development via the renin-angiotensin-aldosterone system thus potentially contributing to differences in AF development.[125]

3.4 Sex differences in impact of co-morbidities

3.4.1 Sex differences in heart failure with preserved ejection fraction and endothelial dysfunction

Heart failure with preserved ejection fraction (HFpEF) and diastolic dysfunction are associated with a high prevalence of AF.[126] This has important implications, as AF is associated with poorer outcome in patients with HFpEF in general and women in particular.[127] Both, similar risk factors

for AF and HFpEF and the hemodynamic consequences of diastolic dysfunction at the atrial level likely play a causative role.[126] Importantly, it has been shown in different populations that women have an increased age-related risk of developing diastolic dysfunction or HFpEF as compared to men.[127-129] At the atrial level, this is reflected by a greater atrial functional decay with age in women, as measured by longitudinal strain rate.[130] The age-related higher risk of diastolic dysfunction in women suggests a relation with estrogen deficiency after menopause. Indeed, animal studies indicate a number of pathways, by which estrogen deficiency can modulate diastolic dysfunction, including enhanced cardiac remodeling, left ventricular hypertrophy and increased arterial stiffness.[38, 131, 132] In a large human population study, arterial stiffness leading to diastolic dysfunction has also been shown to be greater in women.[133] Despite the fact that hormone replacement therapy does not result in survival benefit in large studies, it does lead to improvement of diastolic function in postmenopausal women,[134, 135] which may contribute to the reduced AF incidence observed with estrogen replacement therapy. These studies confirm the role of estrogen deficiency in the etiology of diastolic dysfunction and HFpEF (Table 1).

Women also have a higher rate of microvascular disease compared to men. Most of these patients with microvascular disease are in the peri-menopausal age range (45-60 years), suggesting a similar relation with estrogen deficiency. Patients with microvascular disease are known to have a high prevalence of AF.[136] Interestingly, treatment with ranolazine, which exerts anti-anginal effects but also impacts on cardiac electrophysiology, can reduce the burden of AF in these patients.[136]

3.4.2 Sex differences in pro-inflammatory signaling, role of epicardial fat

Several lines of evidence suggest an association between epicardial fat and AF.[137] Epicardial fat correlates with a higher prevalence of AF, a progression to atrial fibrosis and permanent AF and even a higher recurrence rate after ablation.[138, 139] Moreover, the extent of epicardial atrial tissue

is associated with lower bipolar voltage and electrogram fractionation in electro-anatomic mapping during sinus rhythm.[140] It has been demonstrated that the secretome from human epicardial adipose tissue induces myocardial fibrosis through the secretion of adipo-fibrokines.[141,142] Tissue fibrosis reflects a chronic inflammatory process. In fact, accumulation of fat tissue triggers a chronic low-grade activation of the innate immune system. Epicardial adipocytes are able to release pro-inflammatory adipokines and activate the chemotactic monocyte chemoattractant protein-1 (MCP-1)/C-C chemokine receptor 2 (CCR2) pathways to promote inflammatory macrophage accumulation (Figure 2). The crosstalk between adipocytes and inflammatory cells depends on the release of cytokines (IL-1, IL-6 and TNF-α) by fat tissue macrophages.[141] Other pro-inflammatory adipokines like leptin and resistin are also associated with incident AF in women.[143] Menopausal hormonal changes are related to an increase in epicardial fat and metabolic syndrome incidence,[144] risk factors for AF development and prognosis.[145] Taken together, the association between increased epicardial fat and hormonal changes in postmenopausal women on one hand, as well as the causal link between epicardial fat, chronic inflammation, atrial fibrosis, and AF burden on the other hand, all point to a protective role of female sex hormones against AF development and progression (Figure 2).

4. Clinical implications for future therapies and research

4.1 How can we use the known mechanistic findings for future sex-specific diagnostic and therapeutic strategies?

Based on the mechanistic findings discussed above, several sex-specific therapeutic strategies might complement our current "general" treatment approaches. In the following, we will highlight a few. As many repolarizing ion currents are lower and APD prolonged in female atria and ventricles (section 3.1.2), class III drugs further prolonging APD might be anti-arrhythmic at the atrial level at lower dosages compared to men but carry an inherent more pronounced risk of

ventricular pro-arrhythmia in women even when used at low dose. Non-pulmonary triggered activity is more often found in women (section 3.1.1), suggesting that the success of AF ablation in women might be increased if other triggers apart from pulmonary vein triggers are additionally tackled. As women demonstrate more pronounced AF-associated fibrotic remodelling due to an upregulation of the TGFβ/Smad3 pathway (sections 3.2.1/3.2.3), antifibrotic drugs (such as angiotensin receptor blockers or spironolactone) might be beneficial in women - particularly if specifically targeting this pathway. Similarly, the fact that epicardial adipocyte infiltration and consecutive pro-inflammatory signalling increase in postmenopausal women (section 3.4.2) indicates that complementing classical anti-arrhythmic therapies with anti-inflammatory treatment approaches might be beneficial in older women. Generally, co-morbidities such as microvascular diseases or diastolic dysfunction are more often encountered in women with AF than in men (sections 2.1.3/3.4.1), suggesting that drugs affecting these co-morbidities might be particularly efficient in women, when added to classical anti-arrhythmic therapies. As estrogen has a beneficial / protective effect and the lack of estrogen after menopause a harmful effect on several factors predisposing to AF (Table 1), estrogen-based hormone replacement therapy in postmenopausal women may reduce AF incidence by reducing HFpEF and HFpEF-related AF, by impacting on electrical features and on structural remodeling and might therefore reduce AF burden in this population. However, the exact hormonal preparation might be important for anti-arrhythmic / protective effects as a reduced AF incidence has only been observed with estradiol and estrogen+progesterone but not with conjugated (equine) estrogens.[52]

4.2 What are current gaps of knowledge that we need to close to reach this aim?

As highlighted above, epidemiological and experimental evidence suggests that sex-specific differences in physiological, electrical and structural characteristics of the atria and, particularly, in pathological remodelling of cardiac tissue in atrial fibrillation exist. It is still unclear, however, a) to what extent sex differences are due to direct effects of female sex hormones, b) to what

extent they are mediated by male sex hormone effects, or c) whether they mainly reflect the lack of female sex hormones in a postmenopausal population of women causatively linked to changes in co-morbidities thereby indirectly impacting on AF risk. Epidemiologic data indeed point to a direct protective role for estrogens in this context, but mechanistic concepts are still based on observational reports and insights from animal studies. Here, more detailed mechanistic studies - ideally directly performed in human atrial tissue and cells - are warranted to increase our patho-physiological understanding and to reveal important pathways. This is mandatory to develop targeted, mechanism-based, pathway- and sex-specific therapies - either based on drugs, or on a combination of drugs with more specific ablation strategies.

Acknowledgements

The authors would like to thank Prof. Harry Crijns, Maastricht, NL and Prof. John Camm, London, UK for their thorough assessment and detailed comments on the manuscript.

References

1. Schnabel RB, Yin X, Gona P, Larson MG, Beiser AS, McManus DD, et al. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. *Lancet*. 2015;386:154-62.
2. Heeringa J. Atrial fibrillation: is the prevalence rising? *Europace*. 2010;12:451-2.
3. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Europace*. 2016;18:1609-1678.
4. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ and Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. *JAMA*. 1994;271:840-4.
5. Dagres N, Nieuwlaat R, Vardas PE, Andresen D, Levy S, Cobbe S, et al. Gender-related differences in presentation, treatment, and outcome of patients with atrial fibrillation in Europe: a report from the Euro Heart Survey on Atrial Fibrillation. *Journal of the American College of Cardiology*. 2007;49:572-7.
6. Volgman AS, Manankil MF, Mookherjee D and Trohman RG. Women with atrial fibrillation: Greater risk, less attention. *Gend Med*. 2009;6:419-32.

7. Nattel S and Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *Journal of the American College of Cardiology*. 2014;63:2335-45.
8. Ball J, Carrington MJ, Wood KA, Stewart S and Investigators S. Women versus men with chronic atrial fibrillation: insights from the Standard versus Atrial Fibrillation spEcific managemenT studY (SAFETY). *PLoS One*. 2013;8:e65795.
9. Scheuermeyer FX, Mackay M, Christenson J, Grafstein E, Pourvali R, Heslop C, et al. There Are Sex Differences in the Demographics and Risk Profiles of Emergency Department (ED) Patients With Atrial Fibrillation and Flutter, but no Apparent Differences in ED Management or Outcomes. *Acad Emerg Med*. 2015;22:1067-75.
10. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB and Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation*. 1998;98:946-52.
11. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. *Circulation*. 2014;129:837-47.
12. Ko D, Rahman F, Schnabel RB, Yin X, Benjamin EJ and Christophersen IE. Atrial fibrillation in women: epidemiology, pathophysiology, presentation, and prognosis. *Nature reviews Cardiology*. 2016;13:321-32.
13. Lloyd-Jones DM, Wang TJ, Leip EP, Larson MG, Levy D, Vasan RS, et al. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. *Circulation*. 2004;110:1042-6.
14. Xiong Q, Proietti M, Senoo K and Lip GY. Asymptomatic versus symptomatic atrial fibrillation: A systematic review of age/gender differences and cardiovascular outcomes. *International journal of cardiology*. 2015;191:172-7.
15. Paquette M, Roy D, Talajic M, Newman D, Couturier A, Yang C et al. Role of gender and personality on quality-of-life impairment in intermittent atrial fibrillation. *The American journal of cardiology*. 2000;86:764-8.
16. Ellinor PT, Yoerger DM, Ruskin JN and MacRae CA. Familial aggregation in lone atrial fibrillation. *Human genetics*. 2005;118:179-84.
17. Zoller B, Ohlsson H, Sundquist J and Sundquist K. High familial risk of atrial fibrillation/atrial flutter in multiplex families: a nationwide family study in Sweden. *Journal of the American Heart Association*. 2012;2:e003384.
18. Chen LY, Herron KJ, Tai BC and Olson TM. Lone atrial fibrillation: influence of familial disease on gender predilection. *Journal of cardiovascular electrophysiology*. 2008;19:802-6.
19. Oyen N, Ranthe MF, Carstensen L, Boyd HA, Olesen MS, Olesen SP, et al. Familial aggregation of lone atrial fibrillation in young persons. *Journal of the American College of Cardiology*. 2012;60:917-21.
20. Karst ML, Herron KJ and Olson TM. X-linked nonsyndromic sinus node dysfunction and atrial fibrillation caused by emerin mutation. *Journal of cardiovascular electrophysiology*. 2008;19:510-5.
21. Ravn LS, Hofman-Bang J, Dixen U, Larsen SO, Jensen G, Haunso S, et al. Relation of 97T polymorphism in KCNE5 to risk of atrial fibrillation. *The American journal of cardiology*. 2005;96:405-7.
22. Mohanty S, Mohanty P, Tamaki M, Natale V, Gianni C, Trivedi C, et al. Differential Association of Exercise Intensity With Risk of Atrial Fibrillation in Men and Women: Evidence from a Meta-Analysis. *J Cardiovasc Electrophysiol*. 2016;27:1021-9.
23. Drca N, Wolk A, Jensen-Urstad M and Larsson SC. Physical activity is associated with a reduced risk of atrial fibrillation in middle-aged and elderly women. *Heart (British Cardiac Society)*. 2015;101:1627-30.
24. Ofman P, Khawaja O, Rahilly-Tierney CR, Peralta A, Hoffmeister P, Reynolds MR, et al. Regular physical activity and risk of atrial fibrillation: a systematic review and meta-analysis. *Circ Arrhythm Electrophysiol*. 2013;6:252-6.

25. Everett BM, Conen D, Buring JE, Moorthy MV, Lee IM and Albert CM. Physical activity and the risk of incident atrial fibrillation in women. *Circ Cardiovasc Qual Outcomes*. 2011;4:321-7.
26. Mozaffarian D, Furberg CD, Psaty BM and Siscovick D. Physical activity and incidence of atrial fibrillation in older adults: the cardiovascular health study. *Circulation*. 2008;118:800-7.
27. Elosua R, Arquer A, Mont L, Sambola A, Molina L, Garcia-Moran E, et al. Sport practice and the risk of lone atrial fibrillation: a case-control study. *International journal of cardiology*. 2006;108:332-7.
28. Karjalainen J, Kujala UM, Kaprio J, Sarna S and Viitasalo M. Lone atrial fibrillation in vigorously exercising middle aged men: case-control study. *Bmj*. 1998;316:1784-5.
29. Heidbuchel H, Anne W, Willems R, Adriaenssens B, Van de Werf F and Ector H. Endurance sports is a risk factor for atrial fibrillation after ablation for atrial flutter. *International journal of cardiology*. 2006;107:67-72.
30. Wilhelm M, Roten L, Tanner H, Wilhelm I, Schmid JP and Saner H. Gender differences of atrial and ventricular remodeling and autonomic tone in nonelite athletes. *The American journal of cardiology*. 2011;108:1489-95.
31. Guasch E, Benito B, Qi X, Cifelli C, Naud P, Shi Y, et al. Atrial fibrillation promotion by endurance exercise: demonstration and mechanistic exploration in an animal model. *Journal of the American College of Cardiology*. 2013;62:68-77.
32. Oberhauser V, Schwertfeger E, Rutz T, Beyersdorf F and Rump LC. Acetylcholine release in human heart atrium: influence of muscarinic autoreceptors, diabetes, and age. *Circulation*. 2001;103:1638-43.
33. Mont L, Sambola A, Brugada J, Vacca M, Marrugat J, Elosua R, et al. Long-lasting sport practice and lone atrial fibrillation. *Eur Heart J*. 2002;23:477-82.
34. Agarwal SK, Norby FL, Whitsel EA, Soliman EZ, Chen LY, Loehr LR, et al. Cardiac Autonomic Dysfunction and Incidence of Atrial Fibrillation: Results From 20 Years Follow-Up. *Journal of the American College of Cardiology*. 2017;69:291-299.
35. Lavi S, Nevo O, Thaler I, Rosenfeld R, Dayan L, Hirshoren N, et al. Effect of aging on the cardiovascular regulatory systems in healthy women. *American journal of physiology Regulatory, integrative and comparative physiology*. 2007;292:R788-93.
36. Gautam S, Shankar N, Tandon OP and Goel N. Comparison of cardiac autonomic functions among postmenopausal women with and without hormone replacement therapy, and premenopausal women. *Indian journal of physiology and pharmacology*. 2011;55:297-303.
37. Bauernschmitt R, Malberg H, Wessel N, Brockmann G, Wildhirt SM, Kopp B, et al. Autonomic control in patients experiencing atrial fibrillation after cardiac surgery. *Pacing and clinical electrophysiology : PACE*. 2007;30:77-84.
38. Magnussen C, Niiranen TJ, Ojeda FM, Gianfagna F, Blankenberg S, Njolstad I, et al. Sex Differences and Similarities in Atrial Fibrillation Epidemiology, Risk Factors, and Mortality in Community Cohorts: Results From the BiomarCaRE Consortium (Biomarker for Cardiovascular Risk Assessment in Europe). *Circulation*. 2017;136:1588-1597.
39. Gallagher C, Hendriks JML, Elliott AD, Wong CX, Rangnekar G, Middeldorp ME, et al. Alcohol and incident atrial fibrillation - A systematic review and meta-analysis. *International journal of cardiology*. 2017;246:46-52.
40. Larsson SC, Drca N and Wolk A. Alcohol consumption and risk of atrial fibrillation: a prospective study and dose-response meta-analysis. *Journal of the American College of Cardiology*. 2014;64:281-9.
41. Selmer C, Olesen JB, Hansen ML, Lindhardsen J, Olsen AM, Madsen JC, et al. The spectrum of thyroid disease and risk of new onset atrial fibrillation: a large population cohort study. *Bmj*. 2012;345:e7895.
42. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, et al. Inflammation as a risk factor for atrial fibrillation. *Circulation*. 2003;108:3006-10.

43. Wolbrette DL. Risk of proarrhythmia with class III antiarrhythmic agents: sex-based differences and other issues. *The American journal of cardiology*. 2003;91:39d-44d.
44. Meyer S, Brouwers FP, Voors AA, Hillege HL, de Boer RA, Gansevoort RT, et al. Sex differences in new-onset heart failure. *Clin Res Cardiol*. 2015;104:342-50.
45. Potpara TS, Marinkovic JM, Polovina MM, Stankovic GR, Seferovic PM, Ostojic MC et al. Gender-related differences in presentation, treatment and long-term outcome in patients with first-diagnosed atrial fibrillation and structurally normal heart: the Belgrade atrial fibrillation study. *International journal of cardiology*. 2012;161:39-44.
46. Ko D, Rahman F, Schnabel RB, Yin X, Benjamin EJ and Christophersen IE. Atrial fibrillation in women: epidemiology, pathophysiology, presentation, and prognosis. *Nat Rev Cardiol*. 2016;13:321-32.
47. Rossi R, Grimaldi T, Origliani G, Fantini G, Coppi F and Modena MG. Menopause and cardiovascular risk. *Pathophysiol Haemost Thromb*. 2002;32:325-8.
48. Perez MV, Wang PJ, Larson JC, Virnig BA, Cochrane B, Curb JD, et al. Effects of postmenopausal hormone therapy on incident atrial fibrillation: the Women's Health Initiative randomized controlled trials. *Circ Arrhythm Electrophysiol*. 2012;5:1108-16.
49. Bretler DM, Hansen PR, Lindhardsen J, Ahlehoff O, Andersson C, Jensen TB, et al. Hormone replacement therapy and risk of new-onset atrial fibrillation after myocardial infarction--a nationwide cohort study. *PLoS One*. 2012;7:e51580.
50. Magnani JW, Moser CB, Murabito JM, Nelson KP, Fontes JD, Lubitz SA, et al. Age of natural menopause and atrial fibrillation: the Framingham Heart Study. *Am Heart J*. 2012;163:729-34.
51. Tsai WC, Haung YB, Kuo HF, Tang WH, Hsu PC, Su HM, et al. Hormone replacement therapy and risk of atrial fibrillation in Taiwanese menopause women: A nationwide cohort study. *Scientific reports*. 2016;6:24132.
52. Wong JA, Rexrode KM, Sandhu RK, Moorthy MV, Conen D and Albert CM. Menopausal age, postmenopausal hormone therapy and incident atrial fibrillation. *Heart (British Cardiac Society)*. 2017;103:1954-1961.
53. Tawam M, Levine J, Mendelson M, Goldberger J, Dyer A and Kadish A. Effect of pregnancy on paroxysmal supraventricular tachycardia. *The American journal of cardiology*. 1993;72:838-40.
54. Lee MS, Chen W, Zhang Z, Duan L, Ng A, Spencer HT, et al. Atrial Fibrillation and Atrial Flutter in Pregnant Women-A Population-Based Study. *J Am Heart Assoc*. 2016;5:e003182.
55. Salam AM, Ertekin E, van Hagen IM, Al Suwaidi J, Ruys TPE, Johnson MR, et al. Atrial Fibrillation or Flutter During Pregnancy in Patients With Structural Heart Disease. *Data From the ROPAC (Registry on Pregnancy and Cardiac Disease)*. 2015;1:284-292.
56. Kirbas O, Biberoglu EH, Kirbas A, Daglar HK, Kurmus O, Uygur D et al. P-wave duration changes and dispersion in preeclampsia. *Eur J Obstet Gynecol Reprod Biol*. 2014;183:141-5.
57. Parasuraman R, Gandhi MM and Liversedge NH. Nifedipine tocolysis associated atrial fibrillation responds to DC cardioversion. *Bjog*. 2006;113:844-5.
58. Carson MP, Fisher AJ and Scorza WE. Atrial fibrillation in pregnancy associated with oral terbutaline. *Obstet Gynecol*. 2002;100:1096-7.
59. Ntusi NB, Badri M, Gumedze F, Sliwa K and Mayosi BM. Pregnancy-Associated Heart Failure: A Comparison of Clinical Presentation and Outcome between Hypertensive Heart Failure of Pregnancy and Idiopathic Peripartum Cardiomyopathy. *PLoS One*. 2015;10:e0133466.
60. Magnani JW, Moser CB, Murabito JM, Sullivan LM, Wang N, Ellinor PT, et al. Association of sex hormones, aging, and atrial fibrillation in men: the Framingham Heart Study. *Circ Arrhythm Electrophysiol*. 2014;7:307-312.
61. Lai J, Zhou D, Xia S, Shang Y, Want L, Zheng L et al. Reduced testosterone levels in males with lone atrial fibrillation. *Clinical cardiology*. 2009;32:43-46.

62. O'Neal WT, Nazarian S, Alonso A, Heckbert SR, Vaccarino V and Soliman EZ. Sex hormones and the risk of atrial fibrillation: The Multi-Ethnic Study of Atherosclerosis (MESA). *Endocrine*. 2017;58:91-96.
63. Sharma R, Oni OA, Gupta K, Sharma M, Sharma R, Singh V, et al. Normalization of Testosterone Levels After Testosterone Replacement Therapy Is Associated With Decreased Incidence of Atrial Fibrillation. *Journal of the American Heart Association*. 2017;6.
64. Tsai W-C, Lee T-I, Chen Y-C, Kao Y-H, Lu Y-Y, Lin Y-K, et al. Testosterone replacement increases aged pulmonary vein and left atrium arrhythmogenesis with enhanced adrenergic activity. *International journal of cardiology*. 2014;176:110-118.
65. Tsai WC, Chen YC, Lin YK, Chen SA and Chen YJ. Sex differences in the electrophysiological characteristics of pulmonary veins and left atrium and their clinical implication in atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2011;4:550-9.
66. Tsai WC, Chen YC, Kao YH, Lu YY, Chen SA and Chen YJ. Distinctive sodium and calcium regulation associated with sex differences in atrial electrophysiology of rabbits. *International journal of cardiology*. 2013;168:4658-66.
67. Suenari K, Hu YF, Tsao HM, Tai CT, Chiang CE, Lin YJ, et al. Gender differences in the clinical characteristics and atrioventricular nodal conduction properties in patients with atrioventricular nodal reentrant tachycardia. *J Cardiovasc Electrophysiol*. 2010;21:1114-9.
68. Takigawa M, Kuwahara T, Takahashi A, Watari Y, Okubo K, Takahashi Y, et al. Differences in catheter ablation of paroxysmal atrial fibrillation between males and females. *International journal of cardiology*. 2013;168:1984-91.
69. Goldstein J, Sites CK and Toth MJ. Progesterone stimulates cardiac muscle protein synthesis via receptor-dependent pathway. *Fertil Steril*. 2004;82:430-6.
70. Lizotte E, Grandy SA, Tremblay A, Allen BG and Fiset C. Expression, distribution and regulation of sex steroid hormone receptors in mouse heart. *Cell Physiol Biochem*. 2009;23:75-86.
71. Liu XK, Katchman A, Whitfield BH, Wan G, Janowski EM, et al. In vivo androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. *Cardiovasc Res*. 2003;57:28-36.
72. Furukawa T and Kurokawa J. Non-genomic regulation of cardiac ion channels by sex hormones. *Cardiovasc Hematol Disord Drug Targets*. 2008;8:245-51.
73. Er F, Michels G, Brandt MC, Khan I, Haase H, Eicks M, et al. Impact of testosterone on cardiac L-type calcium channels and Ca2+ sparks: acute actions antagonize chronic effects. *Cell Calcium*. 2007;41:467-77.
74. Odening KE. Another jigsaw piece in the complex picture of hormonal regulation of cardiac repolarization. *Eur Heart J*. 2016;37:651-3.
75. Kurokawa J, Tamagawa M, Harada N, Honda S, Bai CX, Nakaya H et al. Acute effects of oestrogen on the guinea pig and human IKr channels and drug-induced prolongation of cardiac repolarization. *J Physiol*. 2008;586:2961-73.
76. Anneken L, Baumann S, Vigneault P, Biliczki P, Friedrich C, Xiao L, et al. Estradiol regulates human QT-interval: acceleration of cardiac repolarization by enhanced KCNH2 membrane trafficking. *Eur Heart J*. 2016;37:640-50.
77. Drici MD, Burklow TR, Haridasse V, Glazer RI and Woosley RL. Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart. *Circulation*. 1996;94:1471-4.
78. El Gebeily G, El Khoury N, Mathieu S, Brouillette J and Fiset C. Estrogen regulation of the transient outward K(+) current involves estrogen receptor alpha in mouse heart. *Journal of molecular and cellular cardiology*. 2015;86:85-94.
79. Odening KE, Choi BR, Liu GX, Hartmann K, Ziv O, Chaves L, et al. Estradiol promotes sudden cardiac death in transgenic long QT type 2 rabbits while progesterone is protective. *Heart Rhythm*. 2012;9:823-32.

80. Odening KE and Koren G. How do sex hormones modify arrhythmogenesis in long QT syndrome? Sex hormone effects on arrhythmogenic substrate and triggered activity. *Heart Rhythm*. 2014;11:2107-15.
81. El Khoury N, Mathieu S, Marger L, Ross J, El Gebeily G, Ethier N et al. Upregulation of the hyperpolarization-activated current increases pacemaker activity of the sinoatrial node and heart rate during pregnancy in mice. *Circulation*. 2013;127:2009-20.
82. Tang YR, Yang WW, Wang Y, Gong YY, Jiang LQ and Lin L. Estrogen regulates the expression of small-conductance Ca-activated K+ channels in colonic smooth muscle cells. *Digestion*. 2015;91:187-96.
83. Hao X, Li X and Li X. 17beta-estradiol downregulated the expression of TASK-1 channels in mouse neuroblastoma N2A cells. *J Membr Biol*. 2014;247:273-9.
84. Liang B, Soka M, Christensen AH, Olesen MS, Larsen AP, Knop FK, et al. Genetic variation in the two-pore domain potassium channel, TASK-1, may contribute to an atrial substrate for arrhythmogenesis. *Journal of molecular and cellular cardiology*. 2014;67:69-76.
85. Qi XY, Diness JG, Brundel BJ, Zhou XB, Naud P, Wu CT, et al. Role of small-conductance calcium-activated potassium channels in atrial electrophysiology and fibrillation in the dog. *Circulation*. 2014;129:430-40.
86. Chen G, Yang X, Alber S, Shusterman V and Salama G. Regional genomic regulation of cardiac sodium-calcium exchanger by oestrogen. *J Physiol*. 2011;589:1061-80.
87. Yan S, Chen Y, Dong M, Song W, Belcher SM and Wang HS. Bisphenol A and 17beta-estradiol promote arrhythmia in the female heart via alteration of calcium handling. *PLoS One*. 2011;6:e25455.
88. Tsang S, Wong SS, Wu S, Kravtsov GM and Wong TM. Testosterone-augmented contractile responses to alpha1- and beta1-adrenoceptor stimulation are associated with increased activities of RyR, SERCA, and NCX in the heart. *Am J Physiol Cell Physiol*. 2009;296:C766-82.
89. Tsuneda T, Yamashita T, Kato T, Sekiguchi A, Sagara K, Sawada H, et al. Deficiency of testosterone associates with the substrate of atrial fibrillation in the rat model. *J Cardiovasc Electrophysiol*. 2009;20:1055-60.
90. Pfannmüller B, Boldt A, Reutemann A, Duerrschmidt N, Krabbes-Graube S, Mohr F-W and Dhein S. Gender-specific remodeling in atrial fibrillation? *The Thoracic and cardiovascular surgeon*. 2013;61:66-73.
91. Ambrosi CM, Yamada KA, Nerbonne JM and Efimov IR. Gender differences in electrophysiological gene expression in failing and non-failing human hearts. *PLoS ONE*. 2013;8:e54635.
92. Biagetti MO and Quinteiro RA. Gender differences in electrical remodeling and susceptibility to ventricular arrhythmias in rabbits with left ventricular hypertrophy. *HRTHM*. 2006;3:832-839.
93. Hudsmith LE, Petersen SE, Francis JM, Robson MD and Neubauer S. Normal human left and right ventricular and left atrial dimensions using steady state free precession magnetic resonance imaging. *J Cardiovasc Magn Reson*. 2005;7:775-82.
94. Nikitin NP, Witte KK, Thackray SD, Goodge LJ, Clark AL and Cleland JG. Effect of age and sex on left atrial morphology and function. *Eur J Echocardiogr*. 2003;4:36-42.
95. Maceira AM, Cosin-Sales J, Prasad SK and Pennell DJ. Characterization of left and right atrial function in healthy volunteers by cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2016;18:64.
96. Le Ven F, Bibeau K, De Larochelliere E, Tizon-Marcos H, Deneault-Bissonnette S, Pibarot P, et al. Cardiac morphology and function reference values derived from a large subset of healthy young Caucasian adults by magnetic resonance imaging. *Eur Heart J Cardiovasc Imaging*. 2016;17:981-90.

97. Forleo GB, Tondo C, De Luca L, Dello Russo A, Casella M, De Sanctis V, et al. Gender-related differences in catheter ablation of atrial fibrillation. *Europace*. 2007;9:613-20.
98. Cochet H, Mouries A, Nivet H, Sacher F, Derval N, Denis A, et al. Age, atrial fibrillation, and structural heart disease are the main determinants of left atrial fibrosis detected by delayed-enhanced magnetic resonance imaging in a general cardiology population. *J Cardiovasc Electrophysiol*. 2015;26:484-92.
99. Li Z, Wang Z, Yin Z, Zhang Y, Xue X, Han J, et al. Gender differences in fibrosis remodeling in patients with long-standing persistent atrial fibrillation. *Oncotarget*. 2017;8:53714-53729.
100. Sanchez M, Secades L, Bordallo C, Meana C, Rubin JM, Cantabrana B et al. Role of polyamines and cAMP-dependent mechanisms on 5alpha-dihydrotestosterone-elicited functional effects in isolated right atria of rat. *Journal of cardiovascular pharmacology*. 2009;54:310-8.
101. Velasco L, Sanchez M, Rubin JM, Hidalgo A, Bordallo C and Cantabrana B. Intracellular cAMP increases during the positive inotropism induced by androgens in isolated left atrium of rat. *European journal of pharmacology*. 2002;438:45-52.
102. Kocic I, Gruchala M and Petrusewicz J. Pretreatment of male guinea pigs by 17-beta-estradiol induces hypersensitivity of beta-adrenoceptors in electrically driven left atria. *International journal of cardiology*. 2008;129:22-5.
103. Giraud GD, Morton MJ, Davis LE, Paul MS and Thornburg KL. Estrogen-induced left ventricular chamber enlargement in ewes. *The American journal of physiology*. 1993;264:E490-6.
104. Deng Y and Kaufman S. The influence of reproductive hormones on ANF release by rat atria. *Life sciences*. 1993;53:689-96.
105. Frisancho-Kiss S, Davis SE, Nyland JF, Frisancho JA, Cihakova D, Barrett MA, et al. Cutting edge: cross-regulation by TLR4 and T cell Ig mucin-3 determines sex differences in inflammatory heart disease. *J Immunol*. 2007;178:6710-4.
106. Frisancho-Kiss S, Nyland JF, Davis SE, Barrett MA, Gatewood SJ, Njoku DB, et al. Cutting edge: T cell Ig mucin-3 reduces inflammatory heart disease by increasing CTLA-4 during innate immunity. *J Immunol*. 2006;176:6411-5.
107. Li K, Xu W, Guo Q, Jiang Z, Wang P, Yue Y et al. Differential macrophage polarization in male and female BALB/c mice infected with coxsackievirus B3 defines susceptibility to viral myocarditis. *Circ Res*. 2009;105:353-64.
108. Liu L, Yue Y and Xiong S. NK-derived IFN-γ/IL-4 triggers the sexually disparate polarization of macrophages in CVB3-induced myocarditis. *J Mol Cell Cardiol*. 2014;76:15-25.
109. Li Z, Wang Z, Yin Z, Zhang Y, Xue X, Han J, et al. Gender differences in fibrosis remodeling in patients with long-standing persistent atrial fibrillation. *Oncotarget*. 2017;8:53714-53729.
110. Akoum N, Mahnkopf C, Kholmovski EG, Brachmann J and Marrouche NF. Age and sex differences in atrial fibrosis among patients with atrial fibrillation. *Europace*. 2017.
111. Coumel P. Paroxysmal atrial fibrillation: a disorder of autonomic tone? *Eur Heart J*. 1994;15 Suppl A:9-16.
112. Hirose M, Carlson MD and Laurita KR. Cellular mechanisms of vagally mediated atrial tachyarrhythmia in isolated arterially perfused canine right atria. *J Cardiovasc Electrophysiol*. 2002;13:918-26.
113. Chen PS, Chen LS, Fishbein MC, Lin SF and Nattel S. Role of the autonomic nervous system in atrial fibrillation: pathophysiology and therapy. *Circ Res*. 2014;114:1500-15.
114. Harvey RD and Belevych AE. Muscarinic regulation of cardiac ion channels. *Br J Pharmacol*. 2003;139:1074-84.

115. Evans JM, Ziegler MG, Patwardhan AR, Ott JB, Kim CS, Leonelli FM et al. Gender differences in autonomic cardiovascular regulation: spectral, hormonal, and hemodynamic indexes. *J Appl Physiol (1985)*. 2001;91:2611-8.
116. Koenig J and Thayer JF. Sex differences in healthy human heart rate variability: A meta-analysis. *Neurosci Biobehav Rev*. 2016;64:288-310.
117. Jensen-Urstad K, Storck N, Bouvier F, Ericson M, Lindblad LE and Jensen-Urstad M. Heart rate variability in healthy subjects is related to age and gender. *Acta physiologica Scandinavica*. 1997;160:235-41.
118. Insulander P, Juhlin-Dannfelt A, Freyschuss U and Vallin H. Electrophysiologic effects of mental stress in healthy subjects: a comparison with epinephrine infusion. *J Electrocardiol*. 2003;36:301-9.
119. Larsen JA and Kadish AH. Effects of gender on cardiac arrhythmias. *J Cardiovasc Electrophysiol*. 1998;9:655-64.
120. Davidson L, Rouse IL, Vandongen R and Beilin LJ. Plasma noradrenaline and its relationship to plasma oestradiol in normal women during the menstrual cycle. *Clin Exp Pharmacol Physiol*. 1985;12:489-93.
121. Yildirir A, Kabakci G, Akgul E, Tokgozoglu L and Oto A. Effects of menstrual cycle on cardiac autonomic innervation as assessed by heart rate variability. *Ann Noninvasive Electrocardiol*. 2002;7:60-3.
122. Convertino VA. Gender differences in autonomic functions associated with blood pressure regulation. *The American journal of physiology*. 1998;275:R1909-20.
123. Wang TJ, Larson MG, Levy D, Leip EP, Benjamin EJ, Wilson PW, et al. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. *The American journal of cardiology*. 2002;90:254-8.
124. Wijffels MC, Kirchhof CJ, Dorland R, Power J and Allessie MA. Electrical remodeling due to atrial fibrillation in chronically instrumented conscious goats: roles of neurohumoral changes, ischemia, atrial stretch, and high rate of electrical activation. *Circulation*. 1997;96:3710-20.
125. Burstein B and Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. *Journal of the American College of Cardiology*. 2008;51:802-9.
126. Zakeri R, Chamberlain AM, Roger VL and Redfield MM. Temporal relationship and prognostic significance of atrial fibrillation in heart failure patients with preserved ejection fraction: a community-based study. *Circulation*. 2013;128:1085-93.
127. O'Neal WT, Sandesara P, Hammadah M, Venkatesh S, Samman-Tahhan A, Kelli HM et al. Gender Differences in the Risk of Adverse Outcomes in Patients With Atrial Fibrillation and Heart Failure With Preserved Ejection Fraction. *The American journal of cardiology*. 2017;119:1785-1790.
128. Ferreira RG, Nicoara A, Phillips-Bute BG, Daneshmand M, Muehlschlegel JD and Swaminathan M. Diastolic dysfunction in patients undergoing cardiac surgery: the role of gender and age-gender interaction. *J Cardiothorac Vasc Anesth*. 2014;28:626-30.
129. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL and Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006;355:251-9.
130. Liao JN, Chao TF, Kuo JY, Sung KT, Tsai JP, Lo CI, et al. Age, Sex, and Blood Pressure-Related Influences on Reference Values of Left Atrial Deformation and Mechanics From a Large-Scale Asian Population. *Circ Cardiovasc Imaging*. 2017;10.
131. Mori T, Kai H, Kajimoto H, Koga M, Kudo H, Takayama N, et al. Enhanced cardiac inflammation and fibrosis in ovariectomized hypertensive rats: a possible mechanism of diastolic dysfunction in postmenopausal women. *Hypertens Res*. 2011;34:496-502.
132. Pedram A, Razandi M, Lubahn D, Liu J, Vannan M and Levin ER. Estrogen inhibits cardiac hypertrophy: role of estrogen receptor-beta to inhibit calcineurin. *Endocrinology*. 2008;149:3361-9.

133. Russo C, Jin Z, Palmieri V, Homma S, Rundek T, Elkind MS, et al. Arterial stiffness and wave reflection: sex differences and relationship with left ventricular diastolic function. *Hypertension*. 2012;60:362-8.
134. Gokce M, Karahan B, Erdol C, Kasap H and Ozdemirci S. Left ventricular diastolic function assessment by tissue Doppler echocardiography in relation to hormonal replacement therapy in postmenopausal women with diastolic dysfunction. *Am J Ther*. 2003;10:104-11.
135. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML,et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *Jama*. 2002;288:321-33.
136. Yuriivna Osovska N and Vitaliivna Kuzminova N. Ranolazine in treatment of stable angina in woman with atrial fibrillation and intermittent left bundle branch block - a case report. *Pol Merkur Lekarski*. 2016;41:287-292.
137. Gaeta M, Bandera F, Tassinari F, Capasso L, Cargnelutti M, Pelissero G, et al. Is epicardial fat depot associated with atrial fibrillation? A systematic review and meta-analysis. *Europace*. 2017;19:747-752.
138. Wong CX, Sun MT, Odutayo A, Emdin CA, Mahajan R, Lau DH, et al. Associations of Epicardial, Abdominal, and Overall Adiposity With Atrial Fibrillation. *Circ Arrhythm Electrophysiol*. 2016;9.
139. Canpolat U, Aytemir K, Yorgun H, Asil S, Dural M and Ozer N. The Impact of Echocardiographic Epicardial Fat Thickness on Outcomes of Cryoballoon-Based Atrial Fibrillation Ablation. *Echocardiography*. 2016;33:821-9.
140. Zghaib T, Ipek EG, Zahid S, Balouch MA, Misra S, Ashikaga H, et al. Association of left atrial epicardial adipose tissue with electrogram bipolar voltage and fractionation: Electrophysiologic substrates for atrial fibrillation. *Heart Rhythm*. 2016;13:2333-2339.
141. Baker AR, Silva NF, Quinn DW, Harte AL, Pagano D, Bonser RS, et al. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol*. 2006;5:1.
142. Venteclef N, Guglielmi V, Balse E, Gaborit B, Cotillard A, Atassi F, et al. Human epicardial adipose tissue induces fibrosis of the atrial myocardium through the secretion of adipo-fibrokines. *Eur Heart J*. 2015;36:795-805a.
143. Ermakov S, Azarbal F, Stefanick ML, LaMonte MJ, Li W, Tharp KM, et al. The associations of leptin, adiponectin and resistin with incident atrial fibrillation in women. *Heart (British Cardiac Society)*. 2016;102:1354-62.
144. Keller KM and Howlett SE. Sex Differences in the Biology and Pathology of the Aging Heart. *Can J Cardiol*. 2016;32:1065-73.
145. Bernasochi GB, Boon WC, Curl CL, Varma U, Pepe S, Tare M, et al. Pericardial adipose and aromatase: A new translational target for aging, obesity and arrhythmogenesis? *Journal of molecular and cellular cardiology*. 2017;111:96-101.

Tables

Table 1: Sex differences in prevailing mechanisms / diseases predisposing to AF

	Men	**Women**
Prevailing risk factors / diseases predisposing to AF	Coronary heart disease and cardiovascular risk factors	Heart failure, particularly diastolic heart failure (HFpEF)
	Excessive sports (vagal AF)	Hypertension and left ventricular hypertrophy
		Valvular heart disease
	High BMI / metabolic disease (increased epicardial fat)	High BMI / metabolic disease / epicardial fat
	Potential pro-arrhythmic mechanisms increasing AF prevalence in men	**Potential anti-arrhythmic mechanisms reducing AF prevalence in pre-menopausal women**
Hormonal effects impacting on AF prevalence	Detrimental testosterone-effects on atherosclerosis / CAD	Beneficial estrogen-effects on cardiovascular risk factors
	Pro-arrhythmic testosterone-effects on atrial electrical features (shorter APD facilitating reentry)	Anti-arrhythmic estrogen-effects on atrial electrical features (longer atrial APD)
	More pronounced fibrotic remodeling in male animals (testosterone-effect?)	Beneficial estrogen-effects on structural remodeling (attenuation of fibrosis)
		Beneficial estrogen-effects on diastolic function
		Reduction of epicardial fat (by estrogen? indirect evidence: more epicardial fat in post-menopausal women)

Table legend:

AF, atrial fibrillation; HFpEF, heart failure with preserved ejection fraction, BMI, body mass index; CAD, coronary artery disease; APD, action potential duration

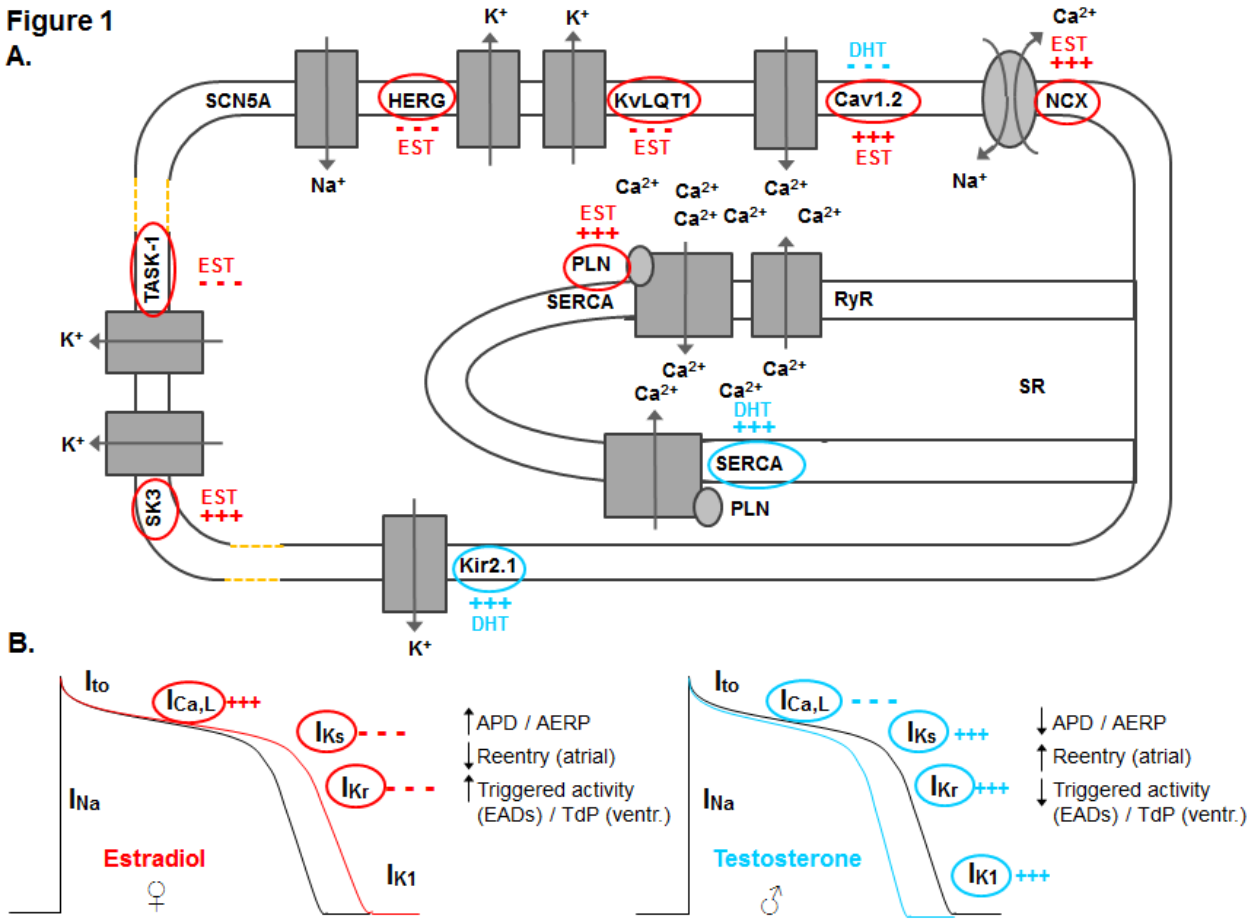

Figure 1:

A. Schematic figure indicating sex hormone effects on cardiac ion channels / currents and calcium handling proteins in cardiomyocytes. Effects on ion channels that have thus far only been demonstrated in non-cardiac tissues are indicated in the left corner separated by orange dotted lines. Estrogen-induced changes are colour-coded in red, testosterone-induced changes in light blue. **B.** Illustration of resulting effects on cardiac repolarization and arrhythmogenic mechanisms. ↑, indicates an increase/prolongation, ↓, indicates a decrease/abbreviation.

EST, estrogen; DHT, testosterone; + + +, increase; - - -, reduction; SR, sarcoplasmic reticulum; APD, action potential duration; AERP, atrial effective refractory period; EAD, early afterdepolarisation; TdP, Torsade-de-Pointes; HERG/IKr; KvLQT1/IKs, Kir2.1/IK1; SCN5A/INa; Cav2.1/ICa,L; SK3; TASK-1

Figure 2

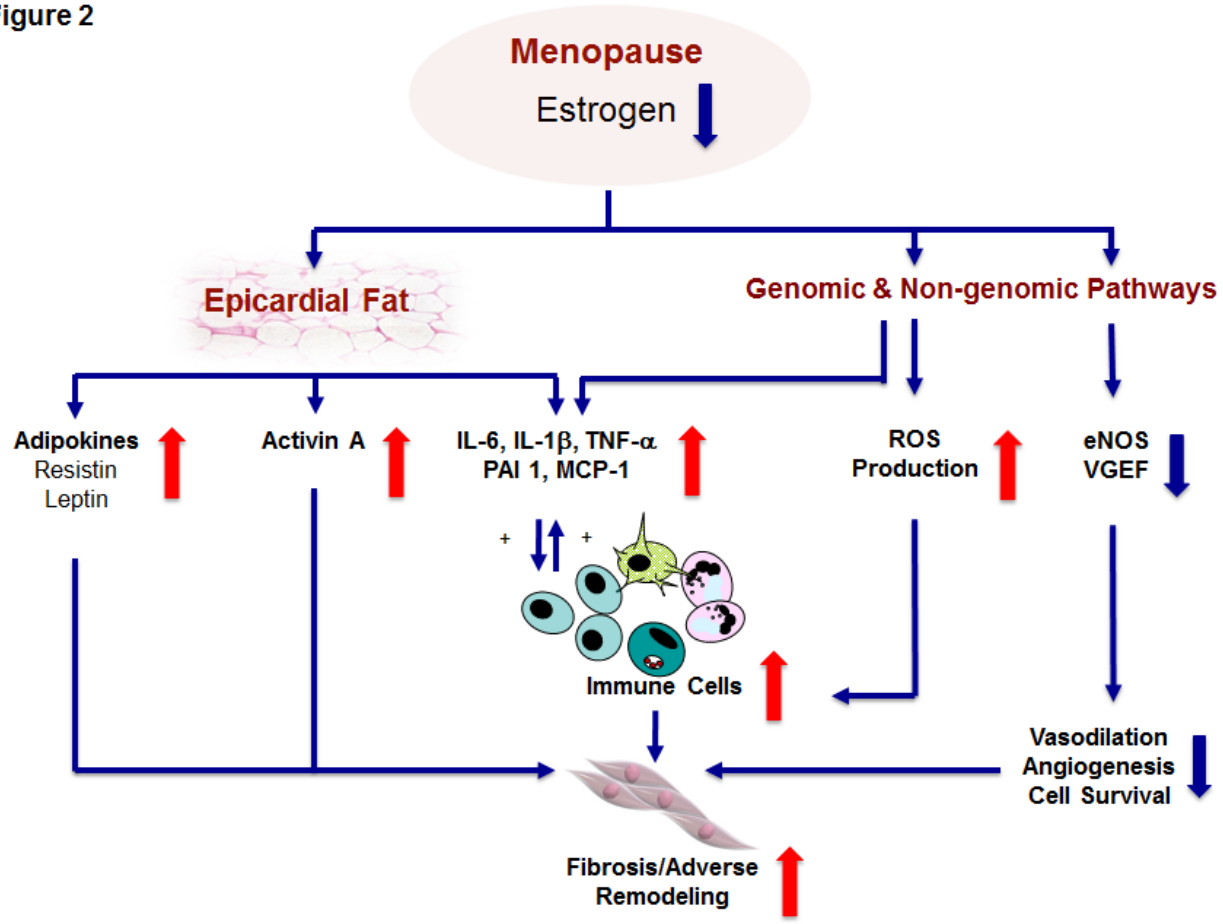

Figure 2:

Schematic figure indicating effects of menopause-associated reduction in estrogen on epicardial fat and related (pro-inflammatory) signalling pathways and fibrotic remodeling mechanisms.

↑, indicates an increase/activation, ↓, indicates a decrease; ROS, reactive oxygen species.

Figure 3

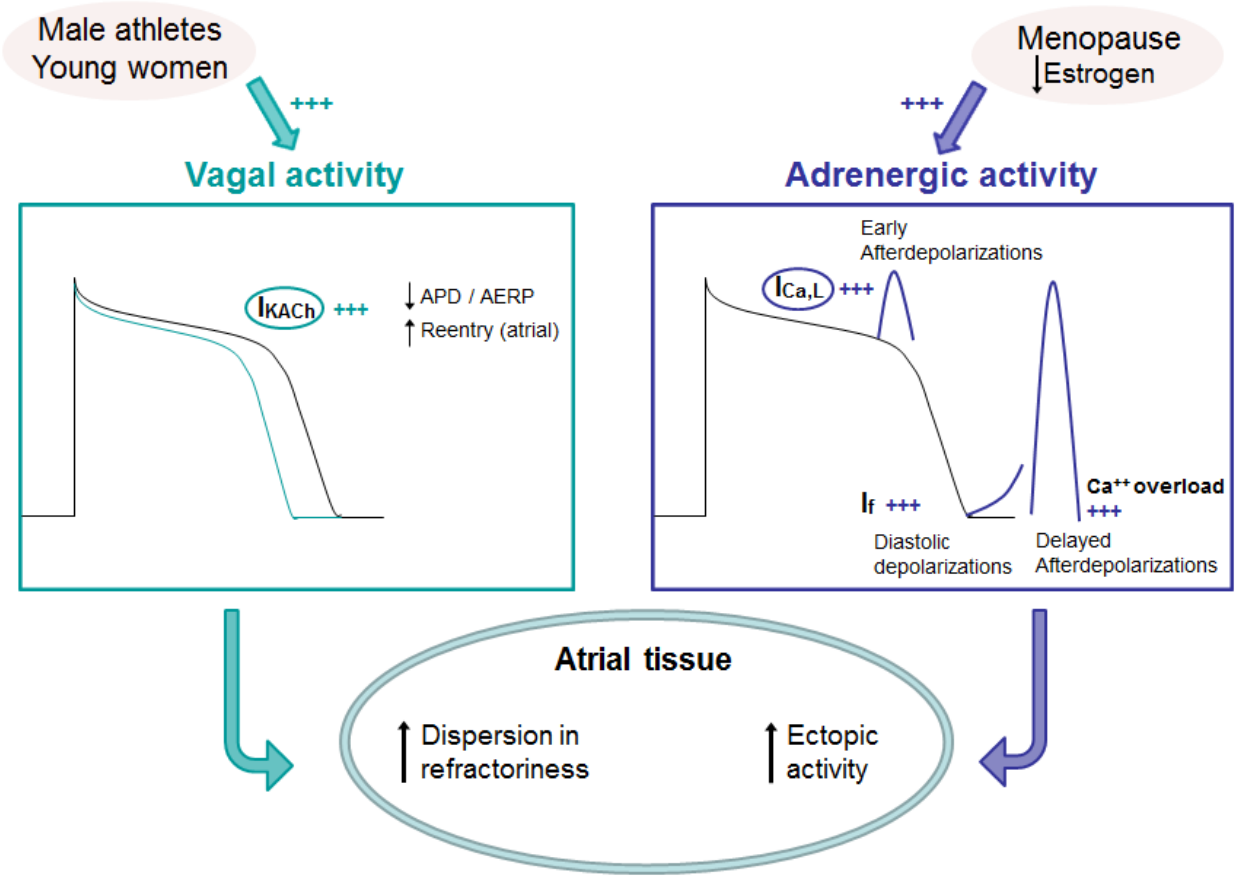

Figure 3:

Schematic figure indicating electrophysiological effects of vagal and sympathetic activity on action potential duration and triggered activity and their changes by endurance sport and menopause. + + +, increase; - - -, reduction; APD, action potential duration; AERP, atrial effective refractory period; ↑, indicates an increase/prolongation, ↓, indicates a decrease/abbreviation.