CHARLES UNIVERSITY IN PRAGUE

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SELECTED DIFFERENCES IN PATHOPHYSIOLOGY OF CARDIOVASCULAR SYSTEM IN WOMEN

Doctoral dissertation

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Selected differences in pathophysiology of cardiovascular system in women

Doctoral dissertation

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ABSTRACT

It has become increasingly apparent in recent years that there are important differences of many cardiovascular disorders including ventricular tachycardias in men and women. Gender differences have been observed in the epidemiology, pathogenesis and clinical presentation of various ventricular arrhythmias. For example, Brugada's syndrome is more common in men than in women, while idiopathic ventricular tachycardia from right ventricular outflow tract (RVOT-VT) or long QT syndrome are more common in women. Females are also more susceptible to drug-induced torsade de pointes (TdP) and in one recent study they were proved to have greater QTc prolongation than males following sotalol administration what can explain the higher incidence of drug-induced TdP seen in females. Significantly higher mortality rate and sudden death rate have been documented in male patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia than in female patients with the same mutation.

Physiological menopause occurs as a part of a woman's normal aging process being based on the natural cessation of estradiol and progesterone production by the ovaries. The dramatic fall in circulating estrogens levels at menopause impacts many tissues including cardiovascular system. During peri-menopausal period the effect of decreasing production of ovarian hormones is modulated by an increase in circulating follicle stimulating hormone and luteinizing hormone levels because of a feed-back stimulation of their secretion. Because the incidence of coronary heart disease (CHD) rises significantly after menopause, it has been hypothesized that women's CHD advantage before menopause (in comparison to men of the same age) could be due to the protective effects of estrogens. Based on this hypothesis many animal and observational studies were designed to elucidate the effect of postmenopausal hormone therapy (HT). However, controversial results have been reported since early nineties until today. While some studies found reduction in the incidence of CHD and in mortality from cardiovascular diseases some other studies failed to provide any evidence for an independent role of estradiol levels in determining CHD in postmenopausal women and some studies even found positive association of exogenous estradiol with the risk of CHD among women above 65 years of age.

Study 1: This study explored possible gender differences in electrophysiological characteristics and catheter ablation outcome in RVOT-VT patients. We have found that females suffering from RVOT-VT had shorter QRS duration, lower right ventricular voltage, and more low voltage zone in the RVOT free wall than males with the same diagnosis. Although the possible mechanisms are not clear, our findings suggest differences in ventricular remodeling between genders in patients with

idiopathic RVOT-VT. The RVOT free wall was the prominent region, which was not associated with different ventricular tachycardia (VT) incidences. Those findings suggest that ROVT low voltage might be the remodeling result after VT, rather than its cause. Regarding an effect of the ablation, the acute success rate, repeated catheter ablation rate and VT recurrence rate were similar in both genders.

Study 2: The aim of this study was to compare the responses of heart rate variability (HRV) with two different types of hormonal substitution therapy (HT) in post-menopausal women (cross-sectional study) and to reveal an effect of HT shortly after beginning of its administration (follow-up study). Significantly lower portion of the low frequency power (LF) was found in premenopausal women when compared to untreated postmenopausal women and men. Treatment by estrogen only was proved to decrease the LF% while no effect on HRV was observed in women treated with combination of estrogen and progesterone. Also the high frequency power (HF) was lower in postmenopausal women than in premenopausal women and women treated with estrogen only while in women treated with combined hormonal therapy the average value did not significantly differ from that of untreated postmenopausal women. The follow-up study also proved increase of HF already after two months of estrogen substitution therapy. These results suggest that higher vagal modulation of heart rate that seems typical for younger women becomes after menopause similar to that of men.

Key words : sex differences, menopausal, cardiovascular disease, pathophysiology of menopause, sex hormones, estrogen, progesterone, androgens, testosterone.

ABSTRAKT

V posledních letech byly prokázány významné rozdíly mezi muži a ženami ve výskytu kardiovaskulárních poruch včetně arytmií. Pohlavní rozdíly byly pozorovány v epidemiologii, patogenezi i klinických projevech různých arytmií. Tak například syndrom Brugadových je běžnější u mužů než u žen, zatímco idiopatická komorová tachykardie z pravého výtokového traktu (RVOT-VT) nebo syndrom dlouhého QT jsou častější u žen. Ženy jsou také citlivější na lékové podmíněné torsade de pointes (TdP) a nedávno zveřejněná studie prokázala větší prodloužení QTc intervalu u žen po podání sotalolu, což by mohlo vysvětlit zmíněnou vyšší incidenci lékové podmíněných TdP u žen. Významně vyšší úmrtnost byla naopak dokumentována u mužů s arytmogenní komorovou kardiomyopatií/dysplasií než u žen se stejnou mutací.

Menopauza je fyziologickou součástí procesu stárnutí u žen a je podmíněna přirozeným poklesem produkce estrogenu a progesteronu ve vaječnících. Dramatický pokles hladiny estrogenů v plasmě má vliv na mnoho tkání včetně kardiovaskulárního systému. Během peri-menopauzálního období je účinek klesající produkce ovariálních hormonů modulován zpětnovazebně podmíněnou zvýšenou sekrecí folikuly stimulujícího hormonu a luteinizačního hormonu. Protože incidence ischemické choroby srdeční (ICHS) významně stoupá po menopauze, byla vyslovena hypotéza, že nižší výskyt ICHS oproti mužům stejného věku je u žen před menopauzou dán protektivním účinkem estrogenů. Na základě této hypotézy byla provedena řada pokusů na zvířatech i observačních studií, které měly potvrdit účinnost postmenopauzální hormonální substituční terapie (HST). Avšak již od počátku devadesátých let byla publikována řada kontroverzních výsledků. Zatímco některé studie nalezly sníženou incidenci ICHS a

sníženou kardiovaskulární mortalitu, jiné studie neprokázaly žádný efekt estrogenové terapie na výskyt ICHS po menopauze a některé studie dokonce nalezly pozitivní korelaci mezi hladinou estradiolu a kardiovaskulární mortalitou u žen nad 65 let věku.

Studie 1: Tato studie zkoumala možné pohlavní rozdíly v elektrofyziologických charakteristikách a výsledcích katetrizační ablace u pacientů s RVOT-VT. Zjistili jsme, že ženy trpící RVOT-VT mají kratší QRS interval, nižší komorovou voltáž a více nízkovoltážních zón na volné stěně RVOT než muži se stejnou diagnózou. Ačkoliv mechanismus těchto rozdílů není jasný, naše výsledky ukazují pohlavní rozdíly v remodelaci komorového myokardu u pacientů s RVOT-VT. Volná stěna RVOT byla oblastí nápadnou tím, že nebyla v korelaci s rozdíly v incidenci komorové tachykardie. Z tohoto nálezu lze odvozovat, že nízké voltáže v této lokalitě jsou pravděpodobně spíše důsledkem než příčinou remodelace myokardu. Pokud se týká efektu katetrizační ablace, byl výskyt opakovaných ablací i výskyt rekurentních komorových tachykardií obdobný u obou pohlaví.

Studie 2: Cílem této studie bylo porovnat odpověď variability srdeční frekvence (HRV) na dva typy HST u postmenopauzálních žen a ukázat případný efekt HST krátce po začátku léčby. Signifikantně nižší výskyt spektrální síly v nízkofrekvenčním pásmu (LF) byl nalezen u léčených žen ve srovnání s neléčenými ženami a s muži stejného věku. HST léčba pouhým estrogenem byla v tomto směru účinná, zatímco žádný účinek HST na LF nebyl prokázán u kombinované HST estrogenem a progesteronem. Spektrální síla ve vysokofrekvenčním pásmu (HF) byla nižší u neléčených postmenopauzálních žen než u žen léčených estrogenovou HST a než u žen premenopauzálních, zatímco od žen léčených kombinovanou HST se významně nelišila. Zvýšené hodnoty HF byly prokázány i v kontrolním měření 2 měsíce po začátku HST estrogenem. Tyto výsledky naznačují, že vagová modulace srdeční frekvence typická pro mladší ženy se po

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CHAPTER 1

INTRODUCTION

Cardiovascular diseases remain the primary cause of death worldwide. In the US, deaths due to cardiovascular disease for women exceed those of men. While cultural and psychosocial factors such as education, economic status, marital status and access to healthcare contribute to sex differences in adverse outcomes, physiological and molecular bases of differences between women and men that contribute to development of cardiovascular disease and response to therapy remain underexplored. Therefore, we need to discover some theories to do the best forecast, diagnosis, treatment and post-therapeutic prognosis between women and men. There are several pathways where sex hormones can effect human being cardiovascular system to produce original gender differential pathophysiology between women and men.

The mechanisms of gender differential pathophysiology of cardiovascular system between women and men that can be the following theories or hypotheses. The rapid signaling cascade activation and long term respond are the two major pathways revealing how sex hormone to effect cardiovascular system whose results can be found out from human being. However, some unclear mechanisms may need reference animal model experiment to hypothesize human being condition.

Firstly, the gene expression theory of human sex hormone to modulate cardiovascular system is described differences between women and men. For instance, the phosphoinositide 3-kinase/serine/threonine protein kinase (PI3K/AKT)-dependent endothelial nitric oxide (NO) synthase (eNOS) activation pathway to protect cardiovascular system can proved by some authors. They publish that progesterone increases nitric oxide (NO) synthesis in human vascular endothelial cells through activation of membrane progesterone receptor-α. (Pang et al. 2015) Using human umbilical vein endothelial cells (HUVECs) as a model which shows that progesterone binds to plasma membranes of HUVECs with the characteristics of membrane progesterone receptors (mPRs). Immunocytochemical and Western blot analyses confirmed that mPRs are expressed in HUVECs and are localized on their plasma membranes. The mPR is the primary receptor mediating the rapid, nongenomic actions of physiologically relevant concentrations of progesterone in human vascular endothelial cells to increase NO synthesis. Progesterone significantly increased endothelial nitric oxide synthase (eNOS) activity and eNOS phosphorylation. Knockdown of mPRα expression by treatment with small-interfering RNA (siRNA) blocked the stimulatory effects of 20 nM progesterone on NO production and eNOS

phosphorylation, whereas knockdown of nPR was ineffective. (Pang et al. 2015) Treatment with PI3K/Akt and mitogen-activated protein (MAP) kinase inhibitors blocked the stimulatory effects of progesterone, eNOS phosphorylation and also prevented progesterone induced increases in Akt and extracellular signal–regulated kinase (ERK) phosphorylation. The results suggest that progesterone stimulation of NO production in HUVECs is mediated by mPRα and involves signaling through PI3K/Akt and MAP kinase pathways. Raise the possibility that mPR could be a useful therapeutic target for the treatment of hypertension. Progesterone actions through mPR in vascular cells and evaluate whether activation of this novel signaling pathway would be an effective means of regulating blood pressure.

Secondly, the hypothesis of cardiac cells contain sex hormone receptors whose dominant activity are tendency differentially towards sexual difference from animal model. Hydrogen sulfide, generated in the myocardium predominantly via cystathionine-γ-lyase (CSE), is cardioprotective. Some authors study has shown that estrogens enhance CSE expression in myocardium of female rats. The present study aims to explore the mechanisms by which estrogens regulate CSE expression, in particular to clarify the role of estrogen receptor subtypes and the transcriptional factor responsible for the estrogenic effects. They found that either the CSE inhibitor or the CSE small interfering RNA attenuated the protective effect of 17β-estradiol (E2) against H2O2- and hypoxia/reoxygenation-induced injury in primary cultured neonatal cardiomyocytes. E2 stimulates CSE expression via estrogen receptor (ER)-α both in cultured cardiomyocytes in vitro and in the myocardium of female mice in vivo. (Wang et al. 2015) A specificity protein-1 (Sp-1) consensus site was identified in the rat CSE promoter and was found to mediate the E2-induced CSE expression. E2 increases ERα and Sp-1 and inhibits microRNA (miR)-22 expression in myocardium of ovariectomized rats. In primary cardiomyocytes, E2 stimulates Sp-1 expression through the ERα-mediated down-regulation of miR-22. It was confirmed that both ERα and Sp-1 were targeted by miR-22. In the myocardium of ovariectomized rats, the level of miR-22 inversely correlated to CSE, ERα, Sp-1, and antioxidant biomarkers and positively correlated to oxidative biomarkers. In summary, this study demonstrates that estrogens stimulate Sp-1 through the ERα-mediated down-regulation of miR-22 in cardiomyocytes, leading to the up-regulation of CSE, which in turn results in an increase of antioxidative defense. Interaction of ERα, miR-22, and Sp-1 may play a critical role in the control of oxidative stress status in the myocardium of female rats.

Thirdly, the hypothesis of Y chromosome effect from animal model. Like autosomal chromosomes (Chrs), the sex chromosomes (ChrX; ChrY) are thought to have once been identical pairs that were free to recombine and exchange genetic material. Over

the course of evolution, ChrY became unique from all other Chrs with the acquisition of a dominant sex-determining gene and subsequent chromosomal inversions that restricted recombination with its homologous ChrX, that led to its degradation. The relatively few protein-coding genes on ChrY are predominantly male-specific genes acquired through transposition and translocation from other Chrs. Some authors indicate that a locus/loci on the Y chromosome may influence LDL levels, independent of testosterone levels. (Charchar et al. 2004) Other authors also demonstrate in animal models that having 2 X chromosomes versus an X and Y chromosome complement drives sex differences in higher HDL cholesterol (HDL-C). It is conceivable that increased expression of genes escaping X-inactivation in XX mice regulates downstream processes to establish sexual dimorphism in plasma lipid levels. (Link et al. 2015)

The female immune response against many infectious pathogens tends to be more robust, leading to a better prognosis in disease outcome. (Case et al. 2013) However, the evolutionary advantage of this heightened female immune response also contributes to their higher risk of developing autoimmune disease. While these sex differences in immunity are predominantly linked to the differential effects of sex hormones on immune cells, ChrY can also influence the immune response and susceptibility. Certainly, the immune respond is also the influence of cardiovascular disease. On the other hand, the lineage of the Y chromosome accounts for up to 15 to 20 mm Hg in arterial pressure. Genes located on the Y chromosome from the spontaneously hypertensive rat (SHR) are associated with the renin-angiotensin system. (Sampson et al. 2014) Given the important role of the renin-angiotensin system in the renal regulation of fluid homeostasis and arterial pressure which hypothesized that the origin of the Y chromosome influences arterial pressure via interaction between the intrarenal vasculature and the renin-angiotensin system. This study demonstrates that the origin of the Y chromosome significantly impacts the renal vascular responsiveness and therefore may influence the long-term renal regulation of blood pressure. Recently, studies on the human Y chromosome have also demonstrated that genetic variation within the male-specific region of the Y chromosome (MSY) could play a part in determining cardiovascular risk in men, confirming the notion that the increased risk for CAD in men cannot be fully explained through common CAD risk factors. (Molina et al. 2016)

Finally, the sex hormone can also combination with neuroendocrine and other neurotransmitters to effect cardiovascular system including blood pressure, heart rate, gender differential anxiety respond and so on. For instance, sex comparisons in the functional and molecular aspects of the HPA axis, through various phases of activity,

including basal, acute stress, and chronic stress conditions. The HPA axis in females initiates more rapidly and produces a greater output of stress hormones. This review focuses on the interactions between the gonadal hormone system and the Hypothalamic-pituitary-adrenal (HPA) axis as the key mediators of these sex differences, whereby androgens increase and estrogens decrease HPA activity in adulthood. In addition to the effects of gonadal hormones on the adult response, morphological impacts of hormone exposure during development are also involved in mediating sex differences. Additional systems impinging on the HPA axis that contribute to sex differences include the monoamine neurotransmitters norepinephrine and serotonin. (Goel et al. 2014) Diverse signals originating from the brain and periphery are integrated to determine the level of HPA axis activity, and these signals are, in many cases, sex-specific. Sex hormones can also exert differential effects on a variety of sensitive tissues like the reproductive tract, gonads, liver, bone and adipose tissue, among others. In the brain, sex hormones act as neuroactive steroids regulating the function of neuroendocrine diencephalic structures like the hypothalamus. In addition, steroids can exert physiological effects upon cortical, limbic and midbrain structures, influencing different behaviors such as memory, learning, mood and reward. In the last three decades, the role of sex hormones on monoamine neurotransmitters in extra-hypothalamic areas related to motivated behaviors, learning and locomotion has been the focus of much research. The purpose of this thematic issue is to present the state of art concerning the effects of sex hormones on the neurochemical regulation of dopaminergic midbrain areas involved in neurobiological and pathological processes. Neonatal exposure to sex hormones or endocrine disrupting chemicals can produce long-term changes on the neurochemical regulation of dopaminergic neurons in the limbic and midbrain areas. (Sotomayor-Zarate et al. 2014)

In the incidence of cardiovascular disease, women tend to present with longer-lasting episodes of atrial fibrillation, with a faster ventricular response and a higher incidence of cardio-embolic complications. The predominance of atrioventricular (AV) nodal reentrant supraventricular tachycardia with a higher prevalence in women–2:1 compared to men. (Rodriguez et al. 1992) The prevalence of the use of antihypertensive agents is higher among middle-aged women than among men. Notably, in the United States, hypertensive women use more diuretics and angiotensin receptor blockers than men, whereas hypertensive men more often receive beta-blockers, calcium channel antagonists, or inhibitors of angiotensin-converting enzyme.(Cadeddu et al. 2016) Drug-induced torsade de pointes and symptomatic long QT syndrome have a female predominance.(Wolbrette et al. 2002) A clear trend to a higher incidence of bleeding complications has been consistently reported in women, which might be related to a

more frequent over-dosage of antithrombotic treatment in women than in men. (Gutiérrez-Chico & Mehilli. 2013) Anticoagulants are less frequently used in women (David & Christine. 2010) Women are at higher risk than men for AF-related thromboembolism off warfarin (Fang et al. 2005) Women with ischemic heart disease and women with left bundle branch block (LBBB) who received cardiac resynchronization therapy-defibrillator device (CRT-D) had the lowest incidence of ventricular tachycardia/ ventricular fibrillation (VT/VF) or death when compared to men. The risk of stroke after left ventricular assist device (LVAD) implantation varies based on sex, with a higher risk in female patients. (Morris et al. 2015)

However, seldom study mentioned that idiopathic ventricular tachycardia from right ventricular outflow tract (RVOT-VT) has any sex different in cardiologic tissues patterns. Our study find RVOT-VT that females had shorter QRS duration, lower right ventricular voltage, and more low voltage zone in the RVOT free wall than males. Although the possible mechanisms are not clear, our findings suggest differences in ventricular remodeling between genders in patients with idiopathic RVOT-VT. The RVOT free wall was the prominent region, which was not associated with different ventricular tachycardia (VT) incidences. Those findings suggest that ROVT low voltage might be the remodeling result after VT, rather than the cause. We also concern that what kind of arrhythmic treatment methods can reduce the sex differences in prognosis. We used 3D mapping ablation which can indeed choice precise position to let prognosis better.

Another important pathophysiology field about women who undergo pre-menopause, menopause and post-menopause, their sex hormones level are quite variant so that possibility of cardiovascular disease will be reversion comparing with men. (Virginia & Sue. 2008) The higher incidence of cardiovascular disease in men than in women of similar age, and the menopause-associated increase in cardiovascular disease in women, has led to speculation that gender-related differences in sex hormones have a key role in the development and evolution of cardiovascular disease. Compelling data have indicated that sex differences in vascular biology are determined not only by gender-related differences in sex steroid levels, but also by gender-specific tissue and cellular differences that mediate sex-specific responses. (Vitale et al. 2009) There are substantial gender differences in the pattern, severity and clinical outcomes of coronary heart disease independent of environmental risk factor exposure. As a consequence, there has been considerable interest in the potential role of sex hormones in atherogenesis, particularly the potential protective effects of estrogen. Over the last decade, compelling evidence has emerged that sex differences in vascular biology are not only determined by gender-related differences in sex steroid levels but also by

gender-specific tissue and cellular characteristics which mediate sex-specific responses to a variety of stimulation. This gender-dependent regulation may have important implications for understanding the basis of the gender gap in atherosclerosis and may eventually lead to the development of sex-specific treatments for cardiovascular disease. This review will summarize the current data for the role of androgens in gender differences in coronary heart disease and cardiovascular biology. (Ng. 2007) After menopause, the decline in estrogen levels accelerates key atherogenic processes, including dyslipidemia, endothelial dysfunction and arterial stiffening, increasing the risk for cardiovascular events. (Haapalahti & Mikkola. 2015) In the healthy vasculature, estrogen has several structural and functional protective effects. The primary indication for menopausal hormone therapy is the treatment of climacteric symptoms, in particular hot flashes. With appropriate timing of estrogen treatment, it is possible to improve the ageing women's vascular health without increase carcinogen risk of women's reproductive systems.

1. THEORETICAL BACKGROUND

The higher incidence of cardiovascular disease in men than in women of similar age, and the menopause-associated increase in cardiovascular disease in women, has led to speculation that gender-related differences in sex hormones have a key role in the development and evolution of cardiovascular disease. Compelling data have indicated that sex differences in vascular biology are determined not only by gender-related differences in sex steroid levels, but also by gender-specific tissue and cellular differences that mediate sex-specific responses. Cardiovascular cells contain functional estrogen and androgen receptors and are targets for sex hormone action, which can influence many physiological and pathological processes, including vascular and myocardial cell homeostasis. However, hormones are important but not unique actors in this issue, further genetic and epigenetic determinants being involved. The effects of gender differences, including sex hormones, on cardiac and vascular cell injury and death and their influence in determining atherosclerosis, heart failure and other main human cardiovascular diseases. (Pierdominici et al. 2011)

1.1 Review of different cardiovascular physiological function control between men and women

The ability to recognize and appreciate from a reproductive standpoint that males and females possess different attributes has been long standing. Only more recently have we begun to look more deeply into both the similarities and differences between men and women, as well as between boys and girls, with respect to the structure and function of other organ systems. This article focuses on the cardiovascular system, with examples of sex differences in the control of coronary function, blood pressure, and volume. Recognizing the differences between the sexes with respect to cardiovascular function facilitates understanding of the mechanisms whereby homeostasis can be achieved using different contributions or components of the living system. Furthermore, recognition of the differences as well as the similarities permits the design of appropriate diagnostic instruments, recognition of sex-specific pathophysiology, and implementation of appropriate treatment of cardiovascular disease in men and women.

1.1.1 Sex-hormone regulation of cardiovascular function

Sex differences at several different locations in the excitation– contraction (Ec)

coupling pathway have been implicated. (Fig.1) Most notably, new studies have shown that cardiomyocytes from female hearts exhibit a marked decrease in the gain of Ec coupling, which translates to a decrease in sarcoplasmic reticulum (SR) Ca2+ release. This has been observed as lower peak Ca^{2+} transients and smaller individual SR Ca^{2+} sparks in myocytes from females, in comparison to males. (Parks & Howlett. 2013) Nonetheless, little is known about the specific signalling pathways implicated in these sex differences, and this limits the ability to translate these findings to new therapeutic strategies.

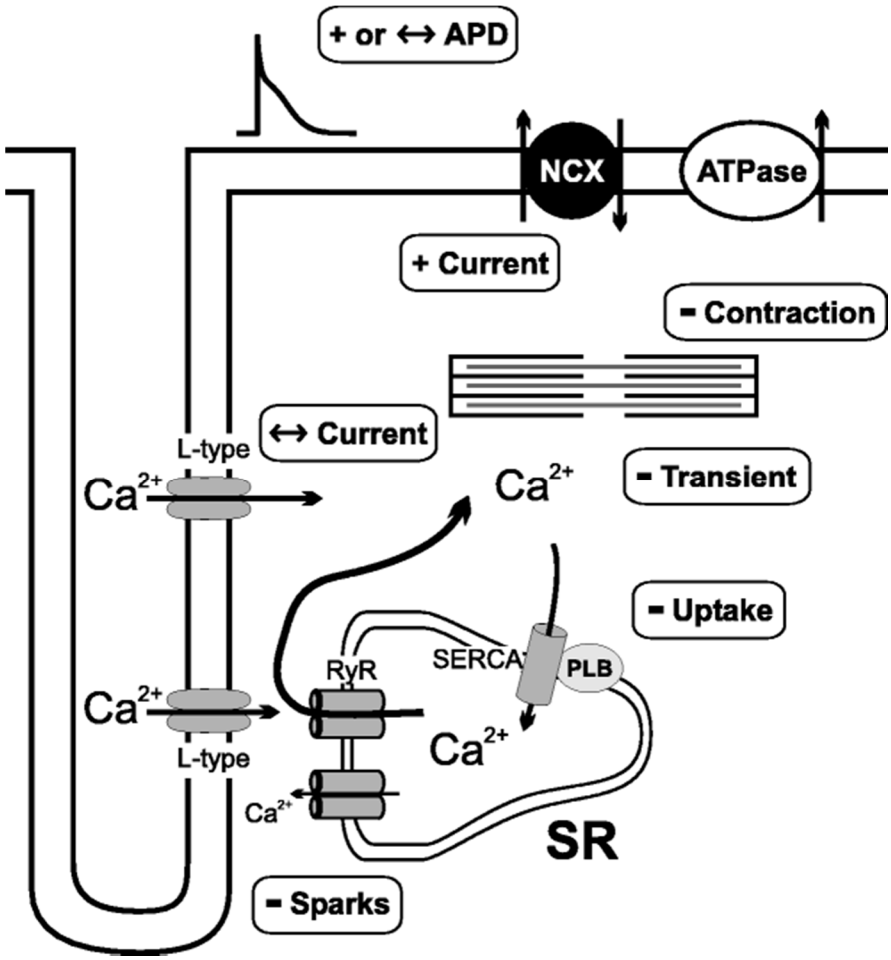


Fig. 1 : Differences in the major components of cardiac excitation–contraction (Ec) coupling in ventricular myocytes from female animals in comparison to males. L-type Ca^{2+} current density does not differ between males and females, although NCX activity is higher in myocytes from females. However, Ca^{2+} transient amplitudes and SR Ca^{2+} spark amplitudes are decreased in females in comparison to males. Therefore, females have lower Ec upling gain (Ca^{2+} transient per unit Ca^{2+} current). Female myocytes have smaller contractions in comparison to males. Contractions are also slower to relax in

female myocytes, which is likely a result of reduced SR Ca^{2+} uptake via SERCA. Additionally, APD is either unchanged or prolonged in myocytes from females in comparison to cells from males. These sex differences in Ec coupling are thought to contribute to reduced contractile function in myocytes from females in comparison to males. *APD* action potential duration, *NCX* Na^{+}–Ca^{2+} exchanger, *PLB* phospholamban, *RyR* ryanodine receptor, *SERCA* sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase, *SR* sarcoplasmic reticulum. (Parks & Howlett, 2013)

Importantly, ovariectomy (OVX) causes a marked increase in the gain of EC coupling, resulting in larger peak Ca^{2+} transients and larger Ca^{2+} sparks. These results strongly suggest that oestrogen suppresses SR Ca^{2+} release and contributes importantly to the reduction in EC coupling gain present in cardiomyocytes from females. OVX also promotes cardiomyocyte Ca^{2+} dysregulation, including elevated SR Ca^{2+} content and larger unitary Ca^{2+} release events. This SR Ca^{2+} overload promotes the spontaneous release of Ca^{2+} from the SR. This could increase susceptibility to a range of different cardiovascular diseases in low oestrogen states, such as in older, post-menopausal women. New research that explores the intracellular signalling pathways involved in these effects could lead to the identification of new targets for the treatment of cardiovascular diseases in older women. (Parks and Howlett, 2013) For example, men have faster rates of repolarization than women, and castrated men have prolonged repolarization while the reverse is seen in women with abnormally high levels of testosterone. This is consistent with evidence that gonadectomy (GDX) increases action potential duration (APD) in individual myocytes in animal models. This may be clinically important as prolongation of the AP can increase the probability of early after depolarizations, which can trigger arrhythmias such as torsades des pointes.

Using both an in vivo and an isolated heart model of ischemia and reperfusion (I/R), we found that females had less injury than males. Posttranslational modifications can modify ROS handling and play an important role in female cardio-protection. Female hearts had increased phosphorylation and activity of aldehyde dehydrogenase (ALDH)2 , an enzyme that detoxifies reactive oxygen species (ROS)-generated aldehyde adducts, and that an activator of ALDH2 reduced I/R injury in males but had no significant effect in females. Myocytes from female hearts had less ROS generation following I/R than males. (Claudia et al. 2010)

Recent studies of blood pressure control and cardiac function in healthy men and women have demonstrated that women and men use the two arms of the baroreflex system differently. At all ages, women were found to have reduced sympathetic activity (reflected by lower total peripheral resistance (TPR) and pulmonary artery (Pa) pressure

and enhanced parasympathetic activity relative to men. Similarly, men were found to have higher plasma norepinephrine levels than women. The consequence, though, was that in response to changes in body position (e.g., in response to fluid shifts), women appeared to be more vulnerable to orthostatic hypotension and fainting. Should the stresses be maintained and the system adapts or remodels, pathophysiology will develop. Given that the different mechanisms put strains on different components of the cardiovascular system, the long-term consequences of repeated and prolonged exposure to stress in the conduit and resistance vessels of males is vessel remodeling, resulting in sustained hypertension with less tissue perfusion. The consequences of reductions in tissue perfusion can be appreciated when recalling that metabolically active cells of an organ are downstream of the constricting arterial vessels. In women, it is the heart rather than the large arterial vessels that takes the burden.

The Frank–Starling law of the heart (also known as Starling's law or the Frank–Starling mechanism or Maestrini heart's law) states that the stroke volume of the heart increases in response to an increase in the volume of blood filling the heart (the end diastolic volume) when all other factors remain constant. In other words, as a larger volume of blood flows into the ventricle, the blood will stretch the walls of the heart, causing a greater expansion during diastole, which in turn increases the force of the contraction and thus the quantity of blood that is pumped into the aorta during systole. The increased volume of blood stretches the ventricular wall, causing cardiac muscle to contract more forcefully (the so-called Frank–Starling mechanisms). The stroke volume may also increase as a result of greater contractility of the cardiac muscle during exercise, independent of the end-diastolic volume. The Frank–Starling mechanism appears to make its greatest contribution to increasing stroke volume at lower work rates, and contractility has its greatest influence at higher work rates. This allows the cardiac output to be synchronized with the venous return, arterial blood supply and humoral length without depending upon external regulation to make alterations.

According to this law : cardiac output (CO) equals to heart rate (HR) times stroke volume (SV)

$$HR \times SV = CO \tag{1}$$

That the frequency of beats (HR; beats/time) multiplied by the amount ejected with each beat (SV; amount) is what determines cardiac output (CO ; amount/time), HR x SV = CO. In studies of large numbers of normal healthy adults, even after accounting for the larger body surface area of men than women, the SV was ~10% smaller and HR

similarly greater, resulting in no difference in cardiac index (volume·time−1·surface area−1). The gradient in pressure across the systemic circulation between the aorta [or mean arterial pressure outside the left heart (Pa)] and the vena cava [coming into the right heart pulmonary vein (Pv)] is a function of the amount of blood pumped into the systemic vasculature CO and the resistance to blood flow offered by the vessels of the tissues [total peripheral resistance (TPR)], as

$$CO=(Pa–Pv)/TPR \tag{2}$$

In the normal circulatory system, Pv is on the order of 5% of Pa and is therefore considered negligible; under these conditions, the relationship can be rewritten to indicate that Pa is determined by the product of CO and TPR, as shown by

$$Pa \sim CO \times TPR \tag{3}$$

Under conditions of cardiovascular stress (e.g., exercise, loud noises, or psychological stress) men respond by increasing mainly vascular resistance (TPR in Eqs. 2 and 3), which is manifested as an increase in mean Pa (blood pressure), whereas women predominantly increase HR (Eqs. 1), thereby increasing CO. In both cases, there is an appropriate cardiovascular response, but there are potentially different outcomes. Recall further that the control of blood pressure involves actions of the autonomic nervous system. On the one hand, there is a sympathetic drive (by analogy, the accelerator) to the heart and periphery. Increases in sympathetic activity in the heart results in both elevated contractility, which leads to increases in SV, and increases in HR. Either or both actions lead to an increase in CO. In the periphery, increases in sympathetic activity results in resistance vessel constriction with an increase in TPR. Reduction or withdrawal of sympathetic activity is analogous to taking one's foot off of the accelerator, thereby reducing SV, HR, and TPR. On the other hand, the parasympathetic (vagal) system (by analogy, the brake) slows the heart; withdrawal of parasympathetic activity unmasks the sympathetic drive. Recent studies of blood pressure control and cardiac function in healthy men and women have demonstrated that women and men use the two arms of the baroreflex system differently. At all ages, women were found to have reduced sympathetic activity (reflected by lower TPR and Pa) and enhanced parasympathetic activity relative to men. Similarly, men were found to have higher plasma norepinephrine levels than women. (Geelen et at, 2002)

1.1.2 Different lipoprotein concentrations and glucose metabolism

The composition of the blood circulating in the body also displays sex-specific differences in the levels of the formed elements, the most obvious being that lower numbers of circulating red blood cells per unit volume of plasma in females than in males. This is manifested as lower hematocrit in women than in men. Both lipid and plasma protein compositions demonstrate sexual dimorphism. With respect to lipids, high-density lipoprotein (HDL) is higher and triglycerides are lower in females than in males; this "antiathrogenic" blood lipid profile is also associated with a lower incidence of cardiovascular disease. Following menopause, the lipid profile of females becomes more athrogenic and is correlated with the higher incidence of heart disease in that population. Less obvious is the lower plasma protein levels in females than in males. (Fig. 2)

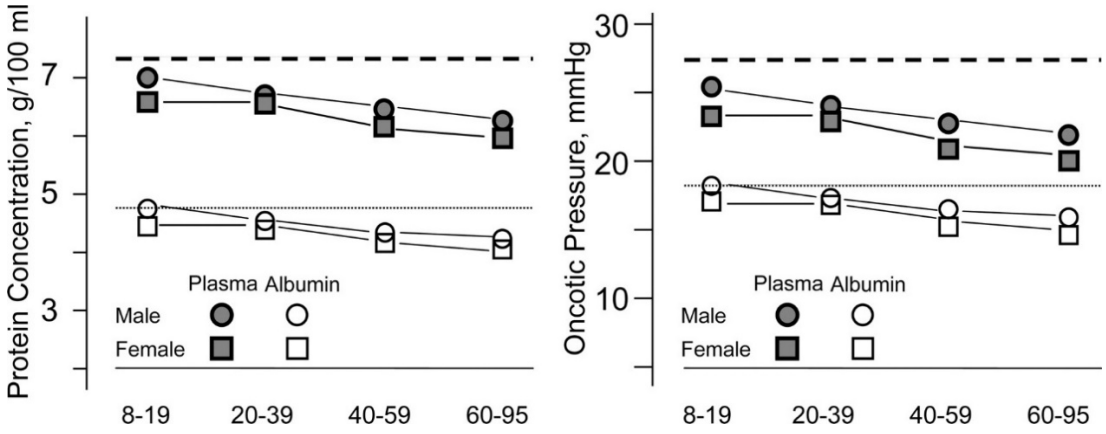


Fig. 2 : Total plasma protein and plasma albumin concentrations for males and females at 4 ages. Data are redrawn from Ciba-Giegy data tables. (*A*). In *B*, the data shown in *A* were used to construct a plot of the net plasma oncotic pressures for the same groups. Dashed lines represent the "normal" values for humans. (Virginia H & Huxley, 2007)

Males appear to be at greater risk of diabetes at younger age and at lower body mass index (BMI) compared to women, but women feature a dramatic increase of their cardio-metabolic risk after menopause. The estimated future years of life lost owing to diabetes is somewhat higher in women than men, with higher increase of vascular death in women, but higher increase of cancer death in men. In women pre-diabetes or diabetes are more distinctly associated with a higher number of vascular risk factors, such as inflammatory parameters, unfavorable changes of coagulation and blood

pressure. Pre-diabetic and diabetic women are at much higher relative risk for vascular disease. Women are more often obese and less physically active, but may even have greater benefit from increased physical activity than males. Whereas men predominantly feature impaired fasting glucose, women often show impaired glucose tolerance. A history of gestational diabetes or the presence of a polycystic ovary syndrome (PCOS) or increased androgen levels in women, on the other hand the presence of erectile dysfunction (ED) or decreased testosterone levels in men are sex specific risk factors for diabetes development. ED is a common feature of obese men with the Metabolic Syndrome and an important predictor of cardiovascular disease. Several studies showed that diabetic women reach their targets of metabolic control glycated hemoglobin (HbA1c), blood pressure and low-density lipoprotein (LDL)-cholesterol less often than their male counterparts, although the reasons for worse treatment outcome in diabetic females are not clear. Furthermore, sex differences in action, pharmacokinetics, and side effects of pharmacological therapy have to be taken into account. (Kautzky-Willer et at,. 2016) Insulin sensitivity of whole-body and leg glucose disposal was studied in 16 young well-matched healthy men and women infused with intra-lipid or saline for 7 hours. (Høeg et al. 2011) Overnight fasted women have higher insulin-stimulated whole body and leg glucose uptake despite a higher intramyocellular triacylglycerol concentration than men. Women also express higher muscle mRNA levels of proteins related to lipid metabolism than men. Intra-lipid infusion causes less insulin resistance of muscle glucose uptake in women than in men. (Høeg et al. 2011) This insulin resistance is not due to decreased canonical insulin signaling, accumulation of lipid intermediates, inflammation, or direct inhibition of glucose transporter (GLUT) activity. Rather, a higher leg lactate release and lower glucose oxidation with intra-lipid infusion may suggest a metabolic feedback regulation of glucose metabolism.

The consequence, though, was that in response to changes in body position (e.g., in response to fluid shifts), women appeared to be more vulnerable to orthostatic hypotension and fainting. Should the stresses be maintained and the system adapts or remodels, pathophysiology will develop. Given that the different mechanisms put strains on different components of the cardiovascular system, the long-term consequences of repeated and prolonged exposure to stress in the conduit and resistance vessels of males is vessel remodeling, resulting in sustained hypertension with less tissue perfusion. The consequences of reductions in tissue perfusion can be appreciated when recalling that metabolically active cells of an organ are downstream of the constricting arterial vessels. In women, it is the heart rather than the large arterial vessels that takes the burden.

1.2 Sex different response activity of autonomic nervous system under certain pathophysiological conditions

In young men, sympathetic nerve activity is directly related to the level of vasoconstrictor tone in the peripheral vasculature. However, in young women this relationship does not exist, suggesting that certain factors (potentially related to the female sex hormones) offset the transfer of sympathetic nerve activity into vasoconstrictor tone in this population. In the present study we show that, in young women, the β-adrenergic receptors (which cause vasodilatation in response to noradrenaline) blunt the vasoconstrictor effect of resting sympathetic nerve activity in young women. This mechanism does not occur in young men or postmenopausal women. It is possible that the β-adrenergic receptors may partially protect young women against the sometimes harmful effects of high sympathetic nerve activity. This may explain why the risk of developing hypertension is greater in young men and postmenopausal women (who have very high sympathetic nerve activity) compared to young women. (Hart et al. 2011)

Table 1 : Summary of sex-related differences under physiologic and pathophysiological condition and the contribution by autonomic nervous system (Dart et al. 2002)

Physiologic and/or Disease state	Sex difference	Autonomic nerve involvement
Disease state		
Prevalence of Raynaud's syndrome	F>M	++
Prevalence of pre-syncope/syncope	F>M	++
Survival of non-CAD heart failure	F>M	++
Survival after myocardial infarction	M>F	±
Risk of ventricular arrhythmias	M>F	+
Left ventricular hypertrophy	Present	±
Central obesity	M>F	+
Physiological state		
Systolic blood pressure rise on aerobic exercise	M>F	±
Hemodynamic response to isometric exercise	M>F	+
Morphology of ECG 'T' wave	Present	±
Tolerance to cold temperature	M>F	+
Tolerance to repeated hypoglycemia	M>F	+

ANS, autonomic nervous system; CAD, coronary artery disease. (++) Strong

evidence, (+) good evidence, (±) conflicting data. M, males; F, females.

The autonomic nervous system plays a major role in the regulation of the cardiovascular system under both physiological and pathophysiological conditions. There is substantial evidence of gender difference in the functioning of the autonomic system, including specific effects of both male and female sex hormones, as summarized in Table 1. As a generalisation, at least in humans, there is a preponderance of sympathetic mediated responses in males and of parasympathetic in females — perhaps related to divergent gender roles pertaining during human evolution. These phylogenic influences play a major role in modulating the course of many current and widely prevalent diseases.

Over the last 12 years, authors used physiological measurements, including muscle sympathetic nerve activity (MSNA), to explore the balance among mean arterial blood pressure, cardiac output and total peripheral resistance (TPR) in normotensive humans. These determinants of blood pressure can vary widely in different subjects and how they vary depends on sex and age. In young men, there is a direct relationship between MSNA and TPR but no relationship with blood pressure. This is because cardiac output is proportionally lower in those with high MSNA and TPR. In contrast, in young women there is no relationship between MSNA and TPR (or cardiac output) ; this is because β-adrenergic vasodilator mechanisms offset α-adrenergic vasoconstriction. Thus, blood pressure is unrelated to MSNA in young women. In older women, β-adrenergic vasodilator mechanisms are diminished, and a direct relationship between MSNA and TPR is seen. (Joyner et al. 2015) In older men, the relationships among these variables are less clear cut, perhaps owing to age-related alterations in endothelial function. With ageing, the relationship between MSNA and blood pressure becomes positive, more so in women than in men. The finding that the physiological control of blood pressure is so different in men and women and that it varies with age suggests that future studies of mechanisms of hypertension will reveal corresponding differences among groups.

The β-adrenergic receptors appear to offset the vasoconstrictor effects of sympathetic nerve activity in young women at rest. This mechanism appears to be minimal or non-existent in young men and in postmenopausal women. Therefore suggest that the β-adrenergic receptors play a fundamental role in resting arterial pressure regulation in young women. Furthermore, these changes in the contributions of β-adrenergic receptors with ageing, combined with an increase in MSNA, probably contribute to the marked increase in risk of hypertension that occurs in women after menopause.

2. Interpretation of cardiac electrophysiology

Cardiac electrophysiology is the science of elucidating, diagnosing, and treating the electrical activities of the heart. The term is usually used to describe studies of such phenomena by invasive (intracardiac) catheter recording of spontaneous activity as well as of cardiac responses to programmed electrical stimulation (PES). These studies are performed to assess complex arrhythmias, elucidate symptoms, evaluate abnormal electrocardiograms, assess risk of developing arrhythmias in the future, and design treatment.

2.1 Application of Cardiac Monophasic Action Potential

Monophasic action potential (Map) recording plays an important role in a more direct view of human myocardial electrophysiology under both physiological and pathological conditions. The procedure of Map measuring can be simply performed using the Seldinger technique, when Map catheter is inserted through femoral vein into the right ventricle or through femoral artery to the left ventricle. The Map method represents a very useful tool for electrophysiological research in cardiology. Its crucial importance is based upon the fact that it enables the study of the action potential (AP) of myocardial cell in vivo and, therefore, the study of the dynamic relation of this potential with all the organism variables. This can be particularly helpful in the case of arrhythmias. There are no doubts that physiological Map recording accuracy is almost the same as transmembrane action potential as was recently confirmed by anisotropic bidomain model of the cardiac tissue. Map recording devices provide precise information not only on the local activation time but also on the entire local repolarization time course. Although the Map does not reflect the absolute amplitude or upstroke velocity of transmembrane APs, it delivers highly accurate information on AP duration and configuration, including early after depolarizations as well as relative changes in transmembrane diastolic and systolic potential changes. Based on available data, the Map probably reflects the transmembrane voltage of cells within a few millimeters of the exploring electrode. Thus Map recordings offer the opportunity to study a variety of electrophysiological phenomena in the in situ heart (including effects of cycle length changes and antiarrhythmic drugs on AP duration). (Yang & Kittnar, 2010) The Map technique is useful for assessing the local electrical activity of the myocardium in contact with the depolarizing electrode. (Tse et al. 2016)

2.1.1 Introduction of Map

Electrocardiography (ECG) is the best-known and the most popular procedure of recording of the electrical activity of myocardium. However, ECG detects just body surface projection of the electrical heart field and cannot reveal local information such as actual cellular depolarization and repolarization process of myocardial tissue. The standard surface electrocardiogram as well as the intracavitary electrocardiographic recordings are not able to provide more precise and more locally oriented information as they represent just the summation of an electric activity of many myocardial cells from relatively big regions of the heart. Moreover, the obtained picture of the electric heart field is distorted by different conductivities and resistances of tissues situated between the source of an electric activity and measuring electrodes. In many situations an advanced knowledge of the entire temporal extension of cellular action potentials would be very helpful what is related particularly to the study of arrhythmias pathogenesis and of mechanisms of antiarrhythmic drugs actions. For such purposes only two methods are available: the cellular impalement technique and the Map method. Thus, MAp recording plays an important role in a more direct view of human.

2.1.2 History and definition of Map

The history of Map started in 1883 when the potentials generated by frog cardiac beats were continuously recorded (Burdon-Sanderson & Page 1883). In one of the described observations one electrode was placed on the intact surface of the heart while the other one on an injured region. Transitory monophasic potential (with only one polarity) was then recorded. Monophasic was the potential in comparison to the (at that time already known) transitory multiphase recordings that have got both positive and negative polarities. This was the origin of the term monophasic action potential whose form was very similar to the cellular action potential later obtained by the cellular impalement technique with microelectrodes. This technique (known from the late 1930s from the giant axon of the squid) was applied to the cardiac cell by Coraboeuf and Weidmann (1949) and by Woodbury *et al.* (1950). Thanks to those experiments, many of the theories developed for the giant axon of the squid could also be applied to cardiac cells, elucidating the role of Na+, K+, and Ca2+ in the processes underlying electrical changes in the myocardial cells. (Coraboeuf and Weidmann 1949, Burgen and Terroux 1953, Orkand and Niedergerk 1964). The first non-traumatic method for recording of Maps was developed and published by Jochim *et al.* (1934). These authors demonstrated that Maps can be obtained simply by pressing an electrode against the

epicardium of the toad ventricle, while another electrode merely touched the nearby epicardium. They also demonstrated that the Map is positive with respect to zero if the pressure electrode is the active one (connected to the positive amplifier input). Unfortunately, their important observations went largely unnoticed for many years. However, the procedures used in their study were both in methodology and interpretation surprisingly similar to the current principle of recording Maps by contact electrode. Franz *et al.* (1986) have revealed the forgotten paper of the Jochim´s team and based on its observations they have produced an electrode-catheter, that using just simple contact with the myocardium, obtained a stable and high-quality Map, eliminating the risks of suction. Suction electrode that captured monophasic potentials with great simplicity, not requiring the production of a specific myocardial lesion, because this was already caused by the suction itself, was introduced by Korsgren *et al.* (1966). In this way, the right ventricle Map of a patient could be recorded, which revealed a clinical application for the technique of Map recording. However, suction presented the risks of air embolism and irreversible mechanical myocardial lesion (Olsson 1972) and thus the contact electrode technique was once again considered to be a useful tool for experimental and clinical cardiac electrophysiology.

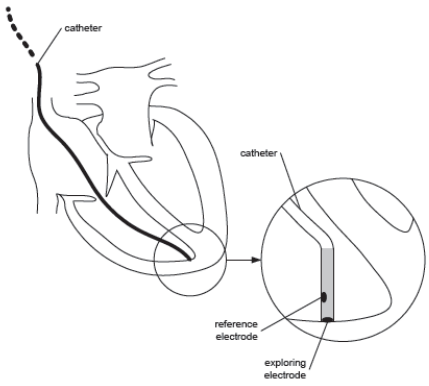


Fig. 3 : The Map catheter is introduced through femoral vein to right ventricle. In the detail: the situation of the electrodes in relation to the myocardium.

The ‘contact electrode technique’ for clinical use was developed between 1980 and 1983 by Franz and coworkers. Besides being simple and more clinically safe, the contact electrode method provides Map recordings that, due to lack of myocardial injury, are stable over time. This allows clinical electrophysiologists to monitor Maps over periods of several hours from the same endocardial site to assess, for instance, the effects of antiarrhythmic drugs or cycle length changes on local myocardial

repolarization. With the contact electrode technique, Map recordings can be obtained from the human endocardium or epicardium without suction but rather by pressing a nonpolarizable electrode gently against the endocardium or epicardium. Catheters and probes for endocardial and epicardial Map recording were developed both for clinical and experimental studies. The application of the epicardial electrode requires direct contact with the epicardium and, consequently, needs a surgical incision for the heart exposure. However, the endocardial electrode can be fixed to the tip of a catheter and installed to the endocardium through blood vessels (Fig. 3), enabling its clinical utilization with a minimum risk for the patient (Leirner and Cestari 1999). Many interesting results also in the field of theoretical electrocardiography were obtained by the Map method, for instance an evidence for the hypothesis of opposite directions of ventricular depolarization and repolarization (Franz *et al.* 1987) thanks to the measurements of Maps from different left ventricular endocardial and epicardial sites during cardiac surgery and catheterization.

2.1.3 Potential applications of the Map

Two hypotheses have been advanced to explain the generation of Map recordings. One hypothesis suggests that Map corresponds to a local electrical activity flowing from the active to inactive regions near the tip of the inactive electrode. An alternative hypothesis suggests that the Map "indifferent electrode" actually records active myocardial tissue from a wide field-ofview. In any case the Map method represents a very useful and agile tool for an electrophysiological research in cardiology. Its crucial importance lies in the fact that it enables the study of the action potential of myocardial cell *in vivo* and, therefore, the study of the dynamic relation of this potential with all the organism variables (Leirner and Cestari 1999). As mentioned earlier it can be particularly helpful in the case of arrhythmias. Using the Map measurement an association between the arrhythmias accompanying the long QT syndrome and the anomalies of duration and temporal dispersion of the MAPs, as well as the presence of postpotentials was found (Gravilescu and Luca 1978). A possible relation of postpotentials to cardiac arrhythmias as well as their developing mechanisms were largely studied using Maps by Zipes (1991). Nevertheless, the myocardial action potential can be affected by many other factors (Slavíček *et al.* 1998):

1) *Cellular hypertrophy* : The development of ventricular arrhythmias was recently found to be correlated with electrophysiological remodeling in isolated ventricular myocytes, including action potential prolongation, increased sodium-calcium exchanger activity, reduced outward potassium currents, sarcoplasmic reticulum

Ca2+defects, and loss of protein kinase A-dependent phospholamban phosphorylation (Ruan *et al.* 2007). However, cardiac hypertrophy is associated in a reverse process with increased mechanical stretch, electrical remodeling and arrhythmogenesis (Michael *et al.* 2009).

2) *Ischemia and reperfusion*: Acute ischemia opens ATP-sensitive potassium channels (KATP) and causes acidosis with hypoxia/anoxia in cardiac muscle. The ensuing repolarizing potassium efflux shortens the action potential. Moreover, accumulation of extracellular potassium is able to partially depolarize the membrane, reducing the upstroke velocity of the action potential and thereby impairing impulse conduction. Both mechanisms are believed to be involved in the development of reentrant arrhythmias during cardiac ischemia (Liu *et al.* 2007). On the other hand, the ischemia-reperfusion can induce significant down-regulation of INa (sodium current) and Ito (transient outward potassium current) and upregulation of ICa-L (L-type calcium current), which may underlie the altered electrical activity and long abnormal transmembrane action potential duration of the surviving ventricular myocytes, thus contributing to ventricular arrhythmias during acute ischemiareperfusion period (Gao *et al.* 2008).
3) *Chemical effects*: In addition to antiarrhythmic drugs a lot of other chemical substances can also change the cardiac action potential. These chemical substrates change cardiac action potential by an alteration of cardiac ion channel behavior. Assessment of potential drugs and disclosure of their action mechanisms has been one of the most frequent uses of the Map method for a few last years.
4) *Thermal effects*: The electrical excitability of cardiac myocytes is determined by sarcolemmal ion currents which flow through ion specific channels. Since function of the ion channels is dependent on temperature, low temperatures are expected to reduce sarcolemmal ion currents and therefore compromise excitability and conductivity of the cardiac myocytes. The changes in the depolarizing sodium current (INa) tend to maintain adequate excitability in the cold, while increased intensity of the rectifying potassium current (IKr) will prevent excessive lengthening of action potential duration in the cold. The ATP-sensitive potassium channel (K(ATP)) opener nicorandil used instead of potassium in hypothermic cardioplegia significantly improves preservation of cardiac function and energetics in the in situ heart preparation. The present study, therefore, examines the effect of nicorandil at different temperatures and the role of sarcolemmal and mitochondrial K(ATP) channels under ex vivo conditions using contractile force (Cf) and action potential duration (APD) as end points. Shortening of APD and activation of sarc K(ATP) by nicorandil were not related to myocardial protection. Thus, the mito

K(ATP) seems to play a significant role in cardioprotection compared to the sarc K(ATP) also when substrate depletion and hypoxia are combined with hypothermia. (Steensrud T et al. 2006)

5) *Mechanical effects* : The electrical activity of the cardiac cell is usually understood to be triggering the mechanical activity in a single direction. However, the mechanical activity could also cause changes in the electrical potential of the cells. This process is called mechano-electrical feedback (Lab 1991). For instance, an isovolumetric contractions against an infinite afterload is causing evident changes in the action potential (Leirner 1992).

There is no doubt that physiological Map recording accuracy is almost the same as transmembrane action potential what was confirmed recently by anisotropic bidomain model of the cardiac tissue (Colli *et al.* 2007). To understand why Map recordings register an approximation of the transmembrane voltage, an ideal system can be described : first it is necessary to consider the potential at the contact electrode as ground. The transmembrane voltage of the region under the electrode is also constant and thus, the intracellular potential is fixed. To reach the indifferent electrode, a path has to be followed that goes on intracellularly under the electrode and then across the membrane which has a time-varying voltage. Therefore, relative to the intracellular potential that is fixed with respect to ground, the extracellular potential will move with the transmembrane voltage (Vigmond 2005). Although the Map does not reflect the absolute amplitude or upstroke velocity of transmembrane APs, it delivers highly accurate information on the AP duration and configuration, including early afterdepolarizations as well as relative changes in transmembrane diastolic and systolic potential changes. It also documents regional electrophysiological phenomena of the heart without interrupting the intrinsic organization of the tissue, and also documents the normal or pathological interrelations between the heart and the body. Thus Map recordings offer the opportunity to study, in the *in situ* heart, a variety of electrophysiological phenomena including effects of cycle length changes and antiarrhythmic drugs on AP duration (Franz 1991). Maps measurement has many interesting applications but two fields dominate: 1) research of arrhythmias and mechanisms underlying their origin and maintenance, and 2) drugs assessment (particularly antiarrhythmic drugs) and their action mechanisms. For instance, investigation of atrial fibrillation using Maps of the atrial myocardium has a very long tradition. Olsson *et al.* (1971) described changes of action potential duration in patients with higher risk of atrial fibrillation relapses after cardioversion almost 40 years ago and research in this field has been continuing till today (Aidonidis *et al.* 2009). Similar tradition can be found in the research of myocardial action potential alterations caused

by antiarrhythmic drugs (Vaughan Williams 1984, Franz 1991, Osaka *et al.* 2009). In last few years many studies have used the Map method for endocardial and epicardial mapping of electrophysiological events in the heart. For instance, Kongstad *et al.* (2005) have measured the activation time, Map duration and end of repolarization time in healthy pigs and they have described both endo- and epicardial dispersion of ventricular repolarization. The same team (Li *et al.* 2002) has also found repolarization gradients over the atrial endocardium. Map recordings can also be used as a validation of other electrophysiological mapping procedures, e.g. a non-contact mapping (Yue *et al.* 2004). The procedure of Map measuring can be simply realized using the Seldinger technique, when Map catheter is inserted through femoral vein into the right ventricle or through femoral artery to the left ventricle. The tip electrode has to be nearly perpendicular to endocardium what allows flexions back and forth with each cardiac contraction-relaxation cycle. The Map catheter lead is connected to an electrophysiologic recording system and the signal can be analyzed by automated computer system (Tsalikakis *et al.* 2003) what allows to calculate 2D or 3D endocardial mapping by Maps.

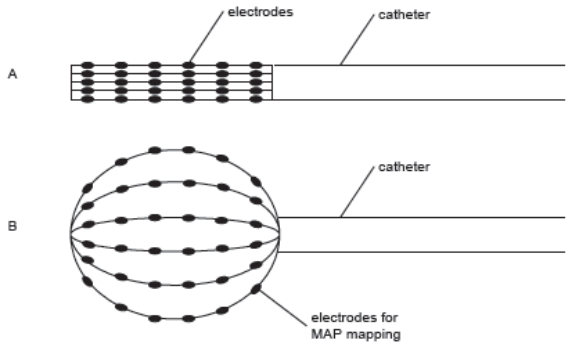


Fig. 4 : The 'Lantern Catheter' is used for transpercutaneous catheterization followed by three-dimensional mapping of the endomyocardial Maps. The catheter in close (A) and open (B) positions.

For real-time endocardial mapping of Maps a Lantern Catheter was designed recently (Cui and Sen 2008). The Lantern Catheter devised according to the invention is used for transpercutaneous catheterization followed by three-dimensional mapping of the endomyocardial Map. Preferably at least 64 points (Ag-AgCI plated electrodes) of Map are recorded simultaneously and the data analyzed by a conventional electrophysiological (EP) analysis system (Fig. 4). The pattern and/or the magnitude of the alteration of the action potential, changes in the action potential duration and/or the

site or sites of 90 % of the action potential duration (APD90), the slowest action potential repolarization and/or depolarization (dv/dt), and/or other parameters can be determined very precisely. Using this real-time 3D mapping, the site and sites of the myocardium with maximum dispersion of these parameters among 64 or more recording sites are supposed to be identified that could enable to identify the pathology of the myocardium even in an early disease stage. The tip electrode has to be nearly perpendicular to endocardium what allows flexions back and forth with each cardiac contraction-relaxation cycle.

The monophasic action potential method represents a very efficient tool for the research in both experimental and clinical cardiology. It enables the study of the myocardial action potentials in vivo and, therefore, the study of the dynamic relation of this potential with all the organism variables. Thus Map recordings offer the opportunity to study, in the in situ heart, a variety of electrophysiological phenomena including effects of cycle length changes, action potential alternans, and antiarrhythmic drugs on electrical processes in myocardium. The recordings can provide systematic data for the design and interpretation of arrhythmia studies in animal models as well as for a mathematical modeling of ionic currents and corresponding electrical events in myocardial cells. As the recordings may reflect cellular calcium abnormalities the method seems to be also a potential source of a marker for identification of patients before heart failure decompensation or at risk for severe arrhythmias. Therefore it can be supposed that the application and interest in the Map technique for our clinical or experimental research.

2.2 Sex difference in arrhythmic patterns

Sex differences in cardiac repolarization and the arrhythmogenic risk of patients with inherited and acquired long-QT syndromes (LQTS) are well appreciated clinically. Women were less likely to experience VT/VF (Ventricular Tachycardia/Ventricular Fibrillation), and had fewer VT/VF episodes, than men. These findings were strongest in patients with evidence of a stable anatomic VT circuit: those with clinical or electro-physiologically induced VT. Enhancing our knowledge of the mechanisms underlying these differences is critical to improve our therapeutic strategies for preventing sudden cardiac death in such patients. The effects of sex hormones on the expression and function of ion channels that control cardiac cell excitation and repolarization as well as key proteins that regulate Ca^{2+} dynamics at the cellular level.

Moreover, it examines the role of sex hormones in modifying the dynamic spatiotemporal (regional and transmural) heterogeneities in action potential duration

(e.g., the arrhythmogenic substrate) and the susceptibility to (sympathetic) triggered activity at the tissue, organ, and whole-animal levels. Finally, it explores the implications of these effects on the management of LQTS patients.(Odening & Koren. 2014) The evidence from studies also published to date show that women have a higher mean resting heart rate, a longer QT interval, a shorter QRS duration, a lower QRS voltage and a shorter P wave duration and PR interval than men. Women have a higher prevalence of sick sinus syndrome, inappropriate sinus tachycardia, atrioventricular nodal reentry tachycardia, idiopathic right ventricular tachycardia, and arrhythmic events in the long-QT syndrome.

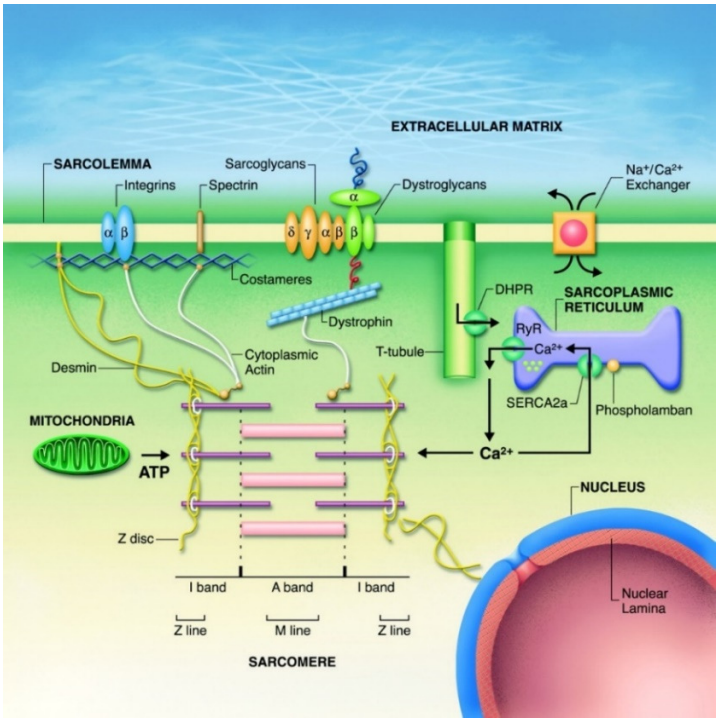


Fig. 5 : Schematic of the components of cardiac myocyte structure. RyR, ryanodine receptor. (Fatkin & Graham. 2002)

Similarly, failure to recognize differences in QRS duration and voltage can make electrocardiographic criteria for ventricular hypertrophy more specific but less sensitive in women. In contrast, men have a higher prevalence of atrioventricular block, carotid sinus syndrome, atrial fibrillation, supraventricular tachycardia due to accessory pathways, Wolff-Parkinson-White syndrome, reentrant ventricular tachycardia, ventricular fibrillation and sudden death, and the Brugada syndrome. (Bernal & Moro, 2006) Two principle mechanisms may have been proposed to explain these differences between the sexes differential : hormonal effects on the expression or function of ion

channels or, conversely, differences in autonomic tone. It is also possible that a combination of these 2 mechanisms may be involved (Table 2). A combined mechanism would lead to greater sympathetic activity and a lower baroreflex response in men of any age as well as to more pronounced parasympathetic or vagal activity in women.

Table 2 : Underlying Mechanisms Responsible for Electrophysiological Differences Between Sexes. (Bernal and Moro, 2006)

Mechanism	Differences
Electrophysiological cell effects	Presence of estrogen receptors
	Modulation of L-type Ca channels
	Modulation of K channels
Autonomic modulation	Physical condition
	Heart rate
	Heart rate variability
	Sensitivity of baroreceptors
	Dispersion of repolarization
	Expression of nitric oxide
Combinations of the above	Dispersion of repolarization
	Long QT syndrome

Not only is the duration of repolarization different for women, the so-called nonspecific repolarization changes in the 12-lead electrocardiogram are much more frequent in women. Similarly, the duration of the QT and QTc interval becomes shorter in men after puberty. The effects of estradiol and progesterone changes occurring in physiological menstrual cycle on ventricular premature beats (VPBs). VPB frequency decreases with estradiol peak in the ovulation period. This suggests that estrogen may have protective effects against ventricular arrhythmias. (Dogan M et al. 2016) Hormonal influences on the membrane ion channels, autonomic tone, or a combination of these factors may all play a role.

2.2.1 Atrial fibrillation

Atrial fibrillation is the most common supraventricular arrhythmia, affecting 0.4% of the population. This arrhythmia is 1.5 times more frequent in men than women. (Benjamin et al.1998) Nevertheless, it is highly prevalent in both sexes and differences between sexes in the incidence tend to even out in subjects over 70 years old. Women tend to present with longer-lasting episodes of atrial fibrillation, with a faster ventricular response and a higher incidence of cardioembolic complications. It shows a greater severity of embolic strokes in women, and so being a women is currently considered as an additional risk factor for thromboembolic events. (Fang et al. 2005)

2.2.2 Supraventricular tachycardia

Inappropriate sinus tachycardia appears almost exclusively in women. It usually affects middle-aged women who are in some way connected to the health profession. Referred for electrophysiological evaluation, found a predominance of atrioventricular (AV) nodal reentrant supraventricular tachycardia with a higher prevalence in women– 2:1 compared to men. However, this ratio was inverted for supraventricular tachycardia due to an AV nodal reentrant mechanism with an accessory pathway circuit, that is, it was 2:1 for men with respect to women. (Rodriguez et al. 1992) Another study has reported electrophysiological differences between sexes for arrhythmias with 2 nodal pathways-the slow refractory pathway periods are shorter in the short pathway and AV cycle block lengths and the tachycardia cycle lengths are also shorter in women. (Liuba I et al. 2006) The incidence of supraventricular paroxysmal tachycardias episodes decreases with age, particularly in women. Some author performed a study in premenopausal women and showed that the incidence of supraventricular tachycardias is greater in the phase of the menstrual cycle in when progesterone concentrations increase (luteal phase). The exact electrophysiological effects of progesterone are not known, but these observations suggest that the hormone may exercise a proarrhythmic effect. Such a proarrhythmic effect has also been described in pregnant women. (Rosano & Panina. 1999) In short, inappropriate sinus tachycardia occurs almost exclusively in women. Supraventricular paroxysmal tachycardias arising from nodal reentry are more prevalent in women. The first episode appears before the subject is 40 years old a third of the cases and the incidence decreases with age. In men, accessory pathways are more common, and serious complications of these arrhythmias are also more frequent.

2.2.3 The Wolff-Parkinson-White syndrome

The incidence of Wolff-Parkinson-White syndrome is 1/3000 in the general population and more frequent in men, occurring at a ratio of 2:1, like AV nodal reentrant tachycardias via an accessory pathway. (Rodriguez et al. 1992) In addition to above mention, the incidence of sudden death in the Wolff-Parkinson-White syndrome is small, and that it is a clinical problem associated also mainly with men aged less than 30 years.(Pappone et al. 2005) Atrial fibrillation associated with this syndrome is also more frequent in men. (Wolbrette et al. 1999 ; Pappone et al. 2005)

2.2.4 Ventricular arrhythmias and sudden death

Differences in ventricular tachycardia and sudden death between the sexes were also reported. After a follow-up of 26 years, the incidence of sudden death increased with the age of the population, with a predominance in men in all age groups and an overall ratio in the incidence of approximately 3:1 compared to woman. (Kannel et al. 1998) It was explained by the epidemiology of the heart disease (in women, it appears 10 years to 20 years later). However, the most common underlying heart disease was ischemic heart disease for both sexes. Sudden death was reported in 40% of the men and 34% of the women with coronary artery disease. The incidence of sudden death is low in subjects of both sexes under 45 years old. Above this age, the incidence doubles with each additional decade of life, starting 20 years later in women. As mentioned earlier, although coronary artery disease is the most common underlying cardiovascular disease, in women, sudden death with no history of this disease is more common, particularly in subjects under 65 years old-below this age, 90% of the cases of sudden death occur with no history of coronary artery disease. (Kannel et al. 1998) A history of myocardial infarction increases the risk of sudden death by 4 in men and by 3 in women. Ten years after the infarction, the risk of sudden death was 5.3% in women and 11.9% in men. The presence of isolated coronary artery disease is predictive of a higher mortality in women regardless of ejection fraction, and the presence of dyskinesia leads to an additional 5-fold increase. Another epidemiological study on sudden death performed in the United States indicates that men have 50% higher age-adjusted risk of sudden death than women. (Zheng et al. 2001) The reason for these differences between the sexes is probably the difference in the incidence of ischemic heart disease. In a retrospective study of the survivors of cardiac arrest referred for electrophysiological study found ischemic heart disease was the underlying cause in 80% of the men and only 45% of the women.(Albert et al. 1996) Another study identified systolic blood pressure, smoking, intraventricular block, ST-T changes, family history of myocardial infarction in relations under 60 years old, body mass greater than 30, and diabetes as

long-term predictors of sudden death in women.(Cupples et al. 1992) The indicate revealed that ventricular premature beats increase the risk in men but not women. (Moss et al. 1991 ; Dittrich et al. 1998) Differences between the sexes exist for inducibility of ventricular arrhythmias with programmed electrical stimulation in electrophysiological studies and it is easier to induce ventricular arrhythmias in men with postinfarction scarring (95%) than in women (72%). In women with no coronary artery disease, such arrhythmias can only be induced 19% of the time. (Freedman et al. 1998 ; Vaitkus et al. 1991) Men showed a trend toward greater risk of fast VT than women. (Choudhary et al. 2016)

2.2.5 Bradyarrhythmias and tachyarrhythmias

Sick sinus syndrome is more frequent in women, whereas and AV block and carotid sinus syndrome are more common in men. (Liu et al. 2001)

Table 3 : Differences in the Incidence of Bradyarrhythmias and Tachyarrhythmias According to Sex* (Liu et al. 2001)

	Predominance in Men	Predominance in Women
Bradyarrhythmia	AV block. Carotid sinus syndrome.	SSS.
Supraventricular tachyarrhythmia	Atrial premature beats. AF. SVT via accessory pathway. WPW.	IST. INT.
Ventricular Tachyarrhythmia	Ventricular premature beats. Re-entrant VT. VF. Sudden death. Brugada syndrome.	Idiopathic RV VT. Congenital ILQTS. Acquired LQTS.

* SSS indicates sick sinus syndrome; AF, atrial fibrillation; LQTS, long QT syndrome; INT, intranodal tachycardia; IST, inappropriate sinus tachycardia;

SVT,supraventricular tachycardia; VT, ventricular tachycardia; VF, ventricular fibrillation; WPW, Wolff-Parkinson-White; RV, right ventricular.

Electrophysiological variations in sinus function have been described, with longer recovery times in men, and variations in AV conduction, with a longer AV block cycle length in men than in women. The AV block cycle length is also longer and there is a greater incidence of lack of retrograde AV conduction (23% in men vs 11% in women) (Liu et al. 2001)

2.2.6 Idiopathic left-ventricular tachycardia

There are 2 different phenotypes, one with repetitive, unsustained episodes of monomorphic ventricular tachycardia, and the other with sustained exercise-induced paroxysmal tachycardia. In both types, QRS morphology is a left bundle-branch block configuration with an inferior axis and the arrhythmia is sensitive to adenosine. The mechanism is presumably related to the activity triggered by delayed post-depolarization in turn mediated by cyclic AMP. A higher prevalence has been reported in women. Idiopathic left-ventricular tachycardia or fascicular tachycardia on the other hand is more prevalent in men. (Nakagawa et al. 2002 ; Lamberti et al. 2002)

2.2.7 Arrhythmogenic right-ventricular dysplasia

It is a condition characterized by replacement of muscle by fibrous or fibrofatty tissue. It is more frequent in young adults, with a ratio of incidence in men compared to women of 2.7:1. The estimated prevalence is 0.02%-0.1% in the general population. Dysplasia is assumed to be the cause of sudden death in young athletes in 5% of the autopsies done in the United States, and this percentage is as high as 25% in the autopsies done in northern Italy. A family history is reported in 50% of the cases, and several of the implicated genes have been identified. (Calkins, 2006 ; Kies et al. 2006)

2.2.8 Congenital and acquired long QT syndrome

The high incidence of arrhythmic events in women, and particularly ventricular tachycardia in torsade de pointes, has been described in association with long QT syndromes, whether congenital or acquired. (Moss et al. 1991 ; Ebert et al. 1998 ; Locati et al. 1998 ; Lehmann et al. 1997 ; Rashba et al. 1998 ; Schwartz et al. 2006) In the

registry on congenital long QT syndrome, 58% of those included were women. (Locati et al. 1998) Female sex is a risk factor for inherited and acquired long-QT (LQT) syndrome and associated with torsade de pointes (TdP) arrhythmias. Various studies have shown that females have a higher risk of a first cardiac event between 15 and 40 years, and observed that women are at higher risk than men of drug-induced TdP by class III anti-arrhythmic drugs and other drugs that block HERG (the human Ether-à-go-go-Related Gene). Animal studies have shown higher-level inward currents in females. These agree with a recent expression-pattern study, where the authors found lower expression-levels of K^+ channel α- (Kir2.3, Kv1.4 and HERG) and β- (minK) subunits in female heart. The differences between male and female HERG were significant in RV only, while the sex differences on Kir2.3, Kv1.4 and minK were significant in both RV and LV. (Yang & Clancy. 2011) The fact that women are at particular risk for drug-induced arrhythmias and that arrhythmia risk rises around the time of puberty, suggests the dominant female hormones estrogen and progesterone modulate arrhythmia vulnerability. While estrogen may exacerbate arrhythmia susceptibility by directly interacting with the drug binding site on the promiscuous HERG subunit and reducing I_{Kr} current and increasing the rate of channel deactivation, progesterone is apparently protective and reduces QT intervals. Male subjects were more likely to suffer syncope and sudden death of unknown cause up until puberty. Thereafter, the predisposition was greater in women. Likewise, women with congenital long QT syndrome were at a higher risk of cardiac events in the period after giving birth. These events could be prevented with administration of beta-blockers. (Lehmann et al. 1997 ; Rashba et al. 1998) The Jervell-Lange-Nielsen syndrome, a variant of the congenital long QT syndrome associated with deafness, is a severe variant of the long QT syndrome caused by mutation of the genes that code for proteins that modulate the current through the IKs channel. In these patients, men are at a higher risk of serious arrhythmic events. Patients with drug induced *torsade de pointes* and found that 70% were women, a percentage that was independent of left ventricular function, electrolytic imbalances, and the basal QT interval. (Makkar et al. 1993) A 4.7-fold increase in the risk of proarrhythmia and sudden death in women was shown. These results were later confirmed in subsequent studies also with Sotalol in a number of patient populations. (Lehmann et al. 1996 ; Kuhlkamp et al. 1997)

Studies suggested that both progesterone and testosterone acutely modulate I_{Ks} and I_{CaL} through phosphoinositide 3-kinase (PI3K)/AKT-dependent endothelial nitric oxide (NO) synthase (eNOS) activation pathwa , resulting in suppressing I_{CaL} currents and increasing I_{Ks} current density.(Yang and Clancy. 2011) It has been recently suggested that the N-terminal truncated isoform of the androgen receptor (AR) 45 plays an

essential role in the heart since the transcript level of the AR45 is high in human heart tissue. An experiment of AR45 effects on the HERG potassium channel demonstrated that AR45 enhanced HERG channels by stabilizing HERG channel protein via Extracellular signal-regulated kinase 1/2 (ERK 1/2) stimulations. Other studies also indicated that the male hormone testosterone (5α-DHT) increased repolarizing K^+ currents density (I_{K1} and I_{Kr}) and acts to protect against arrhythmia initiation. (Liu et al. 2003) During the follicular phase (prior to ovulation) of the menstrual cycle, QT interval is longer than that in the luteal phase (following ovulation) when progesterone is increased. Arrhythmic events associated with acquired and inherited LQTs are significantly reduced during phases where progesterone level is high. Moreover, QT is significantly increased by estrogen hormone replacement therapy in females and susceptibility to drug-induced arrhythmias is exaggerated in the late follicular phase where estrogen level is the highest. In contrast, Burke *et al*. found that in pre-menopausal women the corrected QT (QTc) interval does not greatly change through the menstrual cycle, but QTc is reduced in the luteal phase after autonomic blockade. Furthermore, one study showed that QTc did not change during the menstrual cycle, but its shortening was more pronounced in the luteal phase in women. (Yang et al. 2010) The disparity in these studies may be due to the fact that corrected QT interval measurements were based on a single point or a few points with the individual patient at rest. Such an analysis is unlikely to be sensitive enough to observe significant individual differences in QT intervals as they fluctuate throughout the menstrual cycle since biological variability between patients may be larger than fluctuations in individual patients. In addition, some drug studies demonstrated that females have greatly increased QT intervals compared with males during treatment with d,I-sotalol and quinidine. Therefore, the I_{Kr} blockers seem to increase early after depolarization (EAD) development and prolong repolarization in females, both primary and critical predictors of drug-induced TdP. E2 induces the expression of the progesterone receptor (PGR) mRNA and protein in the myocardium of women but not in that of men. (Kararigas et al. 2010)

2.2.9 Brugada syndrome

Sudden death in patients with Brugada syndrome usually occurs during sleep, particularly in the early hours of the morning, and when patients are in their 30s and 40s, although cases have been described in 1-year-old children and patients aged 77 years. There is a higher prevalence in men, and this prevalence is very marked in certain regions, such as Southeast Asia, where the ratio of men to women with this syndrome

is 8:1. (Sarkozy & Brugada, 2005) Males are more likely to develop Brugada syndrome. (Kurokawa et al. 2012)

2.2.10 Arrhythmias and pregnancy

Some reports in the literature indicate that the incidence of both supraventricular arrhythmias and ventricular ones increases during pregnancy. However, the supporting evidence is limited and based on case studies. (Tawan et al. 1993 ; Wilderhorn et al. 1992 ; Brodsky et al. 1992)

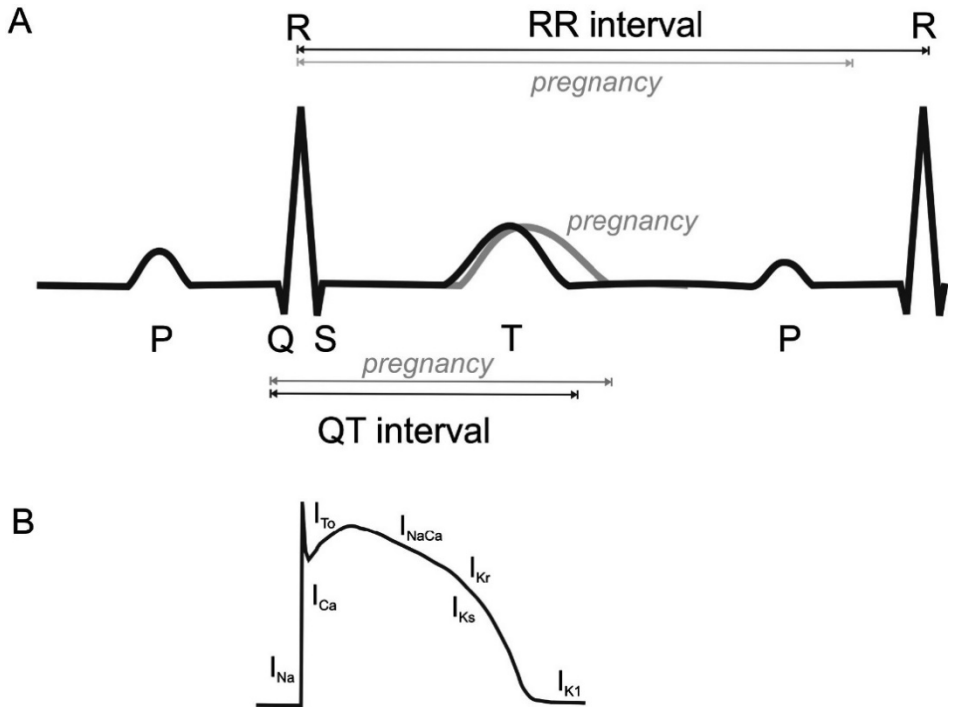


Fig. 6 : The human ECG during pregnancy (A) The major deflections of the human ECG. The P wave is associated with atrial depolarization. The QRS complex is the result of ventricular depolarization, and the T-wave repolarization. The shape, duration and timing, of the T wave are affected by heterogeneity of repolarization of the ventricle. In pregnancy, the RR duration is reduced, i.e. heart rate increases. In addition, the QT interval increases, as does T-wave dispersion. (B) Human ventricular cellular action potentials. The rapid upstroke of cellular action potentials is well co-ordinated across the ventricle, leading to the short QRS duration. Conversely, the repolarization of ventricular action potentials varies across the ventricle, leading to the broad T wave. The dominant ion channels during various parts of the action potential are indicated. (Bett. 2016)

Gestational cardiac hypertrophy and a physical shift of the heart contribute to changes in the ECG. (Fig. 6) There are also electrical changes such as an increased heart rate and lengthening of the QT interval. There is an increased susceptibility to arrhythmias during pregnancy and the postpartum period. (Bett. 2016) Treatments for rhythm disorders during pregnancy can be complicated by the additional risk of fetal damage. Prolonged and continuous treatments with antiarrhythmic drugs should be avoided, at least during the first 3 months of pregnancy.

3. Phenomenon of sex-difference in cardiovascular disease

The incidence and the progression rate of cardiovascular disease and hypertension (CVDH) is markedly higher in men than in age-matched, premenopausal women.(Rechelhoff., 2001) After menopause, this relationship no longer exists, and the incidence as well as the rate of progression of CVDH are very similar in women and men.(Rechelhoff., 2001) Sex differences in CVDH have also been reported in animal models, including the spontaneously hypertensive rat (Rechelhoff., 1999) and Dahl salt-sensitive rat (Rowland, 1992) and ischemia-reperfusion injury. (Wang et al. 2005) Although the mechanisms underlying these sex differences in the incidence and progression of CVDH are largely unknown, the role of sex hormones in modulating the activity of several regulatory systems, including the renin-angiotensin system (RAS), has been suggested. In addition, genetic differences, especially with respect to the RAS , have also been implicated in mediating sex differences in the incidence and progression of CVDH.(Christine, 2005) Sex differences in RAS-regulating aminopeptidase activities, their relationship with sex hormones, and their potential role in controlling blood pressure acting through local and circulating RAS.

3.1 Atherosclerosis and response to intimal injury

Observed that arteries undergo positive remodeling by enlarging outward in order to accommodate plaque and avoid luminal compromise. Proteases produced by inflammatory cells appear to have a role in adventitial remodeling through extracellular matrix (ECM) destruction, (Shah et al. 2007) suggesting that the inflammatory processes are relevant in outward remodeling. Women appear to have more diffuse atherosclerosis, less luminal stenosis, higher incidence of endothelial dysfunction, and a higher prevalence of microvascular dysfunction than men,(Shaw et al. 2006 ; Bairey

Merz et al. 2006) suggesting that women may have greater positive remodeling. (Fig 7) (Merz et al. 2010) Women are less likely to undergo revascularization after acute myocardial infarction (AMI). (Hayashi et al. 1995) Apparent gender differences may interfere with decisions regarding investigation and revascularization of coronary arteries, and perhaps both sex differences and gender differences may affect outcomes after revascularization. (Pelletier et al. 2014)

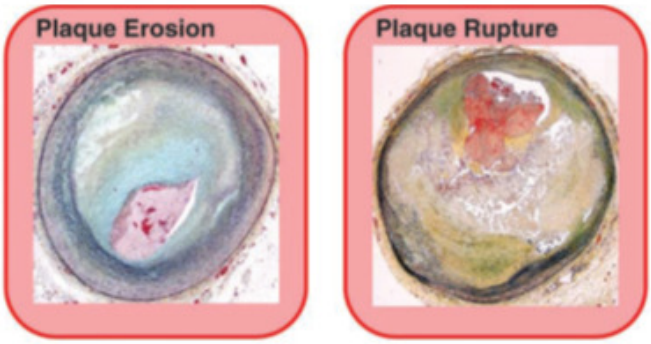


Fig 7 : Younger women: Plague erosion. Thrombus over a base rich in smooth muscle with proteoglycan-rich matrix (necrotic core often absent) : 40% of thrombi in sudden cardiac death. However, men and older women : Plaque rupture. Thin fibrous cap over large necrotic core infiltrated by foamy macrophages: 60% of thrombi in sudden cardiac death. (Merz et al. 2010)

The pathoanatomic substrate for coronary thrombosis also differs between men and women. In men, 80% of coronary thrombi tend to occur because of plaque rupture, whereas in women, 20%–40% of coronary thrombi occur on an intact atherosclerotic plaque with superficial atherointimal erosion. (Burke et al. 1998 ; Farb et al. 1996) This plaque erosion is a common finding in sudden cardiac death (SCD) in younger women who were smokers and postmenopausal women taking hormone replacement therapy (HRT). Plaques that tend to rupture typically (Fig. 8) have a lipid-laden atherosclerotic core with intimal and adventitial inflammation and increased plaque neovascularity. (Libby, 2001)

Inflammatory cells trigger death of smooth muscle cells through apoptosis and produce matrix-degrading enzymes that can induce depletion of the collagen framework, leading to loss of collagen and thinning of the fibrous cap (Shah, 2007) and, eventually, either rupture or erosion. Importantly, lipid-laden plaques have inflammatory cell-derived tissue factor (TF) that is a prototypical trigger for activating the clotting cascade. When a lipid-rich plaque ruptures, TF is immediately exposed to

circulating blood, which, with other factors, stimulates the production of thrombin. This in turn leads to platelet-fibrin thrombus formation.

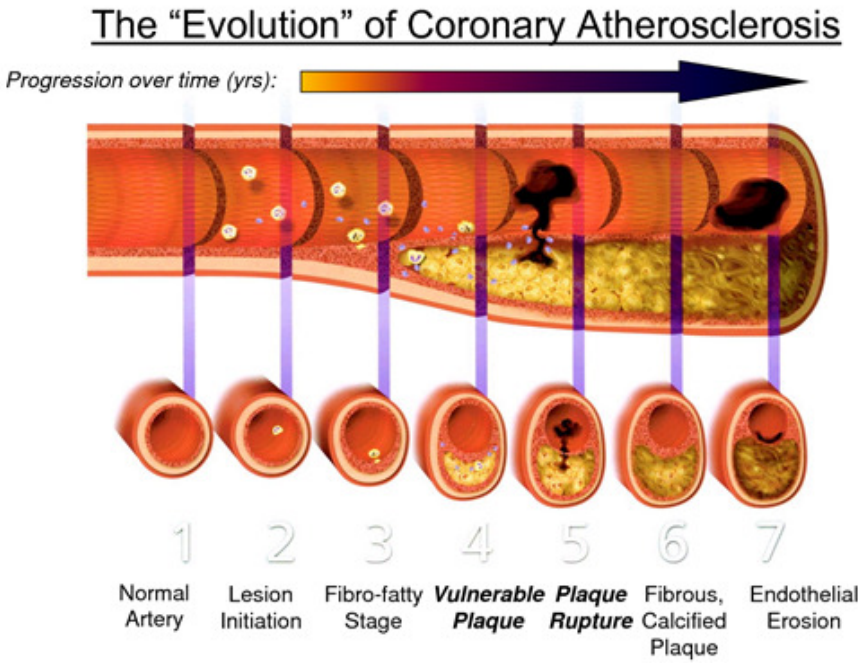


Fig 8 : Initiation, progression, and complication of human coronary atherosclerotic plaque. Top, Longitudinal section of artery depicting "timeline" of human atherogenesis from normal artery to atheroma that caused clinical manifestations by thrombosis or stenosis. Bottom, Cross sections of artery during various stages of atheroma evolution. 1, Normal artery. Note that in human arteries, the intimal layer is much better developed than in most other species. The intima of human arteries contains resident smooth muscle cells often as early as first year of life. 2, Lesion initiation occurs when endothelial cells, activated by risk factors such as hyperlipoproteinemia, express adhesion and chemoattractant molecules that recruit inflammatory leukocytes such as monocytes and T lymphocytes. Extracellular lipid begins to accumulate in intima at this stage. 3, Evolution to fibrofatty stage. Monocytes recruited to artery wall become macrophages and express scavenger receptors that bind modified lipoproteins. Macrophages become lipid-laden foam cells by engulfing modified lipoproteins. Leukocytes and resident vascular wall cells can secrete inflammatory cytokines and growth factors that amplify leukocyte recruitment and cause smooth muscle cell migration and proliferation. 4, As lesion progresses, inflammatory mediators cause expression of tissue factor, a potent procoagulant, and of matrix-degrading proteinases that weaken fibrous cap of plaque. 5, If fibrous cap ruptures at point of weakening, coagulation factors in blood can gain access to

thrombogenic, tissue factor–containing lipid core, causing thrombosis on nonocclusive atherosclerotic plaque. If balance between prothrombotic and fibrinolytic mechanisms prevailing at that particular region and at that particular time is unfavorable, occlusive thrombus causing acute coronary syndromes may result. 6, When thrombus resorbs, products associated with thrombosis such as thrombin and mediators released from degranulating platelets, including platelet-derived growth factor and transforming growth factor-b, can cause healing response, leading to increased collagen accumulation and smooth muscle cell growth. In this manner, the fibrofatty lesion can evolve into advanced fibrous and often calcified plaque, one that may cause significant stenosis, and produce symptoms of stable angina pectoris. 7, In some cases, occlusive thrombi arise not from fracture of fibrous cap but from superficial erosion of endothelial layer. Resulting mural thrombus, again dependent on local prothrombotic and fibrinolytic balance, can cause acute myocardial infarction. Superficial erosions often complicate advanced and stenotic lesions, as shown here. However, superficial erosions do not necessarily occur after fibrous cap rupture, as depicted in this idealized diagram. (Libby, 2001)

The mechanisms of sex differences in this process may enhanced endothelial apoptosis is associated with exposure of inflammatory cell-derived tissue factor (TF) on the luminal side of the coronary artery. A higher prevalence of superficial endothelial erosions is associated with increased sex-specific circulating coagulability. (Sugiyama et al. 2004) Also, systemic inflammatory processes increase anticardiolipin antibodies, which are more prevalent in women. TF that may be originating not from the plaque but from the circulation may also create a prothrombotic state. Atherosclerosis is qualitatively and quantitatively different in women and men; women demonstrate more plaque erosion and more diffuse plaque with less focal artery lumen intrusion. Investigation into these areas aimed at more fully understanding sex-specific mechanisms should be targeted in order to develop tailored therapies.

Some evidences indicate that sex hormones play a role in the development of ischemic heart disease (IHD) in women. Endogenous and exogenous sex hormones influence fat distribution/deposition, insulin resistance, lipid metabolism, coagulation factors, and inflammation measured by high sensitivity C-reactive protein. (Bairey Merz et al. 2006) Vascular dysfunction, in the absence of obstructive disease, is generally more prevalent in women as compared to men, due to these sex hormone differences, and is manifest by more frequent symptoms and evidence of provocative ischemia or altered metabolism. Because of this sex-specific link, some authors also hypothesize that vascular dysfunction is more frequently present in women with

obstructive coronary disease, and may, therefore, contribute to the higher adverse outcomes also experienced by this group as compared with men. There are insulin resistance, the metabolic syndrome, or hypertensives with diastolic dysfunction, potentiating the declining functional capacity in postmenopausal women. (Bairey Merz et al. 2006)

3.2 Specific treatment of sex different cardiovascular disease

The different responses of women and men to cardiovascular drugs reflect sex-specific variances in pharmacokinetic profiles and drug sensitivities coupled to inherent differences in the underlying physiology of each sex. Thus, many common cardiovascular drugs exhibit sex-specific therapeutic and adverse effects. For example, the QT interval of the electrocardiogram is longer in women compared to men, and accordingly, drugs that prolong the QT interval are more likely to cause lethal ventricular arrhythmias in female than male patients. As more clinical drug trials include women subjects, to improve knowledge base for assessing the risk/benefit ratio for cardiovascular drugs in women will enable us to consider gender as one factor in prescribing drugs and adjusting drug loading and maintenance dosages. This will present evidence for sex-related differences in the responses to common cardiovascular drugs including statins, antiplatelet and antithrombotic agents, β-blockers, digoxin, vasodilator therapies, and drugs associated with the Long QT Syndrome. (Stolarz & Rusch. 2015) Sex differences underlying predilection to distinct arrhythmia syndromes must be revealed so that new therapeutic strategies that take gender into account can be applied to at-risk patients. (Kurokawa et al. 2012)

3.2.1 Hypertension

It is a major risk factor for cardiovascular disease and outcomes in women, and antihypertensive therapy is not always successful in achieving control over the blood pressure (BP). Non-optimal control of BP remains a crucial risk factor for cardiovascular mortality, and in women, it could be related to sex-specific factors. Historically, women have been under-represented in clinical trials; therefore, the benefits of clinical outcomes and the safety profiles of antihypertensive therapies have been studied less extensively in women. The reasons for the sex differences in BP levels are multifactorial, implying different roles of the sex hormones, the renin-angiotensin system, sympathetic activity, and arterial stiffness. A complete understanding of the

pathophysiological features of these differences requires further investigation. Nevertheless, the prevalence of the use of antihypertensive agents is higher among middle-aged women than among men. Notably, in the United States, hypertensive women use more diuretics and angiotensin receptor blockers than men, whereas hypertensive men more often receive beta-blockers, calcium channel antagonists, or inhibitors of angiotensin-converting enzyme. (Cadeddu et al. 2016)

3.2.2 Heart failure in electrophysiological remodeling study

In Hf (heart failure), the heart cannot supply an adequate amount of blood to the rest of body. Blood moves to the heart and body at a slower rate, and pressure increases in the heart. In order to sustain cardiac performance, the chambers of the heart stretch to hold more blood to pump through the body by becoming thickened and stiff. For a short period of time, this helps to maintain the blood pressure, but eventually leads to cardiac dysfunction. The common causes of Hf include ischemic heart disease, cigarette smoking, hypertension, obesity, diabetes mellitus, and valvular heart disease. The causes of Hf are difficult to analyze because of differences in gender, race and prevalence of causes changing with age. Clinical data confirm that Hf is more common in patients older than 50 years when testosterone levels are reduced. A number of studies have also found low levels of testosterone in Hf patients, and have shown measurable short-term benefits from testosterone therapy. However, no clear predictive role of testosterone levels has been defined. In addition, clinical trials have shown that the progression of Hf is slower in women than in men, and females have improved survival in Hf. Compared to men, women tend to develop Hf at older ages. Interestingly, women are more likely to develop diastolic Hf with normal left ventricular ejection fraction compared with men. There are a number of recent detailed reviews on ion-channel remodeling in Hf and sex differences in quality of life in Hf patients.

It is well known that Hf causes cardiac functional changes. These changes make the heart prone to arrhythmias and diastolic and systolic contractile dysfunction. One of the important regulators of cardiac contractile function is phospholamban (PLB). During systole, PLB binds to a Ca^{2+} pump and prevents Ca^{2+} from being pumped back into the sarcoplasmic reticulum (SR). During muscle relaxation, PLB is in its phosphorylated state, which removes its inhibitory effect on the SR Ca^{2+}-ATPase (SERCA) and restores low calcium levels in the cytoplasm. In a gene expression study, PLB is found highly expressed in human failing hearts, and may be a mechanism of systolic contractile dysfunction. Notably, in men, the expression levels of PLB are increased. PLB has

also been shown to be phosphorylated by cAMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase. Calmodulin-3 has a lower expression level in men. The activity of the Na^+/K^+-ATPase via its interaction with the Na^+/Ca^{2+} exchanger (NCX) is important for maintaining Ca^{2+} homeostasis in the heart. Hf studies have found reduced expression of Na^+/K^+-ATPase-α1 in human failing heart tissue. This may lead to decreasing Ca^{2+} efflux by NCX, which increases cytoplasmic Ca^{2+} concentration and causes development of Ca^{2+}-dependent arrhythmias. In a sex difference study, it was discovered that men had reduced expression-levels of Na^+/K^+-ATPase-α1. (Yang & Clancy. 2011) In addition, the plasma membrane Ca^{2+}-ATPase isoform was found to be less strongly expressed in Hf mice and in men. Other cardiac functional changes in Hf include action potential duration (APD) prolongation, reduction of cell excitability, increased Na^+/Ca^{2+} exchange, preserved β-adrenergic responsiveness, and reduced outward K^+ currents, which may contribute to APD prolongation. A Hf study in porcine myocytes demonstrated that NCX is more phosphorylated in male pacing-induced failing swine and that β-adrenergic responsiveness was greatly reduced in males compared to females. This study suggested that increased NCX activity could lead to impaired contractile function by decreasing SR Ca^{2+} content and promote the development of arrhythmia triggers. Females may have better survival rates in Hf because they have a smaller NCX current and larger preserved β-adrenergic regulation. In ischemic myocytes, high levels of intracellular Na^+ cause membrane potential changes that enhance Ca^{2+} influx via NCX. This increased influx could lead to Ca^{2+} "overload". Various studies have been conducted to investigate the female gender in cardio-protection during ischemia and suggest a protective role of estrogen in hypertrophied and/or failing myocardium. Acute effects of estrogen at physiological concentration (1 nmol/L) reduced the increase in $[Na^+]_i$ during metabolic inhibition (MI), and suggested that estrogen may regulate Ca^{2+} influx through reverse NCX by lessening the magnitude of the rise in $[Na^+]_i$ during MI in ischemic hearts. (Yang & Clancy. 2011)

3.2.3 Defibrillation therapy

For the pharmacokinetics and -dynamics of drugs a body of evidence does exist to prove the presence of significant sex-related differences. Especially for the major drug metabolizing enzymes, the cytochrome P 450 family, but also for phase II reactions such as glucuronidation, sex-differences were observed.(Thürmann. 2007) However, most of these differences are either clinically not relevant or were not just observed, because they result in slight increases in the frequency of adverse reactions.

Major sex-specific differences were observed for the cardiac electrophysiology, for opiate and benzodiazepine receptors. Women are significantly more likely to experience drug-induced QT-prolongation and torsade-de-pointes arrhythmia. It should also be considered that conditions such as depression, myocardial infarction and heart failure are characterized by sex-specific symptoms and therefore may deserve a sex-specific. Different trials and epidemiological surveys have repeatedly shown that women experience more adverse drug effects than men. Sex differences in cardiac repolarization and the arrhythmogenic risk of patients with inherited and acquired long QT syndromes are well appreciated clinically. Enhancing our knowledge of the mechanisms underlying these differences is critical to improve our therapeutic strategies for preventing sudden cardiac death in such patients. (Odening & Koren. 2014)

3.2.4 Interaction of sex steroid hormone and drugs

It is challenging to determine the role of gender experimentally in complex cardiac functioning since sex effects are multi-factorial and affect cardiac components at different scales of the cardiac system. However, a computational approach can be useful in this respect as it allows study of specific effects in isolation without other perturbations to the system. For example, it is not easy to determine how much a role physiological concentrations of circulating sex steroid hormones play in gender linked arrhythmia susceptibility. Computational models can incorporate the effects of sex hormones measured experimentally and test these changes specifically from non-linear interactions within cells, between cells and among various tissue components that culminate to produce the overall effects of gender on the heart. The simulations can be used to investigate how acute sex hormones and drugs affect system behavior. The tissue simulations to predict the effects of sex steroid hormones on clinically observed QT intervals and on drug-induced LQTS. Estrogen significantly increases susceptibility to drug-induced arrhythmias. (Yang et al. 2010) However, low concentrations of testosterone are sufficient to protect against drug-induced arrhythmias. The estrogen-mediated susceptibility to drug-induced arrhythmia initiation and protective effects of progesterone and testosterone against congenital and drug-induced LQT syndrome.

The different responses of women and men to cardiovascular drugs reflect sex-specific variances in pharmacokinetic profiles and drug sensitivities coupled to inherent differences in the underlying physiology of each sex. Thus, many common cardiovascular drugs exhibit sex-specific therapeutic and adverse effects. For example, the QT interval of the electrocardiogram is longer in women compared to men, and

accordingly, drugs that prolong the QT interval are more likely to cause lethal ventricular arrhythmias in female than male patients. As more clinical drug trials include women subjects, our improved knowledge base for assessing the risk/benefit ratio for cardiovascular drugs in women will enable us to consider gender as one factor in prescribing drugs and adjusting drug loading and maintenance dosages. This will present evidence for sex-related differences in the responses to common cardiovascular drugs including statins, antiplatelet and antithrombotic agents, β-blockers, digoxin, vasodilator therapies, and drugs associated with the Long QT Syndrome. (Stolarz & Rusch, 2015)

3.3 Predict of therapeutic prognosis

Cardiovascular diseases differ between men and women as do outcomes after therapeutic interventions. Female gender appears to suffer from more adverse drug effects [ADE] than the male. For example, women are significantly more likely to experience drug-induced QT-prolongation and torsade de pointes arrhythmia and many other types of ADE. The major sex-specific differences present in pharmacokinetics, especially for the major drug metabolizing enzymes. (Franconi et al. 2011) The manifestation of specific arrhythmia syndromes appears to be sex specific. In particular, female sex is an independent risk factor for development of torsade de pointes (TdP) arrhythmias not only in congenital long QT syndromes but also in acquired long QT syndromes which occur as adverse effects of existing drugs. Males, on the other hand, are more likely to develop Brugada syndrome. Recent clinical and experimental studies suggest that these differences may stem from intrinsic sex differences in cardiac tissue. These include fundamental electrical differences resulting from variable ion channel expression and diverse sex hormonal regulation via long-term genomic and acute nongenomic pathways, and sex differences in drug responses and metabolisms. (Junko. 2014)

3.3.1 The pathways of myocardial ischemia-reperfusion injury are different

Sex different pathways activated during myocardial Ischemia-reperfusion (I-R) injury in male and female myocardium (Fig.9) were mentioned by some authors.(Mihailidou & Ashton. 2014) The relationship between sex and the effects of aldosterone in cardiovascular disease (CVD), an issue of significant need that may lead

to changes in best practice to optimise clinical care and improve outcomes for females with CVD. This modified tissue response may foster a homeostatic environment.

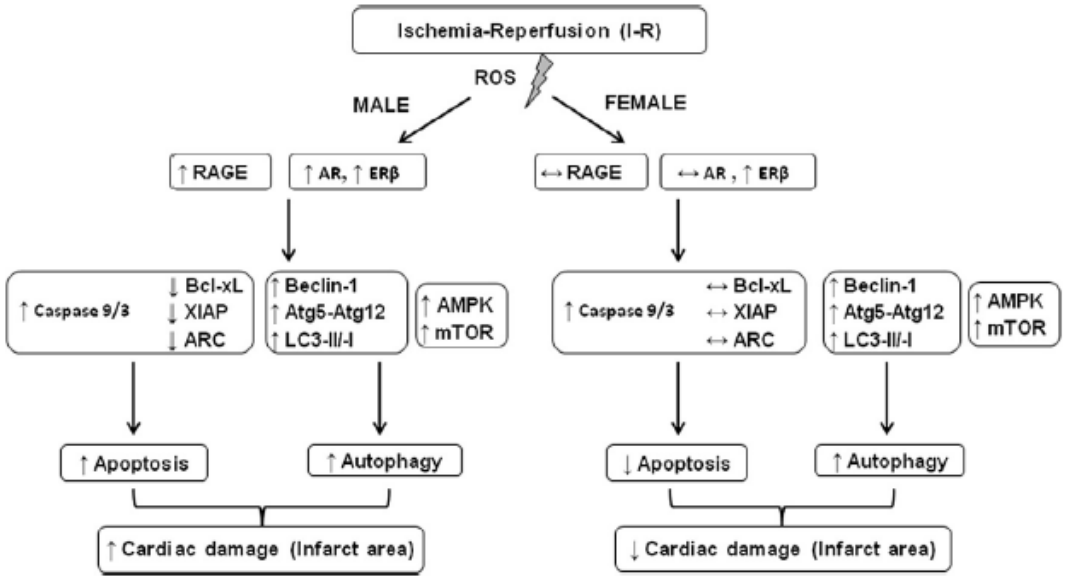


Fig 9 : Above Schematic summary of pathways activated during myocardial I-R injury in male and female myocardium. ROS, reactive oxygen species; RAGE, receptor for advanced glycation end products; AR, androgen receptors; ER, estrogen receptor; Caspase, cysteine-dependent aspartate-directed proteases; Bcl-xL, B-cell lymphoma-extra large; XIAP,X-linked inhibitor of apoptosis protein; ARC, apoptosis repressor with a caspase recruitment domain; Atg, autophagy-related gene; Beclin-1, known as Atg6; LC3,microtubule-associated protein light chain 3; mTOR mammalian target of rapamycin; AMPK, AMP-activated protein kinase ", increased , decreased; M, no changed. (Mihailidou & Ashton. 2014)

Therefore, higher prevalence of co-morbidities in women only partly explains the lack of decrease in mortality rates in younger women (Mihailidou & Ashton. 2014)

3.3.2 Clinical treatment result and attention

Determining the effect of gender on cardiac function will be difficult and require sophisticated methodologies. However, gender differences underlying predilection to distinct arrhythmia syndromes must be revealed so that new therapeutic strategies that take gender into account can be applied to at-risk patients. Although cardiac arrhythmia had long been considered a predominantly male syndrome, it is now clear that

arrhythmia is also a primary cause of mortality in women. Notably, the manifestation of specific arrhythmia syndromes appears to be sex specific. In particular, female sex is an independent risk factor for development of torsade de pointes (TdP) arrhythmias not only in congenital long QT syndromes but also in acquired long QT syndromes which occur as adverse effects of existing drugs. (Kurokawa et al. 2012) Atrioventricular nodal reentry tachycardia has a 2:1 female-to-male predominance, while accessory pathways are twice as frequent in men. (Wolbrette et al. 2002) Although atrial fibrillation is more prevalent in men of all age groups, the absolute numbers of men and women with atrial fibrillation are equal, and the associated morbidity and mortality experienced by women with atrial fibrillation appear to be worse. Women have a lower incidence of sudden cardiac death, and female survivors of sudden cardiac death have a lower frequency of spontaneous or inducible ventricular tachycardia. On the other hand, drug-induced torsade de pointes and symptomatic long QT syndrome have a female predominance. Therefore, greater caution should be used when prescribing QT-prolonging drugs in women. The incidence of arrhythmias is increased during pregnancy, and management of pregnant patients poses a significant challenge. The mechanisms of these gender differences are unclear but may be related to hormonal effects and the shorter QT interval in adult males. Pharmacologic and nonpharmacologic therapies are usually equally efficacious, but the risks of pharmacologic therapy are different in men and women. Atrial fibrillation may be more difficult to treat in women. (Wolbrette et al. 2002)

A clear trend to a higher incidence of bleeding complications has been consistently reported in women, which might be related to a more frequent over-dosage of antithrombotic treatment in women than in men. (Gutiérrez-Chico & Mehilli. 2013) Women are therefore one of the subgroups that might benefit the most from careful dose adjustment of available antithrombotic drugs. Women with Atrial fibrillation (AF) are more likely to develop stroke than men. Anticoagulants are less frequently used in women although women seem to receive a similar benefit. Studies suggest that women are referred to interventional therapies later than men and often with a more complex presentation. (David & Christine. 2010) Women are at higher risk than men for AF-related thromboembolism off warfarin. Warfarin therapy appears be as effective in women, if not more so, than in men, with similar rates of major hemorrhage. Female sex is an independent risk factor for thromboembolism and should influence the decision to use anticoagulant therapy in persons with AF. (Fang et al. 2005)

3.3.3 Sex-specific in therapeutic result of defibrillation

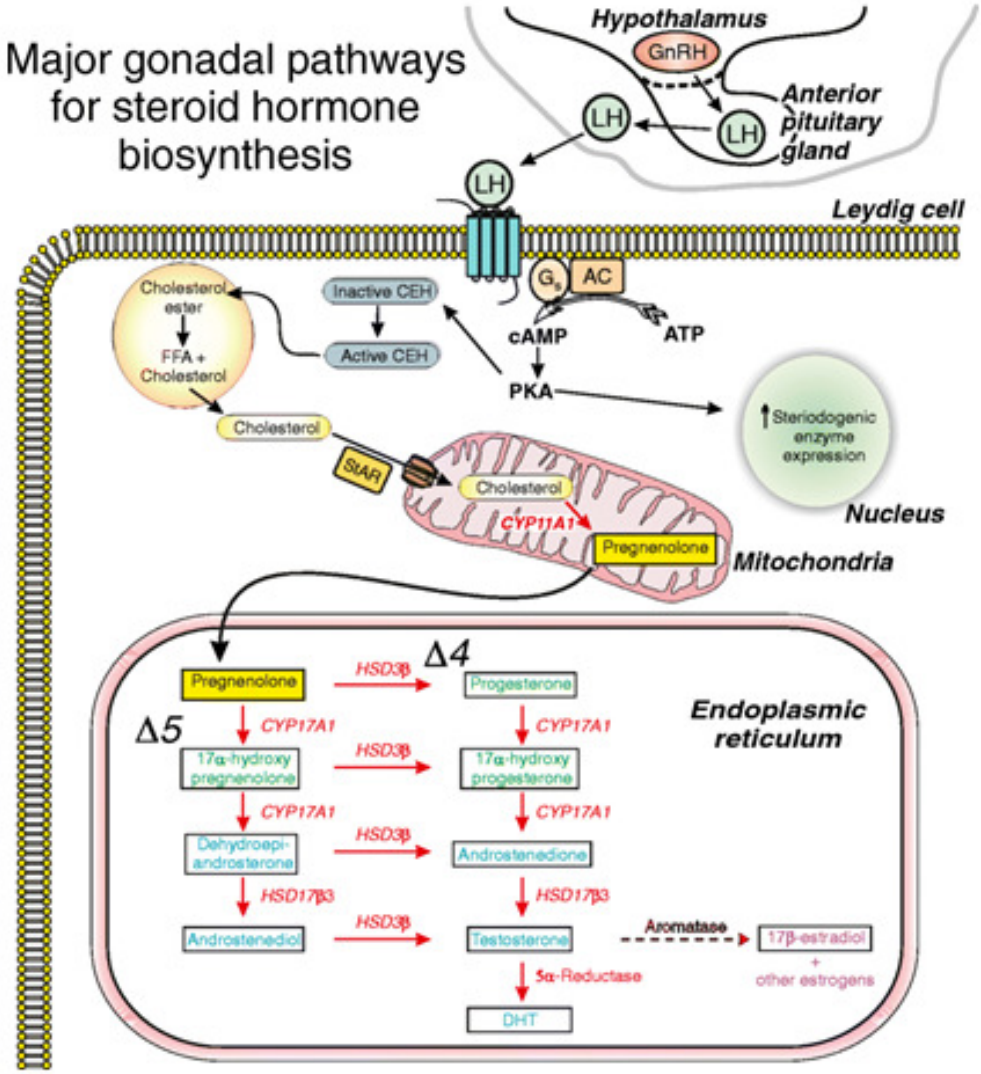
Baseline brain natriuretic peptide (BNP) was found to be an independent predictor of atrial fibrillation (AF) recurrence in male patients undergoing ablation. This correlation between BNP and AF recurrence was not observed in females. (Mohanty et al. 2011) Thus, BNP plays a sex-specific prognostic role in AF. Based on current available data, mortality is greater for women with AF than for men with AF. Women with atrial fibrillation AF have a higher risk of stroke compared with their male counterparts. Women tend to be referred for AF ablation less and later than are men. (Michelena et al. 2010)

Concerning another defibrillation way, there is a higher preponderance of non-ischemic cardiomyopathy (NICM) in women, and most of the ischemic cardiomyopathy (ICM) literature is derived from sub-study analysis. (Mehta et al. 2015) The sex differences in device therapies for ventricular arrhythmias or death in the multicenter automatic defibrillator Implantation trial with cardiac resynchronization therapy (MADIT-CRT) trial revealed that women with ischemic heart disease and women with left bundle branch block (LBBB) who received cardiac resynchronization therapy-defibrillator device (CRT-D) had the lowest incidence of ventricular tachycardia/ ventricular fibrillation (VT/VF) or death when compared to men. The risk of stroke after left ventricular assist device (LVAD) implantation varies based on sex, with a higher risk in female patients. (Morris et al. 2015)

4. Investigate possible mechanisms of sex hormones effect cardiovascular system

The mechanisms of sex differential pathophysiology of cardiovascular system between women and men that can be the following theories or hypotheses because some unclear mechanisms may need reference animal model experiment to hypothesize human being condition. We need to introduce the relationship between cardiovascular system and two major sex hormones (male testosterone and female estrogen) then research their possible mechanisms. Major gonadal pathways for testosterone and estrogens biosynthesis (Fig 10) reveal that testosterone is converted to dihydrotestosterone (DHT) by 5α-reductase, and some are aromatized to 17ß-estradiol. (Ayaz & Howlett. 2015) Male-female differences in intracellular Ca2+ release and contraction in isolated ventricular myocytes. Growing evidence suggests that these differences arise from effects of sex steroid hormones on processes involved in intracellular Ca2+ homeostasis. Myocardial contractile function is modified by testosterone, with a focus on the impact of testosterone on processes that regulate Ca2+ handling at the level of the ventricular myocyte. The idea that testosterone regulates

Ca2+ handling in the heart is important, as Ca2+ dysregulation plays a key role in the pathogenesis of a variety of different cardiovascular diseases. (Ayaz & Howlett. 2015) It also demonstrates that the enzyme aromatase can convert testosterone to the primary estrogen, 17ß-estradiol. This is a minor pathway in the Leydig cell. Interestingly, aromatase also is present in a number of extragonadal sites including adipose tissue, bone, and the brain in both men and women. Furthermore, cardiac-specific expression of aromatase has recently been shown in the adult mouse heart. Conversion of circulating testosterone to 17ß-estradiol by these tissue-based aromatase pathways may increase 17ß-estradiol levels under conditions such as obesity. Furthermore, certain anabolic steroids, including testosterone esters as well as nortestosterone derivatives (e.g., nandrolone decanoate and nandrolene phenpropionate), can be aromatized to estradiol. Indeed, some anabolic steroid users take anti-estrogens to minimize adverse effects (e.g., gynecomastia) associated with the aromatization of testosterone derivatives to estradiol.



(Fig 10 : Major gonadal pathways for testosterone and estrogens biosynthesis. Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus releases luteinizing hormone (LH) from the pituitary. LH binds to LH receptors on Leydig cells, stimulates G_s, and activates the cAMP/protein kinase A (PKA) pathway. PKA promotes the transport of cholesterol into mitochondria and increases transcription of genes involved in testosterone biosynthesis. Cholesterol is converted to pregnenolone, which diffuses into the endoplasmic reticulum for testosterone biosynthesis via Δ^4 and Δ^5 pathways. Testosterone is formed by 17β-hydroxysteroid dehydrogenase 3 (HSD17β3) in the Δ^4 pathway and by 3β-hydroxysteroid dehydrogenase (HSD3β) in the Δ^5 pathway. Testosterone is converted to dihydrotestosterone (DHT) by 5α-reductase, and some are aromatized to 17ß-estradiol. (Ayaz & Howlett. 2015)

Therefore, the effects of testosterone supplementation with derivatives that can be aromatized may actually be due, at least in part, to estradiol rather than androgen. As a consequence, some studies of the effect of androgens on the cardiovascular system use the non-aromatizable androgen DHT, rather than testosterone or its derivatives. (Shilling & Williams. 2000 ; Rutherford et al. 2015) There is evidence that circulating testosterone decreases with age in both men and women. (Ayaz & Howlett. 2015) In aging men, the fall in serum testosterone is largely due to a decrease in the ability of Leydig cells to produce testosterone in response to LH. This arises as a result of age-associated attenuation of the cyclic adenosine monophosphate/ protein kinase A (cAMP/PKA) pathway, leading to less transfer of cholesterol into the mitochondria and a reduction in the production of steroidogenic enzymes. Interestingly, a similar mechanism has been proposed to lead to the age-dependent decrease in testosterone levels in male rats. In women, the age-dependent decline in circulating testosterone is thought to result from a combination of events including ovarian failure plus a reduction in the adrenal production of androgens. Thus, the aging process reduces the amount of testosterone available to interact with androgen receptors in animal models. The action potential duration (APD) is prolonged in the absence of testosterone (Fig 11), an effect mediated by a decrease in magnitude of the repolarizing K+ current, IKur, at least in rodent models. This reduction in IKur is secondary to a decrease in the expression of voltage-gated potassium channel subtype 1.5 (Kv1.5). Ca2+ transients also are smaller and slower in ventricular myocytes from gonadectomy (GDX) animals when compared to sham-operated controls, especially when cells are paced at physiological rates. The evidence reviewed here suggests that chronic testosterone withdrawal influences cardiac Ca^{2+}-handling mechanisms in ventricular myocytes.

Sex hormone regulation of myocardial Ca2+ homeostasis may reveal new targets for the treatment of cardiovascular diseases in all older adults. The idea that testosterone regulates the cardiac action potential and Ca2+ homeostasis at the level of the individual heart cell has a number of important clinical implications. For example, men have faster rates of repolarization than women, and castrated men have prolonged repolarization while the reverse is seen in women with abnormally high levels of testosterone.

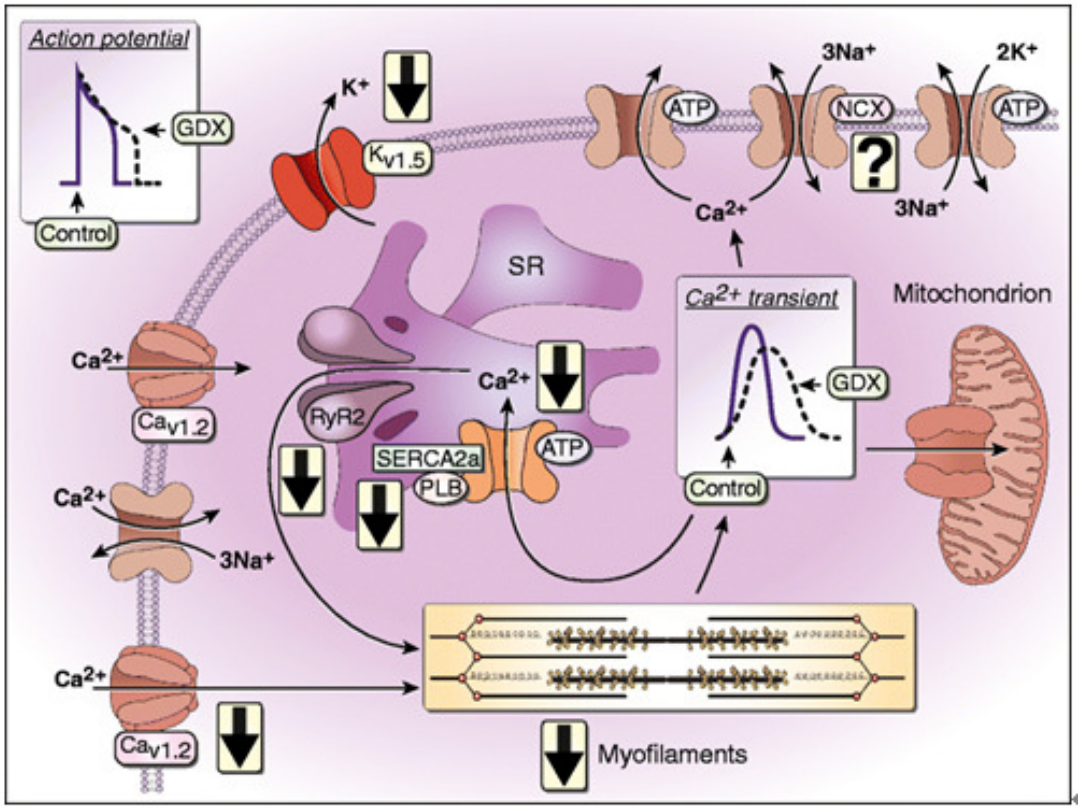


Fig 11 : Impact of GDX on intracellular Ca^{2+}-handling mechanisms in ventricular myocytes isolated from rodent hearts. APD is prolonged by GDX, due to a decrease in repolarizing K^+ currents (I_{Kur}) and a reduction in the expression of Kv1.5. Reduced Ca^{2+} influx along with smaller Ca^{2+} sparks attenuates SR Ca^{2+} release. Ca^{2+} transient decay is slowed by longer APs and slower SR Ca^{2+} uptake mediated by a decrease in phosphorylation of PLB by CaMKII (and possibly PKA). Peak contractions are attenuated through smaller peak Ca^{2+} transients and a decrease in maximal myofilament responsiveness to Ca^{2+}. Contractions are slowed because SR Ca^{2+} uptake is reduced and the slower β-MHC isoform predominates. Whether NCX activity or expression is affected by GDX is not yet clear. ; NCX : Bidirectional Na^+/Ca^{2+} exchanger ; GDX : Gonadectomy. (Ayaz & Howlett. 2015)

This is consistent with evidence that GDX increases APD in individual myocytes in animal models. This may be clinically important as prolongation of the action potential (AP) can increase the probability of early after depolarizations, which can trigger arrhythmias such as torsades des pointes. Furthermore, it is well known that levels of testosterone decline with age, at the same time as the incidence of cardiovascular disease rises. Modifications in myocardial Ca2+ handling and contraction linked to falling testosterone levels in older adults are likely to interact with diseases in the aging heart. For example, the observation that contractions and Ca2+ transients decline in low-testosterone states may promote heart failure with reduced ejection fraction. Intracellular Ca2+ dysregulation also is implicated in the pathogenesis of diseases such as myocardial ischemia and arrhythmias (Doroudgar & Glembotski. 2013) where a decrease in testosterone may influence disease expression. Improved understanding of the cellular mechanisms involved in the effects of testosterone on the heart may reveal mechanisms involved in the increase in susceptibility to cardiovascular diseases in aging and may ultimately help identify new targets for intervention in the treatment of these diseases in both men and women.

The risk for women is misperceived because of the strong conviction that females are “protected” against cardiovascular disease. (Vassalle et al. 2009) This conviction rises from the fact that women are effectively at lower risk of cardiovascular events with respect to men during their first decades of life, being protected by estrogen action in their premenopausal life (Fig. 12).

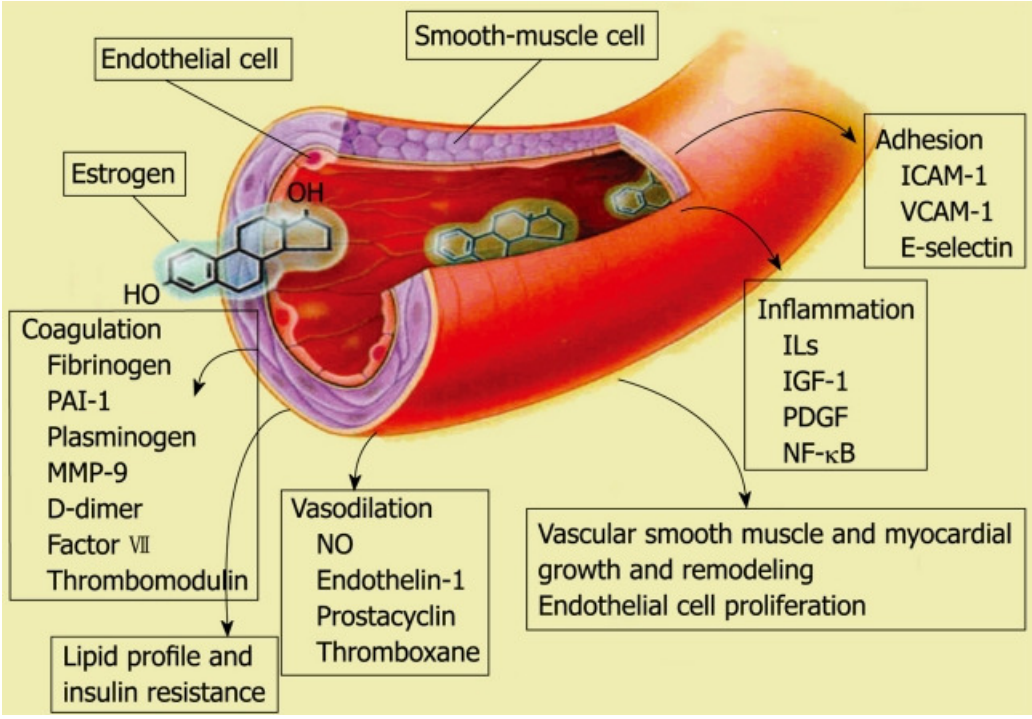


Fig. 12 : Multiple effects of estrogen on the cardiovascular system. (Vassalle et al. 2009) Available from: URL: http://www.ehealthspan.com.

The incidence of cardiovascular disease (CVD) is significantly higher in postmenopausal women when compared to those in the same age. In particular, menopausal status appears to enhance the development of CVD through several unfavourable changes in metabolism and hemodynamic parameters. The mechanisms of gender differential pathophysiology of cardiovascular system between women and men that can be the following theories or hypotheses. The rapid signaling cascade activation and long term respond are the two major pathways revealing how sex hormone to effect cardiovascular system whose results can be found out from human being. However, some unclear mechanisms may need reference animal model experiment to hypothesize human being condition.

4.1 Non-genomic and / or genomic signal pathways

Clinical and basic research has demonstrated that estrogen has a dramatic impact on the response to vascular injury and the development of atherosclerosis. Further work has indicated that this is at least estrogen partially mediated by an enhancement in nitric oxide (NO) production (Fig. 13) by the endothelial isoform of NO synthase (eNOS) due to increases in both eNOS expression and level of activation. (Chambliss & Shaul. 2002). Hydrogen sulfide-mediated cardioprotection (Fig. 14) was also mention by some authors (Lavu et al. 2011). Nowadays, estrogen may involve not only NO production but also H2S-mediated cardioprotection. The combination of H2S and NO cooperatively (Fig. 18) regulate vascular tone will be more noticed by HNO-TRPA1-CGRP signaling pathways. (Eberhardt et al. 2014). We are going to discuss the three pathways individually.

4.1.1 Endothelial nitric oxide synthase (eNOS) activation pathway model

The momentum of evidence in both human studies and animal models indicates that estrogen has potent stimulatory effects on eNOS expression and activity in vascular endothelium. Estrogen has potent nongenomic effects on eNOS activity mediated by a subpopulation of ERα localized to caveolae in endothelial cells, where they are

coupled to eNOS in a functional signaling module (Fig. 13). Emphasizing the dependence on cell surface-associated receptors, these observations provide evidence for the existence of a steroid receptor fast-action complex (SRFC), in caveolae. Estrogen binding to ERα within caveolae leads to Gαi activation, which mediates downstream events. The downstream signaling includes activation of tyrosine kinase-MAPK and Akt/protein kinase B signaling, stimulation of HSP90 binding to eNOS, and perturbation of the local calcium environment, ultimately leading to eNOS phosphorylation and calmodulin-mediated eNOS stimulation. The resulting combination of genomic and nongenomic mechanisms by which estrogen modulates eNOS plays a critical role in vascular health. The current and future challenges in this area of research are numerous. The mechanisms by which estrogen up-regulates eNOS expression warrant further study. In addition to eNOS, estrogen modifies the expression of other endothelial cell genes such as cyclooxygenase type 1 (Jun et al. 1998), and the processes underlying these effects are yet to be determined. Novel endothelial cell gene targets should also be sought. Our knowledge of the nongenomic basis of estrogen action in endothelial cells is also currently limited. Although immunocytochemical analyses suggest that the ligand binding domain of cell surface-associated ERα may be extracellular (Razandi et al. 1999), the orientation of plasma membrane ERα is yet to be elucidated. The mechanisms by which ERα is membrane associated and the processes regulating the relative number of cell surface ERα are not well understood. The basis for ERα-Gαi interaction, which may involve a classical G-protein coupled receptor (GPCR) or an alternative intermediate protein, is also entirely unknown. Furthermore, the proximal signal transduction events following G protein alpha i subunit (Gαi) activation deserve in-depth study.

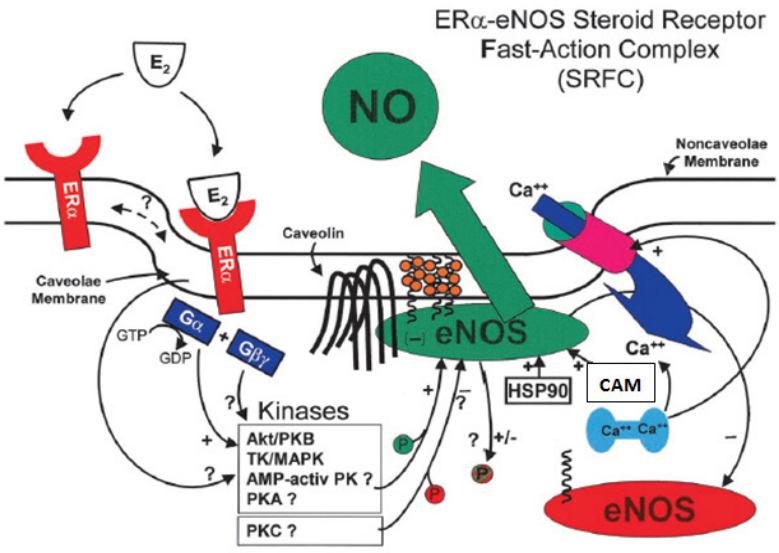


Fig. 13 : Estrogen activation of eNOS involves ERα1 coupling to the enzyme in a SRFC (steroid receptor fast-action complex) in endothelial cell caveolae. eNOS localization to cholesterol-enriched (*orange circles*) caveolae is based on the myristoylation and palmitoylation of the protein (*wavy lines*), and within caveolae eNOS interaction with caveolin attenuates the activity of the enzyme. A subpopulation of ERα1 has also been localized to endothelial cell caveolae, and ERα1 is also found in noncaveolae membranes. Estrogen binding to ERα1 leads to Gαi activation, which mediates downstream events. The downstream signaling includes activation of tyrosine kinase-MAPK and Akt/protein kinase B (PKB) signaling, stimulation of Heat shock protein 90 (HSP90) binding to eNOS, and perturbation of the local calcium environment, ultimately leading to eNOS phosphorylation and calmodulin (CAM)-mediated eNOS stimulation. Soon after E2 exposure, eNOS translocates from the membrane to intracellular sites, resulting in diminished NOS activity (change from *green* to *red*). The potential roles of other kinases and other phosphorylation /dephosphorylation events are yet to be clarified, and the mechanisms dictating the localization of ERα1 to plasma membrane domains are also currently unknown. Nitric oxide synthase (NOS) expressed within the vascular wall is a target of estrogen action. Under normal conditions in younger women, the primary product of estrogen action is NO, which produces a number of beneficial effects on vascular biology. As a woman ages, however, there is evidence for loss of important molecules essential for NO production (e.g., tetrahydrobiopterin, l-arginine). As these molecules are depleted, NOS becomes increasingly "uncoupled" from NO production, and instead produces superoxide, a dangerous reactive oxygen species. A similar uncoupling and reversal of estrogen response occurs in diabetes. However, it is the biochemical environment around NOS that will determine whether estrogen produces a beneficial (NO) or deleterious (superoxide) product, and can account for this dual and opposite nature of estrogen pharmacology. Further, this molecular mechanism is consistent with recent analyses revealing that HRT produces salutary effects in younger women, but mainly increases the risk of cardiovascular dysfunction in older postmenopausal women. (Chambliss & Shaul. 2002)

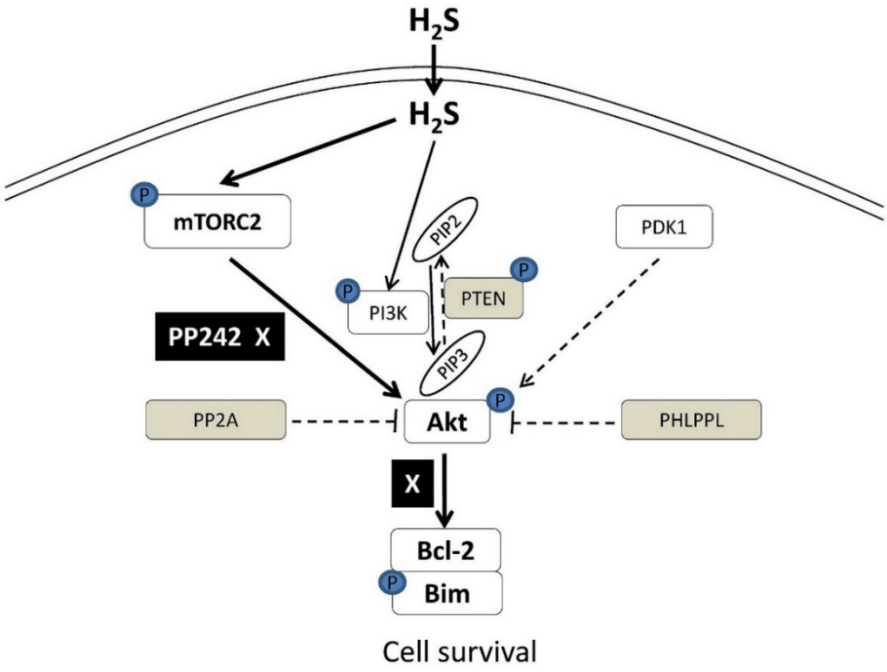
The eNOS modulation by estrogen typifies but a fraction of what may ultimately be a total of four categories of ER action. These categories are (1) membrane-initiated, nongenomic actions; (2) membrane-initiated, genomic actions; (3) non-membrane-initiated, nongenomic actions; and (4) non-membrane-initiated, genomic actions. When one considers that there are not only ER-dependent but also ER-independent

mechanisms of estrogen response in certain paradigms, there are actually eight categories of possible estrogen action.

4.1.2 Hydrogen sulfide-mediated cardioprotection

Hydrogen sulfide (H_2S) was first identified in 1996 as an important endogenous regulator of a wide range of cell functions. Discoveries on the endogenous synthesis of H_2S in the mammalian system and its protective role in combating cellular necrosis, apoptosis, oxidative stress, inflammation as well as promoting angiogenesis and modulation of mitochondrial respiration in the setting of myocardial ischemia and reperfusion injury have prompted vast interest in the possibility of developing new therapies based around mimicry or facilitation of endogenous H_2S for cardioprotection. These observations have inspired rapid development of H_2S-releasing drugs in hopes of swift clinical translation in patients with cardiovascular disease. The protective signaling pathways elicited by H_2S in the heart with an emphasis on the versatile benefits of this gasotransmitter and its potential for clinical translation in patients with cardiovascular disease. (Salloum et al. 2015) In the cardiovascular system H_2S produces three important effects. First, it induces the relaxation of isolated blood vessels and serves as an in vivo regulator of blood pressure. Second, it has negative chronotropic and inotropic effects on heart muscle. Third, H_2S potently protects against ischemia/reperfusion (I/R) injury in myocytes, in isolated hearts and in intact animals. (Salloum et al. 2009) The activation of myocardial Akt is an important mediator of this ischemic cardioprotection. Binding of Phosphoinositide 3, 4, 5 trisphosphate (PIP3), the down-stream product of PI3K, to Akt recruits Akt to membranes where it is subsequently phosphorylated by other kinases. As mTORC2 also phosphorylates Akt, it may be an unrecognized contributor to H_2S cardioprotection. Other potential modulators of Akt activity include (1) the tyrosine phosphatase Phosphatase and Tensin homolog (PTEN) which regulate Akt activity through dephosphorylation of phosphoinositide PIP3 down-stream of Phosphoinositide 3-kinase (PI3K), (2) 3-phosphoinositide dependent protein kinase-1 (PDK1), and (3) PH domain and leucine rich repeat protein phosphatases 2 (PHLPPL or PHLPP2) and protein phosphatase 2A (PP2A) which dephosphorylate and inhibit Akt. (Fig 14) While Akt activation is critical for ischemic cardioprotection, the downstream targets for Akt in this setting remain unresolved. Increasing experimental evidence shows that the Bcl-2 family is a critical mediator of cardiac ischemia/reperfusion injury through activation of myocyte apoptotic signaling. It is not clear whether Akt activated by H2S during ischemia/reperfusion (I/R) might regulate Bcl-2 and Bim which would decrease

apoptosis and thereby contribute to cardioprotection. (Zhou et al. 2014) The mTORC2 can activate Akt in ischemic hearts treated with H2S, and that inhibition of Bim signaling coupled with an increase in Bcl-2 may be intrinsic to the molecular mechanisms of H2S cardioprotection.



(Fig. 14 : A schematic diagram illustrates the mechanisms of H2S-induced cardiac protection. In addition to PI3K regulation, H2S increases cell survival through mTORC2-mediated activation of Akt/Bim and Bcl-2 pro-survival cell signaling pathway. PI3K generates PIP3 from PIP2. PIP3 recruits Akt to the membrane and where Akt is phosphorylated by mTORC2. Solid lines indicate positive and dotted lines negative findings in H2S-induced cardioprotection. PP242 inhibits mTORC2 induced Akt phosphorylation, B-cell lymphoma 2 (Bcl-2) expression and Bim phosphorylation. (Zhou et al. 2014); doi:10.1371/journal.pone.0099665.g008

Discoveries during the last decade on the endogenous synthesis of H_2S in the mammalian system and its protective role in combating cellular necrosis, apoptosis, oxidative stress, inflammation as well as promoting angiogenesis and modulation of mitochondrial respiration in the setting of myocardial ischemia and reperfusion injury have prompted vast interest in the possibility of developing new therapies based around mimicry or facilitation of endogenous H_2S for cardioprotection. (Salloum 2015) These observations have inspired rapid development of H_2S-releasing drugs in hopes of swift clinical translation in patients with cardiovascular disease. H_2S preconditioning

produced delayed cardioprotection against lethal ischemia. Some authors found that neuropeptide substance P (SP)-induced protein kinase C (PKC) isoform activation accelerates the rectification of elevated $[Ca^{2+}]_i$ and thereby increases the susceptibility of cardiomyocytes to ischemia-induced Ca^{2+} overload and consequent series of damages induced by lethal ischemia-reperfusion insults (Fig. 15). These findings disclose a novel mechanism for SP-induced cardioprotection and also provide considerable implication for other PKC-related anti-ischemia interventions. Upon stimuli, PKC isoforms translocate from the cytosol to subcellular membrane regions, a process associated with their activation. Such translocation has been deemed as a hallmark of PKC activation. However, it is completely unknown whether SP can stimulate PKC activation and what the even downstream effects are. PKC activation has been reported to play a role in regulating intracellular calcium handling. (Pan et al .2008) Under the physiological condition, intracellular calcium concentration ($[Ca^{2+}]_i$) is sophisticatedly regulated by several proteins present in the sarcolemmal and sarcoplasmic reticulum (SR) membranes.

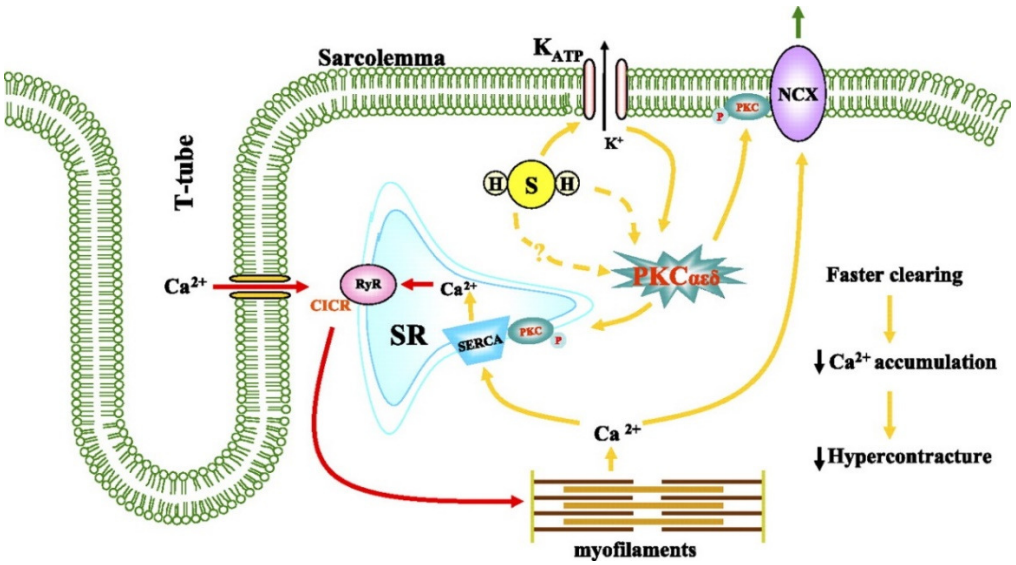


Fig 15 : Proposed signaling pathway for SP-induced cardioprotection (yellow route). H_2S activates different PKC isoforms directly (dashed line) or indirectly through the opening of K_{ATP} or other unknown mechanisms (dashed line). The activated PKC isoforms stimulate the Ca^{2+} handling proteins (i.e., NCX and SERCA) and thereby facilitate the clearing of cytosolic Ca^{2+}. During ischemia, the faster clearing of cytosolic Ca^{2+} induced by SP attenuates the Ca^{2+} accumulation and reduces hypercontracture. (Pan et al .2008); Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) ; Na^+/Ca^{2+} exchanger (NCX).

Upon the arrival of action potential, Ca^{2+} influxes through the L-type Ca^{2+} channel and triggers the opening of the ryanodine receptor (RyR), resulting in further release of Ca^{2+} from the SR, which accomplishes the sharp $[Ca^{2+}]_i$ elevation required for myofibril contraction. In the rat cardiomyocytes, >90% of the Ca^{2+} after contraction is immediately reuptaken by SR via sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), whereas the remaining Ca^{2+} is pumped out of the cell via Na^{+}/Ca^{2+} exchanger (NCX).However, the well-controlled intracellular Ca^{2+} homeostasis is vulnerably disrupted with the advent of ischemia and reperfusion insults. (Pan et al .2008) During ischemia, excessive Ca^{2+} accumulates in the cytosol and leads to a series of severe damages upon reperfusion. For example, once the myofilaments are reenergized by reperfusion, they contract intensely in an extreme and sustained manner (hypercontracture) due to overstimulation of calcium on the contractile apparatus. In single cardiomyocytes, such hypercontracture causes irreversible shortening of the cell length. In tissues, it causes a disruptive change in the myocardium termed contraction band necrosis. (Pan et al .2008) Under this circumstance, a faster clearing of excessive Ca^{2+} from cytosol is therapeutically important because it would potentially attenuate Ca^{2+} overloading during ischemia challenge, reduce the myocyte hypercontracture, and preserve the cardiac function. Since PKC is implicated in the intracellular Ca^{2+} handling, it is worthwhile investigating whether H_2S produces any effect on Ca^{2+} handling, given PKC is activated after SP. Therefore, Hydrogen sulfide, generated in the myocardium predominantly via cystathionine-γ-lyase (CSE), is cardioprotective. Some authors' study has shown that estrogens enhance CSE expression in myocardium of female rats. (Wang et al. 2015)

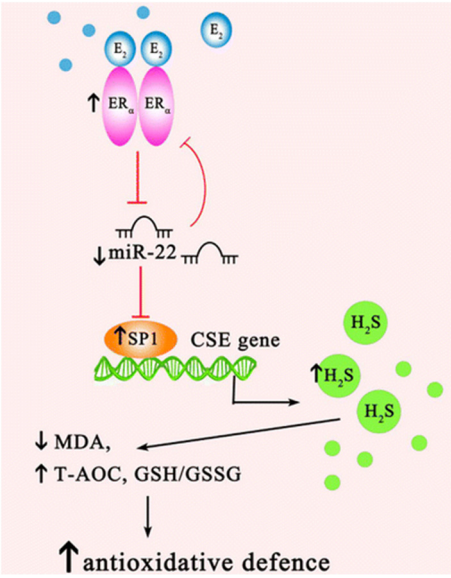


Fig. 16 : A graphical illustration for the possible mechanisms underlying estrogen up-regulating CSE expression, which contributes to estrogenic protection against oxidative stress in cardiomyocytes. Both ERα and Sp-1 are targeted by miR-22; therefore, the down-regulation of miR-22 results in increases of ERα and Sp-1. Estrogens down-regulates miR-22 expression via ERα, thus leading to the up-regulation of Sp-1 expression in cardiomyocytes. Transcriptional factor Sp-1 acts to increase CSE expression as well as H2S production, which finally leads to an increase of antioxidative defense in myocardium. (Wang et al. 2015)

To explore the mechanisms by which estrogens regulate CSE expression, in particular to clarify the role of estrogen receptor subtypes and the transcriptional factor responsible for the estrogenic effects. They found that either the CSE inhibitor or the CSE small interfering RNA attenuated the protective effect of 17β-estradiol (E2) against H2O2- and hypoxia/reoxygenation-induced injury in primary cultured neonatal cardiomyocytes.(Wang et al. 2015) E2 stimulates CSE expression via estrogen receptor (ER)-α both in cultured cardiomyocytes in vitro and in the myocardium of female mice in vivo. A specificity protein-1 (Sp-1) consensus site was identified in the rat CSE promoter and was found to mediate the E2-induced CSE expression. E2 increases ERα and Sp-1 and inhibits microRNA (miR)-22 expression in myocardium of ovariectomized rats. In primary cardiomyocytes, E2 stimulates Sp-1 expression through the ERα-mediated down-regulation of miR-22. It was confirmed that both ERα and Sp-1 were targeted by miR-22. In the myocardium of ovariectomized rats, the level of miR-22 inversely correlated to CSE, ERα, Sp-1, and antioxidant biomarkers and positively correlated to oxidative biomarkers. (Wang et al. 2015) In summary, this study demonstrates that estrogens stimulate Sp-1 through the ERα-mediated down-regulation of miR-22 in cardiomyocytes, leading to the up-regulation of CSE, which in turn results in an increase of antioxidative defense. (Fig.16) Interaction of ERα, miR-22, and Sp-1 may play a critical role in the control of oxidative stress status in the myocardium of female rats.

4.1.3 H_2S-NO-HNO–TRPA1–CGRP pathway

Hydrogen sulfide (H_2S) is a unique gasotransmitter, with regulatory roles in the cardiovascular, nervous, and immune systems. However, some of the vascular actions of H_2S (stimulation of angiogenesis, relaxation of vascular smooth muscle) resemble those of nitric oxide (NO). Although it was generally assumed that H2S and NO exert

their effects via separate pathways, the results of the current study show that H2S and NO are mutually required to elicit angiogenesis and vasodilatation. Exposure of endothelial cells to H2S increases intracellular cyclic guanosine 5'-monophosphate (cGMP) in a NO-dependent manner, and activated protein kinase G (PKG) and its downstream effector, the vasodilator-stimulated phosphoprotein (VASP). Inhibition of endothelial isoform of NO synthase (eNOS) or PKG-I abolishes the H2S-stimulated angiogenic response, and attenuated H2S-stimulated vasorelaxation, demonstrating the requirement of NO in vascular H2S signaling. Conversely, silencing of the H2S-producing enzyme cystathionine-γ-lyase abolishes NO-stimulated cGMP accumulation and angiogenesis and attenuates the acetylcholine-induced vasorelaxation, indicating a partial requirement of H2S in the vascular activity of NO. The actions of H2S and NO converge at cGMP; though H2S does not directly activate soluble guanylyl cyclase, it maintains a tonic inhibitory effect on Phosphodiesterase 5 (PDE 5) , thereby delaying the degradation of cGMP. H2S also activates PI3K/Akt, and increases eNOS phosphorylation at its activating site Ser1177. (Coletta et al. 2012) The cooperative action of the two gasotransmitters on increasing and maintaining intracellular cGMP is essential for PKG activation and angiogenesis and vasorelaxation. H2S-induced wound healing and microvessel growth in matrigel plugs is suppressed by pharmacological inhibition or genetic ablation of eNOS. Thus, NO and H2S are mutually required for the physiological control of vascular function.

Therefore, H_2S can also react with NO to give nitrosyl hydride (HNO) (Fig. 17), and demonstration that H_2S effects could be diminished by either blocking NOS activity or deleting transient receptor potential channel A1 (TRPA1) or calcitonin gene-related peptide (CGRP) are in favour of a new signalling pathway for cardiovascular control. In addition, co-expression of TRPA1 and cystathionine-beta-synthase (CBS) in small to medium-sized sensory neurons and axons together with the recent demonstration of co-expression of TRPA1 and nNOS suggest a structural and functional organization for constitutive HNO generation and subsequent activation of TRPA1-dependent CGRP release. This functional unit is of importance in the regulation of peripheral blood flow (as demonstrated in dura mater and brainstem) and even of systemic blood pressure. In addition, positive inotropic and lusitropic cardiac effects of circulating and/or paracrine CGRP have been reported. The positive inotropic and lusitropic effects of HNO were originally ascribed to CGPR release and completely blocked by the use of CGRP receptor antagonist (Coletta et al. 2012)

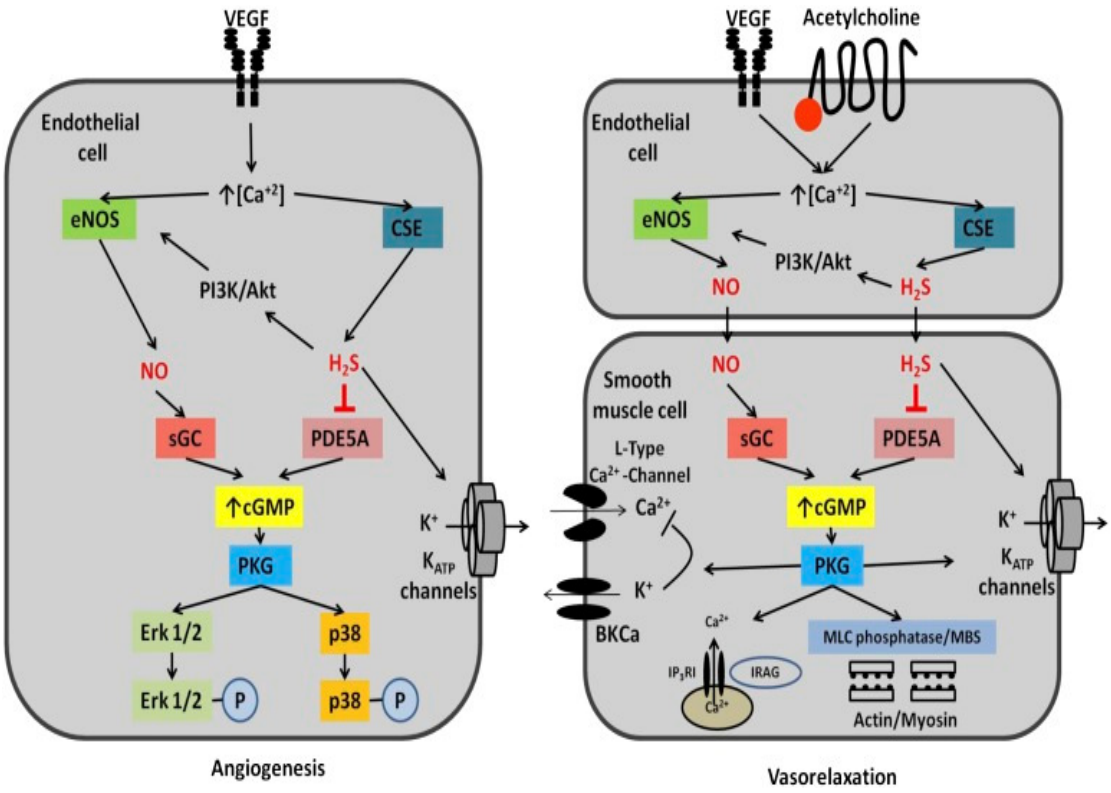


Fig 17 : Proposed pathways of H_2S and NO interaction. Cooperation between NO and H_2S in angiogenesis (*Left*) or endothelium-dependent vasorelaxation (*Right*). Binding of vascular endothelial growth factor (VEGF) or acetylcholine to its receptor on the endothelial cell mobilizes intracellular calcium and activates eNOS as well as CSE (two calcium-dependent enzymes), resulting in a simultaneous elevation of intracellular NO and H_2S levels in endothelial cells (in the context of angiogenesis) or in the smooth muscle cell (in the context of vasorelaxation). NO stimulates guanylyl cyclase, whereas endogenously produced H_2S maintains a tonic inhibitory effect on PDE5, thereby delaying the degradation of cGMP and permitting physiological cGMP signaling. These two simultaneous actions ensure that cGMP has a sufficiently long half-life to activate PKG to stimulate PKG-dependent downstream signaling such as ERK1/2 and p38 in the case of angiogenesis and myosin light chain (MLC) phosphatase (via its myosin-binding subunit, MBS), large-conductance calcium- and voltage-activated potassium channels (BK_{Ca}), and IP_3-R-–associated cG-kinase substrate (IRAG) in the case of smooth muscle relaxation. NO and H_2S are also known to activate K_{ATP} channels, which are also involved in angiogenesis and endothelium-dependent relaxation. (Coletta et al. 2012) ; PDE : phosphodiesterase)

These results explain the CGRP receptor-mediated positive inotropic effects that

have previously been reported from isolated trabecular muscles of the human heart. Also on rat cardiomyocytes, direct CGRP-mediated inotropic and lusitropic effects have recently been demonstrated. Thus, the failing heart may gain particular benefit from CGRP release, as coronary blood flow is increased and afterload (peripheral resistance) reduced by CGRP. Thereby, it would not really matter whether beneficial effects derive from circulating plasma levels of CGRP or from paracrine release from ubiquitous sensory nerves that accompany every peripheral blood vessel.

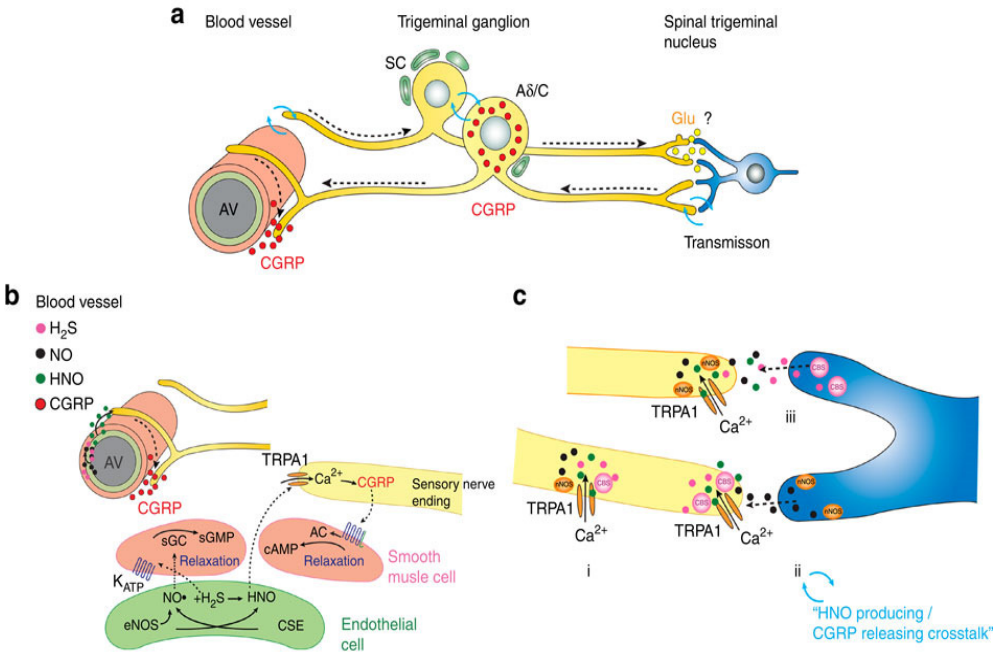


Fig. 18 : The trigeminal system as an example of CGRP-containing nerve fibres and its neuronal and vascular interaction sites. By means of diffusion of H_2S or NO, produced either in the endothelium or in neurons, there are several possibilities of their reaction leading to formation of HNO that targets the cysteines of TRPA1 in close vincinity. (**b**) TRPA1/CGRP expressing nerve endings in the periphery communicate with the smooth muscle cells surrounding the endothelium of blood vessels. Endothelial cells are known to produce NO and H_2S, both of which freely diffuse and activate guanylyl cyclase and K_{ATP} channels, respectively, to induce vasodilatation. However, H_2S and NO also react with each other to give HNO, which could reach paravascular TRPA1-expressing sensory nerve fibres, inducing Ca^{2+} influx and CGRP release. (**c**) Other potential sites of NO–H_2S interaction in neurons: (i) TRPA1 channels are co-expressed with nNOS and CBS in primary afferents forming a functional signalling complex that leads to confined HNO generation and TRPA1 gating upon activation of the gasotransmitter-generating enzymes. In addition, NO (ii) or H_2S (iii) could originate from either side of

a synaptic cleft (or from nearby axons of passage) and freely diffuse into adjacent neurons (or nerve fibres). There, they react with their counterpart producing HNO in vicinity of TRPA1, which leads to its activation, Ca^{2+} influx and release of CGRP. Apart from its vascular functions, CGRP acts as a co-transmitter, facilitating synaptic transmission, which may play a role in migraine headaches. Glu, glutamate; SC, satellite cells. (Eberhardt et al. 2014) TRPA1 : transient receptor potential channel A1 , CGRP : calcitonin gene-related peptide.)

All three, NO, H_2S and HNO are freely diffusible, so several possibilities could be envisioned for TRPA1 activation and CGRP release (Fig. 18) : (1) NO and H_2S produced in endothelium react to give HNO that could diffuse and activate nearby nerve endings expressing TRPA1 and releasing CGRP that relaxes vascular smooth muscles; (2) production of H_2S and NO from colocalized CBS and neuronal nitric oxide synthase (NOS) leads to intracellular HNO formation and TRPA1 activation; (3) taking into account that constitutive levels of NO in neurons are very low it is also plausible that, for instance in the CNS, astrocyte-derived NO, as a paracrine signal, meets with endothelial or neuronal H_2S, forming HNO, which activates TRPA1 in primary afferent peptidergic terminals. Recent work showed that the NO vasodilatory effect on aorta rings is partially blocked by inhibiting H_2S production, *vice versa* H_2S effects are diminished by inhibiting NO production, further strengthening the link between these gasotransmitters. Most of H_2S-induced vasodilation is directly dependent on its reaction with NO to form HNO, as well as on functional presence of TRPA1 and CGRP.

In above summary, sex hormones induce non-genomic and / or genomic signal pathways which can occur alone or both simultaneously. Sex-steroid hormones have been postulated as the major contributors towards these sex related differences. It can be also discussed that current evidence on gender differences in cardiovascular (CV) function and remodeling, and will present the different role of the principal sex-steroid hormones on female heart. The current knowledge concerning the role of sex hormones on the regulation of our daily activities throughout the life, via the modulation of autonomic nervous system, excitation–contraction coupling pathway and ion channels activity from estrogen (Fig.19) and androgens (Fig. 20), to effect female heart. (Salerni et al. 2015)

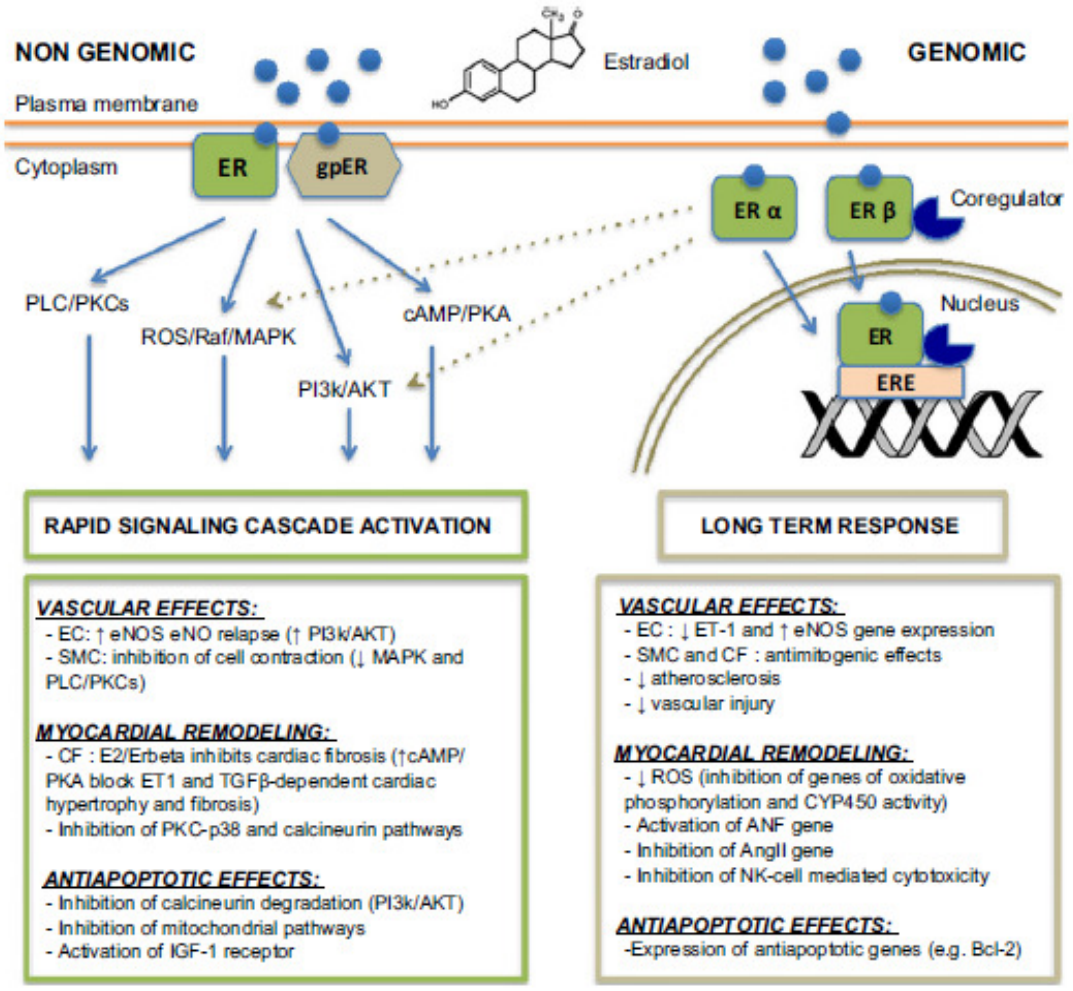


Fig. 19: Above figure means that genomic and nongenomic effects of 17b-oestradiol (E2) on vessels, myocardial remodelling and apoptosis. Dotted arrow: interaction of genomic and nongenomic pathways. ER, oestrogen receptor; gpER, transmembrane G proteincoupled oestrogen receptor; EC, endothelial cell; NO, nitric oxide; SMC,smooth muscle cell; CF, cardiac fibroblast; ROS, reactive oxygen species; ANF, atrial natriuretic factor gene; ET-1, endothelin-1; AngII, angiotensin II; NK, natural killer. (Salerni et al. 2015)

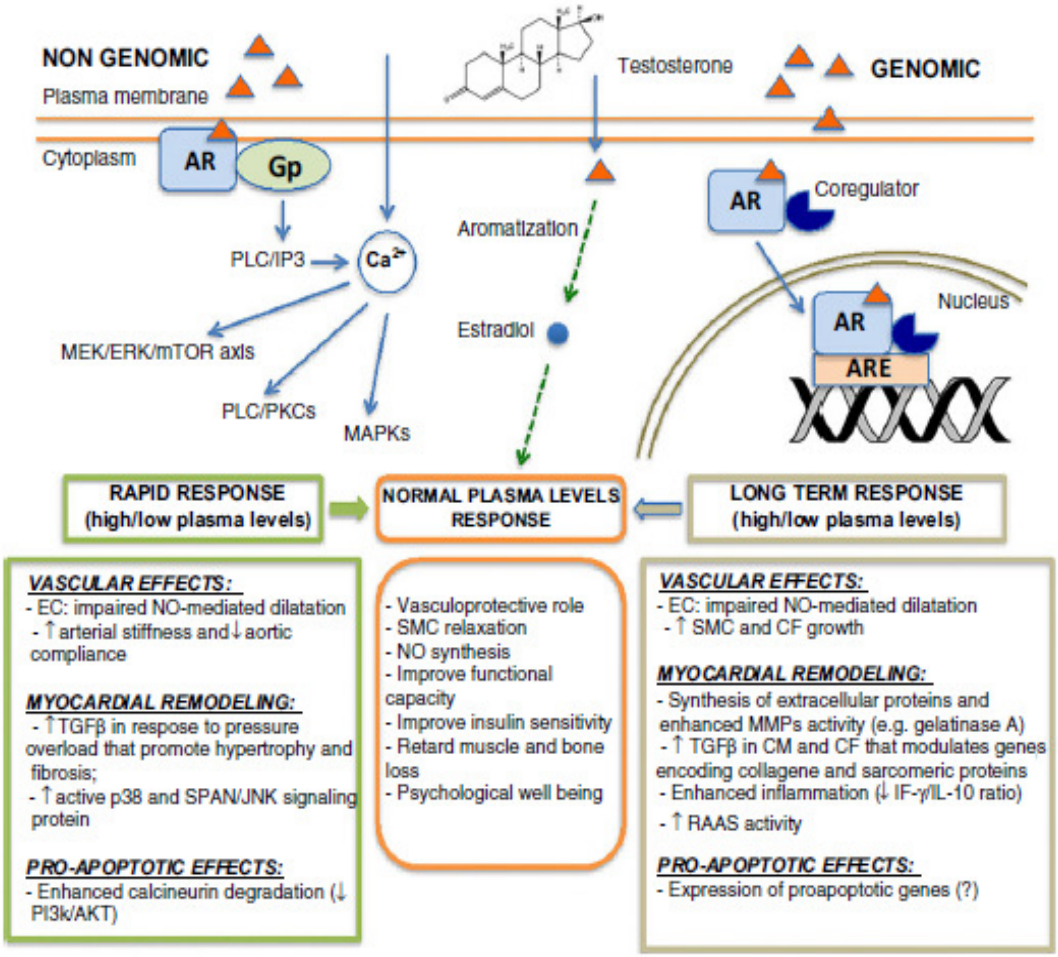


Fig. 20: Above figure means that lights and shadows on the effects of androgens on vessels, myocardial remodelling and apoptosis at different plasma concentrations in females (nongenomic and genomic androgen responses). Dotted arrow: one of the possible vasculoprotective effects of androgens is mediated by aromatization in oestradiol. AR, androgen receptor; Gp, protein G; ARE, androgen response element; EC, endothelial cells; NO, nitric oxide; SMC, smooth muscle cell; MMPs, matrix metalloproteinases; CM, cardiac myocytes; CF, cardiac fibroblasts; RAAS, renin–angiotensin–aldosterone system. (Salerni et al. 2015)

4.2 Hypothesis of cardiac cell contains sex hormone receptors

Clinical studies suggest that estrogen receptors are involved in the development of myocardial hypertrophy and heart failure. Some authors investigated whether human myocardial estrogen receptor alpha (ERα) expression (Mahmoodzadeh et al. 2006) , localization, and association with structural proteins was altered in end stage-failing

hearts.

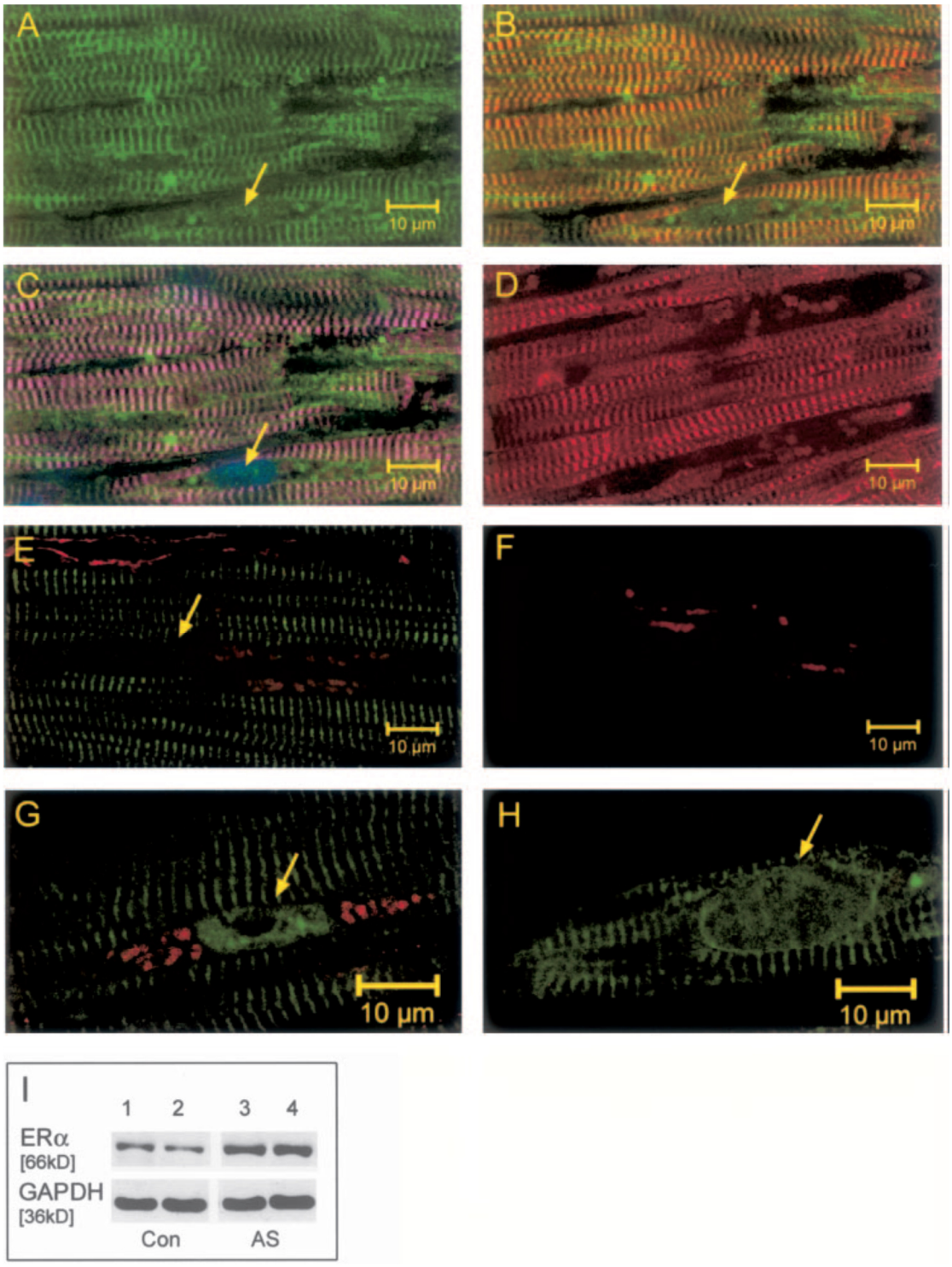


Fig. 21: A to D, Detection of ERα in 5-µm paraffin sections of left ventricle of a control human heart by immunofluorescent staining and confocal laser-scanning microscopy. A to C show the same section stained for ERα (FITC-green) (A); ERα (FITC-green)

and troponin T (Cy3-red) (B); and ERα (FITC-green), troponin T (Cy3-red), and 4', 6-diamidino-2-phenylindole (DAPI) (blue) (C). B and C are merged images. Arrow indicates a nucleus. D, Negative control: myocardial section stained with antibodies directed against troponin T (Cy3-red) and ERα (FITC-green) incubated with its blocking peptide. No nonspecific binding of primary or secondary antibody was detected for ERα. E, F, Detection of ERβ in 5-μm paraffin sections of left ventricle of a control human heart by immunofluorescent staining and confocal laser-scanning microscopy. E and F show serial sections stained for ERβ (FITC-green) and vimentin (Cy3-red) (merged image) (E) and negative control (without primary antibodies) (F). Autofluorescence (lipofuscin) is shown in red. G, ERα nuclear staining. Autofluorescence (lipofuscin) is shown in red. H, ERβ nuclear staining. I, Representative immunoblot. ERα protein is upregulated in left ventricular myocardium of patients with aortic stenosis (1+2, controls; 3+4, aortic stenosis). (Nordmeyer et al. 2004)

For example, hearts from female patients with aortic valve stenosis are characterized by a different form of hypertrophy than male hearts. Sexual hormones and/or their myocardial receptors (Fig. 21) are first-line candidates to explain such differences. (Nordmeyer et al. 2004)

4.3 Hypothesis of male's Y chromosome effect

Studies including genome-wide association studies (GWAS) have clearly demonstrated a genetic influence on Coronary artery disease (CAD). Like autosomal chromosomes (Chrs), the sex chromosomes (ChrX; ChrY) are thought to have once been identical pairs that were free to recombine and exchange genetic material. Recently, studies on the human Y chromosome have also demonstrated that genetic variation within the male-specific region of the Y chromosome (MSY) could play a part in determining cardiovascular risk in men, confirming the notion that the increased risk for CAD in men cannot be fully explained through common CAD risk factors. (Molina et al. 2016) Some authors indicate that a locus/loci on the Y chromosome may influence LDL levels, independent of testosterone levels. (Charchar et al. 2004) Other authors also demonstrate in animal models that having 2 X chromosomes versus an X and Y chromosome complement drives sex differences in higher HDL cholesterol (HDL-C). It is conceivable that increased expression of genes escaping X-inactivation in XX mice regulates downstream processes to establish sexual dimorphism in plasma lipid levels.

(Link et al. 2015)

The female immune response against many infectious pathogens tends to be more robust, leading to a better prognosis in disease outcome. (Case et al. 2013) However, the evolutionary advantage of this heightened female immune response also contributes to their higher risk of developing autoimmune disease. While these sex differences in immunity are predominantly linked to the differential effects of sex hormones on immune cells, ChrY can also influence the immune response and susceptibility. Certainly, the immune respond is also the influence of cardiovascular disease. On the other hand, the lineage of the Y chromosome accounts for up to 15 to 20 mm Hg in arterial pressure. Genes located on the Y chromosome from the spontaneously hypertensive rat (SHR) are associated with the renin-angiotensin system. (Sampson et al. 2014) Given the important role of the renin-angiotensin system in the renal regulation of fluid homeostasis and arterial pressure which hypothesized that the origin of the Y chromosome influences arterial pressure via interaction between the intrarenal vasculature and the renin-angiotensin system. This study demonstrates that the origin of the Y chromosome significantly impacts the renal vascular responsiveness and therefore may influence the long-term renal regulation of blood pressure.

4.4 Combination with neuroendocrine and neurotransmitters

The sex hormone can also combination with neuroendocrine and other neurotransmitters to effect cardiovascular system including blood pressure, heart rate, sex differential anxiety respond and so on. Sex differences in the neuroendocrine and cardiovascular response to psychological stress may contribute to the sex differences in the prevalence of diseases associated with hypothalamic-pituitary-adrenal (HPA) axis reactivity such as cardiovascular disease (CVD), diabetes and hypertension. (Traustadóttir et al.2003) For instance, sex comparisons in the functional and molecular aspects of the hypothalamic-pituitary-adrenal (HPA) axis, through various phases of activity, including basal, acute stress, and chronic stress conditions. (Fig. 22) The HPA axis in females initiates more rapidly and produces a greater output of stress hormones.

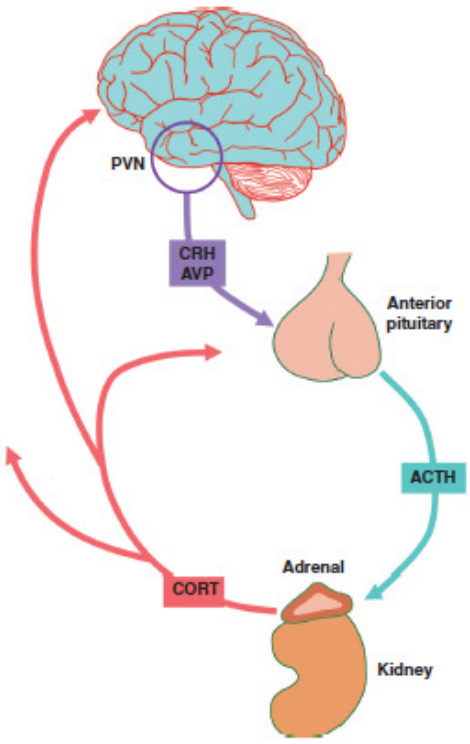


Fig 22 : Schematic diagram of the hypothalamic-pituitary-adrenal (HPA) axis. When a stressor is perceived, the paraventricular nucleus of the hypothalamus (PVN) releases corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which is transported to the anterior pituitary, leading to the release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH stimulates the adrenal cortex to release glucocorticoids (CORT), which have numerous physiological effects. Glucocorticoids also exert negative feedback at the level of the brain and pituitary to dampen excess activation of the HPA axis. (Goel et al. 2014)

This review focuses on the interactions between the gonadal hormone system and the HPA axis as the key mediators of these sex differences, whereby androgens increase and estrogens decrease HPA activity in adulthood. In addition to the effects of gonadal hormones on the adult response, morphological impacts of hormone exposure during development are also involved in mediating sex differences. Additional systems impinging on the HPA axis that contribute to sex differences include the monoamine neurotransmitters norepinephrine and serotonin.(Goel et al. 2014) Diverse signals originating from the brain and periphery are integrated to determine the level of HPA axis activity, and these signals are, in many cases, sex-specific. Sex hormones can also exert differential effects on a variety of sensitive tissues like the reproductive tract, gonads, liver, bone and adipose tissue, among others. In the brain, sex hormones act as

neuroactive steroids regulating the function of neuroendocrine diencephalic structures like the hypothalamus. In addition, steroids can exert physiological effects upon cortical, limbic and midbrain structures, influencing different behaviors such as memory, learning, mood and reward. In the last three decades, the role of sex hormones on monoamine neurotransmitters in extra-hypothalamic areas related to motivated behaviors, learning and locomotion has been the focus of much research. The purpose of this thematic issue is to present the state of art concerning the effects of sex hormones on the neurochemical regulation of dopaminergic midbrain areas involved in neurobiological and pathological processes. Neonatal exposure to sex hormones or endocrine disrupting chemicals can produce long-term changes on the neurochemical regulation of dopaminergic neurons in the limbic and midbrain areas. (Sotomayor-Zarate et al. 2014)

Contributed to sex-related differences in myocardial remodelling (Fig. 23), estrogens may also trigger some of the major sex-based differences observed in cardiac pathophysiology, through unique effects in the different cell types present in the heart. (Fazal et al. 2014). Inflammatory cells, mast cells and cardiac fibroblasts are known to have a detrimental role during cardiac disease and these cell types might also be modulated by sex hormones. For example, estrogens appeared to protect against the significant increases in mast cell density, collagen degradation, ET-1 and TNF-α, induced by volume overload (Montalvo et al. 2012).

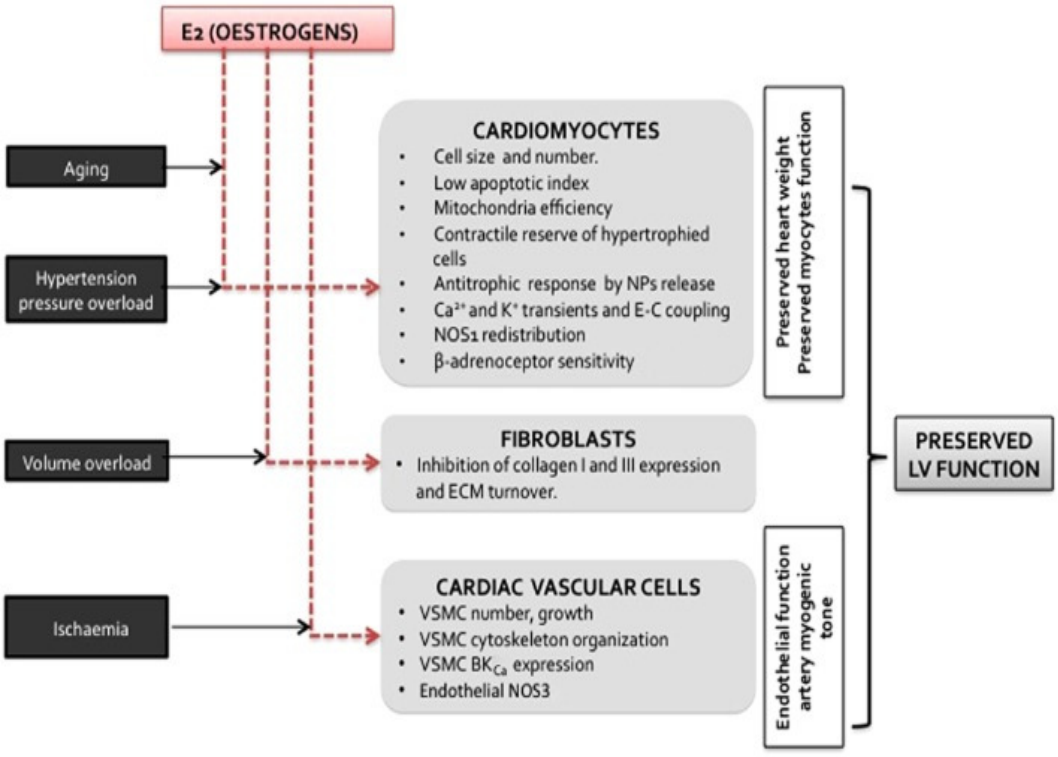


Fig. 23: Summary of the effects of estrogens, according to the cell types in the heart, which can be involved in cardioprotection induced by estrogens. (Fazal et al. 2014)

The sex difference effects on the cardiac fibroblast have been investigated, combining the analysis together with the effect of castration, it was demonstrated that circulating sex hormones contributed to the male sex-related increase in fibrosis and subsequent LV dysfunction after thoracic aorta constriction (TAC) through a mechanism involving TGF-β (Montalvo et al. 2012).

5. Maternal cardiovascular diseases associate with sex hormones activity during pregnancy

Normal pregnancy is associated with adaptive hemodynamic, hormonal, and vascular changes, and estrogen (E2) may promote vasodilation during pregnancy; however, the specific E2 receptor (ER) subtype, post-ER signaling mechanism, and vascular bed involved are needed to research that pregnancy-associated vascular adaptations involve changes in the expression/distribution/activity of distinct ER subtypes in a blood vessel-specific manner. (Mata et al. 2015) The steroid hormone estrogen and its classical estrogen receptors (ERs), ER-α and ER-β, have been shown to be partly responsible for the short- and long-term uterine endothelial adaptations during pregnancy. The ER-subtype molecular and structural differences coupled with the differential effects of estrogen in target cells and tissues suggest a substantial functional heterogeneity of the ERs in estrogen signaling. There are following ERs effect (1) the role of estrogen and ERs in cardiovascular adaptations during pregnancy, (2) in vivo and in vitro expression of ERs in uterine artery endothelium during the ovarian cycle and pregnancy, contrasting reproductive and nonreproductive arterial endothelia, (3) the structural basis for functional diversity of the ERs and estrogen subtype selectivity, (4) the role of estrogen and ERs on genomic responses of uterine artery endothelial cells, and (5) the role of estrogen and ERs on nongenomic responses in uterine artery endothelia. These current knowledge of this very rapidly expanding scientific field with diverse interpretations and hypotheses regarding the estrogenic effects that are mediated by either or both ERs and their relationship with vasodilatory and angiogenic vascular adaptations required for modulating the dramatic physiological rises in uteroplacental perfusion observed during normal pregnancy.(Pastore et al. 2012) Other authors also believed that an increase in ERα expression in endothelial and vascular smooth muscle layers of the aorta and mesenteric artery is associated with

increased ERα-mediated relaxation via endothelium-derived vasodilators and inhibition of Ca(2+) entry into vascular smooth muscle, supporting a role of aortic and mesenteric arterial ERα in pregnancy-associated vasodilation. GPER may contribute to aortic relaxation while enhanced ERβ expression could mediate other genomic vascular effects during pregnancy (Mata et al. 2015)

For plasma testosterone levels, increased 2-fold in testosterone-injected pregnant Sprague-Dawley rats that placental and fetal weights were lower in rats with elevated testosterone as a testosterone group comparing with another control group. Uterine artery blood flow was also lower, and resistance index was higher in the testosterone group. Radial and spiral artery diameter and length, the number of fetoplacental arterial branches, and umbilical artery diameter were reduced in the testosterone group. In addition, markers of hypoxia in the placentas and fetuses were elevated in the testosterone group. The magnitude of changes in placental vasculature and hypoxia was greater in males than in females and was associated with sex-specific alteration of unique sets of genes involved in angiogenesis and blood vessel morphogenesis. (Gopalakrishnan et al. 2016) The results demonstrate that elevated testosterone during gestation induces a decrease in uterine arterial blood flow and fetal sex-related uteroplacental vascular changes, which may set the stage for subsequent sex differences in adult-onset diseases. Therefore, Elevated maternal testosterone levels are shown to cause fetal growth restriction, eventually culminating in sex-specific adult-onset hypertension that is more pronounced in males than in females. (Gopalakrishnan et al. 2016)

5.1 Pregnancy-induced hypertension

Hypertension is the most common medical problem encountered in pregnancy and remains an important cause of maternal and fetal morbidity and mortality. It complicates up to 15% of pregnancies and accounts for approximately a quarter of all antenatal admissions. (Malhotra et al, 2014) The hypertensive disorders of pregnancy cover a spectrum of conditions which preeclampsia poses the greatest potential risk and remains one of the most common causes of maternal death. High blood pressure can present itself in a few different ways during pregnancy. The classification of pregnant hypertensive disorders into 4 common types (Malhotra et al, 2014) : (1) gestational hypertension or pregnancy-induced hypertension (PIH) : It is the development of first time hypertension (a blood pressure greater than 140/90 mm Hg on two separate occasions, more than 6 hours apart) in a pregnant woman after 20 weeks gestation and

not yet reaching the presence of protein in the urine or other signs of preeclampsia. It complicates 6-7% of pregnancies and resolves postpartum. The risk of superimposed preeclampsia is 15-26% (Malhotra et al, 2014), but this risk is influenced by the gestation at which the hypertension develops. (2) Chronic hypertension: It means that women who have high blood pressure (over 140/90 mmHg) before pregnancy, early in pregnancy (before 20 weeks), or continue to have it after delivery. (3) Preeclampsia and eclampsia: Preeclampsia is gestational hypertension and proteinuria (the proteinuria defined as >300 mg/day or > 30 mg/mmol in a single specimen or > 1+ on dipstick) were detected for the first time after 20 week' gestation. Severe preeclampsia involves a blood pressure greater than 160/110 mmHg, with additional medical signs and symptoms. HELLP syndrome is a type of preeclampsia. It is a combination of three medical conditions : hemolytic anemia (microangiopathy), elevated liver enzymes (liver dysfunction) and low platelet count (thrombocytopenia). Eclampsia is the occurrence of seizures superimposed on the syndrome of preeclampsia. Following the seizure there is typically either a period of confusion or coma. (4) Preeclampsia superimposed on chronic hypertension: Onset of new signs or symptoms of preeclampsia after 20 weeks' gestation in a woman, with chronic hypertension.

There are many risk factors of PIH, such as the maternal causes including obesity, age 35 years or more, past history of diabetes mellitus, hypertension, renal diseases, adolescent pregnancy, new paternity, thrombophilias, having donated a kidney ; the pregnancy condition including multiple gestation (twins or triplets, etc.), placental abnormalities, hyperplacentosis, placental ischemia and the family history investigation including pre-eclampsia family history, African American race. However, one of the potential causes of gestational hypertension and preeclampsia is when the spiral arteries do not become fully converted into low-resistance channels. It has been found that this incomplete conversion of spiral arteries increases the resistance to uterine blood flow during pregnancy, and that this occurrence was associated with gestational hypertension.

Plasma testosterone levels are elevated in pregnant women with pre-eclampsia and polycystic ovary syndrome, who often develop gestational hypertension. Some authors tested the hypothesis that increased gestational testosterone levels induce hypertension via heightened angiotensin II signaling. Some authors did pregnant Sprague-Dawley rats were injected with vehicle or testosterone propionate from Gestational Day 15 to 19 to induce a 2-fold increase in plasma testosterone levels, similar to levels observed in clinical conditions like pre-eclampsia. A subset of rats in these two groups was given losartan, an angiotensin II type 1 receptor antagonist by gavage during the course of testosterone exposure. Blood pressure levels were assessed through a carotid arterial catheter and endothelium-independent vascular reactivity through wire myography.

Angiotensin II levels in plasma and angiotensin II type 1 receptor expression in mesenteric arteries were also examined. Blood pressure levels were significantly higher on Gestational Day 20 in testosterone-treated dams than in controls. Treatment with losartan during the course of testosterone exposure significantly attenuated testosterone-induced hypertension. Plasma angiotensin II levels were not significantly different between control and testosterone-treated rats; however, elevated testosterone levels significantly increased angiotensin II type 1 receptor protein levels in the mesenteric arteries. In testosterone-treated rats, mesenteric artery contractile responses to angiotensin II were significantly greater, whereas contractile responses to K^+ depolarization and phenylephrine were unaffected. The results demonstrate that elevated testosterone during gestation induces hypertension in pregnant rats via heightened angiotensin II type 1 receptor-mediated signaling, providing a molecular mechanism linking elevated maternal testosterone levels with gestational hypertension. (Chinnathambi et al. 2014)

The discrete regulation of vascular tone in the human uterine and placental circulations is a key determinant of appropriate uteroplacental blood perfusion and pregnancy success. Humoral factors such as estrogen, which increases in the placenta and maternal circulation throughout human pregnancy, may regulate these vascular beds as studies of animal arteries have shown that 17β-estradiol, or agonists of estrogen receptors (ER), can exert acute vasodilatory actions. One study revealed that uterine and placental arteries were isolated from biopsies obtained from women with uncomplicated pregnancy delivering a singleton infant at term. (Corcoran et al. 2014) The mRNA expression of ERα and ERβ was greater in myometrial arteries than placental arteries. ER-specific agonists, and 17β-estradiol, differentially modulate the tone of uterine versus placental arteries highlighting that estrogen may regulate human uteroplacental blood flow in a tissue-specific manner. (Corcoran et al. 2014) The regulation of vascular tone in the uterine circulation is a key determinant of appropriate utero-placental blood perfusion and successful pregnancy outcome. Estrogens, which increase in the maternal circulation throughout pregnancy, can exert acute vasodilatory actions. Recently a third estrogen receptor named GPER (G protein-coupled estrogen receptor) was identified and several studies have shown vasodilatory effects in several vascular beds. The potential importance of GPER signaling in reducing uterine vascular tone during pregnancy and it can play a role in the regulation of utero-placental blood flow and normal fetus growth. (Tropea et al. 2015)

5.2 Pre-eclampsia and eclampsia

Preeclampsia is gestational hypertension and proteinuria (the proteinuria defined as >300 mg/day or > 30 mg/mmol in a single specimen or > 1+ on dipstick) were detected for the first time after 20 week' gestation. (Malhotra et al, 2014) The disorder usually occurs in the third trimester of pregnancy and worsens over time. In severe disease there may be red blood cell breakdown, a low blood platelet count, impaired liver function, kidney dysfunction, swelling, shortness of breath due to fluid in the lungs, or visual disturbances. Preeclampsia increases the risk of poor outcomes for both the mother and the baby. If untreated, it may result in seizures at which point it is known as eclampsia. Risk factors for preeclampsia include: obesity, prior hypertension, older age, and diabetes mellitus. It is also more frequent in a woman's first pregnancy and if she is carrying twins. The underlying mechanism involves abnormal formation of blood vessels in the placenta (Fig. 24) among many factors. Most cases are diagnosed before delivery. Rarely, preeclampsia may begin in the period after delivery. While historically both high blood pressure and protein in the urine were required to make the diagnosis, some definitions also include those with hypertension and any associated organ dysfunction. Preeclampsia is routinely screened for during prenatal care.

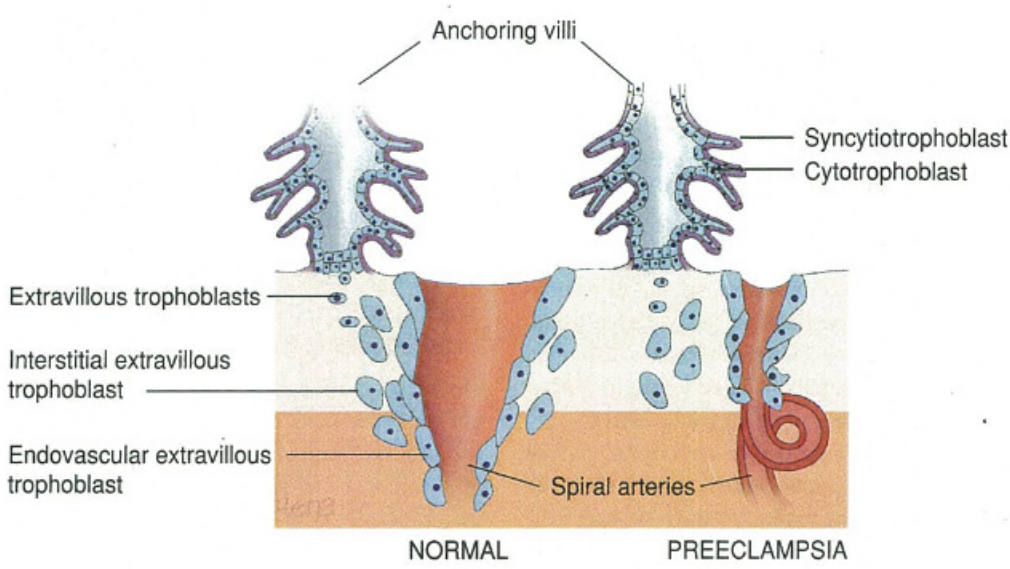


Fig. 24: Schematic representation of normal placental implantation shows proliferation of extravillous trophoblasts from an anchoring villus. These trophoblasts invade the decidua and extend into the walls of the spiral arteriole to replace the endothelium and muscular wall to create a dilated low-resistance vessel. With preeclampsia, there is defective implantation characterized by incomplete invasion of the spiral arteriolar wall by extravillous trophoblasts. (Cunningham et al. 2014) This results in a small-caliber

vessel with high resistance to flow.

The etiology of writings describing eclampsia have been traced as far back as 2200 BC (Lindheimer, 2014). An imposing number of mechanisms have been proposed to explain its cause. These currently considered important include : (1) Placental implantation with abnormal trophoblastic invasion of uterine vessels (2) Immunological maladaptive tolerance between maternal, paternal (placental), and fetal tissues (3) Maternal maladaptarion to cardiovascular or inflammatory changes of normal pregnancy (4) Genetic Factors including inherited predisposing genes and epigenetic influences. The concept of vasospasm with preeclampsia was based on his direct observations of small blood vessels in the nails beds, ocular fundi, and bulbar conjuctivae. Endothelial activation causes vascular constriction with increased resistance and subsequent hypertension. At the same time, endothelial cell damage causes interstitial leakage through which blood constituents, including platelets and fibrinogen are deposited sub-endothelial region of resistance arteries in preeclamptic women. The much larger venous circuit is similarly involved, and with diminished blood flow because of maldistribution, ischemia of the surrounding tissues can lead to necrosis, hemorrhage, and ocher end-organ disturbances characteristic of the syndrome. One important clinical correlate is the markedly attenuated blood volume seen in women with severe Preeclampsia. Women with early preeclampsia, have increased vascular reactivity to infused norepinephrine and angiotensin II. Moreover, increased sensitivity to angiotensin II clearly precedes the onset of gestational hypertension. The 21-amino acid peptides are potent vasoconstrictors, and endorhelin-1 (ET-l) is the primary isoform produced by human endothelium. Treatment of preeclamptic women with magnesium sulfate can lower ET-1 concentrations. (Cunningham et al. 2014)

Any satisfactory theory concerning the etiology and pathogenesis of preeclampsia must account for the observation that gestational hypertensive disorders are more likely to develop in women with the following characteristics: (1) Are exposed to chorionic villi for the first time. (2) Are exposed to a superabundance of chorionic villi, as with twins or hydatidiform mole. (3) Have preexisting conditions of endothelial cell activation or inflammation such as diabetes or renal or cardiovascular disease. (4) Are genetically predisposed to hypertension developing during pregnancy. Regardless of precipitating etiology, the cascade of events leading to the preeclampsia syndrome is characterized by abnormalities that characterized by result in by vascular endothelial damage with resultant vasospasm, transudation of plasma, and ischemic and thrombotic sequelae. The severe disturbances of normal cardiovascular function are common with preeclampsia syndrome. There are related to (1) increased cardiac afterload caused by

hypertension. (2) cardiac preload, which is affected negatively by pathologically diminished hypervolemia of pregnancy and is increased by intravenous crystalloid or oncotic solutions. (3) endothelial activation with inter-endothelial extravasation of intravascular fluid into the extracellular space and importantly into the lungs. (Cunningham et al. 2014)

The potent vasodilator is synthesized from L-arginine by endothelial cells. Withdrawal of nitric oxide (NO) results in a clinical picture similar to preeclampsia in a pregnant animal model. Inhibition of NO synthesis increases mean arterial pressure, decreases heart rare, and reverses the pregnancy-induced refractoriness to vasopressors. In humans, nitric oxide likely maintain low-pressure vasodilated state characteristic of fetoplacental perfusion. (Cunningham, 2014) It appears that syndrome is associated with decreased endothelial nitric oxide synthase expression, thus increasing nitric oxide inactivation. The role of estrogen has potent stimulatory effects on endothelial nitric oxide synthase (eNOS) expression and activity in vascular endothelium (Chambliss & Shaul. 2002) and can prevent preeclampsia that estrogen receptor alpha (ESR1) gene polymorphisms were proved responsible for cardiovascular diseases. One case control study revealed that 2 polymorphic genes of ESR1 are associated with pre-eclampsia. (El-Beshbishy et al. 2015)

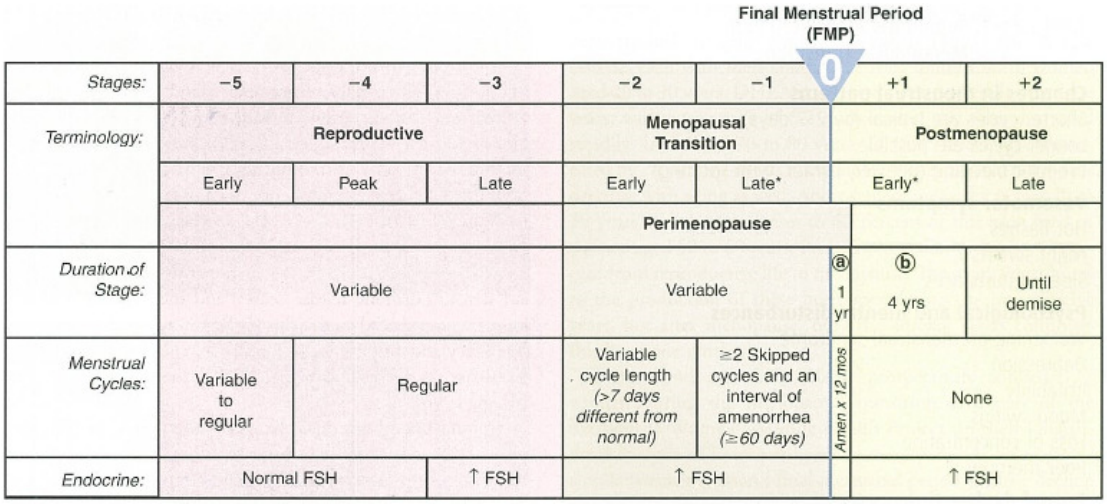
6. Pathophysiology of menopause

In the human embryo, oogenesis begins in the ovary around the third week of gestation. Primordial germ cells appear in the yok sac, migrate to the germinal ridge, and undergo cellular divisions. It is estimated that the fetal ovaries contain approximately 7 million oogonia at 20 weeks' gestation. After 7 months' gestation, no new oocytes are formed. At birth, there are approximately 1-2 million oocytes, and by puberty this number is reduced to 300,000-500,000. Continued reduction of oocyte numbers occurs during the reproductive years through ovulation and atresia. Nearly all oocytes vanish by atresia, with only 400-500 actually being ovulated. Very little is known about oocyte atresia. Animal studies show that estrogens prevent the atretic process, whereas androgens enhance it. (DeCherney et al. 2013)

Menopause is permanent cessation of menstruation following the loss of ovarian activity for 12 months. The average age of women experiencing their final menstrual period is 51.5 years, but cessation of menses due to ovarian failure may occur at any age. It apparently occurs in the human female because of 2 processes. First, oocytes responsive to gonadotropins disappear from the ovary, and second, the few remaining

oocytes do not respond to gonadotropins. Isolated oocytes can be found in postmenopausal ovaries on very careful histologic inspection. Some of them show a limited degree of development, but most reveal no sign of development in the presence of excess endogenous gonadotropins. (DeCherney et al. 2013) Its hormones are stable with long term consequences of hypoestrogenism. Characteristically, it begins with menstrual cycle irregularity and extends to 1 year after permanent cessation of menses. The more correct terminology for this time is menopausal transition. This transition typically develops over a span 4 to 7 years, and the average age at its onset is 47 years. (Hoffman et al. 2012)

The first standardized classification guidelines for female reproductive aging were proposed in 2001 at the Stages of Reproductive Aging workshop (STRAW). The purpose of the STRAW report (Fig. 25) was to clarify the stages and nomenclature of normal female reproductive aging. These staging criteria are intended to be guidelines rather than strictly applied diagnoses. The STRAW report divides reproductive and post-reproductive life into several stages. The anchor for the staging system is the final menstrual period (FMP), and the age range and duration of each stage varies. Five stages precede and two stages follow the FMP. Stage-5 refers to the early reproductive period, stage-4 to the reproductive peak, and stage-3 to the late reproductive period. Stage-2 refers to the early menopausal transition and stage-1 to the late menopausal transition. Stage+1 refers to the 5 years after the FMP, stage + 1a is the first year after FMP, stage + 1b refers to years 2 to 5 post-menopause, and stage +2 refers to the ensuing later postmenopausal years. (Hoffman et al. 2012)



★ Stages most likely to be characterized by vasomotor symptoms

↑= elevated

Fig. 25 : The stages of reproductive aging. (Hoffman et al. 2012) Amen =

Amenorrhea; FSH＝ follicle-Stimulating hormone level.

The transition from ovulatory cycles to menopause typically begins in the late 40s and in early menopausal transition (stage-2). Levels of FSH rise slightly and lead to an increased ovarian follicular response. This rise in FSH levels is attributed to a decrease in ovarian inhibin secretion, rather than to a decrease in estradiol production. (Hoffman et al. 2012) During the menopausal transition, more erratic fluctuations in female reproductive hormones can lead to an array of physical and psychological symptoms such as common symptoms including changes in menstrual patterns, hot flashes, night sweats, irregular menstrual periods, loos libido, vaginal dryness, mood swings ; fatigue, hair loss, sleeping disorder, loss of concentration, irritability, weight gain, osteoporosis, headache, dizziness, urinary incontinence, breast pain, back pain, dry skin and so on.

6.1 Vasomotor symptoms

The most common symptom is relating to thermoregulation even if many symptoms of menopause that may affect quality of life. These vasomotor symptoms can be described as hot flashes and night sweating. All of the published epidemiologic studies and determined chat vasomotor symptoms developed in 11 to 60 percent of menstruating women during the transition. (Hoffman et al. 2012) Some dysfunction of central thermoregulatory centers in the hypothalamus is likely the cause of this common symptom. Vasomotor symptoms (VMS) may be associated with an increased risk of cardiovascular disease. One candidate mechanism may involve alterations in physiological responses to stress. The current study therefore examined the relationship between self-reported VMS bother and cardiovascular, hemodynamic, neuroendocrine, and inflammatory responses to an acute psychosocial stress protocol. VMS bother is associated with an unfavorable hemodynamic and neuroendocrine profile characterized by increased hypothalamic-pituitary-adrenal axis and central sympathetic activation, inflammation, and vasoconstriction. Women who report having hot flushes or night sweats 'often' have an increased risk of developing coronary heart disease (CHD) over a period of 14 years, even after taking the effects of age, menopause status, lifestyle, and other chronic disease risk factors into account. (Herber-Gast et al. 2015) VMS are also associated with decreased bone mineral density (BMD), and moderate-to-severe VMS in particular are independently associated with the risk of osteoporosis in otherwise healthy postmenopausal Korean women. (Ryu et al. 2016)

6.2 Hot flashes

Thermoregulatory and cardiovascular changes that accompany a hot flash have been well documented. An individual hot flash generally lasts 1 to 5 minutes, and skin temperatures rise because of peripheral vasodilation. This change is particularly marked in the fingers and toes, where skin temperature can increase 10 to 15 ℃. Most women sense a sudden wave of heat that spreads over the body, particularly on the upper body and face. Sweating has been observed in women during 90 percent of hot flashes. Five to 9 minutes after a hot flash begins, core temperature decreases 0.1 to 0.9 ℃ due to heat loss from perspiration and increased peripheral vasodilation. If the heat loss and sweating are significant, a woman may experience chills. Skin temperature gradually returns to normal, sometimes taking 30 minutes or longer. (Hoffman et al. 2012) Menopausal hot flashes and night sweats are the most common symptoms of the menopause, and a minority of women find themselves distressing then seek for treatment. Hormone replacement therapy is the most effective treatment for managing these symptoms. HRT is also beneficial in the treatment of other symptoms associated with menopause such as urogenital atrophy and psychological symptoms, and in protecting against the early metabolic changes associated with premature ovarian failure. (Brockie. 2013) Some authors thought that the relationship between hot flashes and cardiovascular disease (CVD) risk observed in the periphery may extend to white matter hyperintensities (WMH) of the brain. (Thurston et al. 2016)

6.3 Cardiovascular disease risk

Before menopause, women have a much lower risk for cardiovascular events compared with men their age. Reasons for protection from CVD in premenopausal women are complex, but a significant contribution can be assigned to the greater high-density lipoprotein (HDL) levels in younger women which is an effect of estrogen. (Hoffman et al. 2012) However, after menopause this benefit disappears over time such that a 70-year-old woman begins co have a risk identical to chat a comparably aged male. The risk of CVD increases exponentially for women as they enter the post-menopause and estrogen levels decline. Total cholesterol and low-density lipoprotein (LDL) levels are lower in premenopausal women than in men. After menopause and with the subsequent decrease in estrogen, this favorable effect on lipids is lost. High-density lipoprotein (HDL) levels decrease and total cholesterol levels increase. After

menopause, the risk of coronary heart disease doubles for women and at approximately age 60, the atherogenic lipids reach levels higher than chose in men. (Hoffman et al. 2012)

Estrogens through their intracellular receptors regulate various aspects of glucose and lipid metabolism. The effects of estrogens in metabolism can be mediated by their receptors located in different areas of the brain such as the hypothalamus, which is involved in the control of food intake, energy expenditure, and body weight homeostasis. Alterations in the metabolic regulation by estrogens participate in the pathogenesis of the metabolic syndrome and cardiovascular diseases in women. The metabolic syndrome is an important disease around the world, consisting in a combination of characteristics including abdominal obesity, dyslipidemia, hypertension, and insulin resistance. It increases the risk of cardiovascular disease and type 2 diabetes. It has been suggested that there is an increase in the incidence of metabolic syndrome during menopause due to estrogens deficiency. In the brain, estrogens through the interaction with their receptors regulate the activity of neurons involved in energy homeostasis, including appetite and satiety. A mechanism insight for the effect of E2 on the maintenance of fat distribution with an increased use of lipids as energy source, which partially promotes fat reduction in abdominal fat. (Fig. 26) This effect occurs via the facilitation of fat oxidation in the muscle by the inhibition of lipogenesis in the liver and muscle through the regulation of peroxisome proliferator-activated receptor γ (PPARγ) and an increase Lipoprotein lipase (LPL) expression.(Lizcano & Guzmán., 2014)

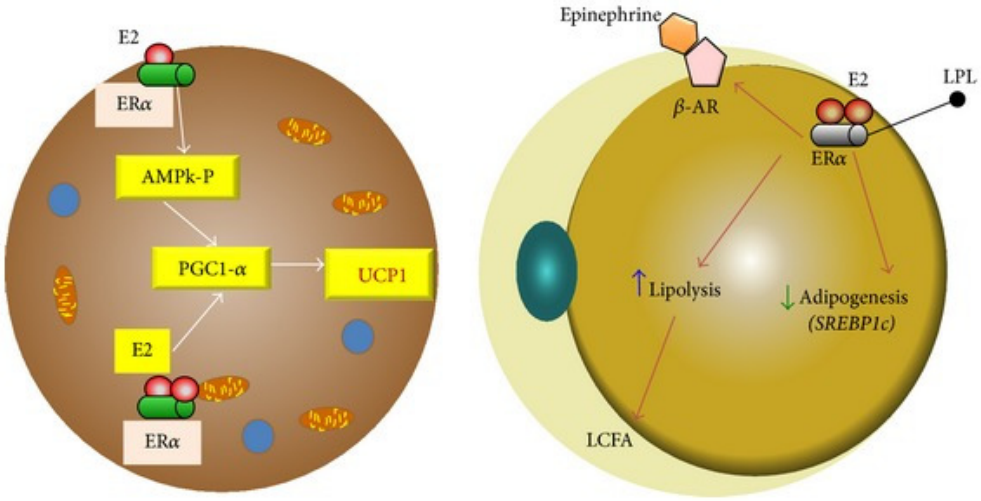


Fig. 26: Estrogen in the fat cell. (a) In brown adipocyte cell the ER alpha receptor can

increase the expression of UCP1 by increasing PGC1alpha coactivator through AMPk and by a direct effect on the receptor coactivator. (b) In white adipocyte ER alpha receptor activation by estrogen reduces lipoprotein lipase and increases beta-adrenergic receptor activity. (Lizcano & Guzmán., 2014) UCP1: uncoupling protein 1; PGC1alpha: peroxisome proliferative activated receptor gamma coactivator 1 alpha; ER: estrogen receptor; AMPk: AMP-activated protein kinase. LPL: lipoprotein lipase; β-AR: adrenergic receptor beta.

E2 also increases muscle oxidative capacity by means of the regulation of acyl-CoA oxidase and uncoupling proteins (UCP2-UCP3), which enhances fatty acid uptake without lipid accumulation. Therefore, E2 improves fat oxidation (Fig. 27) through the phosphorylation of AMPkinase (AMPk) in muscle and myotubes in culture and malonyl-CoA inactivation by increasing the affinity of carnitine palmitoyltransferase. (Lizcano & Guzmán., 2014)

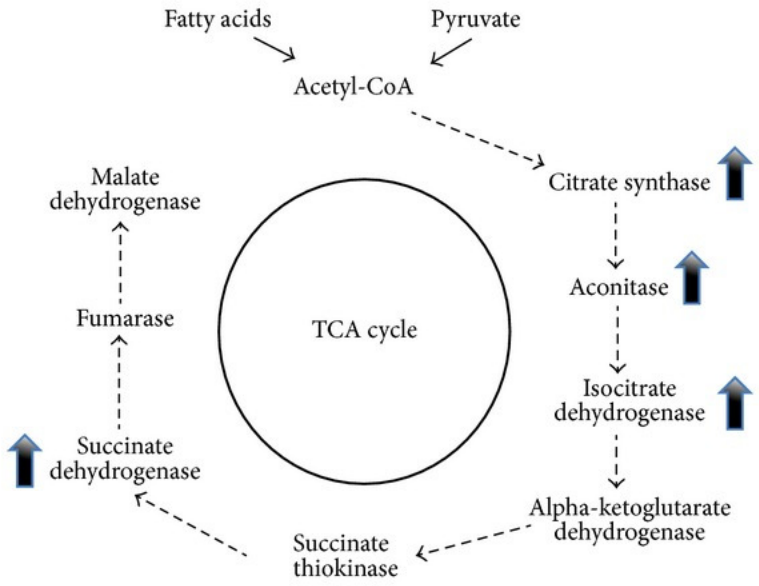


Figure 27: Estradiol availability affects the regulation of enzymes involved in tricarboxylic acid cycle activity.(Lizcano & Guzmán., 2014) E2 enhances the glycolytic/ pyruvate/acetyl-CoA pathway to generate electrons required for oxidative phosphorylation and ATP generation to sustain utilization of glucose as the primary fuel source.

Some areas of the hypothalamus, including the ventromedial (VMN), arcuate (ARC), and paraventricular (PVN) nuclei, regulate physiological events that control weight.

The process by which estrogens regulate the activity of the hypothalamic nuclei is complex. (Fig. 28) Estrogens directly and indirectly modulate the activity of molecules involved in orexigenic action, which induces an increase in food intake. (Lizcano & Guzmán., 2014)

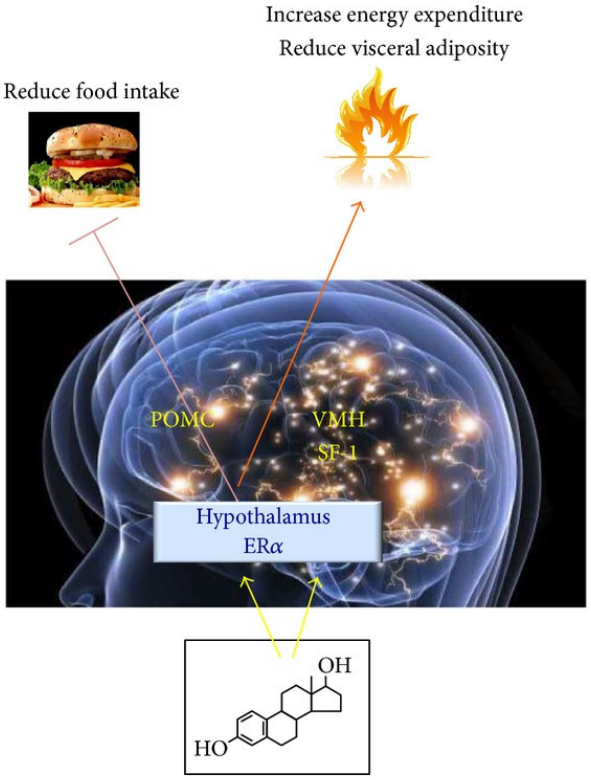


Fig. 28 : Estrogen hypothalamic control of obesity. ER alpha in the brain regulates body weight in both males and females ▸ ER alpha in female SF1 neurons regulates energy expenditure and fat distribution ▸ ER alpha in female POMC neurons regulates food intake. (Lizcano & Guzmán., 2014) POMC: proopiomelanocortin; SF1 : steroidogenic factor-1; VMH : ventromedial nucleus of the hypothalamus.

Estrogens and estrogen receptors regulate various aspects of glucose and lipid metabolism. Disturbances of this metabolic signal lead to the development of metabolic syndrome and a higher cardiovascular risk in women. The absence of estrogens is a clue factor in the onset of cardiovascular disease during the menopausal period, which is characterized by lipid profile variations and predominant abdominal fat accumulation. If the absence of estrogens have a significant effect of obesity in menopausal women. (Lizcano & Guzmán. 2014) Thus, estradiol and their receptors in the hypothalamus play a key role in metabolic syndrome development during menopause.

In addition to above risks, some authors examined the age at menopause is associated

inversely with heart failure (Hf) incidence in the Atherosclerosis Risk In Communities (ARIC) study and summarized all existing data in a meta-analysis. Their results provided evidence that early age at menopause is associated with a modestly greater risk of Hf. (Appiah et al. 2016) It means that not only early age (<45 years) at menopause has been postulated to be associated with increased cardiovascular disease risk but also associated with Hf. They suggested that identification of women with early menopause offers a window of opportunity to implement interventions that will improve overall cardiovascular health during the postmenopausal years.

6.4 Hormone replacement therapy in menopausal women

Hormone replacement therapy (HRT) in menopause is medical treatment in surgically menopausal, perimenopausal and postmenopausal women. Its goal is to mitigate discomfort caused by diminished circulating estrogen and progesterone hormones in menopause. The main hormones involved are estrogen, progesterone and progestin. Some recent therapies include the use of androgens as well. Proprietary mixtures of progestins and conjugated equine estrogens are a commonly prescribed form of HRT. Estrogenic deficiency of menopause is not only responsible to the precocious occurrence of climateric troubles but exposes at increased risk of osteoporosis, metabolic troubles, and cardiovascular complications. Some authors believed for a long time that the hormonal treatment prescription could prevent cardiovascular risk. (Dessapt & Gourdy 2012) The use of HRT and alternative treatment approaches to manage menopausal hot flashes and night sweats. (Brockie. 2013)

Cardiovascular disease (CVD) is more common in men and postmenopausal women (Post-MW) than premenopausal women (Pre-MW). The incidence of CVD in women has shown a rise that matched the increase in the Post-MW population. The increased incidence of CVD in Post-MW has been related to the decline in estrogen levels, and hence suggested vascular benefits of endogenous estrogen. Experimental studies have identified estrogen receptor ERα, ERβ and a novel estrogen binding membrane protein G-protein coupled receptor 30 (GPER) in blood vessels of humans and experimental animals. The interaction of estrogen with vascular ERs mediates both genomic and non-genomic effects. (Fig. 29) Estrogen promotes endothelium-dependent relaxation by increasing nitric oxide, prostacyclin, and hyperpolarizing factor. Estrogen also inhibits the mechanisms of vascular smooth muscle (VSM) contraction including [Ca2+]i, protein kinase C and Rho-kinase.

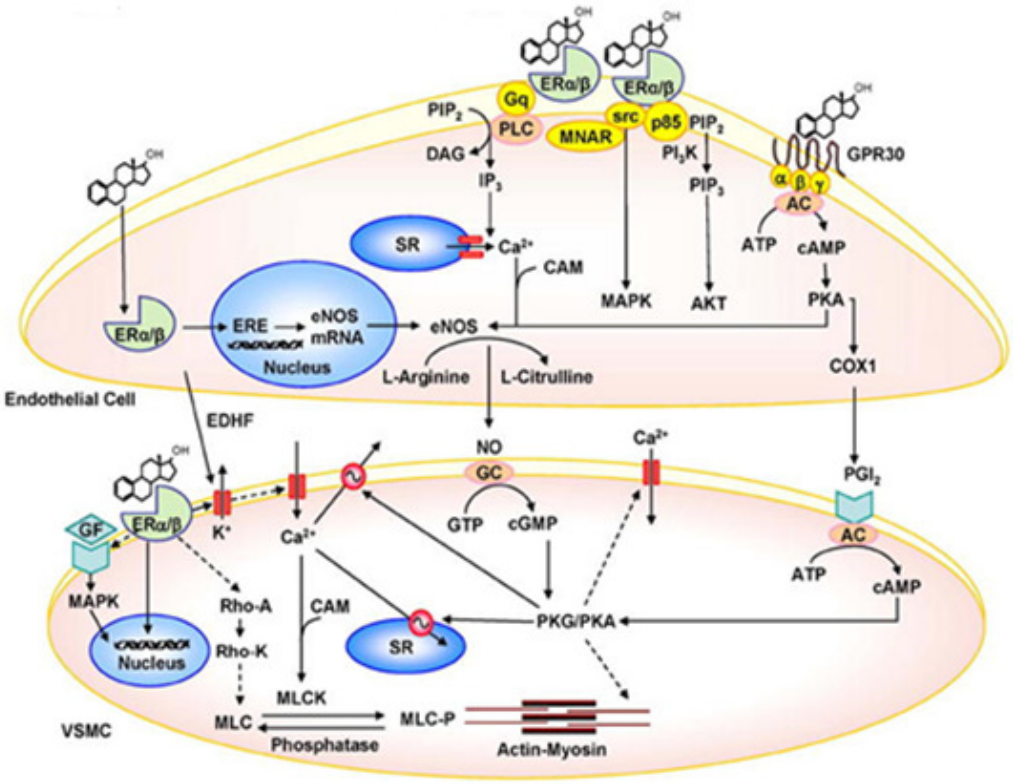


Fig 29 : Genomic and nongenomic vascular effects of estrogen. In the genomic pathway in endothelial cells, E2 binds to cytoplasmic ER leading to ER dimerization and localization to the nucleus where the complex interacts with EREs to increase gene transcription and eNOS expression. In the nongenomic pathway, E2 binds to endothelial ER and activates phospholipase C (PLC), generating inositol 1,4,5-triphosphate (IP_3) and Diacyl glycerol (DAG). IP_3 causes Ca^{2+} release from the endoplasmic reticulum. Ca^{2+} forms a complex with calmodulin (CAM), which activates eNOS. E2/ER also interacts with Src and activates Modulator of Nongenomic Action of ER (MNAR). They interact with the p85 regulatory subunit of PI_3-kinase (PI_3K), which transforms phosphatidylinositol-4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which activates Akt. ER-mediated activation of Akt or MAPK pathway causes phosphorylation and full activation of eNOS, transformation of L-arginine to L-citrulline and production of NO, which causes VSM relaxation. E2 also binds to membrane GPR30 and activates adenylate cyclase (AC) leading to increased cAMP and activation of protein kinase A (PKA), which activates eNOS and COX1 to produce NO and PGI2, respectively. E2 also induces production of EDHF. In the genomic pathway in VSM, E2 binds to ER, inhibiting growth factor (GF) receptors, which are known to activate MAPK translocation to the nucleus. E2 binding to ERs

also stimulates ER translocation to the nucleus where it may affect gene transcription and VSM growth. In the non-genomic pathway, E2 binds to membrane ERs to inhibit the mechanisms of VSM contraction including $[Ca^{2+}]_i$, Ca^{2+}-dependent MLC phosphorylation, protein kinase C (PKC), and Rho-kinase (Rho-K). Endothelial NO and PGI2 activate guanylate cyclase (GC) and AC, respectively, leading to increased cGMP/cAMP and increased activity of protein kinase G and A (PKG and PKA), respectively. PKG/PKA activate Ca^{2+} extrusion via plasmalemmal Ca^{2+} pump and Ca^{2+} uptake by SR, and inhibit Ca^{2+} entry through membrane Ca^{2+} channels. E2 may also bind plasma membrane ERs and activate K^+ channels and other EDHF leading to hyperpolarization and inhibition of membrane Ca^{2+} channels. (Reslan & Khalil. 2012) COX1, cyclooxygenase-1; DAG, diacylglycerol; EDHF, Endothelial derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; ER, estrogen receptor; ERE, estrogen response elements; PGI2, prostacyclin; MAPK, mitogen-activated protein kinase; SR, sacroplasmic reticulum; VSM, vascular smooth muscle.

Additional effects of estrogen on the vascular cytoskeleton, extracellular matrix, lipid profile and the vascular inflammatory response have been reported. In addition to the experimental evidence in animal models and vascular cells, initial observational studies in women using menopausal hormonal therapy (MHT) have suggested that estrogen may protect against CVD. However, randomized clinical trials (RCTs) such as the Heart and Estrogen/ progestin Replacement Study (HERS) and the Women's Health Initiative (WHI), which examined the effects of conjugated equine estrogens (CEE) in older women with established CVD (HERS) or without overt CVD (WHI), failed to demonstrate protective vascular effects of estrogen treatment. Despite the initial set-back from the results of MHT RCTs, growing evidence now supports the 'timing hypothesis', which suggests that MHT could increase the risk of CVD if started late after menopause (Fig. 30), but may produce beneficial cardiovascular effects in younger women during the perimenopausal period. (Reslan & Khalil. 2012) The choice of an appropriate MHT dose, route of administration, and estrogen/progestin combination could maximize the vascular benefits of MHT and minimize other adverse effects, especially if given within a reasonably short time after menopause to women that seek MHT for the relief of menopausal symptoms.

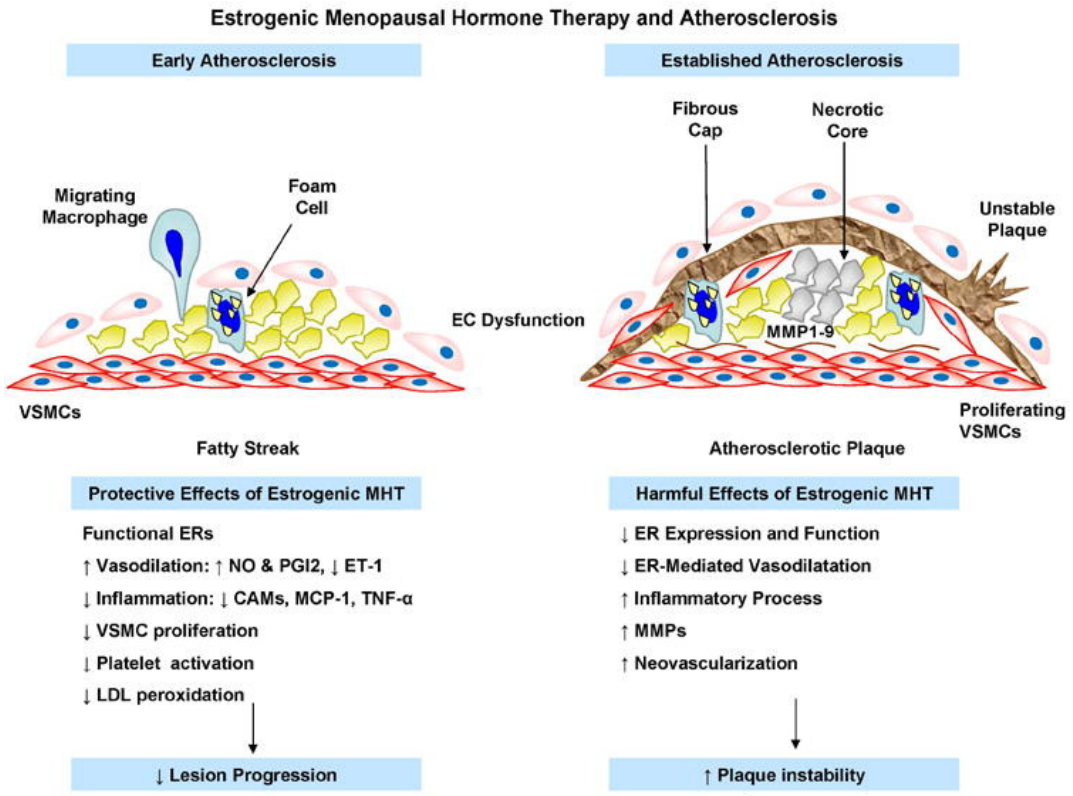


Fig 30: Differential protective effects of estrogenic MHT in early atherogenesis and harmful effects in established atherosclerosis. In early atherogenesis cardiovascular risk factors, hemodynamic forces, and circulating inflammatory factors cause endothelial cell injury resulting in decreased NO production and increased EC permeability. Once injured, the endothelium increases the expression of leukocyte adhesion molecules, which increases the adherence of macrophages and other leukocytes. The increased EC permeability allows entry of leukocytes and lipoproteins into the subendothelial space. Oxidized lipoproteins are taken up by macrophages and SMCs to form foam cells (fatty streak). E2 has beneficial effects on early atherosclerotic lesions by changing the plasma lipid profile, maintaining EC integrity and promoting NO production. In established atherosclerosis foam cells at the central-most position of the developing atheroma become necrotic and form the central lipid core, whereas the shoulder regions contain SMCs, macrophages, and other leukocytes. Platelet-derived growth factor and transforming growth factor-β stimulate SMC migration and collagen formation in the subendothelial space, as well as formation of the fibrous cap. E2 increases MMP expression in established atherosclerosis, causing instability of the fibrous cap and rupture of the plaque. (Reslan & Khalil. 2012) CAMs, cell adhesion molecules; EC, endothelial cell; ET-1, endothelin-1; LDL, low density lipoprotein; MCP-1, monocyte chemotactic protein-1; MMP, matrix metalloproteinase; NO, nitric Oxide; PGI2,

prostacyclin; TNF-α, tumor necrosis factor-α; VSMC, vascular smooth muscle cell.

The choice of an appropriate menopausal hormonal therapy (MHT) dose, route of administration, and estrogen/progestin combination could maximize the vascular benefits of MHT and minimize other adverse effects, especially if given within a reasonably short time after menopause to women that seek MHT for the relief of menopausal symptoms. (Reslan & Khalil. 2012) Postmenopausal women in China had worse cardiovascular risk factors (CRFs) profile than the premenopausal ones, which implied menopause might aggravate the CRFs epidemic and increase the risk of cardiovascular disease beyond effects of aging, which would increase the CVD burden during and after their middle ages. (He et al. 2016)

CHAPTER 2

1. AIMS OF RESEARCH

Cardiovascular organs are not endocrine systems. However, not only the cardiovascular physiology function is quite distinct between men and women but also gender difference in cardiac dominant disease including arrhythmic patterns. Menopause women have hot flashes which causes fast heart rate but can be cured by hormones replacement therapy. It means that somehow relationship between sex hormones and cardiovascular systems.

The aims of the present dissertation are:

- To investigate whether gender differences in electrophysiological characteristics when idiopathic ventricular tachycardia is happened and its prognosis after suitable therapy.
- To elucidate Estrogen can modulate menopausal women's heart rate variability.

We concentrated particularly on these questions:

1. Assume that estrogen has a cardio-protective effect, whether idiopathic ventricular tachycardia from right ventricular outflow tract (RVOT-VT) is a sex difference or not ?
2. How to compare the prognosis of catheter ablation in patients suffering from idiopathic RVOT-VT between men and women ?
3. Do women are tend to have frequent ventricular tachycardia initiation during hormonal flux ?
4. Do sex hormones really effect menopausal women's cardiac rate variability because menopausal hot flashes can be cured by Estrogen ?
5. What is the difference cardiac rhythm between men, pre-menopause and menopause women if give Estrogen or combine with Progesterone ?

2. EXPERIMENTAL PROTOCOL

To solve the questions listed previously we have designed 2 separate experiments covering physiological and pathophysiological situations mentioned. Experiments will be described in in the following chapters in detail.

Brief overview of experimental protocols

Experiment 1

The experiment 1 is a vitro experiment in human which allows to describe the role of discovery on:

- Male patients were found to have higher mean right ventricular (RV) voltage than female patients.
- Analyzing scar zones and low voltage zones at RVOT and RV, females had more low voltage zones at the RVOT free wall as compared with males.
- Females were proved to have shorter QRS duration as compared with males.
- The RVOT free wall as the prominent region, which was not associated with different ventricular tachycardia (VT) incidences.
- ROVT low voltage might be the remodeling result after VT, rather than the cause.
- Prove that successful 3D mapping ablation for RVOT-VT, will produce similar outcome of recurrence rates, and necessary repetitive operations between men and women.

Experiment 2

The experiment 2 is a vitro experiment in human which allows to describe the role of discovery on:

- Proof sex hormones really effect menopausal women's cardiac rate variability because menopausal hot flashes can be cured by Estrogen.
- Distinguish difference cardiac rhythm between men, pre-menopause and menopause women if give Estrogen or combine with Progesterone.

Further on, the current study was conducted to elucidate why gender difference in cardiac electrophysiological characteristics which only occurs on certain arrhythmias

patterns. How to apply hormones replacement therapy to menopausal women. It is also convenient to study cardiac electrophysiology.

3. STUDY 1

GENDER DIFFERENCES IN ELECTROPHYSIOLOGICAL CHARACTERISTICS OF IDIOPATHIC VENTRICULAR TACHYCARDIA ORIGINATING FROM RIGHT VENTRICULAR OUTFLOW TRACT

3.1 Methods and patients of study 1

3.1.1 Study population

93 patients with idiopathic ventricular tachycardia from right ventricular outflow tract (RVOT-VT) (mean age 38.7±15.5 years) were enrolled into the study between January 2011 and September 2013. As shown in Table 4, there was no difference between genders in age of disease onset, diabetes mellitus, hyperlipidemia, previous syncope episodes or family history of ventricular arrhythmias. The diagnosis of VT was documented either by 12-lead resting electrocardiogram (ECG) or by 24-hour ambulatory ECG according to Holter. Cardiac catheterization excluded the possibility of coronary artery disease. All patients were examined using transthoracic echocardiography at the time of diagnosis and all other heart diseases, such as dilated cardiomyopathy, were excluded. Right ventricular (RV) function, regional RV wall motion, as well as left ventricular (LV) function, LV wall motion and LV ejection fraction (EF) were evaluated. RV dysfunction was defined as regional RV akinesia, RV dyskinesia, RV aneurysm, or RV EF less than 40%.

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) was excluded by Task-Force criteria (Marcus et al. 2010). Monomorphic VT was defined as VT with a uniform beat-to-beat surface QRS morphology. Electrocardiographic criteria were employed to identify VT origin, and in all patients included into the study the VTs were confirmed to be of RVOT-VT type (Arya et al. 2007).

	Males (n = 30)	**Females (n = 63)**	**p value**
Age onset (years)	44.8 ± 18.3	36.7 ± 14.1	NS
BMI (kg/m^2)	23.8 ± 2.7	24.0 ± 6.8	NS
Syncope	43.3%	34.9%	NS

Hypertension	20.0%	17.5%	NS
Diabetes mellitus	10.0%	7.9%	NS
Hyperlipidemia	20.0%	6.3%	NS
Family history of ventricular arrhythmias	3.3%	3.2%	NS

Table 4: Baseline characteristics of male and female patients with idiopathic RVOT-VT (BMI, body mass index; RVOT-VT, right ventricular outflow tract-ventricular tachycardia)

	Males (n = 30)	**Females (n = 63)**	**p value**
Atrial arrhythmia	30.3%	14.3%	NS
QTc (ms)	418.6 ± 34.4	432.3 ± 41.2	NS
QTc prolonged (≧ 440 ms)	29.6%	34.5%	NS
RBBB	13.3%	7.9%	NS
QRS width (ms)	99.9 ±19.4	88.4 ± 20.7	0.02
Repolarization abnormalities*	10.0%	19.0%	NS
Clinically documented VT	66.7%	79.4%	NS
LV EF	0.58 ± 0.8	0.57 ± 0.8	NS
LV enlargement	3.4%	1.6%	NS
LV hypokinesis	3.4%	6.3%	NS
RV enlargement	6.9%	6.3%	NS
RV regional dyskinesia	13.8%	20.6%	NS

Table 5 : Electrocardiography and echocardiography characteristics in male and female patients with idiopathic RVOT-VT (EF, ejection fraction; LV, left ventricle; QTc, QT interval corrected for heart rate; RBBB, right bundle branch block; RV, right ventricle; RVOT-VT, right ventricular outflow tract-ventricular tachycardia; VT, ventricular tachycardia.

* T wave inversion in right precordial leads V_1 - V_3.

3.1.2 Electrophysiological study and electromechanical mapping

Standard electrophysiological study was performed on all patients after discontinuation of anti-arrhythmic agents for more than five half-lives. Programmed ventricular electrical stimulation (Hummel et al. 1994) was performed with up to three extrastimuli delivered during sinus rhythm after eight paced ventricular cycle lengths. Induced VT with duration longer than 30 seconds or concomitant hemodynamic compromise was classified as inducible sustained VT. The first test location was at the RV apex, while the next test site was the RVOT if sustained VT was not induced from the previous site. When electrical stimulation failed to induce sustained VT, we used intravenous infusion of isoprenaline (1-4 ug/min) with or without atropine. Sustained VT induced under the use of isoprenaline with or without atropine was defined as catecholamine-sensitive VT. We analyzed the location of the RVOT-VT origin using multiplane fluoroscopy, mainly in the 60∘left anterior oblique projection and the 30° right anterior oblique projection. If the VT origin was located to the anterior or posterolateral attachment of the RVOT the patient was thought to have VT of a septal origin (Tada et al. 2004).

We performed simultaneous electromechanical mapping during electrophysiological study (EPS) using the NavX mapping system (NavX, St. Jude Medical, St. Paul, MN, USA). The mapping procedure included pace mapping during sinus rhythm, endocardial activation mapping, identification of diastolic potentials and entrainment mapping during VT. Entrainment was performed to identify the critical component of the VT circuit and for guidance of selective ablation. We used endocardial activation-sequence mapping to record the earliest endocardial activity and diastolic potentials during VT. Continuous recordings of RV endocardium voltage were done during sinus rhythm, and abnormal areas were defined as voltage setting of $\leqq$1.5 mV on bipolar electrocardiogram. Scar zones were defined by a voltage setting < 0.5 mV, whereas areas with voltage between 0.5 mV and 1.5 mV were evaluated as low voltage zones. This facilitates the delineation of the culprit substrate for VT. We performed catheter

ablation in those patients with inducible VT with standard radiofrequency energy delivered through 4-mm tipped deflectable ablation catheters. Twelve patients did not receive catheter ablation because relevant clinical VT was not induced during EPS. Acute success was defined by the absence of any inducible VT at the end of the catheter ablation procedure via electrical stimulation with or without isoprenaline. ECGs were checked soon after EPS or catheter ablation.

After hospital discharge, the first outpatient follow-up time was arranged two weeks later and further follow-up visits were scheduled at three-month intervals. Surface ECGs and 24-hour holter ECG exams were arranged during serial outpatient follow-up. VT with RVOT origins documented on ECGs or 24-hour holter ECG exams was classified as VT recurrence.

All measurements were performed in the morning between 8 a.m. and 12 a.m. In our study we have used only standard measurement procedures, more detailed description of methods is available in cited papers (Hummel et al. 1994, Tada et al. 2004).

3.1.3 Statistical analysis

Continuous variables are expressed as mean ± standard deviation and the comparisons between continuous data were performed using Student's t test. Comparisons of categorical data were performed using a Chi-square test with a Yates' correction or Fisher's exact test. Statistical significance was established at a p value of < 0.05. All statistical analyses were performed using commercial statistical SPSS version 17.0 software (SPSS, Chicago, IL, USA).

	Males (n = 30)	**Females (n = 63)**	**p value**
Mean RV voltage	3.7 ± 0.9	3.0 ± 0.7	0.03
Low voltage zones RVOT free wall	6.7%	27.0%	0.02
Low voltage zones RVOT septum	10.0%	12.7%	NS
Low voltage zones RV free wall	16.7%	30.2%	NS

Low voltage zones RV septum	6.7%	0.6%	NS

Table 6: Electroanatomical mapping characteristics for male and female patients with idiopathic RVOT-VT (RV, right ventricle; RVOT, right ventricular outflow tract.)

3.2. Results of study 1

3.2.1 Electrocardiographic and echocardiographic differences

No differences existed between genders in respect to percentage of atrial arrhythmias and QTc prolongation. We found an RBBB pattern of QRS in four male patients (13.3%) and five female patients (7.9%), which was not statistically significant. The QRS width was longer in men comparing to women (99.9 ± 19.4 ms vs. 88.4 ± 20.7 ms, p = 0.02). The incidence of T-wave inversion in right precordial leads (V_1 to V_3) indicated no statistical differences between genders. Twenty male patients (66.7%) presented with clinically documented VT, while 50 female patients (79.4%) did. Most of them had monomorphic VT. Only two male patients and three female patients had multiple monomorphic VT or polymorphic VT (Table 5).

There was no difference in LVEF between both genders. Twenty-one percent of male patients had RV dysfunction compared to 19% of female patients (p = 0.85). The percentage of RV dyskinesia was similar for male and female patients (13.8% vs. 20.6%, p = 0.43).

3.2.2 Electrophysiology study and electro-anatomic mapping

Male patients were found to have higher mean RV voltage than female patients (3.7 ± 0.9 mV vs. 3.0 ± 0.7 mV, p = 0.03). Analyzing scar zones and low voltage zones at RVOT and RV, females had more low voltage zones at the RVOT free wall (27% vs. 6.7%, p = 0.02) as compared with males. The percentage of low voltage zones in other areas was similar between both genders (male vs. female, RVOT septum, 10% vs. 12.7%, p = 0.50; RV body free wall, 16.7% vs. 30.2%, p = 0.17; RV body septum, 6.7% vs. 0.6%, p = 0.10). There was no statistical difference in the percentage of scar zone

distribution between male and female (RVOT free wall, 6.7% vs. 15.9%, p = 0.18; RVOT septum, 3.3% vs. 7.9%, p = 0.37; RV body free wall, 13.3% vs. 23.8%, p = 0.24; RV body septum, 6.7% vs. 0, p = 0.10, Table 6)

3.2.3 The outcome after 3D catheter ablation

In total, 81 patients passed catheter 3 dimensions (3D) mapping ablation (23 male and 58 female patients), and the mean follow up time was 26.9 months (26.9 ±32.8 months). The acute success rate was similar in the two studied groups (73.9% vs. 65.5%, p = 0.47). Despite the fact that most patients had monomorphic VT, multifocal ablation sites were needed in 7 male patients and 13 female patients. We tried to analyze the sites where ablation was successful and the correlation with frequencies of scar zone or of low voltage zone and there were no differences between male and female patients. The ablation performed according to the result of pacemap locations was successful in 34.8% of male patients and 36.2% of female patients. The overall VT recurrence rate was likewise similar between the two groups (26.1% vs. 27.6%, p = 0.89). Three male and five female patients had to pass catheter ablation a second time (Table 7).

	Males (n = 23)	**Females (n = 58)**	**p value**
HR (beats/minute)	71.1 ± 12.2	72.0 ± 15.1	NS
Inducible sustained VT	24.1%	23.3%	NS
Catecholamine sensitive	44.8%	55.0%	NS
Multifocal ablation sites	41.2%	33.3%	NS
Scar zone	21.7%	17.2%	NS
Low voltage zone	17.4%	19.0%	NS
Acute success	73.9%	65.5%	NS
VT recurrence	26.1%	27.6%	NS
Repeat ablation	13.0%	8.6%	NS

Table 7 : Ablation results for male and female patients with idiopathic RVOT-VT receiving catheter ablation (HR, heart rate; RVOT-VT, right ventricular outflow tract-ventricular tachycardia; VT, ventricular tachycardia.)

4. STUDY 2

ESTROGEN CAN MODULATE MENOPAUSAL WOMEN'S HEART RATE VARIABILITY

4.1 Methods and patients of study 2

The cross-sectional study was performed on 925 volunteers. The whole cohort was composed of three groups: premenopausal women, postmenopausal women (at least two years from the last menstrual period) and men at the same age as postmenopausal women (Tab. 8).

	n	**age**	**height (cm)**	**weight (Kg)**	**SBP (mmHg)**	**DBP (mmHg)**	**MP age**
men	140	60.8 ± 2.1*	161.8 ± 1.5	68.3 ± 2.3	121.6 ± 2.2	77.1 ± 1.7	-
preMP	140	42.2 ± 1.4	156 ± 1.4*	61.9 ± 4.5	111.4 ± 3.1	71.9 ± 3.1	-
postMP	360	58.2 ± 0.8*	151.4 ± 1.0*	64.4 ± 1.1	121.6 ± 2.0	75.0 ± 1.4	50.1 ± 0.6
E	170	58.2 ± 1.1*	151.5 ± 1.2*	64.2 ± 2.0	120.2 ± 3.3	74.0 ± 1.6	49.1 ± 0.8
E+P	120	56.1 ± 0.8*	153.2 ± 1.5*	64.9 ± 2.4	122.2 ± 2.4	76.6 ± 2.1	47.4 ± 1.2

Table 8 : Characteristics of the groups involved in our study (values are presented as means ± SE; n - number of subjects/group; SBP - systolic blood pressure; DBP - diastolic blood pressure; preMP - premenopausal women; postMP - postmenopausal women without HRT; E - postmenopausal women with estrogen replacement therapy only; E+P - postmenopausal women with combined estrogen and progesterone HRT)

The volunteers were recruited from healthy population who live in the same Datong District of Taipei, Taiwan, R.O.C. The group of postmenopausal women was divided randomly into three subgroups: women without any hormonal treatment, women treated by conjugated estrogen (Premarin at 0.625 mg/day) and women receiving combined hormone replacement (Covina at estradiol 2 mg + norethisterone acetate 1 mg/day). Both subgroups with hormone therapy (HT) were treated continuously, group E (estrogen only) continuously by conjugated estrogen, group E+P repeatedly by estrogen only in first 14 days of 28-days periods and by combination of estrogen and progestin in second halves of 28-days periods.

The follow-up study included only postmenopausal woman with estrogen replacement therapy and postmenopausal woman with combined HT receiving the hormone therapy for at least two consecutive months. The exclusion criteria were cardiovascular diseases, arterial hypertension, diabetes mellitus, asthma, smoking, neurological or psychiatric diseases and any medication that have been reported to affect heart rate (like for instance autonomic blockers). Also postmenopausal women receiving HT or contraceptives before menopausal symptoms were excluded from the study. Before the experiment, objects had no participation in any strenuous exercise, drinking, smoking, drinking caffeinated beverages and taking sleeping pills or tranquilizers.

A detailed overall examination including basic biochemistry was performed before the beginning of the study and all participants were informed about the purpose of the study and all procedures before obtaining their written consent. All ECG examinations were performed between 8 a.m. and 3 p.m. Before every ECG examination the participants were ordered rest in a supine position for at least 5 minutes in a separate quiet room. One electrode was affixed to the sternal edge of the left second intercostal edge, the other one to the midclavicular line in the fourth intercostal space. The examination was realised at standard conditions under complete physical and mental rest, participants were asked to keep laying quietly and breathing normally but not to go to sleep.

ECG signals were recorded by an 8-bit analogue to digital convertor with a sampling rate of 256 Hz, analysed on-line, and stored on the IBM-PC hard drive. Software used

for processing of the signal first identified each QRS complex by a spike detection algorithm and then excluded all premature beats and all beats not corresponding to the standard QRS template (Fig. 31).

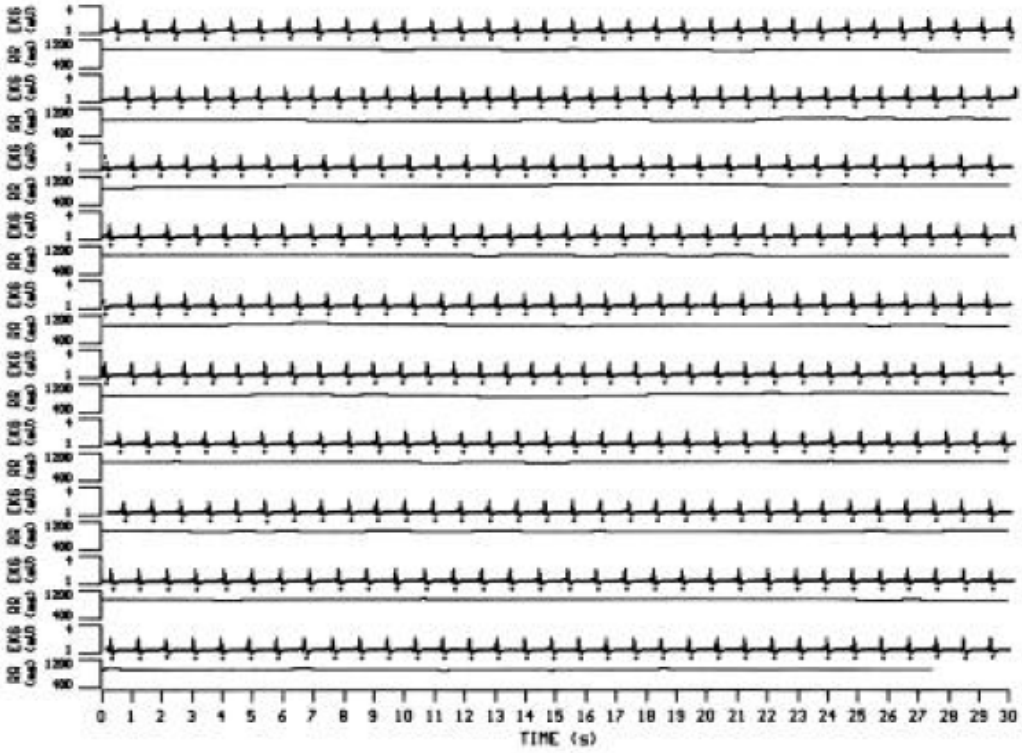


Fig. 31: Sample of continuous tracing of 5-minute raw ECG signals.

Non-parametric spectral analysis of the heart rate variability (HRV) was performed using fast Fourier transformation (FFT) and for attenuation of the leakage effect a Hamming operator was used. Such obtained power spectrum was computed to get standard frequency domain measurements (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996) which include: total variance, low-frequency power (LF), high-frequency power (HF) and low-frequency to high-frequency power ratio (LF/HF). All these variables were then logarithmically transformed in order to correct for the skewness of the distribution (Kuo et al. 1999). The data were expressed as means ± standard error. For statistical analysis one-way ANOVA and Fisher's least significant difference test were used. Differences were considered to be statistically significant at $p < 0.05$.

4.2. Results of study 2

The average heart rate (±SD) in the group of women treated by conjugated estrogen [64.6 (±3.5) min^{-1}] was significantly lower than in premenopausal women [73.1 (±3.9) min^{-1}] and also than in women treated by the combined hormone replacement therapy [81.4 (±4.6) min^{-1}]. The heart rate of women treated by conjugated estrogen was comparable with that of men. No significant difference was observed between premenopausal women and postmenopausal women without any hormonal treatment (Fig. 32).

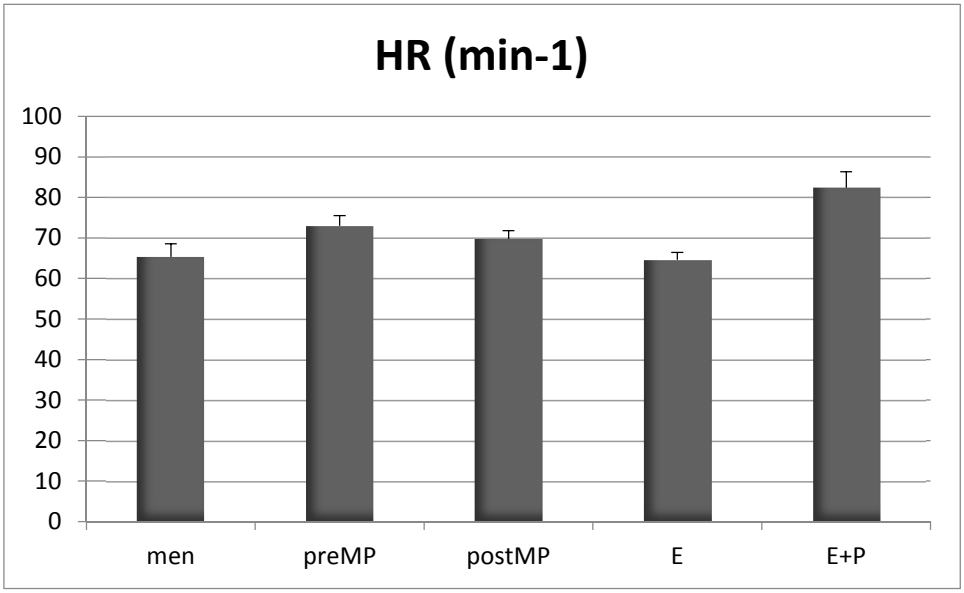


Fig. 32: Average heart rate (±SD) in studied groups (preMP - premenopausal women; postMP - postmenopausal women without HRT; E - postmenopausal women with estrogen replacement therapy only; E+P - postmenopausal women with combined estrogen and progesterone HRT)

Significantly lower portion of the low frequency power (LF%) was found in premenopausal women [46.9 (±2.7) nu] when compared to untreated postmenopausal women [54.3 (±2.9) nu] and men [55.2 (±3.0) nu]. Treatment by estrogen alone significantly decreased the low frequency power ratio [40.1 (±2.1) nu] while no similar effect was observed in women treated with combination of estrogen and progesterone [57.2 (±3.1) nu] (Fig. 33)

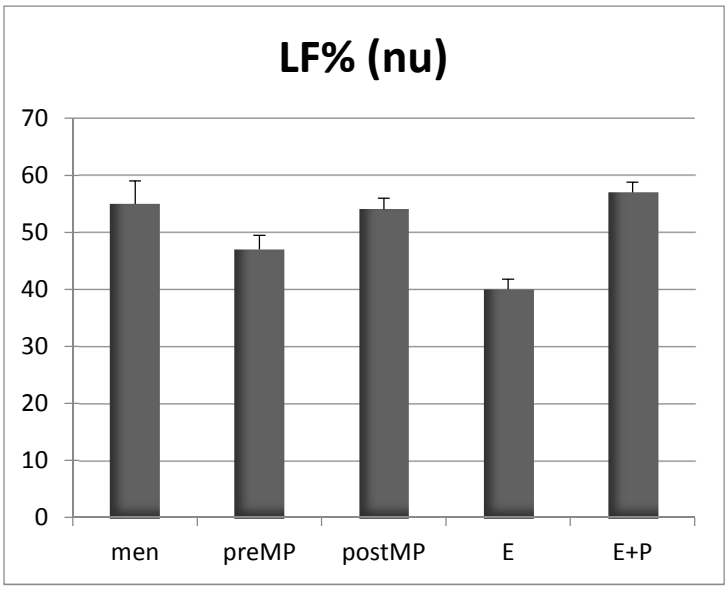


Fig. 33: Average portion of the low frequency power (±SD) in studied groups (preMP - premenopausal women; postMP - postmenopausal women without HRT; E - postmenopausal women with estrogen replacement therapy only; E+P - postmenopausal women with combined estrogen and progesterone HRT)

Also the high frequency power was lower in postmenopausal women [4.16 (±0.16) ms^2] than in premenopausal women [4.79 (±0.22) ms^2] and women treated with estrogen only [4.98 (±0.25) ms^2] while in women treated with combined hormonal therapy the average value [3.99 (±0.21) ms^2] did not significantly differ from that of untreated postmenopausal women (Fig. 34).

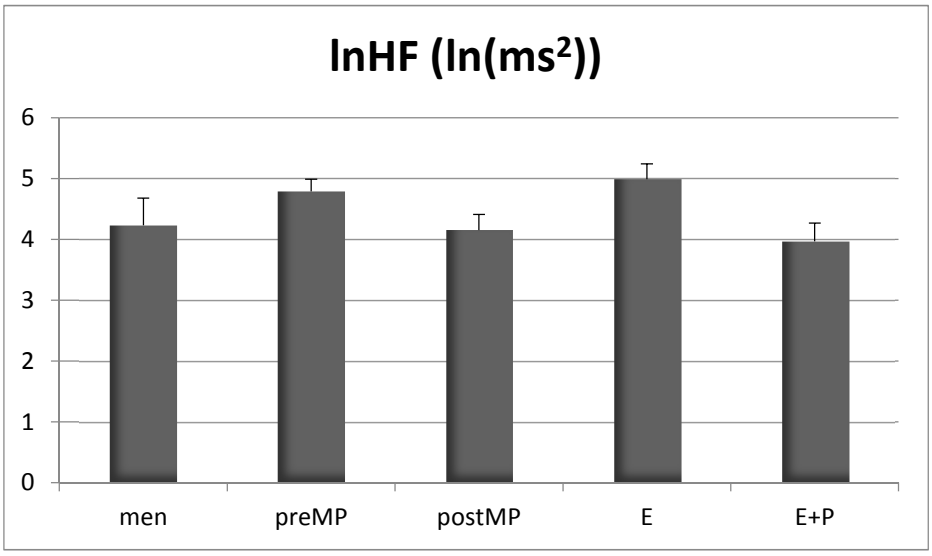


Fig. 34: Average high frequency power after natural logarithm transformation (±SD) in studied groups (preMP - premenopausal women; postMP - postmenopausal women without HRT; E - postmenopausal women with estrogen replacement therapy only; E+P - postmenopausal women with combined estrogen and progesterone HRT)

The corresponding results were consistently found in low-frequency to high-frequency power ratio: the values in premenopausal women [0.04 (±0.12)] and women treated by estrogen [-0.25 (±0.16)] were significantly lower than values found in untreated postmenopausal women [0.36 (±0.12)] and women treated by combined hormonal therapy [0.48 (±0.16)] (Fig. 35).

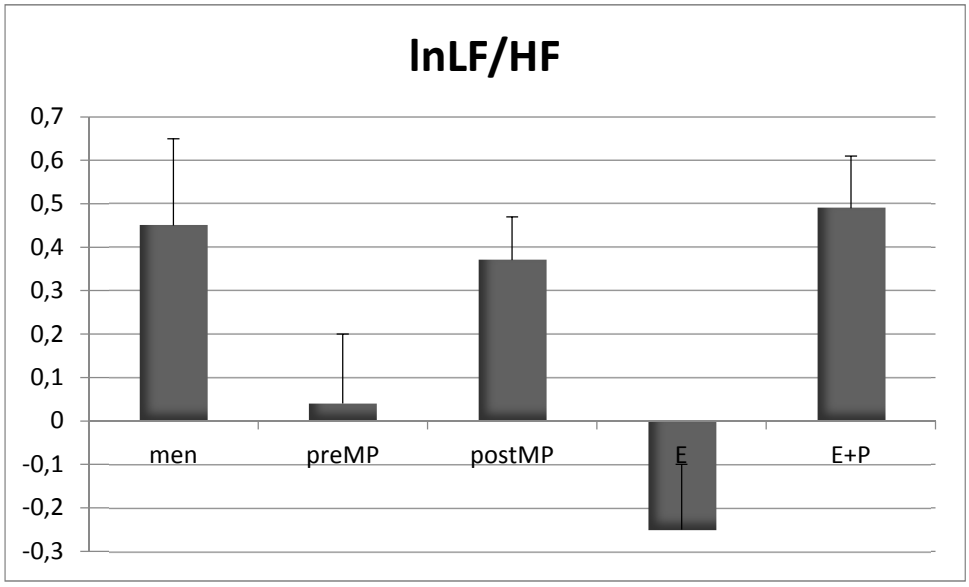


Fig. 35: Average low-frequency to high-frequency power ratio (±SD) in studied groups (preMP - premenopausal women; postMP - postmenopausal women without HRT; E - postmenopausal women with estrogen replacement therapy only; E+P - postmenopausal women with combined estrogen and progesterone HRT)

The follow-up study also proved the increase of high frequency power already after two months of estrogen substitution therapy [4.86 (±0.14) ms^2 vs. 4.19 (±0.15) ms^2].

5. DISCUSSION

In this chapter, the discussion is divided into two parts. They initially focus on interpreting the results from the both of the conducted experiments and are followed by the conclusion with regard to the ultimate outcome of both experiments.

5.1 Study 1

GENDER DIFFERENCES IN ELECTROPHYSIOLOGICAL CHARACTERISTICS OF IDIOPATHIC VENTRICULAR TACHYCARDIA ORIGINATING FROM RIGHT VENTRICULAR OUTFLOW TRACT

5.1.1 Main findings

The present study was focused on gender differences in electro-anatomic characteristics and catheter ablation in RVOT-VT, which according to the authors' best knowledge, have never been previously reported. Females were proved to have more low voltage zone in the RVOT free wall, lower mean RV voltage, and shorter QRS duration as compared with males. The acute success rate, repetitive ablation rate and VT recurrence rate did not differ between genders.

5.1.2 Gender differences in electrophysiological characteristics

Differences in electrophysiological characteristics between genders have been reported in several recent studies. Women have been noted to have higher heart rates at rest, longer corrected QT intervals, shorter sinus node recovery time, and longer ventricular effective refractory periods compared with men (Bernal and Moro 2006). Differences in gender hormones may explain some of these findings, but precisely how is still not well understood. For instance, variations in arrhythmia frequency with respect to the menstrual cycle have been observed. In addition, an increase in arrhythmia frequency or the new onset of arrhythmias has been noted during pregnancy (Yarnoz and Curtis 2008). Differences have also been documented in the incidence and

prevalence of specific arrhythmias, including atrial fibrillation, other various supraventricular tachycardias, and sudden cardiac death. Gender differences in pulmonary vein and left atrium action potential characteristics were noted in the animal study (Tsai *et al.* 2011). In the human beings, female gender with atrial fibrillation could predict the presence of superior vena cava ectopic beats (Lee *et al.* 2005). Women have also a higher prevalence of multiple accessory pathways and orthodromic atrio-ventricular re-entrant tachycardia in the case of pre-excitation syndrome (Huang *et al.* 2011). In atrioventricular nodal re-entrant tachycardia, both the antegrade fast and slow pathways effective refractory periods in women were significantly shorter than those in men (Suenari *et al.* 2010). Gender-specific differences exist in the incidence and age distribution of the various types of VT (Nakagawa *et al.* 2002). Nevertheless, limited information was noted from currently available literature. Women were found to have longer ventricular effective refractory periods in comparison to men (Liu *et al.* 2004). Women have also higher incidence of congenital and acquired long QT syndrome, but less ventricular tachycardia/fibrillation-related sudden cardiac death (Bernal and Moro 2006). In idiopathic ventricular tachycardia including RVOT-VT, gender-specific differences exist in the incidence and age distribution. The incidence of RVOT-VT in female is higher than that in males (Bernal and Moro 2006) but gender was not associated with the outcome after catheter ablation (Tanaka *et al.* 2011). Moreover, the same authors have reported that in the patients with idiopathic ventricular arrhythmias, males are prone to have tachycardia induced cardiomyopathy.

5.1.3 Mechanisms of gender differences

Several mechanisms have been proposed to explain the gender differences in arrhythmias, and one of those is associated with sex hormones (Chen *et al.* 1999). Sex hormones could regulate the expression of cardiac ion channels. Progesterone increases delayed rectifier K+ current (Iks) through the nitric oxide production pathway and prevents cyclic adenosine monophosphate enhancement of L-type Ca2+ current (Rosano *et al.* 1996). Other possible mechanisms are different distributions of ion channels between genders. James *et al.* (2004) reported gender-related differences in ventricular myocyte repolarization in the guinea pig. They have found in their study that IKs and inward rectifier K^+ current were different between genders regardless of menstrual cycle. Gaborit *et al.* (2010) further reported that male and female human hearts had significant differences in ion-channel subunit composition, with female hearts showing decreased expression of a number of repolarizing ion-channels. The autonomic nervous system could also play the role. Autonomic regulation, contributing

to different cardiac electrophysiology (Kapa *et al.* 2010, Yang *et al.* 2013) might explain gender differences in various arrhythmias (Dart *et al.* 2002, Hu *et al.* 2009, Morillo *et al.* 1994). Arg16Gly in β2-adrenoceptor is significantly associated with idiopathic ventricular outflow tract tachycardias in the Chinese Han population (Ran *et al.* 2010) which suggests the possible roles of sympathetic system in the RVOT-VT. In summary, gender differences might be attributed to multiple factors.

5.1.4 The outcome after 3D catheter ablation

The gender differences in outcomes after catheter ablation for different arrhythmias have been recently reported. For instance, outcomes of catheter ablation for atrial fibrillation in women were worse than in men, probably due to later referral and older age in the women in reported study (Santangeli *et al.* 2011). Similar ablation results regarding differences between the genders were observed in atrioventricular nodal and atrioventricular reentrant tachycardia. With regard to idiopathic VTs, no gender-related differences in outcome of catheter ablation have been found (Tanaka *et al.* 2011). This study also shows that successful ablation rates, recurrence rates, and necessary repetitive operations were similar between genders. Idiopathic RVOT-VT is a relatively benign ventricular arrhythmia, and prognosis should rely on underlying conditions and comorbidities, rather than on the arrhythmia itself. Ventura *et al.* (2007) reported decennial follow-up in 133 patients (77 females; 39±13 years) with RVOT-VT for 135±68 months and 127 (95 %) survived, while six (5 %) died but from noncardiac diseases. In this study, ablation was performed in middle-aged groups with relatively few comorbidities and preserved left ventricular function. The modern technique of catheter ablation has a high success rate and low complications, which explains why the acute success rate and recurrence rate didn't differ between the two groups. The recent study also revealed gender difference in mutation carriers in the lamin A/C gene (LMNA) when male had a worse prognosis due to a higher prevalence of malignant ventricular arrhythmias and end-stage heart failure (van Rijsingen *et al.* 2013).

5.1.5 Limitations of the study

In this study, some patients had RV regional dyskinesia on echocardiographic examination, while no cardiac magnetic resonance imaging (MRI) was performed to evaluate the presence of RV dysplasia. However, this is a universal limitation of registry data. According to one previous report, MRI abnormalities could still be detected in patients with idiopathic RVOTVT without other evidence of Arrhythmogenic right

ventricular cardiomyopathy (ARVC) (Joshi and Wilber, 2005).

5.2 Study 2

ESTROGEN CAN MODULATE MENOPAUSAL WOMEN'S HEART RATE VARIABILITY

The present study aimed to compare the responses of HRV with two different types of hormonal substitution therapy (HT) in postmenopausal women (cross-sectional study) and to reveal an effect of HT shortly after beginning of its administration (follow-up study). Simultaneously we compared the HRV between postmenopausal women and men at corresponding age. The characteristics of the groups involved in our study are summarized in Table 1, the only significant differences are in the age of premenopausal women in comparison to all other groups and in the body height of men in comparison to all women groups.

Decline in short-term indexes of HRV is associated both with ageing and with declined estrogen levels after menopause (Neves *et al.* 2007). Higher high-frequency power in premenopausal women in comparison to age-matched men was found by Kuo and al. (1999). This finding was confirmed by Liu *et al.* (2003) and moreover they have proved that these gender-related differences disappear after menopause. Our results fully correspond to these findings as we have not found any difference between postmenopausal women and men, but significant differences between premenopausal women and both men and postmenopausal women.

These results suggest that higher vagal modulation of heart rate that seems to be typical for younger women becomes after menopause similar to that of men. Responsibility for this phenomenon lies fully in ovarian hormones what was reported by Mercuro *et al.* (2000) who searched for effect of oophorectomy in premenopausal women on HRV. In their study comparing healthy women before and after oophorectomy with age-matched women who underwent hysterectomy with ovarian conservation they concluded that surgical menopause induced a decline in cardiac vagal modulation with a recovery of the baseline condition after 3 months of estrogen replacement therapy. This result suggests a crucial role of estrogen in the autonomic nervous control of the heart rate. On the other hand it is known that progesterone has a number of potential adverse effects on the cardiovascular system (Rosano GM 2000, Lantto *et al.* 2012) and our results support the concept that progesterone effect attenuates the benefit of unopposed estrogen replacement therapy in post-menopausal

women. In order to confirm our hypothesis of a reverse effect of progesterone on HRV we used different types of HT. Although there are many possible HT regimens described in literature we choose only two standard prescriptions in order to make the study transparent. For comparison of effects of these two types of HT on the heart rate autonomic control we used spectral analysis of HRV. This method has been used since the late 1960s. Power spectral density analysis provides information on how power (variance) distributes as a function of frequency. (Carter *et al.* 2003). The advantages of nonparametric methods, such as the fast Fourier transform, are the simplicity of the algorithm used and the high processing speed. HRV as a powerful tool for the estimation of cardiac autonomic modulations is associated with three major physiological factors, which primarily reflect changing level of both parasympathetic and sympathetic neural control of the heart: oscillatory fluctuations in blood pressure, frequency oscillations due to thermal regulation and respiration. HRV may therefore be considered an output variable of a feedback network that is continuously monitored and regulated by the autonomic nervous system.

Our study showed that HRV differences among the groups were related to the differences of heart rate. This phenomenon could have both mathematical (shorter RR intervals mean that also their fluctuations are lower and thus also less identifiable) and physiological background (tachycardia is generally associated with increased sympathetic and decreased parasympathetic tone). We suppose that decreased heart rate and simultaneously increased high frequency power and very low value of lnLF/HF, that was identified in our study, fully correspond to the described changes in the tones of the autonomic nervous system. Moreover it was proved that clinical usefulness of HRV is relatively independent on the heart rate differences (Kautzner *et al.* 1998).

Animal studies and observational studies have suggested that the use of HT in postmenopausal women could be beneficial with regards to the development of CHD (Adams *et al.* 1990, Grodstein *et al.* 1996, Arnal *et al.* 2006). On the other hand a possible association between estrogens and higher risk of cardiovascular mortality started to be discussed in many recent studies both with negative (Barret-Connor and Goodman-Gruen. 1995) and positive (Scarabin-Carré *et al.* 2012) conclusions. One possible reason for different results could be the time of measurement. Most published measurements as well as our measurements were performed between 8 a.m. and 3 p.m. when sympathetic activity dominates over the parasympathetic one. One of recently published papers studied the effect of estrogen HT on nocturnal HRV and found that HT has a slightly but distinctively attenuating effect on some nocturnal nonlinear measures of HRV, especially on complexity of heart rate dynamics, suggesting that estrogen HT may have potentiallydeleterious effects on cardiovascular health during

sleep (Virtanen *et al.* 2008). Another possible reason for different results could be different age of postmenopausal women in these studies. While in younger postmenopausal women no association between estradiol levels and cardiovascular risk was found in postmenopausal women over 65 years a positive association between estrogens and progression of atherosclerotic process was identified. Our results proved a positive shift of HRV parameters toward more beneficial values regarding the cardiovascular risk in postmenopausal women treated with estrogens but not in women treated by combined therapy with estrogens and progesteron. But more particularly prospective studies are needed to confirm or definitely reject the theory of protective effects of estrogen HT in postmenopause or to define an age-limit for beneficial effects of this therapy.

6. CONCLUSION

It means that some relationship between sex hormones and cardiovascular systems by above two experiments.

Sex hormones and gender differences have been reported to be associated with the occurrences of ventricular arrhythmias. Some authors investigated the relationship between sex hormones and idiopathic outflow tract ventricular arrhythmias (IOTVA) in adult male patients.(Hu et al. 2009) They suggested that IOTVA might be associated with the reduction of estradiol level even if adult in male patients. IOTVA, including left and right ventricular outflow tract, are dued to cyclic adenosine monophosphate (cAMP)-mediated calcium-dependent delayed after depolarizations. (Jiang et al. 2012) Meanwhile, estrogen replacement therapy could inhibit significantly the count of ventricular arrhythmias in the postmenopausal patients with IOTVA. Their conclusion revealed that estrogen replacement therapy may be a potential therapeutic approach for IOTVA besides postmenopausal patients.(Jiang et al. 2012) IOTVA has been demonstrated to have a unique arrhythmogenic substrate and electropharmacological profile, including ventricular tachycardia from right ventricular outflow tract (RVOT) and LVOT arrhythmias. The most common forms of idiopathic ventricular arrhythmias arise from the RVOT. (Hu et al. 2011) Therefore, we need to know the present study provides further evidence of the gender differences in electroanatomical mapping findings. Females had shorter QRS duration, lower right ventricular voltage, and more low voltage zone in the RVOT free wall than males. Although the possible mechanisms are not clear, our findings suggest differences in ventricular remodeling between genders in patients with idiopathic RVOT-VT. The RVOT free wall was the prominent region, which was not associated with different VT incidences. Those findings suggest that ROVT low voltage might be the remodeling result after VT, rather than the cause. The outcome after catheter ablation was similar between genders, what corresponded with the previous report. (Tanaka *et al.* 2011).

Measurement of heart rate variability (HRV) is an established method to assess the activity of the autonomic nervous system. A literature review was performed a decrease of the vagal dominance on the heart from the follicular to the luteal cycle phase, although some studies asserted no change. The intake of oral contraceptives appeared not to alter the vagal modulation of the heart. (von Holzen et al. 2016) Our research is corresponding with all recent investigations which agreed on a decline of HRV towards higher sympathetic control after menopause. (Von Holzen et al. 2016) Different menopausal hormone therapy approaches showed a supporting impact of estrogen on

HRV in most studies. Further research is needed to demonstrate how this process might be attenuated by different menopausal hormone therapies. (von Holzen et al. 2016) Prescription of hormone replacement therapy (HRT) should never be made only for cardiovascular risk reduction. However, when symptom-related and other indications are present, HRT is appropriate and well tolerated in the early years after menopause with onset at a normal age. (Whayne & Mukherjee. 2015)

Estrogen is important throughout women's whole life. Not only it may regulate human utero-placental blood flow in a tissue-specific manner which can regulate vascular tone in the uterine circulation is a key determinant of appropriate utero-placental blood perfusion and successful pregnancy outcome (Corcoran et al. 2014), but also estrogen can improve menopausal women's heart rhythm. The impact of hot flashes and various forms of hormone therapy on health-related quality of life and sexual well-being in recently postmenopausal women. Estradiol or an estradiol-medroxyprogesterone acetate combination similarly alleviates hot flashes and improves health-related quality of life in relation to elimination of hot flashes. (Savolainen-Peltonen et al. 2014) In a cohort of healthy, drug-naive, postmenopausal women, HRT seems to positively affect glomerular filtration and is associated with lower values of left ventricular mass and aortic root size, thus offering a further mechanism through female hormones exert cardioprotection. (Vitolo et al. 2015)

Multiple clinical studies including randomized trials and observational studies converge with animal experimentation to show a consistency that HRT decreases coronary heart disease (CHD) risk and overall mortality in primary prevention when HRT is started at the time of or soon after menopause. The totality of data supports the "timing" hypothesis that posits that HRT effects are dependent on when HRT is started in relation to age and/or time-since-menopause. The totality of data shows that HRT decreases CHD and overall morality when started in women who are less than 60 years old and/or less than 10 years postmenopausal, providing a "window-of-opportunity". Further evidence shows that women who start HRT when in their 50s and continued for 5-30 years that there is an increase of 1.5 quality-adjusted life-years (QALYs). (Hodis & Mack. 2014) HRT decreases the risk of colon cancer but increases a woman's chance of developing breast cancer. Short-term use of low-dose HRT remains a valid option for management of menopausal symptoms, especially hot flushes. (Takiya & Umland et al. 2003)

LIST OF ABBRIVIATIONS

AC	Adenylate cyclase
AMI	Acute myocardial infarction
ANS	Autonomic nervous system
ACTH	Adrenocorticotropic hormone
ADE	Adverse drug effects
AF	Atrial fibrillation
Ag-AgCI	Silver- silver chloride
Akt	Serine/threonine kinase
AKT	Serine/threonine protein kinase
AMPK	Adenosine monophosphate-activated protein kinase
ANF	Atrial natriuretic factor gene
AngII	Angiotensin II
ALDH	Activity of aldehyde dehydrogenase
Amen	Amenorrhea
AMPk	Adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
AP	Action potential
APD	Action potential duration
APD90	90 % of the action potential duration
ARE	Androgen response element
Atg	Autophagy-related gene
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
AR	Androgen receptor
ARC	Arcuate nuclei
ARC	Apoptosis repressor with a caspase recruitment domain
ARIC	Atherosclerosis risk in communities
ARVC	Arrhythmogenic right ventricular cardiomyopathy
AV	Atrioventricular
AVP	Arginine vasopressin
β-AR	Adrenergic receptor beta
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large

BK_{Ca}	Large-conductance calcium- and voltage-activated potassium channels
BP	Blood pressure
BMD	Bone mineral density
BMI	Body mass index
BNP	Brain natriuretic peptide
Caspase	Cysteine-dependent aspartate-directed proteases
Chrs	Autosomal chromosomes
ChrX	Chromosomes X
ChrY	Chromosomes Y
Ca^{2+}	Calcium ions
$[Ca^{2+}]_i$	Intracellular free calcium concentration
CAD	Coronary artery disease
CHD	Coronary heart disease
CaMKII	Calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CAM	Calmodulin
CAMs	Cell adhesion molecules
CBS	Cystathionine-beta-synthase
CEE	Conjugated equine estrogens
Cf	Contractile force
CF	Cardiac fibroblast
CM	Cardiac myocytes
cGMP	Cyclic guanosine 5'-monophosphate
CGRP	Calcitonin gene-related peptide
CO	Cardiac output
CORT	Glucocorticoids
COX	Cyclooxygenase
CRFs	Cardiovascular risk factors
CRH	Corticotropin-releasing hormone
CRT-D	Cardiac resynchronization therapy-defibrillator device
CSE	Cystathionine-γ-lyase
CV	Cardiovascular
CVD	Cardiovascular disease
CVDH	Cardiovascular disease and hypertension
PDK	3-Phosphoinositide dependent protein kinase
DAG	Diacylglycerol

DAPI	4', 6-Diamidino-2-phenylindole
DBP	Diastolic blood pressure
DHPR	Dihydropyridine receptors
DHT	Dihydrotestosterone
5α-DHT	5α-dihydrotestosterone
3D	3 dimensions
DNA	Deoxyribonucleic acid
Sp-1	Specificity protein-1
E2	17β-estradiol
Ec	Excitation– contraction
EC	Endothelial cell
EAD	Early after depolarization
ECG	Electrocardiography
ECM	Extracellular matrix
ED	Erectile dysfunction
EDHF	Endothelial derived hyperpolarizing factor
EF	Ejection fraction
eNOS	Endothelial nitric oxide synthase
EP	Electrophysiological
EPS	Electrophysiological study
ERE	Estrogen response elements
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERK	Extracellular signal–regulated kinase
ERK1/2	Extracellular signal-regulated kinase 1/2
ET-1	Endothelin-1
FFT	Fast Fourier transformation
FMP	Final menstrual period
FSH	Follicle-Stimulating hormone level
Gαi	G protein alpha i subunit
G_s	Heterotrimeric G protein s
GDX	Gonadectomy
GLUT	Glucose transporter
GnRH	Gonadotropin-releasing hormone
Gp	Protein G
GPCR	G-protein coupled receptor
GPER	G protein-coupled estrogen receptor

gpER	Transmembrane G proteincoupled oestrogen receptor
GWAS	Genome-wide association studies
H_2S	Hydrogen sulfide
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
HDL-C	Higher HDL cholesterol
HELLP	Hemolytic anemia, elevated liver enzymes and low platelet count
HERG	Human ether-à-go-go-Related Gene
HERS	Heart and estrogen/ progestin replacement study
Hf	Heart failure
HF	High-frequency
HF%	High frequency power
HNO	Nitrosyl hydride
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HRT	Hormone replacement therapy
HRV	Heart rate variability
HSD	Hydroxysteroid dehydrogenase
Hsp90	Heat shock protein 90
HUVECs	Human umbilical vein endothelial cells
INa	Sodium current
INT	Intranodal tachycardia
IOTVA	Idiopathic outflow tract ventricular arrhythmias
IP_3	Inositol 1, 4, 5-triphosphate
IST	Inappropriate sinus tachycardia
LDL	Low-density lipoprotein
LQTS	Long-QT syndromes
ICa-L	L-type calcium current
IHD	Ischemic heart disease
IKr	Rectifying potassium current
I_{Kur}	Repolarizing K^+ currents
INa	Sodium current
IRAG	Inositol 1, 4, 5-trisphosphate receptor-associated cGMP kinase substrate
ICM	Ischemic cardiomyopathy (ICM)
I/R	Ischemia and reperfusion
I-R	Ischemia-reperfusion

KATP	Adenosine triphosphate-sensitive potassium channels
Kv	Voltage-gated potassium channel
Kv1.5	Voltage-gated potassium channel subtype 1.5
LBBB	Left bundle branch block
LC	Microtubule-associated protein light chain
LDL	Low-density lipoprotein
LH	Luteinizing hormone
LF	Low-frequency
LF%	Low frequency power
LF/HF	Low-frequency to high-frequency power ratio
LPL	Lipoprotein lipase
LV	Left ventricular
LVAD	Left ventricular assist device
LVEF	Left ventricular ejection fraction
MAP	Mitogen-activated protein
Map	Monophasic action potential
Maps	Monophasic action potentials
MAPK	Mitogen-activated protein kinase
MBS	Myosin-binding subunit
MLC	Myosin light chain
MHC	Myosin heavy chain
miR	Inhibits micro ribonucleic acid
MADIT-CRT	Multicenter automatic defibrillator Implantation trial with cardiac resynchronization therapy
MCP	Monocyte chemotactic protein Menopausal hormonal therapy
MMP	Matrix metalloproteinase
MMPs	Matrix metalloproteinases
MNAR	Modulator of nongenomic action of estrogen receptor
mPRs	Membrane progesterone receptors
MRI	Magnetic resonance imaging
MSNA	Muscle sympathetic nerve activity
MSY	Male-specific region of the Y chromosome
mTOR	Mammalian target of rapamycin
mTORC2	Mammalian target of rapamycin complex 2
Na^+	Sodium ions
$[Na^+]_i$	Intracellular free sodium concentration
NCX	Bidirectional Na^+/Ca^{2+} exchanger

NICM	Non-ischemic cardiomyopathy
NK	Natural killer.
NO	Nitric oxide
NOS	Nitric oxide synthase
nPR	Nuclear progesterone receptors
OVX	Ovariectomy
Pa	Pulmonary artery
PDE	Phosphodiesterase
PGC	Peroxisome proliferative activated receptor gamma coactivator
PHLPP	PH domain and leucine rich repeat protein phosphatase
PHLPP2	PH domain and leucine rich repeat protein phosphatase 2
PHLPPL	PH domain and leucine rich repeat protein phosphatase like
PI3K	Phosphoinositide 3-kinase
PIP3	Phosphatidylinositol 3, 4, 5-trisphosphate
PCOS	Polycystic ovary syndrome
PLB	Phospholamban
PLC	Phospholipase C
PGR	Progesterone receptor
PES	Programmed electrical stimulation
PPARγ	Peroxisome proliferator-activated receptor γ
Pv	Pulmonary vein
PGI2	Prostacyclin
PI3K	Phosphoinositide 3-kinase
PIP_2	Phosphatidylinositol-4, 5-bisphosphate
PI3P	Phosphatidylinositol-3, 4, 5-trisphosphate
PIH	Pregnancy-induced hypertension
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
PKG	Protein kinase G
PLB	Phospholamban
POMC	Proopiomelanocortin
PP	Protein phosphatase
PreMP	Premenopausal women
Pre-MW	Premenopausal women
postMP	Postmenopausal women without HRT
Post-MW	Postmenopausal women

PTEN	Phosphatase and tensin homolog
PVN	Paraventricular nucleus of the hypothalamus
QALYs	Quality-adjusted life-years
QTc	Corrected QT
RAGE	Receptor for advanced glycation end products
RAAS	Renin–angiotensin–aldosterone system.
RAS	Renin-angiotensin system
RBBB	Right bundle branch block
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RV	Right ventricular
RVOT	Right ventricular outflow tract.
RVOT-VT	Ventricular tachycardia from right ventricular outflow tract
ARVC/D	Arrhythmogenic right ventricular cardiomyopathy/dysplasia
RyR	Ryanodine receptor
SC	Satellite cells
SCD	Sudden cardiac death
RCTs	Randomized clinical trials
SERCA	Sarcoplasmic/endoplasmic reticulum calcium ions-adenosine triphosphatase
SF1	Steroidogenic factor-1
SRFC	Steroid receptor fast-action complex
siRNA	Small-interfering ribonucleic acid
SHR	Spontaneously hypertensive rat
SMC	Smooth muscle cell
SR	Sarcoplasmic reticulum
SP	Neuropeptide substance P
Sp-1	Specificity protein-1
SPSS	Statistical product and service solutions
SSS	Sick sinus syndrome
STRAW	Stages of Reproductive Aging workshop
SV	Stroke volume
SVT	Supraventricular tachycardia
TAC	Thoracic aorta constriction
TdP	Torsade de pointes
TF	Tissue factor
TGF-β	Transforming growth factor beta

TNF-α	Tumor necrosis factor-α
TPR	Total peripheral resistance
TRPA	Transient receptor potential channel A
UCP	Uncoupling proteins
VASP	Vasodilator-stimulated phosphoprotein
VEGF	Vascular endothelial growth factor
VF	Ventricular Fibrillation
VMH	Ventromedial nucleus of the hypothalamus
VMS	Vasomotor symptoms
VMN	Ventromedial nuclei
VPBs	Ventricular premature beats
VSM	Vascular smooth muscle
VSMC	Vascular smooth muscle cell
VT	Ventricular Tachycardia
WHI	Women's health initiative
WMH	White matter hyperintensities
WPW	Wolff-Parkinson-White
XIAP	X-linked inhibitor of apoptosis protein

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