The Effects of 17 - Beta Estradiol on the Male Rat's Cardiovascular System

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ABSTRACT

Estrogen is known to have cardio protective effects in women. However, there are contradictory data about the cardiovascular effects of estrogen in men in the literature. The aim of this study is to investigate the effects of castration and estrogen treatment after castration on the heart tissues of male rats by immunohistochemically detecting various molecules (growth factors and receptors, chemokine's and sex hormone receptors) that play a role in cardiovascular diseases. For this purpose, three experimental groups consisting of 21 male rats were formed in the study: control (C), castrated (M -) and post - castration 17 - beta estradiol (E_2) treatment (MX) groups. The heart tissues obtained from all groups stained immunohistochemically with primer antibodies for transforming growth factor - β_1 (TGF β_1), transforming growth factor - β receptor I (TGF βRI), transforming growth factor - β receptor II (TGFβRII), platelet derived growth factor (PDGF), platelet derived growth factor receptor - α (PDGFR - α), platelet derived growth factor receptor - β (PDGFR - β), insulin - like growth factor - I (IGF - I), insulin - like growth factor - I receptor (IGF - IR), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), endothelial Nitric Oxide Synthetase (eNOS), endothelia - I (ET - I), estrogen receptors (ER - α, ER - β) and Androgen Receptor (AR). The results of the study showed that E_2 treatment decreased the expressions of PDGF, PDGFR - β, IGF - I, bFGF and AR, and increased the expressions of TGFβRII, eNOS and ER - α. In conclusion, present study proposes a protective role for estrogens in male cardiovascular system and reveals possible molecular mechanisms for this role.

KEYWORDS
Cardiovascular system, 17 - Beta estradiol, Endothelium, Growth factors, Immunohistochemistry, Orchiectomy

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INTRODUCTION

Gender-based differences exist in the incidence of Cardiovascular Diseases (CVDs) such as Coronary Heart Disease (CHD), cardiac hypertrophy, cardiac remodeling after Myocardial Infarction (MI). The risk of CVDs is higher in men and postmenopausal women than in premenopausal women suggesting the beneficial effects of estrogens and / or the detrimental effects of androgens on Cardiovascular System (CVS).[1] Indeed, the cardio protective effects of estrogens in female have been proved in many previous studies.[2,3] However, elevated estradiol levels in the male patients with CHD have led to argument that estrogens may have opposite effects in men.[4] However, the association of sex steroids with the CVDs is controversial and there is limited literature on the cardiovascular effects of sex hormones on the opposite sex. The dysfunction of the vascular endothelium, Vascular Smooth Muscle Cells (VSMCs) and cardiomyocytes, imbalance in growth factors and cytokines, angiogenesis and apoptosis play an important role in the pathogenesis of CVDs. The abnormal proliferation or hypertrophic growth, the migration and the extracellular matrix production of VSMCs or cardiomyocytes are key events in these processes.[5] Estrogens are involved in the protection of normal endothelial function and in the regulation of growth and contractile function of VSMCs and cardiomyocytes. Estrogens are known to inhibit the proliferation and extracellular matrix production of VSMCs to induce the relaxation of VSMCs and cardiomyocytes and to increase the heart weight and cardiomyocyte volume in healthy heart.[6-7] In pathological conditions, estrogens are seen to suppress ventricular hypertrophy with an opposite effect.[8] Estrogens carry out these effects possibly through modifications of the mediators derived endothelial such as no, Endothelium - Derived Hyperpolarizing Factor (EDHF) and ET - I and / or the growth factors such as PDGF, TGFs, bFGF and IGF - I or directly independently from the endothelium.[9-11] Many studies on the role of growth factors in the pathogenesis of atherosclerosis have suggested that the growth factors and their receptors are major mediators of the proliferation and migration of VSMCs, contributing to the development of CHD. PDGF and bFGF are seen to be mainly responsible for the stimulating effects of VSMCs resulting in intimal thickening. PDGF signals are mediated by structurally related α - and β - receptors.[12] TGF - β_1 acts as a long term growth regulator rather than a specific growth stimulator / inhibitor. It primarily contributes to CHD by inducing the migration and extracellular matrix production of VSMCs. TGF - β_1 gives signals *via* two receptor complex including heterogenic type I and type II receptor on CVS.[13] VEGF and bFGF are known to induce angiogenesis - related functions such as endothelial cell proliferation and migration.[14,15] IGF - I has growth factor - like effect in the long - term on the cell proliferation and it stimulates cardiac growth and improves cardiac function. IGF type I receptor mediates the mitogenic effects of IGF.[16] The cardiovascular effects of estrogens are mediated by E_2.[17] The physiological effects of E_2 on CVS are mediated by two intracellular receptors, ER - α and ER - β, expressing in the endothelial cells, VSMCs and cardiomyocytes.[18] Normal ER function has been known to be required in both males and females for normal cardiovascular development and function. The decreased number of endothelial ERs has been suggested to may be a risk factor for CVDs.[19] ER - α has been reported to mediate most of the protective effects of estrogen on injured blood vessels in mouse models .[20] However, ER - β is the receptor form that is predominantly expressed in human VSMCs, particularly in women and in the myocardium.[21] ARs have been identified in endothelial cells, VSMCs, macrophages and cardiomyocytes in both male and female, but little is known about their functions in cardiovascular physiology.[22] We aimed to investigate the effects of female sex hormone (E_2) in the male rat heart on key molecules regulate proliferation, migration, endothelial function, vascular tone regulation and angiogenesis that play role in the pathogenesis of CVDs. Thus, this study will partly shed light on the regulatory mechanisms of cardiovascular actions of sex hormones and the basis of gender differences in the CVDs.

MATERIAL AND METHODS

Design of Experimental Groups and Induction of Orchiectomy

Experiments were started following the approval of the Ethical Committee of Selcuk University (Project number: 2010 / 005) and they were carried out on 21 male Wistar Albino rats. Rats were housed at ambient temperature (23 ± 2 $^{\circ}$ C) and humidity on a 12 / 12 h light / dark cycle. All animals received food and water ad libitum. Unless otherwise stated all chemicals used were purchased from Sigma (Sigma - Aldrich, Munich, Germany). Induction of orchiectomy was done like in elsewhere.[23] Briefly, under the anesthesia ketamine [(0.15 ml / 100 g) and rompun (0.07 ml / 100 g)] and aseptic conditions approximately 1 cm skin incision was made at the tip of the scrotum, then 5 cm incision was made into each sac at the tip of each testis. Then the cauda epididymis was pulled out and following to the vas deferens and spermatic blood vessels were ligation the testes were removed. Sham operation was performed in which the testis and epididymis were only pulled out and then replaced and the blood vessels were left intact. In order to test the effects of estradiol on the male heart, three experimental groups were planned: 1: Control group (Con, N = 5) was sham operated. 2: To assess the effect of absence of testosterone, rats were castrated and received vehicle till the end of experimental period (M -, N = 8). 3: To observe the effects of estradiol on male rats, the third group received 5 g / 100 mg / day estradiol injections starting from the day after castration (MX, N = 8). These doses of estradiol were shown to supply normal serum hormone levels. Estradiol injections were prepared inside vehicle (sesame oil, 0.1 ml / 100 g / day) and all the injections were done intraperitoneally. Experiments were carried out for 30 days. No infection was observed during follow - up.

Immunohistochemical Analysis

Under the intraperitoneal (ip) ketamine (0.15 ml / 100 g) and rompun (0.07 ml / 100 g) anesthesia, rats were sacrificed on the 30th day and the heart tissues were rapidly excised. The heart tissues were fixed in 10 % buffered formaldehyde solution and subsequently embedded in paraffin following standard procedures. 4 - 5 µm thickness sequential sections were taken from paraffin blocks. The sections were then deparaffinized and rehydrated. The sections were stained with primary antibodies for TGFβ1, TGFβRI, TGFβRII, PDGF, PDGFR - α, PDGFR - β, IGF - I, IGF - IR, bFGF, VEGF, eNOS, ET - I, ER - α, ER - β and AR. Briefly, sections were subjected to heat - mediated antigen retrieval and incubated with 3 % hydrogen peroxide to block endogenous peroxidase activity. Sections were then treated with protein blocking solution to block nonspecific staining. Subsequently, the sections were incubated with primary antibodies overnight at 4°C and were then treated with biotinylated secondary antibodies followed by incubation with peroxidase - conjugated streptavidin. All steps were followed by washing in phosphate buffer solution. Immunoreaction was visualized by incubating the sections with 3, 3 – Diaminobenzidine (DAB) chromogenic. Finally, the sections were counterstained with hematoxylin, dehydrated and mounted. Negative controls were carried out without primary antibodies. The results of the immunohistochemical staining were independently evaluated by two observers under the light microscopy and digital images were recorded. The expressions of the molecules were scored according to the intensity of the staining, as follows: negative staining; 0, weakly positive staining; 1 +, moderately positive staining; 2 +, strongly positive staining; 3 +.

Statistical Analysis

Results were expressed as mean ± standard deviation. Comparisons between multiple groups were analyzed by Kruskal - Wallis test. Mann-Whitney U test was applied for comparisons between two groups. Results were considered significant with a probability level of less than 0, 05.

RESULTS

General Characteristics of Experimental Group of Animals

During the follow up period no infections were seen and none of the animal was died. But as it was expected body weights were found to be changed when compared to initial values (Con: 326,80 ± 23,41 – 330,40 ± 17,13; M-: 353,78 ± 6,76 – 285,33 ± 13,11; MX: 358,20 ± 11,54 – 293,00 ± 18,40, p < 0,05).

Immunohistochemical Results

Immunohistochemically, orchiectomy resulted in the significant depression in the expressions of TGF - β1 and TGFβRI whereas ineffective on the expression of TGFβRII compared to initial quantities. E_2 treatment could not change the orchiectomy induced reduction but increased the expression of TGFβRII even above the control group quantities. For about the expressions of PDGF, PDGFR - α and PDGFR - β orchiectomy resulted in suppression, ineffective and unchanged, respectively. E_2 treatment not only further depressed the expression of PDGF but also decreased the expression of PDGFR - β. Both the orchiectomy and E_2 treatment produced suppression on the expression of IGF - I. The expression of IGF - IR was stimulated by orchiectomy, E_2 treatment did not change that. The expression of bFGF was suppressed by orchiectomy and E_2 treatment. The expression of VEGF was induced by orchiectomy whereas it was not changed by E_2 treatment. Orchiectomy and E_2 treatment resulted in the significant depression and induction in the expression of eNOS, respectively. The expression of ET - I unchanged between the groups. The expression of ER - α was not affected by orchiectomy, whereas the expression of ER - β was increased by orchiectomy. E_2 treatment induced the expression of ER - α, did not change the expression of ER - β. Orchiectomy resulted in the AR up regulation and E_2 treatment caused to AR down regulation, which were below the control group quantities. Figure 1 and 2 demonstrate the comparisons of molecule expressions between the experimental groups and the results of immunohistochemical staining (Figures 3-5).

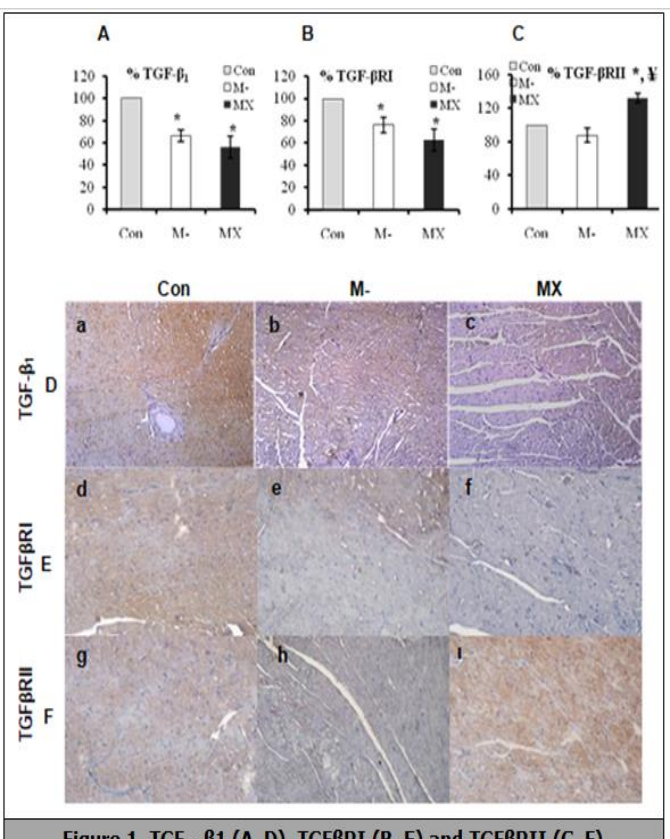

Figure 1. TGF - β1 (A, D), TGFβRI (B, E) and TGFβRII (C, F) Expressions in the Heart Tissue of Experimental Group of Animals.

Figure Represents: A, B, C: Mean average values expressed as mean ± SEM. *Represents the degree of significance (p < 0.05) compared to control group animals. ¥Represents the degree of significance (p < 0.05) compared to M - group animals. A, D, G: Control group, B, E, H: Orchiectomized male group (M -), c, f, ı: Orchiectomies and 17 beta estradiol treated group (MX). Positive staininig for molecules were seen brown in color. Magnification: x 20.

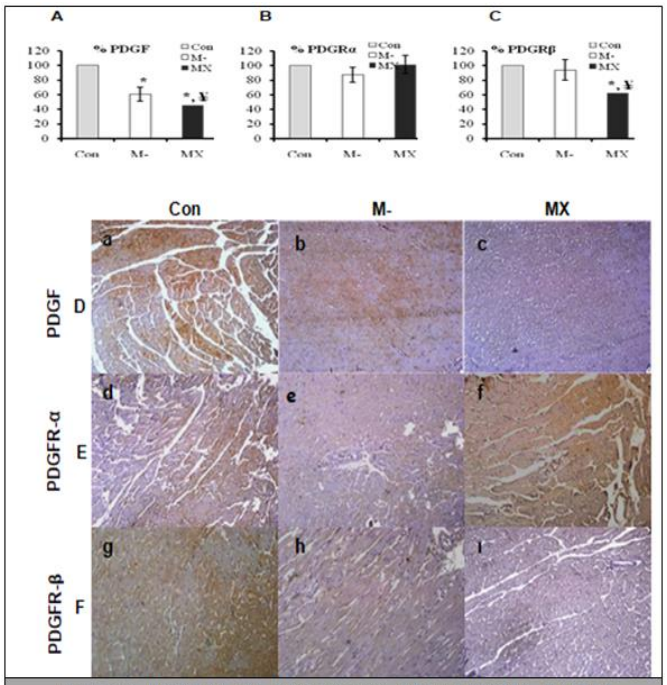

Figure 2. PDGF (A, D), PDGFR-α (B, E) and PDGFR-β (C, F) Expressions in the Heart Tissue of Experimental Group of Animals.

Figure represents: A, B, C: Mean average values expressed as mean ± SEM. *Represents the degree of significance (p < 0.05) compared to control group animals. ¥Represents the degree of significance (p < 0.05) compared to M - group animals. A, D, G: Control group, B, E, H: Orchiectomized male group (M -), c, f, ı: Orchiectomies and 17 beta estradiol treated group (MX). Positive stainiig for molecules were seen brown in color. Magnification: x 20.

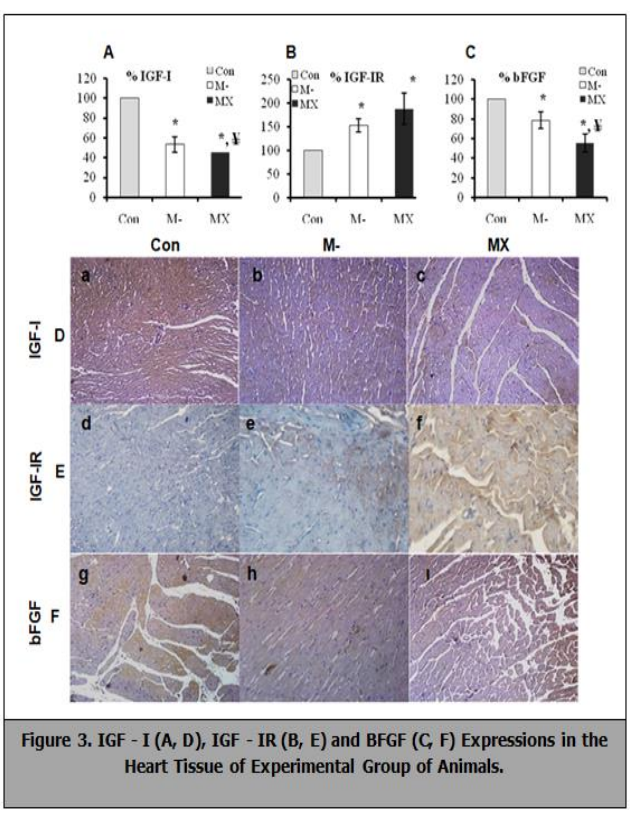

Figure 3. IGF - I (A, D), IGF - IR (B, E) and BFGF (C, F) Expressions in the Heart Tissue of Experimental Group of Animals.

Figure represents: IGF - I (A, D), IGF - IR (B, E) and bFGF (C, F) expressions in the heart tissue of experimental group of animals. A, B, C: Mean average values expressed as mean ± SEM. *Represents the degree of significance (p < 0.05) compared to control group animals. ¥Represents the degree of significance (p < 0.05) compared to M - group animals. a, d, g: Control group, b, e, h: Orchiectomized male group (M -), c, f, ı: Orchiectomized and 17 beta estradiol treated group (MX). Positive stainiig for molecules were seen brown in color. Magnification: x 20.

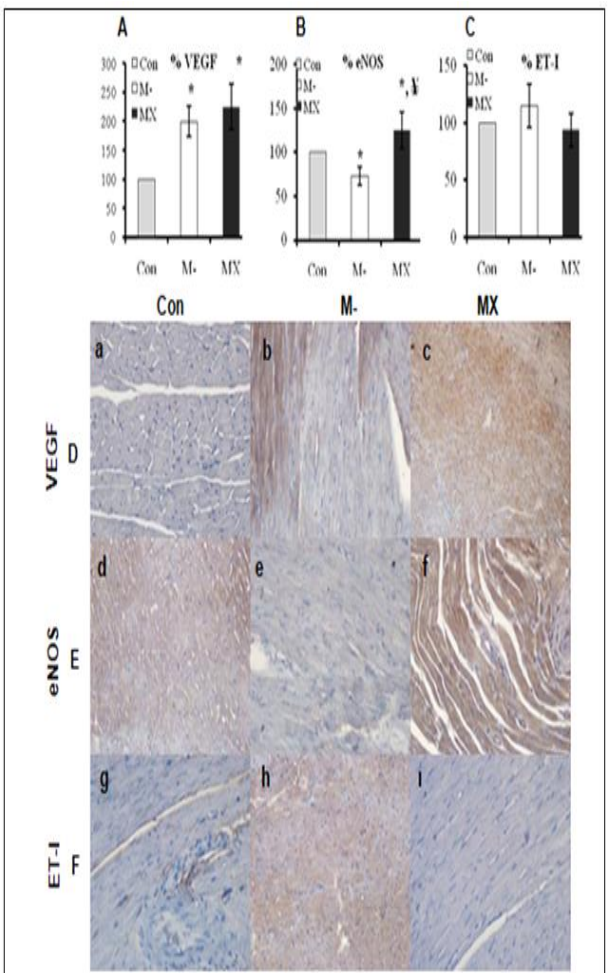

Figure 4. VEGF (A, D), eNOS (B, E) and ET-I (C, F) Expressions in the Heart Tissue of Experimental Group of Animals.

Figure represents: A, B, C: Mean average values expressed as mean ± SEM. *Represents the degree of significance (p < 0.05) compared to control group animals. Represents the degree of significance (p < 0.05) compared to M - group animals. a, d, g: Control group, b, e, h: Orchiectomized male group (M -), c, f, ı: Orchiectomized and 17 beta estradiol treated group (MX). Positive stainiig for molecules were seen brown in color. Magnification: x 20.

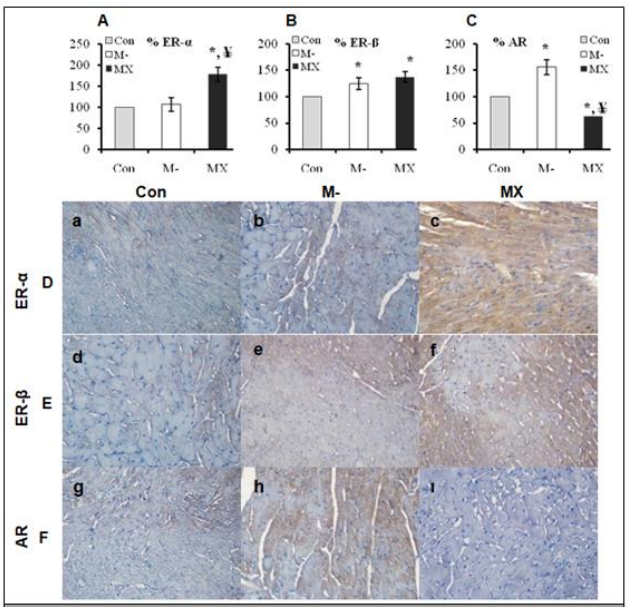

Figure 5. Estrogen receptors, ER - α (A, C), ER - β (B, D) and Androgen Receptor, AR Expressions in the Heart Tissue of Experimental Group of Animals.

Figure represents: A, B: Mean average values expressed as mean ± SEM. * Represents the degree ofsignificance ($p < 0.05$) compared to control group animals. ¥ Represents the degree of significance ($p < 0.05$) compared to M group animals. a, d, g: Control group, b, e, h: Orchiectomized male group (M-), c, f, ı: Orchiectomized and 17 beta estradiol treated group (MX). Positive staininig for molecules were seen brown in color. Magnification: x 20.

DISCUSSION

It has been shown in many previous studies that gender plays an important role on the CVS and there are basic gender differences in the emergence of CVDs, suggesting the gender - dependent differences in the pathogenesis of these processes.[24] Sexual differences have been explained by the different endogenous sex steroid hormone levels in men and women. These differences have suggested that estrogens have protective effects and / or androgens have stimulatory effects against CVDs. In some epidemiological and experimental studies, the cardio protective effect of estrogens in female has been evidenced. However, Coronary Drug Project has published in the 70's that estrogen replacement therapy administered to men with CHD has the adverse effects.[25] In addition, elevated serum estradiol levels in male patients with CHD (4) suggest the hypotheses that hyperestrogenemi may be a risk factor for CHD in men. Phillips has proposed estrogen - androgen paradox endogenous sex hormones may relate both to CVDs and its risk factors oppositely in women and men.[26] However, the further studies have evidenced that estrogen replacement have positive effects on the lipid profiles and decreases the long - term cardiovascular morbidit in the men with androgen deprivation. [27,28] Therefore, the cardiovascular effects of sex hormones are not fully known currently. This study demonstrated that E_2 may have some protective effects on the male CVS through the modification of various molecules, induction of ER - α, eNOS and TGFβRII that are mediated the cardio protective effects, the reduction of AR, IGF - I, bFGF, PDGF and PDGFR - β that are mediated the development of CVDs. The cardio protective effects of estrogens have been associated with promotion of endothelium - dependent relaxation *via* increased NO synthesis and activity decreased ET - I plasma levels and ET - I mRNA expression. [29,30] The transdermal application of estradiol has improved arterial endothelium - dependent vasodilatation in women, but not in men.[31] However, in latter studies, estradiol has caused to vasodilatation by inducing of endothelial NO production in male rats.[32] The gender differences in the heart function and the regulatory mechanisms of sex hormone effects on the myocardium are less well known E_2 increase the expressions of eNOS, ER - α and particularly ER - β and to be prerequisite of ER - β activation for estrogen - dependent upregulation of eNOS in the myocardium of both female and male rats. Whereas we found that E_2 significantly increased the expressions of eNOS and ER - α, but not ER - β in the myocardium of male rats. Our findings suggest that ER - α is mediated the up regulation of eNOS by estrogen, but ER - β activation is not requisite for eNOS up regulation. We also firstly reported that E_2 did not effect on the expression of ET - I in the male myocardium. Our findings suggest that eNOS but not ET - I, plays a role in estrogen induced - endothelium - dependent relaxation in males. PDGF inhibitors have been shown to inhibit the activation, migration and proliferation of VSMCs that occur in intimal thickening. Interestingly, the PDGF receptor subtypes are differentially regulated in neointima formation. The PDGFR - α has been observed to promote hypertrophy of VSMCs, whereas a mitogenic response is mediated only through the PDGFR - β. In addition to the importance of PDGF in vascular remodeling, PDGF has been shown to be a potent mitogen for cardiac fibroblasts.[33] A study investigated the effect on the PDGF signaling of estrogen / ER activity has demonstrated that E_2 reduced PDGF - induced VSMC proliferation and migration, but did not change the PDGFR - β expression, achieved this effect by modifying the PDGF signals at the postreceptor level.[34] We found that E_2 reduced PDGFR - β expression as well as PDGF expression, but did not alter the expression of PDGFR - α. E_2 - induced the down regulations of PDGF and PDGFR - β which are mediated the proliferation of VSMCs, point outs the inhibitory effect of estrogens against to intimal hyperplasia and atherosclerosis. TGF - β_1 overexpression has resulted in cardiac hypertrophy which is characterized by both interstitial fibrosis and hypertrophic growth of cardiomyocytes.[35] TGF - β_1 also has been reported to inhibit the growth of normal VSMCs, with a little induction of collagen synthesis, whereas to stimulate the growth of vascular lesion cells, with markedly increase collagen synthesis. The cells in vascular lesions have been suggested to become resistant to the ant proliferative effect of TGF - β_1 due to the change of their TGF - β_1 receptor profiles. TGF - β_1 has been shown to induce the contractile protein expression, but does not increase the extracellular matrix production through type II receptor of TGF - β_1 which is abundant in VSMC of normal vessels. Whereas type I receptor has increased in diseased vessels and induced the extracellular matrix production and the fatty streak lesion formation.[36] Estrogens have been reported to inhibit the proliferation and extracellular matrix production of VSMCs the hypertrophy of cardiomyocytes and the growth of cardiac fibroblasts gender -

independently. However, the regulatory pathways mediating these effects of estrogens are not fully known. It seems reasonable that TGF - β_1 mediates the cardiovascular effects of estrogens. However, little is known the effect on the TGF - β signaling of estrogen / ER activity in cardiovascular cells. We found that E_2 insignificantly decreased in the expressions of TGF - β_1 and TGFβRI which are mediated to inductive effect of TGF - β_1 on the growth of cardiovascular cells. On the other hand, interestingly, E_2 caused to increase the TGFβRII expression which are mediated the cardio protective effect of TGF - β_1, suggesting a beneficial role for E_2 in males. Estrogens have reported to re - organize the collateral vessels and to increase the perfusion in the myocardium by regulating the angiogenic signal pathways and the growth factors.[37] Several actions of estrogen may promote angiogenesis, including increased levels of VEGF, bFGF.[38,39] Although it is well known that estrogens exhibit the angiogenic properties under hypoxic conditions.[40] however under normoxic conditions, the angiogenic effect of estrogens is highly contradictory. Furthermore, the effect of estrogen signals on VEGF in the males CVS is unclear. We found that E_2 significantly did not alter the cardiac VEGF expression, indicating that the angiogenic effect of E_2 is not *via* VEGF in male heart. Epidemiological and experimental studies have proposed the role for IGF - I in the development of CVDs in bi - directional, as a protective or an inductive.[41,42] It has been reported that estrogen increased the cardiomyocyte size and IGF - I expression in healthy heart *via* ER - α than rather ER - β in female mice, suggesting the cardiac growth stimulatory effect of E_2 under physiological conditions. Whereas E_2 has inhibited the ventricular hypertrophy by reducing the cardiac IGF - I expression under pathological conditions. However, the effects of estrogen on IGF - I and IGF - IR in the males are largely unknown. We demonstrated suppressor effect of E_2 on IGF - I in the male heart under normal conditions. This effect is probably to occur *via* ER - α in the male heart. Interestingly, E_2 did not change IGF - IR expression, suggesting that cardiac effects of IGF - I occur in a different pathway, except at the receptor level. bFGF has been reported to have beneficial cardiovascular effects by providing the vascularization in CHD through the angiogenesis, but, also to contribute to CHD by causing the proliferation and migration of VSMCs participating in the atherosclerotic plaque structure. In the literature, there are contradictory data on the effects of estrogen on bFGF production in cardiovascular cells, that both the stimulating and inhibiting.[43,44] E_2 - induced bFGF stimulation has been argued to contribute to the cardioprotective effects of estrogen. However, present study revealed the inhibitory effect of E_2 on bFGF in the male heart. According to our results, E_2 only stimulated eNOS expression responsible for NO production from angiogenesis - related molecules such as NO, VEGF and bFGF, suggesting that eNOS mediates the angiogenic effect of estrogen in males.

CONCLUSION

Present study demonstrated that estrogen may have some positive effects on the male CVS by modifying various molecules known to be associated with CVDs. In the literature, there are highly conflicting data about the cardiovascular effects of estrogen in men. Our findings do not support the argument that estrogen can lead to CVD in men as claimed previously. E_2 - induced eNOS, TGFβRII and ER - α increase and IGF - I, bFGF, PDGF and PDGFR - β and AR decrease may contribute to cardioprotective effects of estrogen. E_2 possibly carries out at least a part of the cardiovascular effects through the downregulation of AR and / or the up regulation of ER - α. In conclusion, this study suggests a protective role for estrogens and demonstrates the possible molecular mechanisms for this role in the male CVS. Therefore it may contribute to the development of new strategies in the treatment and prevention of CVDs in men. However, the biology of sex hormones and the regulatory mechanisms of their cardiovascular actions are highly complex and there is a need to further studies on this issue.

REFERENCES

1. Hayward CS, Kelly RP, Collins P. The roles of gender, the menopause and hormone replacement on cardiovascular function. Cardiovasc Res 2000;46:28-49.
2. Adams MR, Kaplan JR, Manuck SB, et al. Inhibition of coronary artery atherosclerosis by 17 - beta estradiol in ovariectomized monkeys. Lack of an effect of added progesterone. Arteriosclerosis 1990;10(6):1051-1057.
3. Mori T, Durand J, Chen Y. Effects of short-term estrogen treatment on the neointimal response to balloon injury of rat carotid artery. Am J Cardiol 2000;85(10):1276-1279.
4. Phillips GB, Castelli WP, Abbott RD, et al. Association of hyperestrogenemia and coronary heart disease in men in the Framingham cohort. Am J Med 1983;74(5):863-869.
5. Ross R. The pathogenesis of atherosclerosis an update. N Engl J Med 1986;314(8):488–500.
6. Tostes RC, Nigro D, Fortes ZB, et al. Effects of estrogen on the vascular system. Braz J Med Biol Res 2003;36:1143–1158.
7. Karanigas G, Nguyen BT, Jarry H. Estrogen modulates cardiac growth through an estrogen receptor α-dependent mechanism in healthy ovariectomized mice. Mol Cell Endocrinol 2014;15;382(2):909-914. 8. Tivesten A, Bollano E, Nyström HC, et al. Cardiac concentric remodelling induced by non-aromatizable (dihydro-) testosterone is antagonized by oestradiol in ovariectomized rats. J Endocrinol 2006;189(3):485-91.
9. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. Am J Physiol Regul Integ Comp Physiol 2004;286(2):233–249.
10. Wu Q, Zhao Z, Sun H, et al. Oestrogen changed cardiomyocyte contraction and beta-adrenoceptor expression in rat hearts subjected to ischemia–reperfusion. Exp Physiol 2008;93:1034-1043.
11. Crews JK, Khalil RA. Gender-specific inhibition of Ca^{2+} entry mechanisms of arterial vasoconstriction by sex hormones. Clin Exp Pharmacol Physiol 1999;26(9):707–715.
12. Davies MG, Owens EL, Mason DP, et al. Effect of platelet-derived growth factor receptor-a and -b blockade on flow-induced neointimal formation in endothelialized baboon vascular grafts. Circ Res 2000;86:779–786.
13. Bobik A. Transforming Growth Factor-ßs and Vascular Disorders. Arterioscler Thromb Vasc Bio 2006;26:1712.
14. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–676.
15. Efthimiadou A, Asimakopoulos B, Nikolettos N, et al.

The angiogenetic effect of intramuscular administration of b-FGF and a-FGF on cardiac muscle: the influence of exercise on muscle angiogenesis. J Sports Sci 2006;24:849–854.
16. McMullen JR, Shioi T, Huang WY, et al. The insülin like growth factor 1 receptor induces physiological heart growth *via* the phosphoinositide 3-kinase (p 110 alpha) pathway. J Biol Chem 2004;279:4782–4793.
17. Dubey RK, Gillespie DG, Jackson EK, et al. 17 Beta-estradiol, its metabolites, and progesterone inhibit cardiac fibroblast growth. Hypertension 1998;31(11 Pt 2):522-528.
18. Mendelsohn ME. Genomic and nongenomic effects of estrogen in the vasculature. Am J Cardiol. 2002;90:3–6.
19. Rubanyi GM, Freay AD, Kauser K, et al. Vascular estrogen receptors and endothelium-derived nitric oxide production in the mouse aorta. Gender difference and effect of estrogen receptor gene disruption. J Clin Invest 1997;99:2429–2437.
20. Pare G, Krust A, Karas RH, et al. ER-alfa mediates the protective effects of estrogen against vascular injury. Circ Res 2002;90:1087–1092.
21. Nuedling S, Karas RH, Mendelsohn ME, et al. Activation of estrogen receptor-beta is a prerequisite for estrogen-dependent upregulation of nitric oxide synthases in neonatal rat cardiac myocytes. FEBS Letters 2001;502:103-108.
22. Kaushik M, Sontineni SP, Hunter C. Cardiovascular disease and androgens: a review. Int J Cardiol 2010;25;142(1):8-14.
23. Ayaz M, Akand M, Kucukbagriacik Y, et al. The effects of estradiol on cardiac muscle electrophysiology in orchiectomized rat model: a new insight to side effects caused by castration. Eur Rev Med Pharmacol Sci 2015;19(15):2866-74.
24. Kalin MF, Zumoff B. Sex hormones and coronary disease: a review of the clinical studies. Steroids 1990;55:330–352.
25. Berge KG, Canner PL. The Coronary Drug Project Research Group: Initial findings leading to modifications of its research protocol. JAMA 1970;214:1303–1313.
26. Phillips GB. Is Atherosclerotic Cardiovascular Disease an Endocrinological Disorder? The Estrogen-Androgen Paradox. J Clin Endocrinol Metab 2005;90:2708–2711.
27. Purnell JQ, Bland LB, Garzotto M, et al. Effects of transdermal estrogen on levels of lipids, lipase activity, and inflammatory markers in men with prostate cancer. J Lipid Res 2006;47:349–355.
28. Ockrim JL, Lalani el N, Aslam M, et al. Changes in vascular flow after transdermal oestradiol therapy for prostate cancer: a mechanism for cardiovascular toxicity and benefit? BJU International 2006;97:498–504.
29. Weiner CP, Lizasoain I, Baylis SA, et al. Induction of calcium-dependent nitric oxide synthesis by sex hormones. Proc Natl Acad Sci 1994;94:5212–5216.
30. Tan Z, Wang TH, Yang D, et al. Mechanisms of 17 beta-estradiol on the production of ET-1 in ovariectomized rats. Life Sci 2003;10;73(21):2665-2674.
31. Kawano H, Motoyama T, Kugiyama K, et al. Gender difference in improvement of endothelium dependent vasodilation after estrogen supplementation. J Am Coll Cardiol 1997;30:914-919.
32. Fenoy FJ, Hernandez ME, Hernandez M, et al. Acute effects of 2-methoxyestradiol on endothelial aortic No release in male and ovariectomized female rats. Nitric Oxide 2010;23(1):1219.
33. Rosenkranz S, Kazlauskas A. Evidence for distinct signaling properties and biological responses induced by the PDGF receptor α and β subtypes. Growth Factors 1999;16:201–216.
34. Kappert K, Caglayan E, Huntgeburth M, et al. 17 β - Estradiol attenuates PDGF signaling in vascular smooth muscle cells at the postreceptor level. Am J Physiol Heart Circ Physiol 2006;290:538–546.
35. Rosenkranz S, Flesch M, Amann K, et al. Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-beta(1). Am J Physiol Heart Circ Physiol 2002;283:1253–1262.
36. McCaffrey TA, Consigli S, Du B, et al. Decreased type II/type I TGF-b receptor ration in cells derived from human atherosclerotic lesions. J Clin Invest 1995;96:2667–2675.
37. Lamping KG, Christensen LP, Tomanek RJ. Estrogen therapy induces collateral and microvascular remodeling. Am J Physiol Heart Circ Physiol 2003;285:2039–2044.
38. Jesmin S, Sakuma I, Hattori Y, et al. Regulatory molecules for coronary expressions of VEGF and its angiogenic receptor KDR in hypoestrogenic middle aged female rats. Mol Cell Biochem 204;259:189–196.
39. Rusnati M, Casaroti G, Pecorelli S, et al. Estro-progestinic replacement therapy modulates the levels of basic fibroblast growth factor (bFGF) in postmenopausal endometrium. Gynecol Oncol 1993;48:88–93.
40. Kyriakides ZS, Petinakis P, Kaklamanis L, et al. Intramuscular administration of estrogen may promote angiogenesis and perfusion in a rabbit model of chronic limb ischemia. Cardiovasc Res 2001;49:626–633.
41. Akanji AO, Suresh CG, Al-Radwan R, et al. Insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein (IGFBP)-3 levels in Arab subjects with coronary heart disease. Scand J Clin Lab Invest 2007;67(5):553-559.
42. Hirai H, Kanaya R, Maeda M, et al. The role of insulin growth factor on atherosclerosis and endothelial function: the effect on hyperlipidemia and aging. Life Sci 2011;88:425–431.
43. Albuquerque ML, Akiyama SK, Schnaper HW. Basic fibroblast growth factor release by human coronary artery endothelial cells is enhanced by matrix proteins, 17 beta-estradiol, and a PKC signaling pathway. Exp Cell Res 1998;25;245(1):163-169.
44. Suzuki A, Mizuno K, Ino Y, et al. Effects of 17 beta-estradiol and progesterone on growth-factor-induced proliferation and migration in human female aortic smooth muscle cells *in vitro*. Cardiovasc Res 1996;32(3):516-523.