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Review

Female Sex Hormones Contribute to Vulnerability to Athero-Sclerosis: Overview of Mechanisms and Specific Risk Factors

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Abstract: Atherosclerosis poses a significant challenge to the general population, with its prevalence particularly prominent among older individuals. Age, however, is not the sole determinant of risk, as gender also plays a crucial role. Sex-related disparities exist not only in the incidence of atherosclerosis but also in its progression and development. The intricate interplay of hormones in the female reproductive system contributes to its proper functioning. Perturbations in this hormonal system can give rise to disorders and conditions that influence the susceptibility to various diseases, including cardiovascular disorders. Such conditions encompass both natural occurrences like menopause as well as pathological conditions such as polycystic ovarian syndrome. This comprehensive review delves into the examination of hormonal imbalances in females as risk factors and compiles pertinent information regarding the role of key hormones, particularly estrogen and its derivatives, in the context of atherosclerosis. The review article not only highlights the impact of hormonal imbalances on the risk of developing atherosclerosis but also provides comprehensive insights into the specific mechanisms underlying estrogen's role in the disease process. Additionally, it explores the influence of other major hormones, expanding the understanding of their contributions to atherosclerosis. To select the initial literature sources, we searched the PubMed database with the following keywords and their combinations: "Female hormones", "atherosclerosis", "sex as a risk factor", and "estrogen". Using this search, we chose several papers, from the analysis of which we started this review. Moving through these papers, we developed the structure of the review.

Keywords: Progesterone, Atherosclerosis, Pregnancy, Women's Health, Sex Hormones

Introduction

Atherosclerosis is a chronic condition characterized by the formation of fibrous atheromas or plaques on the walls of arteries. The stages of this process involve the accumulation of lipids in the arterial wall, inflammation, thickening of the intima, and fibrosis, which leads to the stiffening of the arterial wall and eventually result in plaque erosion or rupture (Rafieian-Kopaei *et al*., 2014). Plaque rupture can obstruct blood flow in the vessel, leading to ischemic heart disease or myocardial infarction. This process often remains asymptomatic for many years until clinical symptoms become apparent. Atherosclerosis, being a leading cause of mortality worldwide, contributes to the development of disorders such as stroke, myocardial infarction, peripheral artery disease, and aortic aneurysms (Libby *et al*., 2019). However, in the younger population (40-60 years), atherosclerosis or coronary artery disease primarily affects men, while women generally develop atherosclerosis after menopause (Fairweather, 2014). Although the prevalence of atherosclerosis is lower in women, autoimmune conditions like rheumatoid arthritis, systemic sclerosis, systemic lupus

erythematosus, as well as hypertension, can significantly increase the risk of atherosclerosis in women (Kurmann and Mankad, 2018). These findings suggest that the immune mechanisms underlying atherosclerosis may differ in men and women (Fairweather, 2014).

In women, myocardial infarction death rates are higher than in men, which is partially attributed to the development of atherosclerosis. Other factors include unexpected adverse effects of certain medications and post-surgical complications that are more prevalent in women, as well as vascular devices that are often better suited for men than women (Harvey *et al*., 2015). Furthermore, atherosclerosis in women often remains asymptomatic for a longer duration. The absence of chest pain or noticeable vessel occlusion in women often leads to misdiagnosis and delayed treatment. Additionally, there are significant differences in the inflammatory infiltrate in coronary artery disease between men and women (Pathak *et al*., 2017). Despite extensive research on atherosclerosis, there is a growing recognition that the immune mechanisms underlying the development of this condition may differ between men and women, suggesting the need for gender-specific approaches in understanding and managing atherosclerosis-related disorders.

Gender-Specific Risk-Factors

There are a number of gender-specific conditions that can promote the development of CVD in women, such as menopause, preeclampsia, Polycystic Ovary Syndrome (PCOS), and gestational diabetes (Vakhtangadze *et al*., 2021).

Polycystic Ovary Syndrome

This disorder affects approximately 10% of women during the reproductive years. PCOS with a complete phenotype includes such symptoms as hyperandrogenism, polycystic ovaries, and ovulatory dysfunction. A study by Talbott *et al.* (2004) reported an association between PCOS and intimate media thickness due to metabolic consequences of PCOS. Lifelong exposure to the cardiovascular risk profile in PCOS can result in the early development of atherosclerosis in women. Additionally, PCOS may have other independent effects that are not due to the abovementioned factors related to this condition (El Hayek *et al*., 2016; Rosenfield and Ehrmann, 2016).

Preeclampsia/Eclampsia

Preeclampsia/eclampsia is considered to be a serious risk factor specific for women as it may severely complicate pregnancy. It has also appeared that those females who have been affected by this disorder are at a high risk of developing atherosclerotic conditions at a later age (Gupte and Wagh, 2014).

A systematic review with meta-analysis (Wu *et al*., 2017) demonstrated that women with preeclampsia four times more often develop cardiac failure and have a two-fold higher incidence of stroke, myocardial ischemia, and death caused by coronary heart disease or cardiovascular disease (Wu *et al*., 2017). A study conducted by Haukkamaa *et al*. (2009). Argues that preeclampsia may be an independent risk factor for atherosclerotic plaques (Haukkamaa *et al*., 2009). The research carried out by Kessous *et al*. (2015) suggests that preeclampsia during pregnancy independently contributes to the development of long-term atherosclerosis. Women suffering from acute and recurrent preeclampsia are at a higher risk of developing atherosclerotic disease (Kessous *et al*., 2015).

Gestational Diabetes

Type 1 diabetes or gestational diabetes in young women is another gender-related risk factor. While diabetes associated with other factors causes about 30% of the age-associated growth in the risk of CHD in male patients, in women the risk grows up to 60% (Kramer *et al*., 2019).

Menopause

Another factor contributing to sex differences in atherosclerotic morbidity can be liver estrogen signaling which promotes steps of Reverse Cholesterol Transport (RCT) in the liver (Palmisano *et al*., 2018). The importance of estrogen for the progress of RCT in its early stages in humans may be disputable. Estradiol esters from high-density lipoprotein can improve cholesterol efflux capacity from macrophages. There is a high probability that increased levels of VLDL-TG in menopause cause an increase in efflux of cholesterol from HDL due to estrogen deficiency in menopause compared to the premenopausal period (Corcoran *et al*., 2011).

Anti-Atherosclerotic Effect of Female Sex Hormones

Epidemiological studies showed that overall morbidity and mortality from CVD are lowest in premenopausal women compared to postmenopausal women of the same age as men, suggesting that female sex hormones can be protective against cardiovascular diseases. This notion was confirmed by the evidence that estrogen administration in postmenopausal women ameliorated the course of the disease (Ueda *et al*., 2021). Several studies on animal models demonstrated that estrogen treatment reduced experimentally induced atherosclerosis (Berntsen *et al*., 2021; Goetz *et al*., 2018).

According to a study by Rhee *et al*. (1977), estrogen decreased surgery-induced hyperplasia in rabbit aorta.

Balloon injury-related intimal thickening can be reduced by estradiol. Diet-induced atherosclerosis in rats and rabbits can also be ameliorated by estrogen (Rhee *et al*., 1977).

Studies in macaques revealed lower prevalence of atherosclerosis in premenopausal macaques than in male macaques. However, Ovariectomy (OVX)-) induced menopause in combination with a moderately atherogenic diet led to progressive development of atherosclerosis (Kaplan *et al*., 2009). An Estrogen Replacement Therapy (ERT) initiated at the same time as ovariectomy showed a significant anti-atherosclerotic effect, while delayed treatment had low or no effect (Guo *et al*., 2020).

Several influential interventional studies in humans also demonstrated an inverse correlation between CVD development and estrogen administration in postmenopausal women, confirming the role of estrogen in reducing the CVD risk (Iorga *et al*., 2017). A number of trials (The estrogen in the prevention of atherosclerosis trial and "ELITE" the early versus late intervention trial with estradiol) showed that unopposed ERT with micronized 17-estradiol (1 mg/day) decreased Carotid Intima-Media Thickness (CIMT) in women compared to placebo-administered controls (Mehta *et al*., 2021). Women administered with estradiol within the first 6 years of menopause demonstrated lower mean CIMT than the late intervention group who received the treatment 10 or more years after menopause. Furthermore, a positive association between higher estradiol plasma levels following the therapy and lower subclinical atherosclerosis was only observed in the early intervention group once again confirming the role of timing in Hormone Therapy (HT) (Sriprasert *et al*., 2019). A concurrent cohort multi-ethnic study of atherosclerosis also revealed a reverse correlation between estradiol levels and the risk of atherosclerotic CVD in postmenopausal women (Prabakaran *et al*., 2021).

Natural progesterone administration contributes to estrogen-related effects in reducing CVD in postmenopausal women. However, this does not apply to synthetic Medroxyprogesterone Acetate (MPA). 4-week estradiol-only therapy (18 days 1 mg/day dosage and 2 mg/day for the next 10 days) postponed the onset of ST depression on an ECG in a treadmill exercise stress test (Nie *et al*., 2022). This effect was further enhanced in estradiol-treated subjects administered transvaginal progesterone gel (90 mg every two days), but not oral MPA (10 mg/day).

Negative lipid profile changes belong to the main factors accelerating atherogenesis (Furness *et al*., 2004). Thus, an additional beneficial effect of progesterone in increasing High-Density Lipoprotein Cholesterol (HDL-C) and triglycerides and decreasing Low-Density Lipoprotein Cholesterol (LDL-C) in estrogen-treated patients, was investigated. The postmenopausal estrogen/progestin interventions showed a superior increase in HDL-C in patients administered with unopposed estrogen (0.625 mg/day) in combination with cyclic Micronized Progesterone (MP) (200 mg/day) compared to patients treated with unopposed estrogen and MPA (Jiang and Tian, 2017). Unopposed estrogen plus cyclic MP treatment resulted in higher triglyceride levels and lower LDL-C levels compared to matched placebo-treated controls. The multi-center clinical trial on hormone replacement treatment in China demonstrated similar outcomes (Jiang and Tian, 2017).

Still, a number of interventional studies showed divergent results. The heart and estrogen-progestin replacement study evaluated a group of postmenopausal women with CHD administered with unopposed estrogen (0.625 mg/day) and MPA (2.5 mg/day) (Shufelt and Manson, 2021). The study revealed no significant reduction in either nonfatal MI prevalence and CHD-related death rate or secondary outcomes such as resuscitated cardiac arrest or stroke. The estrogen replacement and atherosclerosis trial involved women with epicardial coronary stenosis (Hashemzadeh *et al*., 2020; Wellons *et al*., 2012). The same regimen or estrogen-only treatment resulted in a prominent increase in HDL-C levels and a decrease in LDL-C levels. However, no reduction in the development of coronary atherosclerosis was observed (Li *et al*., 2021). The Kronos early estrogen prevention study demonstrated no CIMT reduction in patients treated with unopposed estrogen (0.45 mg/day) and transdermal 17-estradiol (50 g/day, concomitant progesterone 200 mg/day was given at the beginning 12 days of each month), although recent-initiated healthy postmenopausal women did not demonstrate negative consequences following ERT. There is still a need for more research on ERT effects on various forms of atherosclerosis in order to find optimal regimens (Miller *et al*., 2019).

Follicle Stimulating Hormone (FSH)

A remarkable inverse correlation has been reported between FSH concentrations and atherosclerosis without evident manifestations in women between 64 and 73 years of age in a recent study. It is worth mentioning that the findings of the study have been adjusted for confounding factors such as sex hormone-binding globulin or estradiol levels, the prevalence of cardiometabolic impairments, or adiposity, unlike earlier trials (Gregersen *et al*., 2019). Furthermore, unlike previous studies, this time only

postmenopausal females were involved, which rules out the possibility of uneven concentrations of estradiol and FSH (Soares *et al*., 2020).

In order to improve normality, the IMT data of the study was transformed, which complicated the evaluation of the revealed associations between FSH and IMT (Bertone-Johnson *et al*., 2017). The unadjusted mean IMT difference between the greatest and lowest values of FSH in analyses of untransformed data was equal to 0.09, a difference of 9,6%. Obese women demonstrated a 3.1% higher unadjusted IMT compared to normal-weight women (P = 0.20), while individuals with hypertension had a 9.8% higher mean IMT compared to women with normal blood pressure. Thus, the level of difference in mean IMT between individuals with the highest and lowest FSH levels is similar to that of other well-known proatherogenic factors (Freeman *et al*., 2010).

The study of women's health across the nation also included some research on the correlation between FSH and IMT. A cross-sectional study of patients between 45-58 years (mean age was ~50 years) did not reveal any association between FSH and IMT. The study assessed the trajectory of FSH and estradiol change throughout the perimenopause (El Khoudary *et al*., 2019). Later El Khoudary *et al*. (2019) measured the correlation between the trajectory and IMT 8 years following the menopausal transition (mean age was ~59 years). Following adjustment, the data demonstrated significantly higher IMT in individuals with the most typical trajectory of FSH growth (n = 431) compared to women with the lowest trajectory (n = 53) (El Khoudary *et al*., 2019). However, IMT in individuals with the highest trajectory (n = 372) was not much greater, which indicates a non-linear relationship. The study did not show any correlation between IMT and estradiol pattern. Interestingly, FSH levels were changed 10 years following the last menstruation, which illustrates the length of postmenopausal hormonal transition. That is why it is very hard to estimate the correlation between hormones and cardiovascular health in women throughout this period. The association between FSH and atherosclerosis may be different before and after the hormonal changes are complete (El Khoudary *et al*., 2019).

Only several works have estimated FSH in later-age patients. Shaw *et al*. revealed a significant decrease in FSH levels as well as in FSH reaction to GnRH in women aged 70-77 years (27 years after the last menstrual period on average) compared to women aged 48-57 (4 years after the last menstrual period on average), though no difference in estradiol levels was observed (Shaw *et al*., 2009).

FSH structure may vary between women before and after menopause. The half-life of the molecules depends on the isoforms of sulfonated N-acetylgalactosamine and sialic acid residues. Postmenopausal isoforms of FSH are characterized by greater concentrations of sialic acid and thus are more negatively charged and have a longer half-life *in vivo*. The prevalence of isoforms depends on age and differs from individual to individual (Wide and Eriksson, 2013).

The negative correlation between FSH and IMT reported in the recent study could possibly be explained by estradiol levels and obesity. Adiposity in postmenopausal women is associated with higher estradiol, since androgens are aromatized to estrogens in adipose tissues. Extragonadal estradiol suppresses FSH expression, thus obese women tend to have higher FSH levels (Mair *et al*., 2020). Inverse associations between FSH and adiposity have been observed in several different studies. However, in the recent study, the inverse associations between FSH and IMT persisted even following adjustment for the confounding factors and thus cannot be a result of an interplay between adiposity and estradiol levels (Barrett-Connor, 2013).

This relationship may be also affected by activities and follistatin. While activins A, B, and AB promote FSH synthesis, follistatin suppresses FSH secretion by binding activin. Together, activins and follistatin mediate adipogenesis, insulin resistance, inflammatory processes, and atherosclerosis. Polycystic ovary syndrome is also connected with dysregulation of follistatin-activins interaction (Teede *et al*., 2013). In addition to anovulation and hyperandrogenism, the condition has been associated with higher follistatin levels, reduced FSH levels, adiposity, and insulin resistance. Thus, these hormones may connect the reproductive and metabolic manifestations of this disorder. During the menopausal transition, follistatin levels decrease and bioavailability of activin increases, presumably leading to higher FSH levels in postmenopausal women. The findings of the recent study may reflect the results of hormonal interaction between activins and follistatin that affect atherosclerosis and insulin resistance in late menopause, leading to decreased sensitivity of the hypothalamic-pituitary-gonadal axis (Teede *et al*., 2013).

Mechanisms Behind the Anti-Atherogenic Effects of Estrogens

Figure 1 provides a schematic summary of antiatherosclerotic effects on various cell types. To illustrate the negative effects of estrogens, we also provided Fig. 2.

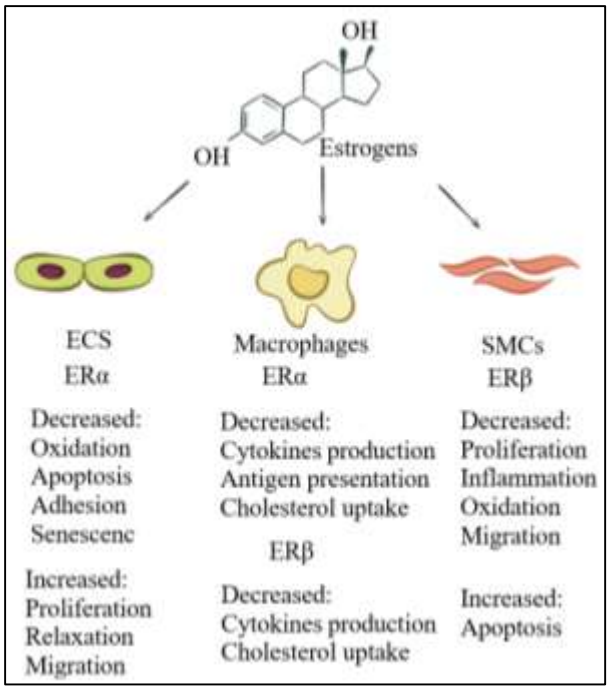

Fig. 1: Summary of anti-atherosclerotic effects of estrogens on cells of different types

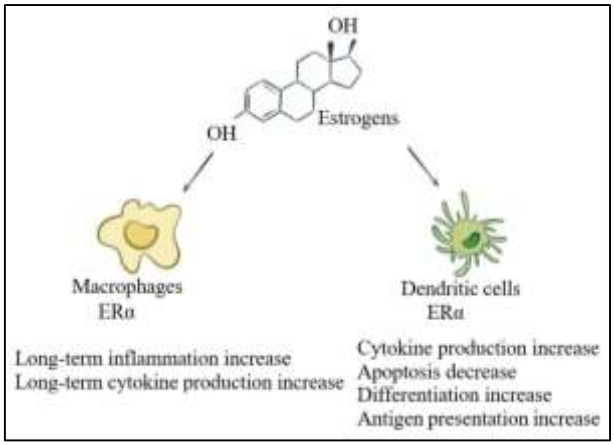

Fig. 2: Summary of pro-atherosclerotic effects of estrogens on cells of different types

The Role of Endothelium

Numerous studies have reported that Estradiol (E2), its metabolites as well as synthetic estrogens have a beneficial effect on endothelial function. This effect is reached through a number of various mechanisms. Firstly, E2 promotes Nitric Oxide (NO) production. In Estrogen Receptor Alpha (ERα) knockout mice E2 does not stimulate eNOS activity and NO-related vasorelaxation and these effects are mimicked by highly selective ERα agonists, which indicates that ERα plays a crucial role in E2-dependent NO production (Fuentes and Silveyra, 2019). However, when exposed to E2, endothelial cells demonstrate S-nitrosylation during which NO modulates protein functions directly. This process involves Estrogen Receptor Beta (ERβ). Furthermore, vascular constriction can be blocked by 2-methoxyestradiol (2-ME, which is not related to ERα or ERβ) through increased eNOS and local NO production. Secondly, E2 promotes prostacyclin (PGI2) release in the endothelium leading to a transfer from vasoconstriction to vasodilation as a result of increased expression of both cyclooxygenase 1 and/or 2 and prostaglandin synthase. Both ERα, ERβ, and 2-ME can mediate this process (Iwakiri, 2011). Thirdly, estrogen blunts endothelial activation, preventing endothelial dysfunction. Under exposure to pro-atherogenic factors like Lysophosphatidylcholine (LPC), IFNγ, Lipopolysaccharide (LPS), IL1B, and TNFα, E2 and its metabolites inhibit the release of adhesion molecules in the endothelium. At the same time, E2 decreases release of α4β1 and αLβ2 integrins which conjugate withVCAM1 and ICAM-1 during inhibition of RAC-1 activity. Subsequently, estrogen prevents monocyte and neutrophil migration into endothelium under *in vitro* conditions. Furthermore, estrogen downregulates NADPH oxidase activity, reducing intracellular expression of ROS which could promote the release of adhesion molecules. Both ERα, ERβ, and 2-ME can mediate this process (Thor *et al*., 2010).

In addition to enhancing endothelial function, estrogens support endothelial integrity by increasing its barrier function and reducing permeability to LDLs. As evidenced by many studies, E2 also promotes the proliferation and survival of endothelial cells due to its mitogenic effect. Furthermore, cell apoptosis initiated by TNFα, H_2O_2, or oxidized LDL can be prevented if the cells are exposed to E2 (Robert *et al*., 2021). Moreover, estrogens inhibit mitochondrial ROS release in the endothelium and ROS-related apoptosis through an interaction with cytochrome c release. Finally, estrogens increase telomerase activity by promoting eNOS-ERα complex formation. The complex interacts with the hTERT gene promoter and promotes hTERT transcription. Thus, estrogens are involved in preventing cellular senescence (Du *et al*., 2017).

These protective effects of E2 on endothelium can be emulated in animal models in the setting of atherosclerosis. E2 has been shown to hamper the negative effects of hypercholesterolemia on vasorelaxation as well as preventing fatty streak formation (Niță *et al*., 2021).

A study involving ovariectomized Apoe KO mice showed that an increase in NO bioavailability does not affect the development of atherosclerosis, suggesting that in early stages E2 has an anti-atherogenic effect that does

not depend on NO. However, in a study involving female rabbits on a pro-atherogenic diet, long-term administration of NOS inhibitors caused chronic endothelial dysfunction which aggravated atherosclerosis. The protective effect of E2 was abolished in this setting in intact but not in de-endothelialized vessels (Rodrigues *et al*., 2022).

There was reported a major inverse correlation between no production and atherogenesis in hypercholesterolemic rabbits with or without endothelial dysfunction. In a similar study, hypercholesterolemia-related adhesion of monocytes and their migration into the endothelium was more prevalent in male than in female animals. Oophorectomy aggravated this process, while E2 administration of female animals reversed it. As evidenced by later studies, regulation of endothelial VCAM1 release plays an important role in estrogen-exerted atheroprotection (Zhou *et al*., 2019).

Holm *et al*. initially revealed the pivotal role of endothelium for the anti-atherogenic effect of estrogens in Holm *et al*. (1997). The study showed that estrogens lost their atheroprotective effect in rabbits with hypercholesterolemia following the destruction of endothelium with a balloon catheter. These findings were confirmed by Billon-Galés *et al*. (2009). By means of CreLox recombination, they showed that ERα elimination in the endothelium leads to a complete abolition of the anti-atherogenic effect of E2 in ovariectomized LDLR-deficient murine models, following the suppression of E2-related no expression (Billon-Galés *et al*., 2009). These data prove that the endothelial monolayer should be the main target for estrogen treatment of vascular diseases and that the anti-atherogenic effect of E2 is mediated primarily by ERα which likely regulates estrogen-induced VCAM1 suppression in the endothelium.

The Role of Macrophages, Dendritic Cells and Lymphocytes

The number of macrophages in the arterial wall depends on the inverse processes of proliferation and apoptosis. The development of atherosclerotic plaques can be promoted by M-CSF-a cytokine that mediates the transition of monocytes to macrophages and growth of macrophages-as well as by suppression of macrophage apoptosis, while M-CSF absence, on the contrary, has an anti-atherogenic effect. However, in advanced lesions, apoptotic macrophages coalesce over time into a necrotic core which ultimately leads to plaque rupture and atherothrombotic events (Checkouri *et al*., 2021).

In regard to E2's influence on macrophage-related cholesterol homeostasis, currently available data demonstrate that E2 reduces cholesterol ester content. This has been proved by a study involving murine models. E2 inhibits acylCoA-cholesterol transferase and stimulates neutral cholesterol ester hydrolase. Furthermore, E2 decreases macrophage uptake of modified LDL. This effect depends on ERα-induced suppression of scavenger receptor CD36. Additionally, ERα-deficiency affects cholesterol efflux in macrophages mediated by ABCA1 and APOE proteins (Hai *et al*., 2018). ERα-deficient macrophages export less cholesterol after incubation with HDL, while E2 promotes cholesterol efflux when associated with HDL particles. According to numerous studies, E2 is carried in HDL in the form of fatty acyl esters. Lecithin-cholesterol acyltransferase (LCAT) catalyzes their production. LDLR- and/or SR-BI mediate the internalization of the esters and then they are hydrolyzed in the cell in order to unfold their effects. Additionally, Cholesteryl Ester Transfer Protein (CETP) transports E2 esters to LDL particles where they moderate oxidation caused by free radicals expressed by activated macrophages. Thus, E2 has a favorable effect on cholesterol homeostasis and reduces oxidation of LDL particles, decreasing LDL retention in the vessel wall (Jebari-Benslaiman *et al*., 2020).

In addition to enhancing cholesterol homeostasis, E2 was shown to have an indirect effect on macrophage proliferation. Studies involving Apoe-deficient mice demonstrated that E2 can modulate M-CSF expression in bone marrow and aorta. Additionally, E2 upregulates Fas and Fas Ligand (FasL (FASLG)) and activates caspases 8 and 3 which promotes apoptosis of monocytes, cholesterol-loaded macrophages, and macrophage-derived osteoclasts, resulting in lower lesion cellularity in mouse models of atherosclerosis (Farahi *et al*., 2021). Thus, E2 may have an anti-atherogenic effect in the early stages of atherosclerosis by reducing the amount of macrophages in the vessel intima. In one of the instances E2 also suppressed macrophage apoptosis in an ERα-mediated process depending on higher BCL2 expression. This effect can be favorable in treating late-stage atherosclerosis as it hampers necrotic core formation (Rentz *et al*., 2020).

Equivocal outcomes have been achieved in regard to the effect of estrogens on inflammation. Macrophages, monocyte-derived macrophages, primary microglia and microglial cell lines, E2, ER agonists, and phytoestrogens were shown to reduce pro-inflammatory activation, as evidenced by decreased iNOS release, reduce the synthesis of pro-inflammatory cells, as well as matrix metalloproteinase 9 both in mice models and in humans. E2 was also shown to abolish MHC-II release in macrophages in response to INFγ (Villa *et al*., 2016).

E2 was reported to have an impact on several inflammatory pathways, such as nuclear translocation, phosphorylation, mobilization of Signal Transducers and Activators of Transcription (STAT) 1 and 3, and inhibition of the transcription factor NF-κB activation

through both genomic and nongenomic action. E2 impact on iNOS release, TNFα expression, and NF-κB activation was partially inhibited in macrophages without Peroxisome Proliferator-Activated Receptor α (PPARα), suggesting that PPARα is involved in the anti-inflammatory action of E2 and highlighting its value as a therapeutic target in atherosclerosis treatment with fibrates (Marino *et al*., 2006).

Several studies involving animal models of inflammation confirmed the anti-inflammatory impact of estrogens on macrophages, such as abolishment of NF-κB translocation and prevention of glial reactivity in rats with chronic spinal cord trauma. A study in rats and mice with trauma hemorrhage showed that both E2 and ER agonists restored normal activation of NF-κB and cytokine expression through an ERα-mediated mechanism (Giraud *et al*., 2010). Furthermore, E2 suppressed MHC-II antigen expression and thus reduced macrophage recruitment into the allograft intima and transplant atherosclerosis in rats and rabbits that underwent artery transplantation. In a recent study, ERα-deficient macrophages isolated from animal models lacking ERα in myeloid cells were shown to be refractory to the anti-inflammatory effect of IL4, as evidenced by higher release of immune cell activity markers and chemokines. Moreover, the atherosclerotic lesion area in LDLR knockout models with myeloid-specific ERα deficiency was two times larger (Yu *et al*., 2020).

Controversially to these results (Rettew *et al*., 2009) estrogens may contribute to inflammation in a mouse model of sepsis (Rettew *et al*., 2009). Ovariectomy removed endogenous estrogens in the model and reduced the expression of pro-inflammatory cytokines, subsequently decreasing TLR4 expression on macrophages. E2 replacement had the opposite effect after the *in vivo* LPS challenge. Calippe *et al*. (2010) also reported that E2 administration promoted the release of iNOS, IL1B, IL6, and IL12p40 by peritoneal macrophages after *ex vivo* LPS (Calippe *et al*., 2010). Additionally, E2 administration inhibited AKT phosphorylation in LPS-activated macrophages and Phosphoinositide Kinase-3 (PI3K) activity. At the same time, it increased NF-κB p65 transcriptional activity. Later studies showed that ERα gene disruption in macrophages annihilates this effect of E2 on peritoneal macrophages, suggesting that E2 targeted these cells directly. The same group reported that optimal dendritic cell function is mediated by ERα signaling, as evidenced by the expression of MHC-II, CD86, and the release of pro-inflammatory cytokines (Mittal and Roche, 2015). Furthermore, E2 promoted mobilization of dendritic cells and Th1 reaction that promoted inflammation and thus aggravated experimental myasthenia gravis. In addition, E2 was demonstrated to enhance the GM-CSF-induced transition of progenitor cells in the bone marrow into DCs, suppress apoptosis, and increase the expression of pro-inflammatory cytokines. It is still unclear why estrogens have different effects on macrophages and dendritic cells in regard to inflammation *in vitro* and *in vivo*. The duration of the treatment may be one of the factors influencing the results of the studies (Bhattacharya *et al*., 2015).

Rayner *et al*. (2008) presented some new findings, suggesting that estrogens increase the macrophage expression of Heat-Shock Protein 27 (HSP27). HSP27 prevents the uptake of lipoproteins that contain a lot of cholesterol, inhibits foam cell formation, and promotes macrophage response that reduces inflammation (Rayner *et al*., 2008). Studies reported that female but not male murine models with excessive HSP27 presented reduced atherosclerosis. An inverse correlation between HSP27 and E2 levels in plasma was documented. E2 treatment of atherosclerosis was shown to be more efficient in the abovementioned model than wild-type mice and the atheroprotective action of E2 in these mice could be recapitulated by ERβ-specific agonist, but not ERα-specific agonist. These data indicate a completely new mechanism of anti-atherogenic E2 action involving ERβ, which could be a potential therapeutic target in the treatment of atherosclerosis (Madeira *et al*., 2013).

Involvement of Smooth Muscle Cells

In addition to endothelial cells and macrophages, the development of atherosclerosis also depends on Vascular Smooth Muscle Cells (VSMC). Following injury of the intima, VSMCs change their phenotype and become activated. They move from media to intima and increase proliferation. VSMCs contribute to plaque formation in the early stages by releasing lipid uptake receptors and participating in foam cell formation. Furthermore, like endothelial cells, they release pro-inflammatory cytokines, chemokines, free radicals, and adhesion molecules. At the same time, VSMCs are a source of intracellular matrix and thus prevent plaque rupture in the later stages of atherosclerosis (Hu *et al*., 2019).

There is substantial evidence of the significant effect of E2 and estrogens on vascular smooth muscle cells. *In vitro* studies have shown that E2 inhibits VSMC proliferation caused by FGF, PDGF, Ang-II, or LPS and leads to G1 arrest. Concomitantly E2 reduced the release of cell cycle regulators such as cyclin D and phosphorylation of Rb protein and decreased activation of kinases MEK1/2 (MAP2K1/2) and ERK1/2 (MAPK3/6) (Xia *et al*., 2020). In addition, E2 promoted VSMC apoptosis through p42/44 and/or p38 MAPK activation and BAX upregulation and suppressed VSMC migration toward pro-atherogenic chemoattractants. Furthermore, estrogens decreased pro-inflammatory VSNC activation and reduced MCP1, COX2, and endothelin 1 release (Wei *et al*., 2019).

Animal models with vascular injury presented with decreased infiltration of neutrophils following E2 administration which may be due to inhibition of TNFα-induced release of Cytokine-Induced Neutrophil Chemoattractant (CINC)-2β. Moreover, estrogens promote the expression of antioxidative enzymes and suppress NADPH oxidase, thus reducing the oxidative stress in VSMCs. Under *in vivo* conditions, all these effects translate into decreased formation of neointima which has been confirmed by several studies involving ovariectomized mouse models with ligation-injury of carotid arteries as well as in rat carotid arteries and balloon-injured rabbit aortas (Takano and Nakagawa, 2001).

The ER mediator of the anti-atherogenic effects of E2 in CSMC is still unclear. The pro-apoptotic effect of E2 as well as E2-related increase in superoxide dismutase expression are likely mediated by ERα. However, E2-induced suppression of ERK1/2 activity and proliferation in porcine VSMC was abolished as a result of antisense therapy. Similarly, the suppressive action of E2 on TNFα-induced CINC-2β release and Ang-II-induced VSMC proliferation was abolished by ERβ but not by ERα inhibitors. Furthermore, rats with balloon injury presented with increased ERβ mRNA expression, and local treatment with selective ERβ agonists was more effective than ERα agonists in decreasing the formation of neointima. These findings suggest that the effect of estrogens on VSMCs is primarily mediated by Erβ (Ambhore *et al*., 2018).

In addition, G Protein-Coupled Receptor 30 (GPR30) was reported to be released on VSMC in order to regulate vascular tone. The activation of the receptor suppressed VSMC proliferation and NADPH oxidase activation and mediated the proapoptotic effect of E2. GPR30 activation in VSMC may thus be considered one of the atheroprotective cellular effects of E2 (Zha *et al*., 2020).

Finally, 2-ME was demonstrated to mitigate the formation of injury-induced neointima in rat models. *In vitro* studies reported that 2-ME not only suppresses VSMC proliferation at the G0/G1 and G2/M cell cycle phase but also reduces cyclin D and B and increases p27. However, these effects were abolished in COMT-deficient VSMC, as they do not convert E2 to 2-ME, which indicates that the E2 effects on VSMC are partly mediated in a way that does not involve classical ERs (Chiu *et al*., 2022).

Limitations and Future Perspectives

However, despite all the valuable insights from numerous studies, researchers in the field of female sex hormones and their influence on atherosclerosis have various limitations. First of all, many studies examining the relationship between female sex hormones and atherosclerosis rely on observational designs, such as cohort studies or cross-sectional analyses. While these studies can provide valuable insights, they cannot establish a cause-and-effect relationship. Randomized Controlled Trials (RCTs) would be ideal to demonstrate causality but can be challenging to conduct in this context. Also, studies exploring the influence of female sex hormones on atherosclerosis may suffer from small sample sizes, making it challenging to generalize the findings to larger populations. Additionally, the majority of the existing research may have focused on a specific demographic or ethnic group, limiting the generalizability to other populations. Another factor is hormonal variability: The levels and ratios of female sex hormones can vary significantly over a woman's lifespan and menstrual cycle. Failing to account for such hormonal fluctuations in study designs may lead to incomplete or biased conclusions about the influence of hormones on atherosclerosis. Atherosclerosis is a multifactorial disease influenced by various factors, including age, lifestyle, diet, genetics, medications, and comorbidities. It's important to control for these confounding factors adequately to attribute any observed effects specifically to female sex hormones. Addressing these limitations within future research endeavors will contribute to a more robust understanding of the intricate relationship between female sex hormones and atherosclerosis, assisting in the development of effective prevention and treatment strategies.

Here are some strategies for future research in the field of the influence of female sex hormones on atherosclerosis: Longitudinal studies can help establish temporal relationships and provide insights into the long-term effects of hormone fluctuations on cardiovascular health; inclusion of diverse groups of women in future research efforts to account for potential ethnic, racial and cultural differences in the influence of female sex hormones on atherosclerosis will enhance the generalizability of findings and support personalized approaches to healthcare; multi-omics technologies, such as genomics, epigenomics, transcriptomics, proteomics, and metabolomics, can help with a more comprehensive understanding of the molecular mechanisms linking female sex hormones to atherosclerosis. Integrating these data can provide insights into the complex interplay between hormones, genetic factors, and metabolic pathways.

Conclusion

Women's health is undeniably influenced by their hormonal background, encompassing not only the reproductive system but the entire body. Disturbances in hormone production and functioning can significantly impact susceptibility to various diseases. When it comes to atherosclerosis and subsequent cardiovascular diseases, the anti-atherogenic effect of estrogen emerges as a crucial factor. The protective effects of estrogen against atherosclerosis are mediated through multiple mechanisms, prominently involving inflammation and its modulation of key inflammatory molecules within various cell types, such as macrophages, dendritic cells, and endothelial cells.

Studies have revealed that estrogen exerts its anti-atherogenic effects by regulating the expression of pro-inflammatory cytokines, mitigating the adhesion and migration of monocytes, reducing oxidative stress, and modulating the synthesis and degradation of extracellular matrix components. Several experimental and clinical investigations have provided evidence supporting the role of estrogen in promoting favorable vascular health and preventing atherosclerosis progression.

Furthermore, our review extensively examined the risks associated with female hormonal imbalances in the context of atherosclerosis. It is important to acknowledge that hormonal disturbances, such as those seen in Polycystic Ovary Syndrome (PCOS) or hormonal replacement therapies, can confer an increased risk of atherosclerosis and cardiovascular diseases. Irregularities in hormone levels, particularly imbalances in estrogen and its interactions with other hormones can disrupt the delicate physiological equilibrium and contribute to the development of atherosclerotic lesions. Understanding the intricate relationships between hormones and atherosclerosis is indispensable for elucidating the underlying mechanisms and devising targeted therapeutic strategies.

Conclusively, the impact of female hormones on the risk of atherosclerosis cannot be ignored, given the significant anti-atherogenic effects of estrogen observed through modulating inflammation and its related molecules. Further research in this field holds immense potential to unravel novel therapeutic avenues for attenuating atherosclerosis and enhancing cardiovascular health in women.

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Author's Contributions

Anastasia Vladimirovna Poznyak: Wrote the original draft.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Ambhore, N. S., Katragadda, R., Kalidhindi, R. S. R., Thompson, M. A., Pabelick, C. M., Prakash, Y. S., & Sathish, V. (2018). Estrogen receptor beta signaling inhibits PDGF induced human airway smooth muscle proliferation. *Molecular and Cellular Endocrinology*, *476*, 37-47. https://doi.org/10.1016/j.mce.2018.04.007

Barrett-Connor, E. (2013). Menopause, atherosclerosis and coronary artery disease. *Curr Opin Pharmacol*, *13*(2):186-191. https://doi.org/10.1016/j.coph.2013.01.005

Berntsen, C. F., Rootwelt, P., & Dahm, A. E. A. (2021). Bias in animal studies of estrogen effects on cardiovascular disease: A systematic review and meta-analysis. *Research and Practice in Thrombosis and Haemostasis*, *5*(4), e12507. https://doi.org/10.1002/rth2.12507

Bertone-Johnson, E. R., Virtanen, J. K., Niskanen, L., Nurmi, T., Ronkainen, K., Voutilainen, S., ... & Tuomainen, T. P. (2017). Association of follicle-stimulating hormone levels and risk of type 2 diabetes in older postmenopausal women. *Menopause*, *24*(7), 796-802. https://doi.org/10.1097/GME.0000000000000834

Bhattacharya, P., Thiruppathi, M., Elshabrawy, H. A., Alharshawi, K., Kumar, P., & Prabhakar, B. S. (2015). GM-CSF: An immune modulatory cytokine that can suppress autoimmunity. *Cytokine*, *75*(2), 261-271. https://doi.org/10.1016/j.cyto.2015.05.030

Billon-Galés, A., Fontaine, C., Douin-Echinard, V., Delpy, L., Berges, H., Calippe, B., ... & Arnal, J. F. (2009). Endothelial estrogen receptor-α plays a crucial role in the atheroprotective action of 17β-estradiol in low-density lipoprotein receptor-deficient mice. *Circulation*, *120*(25), 2567-2576. https://doi.org/10.1073/pnas.0808742106

Calippe, B., Douin-Echinard, V., Delpy, L., Laffargue, M., Lélu, K., Krust, A., ... & Gourdy, P. (2010). 17β-estradiol promotes TLR4-triggered proinflammatory mediator production through direct estrogen receptor α signaling in macrophages *in vivo*. *The Journal of Immunology*, *185*(2), 1169-1176. https://doi.org/10.4049/jimmunol.0902383

Checkouri, E., Blanchard, V., & Meilhac, O. (2021). Macrophages in atherosclerosis, first or second row players? *Biomedicines*, *9*(9), 1214. https://doi.org/10.3390/biomedicines9091214

Chiu, C. W., Hsieh, C. Y., Yang, C. H., Tsai, J. H., Huang, S. Y., & Sheu, J. R. (2022). Yohimbine, an α2-adrenoceptor antagonist, suppresses PDGF-bb-stimulated vascular smooth muscle cell proliferation by downregulating the plcγ1 signaling pathway. *International Journal of Molecular Sciences*, *23*(14), 8049. https://doi.org/10.3390/ijms23148049

Corcoran, M. P., Lichtenstein, A. H., Meydani, M., Dillard, A., Schaefer, E. J., & Lamon-Fava, S. (2011). The effect of 17β-estradiol on cholesterol content in human macrophages is influenced by the lipoprotein milieu. *Journal of Molecular Endocrinology*, *47*(1), 109. https://doi.org/10.1530/JME-10-0158

Du, X., Fu, X., Yao, K., Lan, Z., Xu, H., Cui, Q., & Yang, E. (2017). Bcl-2 delays cell cycle through mitochondrial ATP and ROS. *Cell Cycle*, *16*(7), 707-713. https://doi.org/10.1080/15384101.2017.1295182

El Hayek, S., Bitar, L., Hamdar, L. H., Mirza, F. G., & Daoud, G. (2016). Polycystic ovarian syndrome: An updated overview. *Frontiers in Physiology*, *7*, 124. https://doi.org/10.3389/fphys.2016.00124

El Khoudary, S. R., Greendale, G., Crawford, S. L., Avis, N. E., Brooks, M. M., Thurston, R. C., ... & Matthews, K. (2019). The menopause transition and women's health at midlife: A progress report from the Study of Women's Health Across the Nation (SWAN). *Menopause (New York, NY)*, *26*(10), 1213. https://doi.org/10.1097/GME.0000000000001424

Fairweather, D. (2014). Sex differences in inflammation during atherosclerosis. *Clinical Medicine Insights: Cardiology*, *8*, CMC-S17068. https://doi.org/10.4137/CMC.S17068

Farahi, L., Sinha, S. K., & Lusis, A. J. (2021). Roles of macrophages in atherogenesis. *Frontiers in Pharmacology*, *12*, 785220. https://doi.org/10.3389/fphar.2021.785220

Freeman, E. W., Sammel, M. D., Lin, H., & Gracia, C. R. (2010). Obesity and reproductive hormone levels in the transition to menopause. *Menopause (New York, NY)*, *17*(4), 718. https://doi.org/10.1097/gme.0b013e3181cec85d

Fuentes, N., & Silveyra, P. (2019). Estrogen receptor signaling mechanisms. *Advances in Protein Chemistry and Structural Biology*, *116*, 135-170. https://doi.org/10.1016/bs.apcsb.2019.01.001

Furness, S., Roberts, H., Marjoribanks, J., Lethaby, A., Hickey, M., & Farquhar, C. (2004). Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. *Cochrane Database of Systematic Reviews*, (3). https://doi.org/10.1002/14651858.CD000402.pub4

Giraud, S. N., Caron, C. M., Pham-Dinh, D., Kitabgi, P., & Nicot, A. B. (2010). Estradiol inhibits ongoing autoimmune neuroinflammation and NFκB-dependent CCL2 expression in reactive astrocytes. *Proceedings of the National Academy of Sciences*, *107*(18), 8416-8421. https://doi.org/10.1073/pnas.0910627107

Goetz, T. G., Mamillapalli, R., Sahin, C., Majidi-Zolbin, M., Ge, G., Mani, A., & Taylor, H. S. (2018). Addition of estradiol to cross-sex testosterone therapy reduces atherosclerosis plaque formation in female ApoE−/−mice. *Endocrinology*, *159*(2), 754-762. https://doi.org/10.1210/en.2017-00884

Gregersen, I., Høibraaten, E., Holven, K. B., Løvdahl, L., Ueland, T., Mowinckel, M. C., ... & Sandset, P. M. (2019). Effect of hormone replacement therapy on atherogenic lipid profile in postmenopausal women. *Thrombosis Research*, *184*, 1-7. https://doi.org/10.1016/j.thromres.2019.10.005

Guo, H., Liu, M., Zhang, L., Wang, L., Hou, W., Ma, Y., & Ma, Y. (2020). The critical period for neuroprotection by estrogen replacement therapy and the potential underlying mechanisms. *Current Neuropharmacology*, *18*(6), 485-500. https://doi.org/10.2174/1570159X18666200123165652

Gupte, S., & Wagh, G. (2014). Preeclampsia-eclampsia. *The Journal of Obstetrics and Gynecology of India*, *64*, 4-13. https://doi.org/10.1007/s13224-014-0502-y

Hai, Q., Ritchey, B., Robinet, P., Alzayed, A. M., Brubaker, G., Zhang, J., & Smith, J. D. (2018). Quantitative trait locus mapping of macrophage cholesterol metabolism and CRISPR/Cas9 editing implicate an ACAT1 truncation as a causal modifier variant. *Arteriosclerosis, Thrombosis and Vascular Biology*, *38*(1), 83-91. https://doi.org/10.1161/ATVBAHA.117.310173

Harvey, R. E., Coffman, K. E., & Miller, V. M. (2015). Women-specific factors to consider in risk, diagnosis and treatment of cardiovascular disease. *Women's Health*, *11*(2), 239-257. https://doi.org/10.2217/WHE.14.64

Hashemzadeh, M., Romo, R., Arreguin, J. M., & Movahed, M. R. (2020). The effects of estrogen and hormone replacement therapy on cardiovascular systems. *Future Cardiology*, *17*(2), 347-353. https://doi.org/10.2217/fca-2020-0054

Haukkamaa, L., Moilanen, L., Kattainen, A., Luoto, R., Kähönen, M., Leinonen, M., ... & Kaaja, R. (2009). Pre-eclampsia is a risk factor of carotid artery atherosclerosis. *Cerebrovascular Diseases*, *27*(6), 599-607. https://doi.org/10.1159/000216834

Holm, P., Stender, S., Andersen, H. O., Hansen, B. F., & Nordestgaard, B. G. (1997). Antiatherogenic effect of estrogen abolished by balloon catheter injury in cholesterol clamped rabbits. *Arteriosclerosis, Thrombosis and Vascular Biology*, *17*(8), 1504-1511. https://doi.org/10.1161/01.ATV.17.8.1504

Hu, D., Yin, C., Luo, S., Habenicht, A. J., & Mohanta, S. K. (2019). Vascular smooth muscle cells contribute to atherosclerosis immunity. *Frontiers in Immunology*, *10*, 1101. https://doi.org/10.3389/fimmu.2019.01101

Iorga, A., Cunningham, C. M., Moazeni, S., Ruffenach, G., Umar, S., & Eghbali, M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biology of Sex Differences*, *8*(1), 1-16. https://doi.org/10.1186/s13293-017-0152-8

Iwakiri, Y. (2011). S-nitrosylation of proteins: A new insight into endothelial cell function regulated by eNOS-derived no. *Nitric Oxide*, *25*(2), 95-101. https://doi.org/10.1016/j.niox.2011.04.014

Jebari-Benslaiman, S., Uribe, K. B., Benito-Vicente, A., Galicia-Garcia, U., Larrea-Sebal, A., Alloza, I., ... & Martín, C. (2020). Cholesterol efflux efficiency of reconstituted HDL is affected by nanoparticle lipid composition. *Biomedicines*, *8*(10), 373. https://doi.org/10.3390/biomedicines8100373

Jiang, Y., & Tian, W. (2017). The effects of progesterones on blood lipids in hormone replacement therapy. *Lipids in Health and Disease*, *16*, 1-8. https://doi.org/10.1186/s12944-017-0612-5

Kaplan, J. R., Chen, H., & Manuck, S. B. (2009). The relationship between social status and atherosclerosis in male and female monkeys as revealed by meta-analysis. *American Journal of Primatology: Official Journal of the American Society of Primatologists*, *71*(9), 732-741. https://doi.org/10.1002/ajp.20707

Kessous, R., Shoham-Vardi, I., Pariente, G., Sergienko, R., & Sheiner, E. (2015). Long-term maternal atherosclerotic morbidity in women with pre-eclampsia. *Heart*, *101*(6), 442-446. https://doi.org/10.1136/heartjnl-2014-306571

Kramer, C. K., Campbell, S., & Retnakaran, R. (2019). Gestational diabetes and the risk of cardiovascular disease in women: A systematic review and meta-analysis. *Diabetologia*, *62*, 905-914. https://doi.org/10.1007/s00125-019-4840-2

Kurmann, R. D., & Mankad, R. (2018). Atherosclerotic vascular disease in the autoimmune rheumatologic woman. *Clinical Cardiology*, *41*(2), 258-263. https://doi.org/10.1002/clc.22916

Li, S., Ma, L., Song, Y., Zheng, J., Cai, Y., Xu, H., ... & Zhou, J. (2021). Effects of hormone replacement therapy on glucose and lipid metabolism in peri-and postmenopausal women with a history of menstrual disorders. *BMC Endocrine Disorders*, *21*(1), 121. https://doi.org/10.1186/s12902-021-00784-9

Libby, P., Buring, J., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., & Tokgözoğlu, L. (2019). Lewis EF. *Atherosclerosis. Nature Reviews Disease Primers*, *5*(1), 56. https://doi.org/10.1038/s41572-019-0106-z

Madeira, M., Mattar, A., Logullo, A. F., Soares, F. A., & Gebrim, L. H. (2013). Estrogen receptor alpha/beta ratio and estrogen receptor beta as predictors of endocrine therapy responsiveness-a randomized neoadjuvant trial comparison between anastrozole and tamoxifen for the treatment of postmenopausal breast cancer. *BMC Cancer*, *13*(1), 1-12. https://doi.org/10.1186/1471-2407-13-425

Mair, K. M., Gaw, R., & MacLean, M. R. (2020). Obesity, estrogens and adipose tissue dysfunction-implications for pulmonary arterial hypertension. *Pulmonary Circulation*, *10*(3), 2045894020952019. https://doi.org/10.1177/2045894020952023

Marino, M., Galluzzo, P., & Ascenzi, P. (2006). Estrogen signaling multiple pathways to impact gene transcription. *Current Genomics*, *7*(8), 497-508. https://doi.org/10.2174/138920206779315737

Mehta, J., Kling, J. M., & Manson, J. E. (2021). Risks, benefits and treatment modalities of menopausal hormone therapy: Current concepts. *Frontiers in Endocrinology*, *12*, 564781. https://doi.org/10.3389/fendo.2021.564781

Miller, V. M., Naftolin, F., Asthana, S., Black, D. M., Brinton, E. A., Budoff, M. J., ... & Harman, S. M. (2019). The Kronos Early Estrogen Prevention Study (KEEPS): What have we learned?. *Menopause (New York, NY)*, *26*(9), 1071. https://doi.org/10.1097/GME.0000000000001326

Mittal, S. K., & Roche, P. A. (2015). Suppression of antigen presentation by IL-10. *Current Opinion in Immunology*, *34*, 22-27. https://doi.org/10.1016/j.coi.2014.12.009

Nie, G., Yang, X., Wang, Y., Liang, W., Li, X., Luo, Q., ... & Liang, X. (2022). The effects of menopause hormone therapy on lipid profile in postmenopausal women: A systematic review and meta-analysis. *Frontiers in Pharmacology*, *13*, 850815. https://doi.org/10.3389/fphar.2022.850815

Niță, A. R., Knock, G. A., & Heads, R. J. (2021). Signalling mechanisms in the cardiovascular protective effects of estrogen: With a focus on rapid/membrane signalling. *Current Research in Physiology*, *4*, 103-118. https://doi.org/10.1016/j.crphys.2021.03.003

Palmisano, B. T., Zhu, L., Eckel, R. H., & Stafford, J. M. (2018). Sex differences in lipid and lipoprotein metabolism. *Molecular Metabolism*, *15*, 45-55. https://doi.org/10.1016/j.molmet.2018.05.008

Pathak, L. A., Shirodkar, S., Ruparelia, R., & Rajebahadur, J. (2017). Coronary artery disease in women. *Indian Heart Journal*, *69*(4), 532-538. https://doi.org/10.1016/j.ihj.2017.05.023

Prabakaran, S., Schwartz, A., & Lundberg, G. (2021). Cardiovascular risk in menopausal women and our evolving understanding of menopausal hormone therapy: Risks, benefits and current guidelines for use. *Therapeutic Advances in Endocrinology and Metabolism*, *12*, 20420188211013917. https://doi.org/10.1177/20420188211013917

Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradaran, A., & Nasri, H. (2014). Atherosclerosis: Process, indicators, risk factors and new hopes. *International Journal of Preventive Medicine*, *5*(8), 927. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4258672/

Rayner, K., Chen, Y. X., Hibbert, B., White, D., Miller, H., Postel, E. H., & O'Brien, E. R. (2008). Discovery of NM23-H2 as an estrogen receptor β-associated protein: Role in estrogen-induced gene transcription and cell migration. *The Journal of Steroid Biochemistry and Molecular Biology*, *108*(1-2), 72-81. https://doi.org/10.1016/j.jsbmb.2007.07.006

Rentz, T., Wanschel, A. C., de Carvalho Moi, L., Lorza-Gil, E., De Souza, J. C., Dos Santos, R. R., & Oliveira, H. C. (2020). The anti-atherogenic role of exercise is associated with the attenuation of bone marrow-derived macrophage activation and migration in hypercholesterolemic mice. *Frontiers in Physiology*, *11*, 599379. https://doi.org/10.3389/fphys.2020.599379

Rettew, J. A., Huet, Y. M., & Marriott, I. (2009). Estrogens augment cell surface TLR4 expression on murine macrophages and regulate sepsis susceptibility *in vivo*. *Endocrinology*, *150*(8), 3877-3884. https://doi.org/10.1210/en.2009-0098

Rhee, C. Y., Spaet, T. H., Stemerman, M. B., Lajam, F., Shiang, H. H., Caruso, E., & Litwak, R. S. (1977). Estrogen suppression of surgically induced vascular intimal hyperplasia in rabbits. *The Journal of Laboratory and Clinical Medicine*, *90*(1), 77-84. https://europepmc.org/article/med/874374

Robert, J., Osto, E., & von Eckardstein, A. (2021). The endothelium is both a target and a barrier of HDL's protective functions. *Cells*, *10*(5), 1041. https://doi.org/10.3390/cells10051041

Rodrigues, K. E., Azevedo, A., Gonçalves, P. R., Pontes, M. H., Alves, G. M., Oliveira, R. R., ... & Prado, A. F. (2022). Doxycycline Decreases Atherosclerotic Lesions in the Aorta of ApoE-/-and Ovariectomized Mice with Correlation to Reduced MMP-2 Activity. *International Journal of Molecular Sciences*, *23*(5), 2532. https://doi.org/10.3390/ijms23052532

Rosenfield, R. L., & Ehrmann, D. A. (2016). The pathogenesis of Polycystic Ovary Syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocrine Reviews*, *37*(5), 467-520. https://doi.org/10.1210/er.2015-1104

Shaw, N. D., Srouji, S. S., Histed, S. N., McCurnin, K. E., & Hall, J. E. (2009). Aging attenuates the pituitary response to gonadotropin-releasing hormone. *The Journal of Clinical Endocrinology and Metabolism*, *94*(9), 3259-3264. https://doi.org/10.1210/jc.2009-0526

Shufelt, C. L., & Manson, J. E. (2021). Menopausal hormone therapy and cardiovascular disease: The role of formulation, dose and route of delivery. *The Journal of Clinical Endocrinology and Metabolism*, *106*(5), 1245-1254. https://doi.org/10.1210/clinem/dgab042

Soares, A. G., Kilpi, F., Fraser, A., Nelson, S. M., Sattar, N., Welsh, P. I., ... & Lawlor, D. A. (2020). Longitudinal changes in reproductive hormones through the menopause transition in the Avon Longitudinal Study of Parents and Children (ALSPAC). *Scientific Reports*, *10*(1), 21258. https://doi.org/10.1038/s41598-020-77871-9

Sriprasert, I., Hodis, H. N., Karim, R., Stanczyk, F. Z., Shoupe, D., Henderson, V. W., & Mack, W. J. (2019). Differential effect of plasma estradiol on subclinical atherosclerosis progression in early vs late postmenopause. *The Journal of Clinical Endocrinology and Metabolism*, *104*(2), 293-300. https://doi.org/10.1210/jc.2018-01600

Takano, K., & Nakagawa, H. (2001). Contribution of cytokine-induced neutrophil chemoattractant CINC-2 and CINC-3 to neutrophil recruitment in lipopolysaccharide-induced inflammation in rats. *Inflammation Research*, *50*, 503-508. https://doi.org/10.1007/PL00000226

Talbott, E. O., Zborowski, J. V., Boudreaux, M. Y., McHugh-Pemu, K. P., Sutton-Tyrrell, K., & Guzick, D. S. (2004). The relationship between C-reactive protein and carotid intima-media wall thickness in middle-aged women with polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism*, *89*(12), 6061-6067. https://doi.org/10.1210/jc.2003-032110

Teede, H., Ng, S., Hedger, M., & Moran, L. (2013). Follistatin and activins in polycystic ovary syndrome: Relationship to metabolic and hormonal markers. *Metabolism*, *62*(10), 1394-1400. https://doi.org/10.1016/j.metabol.2013.05.003

Thor, D., Zhang, R., Anderson, L., Bose, D. D., Dubé, G. P., & Rahimian, R. (2010). Effects of 17 β-estradiol on lipopolysacharride-induced intracellular adhesion molecule-1 mRNA expression and Ca2+ homeostasis alteration in human endothelial cells. *Vascular Pharmacology*, *53*(5-6), 230-238. https://doi.org/10.1016/j.vph.2010.09.001

Ueda, K., Fukuma, N., Adachi, Y., Numata, G., Tokiwa, H., Toyoda, M., ... & Takimoto, E. (2021). Sex differences and regulatory actions of estrogen in cardiovascular system. *Frontiers in Physiology*, *12*, 738218. https://doi.org/10.3389/fphys.2021.738218

Vakhtangadze, T., Singh Tak, R., Singh, U., Baig, M. S., & Bezsonov, E. (2021). Gender differences in atherosclerotic vascular disease: From lipids to clinical outcomes. *Frontiers in Cardiovascular Medicine*, *8*, 707889. https://doi.org/10.3389/fcvm.2021.707889

Villa, A., Vegeto, E., Poletti, A., & Maggi, A. (2016). Estrogens, neuroinflammation and neurodegeneration. *Endocrine Reviews*, *37*(4), 372-402. https://doi.org/10.1210/er.2016-1007

Wei, M., Liu, Y., Zheng, M., Wang, L., Ma, F., Qi, Y., & Liu, G. (2019). Upregulation of protease-activated receptor 2 promotes proliferation and migration of human Vascular Smooth Muscle Cells (VSMCs). *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, *25*, 8854. https://doi.org/10.12659/MSM.917865

Wellons, M., Ouyang, P., Schreiner, P. J., Herrington, D. M., & Vaidya, D. (2012). Early menopause predicts future coronary heart disease and stroke: The Multi-Ethnic Study of Atherosclerosis (MESA). *Menopause (New York, NY)*, *19*(10), 1081. https://doi.org/10.1097/gme.0b013e3182517bd0

Wide, L., & Eriksson, K. (2013). Dynamic changes in glycosylation and glycan composition of serum FSH and LH during natural ovarian stimulation. *Upsala Journal of Medical Sciences*, *118*(3), 153-164. https://doi.org/10.3109/03009734.2013.782081

Wu, P., Haththotuwa, R., Kwok, C. S., Babu, A., Kotronias, R. A., Rushton, C., ... & Mamas, M. A. (2017). Preeclampsia and future cardiovascular health: A systematic review and meta-analysis. *Circulation: Cardiovascular Quality and Outcomes*, *10*(2), e003497. https://doi.org/10.1161/CIRCOUTCOMES.116.003497

Xia, X., Zhou, C., He, X., Liu, C., Wang, G., & Sun, X. (2020). The relationship between estrogen-induced phenotypic transformation and proliferation of vascular smooth muscle and hypertensive intracerebral hemorrhage. *Annals of Translational Medicine*, *8*(12). https://doi.org/10.21037/atm-20-4567

Yu, Q. Q., Cheng, D. X., Xu, L. R., Li, Y. K., Zheng, X. Y., Liu, Y., ... & Zhao, S. H. (2020). Urotensin II and urantide exert opposite effects on the cellular components of atherosclerotic plaque in hypercholesterolemic rabbits. *Acta Pharmacologica Sinica*, *41*(4), 546-553. https://doi.org/10.1038/s41401-019-0315-8

Zha, B., Qiu, P., Zhang, C., Li, X., & Chen, Z. (2020). GPR30 promotes the phenotypic switching of vascular smooth muscle cells via activating the AKT and ERK pathways. *OncoTargets and Therapy*, 3801-3808. https://doi.org/10.2147/OTT.S244128

Zhou, Y., Zhang, N. R., Zheng, Z. N., Yang, Y., Lyu, B. F., Wang, H. L., & Jin, S. Q. (2019). Regular transient limb ischemia prevents atherosclerosis progression in hypercholesterolemic rabbits. *Chinese Medical Journal*, *132*(09), 1079-1086. https://doi.org/10.1097/CM9.0000000000000204