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Sex-specific and hormone-related differences in vascular remodeling in atherosclerosis

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Abstract

Atherosclerosis, a lipid-driven inflammatory disease, is the main underlying cause of cardiovascular diseases (CVDs) both in men and women. Sex-related dimorphisms regarding CVDs and atherosclerosis were observed since more than a decade ago. Inflammatory mediators such as cytokines, but also endothelial dysfunction, vascular smooth muscle cell migration and proliferation lead to vascular remodeling but are differentially affected by sex. Each year a greater number of men die of CVDs compared to women and are also affected by CVDs at an earlier age (40-70 years old) while women develop atherosclerosis-related complications mainly after the menopause (60+ years). The exact biological reasons behind this discrepancy are still not well understood. From the numerous animal studies on atherosclerosis, only a few include both sexes and even less investigate and highlight the sex-specific differences that may arise. Endogenous sex hormones such as testosterone and estrogen modulate the atherosclerotic plaque composition as well as the frequency of such plaques. In men, testosterone seems to act like a double-edged sword as its decrease with aging correlates with an increased risk of atherosclerotic CVDs, while testosterone is also reported to promote inflammatory immune cell recruitment into the atherosclerotic plaque. In premenopausal women estrogen exerts anti-atherosclerotic effects, which decline together with its level after menopause resulting in increased CVD risk in aging women. However, the interplay of sex hormones, sex-specific immune responses and other sex-related factors is still incompletely understood. This review highlights reported sex-differences in atherosclerotic vascular remodeling and the role of endogenous sex-hormones in this process.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally both in men and women. The most important underlying pathology of CVDs is atherosclerosis, an inflammatory disease, driven by lipids and fostering the development of plaques in the intimal layer of the arterial wall. Atherosclerosis is initiated by endothelial dysfunction which increases the permeability of the endothelial barrier allowing low density lipoprotein (LDL) and its modified versions like oxidized LDL (oxLDL) to invade the intimal layer (1). These modified lipids further promote an inflammatory response and upregulation of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and cytokines like monocyte recruitment protein 1 (MCP-1 also known as CCL2) on the surface of endothelial cells (ECs). Thereby, recruitment of monocytes and other inflammatory cells namely neutrophils and T cells from the circulation into the arterial wall is promoted (2). Once inside the vessel wall, monocytes differentiate into macrophages which engulf oxLDL and develop into lipid-laden foam cells. Overloaded foam cells will eventually undergo apoptosis or necrosis forming a necrotic core (2). More advanced lesions form a fibrous cap, which is a barrier built up by vascular smooth muscle cells (VSMCs) migrating from the media into the intimal layer of the vasculature. These VSMCs mostly reside directly underneath the EC lining and play an important role in plaque stability. In atherosclerosis, VSMCs change their phenotype from contractile, which is essential for vascular tone and function, into a synthetic phenotype which promotes their migration, proliferation, and production of extracellular matrix (ECM) which supports the stability of the plaque (3).

Vascular wall remodeling is one characteristic of the pathophysiology of atherosclerosis and refers to structural and functional alterations of the vascular wall (4). This vascular remodeling is caused by the interplay of EC dysfunction, VSMC migration and proliferation, foam cell formation and increased presence of inflammatory mediators such as cytokines. The integrity and stability of the atherosclerotic plaque is also affected by adverse vascular remodeling causing plaque rupture fostering thrombus formation. Plaques which are prone to rupture are described to be smaller (30-40% vessels stenosis) compared to stable lesions and generally contain a large lipid core, a thin fibrous cap, and numerous inflammatory cells (5). Moreover, also plaque erosion can occur which mainly occurs in plaques that are characterized by a thick fibrous cap, large amounts of extracellular matrix, presence of neutrophil extracellular traps (NETs) and fewer inflammatory cells within the lesion. Plaque erosion is initiated by shear stress which induces Toll-like receptor (TLR)-2 expression on ECs causing EC desquamation and apoptosis. EC detachment attracts neutrophils and promotes NET release. These NETs can subsequently trap circulating platelets leading to a platelet rich “white” thrombus formation (5). Hence, plaque rupture but also plaque erosion may eventually cause major cardiovascular events like myocardial infarction (MI) or stroke (6). Literature suggests that vascular remodeling is not only affected by classical risk factors like dyslipidemia and age but also depends on sex differences. Therefore, this review aims to summarize differences described in vascular remodeling in atherosclerosis between males and females and the implication of sex-hormones in this process.

2. Role of biological sex in atherosclerosis

Risk factors for atherosclerosis and disparity between men and women

CVD is the leading cause of death for both men and women worldwide (7). According to the World Health Organization (WHO), nearly 18 million people have died from CVD in 2019 and 85% of these deaths were caused by a stroke or heart attack (8). More men die from atherosclerotic CVD and develop the disease at a younger age (40–60 years), while women usually develop CVD 7 to 10 years later than men (9-11). Women usually develop atherosclerosis following menopause which results in more women suffering from atherosclerotic CVD at an older age compared to men (11). Based on the Global Burden of Cardiovascular Disease study, 9,6 million men and 8,9 million women died worldwide from CVD in 2019 (12). There are well-known risk factors for developing CVD such as smoking, hypertension, dyslipidemia, diabetes, physical inactivity and obesity (13). Already in these risk factors, some sex disparity can be observed. For example, smoking is one of the most important CVD risk factors that lead to EC dysfunction. While slightly more men smoke than women (15% vs 13%), smoking is more harmful to the cardiovascular system of women (14). Women smokers have 25% higher risk of coronary heart disease (CHD) compared to men who also smoke. The reason for this sex-difference has not been sufficiently investigated (15,16). In addition, compared to men who smoke, female smokers have more than 50% increase of relative risk of MI as revealed in a prospective study including approximatively 25,000 persons followed over 13 years (17). Another leading CVD risk factor is diabetes. Men have a higher prevalence of diabetes mellitus type 2 (T2DM) compared to women (14,6% vs 9,1%) (18,19). Nevertheless, T2DM only doubles the risk of CVD mortality from ischemic heart disease or ischemic stroke in men while it **triples** it in women (20). Although women have more favorable CVD profiles without diabetes compared to men, if developing T2DM, women CVD risk factors seem to worsen more rapidly, including greater changes in blood pressure and worse lipid profiles than men and thereby lead to a greater CVD-mortality rate in diabetic women (21,22).

Atherosclerosis and sexual immune dimorphism

The adaptive immune system is involved in T2DM development. Sex dimorphism in lymphocytes including T cell subsets such as CD4+ T cells are already described (23,24). For example, women have higher CD4+ T cell counts and higher CD4/CD8 ratios in the circulation compared with age-matched men, while men have greater CD8+ T cell counts. Surprisingly, there is also a large disparity in gene expression in CD4+ T cells between men and women with T2DM (25). Among patients with coronary artery disease (CAD) and T2DM, men have a higher C-C chemokine receptor type 2 (CCR2)+ effector memory (Em), Matrix metalloproteinase (MMP)-9 (MMP9)+ and programmed death-ligand 1 (PDL1)+ Em CD4 T cell frequency compared with women as revealed by single cell RNA sequencing and CITE-sequencing (25). Of note, all of the above cited CD4+ T cells subsets are significantly lower in patients with CAD then without CAD, independently of the sex (25).

Clinical manifestation

Hypertension also greatly affects atherosclerotic CVD risk. Although generally, men have higher blood pressure at a younger age (adolescence) compared to women, both sexes display an increase of blood pressure during aging. In women the increase of blood pressure is particularly apparent from 30 to 60 years of age, while under the age of 55 years old, men have a higher incidence of hypertension (26). Beyond 60 years, hypertension is even more prevalent in women (27,28). A prospective UK Biobank cohort study which enrolled around 500,000 individuals revealed that women with hypertension have 80% higher risk of MI than men with the same condition (29). In addition to these common risk factors, women are subject to other sex-specific risks that greatly increase the possibility of developing atherosclerosis such as polycystic ovary syndrome (PCOS) or preeclampsia-eclampsia (21). In line, based on the Heart Disease and Stroke Statistics, women have a higher lifetime stroke risk compared to

men (7). Although there is growing evidence and awareness of sex-differences in the prevalence of CVD development and mortality, women are still subjected to delayed diagnosis and treatment (30). Taken together, young men have a worse risk factor profile compared to young women, resulting in increased adverse CVD events already earlier in life. However, the impact of CVD risk factors once they occur on developing CVD is higher in women (18).

Atherosclerotic plaque morphology

Moreover, plaque morphology in men and women seems to be significantly different as underlined by earlier work. Burke *et al*. examined the cause of sudden death in 51 women and revealed that in 35% of the cases plaque erosion (defined as plaque lesion without plaque rupture with a VSMC-rich intima) followed by acute thrombus formation was the underlying cause. Notably, only 15% of deaths were caused by plaque rupture (31). Furthermore, Yahagi *et al*. noted plaque dimorphisms between men and women (32). Plaques in young women had thicker fibrous caps, while plaques in older women tend to have larger necrotic cores. Moreover, the same study noted that thrombi in 80% of women younger than 50 years old were caused by plaque erosion while in women older than 50 years only 47% of the thrombi were caused by plaque erosion. Overall, when comparing women with men at all ages, plaque erosion was more frequent in women than men (58% vs. 24%) and plaque rupture was more frequent in men than women (71% vs. 33%) (**Figure 1**) (32).

Insight from clinical studies

Moreover, sexual dimorphism is also observed in the atherosclerotic plaque burden and degree of stenosis. The *Brazilian Longitudinal Study of Adult Health* (ELSA-Brasil) study revealed that, independent of the race and other risk factors associated with the disease, the carotid intima-media thickness (cIMT) was greater in men than in women (33). Similarly, the Tromsø study observed a greater plaque number, plaque area and plaque size in men than in aged-, body mass index-, blood pressure-, smoking- and diabetes mellitus-matched women (34). Additionally, a recent optical coherence tomography (OCT) study on 103 CAD patients (77 men and 26 women) found out that men with non-culprit plaques have a higher lipid index and larger lipid core compared to women (35). Here again, men had a greater number of plaques and women suffering from CAD were in average ten years older than men with the same disease (35).

These findings suggest that men and women suffer from a different pathophysiological modulation which should also be reflected in the used therapeutic approaches (18,36). One reason for the observed differences could be the differential action of sex hormones such as estrogen and testosterone (11). Therefore, the following section will describe these hormone- related differences in more detail.

3. Sex hormones and their effect on atherosclerosis

In humans, women tend to develop atherosclerosis at an older age, after menopause, compared to men, making the impact of sex steroid hormones on atherosclerosis an interesting research topic. However, the scientific data on sex hormones is very contradictory and difficult to interpret. Previous studies have shown that estrogen is atheroprotective in young women and in ovariectomized female mice and rats treated with estrogen due to anti-inflammatory and vasoprotective effects (37-40). However, in the most frequently used atherosclerotic mouse models, *Apolipoprotein E* knock-out (*Apoe*$^{-/-}$) and *low-density lipoprotein receptor* knock-out (*Ldlr*$^{-/-}$), female mice up to 6 months old, fed normal chow or atherosclerotic diet, have in general a greater atherosclerotic lesion size and overall burden compared with their male counterparts (18). Conversely, with aging (> 6 months old) male mice (*Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$) have larger or equally sized plaques, suggesting that older animals should be used to study atherosclerosis in mice to better compare obtained results to the human setting. Unfortunately, studies directly comparing male and female lesion sizes at different time points are rather scarce and therefore

these reported differences may be (statistically) overemphasized (18). Moreover and in contrast, in older human subjects, estrogens tend to be proatherogenic due to pro-inflammatory and vasotoxic effects (41). Testosterone levels also decrease in aging men and this decrease has been linked to increased CVD risk. However, in atherosclerotic mice, testosterone seems to increase inflammation and promotes inflammatory cells migration, further demonstrating the complex relation between sex hormones and atherosclerosis and vascular remodeling.

The following paragraphs will describe the role of female and male sex hormones in atherosclerosis associated vascular remodeling.

3.1 Female sex hormones in atherosclerosis remodeling

Estrogen, androgen and progesteron hormones can all bind to extracellular and intracellular receptors which act via ligand dependent, ligand-independent, genomic, or non-genomic mechanisms. Intracellular pathways involve the stimulation or inhibition of gene transcription factors by binding to *estrogen response elements* (EREs) or *androgen response elements* (AREs). Extracellular and intracellular *estrogen receptors* (ERs) include ERα, ERβ and G protein-coupled receptor 30 (GPR30) and they are present on both innate and adaptive immune cells such as B cells, T cells and monocytes as well as on cardiovascular cells like vascular ECs, VSMCs, cardiac fibroblasts and cardiomyocytes (11,42). ERs are higher expressed on female coronary artery VSMCs compared to male cells, but decrease with age and after menopause (43). For example, estrogen signaling via ERα is protective against vascular injury, remodeling, and fibrosis after MI in a cardiomyocyte specific ERα overexpression model in female mice compared to male mice (44). Furthermore, estrogen replacement therapies genuinely decrease atherosclerotic plaque size and prevent vascular remodeling (45,46).

Effect of estrogen on the inflammatory immune response in atherosclerosis

Signaling through ERβ is also implicated in the regulation of arterial tone and blood pressure (11). Furthermore, estrogen decreases the expression of pro-inflammatory TNFα (**Figure 1 and Table 1**) (47). TNFα is a cytokine implicated in promoting vascular remodeling by increasing adhesion molecule expression on ECs as well as EC permeability, upregulation of matrix degradation and VSMCs proliferation (48). In line with this, ovariectomized female control rats had a significant increase of serum TNFα compared to ovariectomized animals with estrogen-replacement treatment (estrogen pellet:1.5 mg/pellet). Moreover, endothelium-dependent vasorelaxation was reduced in the ovariectomized and hence estrogen-deficient rats compared to control rats undergoing estrogen-replacement treatment. Combined, these result support the importance of TNFα in causing vascular dysfunction associated with estrogen-deficiency (47). Two studies using ovariectomized rats which underwent balloon-injury in the carotid arteries and were treated with 17β-estradiol (E2) (daily injection of 20 $\mu g \cdot kg^{-1} \cdot d^{-1}$), a sex hormone that also represents estrogen, could show that this treatment reduced neutrophil and monocyte trafficking and infiltration by attenuating the expression of *cytokine-induced neutrophil chemoattractant* (CINC-2α) and MCP-1, compared to vehicle, medroxyprogesterone acetate (MPA; inhibits the protective effect of estrogen on neointima formation) treated rats, 24 hours post-injury (**Table 1**) (38,49). Two hours following balloon injury, 17β-estradiol-treated ovariectomized rats showed a significant decrease of mRNA expression of the adhesion molecules ICAM1, VCAM1 and P-selectin as well as a decrease of pro-inflammatory IL-1β and IL-6 in injured carotids arteries (38). IL-1β and IL-6 are both pro-inflammatory cytokines that promote vascular remodeling by increasing EC dysfunction, leukocyte recruitment to the intima, VSMC migration and proliferation and lesional collagen deposition (48). However, 24 hours post-injury the significant decrease remained only for P-selectin and IL-6 suggesting that estrogen especially helps to reduce early inflammatory processes (38).

Therefore, estrogen mitigates the initial inflammation process seen in atherosclerosis by decreasing adhesion molecule and pro-inflammatory cytokines and chemoattractant molecules expression as well as decreasing the infiltration of neutrophils and monocytes into the plaque. Estrogen has also an inhibitory effect on the expression of certain growth factors affecting vascular remodeling such as insulin-like growth factor 1, platelet-derived growth factor (PDGF)-A and its receptor PDGF-Rα on VSMCs in a rat model of aortic injury leading to increased VSMC proliferation and intima/media thickening (**Table 1**) (50). Taken together, pre-menopausal estrogen seems to protect against atherosclerotic vascular remodeling by decreasing adhesion molecules expression and therefore inflammatory cell recruitment as well as decreasing the expression of pro-inflammatory mediators such as TNFα, IL-1β and IL-6 (**Figure 1**). Estrogen also decreases the negative effect of MMP-12, an important elastase which contributes to arterial stiffening (**Table 1**). Indeed, it has been recently shown *in vitro* that the uptake of oxLDL promotes the release of MMP12 by macrophages and treatment with E2 decreases MMP12 gene expression and secretion in human macrophages (51). In the same study, both *Ldlr*$^{-/-}$*MMP12*$^{+/+}$ and *Ldlr*$^{-/-}$*MMP12*$^{-/-}$ female mice fed a high-fat diet for 16 weeks had a significant decrease of aortic plaque macrophage content compared with males due to greater estrogen level in females (51). In line, lower estrogen levels after menopause are related to altered vascular function, enhanced inflammation, and up-regulation of other hormonal systems such as the renin–angiotensin–aldosterone system and reduced nitric oxide-dependent vasodilation (52,53).

Clinical manifestation and therapies

Menopause goes hand in hand with 10–15% higher circulating LDL-cholesterol and triglyceride levels and a reduction in high density lipoprotein (HDL)-cholesterol (54). Together with this less favorable lipid profile after menopause, various studies also demonstrated a rise in blood pressure which may be a direct effect of hormonal changes on the vasculature and metabolic changes related to ageing (55-57). Endothelial dysfunction starts in early menopause even before signs of subclinical atherosclerosis (58) and while healthy endothelium is sensitive to the vasodilator properties of estrogens, this reverses when vascular stiffness and atherosclerotic disease develops over time (59). All of these detrimental phenomena contribute to an increase rate of MI in women after menopause (60). Therefore, estrogen supplementation therapies have been investigated since the early 1990s, but their benefits remain debateable (11). Evidence suggests that hormone therapy can be effective in reducing CVD risk when it is started during or shortly after menopause (61), but there are side-effects to be considered like an increased risk of breast cancer (62). Modern estrogen supplementation therapies contain lower doses of systemic and vaginal estrogens (63) but oral, not transdermal estrogen supplementation increases the risk of venous thromboembolism (64). Hence, careful evaluation is needed to weigh the risks and benefits of estrogen supplementation to decrease CVD in women after menopause.

3.2 Male sex hormones in atherosclerosis remodeling

Testosterone, the most important sex hormone in men, is a steroid from the androgen hormone family and binds to intracellular androgen receptors (ARs). Testosterone levels in men decrease with aging and this decrease has been linked with an increase in CVD and CVD-associated mortality (65). Testosterone circulates in two forms in the serum, either in its inactive form which is bound to *sex hormone binding globulin* (SHBG) and is unable to bind to ARs (68% of total serum testosterone) or in its active form which can bind to albumin or circulate freely in the blood (66,67).

Effect of testosterone on the inflammatory immune response in atherosclerosis

Testosterone increases neutrophils, mast cell and macrophage numbers and their activation resulting in foam cell formation in atherosclerotic plaques. Furthermore, testosterone affects vascular remodelling

by stimulating the release of pro-inflammatory cytokines IL-1β, IL-6 and TNFα, that are, as mentioned above, also involved in the vascular remodelling process, leading to thrombus formation and MI in men (68-70) (**Figure 1 and Table 1**). Testosterone also induces *tissue inhibitor of metalloproteinase 1* (TIMP-1) and hypothalamic serpin A 3n expression, which both are implicated in the balance of degradation and synthesis of the ECM (**Table 1**) (71). Elevated serum levels of TIMP-1 and serpin A 3n have been linked to cardiac fibrosis (**Table 1**) (72,73). On the other hand, testosterone deficiency leads to an increase in atherosclerotic lesion areas, foam cell accumulation, IMT as well as serum lipid levels in mini pigs fed 12 weeks with a high-fat and high cholesterol diet (74).

Ikeda *et al*. examined the role AR in angiotensin II (Ang II)-induced vascular remodeling (75). Ang II is an important vasoactive peptide that increases vascular wall tension by causing vasoconstriction. Its stimulation leads to an increase of free radicals via *nicotinamide adenine dinucleotide phosphate* (NADPH) oxidase activation and thereby promotes vascular remodeling (76). AR knockout (*ARKO*) and WT mice on a C57BL/6J background were infused with Ang II at 2.0 mg/kg per day for 14 days by a subcutaneously implanted osmotic minipump. Treated *ARKO* mice showed a significant increase in medial thickness and perivascular fibrosis of the coronary artery and aorta compared to untreated animals (75). In addition, collagen I and collagen III gene expression as well as superoxide production was only increased in the Ang II treated *ARKO* mice compared to all other groups (Ang II treated wild type (WT) and untreated mice) (75). Furthermore, Ang II promotes vascular transforming growth factor β (TGFβ) expression in *ARKO* mice compared to male WT mice. Based on these findings, AR seems to have a vascular protective action and counteracts Ang II-induced vascular remodeling (75). Another study investigated AR deletion in monocytes/macrophages, ECs and VSMCs in $Ldlr^{-/-}$ mice. Only monocyte/macrophage-deficient *ARKO* $Ldlr^{-/-}$ mice showed a decrease in atherosclerosis compared to control $Ldlr^{-/-}$ mice after 16 weeks of high cholesterol diet feeding (77). Furthermore, these monocyte/macrophage-deficient *ARKO* $Ldlr^{-/-}$ mice had a significant decrease of macrophage content and collagen deposition as well as increased VSMC content in the aortic root. However, mice with an AR knockout in ECs or VSMCs did not manifest any differences in lesion size or changes in vascular wall composition. *In vitro* experiments revealed that AR expression on monocytes promotes their migration, adhesion to ECs and differentiation into foam cells (77). It seems that systemic AR deficiency and monocytes/macrophage specific AR deficiency, have opposing effects on atherosclerosis suggesting that ARs affect each cell type differently which also reflects on their contribution to the disease progression (78).

Effect of testosterone on cytokines in atherosclerosis

Regarding the cytokine profiles that are also implicated in remodeling such as IL-1β, IL-6 and TNFα, testosterone supplementation seems to decrease the expression of pro-inflammatory cytokines while promoting the production of anti-inflammatory cytokines such as IL-10 (79,80). However, these results are controversial as some studies have found that testosterone treatment decreases TNFα, IL-1β, IL-6 and hs-CRP expression, while others did not (80-85). A recent study from Bernardi *et al*. analyzed the cytokine profile of 104 healthy adults (20-49 years old), including pro-atherogenic cytokines such as IL-1β, IL-6, TNFα and anti-atherogenic cytokines such as IL-10 and IL-1rA (**Table 1**). They showed that men have higher circulating levels of IL-1β, IL-6 and TNFα compared to age-matched women. These pro-inflammatory cytokines were also all significantly associated with testosterone and testosterone/estradiol ratio. This phenomenon was only observed in men, while in woman there was no correlation between there level of testosterone and pro-inflammatory cytokines (86,87).

Clinical manifestation and therapies

Testosterone replacement therapy (TRT) is widely investigated to lower atherosclerotic CVD risk in men. However, despite the numerous clinical trials, the results are still unclear. Some studies have found

that TRT worsens CVD risk by increasing adverse outcomes such as MI or stroke (88-91). In contrast, others have either observed neutral or beneficial effects of TRT on CVD (92-97). Regarding adverse atherosclerotic remodeling, several clinical studies have noted that a lower level of androgens correlate with an increase in cIMT in men from 40-70 years of age (98,99). However, in a randomized clinical trial, investigating the long-term effect of testosterone administration on subclinical atherosclerosis in men older than 60 years old, no significant differences in the rate of changes in cIMT after 3 years of daily testosterone treatment compared to placebo were found (100). The TRAVERSE (Testosterone Replacement Therapy for Assessment of Long-term Vascular Events and Efficacy ResponSE in Hypogonadal Men) trail is the latest randomized clinical trial on this topic that is currently ongoing. It started in 2018 and tested a topically administered testosterone gel versus placebo gel on more then 5000 symptomatic hypogonadal men with increased risk for atherosclerotic CVD. The completion of the study was estimated to be due in June 2022 but seems to be delayed (101). To conclude, the detrimental or beneficial effect of TRT is still ambiguous and further investigations are needed.

4. Perspectives and Conclusions

It is undeniable that there are sex-differences in atherosclerosis and subsequent development of CVDs. However, , the scientific data on sex hormones is very contradictory and difficult to interpret, more studies are needed to fully understand the underlying mechanisms that lead to the observed sex-dimorphism. Men develop atherosclerosis earlier, have larger plaque size and a higher plaque burden compared with women. Women tend to have a thicker fibrous cap, larger necrotic core and are more susceptible to suffer from thrombus formation via plaque erosion. On the other hand, men have a thinner fibrous cap, a smaller necrotic core and plaque rupture is the major cause of arterial thrombosis. Looking at the impact of sex hormones on atherosclerotic vascular remodeling, estrogen in pre-menopausal women prevents vascular inflammation and ensures a proper vascular tone. Post-menopause women have a higher risk of atherosclerotic CVDs potentially mainly due to a decrease of estrogen level. Based on the effect of estrogen observed in women as well as in studies using animal models, there is a growing interest to investigate hormone therapy to prevent CVDs in aging women. However, careful evaluation of estrogen supplementation therapies in women after menopause is needed to weigh out the risks of for example breast cancer and thrombosis against the benefits of CVD risk lowering. Studies on testosterone in human and animal atherosclerotic vascular disease are conflicting on the role of testosterone. The decrease of testosterone levels in aging men is associated with a higher risk of CVDs and testosterone supplementation therapy was described to correlate with a decrease of inflammation. Yet, adverse effects of testosterone on vascular remodeling by promoting pro-inflammatory cytokine production are also reported.

Taken together and consistent with many other studies, sex hormones alone do not seem to explain the sex differences in cytokine release and immune response in atherosclerosis and vascular wall remodeling (102-106). Although the exact reason of this sex-related phenomenon remains unclear. It is hypothesized that in women, it may be due to the general increase of low-grade inflammation that comes with aging or the accumulation and disbalance of O-GlcN-acylation of certain proteins that would lead to a loss of estrogen-induced vasoprotection (107). However, how these conditions specifically affect ERE signaling is unknown and this question still needs to be addressed (107). It can also be assumed that life-long testosterone exposure in men differentially impacts on the vascular wall architecture compared to estrogen exposure of the vessel wall in women. In other words, although estrogen levels significantly and more rapidly decline in menopausal women, while testosterone levels in men more gradually decrease with age, this variance in hormonal vascular "imprinting" echoes on CVD risk and lesion composition throughout the entire life of both sexes.

In addition to sex-related dimorphisms in CVD, also gender-related differences need to be considered when diagnosing and treating CVD. The term "sex" refers to pure biological attributes such as physical and physiological features including hormones but also chromosomes, gene expression and reproductive anatomy. "Gender", on the other hand, refers to the socially constructed roles, behaviors, opportunities, expectations, expressions, and identities of females in society which may also affect disease course (108). Indeed, there is a growing number of gender-related variables that are or may be involved in the prevalence of CVD development and outcome. For example, the relationship between family roles and prevalence of CHD suggests that women living both with their spouse and children had two times higher risk of CHD compared with women living only with a spouse. While married men have a decreased risk of suffering from MI compared with married women, men living alone have an increased risk of fatal MI (109,110). Of note, even though men develop CVD earlier in life, women catch up after menopause and therefore the lifetime risk of CVD is similar for both men and women if estimated for a total life span (111,112). Yet, women are five times less likely to have a diagnosis considering heart disease as main health issue or leading cause of death and they are significantly less likely to have ever received a cardiovascular screening test as well (113). Hence, diagnostic and treatment inequalities between men and women need to be urgently improved.

In conclusion, CVD and atherosclerosis pathophysiological development differ between males and females. There are sex-differences based on the effect of hormones on atherosclerotic plaque morphology and arterial wall remodeling as well as gender-based differences in the prevalence of CVD risks. Better understanding of both is essential for a better diagnosis, treatment, and further study guidelines.

5. Author Contributions

AY, EV and YD performed literature research, drafted the manuscript, and made the figures. IB, SB, and MS wrote the manuscript and provided corrections. All authors have read and agreed to the published version of the manuscript. All authors contributed to the article and approved the submitted version.

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7. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

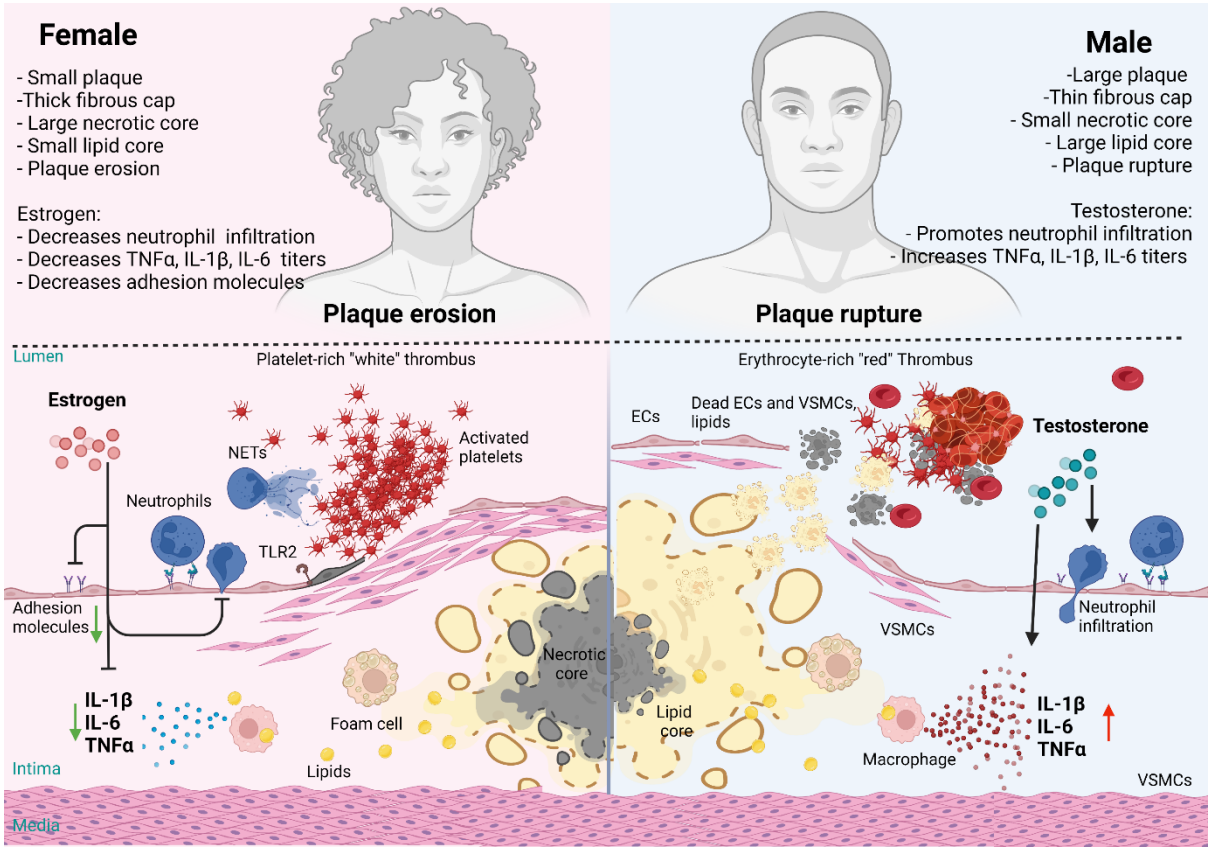

Figure 1. Simplified schematic overview of the main relative sex differences in atherosclerotic plaque remodeling. *Female (left):* Plaque erosion is more dominant in female patients and is initiated by shear stress promoting activation of surface TLR2 on ECs. EC apoptosis fosters their detachment which subsequently leads to the exposure of the fibrous cap to circulating neutrophils. This promotes release of NETs from neutrophils, activation of platelets and platelet-rich "**white**" thrombus formation (6). However, due to the thick fibrous cap vascular integrity remains intact, and the plaque remains stable (114). In addition, atherosclerotic plaques in female humans are characterised by a larger necrotic core compared to males while in female mice estrogen decreases adhesion molecules expression, decreases neutrophil infiltration and pro-inflammatory cytokines such as TNFα, IL-1β and IL-6 secretion and therefore halting atherosclerosis (6,47,48). After menopause, estrogen level decreases and its vasoprotective effects are lost (37-40). *Male (right):* In male humans, plaque rupture is the main cause of thrombus formation induced by fibrous cap burst leading to physical disruption of the vascular wall integrity and to the exposure of highly thrombotic components from the plaque to the blood and to a so-called "**red**" thrombus formation. This phenomenon occurs when the plaques have a thin fibrous cap and a large lipid core therefore the plaque is more vulnerable and more likely to burst (115). Testosterone also affects the plaque remodelling by enhancing neutrophil, mast cell and macrophage infiltration into the intima layer of the vasculature as well as promoting IL-1β, IL-6 and TNFα secretion all fostering atherosclerotic lesion development in animal models (68-70). (This figure was made with biorender.com)

Table 1: Summary of the sex hormone related effects on the pathophysiology of atherosclerosis.

Sex hormone	Increase/decrease	Effect and mediators		References
Estrogen	⬆	vasorelaxation		*(47)*
	⬇	Pro-inflammatory cytokines	TNFα	*(47)*
			IL-6	*(38,48)*
			IL-1β	(38)
	⬇	Immune cell trafficking and infiltration	Neutrophils	*(38,49)*
			Monocytes	*(38,49)*
	⬇	Adhesion molecules	ICAM1	*(49)*
			VCAM1	*(49)*
			P-selectin	*(38,49)*
	⬇	Growth factors	Insulin-like growth factor 1	*(50)*
			Platelet-derived growth factor	
	⬇	Arterial stiffening	Matrix metalloproteinase 12 (MMP12)	*(51)*
Testosterone	⬆	Immune cell trafficking and infiltration	Neutrophils	*(68)*
			Mast cell	*(11)*
			Macrophage	(77)
	⬆	Pro-inflammatory cytokines	TNFα	*(70,87)*
			IL-6	
			IL-1β	
	⬇	Pro-inflammatory cytokines	TNFα	*(70,79,80)*
			IL-6	
			IL-1β	
	⬆	Anti-inflammatory cytokines	IL-10	*(80)*
			IL-1rA	
	⬆	Cardiac fibrosis	tissue inhibitor of metalloproteinase 1 (TIMP-1)	*(71-73)*
			Serpin A 3n	

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