JVR
Journal of
Vascular Research

Review Article

J Vasc Res 2022;59:1–15
DOI: 10.1159/000518787

Received: May 17, 2021
Accepted: July 28, 2021
Published online: September 14, 2021

Microvessel Density: Integrating Sex-Based Differences and Elevated Cardiovascular Risks in Metabolic Syndrome

Angelina Wong Shu Qing Chen Brayden D. Halvorson Jefferson C. Frisbee

Department of Medical Biophysics, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada

Keywords
Metaboloic syndrome · Microvessel density · Sex differences · Cardiovascular risk

Abstract
Metabolic syndrome (MetS) is a complex pathological state consisting of metabolic risk factors such as hypertension, insulin resistance, and obesity. The interconnectivity of cellular pathways within various biological systems suggests that each individual component of MetS may share common pathological sources. Additionally, MetS is closely associated with vasculopathy, including a reduction in microvessel density (MVD) (rarefaction) and elevated risk for various cardiovascular diseases. Microvascular impairments may contribute to perfusion-demand mismatch, where local metabolic needs are insufficiently met due to the lack of nutrient and oxygen supply, thus creating pathological positive-feedback loops and furthering the progression of disease. Sexual dimorphism is evident in these underlying cellular mechanisms, which places males and females at different levels of risk for cardiovascular disease and acute ischemic events. Estrogen exhibits protective effects on the endothelium of premenopausal women, while androgens may be antagonistic to cardiovascular health. This review examines MetS and its influences on MVD, as well as sex differences relating to the components of MetS and cardiovascular risk profiles. Finally, translational relevance and interventions are discussed in the context of these sex-based differences.

Introduction

Microvessels are a distinctive subclass within the circulatory system, generally characterized by vessel diameters being <150 μm – this typically includes arterioles, capillaries, and venules [1]. However, modern definitions place a heavier emphasis on vessel function, rather than structure. The distinction of microvessels is therefore dependent on an isolated vessel's ability to respond to changes in internal pressure. Thus, most arterioles with intact myogenic responses are considered microvessels [2]. This definition also extends to smaller arteries, even if their diameter is above the traditional threshold. For the

Angelina Wong and Shu Qing Chen contributed equally to the manuscript.

karger@karger.com
www.karger.com/jvr

Correspondence to:
Jefferson C. Frisbee, jfrisbee@uwo.ca

Karger

purposes of addressing microvascular remodeling, the term "microvessel" will be used to describe vessels <20 μm in diameter, which includes distal arterioles, capillaries, and proximal venules.

Gas and nutrient exchange, occurring at the microvascular level, is responsible for ensuring homeostasis and adequate tissue oxygenation [3] with arterioles, capillaries, and venules each playing a different role. Arterioles primarily regulate perfusion and convective transport of substrates, capillaries facilitate the majority of exchange through diffusive transport, and venules act as collecting vessels [3]. Perfusion regulation is conducted by a delicate balance of mechanisms namely concentration gradients and blood flow velocity, which dictate the net flux of solutes at the capillary-tissue boundary [4]. Concentration gradients play a large role in flux relating to diffusive transport with higher gradients leading to increased flux, while blood flow velocity is associated to convective transport [5]. Substrate flux into the tissue is largely dependent on local metabolic demand and possible downstream metabolite effects [4]. Physiological or anatomical changes to microvessels may lead to impaired tissue perfusion characterized by increased tissue oxygenation heterogeneity and insufficient perfusion-demand matching.

Microvessel Rarefaction

Microvessel density (MVD) is defined as the number of microvessels per unit tissue volume or, more commonly, per tissue cross-sectional area [3]. A reduction in MVD is termed microvascular rarefaction and often precedes diagnosable symptoms related to specific cardiovascular diseases. In particular, rarefaction is closely associated with cases of moderate hypertension and type 2 diabetes mellitus (T2DM). The obese Zucker rat, a hyperphagic model for metabolic syndrome (MetS), has displayed skeletal muscle rarefaction of up to 20–25% when compared to the control model of age- and sex-matched lean Zucker rats [6]. Microvascular rarefaction is often found in multiple tissue types after initial onset due to the migratory nature of endothelial inflammation [7]. This is evident in the cerebral cortex tissue of obese Zucker rats, where a 20% rarefaction compared to control developed in parallel with rarefaction in the skeletal muscle [6]. The results from this study suggest that a reduced bioavailability of the vasodilator nitric oxide (NO) corresponds to rarefaction and may be an underlying mechanistic contributor to this vascular outcome in metabolic dysfunction [8]. Other vasodilator factors such as the endothelium-derived hyperpolarizing factor may also play a significant role in maintaining the vascular tone [9].

Clinical research in hypertensive patients have found that individuals with primary hypertension exhibit microvessel rarefaction in cutaneous tissue, whereas previous theories mainly focused on decreases in arteriolar diameter [10]. The observed rarefaction typically occurs first as a structural abnormality, suggesting that vessel rarefaction is likely to be a contributor to hypertension, rather than an outcome – although it is certainly possible that these operate in a feedback manner. This is further exemplified in animal model studies, where antihypertensive medications were seen to attenuate microvascular rarefaction when given prior to hypertension development [11]. Impairment of pro-angiogenic endothelial progenitor cells has been suggested as a contributing factor for hypertensive-associated rarefaction and may become a target for therapeutic intervention [12].

Rarefaction can be classified as functional (vessel receives little to no flow) or structural/anatomical (vessel physically deteriorates until it becomes anatomically absent) [13]. With functional rarefaction, nonperfused vessels at rest still have the potential to participate in perfusion when responding to increased metabolic demands. In comparison to anatomical rarefaction, functional rarefaction allows for flexibility and adjustment to increased demands, which may be a key factor in cardiovascular fitness and endurance [14].

The relatively permanent loss of functional vessels associated with structural rarefaction reduces the upper limit of metabolic demand that a tissue can sustain. In conditions of increased demand, remaining functional vessels may be over perfused to compensate for lost vessels, subjecting them to increased pressures in relation to Darcy's law of flow [5]. Mathematical models have suggested that rarefaction of arterioles can increase peripheral resistance by approximately 20% in the vascular bed [15]. However, actual rarefaction in vivo may be larger if overperfusion activates a myogenic contraction response [15], where vessels actively constrict in response to elevated blood pressure. Decreases in functional microvessels decrease the overall efficiency of oxygen and nutrient delivery, resulting in unmet metabolic demands for certain local tissue beds. Organs are then insufficient in functional units, increasing the risk of hypoxia as it can be seen in cases of hypertensive end-organ damage and diabetic limb amputations.

Increased heterogeneous perfusion from microvascular rarefaction also limits the delivery and thus the function of most signaling molecules – including insulin. Microvascular rarefaction is linked to insulin resistance due to the in-

terdependency between insulin signaling, glucose uptake, and distribution of pro-angiogenic factors [15]. A key pro-angiogenic factor is the vascular endothelial growth factor (VEGF), which plays a critical role in angiogenesis and vascular development. Both animal and human studies assessing insulin sensitivity found increased levels of VEGF in diabetic models [16], while another study found that VEGF-knockout mice exhibited up to 60% rarefaction within skeletal muscle [17]. This demonstrates the critical role VEGF plays in preventing or reversing rarefaction, which is often associated with insulin resistance. Overall decreases in perfusion associated with severe rarefaction will also impair delivery of VEGF, further inhibiting angiogenesis. In such cases, functional vessels are continuously being lost, while the formation of new vessels is being impaired, resulting in additional net loss of functional units.

Although microvessel remodeling can indicate oncoming disease, there is considerable uncertainty given the variance in vessels dependent on species, sex, organ type, genetic predisposition, and experimental conditions. Additionally, most in vivo tissue beds do not conform to simple models where all vessel units share the same properties and behavior of perfusion. Models may also overlook angiogenesis, where new vessels are created through pro-angiogenic factors, which alter net perfusion and vessel pathways. The heterogeneous and complicated nature of pressure-flow regulation can increase the risk of developing rarefaction, making it difficult to predict with quantitative modeling [18].

Metabolic Syndrome

MetS is closely associated with the progression of microvascular rarefaction [7]. A diagnosis of this multipathological state relies on the combined presences of its individual components. Although many definitions of MetS exist, the most clinically used was proposed by the National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) [19]. NCEP ATP III identified 6 components of MetS: hypertension, insulin resistance, abdominal obesity, atherogenic dyslipidemia, a proinflammatory state, and a prothrombotic state (Fig. 1). For comparison, the American Heart Association has defined ideal cardiovascular health based on 4 measurements: body mass index (BMI), untreated total cholesterol, untreated blood pressure, and untreated fasting glucose. For an ideal nonsmoking individual, the following measurements indicate good health: BMI <25 kg/m^2, untreated total cholesterol <200 mg/dL, untreated blood pressure <120/<80 mm Hg, and untreated fasting glucose <100 mg/dL [20].

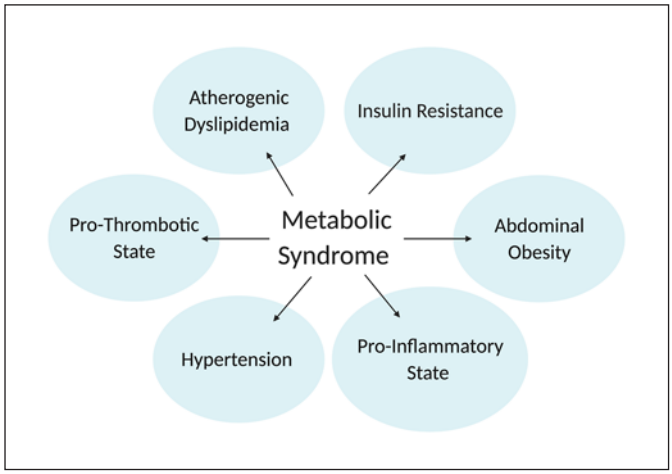

Fig. 1. Definition of MetS according to the NCEP ATP III, identifying 6 components that include hypertension, insulin resistance, abdominal obesity, atherogenic dyslipidemia, a proinflammatory state, and a prothrombotic state. MetS, metabolic syndrome; NCEP ATP III, National Cholesterol Education Program's Adult Treatment Panel III.

The interconnecting cellular mechanisms and pathways responsible for such symptoms suggest that they may be contingent on one another [21], and their co-occurrences can indicate an increased risk for various cardiovascular diseases and T2DM. For instance, peripheral vascular disease is correlated with each individual component of MetS, yet a patient's risk for developing peripheral vascular disease is substantially higher when they are experiencing MetS [22].

Insulin resistance is often regarded as the direct contributing pathophysiology to MetS and its components [23]. Insulin is typically secreted by beta cells within the pancreas and regulates blood glucose levels by promoting glucose uptake through 3 pathways: (1) binding to glucoreceptors on endothelial cells which leads to vasodilation and increased blood flow, (2) moving glucose into the interstitial space through an insulin receptor-dependent process, and (3) promoting the movement of glucose transporters to the cell surface membrane [24].

Insulin resistance is associated with elevated levels of excess fatty acids due to increased lipolysis and inflammation [25]. For example, elevated nonesterified fatty acids concentrations in obesity are thought to arise from increased adipose tissue (AT) mass. The process of fatty acid mobilization from AT – normally suppressed by insulin – becomes insulin resistant itself, further increasing

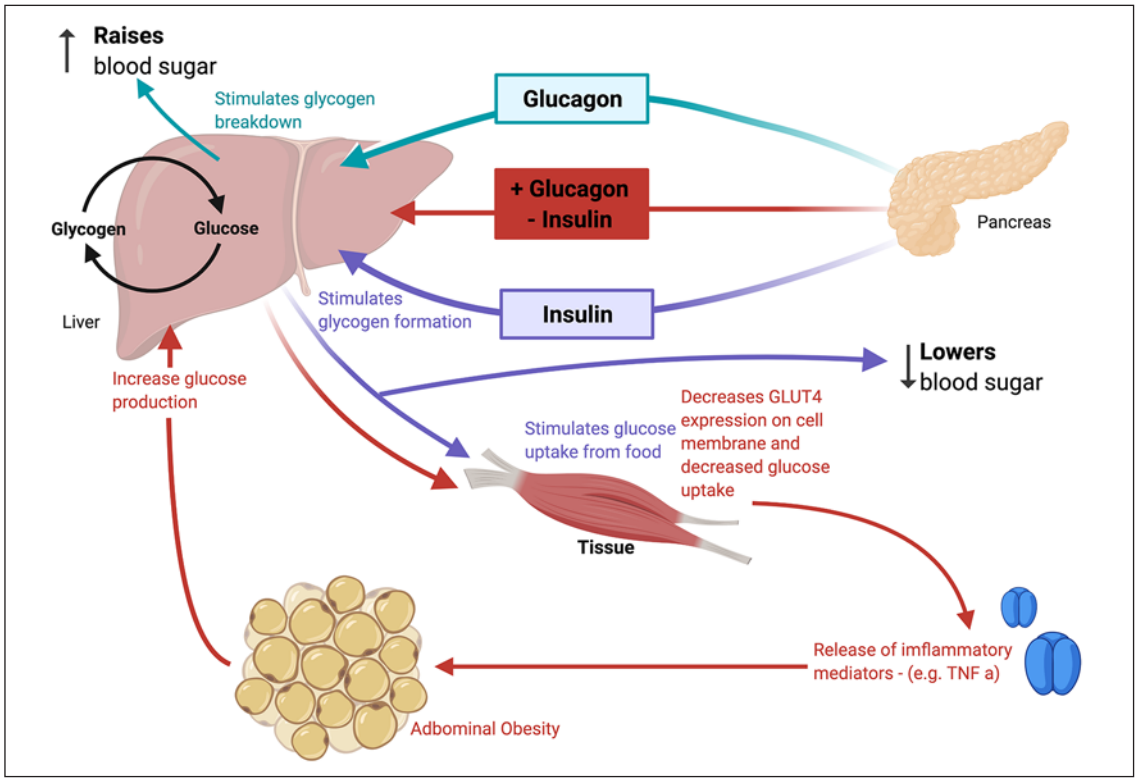

Fig. 2. In normal states, glucagon and insulin help to regulate blood glucose levels via the glucagon and insulin pathways. However, in insulin-resistant models, beta cell dysfunction in the pancreas leads to increased levels of glucagon and decreased levels of insulin. The high glucagon levels stimulate additional breakdown of glycogen into glucose. Combined with decreased expression of GLUT4 protein and increased inflammatory mediators, a positive cycle of hyperglycemia ensues. TNF-α, tumor necrosis factor-alpha.

lipolysis and potentially leading to a vicious cycle [25]. To compensate, insulin secretion is upregulated to maintain blood glucose levels, which stunts glycogenesis and glucose transport in skeletal muscle. This reduces the effectiveness and sensitivity of insulin signaling pathways, resulting in elevated bloodstream insulin levels. Insulin sensitivity is also influenced by hormones such as glucagon and catecholamines, which play key roles in its metabolic pathways [25]. In insulin-resistant models, high levels of glucagon promote glycogenolysis and gluconeogenesis, while high levels of catecholamines are expected to promote lipolysis (Fig. 2). The manifestation of insulin resistance is also believed to occur at the cellular level via post-receptor defects in insulin signaling. Suggested mechanisms include downregulation/dysfunction in the phosphorylation of the insulin receptor or GLUT4 function and recruitment; however, the insulin resistance pathways in humans still lack clarity [26].

Proinflammatory and prothrombotic states seen in MetS suggest that inflammation may be a key pathophysiology. Inflammation is associated with obesity and shares overlapping pathways used to govern metabolic and immune functions [27]. In obesity and other metabolic dysfunctions, the body undergoes a process called metaflammation, a chronic and global low-grade state of inflammation. In obese patients, nutrient and metabolic surplus causes increased macrophage levels, excess cytokines, and irregularities in the signaling pathways of AT, all of which are predicted to play a role in microvessel rarefaction. This effect is further exacerbated by the release of plasminogen activator inhibitor-1 (PAI-1) and adiponectin as elevated levels of PAI-1 contribute to the prothrombotic state by hypofibrinolysis [27]. Decreased activation of the fibrinolytic system at sites of injury or inflammation results in insufficient removal of unwanted fibrin in a thrombus, influencing the development of atherosclerotic lesions [28]. Increased levels of PAI-1 may predispose patients to formation of atherosclerotic plaques that are prone to rupture and decreased cell migration [27].

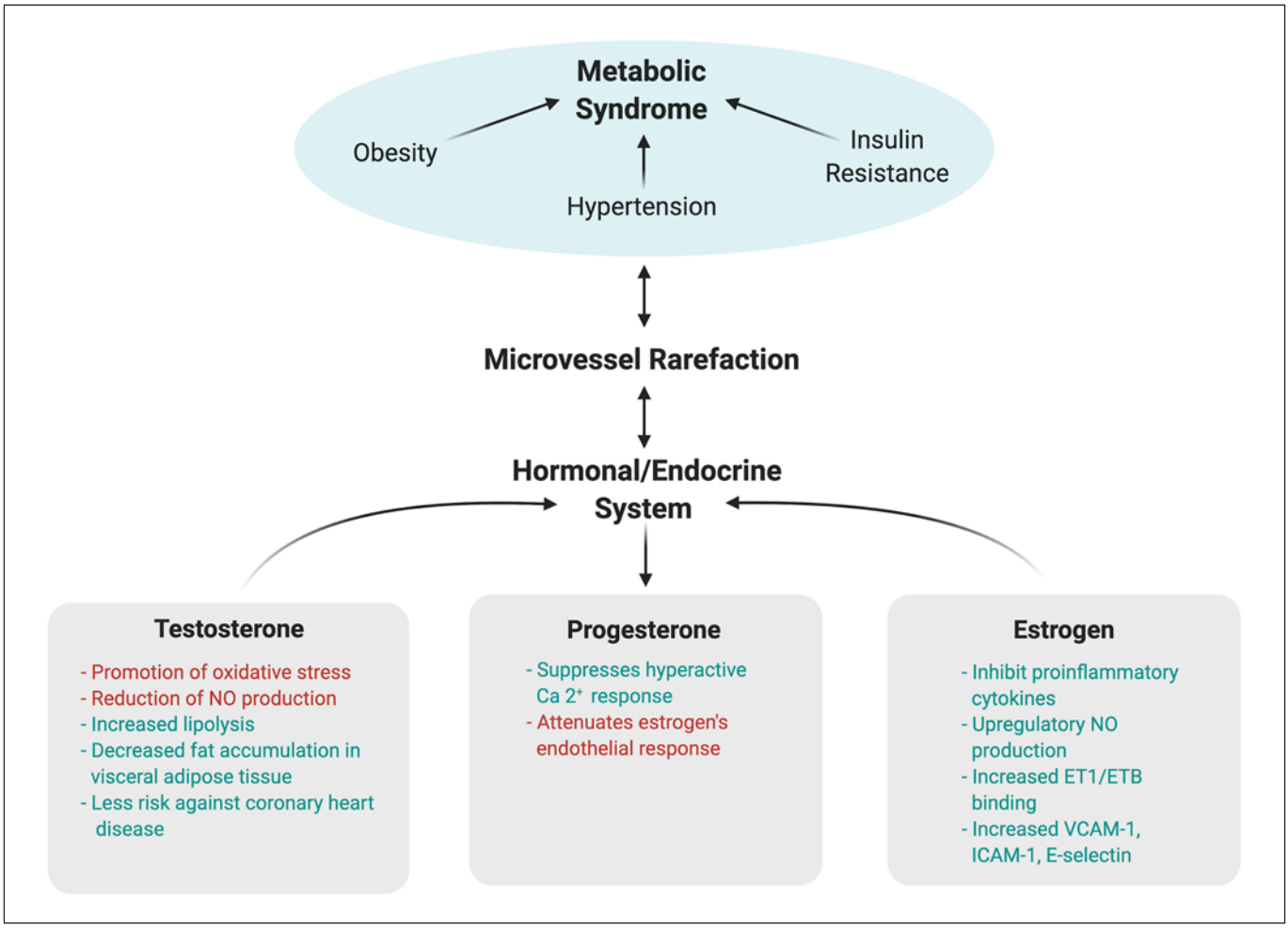

Fig. 3. The effects of cellular physiology on a pathophysiology viewpoint and a clinical viewpoint. Green-colored cellular changes cause protective effects at the pathophysiology level, while red-colored cellular changes cause antagonistic effects at the pathophysiology level. Different contributions of protective and antagonistic effects may accumulate to clinical conditions clustering to components of MetS. MetS, metabolic syndrome; NO, nitric oxide.

Tumor necrosis factor-alpha is a proinflammatory cytokine that is released to activate inflammatory signals and is an inhibitor for insulin activity, thus playing a critical role in insulin-mediated microvessel rarefaction. Clinical trials have demonstrated that excess tumor necrosis factor-alpha leads to decreased insulin sensitivity and glucose homeostasis, accumulating to insulin resistance over time [29]. Insulin resistance has been shown to affect the microvasculature, which contributes to common clinical symptoms such as the tingling or numbing sensations in the limbs. Under normal conditions, elevated levels of insulin will increase the capillary density available for perfusion in order to increase accessible tissue surface area for glucose metabolism – this response is blunted in patients with insulin resistance [30].

The capillary permeability-surface (PS) area product is used to describe the capacity of a substance to reach interstitial fluid, which is dependent on the permeability, recruitment, and surface area of capillaries. PS values for blood glucose can help determine the relationship between insulin resistance and MVD – the higher the PS values, the greater the density. A recent investigation of muscle capillary permeability showed an expected increase in PS for glucose and glucose uptake after an oral glucose load, yet this response was absent in insulin-resistant patients [31]. On a cellular level, reduced PS scores can be attributed to changes in metabolic mechanisms,

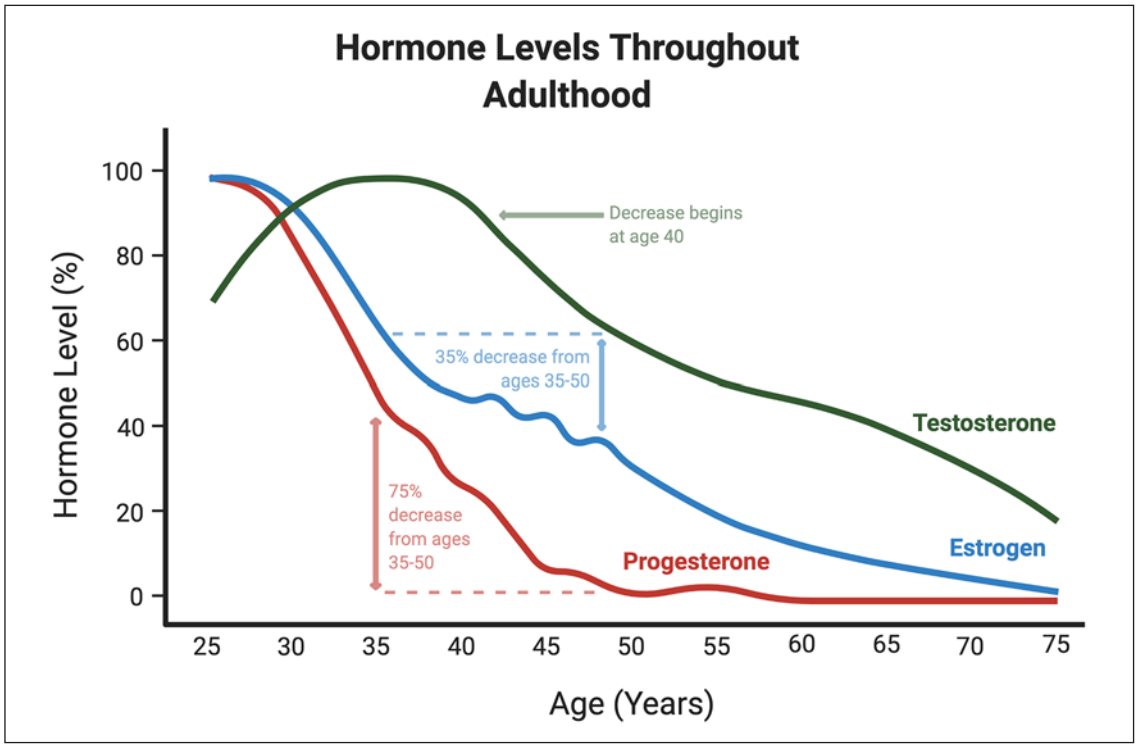

Fig. 4. Hormone levels with age as a proportion to maximum levels during adulthood. Estrogen and progesterone levels pertain to adult females, and testosterone levels pertain to adult males.

such as a reduction in NO bioavailability. Other potential theories include decreased levels of vasodilators, increased activity of vasoconstrictors (including endothelin [ET] or angiotensin-II), heightened response to alpha-adrenergic signaling, and increased reactive oxygen species (ROS) through activation of inflammatory pathways [32, 33].

On a cellular level, hypertension suggests increased peripheral resistance which may be a result of reduced NO-dependent vasodilation – caused by high levels of ROS; reduced NO bioavailability is a key outcome to microvessel rarefaction. In a proinflammatory state, ROS are produced in endothelial cells, smooth muscle cells, and inflammatory cells within the arterial wall, effectively reducing NO bioavailability, which prolongs vasoconstriction. Over time, this constricted state may lead to microvascular rarefaction, potentially reducing glucose uptake and causing insulin resistance [34].

Thus, the influences of insulin resistance on the microvasculature are difficult to separate from the effects of hypertension. Capillary density and vasodilatation are strongly correlated to insulin sensitivity but also inversely related to blood pressure, suggesting that hypertension and insulin resistance may collaboratively impact MVD [35]. The dissociation between obesity and primary insulin resistance in MetS patients is also difficult. An increased level of proinflammatory cytokines and ROS is linked with lipolysis, a progenitor of the insulin resistance pathway and may be related to hyperglycemia [36]. This outlines how interconnected the various components of MetS are at a cellular level in terms of metabolic processes.

Sex Differences

As mentioned previously, a distinctive characteristic associated with MetS is structural and histological remodeling of the microcirculation, including changes such as vascular smooth muscle atrophy, endothelial dysfunction, and decreased MVD. Hypertension is associated with approximately 10–30% rarefaction in skeletal muscle, with varying degrees of rarefaction occurring in other organs such as the renal and cerebral tissues [37]. However, a noteworthy point is that large proportions of translational and clinical research concerning MetS are performed exclusively on males. Sex is often controlled in these stud-

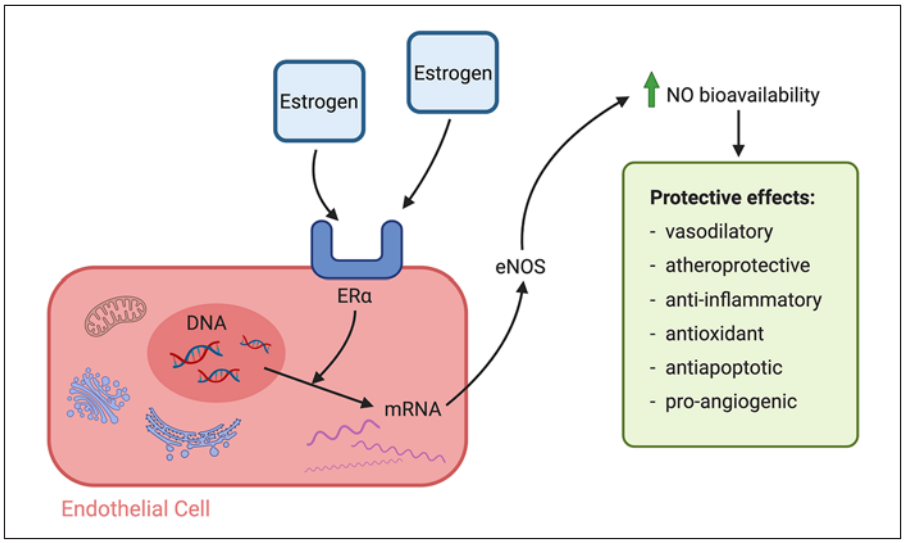

Fig. 5. Estrogen exhibits protective effects on the endothelium, promoting NO bioavailability and vasodilation. Specifically, estradiol is a regulator of VEGF and other pro-angiogenic growth factors. NO, nitric oxide; VEGF, the vascular endothelial growth factor; eNOS, nitric oxide synthase.

ies, and the preference for males may be an issue of experimental and economical practicality as females can be more variable due to hormonal cycles. Additionally, previous research suggests that female sex hormones exert a protective effect on vasculopathy, which introduces a larger variance when comparing male to female parameters [19]. Yet, findings based on male-only samples are still applied to both sexes without consideration of sex-dependent differences. These sexual dimorphisms and their effects on vascular health are dependent on both sex hormones and other biological causes. However, the focus of this review is on the implications of sex hormones, primarily estrogen, progesterone, and testosterone, on MVD in the setting of health and metabolic disease (Fig. 3).

Estrogen

Estrogen is the primary female sex hormone that appears in 3 bioidentical forms: estrone, estradiol, and estriol. Like other steroids, estrogen is highly lipid-soluble, allowing it to be readily diffusive in various cellular interactions [38]. Estrogen acts on both nuclear and extranuclear receptors such as estrogen receptor alpha (ERα), ERβ, and G-protein-coupled estrogen receptors [39]. In premenopausal women, estrogen is largely produced in the ovaries, corpus luteum, and placenta, with organs such as the liver, heart, skin, and brain, producing smaller amounts [40]. Often overlooked, AT is a key site for the peripheral production and metabolism of estrogens in women [41]. As primary estrogen production occurs in the reproductive organs, estrogen levels can change drastically throughout a woman's life, with estradiol levels of an average of 40 pg/mL before menopause – to <18 pg/mL after menopause. Larger fluctuations can occur during the menstrual cycle and pregnancy/childbirth, where estradiol levels range from 30 to 400 pg/mL and 200 to 7,000 pg/mL, respectively [42]. These fluctuations in estradiol levels are used to promote receptor mediated uptake of low-density lipoprotein (LDL) cholesterol for placental steroid production, increasing uteroplacental blood flow and preparing the breast for lactation [43]. When menopause occurs, the ovaries decrease estradiol production while adrenal glands continue estrone synthesis; this causes estrone levels to remain unchanged, yet plasma estradiol levels fall significantly (Fig. 4). When these hormonal changes stabilize near the end of menopause, a general decrease in estrogen level and expression of estrogen receptors is observed. During pre-menopause, receptors such as G-protein-coupled estrogen receptors are widely expressed in diverse cell types, helping to regulate metabolism [39]. However, in postmenopause, Park et al. [44] concluded that there was an overall decrease in ERα and ERβ expression in abdominal and femoral subcutaneous AT. In conjunction with decreasing estrogen levels, changes in receptor expression can promote metabolic changes, subjecting women in the latter half of their life to a higher risk for cardiovascular disease and obesity.

Recent research has established that premenopausal women exhibit more resistance against hypertension than other cardiovascular diseases, thereby also increasing resistance against associated rarefaction [45]. In model of hypertension, male Sprague-Dawley rats displayed significant rarefaction in the gastrocnemius muscle when compared to that of their female counterparts, [46]. Al-

though females are not entirely protected against hypertension, both human and animal studies have found that hypertension in females occur later and to a lesser degree, when compared to their male counterparts [3]. Other research studied female rats with ovaries and ovariectomized female rats with hormone replacements and found that both have more dilated arterioles than male rats or ovariectomized female rats without hormone therapy [47]. These results elucidate estrogen's regulatory effects on vascular tone through estrogen receptors within the endothelium and smooth muscle layers of vessels. By interacting with these receptors, estrogen promotes the release of strong vasodilator agents such as NO [48]. Chakraparti et al. [49] reviewed the endothelial NO synthase (eNOS) activity induced by exogenous estradiol on ERα. Estrogen's effects on increasing NO bioavailability are also associated with increased dilation and an anti-inflammatory, antioxidant, antiapoptotic, and pro-angiogenic environment (Fig. 5) [50]. Although the specific mechanisms underlying rarefaction are not understood, it is well established that estrogen levels directly contribute to the extent of rarefaction in the tissue. Postmenopausal women are more at risk for developing heart diseases involving the microvasculature due to their decreasing estrogen levels. Without high levels of estrogen, ERα and ERβ ligand binding is unable to upregulate transcription of VEGF and stimulate angiogenesis [51].

Blood volume, heart rate, stroke volume, and cardiac output are all known to increase during pregnancy, yet there is a significant decline in vascular resistance. A likely explanation for this seemingly contradictory relationship is the radically elevated levels of estrogen during pregnancy, potentially increasing estrogen's vasoprotective effects [52]. Other studies have yielded similar findings, suggesting that endogenous estrogen in premenopausal females is very likely to have protective effects when intact [3].

However, this protective mechanism dissipates in females after menopause occurs, and postmenopausal women display similar cardiovascular risk profiles to that of age-matched men [19]. Animal studies have found that females may have other inherent cardiovascular advantages on top of the protective mechanisms provided by estrogen. Even after ovariectomy, females were still protected against renal vasoconstriction and glomerular sclerosis. This suggests that protective mechanisms other than estrogen exist in females [53].

Progesterone

Along with estrogen, progesterone is a sex hormone that naturally occurs as progestin in both females and males but in higher levels among females. It is synthesized by the ovaries in females and in the testes and adrenal cortex in males. Although there is less circulating progesterone within the male body, its functions may be equally important to that of females [54]. Progesterone elicits its effects by binding to the progesterone receptor (PR) and other associated response elements, to activate an intricate intracellular signaling pathway and induce genomic or nongenomic responses [55]. As previously discussed, menopause in addition to a decline of estrogen levels, there is a simultaneous decline in progesterone which may contribute to the associated increased risk of cardiovascular and metabolic disease [54]. Data from many studies have demonstrated that the positive correlation between ERα and PR expression continues during postmenopause, resulting in a decreased expression of both ERα and PR [56].

Some studies have demonstrated that progesterone may exhibit an antagonistic effect against estrogen. For instance, progesterone can inhibit estrogen's beneficial effects on bone structure by decreasing serum levels of 1,25-dihydroxyvitamin D [57]. However, progesterone's inhibitory effects on estrogen may be crucial in maintaining hormonal balance and homeostasis. For example, in the absence of progesterone, estrogen can overstimulate uterine lining growth, increasing the risk of endometrial cancer [58]. Progesterone combats this by inducing endometrium shedding during each menstrual cycle and theoretically prolongs the premenopausal years in females. The mechanism of how progesterone and estrogen coexist and regulate each other is not clearly known, but it is evident that they are both essential [58]. Therefore, most hormone therapies combine estrogen with progesterone to reduce the risks of disrupting this balance.

With regards to vascular health, studies on progesterone have yielded contradictory findings. Progestin is believed to induce both vascular smooth muscle relaxation and contraction. A study on in vitro-isolated dog coronary artery rings found that progesterone attenuates estrogen-induced responses in the endothelium, inducing vascular constriction [59]. However, another study concerning ovariectomized monkeys showed that progesterone could play a similar vasorelaxant role to estradiol-17β. In this study, the ovariectomized monkeys displayed hyperactive vascular muscle cells with increased Ca^{2+} and protein kinase C levels, both of which were suppressed when the monkeys received estrogen or progesterone [53]. This suggests that progesterone, similarly to estrogen, may induce vasodilation. Studies have demonstrated that progesterone enhances functional recovery after an

ischemic stroke by increasing the number of newly generated neurons in the infarct region. However, this progesterone therapy has no impact on MVD [60].

Androgens

Testosterone is the primary androgen that exists in 2 different forms: an active free form which binds to androgen receptors (ARs) and an inactive form that cannot bind to ARs until activated. The AR initiates a diverse range of biological actions that play roles in the development and maintenance of various biological systems [61]. Although testosterone and ARs are expressed in both sexes, males have much higher circulating testosterone in comparison to females [62]. When referring to testosterone, it is usually the total testosterone or sum of both active and inactive forms that are involved. Testosterone levels peak in adulthood (around age 30) and steadily decline by 1–2% every year after this peak [63]. In males, a small amount of circulating testosterone is converted into estradiol, which also decreases with age in proportion with testosterone. Thus, metabolic changes attributed to testosterone deficiency might be partly or entirely due to the accompanying decline in estradiol (Fig. 4) [64].

There are contradictory findings, perhaps due to the presence of active and inactive forms of testosterone, demonstrating how testosterone levels may impact risk levels for cardiovascular diseases [65, 66]. Thus, the exact mechanisms of how testosterone deficiency may affect cardiovascular health remain unknown. One hypothesis is that testosterone bioavailability can reduce the risk associated with insulin resistance and obesity. Studies have reported that patients with high levels of bioavailable testosterone displayed less risk for major cardiovascular events and coronary heart disease, while men with lower testosterone levels were associated with increased adiposity and a higher risk of T2DM [67]. This is further supported by studies relating diabetic male patients with their relatively low testosterone levels when compared to their nondiabetic peers [68]. Decreased levels of the sex hormone binding globulin, responsible for transportation of sex hormones, are also prevalent in diabetic men [69, 70]. Previous studies have suggested that castration reduces endothelial cell numbers and the endothelial cell proliferation rate in the rat prostate and can be normalized by testosterone treatment. This study indicates that the vasculature could be regulated by androgens [71]. Using castrated rats, Franck-Lissbrant et al. [72] demonstrated that the lack of testosterone resulted in an involution of the vasculature in the prostate tissue. Although MVD was not directly measured, epithelial cell weights and blood flow was significantly reduced, suggesting a marked drop in vascular density [72].

Unsurprisingly, lower levels of testosterone are also associated with obesity. Ohlsson et al. [67] conducted a study on 2,416 men which demonstrated that the average BMI of the subjects with high levels of total testosterone was lower (24.9 compared to 28.1) than subjects with lower levels of total testosterone. Furthermore, higher levels of testosterone have been shown to increase lipolysis and decrease fat accumulation in visceral AT, lowering BMI index values. The production of testosterone is triggered by insulin levels in the bloodstream but the exact interactions between testosterone level and obesity are largely unknown [73]. Castration of male rats has been found to exhibit vasoprotective effects, indicating that the presence of androgens may be more detrimental than the absence of estrogen in terms of risk for cardiovascular disease [74].

Other Sex Hormones

Other sex hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) can also impact an individual's risk for MetS and cardiovascular diseases. Studies have found that there is a 30–40% decrease in LH and FSH levels between the ages 50 and 75 [75]. Coupled with a decreased response to GnRH, levels of LH and FSH continue to decrease postmenopause. As a result, any elevated changes in LH postmenopause may be used as a clinical indicator. Recent research has revealed that high LH levels are positively associated with established diabetic kidney disease among Chinese men and postmenopausal women [76]. Before menopause, elevated LH levels are also associated with increases angiogenic potential. During female ovulation, the surge in LH levels promotes angiogenesis in the luteinizing follicle, allowing the blood vessels to dilate and increase blood flow to the follicle [77]. However, the impact of LH and FSH on vascular function and remodeling is not clearly understood due to the complex nature of these sex hormones and still needs to be further explored.

Discrepancy in Risk Factor Profiles

Although the extent of estrogen's protective effects are not yet clear, and it is still unknown how low testosterone is related to cardiovascular fitness, there is a clear discrepancy in cardiovascular risk factor profiles due to sex. On average, premenopausal women have lower blood pressure than their male counterparts, regardless of ethnicity [78]. Many studies have found that hypertension prevalence is lower in premenopausal women, and this is like-

ly accredited to both the higher concentration and intact protective effects of estrogen associated with younger females [79, 80]. This is further exemplified as menopausal women begin to have comparable blood pressure profiles to males, during a time when estrogen production decreases significantly [81]. When given estrogen therapy, some postmenopausal women experienced a decreased risk for coronary artery disease (CAD). Other studies have shown that hormonal therapy with menopausal women only lowered their blood pressures slightly, suggesting that hypertension is better correlated to an increase of androgens rather than the decreased estrogen experienced by men [78].

Androgens may increase blood pressure through the renin-angiotensin system as the promotion of oxidative stress and reduction of NO production increases vasoconstriction while decreasing vasodilation [82]. Sex-specific phenotypes arise prior to sexual maturity, even in the absence of sex hormones, and this sex-specific gene expression in cells may explain why hormone therapy can have limited effectiveness. Androgens also upregulate thromboxane receptors and macrophage formation, placing males at a higher risk for atherosclerosis [83]. Therefore, women with the intact sex protection mechanisms of estrogen are unlikely to experience hypertension or risk for hypertension to the same extent as males, even if other genetic and lifestyle factors are similar.

Dimorphisms Explained: Mechanisms and Pathways at the Cellular Level

Estrogen's atheroprotective effects may be due to the presence of its receptors found in vascular smooth muscle cells; binding of estrogen with these receptors stimulates the release of NO from the endothelium, effectively increasing vessel diameter [84]. Other studies support this, finding direct correlations of estrogen to increased overall NO production, possibly explaining why myogenic constriction is experienced to lesser degrees in females [85]. Sexual dimorphism within the NO production system is particularly evident in renal hemodynamics as the kidney is responsible for producing NO synthase (NOS) and consequently NO. Estrogen has upregulatory effects for NOS and NO at both genomic and cellular levels. With age, the presence of NO synthase (NOS) inhibitors increases, limiting NO bioavailability. The combination of increased NOS inhibitor activity with decreasing levels of estrogen may contribute to a reduced NO production in postmenopausal women [86, 87].

Estrogen also exhibits properties relating to inhibiting atherosclerosis [88]. For example, estrogen inhibits expression of VCAM-1, E-selectin, and ICAM-1 on the endothelial surface, which prevents monocyte adherence to vessel walls. Studies have demonstrated that estrogen is associated with decreased levels of LDL cholesterol and increased levels of high-density cholesterol (HDL) and triglycerides [48]. Additionally, estrogen may also inhibit expression of proinflammatory and mitogenic cytokines, preventing deposition of atherosclerotic plaque [89, 90]. Pressure-induced myogenic constriction of arterioles is experienced differently between males and females, and estrogen's resilience against atherosclerosis may partly account for the greater compliance in female vessels [48].

Another clear example of sexual dimorphism can be seen in the ET system. ET-1 is described as a potent vasoconstrictor, and males tend to have higher plasma ET-1 concentrations. Studies have found that testosterone increases ET-1 concentrations, while estrogen and progesterone do the opposite. Males also have greater ET-1/ETA receptor activation, while females have greater ET-1/ETB receptor activation. Although ETA and ETB are both G protein-coupled receptors found on vascular smooth muscle cells, ETA promotes vasoconstriction, inflammation, and oxidative stress, while ETB promotes NO production and vasodilation. The ET system plays a larger role during pregnancy, when ETB activity is stimulated by the pregnancy hormone relaxin, increased estrogen, VEGF, and placental growth factors. Dysfunctional ET activity during pregnancy has been linked to preeclampsia, solidifying the effects of ET's on blood pressure regulation [91].

Translational Relevance

A recent study of MetS on the elderly population demonstrated that males and females differed significantly in prevalence for all MetS components. Notably, females experienced increased prevalence of abdominal obesity and low HDL cholesterol levels, while males were more prone to both high blood pressure and blood sugar levels [92]. It is important to note that this study was done exclusively on 66-year-old subjects, when the large majority of female subjects have experienced menopause. Pre-menopausal women generally accumulate less abdominal visceral fat than men [93], which reinstates the significant vasoprotective effects of female sex hormones when intact. As mentioned in previous sections, the vasoprotective effects of estrogen may also play a role in resistance against rarefaction [83]. These findings suggest that sex

differences should be considered to optimize treatment and prevention plans for MetS and its components. Considering the interconnected nature of MetS, alleviating any single component may be vital in improving the prognoses of other components. If interventions and treatment can be more personalized to each patient, the core pathophysiology experienced by that individual can be targeted. Recognition and further understanding of sex differences is crucial for increasing the efficiency of treatment in clinical settings.

Interventions

Dietary Interventions

Studies have shown that sex may play a protective role against high-sugar diets. When comparing female rats that were fed on a control versus high-fructose diet, there was no significant difference in blood pressure. Contrary to their male counterparts, the female rats did not develop hypertriglyceridemia or insulin resistance on a high carbohydrate diet. However, when male rats were fed a high-fructose diet, the effects of insulin were blunted, suggesting that high-sugar diets may be more detrimental to males [94]. Insulin exhibited no effect on vascular smooth muscle contraction in female rats but was found to reduce contraction in control male rats. This suggests that insulin treatments may be more effective in males, relative to female counterparts.

Other studies have suggested that high-sodium diets may account for hypertension more in women than men. Low-sodium interventions decreased blood pressure significantly more in women, especially those with higher baseline pressures and older age [95]. This suggests that sodium interventions are most appropriate for aging females with moderate hypertension. In contrast to the previous study, females may benefit more from reducing sodium intake, while males may benefit more from reducing sugars.

Exercise Interventions

In conjunction with dietary interventions, men and women also demonstrate different cellular responses to exercise. In men, more myocardial glucose is used to support metabolic demands, while more fatty acids are used in women [96]. Additionally, women are more resistant to exercise fatigability for isometric tasks, suggesting greater endurance than men; however, men demonstrate greater strengths involved in dynamic tasks [97]. Studies regarding weight loss interventions have found that although both sexes will experience weight loss, men lose relatively more weight than women with the same diet and exercise prescription [98]. When combined with caloric restrictions, 30–45 min of moderate exercise for 4–5 days per week resulted in men losing significantly more weight [99]. Relative to dietary interventions, exercise interventions exhibited lesser degrees of sexual discrepancy in decreasing mild hypertension [100]. These findings suggest that exercise regimens are most effective when combined with dietary interventions, and with current treatment plans, men may experience a greater benefit with lifestyle changes. Nonetheless, many studies consistently find that exercise promotes the release of VEGF and other pro-angiogenic factors, regardless of sex [101]. This upregulation may induce angiogenesis in skeletal muscle, increasing MVD and overall circulation.

Hormone Replacement Therapy

Hormone-based therapy is an emerging field of therapeutics for combatting cardiovascular complications. Varying doses of estrogen are often recommended to some premenopausal women as a preventative measure against future menopausal symptoms. Specifically, estrogen therapy is believed to reduce the risk of cardiovascular disease, breast cancer, impaired cognition, and osteoporosis [48]. Estrogen therapy has been demonstrated to significantly reduce body weight, BMI, visceral fat, apelin, and lipid profiles in ovariectomized rats [102]. Furthermore, several studies have reported that estrogen therapy decreases serum levels of both total and LDL cholesterol, while raising HDL and triglycerides, primarily by influencing the expression of hepatic apoprotein genes [48]. These results suggest that estradiol replacement therapy may help attenuate certain components of MetS.

However, over the last few years, observational studies have suggested that hormone-based therapy increases the risk for both deep vein thrombosis and embolism [103]. Studies have shown a two to fourfold increased risk for venous thromboembolic events in women taking estrogens [104]. On the other hand, recent research in male-to-female transitions indicates that long-term estrogen therapy appears to improve overall vascular function – indicating that lesser amounts of estrogen therapy may be beneficial to males. This suggests that the vasoprotective effects of estrogen attributed to endothelium-derived NO may be sex-independent [105].

In recent years, testosterone replacement therapy has been steadily increasing, but the relationship between circulating testosterone and various aspects of cardiovascular health still lacks clarity. A study examining the relationship between heart angina and testosterone therapy found that administering testosterone improved myocar-

dial ischemia in male patients with CAD by inducing arterial vasodilation. Both acute and chronic testosterone therapy showed improvements of myocardial ischemia, and there were no significant differences between intravenous or transdermal formulation of testosterone [106]. Other studies are aligned with these results, where intracoronary infusion of testosterone was found to dilate coronary arteries in men with CAD [107]. Other experimental studies suggest that the most probable mechanism by which testosterone acts on vascular smooth muscle cells is via modulation of non-ATP-sensitive potassium ion channels, calcium-activated potassium ion channels, voltage-sensitive potassium ion channels, and finally L-type calcium ion channels [108].

Testosterone replacement therapy has also shown potential in improving glycemic control. Several authors have demonstrated that testosterone therapy in diabetic men may help with decreasing fasting plasma glucose and homeostatic insulin resistance [109]. A common encounter in testosterone therapy involves an increase in hemoglobin levels, with the most recent meta-analysis study reporting a significant increase in hemoglobin and hematocrit levels, without significant risk for increased cardiovascular events [110].

Conclusions

The need for more sex-specific pathophysiologic analysis in focused studies on therapies such as exercise tolerance, endothelial function, and inflammatory response is more prominent now than ever. Translational and clinical research should continue to assess the potential use of hormone therapy in men and women in relation to cardiovascular risk profiles, while monitoring both short-term and long-term outcomes. The efficacy in various lifestyle interventions should also be further studied in conjunction with hormone status and/or therapy. Greater attention to individualized medicine is a crucial step for optimizing healthcare strategies. Our review demonstrates the urgent need for a better understanding of sex-related factors regarding MetS, its individual components, and interventions.

Statement of Ethics

This review manuscript contains no original data arising from animal or human studies.

Conflict of Interest Statement

The authors declare no conflict of interest.

Funding Sources

This work was supported by CIHR #389769 and NSERC RG-PIN-2018-05450.

Author Contributions

Each author has (1) made substantial contributions to the conception or design of the work or to the acquisition, analysis, or interpretation of the data for the work; (2) participated in drafting of the work or revising it critically for important intellectual content; (3) approved the final version to be published; and (4) agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

References

1 Serné EH, de Jongh RT, Eringa EC, Ijzerman RG, de Boer MP, Stehouwer CD. Microvascular dysfunction: causative role in the association between hypertension, insulin resistance and the metabolic syndrome? Essays Biochem. 2006;42:163–76.
2 Levy BI, Ambrosio G, Pries AR, Struijker-Boudier HA. Microcirculation in hypertension: a new target for treatment? Circulation. 2001;104(6):735–40.
3 Huxley VH, Kemp SS. Sex-specific characteristics of the microcirculation. Adv Exp Med Biol. 2018;1065:307–28.
4 Pittman RN. Regulation of tissue oxygenation. Morgan & Claypool Life Sciences; 2011.
5 Langsdorf LJ, Zydney AL. Diffusive and convective solute transport through hemodialysis membranes: a hydrodynamic analysis. J Biomed Mater Res. 1994;28(5):573–82.
6 Frisbee JC, Goodwill AG, Frisbee SJ, Butcher JT, Brock RW, Olfert IM, et al. Distinct temporal phases of microvascular rarefaction in skeletal muscle of obese Zucker rats. Am J Physiol Heart Circ Physiol. 2014;307(12):H1714–28.
7 Goligorsky MS. Microvascular rarefaction: the decline and fall of blood vessels. Organogenesis. 2010;6(1):1–10.
8 Frisbee JC. Remodeling of the skeletal muscle microcirculation increases resistance to perfusion in obese Zucker rats. Am J Physiol Heart Circ Physiol. 2003;285(1):H104–11.
9 Garland CJ, Hiley CR, Dora KA. EDHF: spreading the influence of the endothelium. Br J Pharmacol. 2011;164(3):839–52.
10 Struijker Boudier HA, le Noble JL, Messing MW, Huijberts MS, le Noble FA, van Essen H. The microcirculation and hypertension. J Hypertens Suppl. 1992;10(7):S147–56.
11 Antonios TF. Microvascular rarefaction in hypertension: reversal or over-correction by treatment? Am J Hypertens. 2006;19(5):484–5.
12 Liang J, Li Y, Chen L, Xia W, Wu G, Tong X, et al. Systemic microvascular rarefaction is correlated with dysfunction of late endothelial progenitor cells in mild hypertension: a Substudy of EXCAVATION-CHN1. J Transl Med. 2019;17(1):368. [published correction appears in: J Transl Med. 2020 Jan 16;18(1):26].

13 Serné EH, Gans RO, ter Maaten JC, Tangelder GJ, Donker AJ, Stehouwer CD. Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. Hypertension. 2001;38(2):238–42.
14 Fry BC, Roy TK, Secomb TW. Capillary recruitment in a theoretical model for blood flow regulation in heterogeneous microvessel networks. Physiol Rep. 2013;1(3):e00050.
15 Greene AS, Tonellato PJ, Lui J, Lombard JH, Cowley AW Jr. Microvascular rarefaction and tissue vascular resistance in hypertension. Am J Physiol. 1989;256(1 Pt 2):H126–31.
16 Schrijvers BF, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int. 2004;65(6):2003–17.
17 Bonner JS, Lantier L, Hasenour CM, James FD, Bracy DP, Wasserman DH. Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. Diabetes. 2013;62(2):572–80.
18 Feihl F, Liaudet L, Waeber B, Levy BI. Hypertension: a disease of the microcirculation? Hypertension. 2006;48(6):1012–7.
19 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation. 2002; 106(25):3143–421.
20 Patterson F, Zhang G, Davey A, Tan Y, Ma GX. American Heart Association's ideal cardiovascular health metrics in under-represented Asian Americans. J Community Health. 2016;41(6):1282–9.
21 Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009; 2(5–6):231–7.
22 Frisbee JC, Goodwill AG, Butcher JT, Olfert IM. Divergence between arterial perfusion and fatigue resistance in skeletal muscle in the metabolic syndrome. Exp Physiol. 2011; 96(3):369–83.
23 Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595–607.
24 Keske MA, Premilovac D, Bradley EA, Dwyer RM, Richards SM, Rattigan S. Muscle microvascular blood flow responses in insulin resistance and ageing. J Physiol. 2016;594(8): 2223–31.
25 Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes. 2011;60(10):2441–9.
26 Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19–39.
27 Alessi MC, Poggi M, Juhan-Vague I. Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. Curr Opin Lipidol. 2007; 18(3):240–5.
28 Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. Cardiovasc Ther. 2010;28(5):e72–91.
29 Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. Diabetes. 2005;54(10):2939–45.
30 Goyal R, Jialal I. Diabetes mellitus type 2. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2020 Nov 20.
31 Gudbjörnsdóttir S, Sjöstrand M, Strindberg L, Lönnroth P. Decreased muscle capillary permeability surface area in type 2 diabetic subjects. J Clin Endocrinol Metab. 2005;90(2): 1078–82.
32 Kibel A, Selthofer-Relatic K, Drenjancevic I, Bacun T, Bosnjak I, Kibel D, et al. Coronary microvascular dysfunction in diabetes mellitus. J Int Med Res. 2017;45(6):1901–29.
33 Picchi A, Capobianco S, Qiu T, Focardi M, Zou X, Cao JM, et al. Coronary microvascular dysfunction in diabetes mellitus: a review. World J Cardiol. 2010;2(11):377–90.
34 Kawasaki R, Cheung N, Wang JJ, Klein R, Klein BE, Cotch MF, et al. Retinal vessel diameters and risk of hypertension: the Multiethnic Study of Atherosclerosis. J Hypertens. 2009;27(12):2386–93.
35 Serné EH, Stehouwer CD, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJ, et al. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. Circulation. 1999;99(7):896–902.
36 Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2004;109(3):433–8.
37 Henrich HA, Romen W, Heimgärtner W, Hartung E, Bäumer F. Capillary rarefaction characteristic of the skeletal muscle of hypertensive patients. Klin Wochenschr. 1988; 66(2):54–60.
38 Ruggiero RJ, Likis FE. Estrogen: physiology, pharmacology, and formulations for replacement therapy. J Midwifery Womens Health. 2002;47(3):130–8.
39 Sharma G, Prossnitz ER. G-protein-coupled estrogen receptor (GPER) and sex-specific metabolic homeostasis. Adv Exp Med Biol. 2017;1043:427–53.
40 Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med. 2013; 19(3):197–209.
41 Hetemäki N, Mikkola TS, Tikkanen MJ, Wang F, Hämäläinen E, Turpeinen U, et al. Adipose tissue estrogen production and metabolism in premenopausal women. J Steroid Biochem Mol Biol. 2021;209:105849.
42 Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynecol. 2009;114(6):1326–31. [published correction appears in: Obstet Gynecol. 2010 Feb;115(2 Pt 1):387].
43 Corcoran JJ, Nicholson C, Sweeney M, Charnock JC, Robson SC, Westwood M, et al. Human uterine and placental arteries exhibit tissue-specific acute responses to 17β-estradiol and estrogen-receptor-specific agonists. Mol Hum Reprod. 2014;20(5):433–41.
44 Park YM, Erickson C, Bessesen D, Van Pelt RE, Cox-York K. Age- and menopause-related differences in subcutaneous adipose tissue estrogen receptor mRNA expression. Steroids. 2017;121:17–21.
45 Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, et al. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med. 1991;325(11): 756–62.
46 Papanek PE, Rieder MJ, Lombard JH, Greene AS. Gender-specific protection from microvessel rarefaction in female hypertensive rats. Am J Hypertens. 1998;11(8 Pt 1):998–1005.
47 Huang A, Sun D, Koller A, Kaley G. Gender difference in myogenic tone of rat arterioles is due to estrogen-induced, enhanced release of NO. Am J Physiol. 1997;272(4 Pt 2):H1804–9.
48 Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999;340(23):1801–11.
49 Chakrabarti S, Morton JS, Davidge ST. Mechanisms of estrogen effects on the endothelium: an overview. Can J Cardiol. 2014;30(7): 705–12.
50 Smiley DA, Khalil RA. Estrogenic compounds, estrogen receptors and vascular cell signaling in the aging blood vessels. Curr Med Chem. 2009;16(15):1863–87.
51 Sickinghe AA, Korporaal SJA, den Ruijter HM, Kessler EL. Estrogen contributions to microvascular dysfunction evolving to heart failure with preserved ejection fraction. Front Endocrinol. 2019;10:442.
52 White RE. Estrogen and vascular function. Vascul Pharmacol. 2002;38(2):73–80.
53 Minshall RD, Stanczyk FZ, Miyagawa K, Uchida B, Axthelm M, Novy M, et al. Ovarian steroid protection against coronary artery hyperreactivity in rhesus monkeys. J Clin Endocrinol Metab. 1998;83(2):649–59.
54 MacLusky NJ, McEwen BS. Progestin receptors in rat brain: distribution and properties of cytoplasmic progestin-binding sites. Endocrinology. 1980;106(1):192–202.
55 Thomas P, Pang Y. Membrane progesterone receptors: evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells. Neuroendocrinology. 2012;96(2):162–71.
56 Taube M, Höckenström T, Isaksson M, Lindgren PR, Bäckström T. Effects of sex steroids on survival and receptor expression in ovarian epithelial tumour cells. Int J Oncol. 2003; 22(6):1257–62.

57 Bikle DD, Halloran BP, Harris ST, Portale AA. Progestin antagonism of estrogen stimulated 1,25-dihydroxyvitamin D levels. J Clin Endocrinol Metab. 1992;75(2):519–23.
58 Rodriguez AC, Blanchard Z, Maurer KA, Gertz J. Estrogen signaling in endometrial cancer: a key oncogenic pathway with several open questions. Horm Cancer. 2019;10(2–3):51–63.
59 Miller VM, Vanhoutte PM. Progesterone and modulation of endothelium-dependent responses in canine coronary arteries. Am J Physiol. 1991;261(4 Pt 2):R1022–7.
60 Jiang C, Zuo F, Wang Y, Lu H, Yang Q, Wang J. Progesterone changes VEGF and BDNF expression and promotes neurogenesis after ischemic stroke. Mol Neurobiol. 2016. [published online ahead of print, 2016 Jan 8].
61 Kim J, Park J, Kim N, Park HY, Lim K. Inhibition of androgen receptor can decrease fat metabolism by decreasing carnitine palmitoyltransferase I levels in skeletal muscles of trained mice. Nutr Metab. 2019;16:82.
62 Handelsman DJ, Hirschberg AL, Bermon S. Circulating testosterone as the hormonal basis of sex differences in athletic performance. Endocr Rev. 2018;39(5):803–29.
63 Araujo AB, O'Donnell AB, Brambilla DJ, Simpson WB, Longcope C, Matsumoto AM, et al. Prevalence and incidence of androgen deficiency in middle-aged and older men: estimates from the Massachusetts Male Aging Study. J Clin Endocrinol Metab. 2004;89(12):5920–6.
64 Ishikawa T, Glidewell-Kenney C, Jameson JL. Aromatase-independent testosterone conversion into estrogenic steroids is inhibited by a 5 alpha-reductase inhibitor. J Steroid Biochem Mol Biol. 2006;98(2–3):133–8.
65 Rosano GM, Sheiban I, Massaro R, Pagnotta P, Marazzi G, Vitale C, et al. Low testosterone levels are associated with coronary artery disease in male patients with angina. Int J Impot Res. 2007;19(2):176–82.
66 Dobrzycki S, Serwatka W, Nadlewski S, Korecki J, Jackowski R, Paruk J, et al. An assessment of correlations between endogenous sex hormone levels and the extensiveness of coronary heart disease and the ejection fraction of the left ventricle in males. J Med Invest. 2003;50(3–4):162–9.
67 Ohlsson C, Barrett-Connor E, Bhasin S, Orwoll E, Labrie F, Karlsson MK, et al. High serum testosterone is associated with reduced risk of cardiovascular events in elderly men. The MrOS (Osteoporotic Fractures in Men) Study in Sweden. J Am Coll Cardiol. 2011;58(16):1674–81.
68 Daubresse JC, Meunier JC, Wilmotte J, Luyckx AS, Lefebvre PJ. Pituitary-testicular axis in diabetic men with and without sexual impotence. Diabete Metab. 1978;4(4):233–7.
69 Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, et al. Type 2 diabetes mellitus and testosterone: a Meta-Analysis Study. Int J Androl. 2011;34(6 Pt 1):528–40.
70 Laaksonen DE, Niskanen L, Punnonen K, Nyyssönen K, Tuomainen TP, Valkonen VP, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care. 2004;27(5):1036–41.
71 English HF, Drago JR, Santen RJ. Cellular response to androgen depletion and repletion in the rat ventral prostate: autoradiography and morphometric analysis. Prostate. 1985;7(1):41–51.
72 Franck-Lissbrant I, Häggström S, Damber JE, Bergh A. Testosterone stimulates angiogenesis and vascular regrowth in the ventral prostate in castrated adult rats. Endocrinology. 1998;139(2):451–6.
73 Dhindsa S, Prabhakar S, Sethi M, Bandyopadhyay A, Chaudhuri A, Dandona P. Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. J Clin Endocrinol Metab. 2004;89(11):5462–8.
74 Baylis C. Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. J Clin Invest. 1994;94(5):1823–9.
75 Hall JE. Neuroendocrine physiology of the early and late menopause. Endocrinol Metab Clin North Am. 2004;33(4):637–59.
76 Zhou Y, Shen L, Dong B, Liu C, Lv W, Chi J, et al. Elevated circulating luteinizing hormone levels are associated with diabetic macroalbuminuria in Chinese men and postmenopausal women: a Cross-Sectional Study. J Diabetes. 2020;12(11):819–33.
77 Trau HA, Davis JS, Duffy DM. Angiogenesis in the primate ovulatory follicle is stimulated by luteinizing hormone via prostaglandin E2. Biol Reprod. 2015;92(1):15.
78 Sandberg K, Ji H. Sex differences in primary hypertension. Biol Sex Differ. 2012;3(1):7.
79 Sadeghi M, Khalili M, Pourmoghaddas M, Talaei M. The correlation between blood pressure and hot flashes in menopausal women. ARYA Atheroscler. 2012;8(1):32–5.
80 Maas AH, Franke HR. Women's health in menopause with a focus on hypertension. Neth Heart J. 2009;17(2):68–72.
81 Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA. 2007;297(13):1465–77. [published correction appears in: JAMA. 2008 Mar 26;299(12):1426].
82 Maranon R, Reckelhoff JF. Sex and gender differences in control of blood pressure. Clin Sci. 2013;125(7):311–8.
83 Huxley VH, Wang J. Cardiovascular sex differences influencing microvascular exchange. Cardiovasc Res. 2010;87(2):230–42.
84 Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation. 1994;89(5):1943–50.
85 Forte P, Kneale BJ, Milne E, Chowienczyk PJ, Johnston A, Benjamin N, et al. Evidence for a difference in nitric oxide biosynthesis between healthy women and men. Hypertension. 1998;32(4):730–4.
86 Baylis C. Changes in renal hemodynamics and structure in the aging kidney; sexual dimorphism and the nitric oxide system. Exp Gerontol. 2005;40(4):271–8.
87 Erdely A, Greenfeld Z, Wagner L, Baylis C. Sexual dimorphism in the aging kidney: effects on injury and nitric oxide system. Kidney Int. 2003;63(3):1021–6.
88 Mendelsohn ME. Nongenomic, ER-mediated activation of endothelial nitric oxide synthase: how does it work? What does it mean? Circ Res. 2000;87(11):956–60.
89 Caulin-Glaser T, Watson CA, Pardi R, Bender JR. Effects of 17beta-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest. 1996;98(1):36–42.
90 Hou X, Pei F. Estradiol inhibits cytokine-induced expression of VCAM-1 and ICAM-1 in cultured human endothelial cells via AMPK/PPARα activation. Cell Biochem Biophys. 2015;72(3):709–17.
91 Gillis EE, Sasser JM, Sullivan JC. Endothelin, sex, and pregnancy: unique considerations for blood pressure control in females. Am J Physiol Regul Integr Comp Physiol. 2016;310(8):R691–6.
92 Lee S, Ko Y, Kwak C, Yim ES. Gender differences in metabolic syndrome components among the Korean 66-year-old population with metabolic syndrome. BMC Geriatr. 2016;16:27. [published correction appears in: BMC Geriatr. 2016;16:71].
93 Nauli AM, Matin S. Why do men accumulate abdominal visceral fat? Front Physiol. 2019;10:1486.
94 Galipeau D, Verma S, McNeill JH. Female rats are protected against fructose-induced changes in metabolism and blood pressure. Am J Physiol Heart Circ Physiol. 2002;283(6):H2478–84.
95 He J, Gu D, Chen J, Jaquish CE, Rao DC, Hixson JE, et al. Gender difference in blood pressure responses to dietary sodium intervention in the GenSalt Study. J Hypertens. 2009;27(1):48–54.
96 Gerdts E, Regitz-Zagrosek V. Sex differences in cardiometabolic disorders. Nat Med. 2019;25(11):1657–66.
97 Hunter SK. The relevance of sex differences in performance fatigability. Med Sci Sports Exerc. 2016;48(11):2247–56.
98 Williams RL, Wood LG, Collins CE, Callister R. Effectiveness of weight loss interventions – is there a difference between men and women: a systematic review. Obes Rev. 2015;16(2):171–86.
99 Stevens VJ, Corrigan SA, Obarzanek E, Bernauer E, Cook NR, Hebert P, et al. Weight loss intervention in phase 1 of the trials of hypertension prevention. The TOHP Collaborative Research Group. Arch Intern Med. 1993;153(7):849–58.

100 Ishikawa K, Ohta T, Zhang J, Hashimoto S, Tanaka H. Influence of age and gender on exercise training-induced blood pressure reduction in systemic hypertension. Am J Cardiol. 1999;84(2):192–6.
101 Gorski T, De Bock K. Metabolic regulation of exercise-induced angiogenesis. Vasc Biol. 2019;1(1):H1–8.
102 Babaei P, Dastras A, Tehrani BS, Pourali Roudbaneh S. The effect of estrogen replacement therapy on visceral fat, serum glucose, lipid profiles and apelin level in ovariectomized rats. J Menopausal Med. 2017;23(3):182–9.
103 Rousseau ME. Evidence-based practice in women's health: hormone therapy for women at menopause. J Midwifery Womens Health. 2001;46(3):167–80.
104 Varas-Lorenzo C, García-Rodríguez LA, Cattaruzzi C, Troncon MG, Agostinis L, Perez-Gutthann S. Hormone replacement therapy and the risk of hospitalization for venous thromboembolism: a Population-Based Study in Southern Europe. Am J Epidemiol. 1998;147(4):387–90.
105 New G, Timmins KL, Duffy SJ, Tran BT, O'Brien RC, Harper RW, et al. Long-term estrogen therapy improves vascular function in male to female transsexuals. J Am Coll Cardiol. 1997;29(7):1437–44.
106 English KM, Steeds RP, Jones TH, Diver MJ, Channer KS. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a Randomized, Double-Blind, Placebo-Controlled Study. Circulation. 2000;102(16):1906–11.
107 Webb CM, Adamson DL, de Zeigler D, Collins P. Effect of acute testosterone on myocardial ischemia in men with coronary artery disease. Am J Cardiol. 1999;83(3):437–A9.
108 O'Connor EK, Ivey JR, Bowles DK. Differential effects of androgens on coronary blood flow regulation and arteriolar diameter in intact and castrated swine. Biol Sex Differ. 2012;3(1):10.
109 Jones TH, Arver S, Behre HM, Buvat J, Meuleman E, Moncada I, et al. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 Study). Diabetes Care. 2011;34(4):828–37.
110 Fernández-Balsells MM, Murad MH, Lane M, Lampropulos JF, Albuquerque F, Mullan RJ, et al. Clinical review 1: adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2010;95(6):2560–75.