Published in final edited form as:

Curr Drug Metab. 2010 October ; 11(8): 693–714.

Impact of Sex Hormone Metabolism on the Vascular Effects of Menopausal Hormone Therapy in Cardiovascular Disease

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Abstract

Epidemiological studies have shown that cardiovascular disease (CVD) is less common in premenopausal women (Pre-MW) compared to men of the same age or postmenopausal women (Post-MW), suggesting cardiovascular benefits of estrogen. Estrogen receptors (ERs) have been identified in the vasculature, and experimental studies have demonstrated vasodilator effects of estrogen/ER on the endothelium, vascular smooth muscle (VSM) and extracellular matrix. Several natural and synthetic estrogenic preparations have been developed for relief of menopausal vasomotor symptoms. However, whether menopausal hormone therapy (MHT) is beneficial in postmenopausal CVD remains controversial. Despite reports of vascular benefits of MHT from observational and experimental studies, randomized clinical trials (RCTs), such as the Heart and Estrogen/progestin Replacement Study (HERS) and the Women's Health Initiative (WHI), have suggested that, contrary to expectations, MHT may increase the risk of CVD. These discrepancies could be due to age-related changes in sex hormone synthesis and metabolism, which would influence the effective dose of MHT and the sex hormone environment in Post-MW. Age-related changes in the vascular ER subtype, structure, expression, distribution, and post-ER signaling pathways in the endothelium and VSM, along with factors related to the design of RCTs, preexisting CVD condition, and structural changes in the blood vessels architecture have also been suggested as possible causes of MHT failure in CVD. Careful examination of these factors should help in identifying the causes of the changes in the vascular effects of estrogen with age. The sex hormone metabolic pathways, the active versus inactive estrogen metabolites, and their effects on vascular function, the mitochondria, the inflammatory process and angiogenesis should be further examined. Also, the genomic and non-genomic effects of estrogenic compounds should be viewed as integrated rather than discrete responses. The complex interactions between these factors highlight the importance of careful design of MHT RCTs, and the need of a more customized approach for each individual patient in order to enhance the vascular benefits of MHT in postmenopausal CVD.

Keywords

estrogen; phytoestrogens; estrogen receptor; endothelium; vascular smooth muscle; hypertension; progesterone; testosterone

INTRODUCTION

Cardiovascular disease (CVD), such as coronary artery disease (CAD) and hypertension (HTN), is more common in men than in premenopausal women (Pre-MW) of the same age,

suggesting cardiovascular benefits of estrogen [1–3]. Epidemiological studies have shown that death due to CAD is delayed by ~5 years in Pre-MW as compared to men [4]. Also, Pre-MW are 4–5 times less likely than men to have ischemic heart disease [5]. With aging the incidence of CVD becomes higher in women than in men. The increased risk of CVD in postmenopausal women (Post-MW) has been linked to the decrease in plasma estrogen levels, thus prompting further investigation of the effects of estrogen on the cardiovascular system.

Estrogen is the predominant sex hormone in women, affecting the development and function of the reproductive system, the uterus and mammary glands. In Pre-MW, estrogen is primarily synthesized in the ovaries and carried in the blood mainly by sex hormone binding globulin (SHBG), and with less affinity by albumin. Estrogen is commonly used as a contraceptive, and is a major component of menopausal hormone therapy (MHT) administered after naturally occurring menopause or following surgical menopause in women undergoing bilateral oophorectomy [6]. Premarin, a conjugated equine estrogen (CEE), was the first preparation approved by the Food and Drug Administration for treatment of menopausal symptoms such as hot flashes, night sweats, vaginal dryness and atrophy. Many estrogen preparations are now available for treatment of distressing menopausal symptoms, and for alleviating osteoporosis [6].

Estrogen produces its effects by binding to estrogen receptors (ERs) in the reproductive system, bone, brain, adipose tissue and musculoskeletal system. ERs have also been identified in numerous vascular beds of humans and experimental animals [7]. Experimental studies have demonstrated vasodilator effects of estrogen on endothelial cells (ECs), vascular smooth muscle (VSM) and extracellular matrix (ECM) via both genomic and non-genomic pathways [3,7]. Effects of estrogen on the inflammatory process, oxidative stress, and angiogenesis have also been reported [8]. Estrogen is also associated with white adipose tissue mass with consequent changes in circulating lipid levels and inflammatory cytokines, pointing to another mechanism by which estrogen might benefit vascular health [9–11].

Despite the ample experimental evidence of vascular benefits of estrogen, randomized clinical trials (RCTs) such as the Heart and Estrogen/progestin Replacement Study (HERS), HERS II, and Women's Health Initiative (WHI) have suggested that, contrary to expectations, MHT may increase the risk of thromboembolism and CVD in Post-MW. The purpose of this review is to discuss reports published in the Pubmed database on how changes in estrogen metabolism could modify its effects on ECs and VSM and consequently contribute to the failure of MHT in CVD. The various pathways of estrogen synthesis, metabolism and pharmacokinetics and the changes in these pathways with age will be described. The role of the estrogen forms, dosage, route of administration and metabolites will be discussed. The efficiency of natural, conjugated, and semi-synthetic estrogens, phytoestrogens, selective estrogen receptor modulators (SERMs), and selective ER agonists will be compared [3]. The age-related changes in the vascular ER subtype, amount, structure and post-ER signaling in ECs and VSM will also be highlighted. The interaction of estrogen with other sex hormones such as progesterone and testosterone and how this may alter the effects of administered estrogen will also be considered. Lastly, selected MHT RCTs will be discussed in order to identify the potential factors responsible for MHT failure and how these factors are being considered in more recent RCTs so as to enhance the benefits of MHT in postmenopausal CVD.

SYNTHESIS OF ESTROGENS

In Pre-MW, natural estrogens are synthesized in the ovaries from cholesterol. Estrogens have the basic ring structure of all steroids, i.e. the cyclopentanoperhydrophenanthrene

nucleus (Fig. 1). Natural estrogens include estrone (E1), estradiol (E2) and estriol (E3) [2]. They have 4 rings A, B, C, and D, a hydroxyl group (OH) at C3, and either an OH or a ketone group at C17 [12] (Fig. 1). The critical moiety in estrogenic compounds is the 3-OH A ring. In Pre-MW E2 is the predominant form and has maximal estrogenic activity, whereas in Post-MW the bulk of estrogen is in the form of E1 and E1-sulfate. E1 is weaker than E2 because it is devoid of OH group at the 17 β position, and its estrogenic potency is mainly due to its conversion to E2. E3 is a weak estrogen that binds to ER for a short period of time [13].

While the ovary is the primary source of circulating E2 in Pre-MW, most of E1 and E3 is formed in the liver from E2 or in peripheral tissues from androstenedione [14] (Fig. 2). Steroidogenic enzymes involved in estrogen synthesis are also localized in the smooth endoplasmic reticulum of testicular Sertoli and Leydig cells, adipose stroma, preimplantation blastocysts, and the brain [15]. During pregnancy, the fetal adrenal glands, a component of fetoplacental steroid biosynthesis, produce dehydroepiandrosterone sulfate (S-DHEA), which is transformed into 16 α -hydroxydehydroepiandrosterone sulfate (S-16 α -OH-DHEA) and further processed in the fetal liver and desulfated in the placenta to 16 α -OH-DHEA, then aromatized in the syncytiotrophoblasts to E3 (Fig. 3) [14].

In Post-MW the major precursors of estrogen in peripheral tissues are circulating androstenedione, testosterone and E1. The androstenedione produced in the ovaries and adrenal glands is transformed into estrogens in peripheral tissues with aromatase activity including blood vessels [15] (Fig. 2). Polymorphisms in the genes encoding steroidogenic enzymes could influence estrogen production, and careful evaluation of these polymorphisms may help in the design of a more individualized MHT approach in Post-MW [8,16] (Table 1). Polymorphisms in genes encoding other enzymes or hormones may also influence the effects of estrogen in aging women. For instance, in Post-MW, the angiotensin-converting enzyme (ACE) Insertion/Deletion (I/D) polymorphism may be associated with endothelial dysfunction [17], and the influence of this polymorphism on the effects of estrogen on the endothelium needs to be studied.

SERUM LEVELS OF ESTROGENS

There is a considerable inter-individual variability among Pre-MW in estrogen synthesis and metabolism, the duration of the various phases of the ovarian cycles, and the serum levels of sex steroids. In normally cycling women, the ovarian follicle secretes 70 to 500 μ g E2 per day, producing plasma estrogen levels of 210 pmol/L (58 pg/mL) in early follicular, 720 pmol/L (196 pg/mL) in late follicular and 490 pmol/L (129 pg/mL) in late luteal phase. In menopausal women, ovarian production of E2 is markedly diminished and the plasma estrogen level is reduced to <100 pmol/L (0 to 60 pg/mL), mostly in the form of E1 (Table 2) [13,18]. Likewise, owing to the large differences in the rates of estrogen synthesis, absorption and metabolism among MHT users, there are marked inter-individual variations in the time course and the serum levels of sex steroids in Post-MW treated with the same estrogen preparation. The inter-individual variations may involve genetic or acquired differences in the intestinal absorption and hepatic metabolism. Intra-individual day to day variations may be caused by external factors such as diet, alcohol or drug consumption, smoking, physical activity and stress, which may cause rapid transient changes in the peripheral or splanchnic blood flow, absorption and metabolism. Certain diseases such as disorders of the thyroid gland may also affect estrogen metabolism [13].

MHT PREPARATIONS and ROUTE OF ADMINISTRATION

Various MHT options are available for treatment of vasomotor symptoms using different routes of administration including oral, topical, transdermal patches, injection, and vaginal

preparations (Table 3). Estrogen preparations vary in their metabolism, vascular effects, and their efficiency as MHT.

Oral estrogen is convenient, non-invasive and reversible, but has low bioavailability because of its hepatic degradation. The oral route affects the estrogen-dependent hepatic enzymes and causes increases in serum levels of SHBG, cortisol-binding globulin, thyroxine-binding globulin, angiotensinogen, high-density lipoprotein cholesterol (HDL-c), triglycerides and apolipoprotein A, and a decrease in low-density lipoprotein cholesterol level (LDL-c) [13]. Oral estrogen may have prothrombotic effects and increase thrombin generation, resistance to activated protein C, inflammatory responses, and C-reactive protein (CRP) levels [21].

Topical estrogen emulsions are highly lipophilic preparations that are absorbed through the skin and remain localized to the tissue that they are applied to. Also, vaginal MHT pessaries allow estrogen to act mainly on the reproductive system, and hence have few effects on the liver and vascular tissues. Although intended for localized effects, some of the estrogen in the topical emulsions may reach the systemic circulation. While the beneficial effects of estrogen on the lipid profile are usually lost in the topical preparations, they may decrease the risk of thrombotic events.

Transdermal estrogen patches also have vasodilator benefits, but with less risk of increased vascular inflammation and atherosclerosis than oral preparations. Another advantage of transdermal estrogen over the oral and topical routes is that it provides a controlled release of estrogen into the circulation. However, transdermal estrogens may cause more irregular and breakthrough bleeding over the course of sequential and continuous therapies than their oral counterparts, and therefore require individual adjustment and continuous monitoring in order to maximize benefits and reduce side-effects [22]. Thus, both oral and transdermal MHT may reduce the development of atherosclerosis in Post-MW, provided that the endothelium is functionally intact. However, for some parameters such as lipid profile, lipoproteins, glucose and insulin metabolism there are greater benefits from oral estrogen, while for other parameters such as hemostatic changes and effects on CRP there are more advantages from transdermal estrogen. Patients with lipid and lipoprotein abnormalities and impaired glucose tolerance may have more benefits from oral estrogen. On the other hand, the risk of deep venous thrombosis or pulmonary thromboembolism is less with transdermal preparations [23]. Recent studies also suggest potential advantages of the non-oral estrogens in the control of blood pressure [24]. However, for the majority of patients who have none of these risk factors it may come down to personal preference [22].

MHT DOSE

Due to the decrease in plasma levels of estrogen during menopause, MHT was originally designed to restore plasma estrogen to the levels observed in Pre-MW. However, estrogens are highly lipophilic compounds, and their levels in the plasma may not reflect those in the vascular tissue. Also, the plasma levels of estrogen are highly variable among individuals due to different pharmacokinetic factors. The hormone pharmacokinetics and volume of distribution may change even further during menopause, particularly in women with liver or kidney disease. Thus, an estrogen dose that may mimic the estrogen levels in Pre-MW could result in excessive plasma levels and produce procarcinogenic effects in Post-MW. The effective dose of estrogen may also be affected by the cytokine milieu. For example, interleukin-1 β may affect the expression of enzymes that influence the local production of E2 by inhibiting sulfatase and stimulating the expression of estrogen sulfotransferase [25].

Observational studies have suggested that a lower oral dose of MHT (0.3125 mg CEE) may be better than the original dose that used to be given to approximate the plasma estrogen levels in pre-MW (0.625 mg CEE). While both doses may have the same effect on

lipoproteins, flow-mediated vasodilation and plasma levels of plasminogen activator inhibitor-1 (PAI-1), the lower dose does not increase IL-6, CRP or prothrombin fragment 1+2, and causes less reduction in anti-thrombin III than the conventional dose [26,27].

MHT PHARMACOKINETICS

Estrogen Absorption

Oral E2 is often used in a microcrystalline form in order to increase the compound surface, and its absorption and bioavailability [13]. Esterification of the C17 OH group of E2 with valeric acid to produce E2-valerate could decrease its metabolism to E1. E2-valerate is hydrolyzed in the intestinal tract and the resulting E2 is rapidly absorbed. Therefore, after oral administration of micronized E2 or E2-valerate, the pharmacokinetics of E2 is similar and the bioavailability is about 5% [22]. By definition, topical estrogens are designed to produce localized effects, but a fraction may be absorbed into the blood stream and produce systemic effects.

Estrogen Metabolism

Estrogens are largely transformed into less active metabolites that are excreted in urine and feces. Estrogen metabolism includes oxidation (hydroxylation) by cytochrome P450s (CYPs), glucuronidation by UDP-glucuronosyltransferase, sulfation by sulfotransferase, oxidation by peroxidases followed by reduction by NADPH reductase, and O-methylation by catechol O-methyltransferase (COMT). While estrogen metabolism occurs mainly in the liver, where most CYPs are expressed, it also occurs in the uterus, breast, kidney, brain, and pituitary [28]. There are two main pathways of oxidative metabolism: 1) the hydroxylation of ring A at C2 or C4 results in the formation of catechol-estrogens hydroxyl metabolites, which are further converted to methoxy metabolites by COMT, and 2) the hydroxylation of C16 α of ring D leads to 16 α -OH-E1 which is converted by 17 β -hydroxysteroid dehydrogenase (17 β -HSD) into E3 (Fig. 4). In healthy subjects, ring A metabolism outweighs ring D metabolism, whereas in overweight individuals ring D metabolism is enhanced [3]. Also, normally, the concentration of 2-OH metabolites is higher than that of 4-OH metabolites [13]. Estrogens are also oxidized and hydroxylated in ring B at C6 α , C6 β , C7 α and C7 β , in ring C at C11 β and C14 α , in ring D at C15 α , and at the angular C18 methyl group [13,28].

Conjugation of estrogens by intestinal and hepatic enzymes gives rise to E1- and E2-sulfates, which are found in the plasma, and E1- and E2-glucuronides, which are the primary forms excreted in bile and urine. The estrogen conjugates in the bile are extensively hydrolyzed in the colon by bacterial enzymes and undergo enterohepatic circulation [13,28].

As part of a metabolic redox system, the 2- and 4-OH-estrogens can be converted to reactive semiquinones and quinones, which may be carcinogenic (Fig. 4) [28]. The 2- and 4-methoxy-estrogens are also transformed into quinones.

E2 metabolism depends on the stage of the menstrual cycle, menopausal status, ethnic background, genetic polymorphisms, drugs and environmental factors [14,24]. Both the catechol-estrogens and their methoxy-derivatives exert considerable estrogenic effects, but the catechol-estrogens are more active than the methoxy-derivatives [13]. The hydroxylated metabolites of E1 and E2 have distinct ring positions and different estrogenic activity. 2-OH-E1 has weak estrogenic activity whereas 4-OH-E1 and 16 α -OH-E1 have strong estrogenic activity [29]. The serum levels, the ER binding and the vascular effects of these metabolites have not been fully characterized. Also, the changes in the effects of these metabolites with aging need to be further examined. 2-OH-E1 and 2-OH-E2 have very low concentrations in the systemic circulation as compared to 16 α -OH-E1 [30], probably due to

rapid conjugative metabolism (O-methylation, glucuronidation, sulfonation) followed by urinary excretion. For example, methoxy-E2s are derived by further metabolism of 2-OH-E2 and 4-OH-E2 by COMT. Both 2-OH-E1 and 2-OH-E2 bind to ERs, but with reduced affinity [30].

4-Hydroxylation is a dominant pathway for catechol-estrogen formation in several extrahepatic target tissues. For example, E2 4-hydroxylase activity is expressed in rat pituitary [31] and in the human uterus [32]. Selective expression of E2 4-hydroxylase activity in target cells does not inactivate the parent estrogen, but may be a mechanism for maintaining strong hormonal activity in these cells or for exerting other unknown biological effects that are not shared with E2. 4-OH-E2 is similar to E2 in its ability to bind to and activate ERs [33]. Interestingly, the interaction of 4-OH-E2 with ER occurs with a reduced dissociation rate and therefore may last longer than E2. 4-OH-E2 has stronger activity than E2 in inducing progesterone receptor formation in the rat pituitary, whereas 4-OH-E2 and E2 induce similar expression of nuclear ER. 4-OH-E2 also serves as a co-oxidant, and strongly stimulates the metabolic co-oxidation of arachidonic acid to prostaglandins in the uterus during pregnancy [34]. 16 α -OH-E2, like 4-OH-E2, retains potent hormonal activity on ERs [35].

Some studies have suggested that the concentration of 2-OH-E1 and 16 α -OH-E1 in urine may reflect the activity of the 2- and 16 α -hydroxylation pathways and that the ratio of the two metabolites could be a good predictor of systolic blood pressure [36]. However, these findings can not be generalized, as there may be heterogeneity in the population. Importantly, after adjustments are made for age and menopausal status, only race could be a predictor of 2-OH-E1/16 α -OH-E1 ratio, and hence systolic blood pressure, as the 2-OH-E1 levels are significantly higher during the follicular phase and mid-cycle among African-American compared with Caucasian women [37,38].

Thus, the gonadal steroid hormone metabolism allows extensive inter-conversion of sex hormone precursors and metabolites, dynamic inter-conversion among E2, E1 and the inactive conjugates E2-sulfate and E1-sulfate [3,13]. The effect of aging on the metabolism of endogenous and exogenous estrogen needs further elucidation. Also, the amount and activity of steroid-metabolizing enzymes may need to be carefully evaluated in Post-MW in order to determine the cause of loss of vascular benefits of MHT.

Estrogen Transport

The majority of the circulating hormones are bound to serum proteins. In women, about 37% of E2 is bound with high affinity to SHBG and 61% with low affinity to albumin, while only 2% is free. Both the free and albumin-bound fractions are biologically active, but are also subject to metabolism [13]. The half-life of E2 and E1 is 20 to 30 min [13]. Estrogen metabolites also bind to serum proteins at variable rates. While the 2-OH metabolite of estrogen has little affinity for SHBG, 2-methoxy-E2 has more than twice the affinity for SHBG when compared to E2 [39]. Also, 99% of the E1-sulfate conjugate is bound with a relatively high affinity to albumin, which gives it a relatively long half-life of 10 to 12 hours [13].

Estrogen Excretion

Estrogen is excreted mainly by metabolic conversion to hormonally inactive or less active water-soluble metabolites in the urine and/or feces [28]. The major estrogen metabolites excreted in urine are the 2-hydroxy products 2-OH-E1 and 2-OH-E2, 2-methoxy-E1, non-metabolized E1 and 16 α -hydroxy products (16 α -OH-E1 and 16 α -OH-E2) (Fig. 4) [40]. The

relative excretion of estrogen metabolites in urine versus feces could be helpful in predicting the effect of renal or hepatic impairment on the estrogen levels.

OTHER COMPOUNDS WITH ESTROGENIC ACTIVITY

Conjugated Equine Estrogens (CEE) and Semi-Synthetic Estrogens

CEE are derived from the urine of pregnant mares, and are a complex formulation containing multiple estrogens that are not secreted by the human ovary, in addition to other biologically active steroids [41]. The first form of CEE to be used was Premarin, which consists of E1-sulfate, equilin and equilenin. Equilin and equilenin have one or two additional double bonds in ring B, which increase their estrogenic activity [13]. Although the balance of the inter-conversion between E2 and its metabolites is largely shifted to E2- and E1-sulfate, the balance between dihydroequilin-17 β or dihydroequilenin-17 β and its respective metabolites is nearly equal, resulting in a higher hormonal activity of the equine preparations [42]. Also, MHT with CEE may cause unanticipated decrease in free E2. During oral treatment with 1 mg E2 or 0.625 mg CEE, the serum levels of SHBG are increased by 50% and 100%, and free E2 decreases from 1.3% to 1.2% and 1.0%, respectively [17]. Since equilin and dihydroequilin-17 β bind to SHBG and albumin with lower affinity, their clearance is much faster than that of the respective sulfates [13]; however, other pharmacokinetic factors could also be involved.

Chemical alterations in natural estrogens to produce semi-synthetic estrogens may increase their oral effectiveness. For example, ethinyl substitution at C17 to produce ethinyl-E2 minimizes the first-pass hepatic metabolism. Ethinyl-E2 is more active than natural estrogens because the 17 α -ethinyl group prevents the oxidation of the 17 β -OH group, and is able, after the oxidative formation of a very reactive intermediate, to irreversibly inhibit CYPs.

Synthetic estrogens include diethylstilbestrol, chlorotrianisene, dienestrol, fosfestrol, mestranol, poly-E2 phosphate and quinestrol [3]. Their structure allows them to bind to ERs, but their inability to fit exactly in the ER ligand-binding domain prevents the correct interaction necessary to exert the same biological effects as E2. Esters like E2-valerate, E3-succinate, or ethinyl-E2 sulfonate, and ethers like mestranol or quinestrol are readily hydrolyzed to the active hormones and have short half-lives. Diethylstilbestrol is a potent estrogen that has a stiff plane with two hydroxyl groups in a distance similar to that of E2 [13], allowing its binding to ER.

CEEs and synthetic estrogens might not mimic E2 to the fullest capacity because the cardiovascular effects of E2 are mediated via both ER-dependent and ER-independent mechanisms. Importantly, equine estrogens may actually impair nitric oxide (NO) production and endothelial NO synthase (eNOS) transcription in human endothelial cells (ECs), and consequently may be less beneficial than natural 17 β -E2, which enhances vasodilation and NO release [43]. Also, the metabolism of synthetic estrogens has not been thoroughly explored to fully comprehend their effects on the vasculature of Post-MW.

Phytoestrogens

Phytoestrogens are polyphenolic non-steroidal compounds synthesized by plants and possess estrogenic activity [3]. They are found in a variety of foods and plants particularly in soybeans, red clover, and wheat grains [44]. The major phytoestrogens are classified into flavonoids, stilbenoids, and lignans (Fig. 5). The flavonoids are further divided into major flavonoids (flavones, flavonols and flavanones), and isoflavonoids (isoflavones and coumestanes). Stilbenoids include stilbenes such as resveratrol, which is found in red wine and peanuts, but only its trans form has estrogenic activity. Plant lignans include

secoisolariciresinol and matairesinol, which are not estrogenic by themselves. Secoisolariciresinol is converted by intestinal microflora to enterodiol and subsequently to enterolactone. Matairesinol is converted directly to enterolactone. Enterodiol and enterolactone, also called enterolignans, are phytoestrogens with structural similarity to endogenous estrogens. Dietary sources of lignans include flaxseed, whole grain bread, vegetables, and tea [44]. Mycoestrogens include resorcylic acid lactone compounds such as zearalenone, α -zearalenol and their derivatives. They have estrogenic effects and are not found in food plants, but are secondary mold metabolites of fungal species, and therefore are called mycoestrogens [45], although some reports classify them as phytoestrogens [46].

Xenoestrogens

Xenoestrogens, or estrogen mimics, are compounds with variable estrogenic activity that are present in our surroundings and are ingested or inhaled. They are found in food, pesticides (methoxychlor, endosulfan, lindane), herbicides (atrazine), polychlorinated biphenyls (DDT, dieldrin, heptachlor), and plastics. These compounds are highly fat-soluble, not biodegradable or well-excreted, and accumulate in the adipose tissue of humans and animals. They bind to ERs in both females and males, and interfere with the natural biochemical and hormonal activity. Accumulation of xenoestrogens in the body with age may contribute to the adverse effects seen in MHT users, highlighting the importance of customizing MHT depending on the individual's past and current exposure to xenoestrogens.

Selective Estrogen Receptor Modulators (SERMs)

SERMs such as raloxifene, toremifene, tamoxifen, clomifene and idoxifene are non-steroidal molecules that bind with high affinity to ERs, but have distinct effects depending on the drug's structure and target tissue (Fig. 5) [47]. PhytoSERMs such as Femarelle® (DT56a) are botanically-derived substances that may have beneficial effects similar to those of synthetic SERMs [48]. SERMs range in activity from purely estrogenic and purely anti-estrogenic to combined partial estrogenic in some tissues and anti-estrogenic or no activity in other tissues [49]. For some SERMs, the agonist/antagonist activity and tissue selectivity may be related to the ratio of co-activator/co-repressor proteins in the target cells, and the ER conformation induced by drug binding. This, in turn, determines how strongly the drug/receptor complex recruits co-activators, resulting in agonism, as compared to co-repressors, which result in antagonism [3]. While E2 induce a conformation of the ER ligand-binding domain and promote co-activator binding, the bulky side chains of SERMs may prevent the ligand-induced ER conformation [50]. By blocking the ER transcriptional activation function-2 (AF-2), SERMs may act as antagonists in cells that depend mainly on this site for activity. However, in tissues where the other transcriptional activation function AF-1 is active, SERMs may act as agonists. Compounds with no phenolic OH group (e.g. tamoxifen) are prodrugs that are converted to the active ER agonist or antagonist (e.g. 4-OH-tamoxifen) in the intestinal tract and liver.

Selective ER agonists and antagonists

Agonists such as propylpyrazole triol (PPT, 410-fold selectivity for ER α over ER β), diarylpropionitrile (DPN, selective for ER β) and G1 (selective for GPR30 or GPER), and antagonists such as methyl-piperidinopyrazole (MPP, selective for ER α) act selectively on certain ERs, while fulvestrant (ICI 182,780) is a non-selective ER antagonist (Fig. 5).

ESTROGEN RECEPTOR (ER)

There are four distinct ERs: two ligand-activated transcription factors (ER α and ER β), one G-protein coupled receptor (GPR30 or GPER) [3,8], and a less defined ER-X in the brain

[51]. The two ERs most often described in the vasculature are ER α and ER β [7]. ER α and ER β are members of the nuclear receptor superfamily which includes the thyroid hormone, retinoic acid and vitamin D receptors [52]. The ESR1 gene which encodes ER α is located on chromosome 6q(25.1), while the ESR2 gene which encodes ER β is located on 14q(23–24.1) [12].

Like all steroid hormone receptors, ERs are organized into domains A-B, C, D, E, and F (Fig. 6). The A-B domain contains a ligand-independent transcriptional activation function-1 (AF-1). Domain C is the DNA-binding domain (DBD) and contains two zinc fingers that are highly conserved in all steroid hormone receptors. Domain D is the hinge region, and has significant amino acid variability among ER subtypes. Domain E contains the ligand-binding domain (LBD) and the hormone-dependent transcriptional activation function-2 (AF-2) [3]. The LBD has twelve helices (H1-H12), folded into a three-layered anti-parallel α -helical sandwich comprising a central core layer of three α -helices (H5–6, H9, and H10) sandwiched between two additional layers of α -helices (H1–4) and (H7, H8, and H11). This helical arrangement creates a ‘wedge-shaped’ molecular scaffold that maintains a sizeable ligand-binding cavity at the narrower end of the domain, into which the hormone binds [53]. Domain F is a variable region that includes the sequence for H12 of ER, and may account for the difference in the ER response to E2 and SERMS [54]. ER β is highly homologous with ER α in the DBD (95 % homology) and LBD (55 % homology), but has a unique A-B domain. GPER is structurally dissimilar to ER α and ER β , but binds to E2 with high affinity and low capacity at the plasma membrane, endoplasmic reticulum, Golgi apparatus and nuclear membrane [55–58]. The molecular weights of ER α , ER β and GPER are 45–66, 53–59 and 40 kDa, respectively [59–61].

ER Polymorphisms

Polymorphisms in ER genes may account for differences in ER function and may modify the outcome of MHT. A number of polymorphic sites of both ER α and ER β gene loci have been identified in humans [50,62], and may affect vascular health in males and females.

For ER β , two tightly linked polymorphisms may affect the risk of myocardial infarction in women, with the rs1271572 variant T allele associated with increased risk and the rs1256049 variant associated with decreased risk [63]. No significant relationship was found in men. On the other hand, the community-based Framingham Heart Study offspring cohort revealed that the rs944460 polymorphism of ER β may affect pulse pressure in men, but not in women [64]. Some polymorphisms of ER β , however, may have an effect that is independent of gender. A case-control study conducted in Brazil has revealed that the rs4986938 polymorphism of ER β shows a higher frequency in both male and female patients with premature CAD [65].

For ER α , several polymorphisms such as IVSI-401 polymorphism C/C genotype have been associated with enhanced effects of MHT to increase the levels of HDL-c in Post-MW [66]. However, studies have not been consistent in showing these relationships because of several confounding factors such as menopausal status and the use of contraceptive pills and MHT, making it difficult to interpret the data [67]. ER α polymorphisms have also been associated with the risk of myocardial infarction and stroke in men [67–69]. Conversely, a polymorphism in the promoter region of ER α is reported to be associated with augmentation of endothelial-dependent vasorelaxation and reduction in LDL cholesterol [70].

ER Expression

The decrease in the protective effects of estrogen with age may be due to decreased number of ERs in the arterial wall [71,72]. We have previously shown no significant difference in

ER expression in the aorta of aging and adult ovariectomized spontaneously hypertensive rats (SHR), and suggested that age-related decrease in the vascular effects of estrogen may not only be related to decreased amount of ERs, but also to changes in the post-ER signaling mechanisms [73]. However, these studies used only E2 as an ER agonist, and studies with other estrogenic compounds and metabolites need to be conducted.

Studies on human right atrium, aorta, internal mammary artery, saphenous vein, coronary atherectomy samples, as well as cultured aortic VSM cells have suggested that methylation of ER α gene is associated with atherosclerosis and increased proliferation of aortic VSM cells. Also, methylation of ER α gene could lead to decreased expression and responsiveness of ER α in the vascular system with aging [72,74].

ER expression is reduced in VSM cells of women with severe compared to milder cases of atherosclerosis [75]. Since atherosclerosis damages the arterial wall, it could also damage and down-regulate ERs in the vasculature of Post-MW. Low ER expression in the aorta of Post-MW and decreased activity of MHT may promote progression of atherogenesis, leading to a vicious cycle of ER disruption and worsening of atherosclerosis.

ER expression may also be dependent on the duration of estrogen treatment. In cultured ovine ECs, short-term treatment (2 hr) with E2 down-regulated ER expression. Longer exposure to E2 for 6 hr increased the expression of ER α , but down-regulated ER β [76]. Also, chronic *in vivo* treatment with physiological levels of E2 upregulated ER α protein in cerebral blood vessels [60]. The regulation of ER expression by estrogen could be helpful in the interpretation of the cardiovascular effects of MHT.

ER Distribution

ER α is expressed in the uterus, vagina, ovaries, mammary gland, and hypothalamus [77]. ER β is expressed highly in the ovaries and prostate, with smaller amounts in lung, brain, and bone [8]. ERs are also widely expressed in the vascular system, specifically in ECs and VSM of humans and experimental animals [7,78,79]. Although both ER α and ER β are expressed in ECs and VSM, some studies suggest that ER β predominates in ECs, whereas ER α expression is greater in VSM [7]. GPER is widely distributed in the brain and peripheral tissues [58]. GPER has also been found in human internal mammary arteries and saphenous veins [58,80], but its functional role needs further investigation. GPER may function in concert with ER α , perhaps serving to assemble a complex essential for rapid estrogen signaling [81].

EFFECTS OF ESTROGEN

Genomic Effects

Estrogen induces genomic responses through effects on gene transcription. Estrogen binds to cytosolic ER, which in turn dissociates from heat shock protein 90 (HSP-90) (Fig. 7). The estrogen-ER complex translocates to the nucleus, where it undergoes homodimerization or heterodimerization [82], and binds to the estrogen receptor elements (ERE) [83]. Regions other than EREs on the genome such as the estrogen responsive sequence TGF- α may also respond to estrogen, but do so with less affinity [84].

The interaction of a ligand with ER initiates the recruitment and binding of several proteins, facilitating the binding of the ER complex to specific DNA sequences, and thereby upregulating transcription of their target genes [7]. The transcriptional responses following ligand activation of nuclear receptors, including ERs, are context specific [85]. The ER-associated proteins provide a first level of specificity. A second level of target tissue specificity is provided by cis-acting elements specific to the genes that are regulated by

estrogen [7]. These specific mechanisms may be altered with aging. For instance, age may alter the balance of ER and NF- κ B which share co-activator proteins such as p300 [86]. NF- κ B is a transcription factor with a p65 protein component that plays a role in regulating the immune response to infection. In coronary artery VSM cells, an increase in p300 levels with aging may reduce the ability of estrogen to repress NF- κ B p65 transcriptional activation of inflammatory genes [86].

E2 may increase the expression of proliferative transcription factors such as *fos*, *myc* and *jun*. E2 may also augment the expression of Egr-1, which serves a protective function in response to acute and chronic vascular injury, and the expression of COX-2 gene, which is involved in the synthesis of the vasodilator prostacyclin (Fig. 7) [87].

Non-Genomic Effects

Estrogen induces rapid vasodilation that occurs within minutes and does not involve gene expression. Acute administration of estrogen in female or male patients improves vasodilatory responses and ameliorates myocardial ischemia [88,89]. Also, studies on dogs *in vivo* and on isolated rat and rabbit hearts have shown that acute treatment with estrogen lowers coronary vascular resistance and enhances coronary blood flow [90,91]. The rapid vasodilator effects of estrogen are mediated via ER α and ER β , but could also involve GPER (Fig. 8).

Integration between genomic and non-genomic ER-Mediated mechanisms

Estrogen may induce post-transcriptional modification (RNA stability) and post-translational modulation of proteins. The amount of mRNA in a cell represents the sum of its gene transcription and degradation. Increased mRNA stability prolongs the expression of a gene and the production of the corresponding protein. Estrogen, by transcriptional regulation of its mRNA stabilizing factor, may autoregulate the mRNA stability of its own ER in some tissues [92–94]. Also, SERMS may have different efficacy due to differences in the ability of the SERM-bound receptor complex to alter ER expression [8].

Estrogen-induced regulation of enzymes that affect the biological half-life of other enzymes, co-factors or receptors may represent another integrated mechanism by which estrogen influences vascular responses to cytokines, hormones or environmental stimuli such as hypoxia [8]. Importantly, estrogen-induced membrane-initiated cell signaling (non-genomic) may potentiate nuclear-initiated estrogen signaling (genomic) [8,87]. Ca²⁺ channels and a number of kinase cascades may be involved in this transcriptional potentiation. For instance, estrogen acutely increases Ca²⁺ concentration in ECs and NO release that leads to relaxation of vascular rings, but could also lead to inhibition of VSM cell proliferation [8]. The net effects of estrogen on the vasculature represent its combined effects on ECs, VSM, and ECM.

Estrogen and Endothelial Cells (ECs)

The EC monolayer is uniquely positioned at the interface between the blood and the vessel wall. By targeting the endothelium, estrogen could have significant effects in the circulation. Estrogen causes endothelium-dependent vasodilation by increasing the release of NO, prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) [3,7]. Estrogen may also decrease the release of endothelin (ET-1) and angiotensin II (AngII), which are potent vasoconstrictors and procoagulants.

Estrogen increases endothelial NO synthase (eNOS) activity, resulting in NO-mediated increase in cyclic guanosine monophosphate (cGMP) in VSM cells and vasodilation. Estrogen-induced eNOS activation occurs through more than one signal transduction

pathway [7]. ER in the plasma membrane interacts with scaffolding proteins such as striatin and Src-homology and collagen homology adapter proteins [95]. Lipid modifications and palmitoylation of ERs could also target ERs to lipid rafts [96]. In ECs, ER-centered protein complexes are associated with caveolae (Fig. 8). ER α , in particular, interacts with caveolin-1, a structural protein in EC caveolae [97]. Studies have shown that estrogen acutely activates intracellular pathways that change $[Ca^{2+}]_i$ concentration in ECs, leading to the release of eNOS from its binding to caveolin-1, increased NO production and relaxation of vascular rings [8]. Estrogen also activate phosphatidylinositol 3-kinase/Akt pathway, leading to phosphorylation and activation of eNOS, and increased NO production [98]. E2 also increases eNOS activity by causing rapid ER-dependent activation of mitogen-activated protein kinase (MAPK) [7].

Like E2, 2-methoxy-E2 increases NO production in aortic ECs in male and ovariectomized female rats [99]. 2-OH-E2 and 2-methoxy-E2 also stimulate the generation of PGI₂ [100]. Some studies that EDHF may be less important in male rat, suggesting gender differences in estrogen's effect on EDHF-mediated vasodilation [101].

E2 induces endothelium-dependent vasodilation in the brachial artery of Post-MW [102]. E2 also promotes proliferation of ECs via cytosolic and nuclear ERs as well as phosphorylation and activation of MAPK-dependent pathways. With age, there is decreased release of endothelial NO and EDHF and increased ET-1 production. This favors a vasoconstrictive and procoagulant state that promotes VSM growth. These processes may be exaggerated in disease states such as HTN and diabetes causing an increased risk of cardiovascular events.

Estrogen metabolites such as catechol-E2s and methoxy-E2s may affect the growth and function of ECs in a concentration-dependent manner. Low concentrations of 2- and 4-OH-E2 and 2- and 4-methoxy-E2 induce proliferation of cultured vascular ECs, whereas higher concentrations of 2-OH-E2 and 2-methoxy-E2 inhibit EC proliferation and angiogenesis. 2-Methoxy-E2 may also induce apoptosis in actively growing ECs [103–105].

Estrogen and Vascular Smooth Muscle (VSM)

Estrogen causes relaxation of endothelium-denuded vascular segments, suggesting direct effects on VSM contraction mechanisms [2]. Our laboratory has demonstrated sex differences in vascular contraction. The contraction to the α -adrenergic receptor agonist phenylephrine (Phe) is greater in aortic segments of intact male than female rats, and in ovariectomized than intact female rats [106]. E2 also causes relaxation of prostaglandin F₂ α (PGF₂ α)-induced contraction in endothelium-denuded porcine coronary artery [107].

E2, via ERs, reduces injury-induced proliferation of VSM cells in vascular lesions. E2 also attenuates the formation of vascular lesions in response to vascular injury in ER α or ER β knockout mice. It has been suggested that local metabolism of E2 to 2- and 4-OH-E2 with little affinity for ERs may mediate the ER-independent antimitogenic effects of E2 on VSM cells. P450- and COMT-derived E2 metabolites also inhibit the activity of human aortic and coronary artery VSM cells [108–110]. These findings suggest that E2 metabolism may be an important determinant of the vascular effects of E2, and that nonfeminizing E2 metabolites may confer cardiovascular protection regardless of gender [110].

When compared to estrogen, the potencies of estrogen metabolites in inhibiting migration, collagen synthesis and proliferation of human and rat aortic VSM cells are: 2-methoxy-E2 > 2-OH-E2 > E2 > 4-methoxy-E2 > E1, E3, 2-OH-E1, 16-OH-E1, 4-methoxy-E1 [100,109–111].

Estrogen and Inhibition of Mechanisms of VSM Contraction

In vascular strips incubated in Ca^{2+} -free solution, Phe causes contraction due to Ca^{2+} release from the intracellular storage sites. The Phe-induced contraction in Ca^{2+} -free solution is not different between intact and ovariectomized female rats, suggesting that endogenous estrogen does not affect the intracellular Ca^{2+} release mechanisms [106]. Membrane depolarization by high KCl solution stimulates the influx of extracellular Ca^{2+} through voltage-gated channels. KCl-induced contraction is less in intact females than intact males or ovariectomized female rats. Also, Phe- and high KCl-induced Ca^{2+} influx is reduced in the aorta of intact female compared with male rats. The decreased vasoconstriction in intact female rats could be due to long-term effects of estrogen on the expression and permeability of voltage-gated Ca^{2+} channels [106]. E2 causes relaxation of high KCl-induced coronary artery contraction, and inhibits $\text{PGF}2\alpha$ and high KCl-induced Ca^{2+} influx, suggesting inhibition of the Ca^{2+} entry mechanism of VSM contraction [107]. In arterial VSM, estrogen may activate ATP-sensitive K^+ channels or Ca^{2+} -activated K^+ channels, leading to membrane hyperpolarization and decreased Ca^{2+} entry (Fig. 8) [112,113]. Estrogen may also promote efflux of Ca^{2+} via plasmalemmal Ca^{2+} pump in VSM [114]. The 2-OH and 2-methoxy metabolites of E2 induce nongenomic inhibition of agonist-induced contraction and influx of extracellular Ca^{2+} , with different potencies compared with E2 [115].

Intracellular Ca^{2+} is reduced to a greater extent in aortic VSM cells from estrogen-replaced ovariectomized spontaneously hypertensive rats (SHR) compared to males or ovariectomized female SHR, demonstrating that the vascular protective effects of estrogen may be enhanced in hypertensive states [116].

Protein kinase C (PKC) is a family of Ca^{2+} -dependent and -independent isoforms, expressed in varying proportions in different vascular beds. During VSM activation, certain PKC isoforms translocate to the cell surface and trigger a protein kinase cascade that enhances the myofilament force sensitivity to Ca^{2+} and promotes VSM contraction (Fig. 8) [117]. Our previous studies have shown that the expression of α -, δ - and ζ -PKC is reduced in the aorta of intact female compared to intact males and ovariectomized female rats, suggesting potential effects of estrogen on the expression/activity of PKC in VSM [118].

Rho-kinase is known to inhibit myosin light chain (MLC) phosphatase and to enhance the VSM myofilament sensitivity to $[\text{Ca}^{2+}]_i$. Rho-kinase is upregulated in CVD and may play a role in the pathogenesis of coronary arteriosclerosis and vasospasm [119]. Estrogen may inhibit Rho-kinase expression and activity. For instance, the expression of Rho-kinase may involve a PKC/NF- κB pathway that is inhibited by estrogen [120]. Also, the vasodilator response to the Rho-kinase inhibitor Y-27632 is similar in ovariectomized female and male rats, and E2 treatment of ovariectomized rats normalizes the vasodilator effects of Y-27632 to those observed in intact females [121]. Also, in cultured human coronary VSM cells, treatment with E2 causes a decrease in Rho-kinase mRNA expression [122].

The decrease in estrogen levels in aging women is likely to cause an increase in the expression of Ca^{2+} channels and decreased Ca^{2+} pumps, leading to increased VSM Ca^{2+} and vasoconstriction. Also, an age-related increase in VSM PKC and Rho-kinase expression may increase cell growth and promote vasoconstriction and thereby increase the incidence of CVD.

Estrogen and Extracellular Matrix (ECM)

Estrogen may play a role in ECM remodeling and reduction of ECM degradation, and the decreased estrogen in menopause may promote ECM degradation. Matrix metalloproteinases (MMPs), a family of zinc-binding proteolytic enzymes, are involved in vascular remodeling and the various stages of atherosclerotic progression [123]. Increased

MMP activity occurs in cancer, arthritis and CVD. Also, MMP-induced ECM degradation within the atherosclerotic plaque may be involved in plaque instability and cardiovascular events. Studies suggest that changes in the levels of MMP-2, -9 and -10 in women receiving MHT may contribute to the potential risk of cardiovascular events and cancer [124]. Also, estrogen via ER α and G α 13 may cause activation of RhoA/Rho-Kinase and phosphorylation of the actin-regulatory protein moesin, and thereby cause remodeling of the actin cytoskeleton and hence EC migration [125].

Estrogen and Lipid Profile

ER α and ER β are expressed in both subcutaneous and visceral fat tissues [126]. It has been shown that Pre-MW have a less atherogenic lipid profile than men due to higher HDL and lower triglyceride levels, which are closely associated with central fat accumulation. Natural and surgical menopause are associated with rapid adverse changes in lipid metabolism within 3 months of menopause [127]. Also, in aromatase knockout mice, E2 treatment markedly decreases visceral fat mass, leading to beneficial changes in the lipid profile [9]. 2-methoxy-E2 also inhibits adipocyte proliferation [10]. These findings suggest that regulation of fat mass may be another mechanism by which estrogen affects vascular health [11].

Estrogen and the Inflammatory Response

The immuno-inflammatory system plays a key role in the development of fatty streak deposit and atherosclerotic plaques, and is a major target of estrogen. E2 prevents fatty streak deposit through an NO-independent mechanism. In ECs, estrogen may activate PI3K/Akt pathway and in turn it increases the function of P38-MAPKAP-2 kinase that phosphorylates HSP-27, a pathway that has been shown to reduce atherosclerosis [128]. The atheroprotective effects of E2 are mediated by ER α , independent of ER β [129]. The anti-inflammatory and atheroprotective effects of E2 are absent in mice deficient in T and B lymphocytes, and are lost in older and estrogen-deficient female animals [130]. One study examined whether age modifies the effects of estrogen on vascular inflammation induced by LPS in ovariectomized female rats. It was demonstrated that the protective anti-inflammatory effect of estrogen on cerebral blood vessels observed in young adult rats was attenuated in aging rats, which exhibited a greater cerebrovascular response to inflammatory stimuli [130]. Studies have also shown that estrogen administration elevates cortisol levels in Post-MW, and this effect is moderated by progestins [131]. Estrogen may also decrease the expression of CRP, an inflammatory biomarker that correlates with the extent of vascular injury [132,133]. E2 also increases protein S-nitrosylation in human umbilical vein ECs directly via ER α and indirectly by eNOS upregulation, and in turn prevents AngII-induced upregulation of intercellular cell adhesion molecule-1 [134]. Estrogen metabolites can also affect inflammation and thereby benefiting vascular health. For example, 2-methoxy-E2 has been shown to reduce atherosclerosis by inhibiting monocyte adhesion to aortic ECs of ovariectomized female rats [135].

While estrogen may have vascular protective effects [136], it may also promote proinflammatory processes [137]. One study has evaluated serum C3, C4, IgG and IgM levels in healthy Post-MW receiving short-term MHT (CEE plus MPA) and in untreated women, and concluded that MHT might be involved in the development of CVD through inflammatory mechanisms [138]. Estrogen may also increase interferon- γ levels, which could enhance plaque destabilization [129].

Estrogen as an Antioxidant

Estrogen has antioxidant properties. It increases NO bioavailability in ECs, and regulates superoxide dismutase in VSM cells [7,139]. Even in the presence of an unfavorable lipid pattern, estrogen and SERMS such as raloxifene may protect against atherosclerosis by

scavenging free oxygen radicals and inhibiting LDL oxidation [140]. Human coronary artery VSM cells infected with human cytomegalovirus (CMV) demonstrate an increase in reactive oxygen species (ROS) and activation of genes involved in viral replication and inflammation. Estrogens with an A ring OH-group promote ER-independent anti-CMV effects by inhibiting ROS generation, NF- κ B activation, NF- κ B-dependent transcription, and viral replication. Thus, to the extent that chronic infection of the vascular wall with CMV may contribute to atherogenesis, the antioxidant actions of estrogen may be of therapeutic importance [141].

Studies of the effects of estrogen on oxidative stress and mitochondrial function have centered on ECs in the brain because they have high energy demand and large number of mitochondria. Estrogen has many antioxidant effects on the mitochondria by increasing the expression of cytochrome c, subunits I and IV of complex IV and manganese superoxide dismutase, and these effects are mediated via ER α . Also, estrogen treatment increases the expression of nuclear respiratory factor-1 (NRF-1), a key regulator of nuclear-encoded mitochondrial genes of oxidative phosphorylation (Fig. 7) [142]. By decreasing mitochondrial production of ROS while sustaining robust oxidative phosphorylation, estrogen may lessen accumulation of mitochondrial DNA mutations over a lifespan. Therefore, while estrogen administration after a significant period without estrogen exposure may protect against future mitochondrial damage, it may not reverse damage accumulated during estrogen deficient periods. This mechanism might have contributed to the lack of benefit of estrogen on CVD in MHT clinical trials [8,143].

The E2 metabolites 2-OH-E2 and 2-methoxy-E2 have antioxidant properties that are more potent than vitamin E and E2, which prevent NO oxidation and potentiate the vasodilatory activity of NO released under basal conditions, and thus benefiting vascular health [111,144].

Estrogen and Angiogenesis

Angiogenesis, the formation of new blood vessels from existing blood vessels, involves several processes including degradation of the existing vascular basement membrane, proliferation and migration of ECs into tubular structures, and formation of new matrix around neovessels. Angiogenesis requires fibroblast growth factor-2, vascular endothelial growth factor, NO, and integrins. Circulating bone marrow-derived endothelial progenitor cells (BM-EPC) may also provide a source of ECs for the new blood vessels [145]. Interestingly, estrogen deficiency in animals and humans reduce the number of circulating BM-EPCs, and estrogenic treatment of ovariectomized animals and post-MW increases the number of BM-EPCs [146]. Estrogen may also slow down the aging of BM-EPC by increasing telomerase activity [147]. Estrogenic regulation of these processes is evidenced by neovascular development in the uterus of ovulating women. These processes are also involved in wound healing, repair of damaged organs, and restoration of blood supply to ischemic tissue and tumor growth, and estrogen may play a role in these conditions [148,149]. This is supported by reports that estrogen promotes the reendothelialization of the carotid artery after endovascular injury [150].

Vascular Effects of Other Estrogenic Compounds

While many studies have examined the vascular effects of E2, the effects of other estrogenic compounds are less clear. SERMs are promising estrogenic compounds, but they have both agonist and antagonist effects. *Ex vivo* studies on rat renal, pulmonary artery and porcine arterial rings have shown that raloxifene induces endothelium-independent relaxation by inhibiting Ca²⁺ influx through voltage-gated channels [151–153]. Also, in ovariectomized

rats, raloxifene demonstrates long-term protective properties against myocardial ischemia-reperfusion injury [154].

Food containing phytoestrogens is consumed in significant quantities in cultures with lower rate of menopausal symptoms, osteoporosis, cancer and CVD [155]. Isoflavones stimulate the activity of eNOS and induce vasodilation via NO [156]. Isoflavones are also antithrombotic and antiatherogenic. A meta-analysis of 38 controlled human studies of soy consumption suggested positive effect on lipid profiles, including reduction in levels of LDL-c and triglycerides and increased HDL-c [157]. Other studies have found no clear effect of flavonoid-rich food on blood pressure and EC function [158]. Also, no studies have examined the effects of phytoestrogens on clinical end points of CVD such as myocardial infarction.

Genistein is an isoflavone found in soy with estrogenic activity that depends on its serum concentration, and on the individual's endogenous estrogen levels and gender. Genistein binds both ER α and ER β , although ER β is bound with higher affinity [159]. Genistein enhances serum antioxidative ability and inhibits the development of atherosclerosis in LDL receptor knockout mice, although it does not affect serum cholesterol or lipid profiles. Genistein also decreases the expression of NF- κ B and vascular cell adhesion molecule (VCAM)-1 in mouse aorta and has less thrombotic effects than oral estrogen [160,161].

Lignan, a phytoestrogen with weak estrogenic and anti-estrogenic properties, has diverse bioactivities including antioxidation. High dietary intake of lignans by Post-MW may lead to better metabolic profile including higher insulin sensitivity, low glycaemia, high glucose disposal rate, and lower adiposity measures [162].

Resveratrol (trans-3,5,4'-trihydroxystilbene), a polyphenol found in grape skin and red wine, has been shown to induce endothelium-dependent relaxation in human internal mammary artery [163]. In mice, resveratrol treatment may attenuate oxidative stress, improve acetylcholine induced vasodilation and inhibit apoptosis in branches of the femoral artery [164]. Conversely, resveratrol may abrogate the antimitogenic effects of estradiol on VSM, and this necessitates further investigation of the vascular effects of resveratrol [165].

Secoisolariciresinol, a phytoestrogen isolated from flaxseed, has been shown to reduce hypercholesterolemic atherosclerosis by 73% in rabbit aorta [166].

Phytoestrogens differ in their estrogenic activity; the less their structural similarity to E2, the less is their binding affinity to ERs and the weaker is the biological effect [13]. Large doses of phytoestrogens may need to be administered in order to attain adequate response. For example, a dose of 500 mg secoisolariciresinol diglucoside/day for 8 weeks may be needed to observe positive effects on cardiovascular risk factors in patients [167]. Also, because the phytoestrogens' source is mainly dietary, their doses and plasma levels may not be efficiently monitored. Additionally, most plant lignans require activation and conversion to secondary metabolites by the intestinal microflora. Therefore, persons taking broad-spectrum antibiotics or having a clinical condition that suppresses the intestinal flora may not have adequate response to lignans. Thus, absorption and metabolism play a major role in the actions of phytoestrogens. Also, the benefits of phytoestrogens are subtle and not seen in all individuals, and their stability and longer half-life in the body raise concerns of potential toxicity. Therefore, phytoestrogens may not be recommended as a primary preventive intervention to reduce the risk of CVD. However, women may be advised that phytoestrogens used for treatment of menopausal symptoms may have additional cardiovascular benefits by reducing risk factors of atherosclerosis such as hyperlipidemia and HTN [13].

Studies with selective ER agonists suggest that ER α mediates many of the vascular benefits of estrogen [168]. More specific ER agonists need to be developed and their vascular effects need further characterization in order to enhance their benefits in Post-MW with CVD.

ESTROGEN INTERACTION WITH OTHER SEX HORMONES

Progestins

The plasma levels of progesterone range between 1.5 nmol/L in the follicular phase to 40 nmol/L in the mid-luteal phase, and decrease to <2 nmol/L along with the decrease in estrogen levels in the early stages of menopause. Progesterone shares many of the effects of E2 including anti-atherosclerotic effects, decreased LDL, and increased HDL [2,3]. Progesterone promotes eNOS expression, NO production, and NO-mediated relaxation in rat aorta and ovine uterine artery and stimulates PGI₂ production by direct non-genomic COX activation. Progesterone also stimulates pulmonary vasodilation via endothelium-dependent and -independent pathways, and causes rapid relaxation of agonist- and KCl-induced contraction in endothelium-denuded porcine coronary artery, although with less potency than E2 [107]. The effect of progesterone on VSM [Ca²⁺]_i is not clearly defined, but it decreases PGF2 α - and high KCl-induced Ca²⁺ influx and [Ca²⁺]_i in porcine and rabbit coronary VSM [169].

However, in canine coronary arteries, progesterone counteracts the stimulatory effects of estrogen on NO production [170]. Also, in ovariectomized mice, the vasoprotective effect of estrogen on antioxidant enzyme expression and activity is antagonized by progesterone, resulting in increased NADPH oxidase activity and ROS [171]. Progesterone can also affect the amount of ERs, and progesterone receptor A may act as a ligand-dependent transrepressor of ERs [172]. Progesterone may also promote vasoconstriction by upregulating angiotensin type₁ receptor mRNA and protein [173]. In the Postmenopausal Estrogen/Progestin Intervention (PEPI) trial, the increase in HDL-c was greater in women taking E2 only compared with users of E2+MPA [174]. Also, in the Heart and Estrogen/Progestin Replacement Study (HERS), which examined the effect of CEE+MPA in Post-MW with CAD, treatment for an average of 4.1 years did not reduce the overall rate of events associated with CAD and increased the rates of thromboembolic events [175]. There was an increase in coronary events in the first year in the treated women and a decrease in coronary events in years 4 and 5. These observations may be due to nonvascular possibly hepatic effects of CEE, MPA or both that may have altered coagulation profiles and increased thrombotic events in a subgroup of the women studied. MPA may also oppose the vasodilatory effect of estrogen on coronary arteries, increase the progression of coronary artery atherosclerosis, accelerate the LDL-c uptake in the plaque, increase the thrombogenic potential of atherosclerotic plaques, and promote insulin resistance and hyperglycemia. Since MPA is a substrate for CYPs and is hydroxylated, MPA may compete with E2 for CYPs and inhibit metabolism of E2 to OH-E2 and methoxy-E2 [176].

While the vascular benefits of postmenopausal estrogen treatment may be attenuated by MPA, they are differentially affected by other progestins [177]. Natural progesterone, but not some synthetic progestins such as 19-nortestosterone derivatives, displays a favorable action on blood vessels [24]. In contrast, progestins with high androgenic potency attenuate the beneficial effects of estrogens on the lipid profile and vasomotion. Therefore, in women with established CHD, MHT may not protect against further episodes when the progestin selected possesses androgenic properties [178]. Synthetic progestins could affect NO synthesis and counteract the favorable effect of E2 on the endothelium, and in the long-term could contribute to the development of HTN. On the other hand, the use of natural progesterone might offer added safety [24]. Natural progesterone is also natriuretic and exhibits a mineralocorticoid antagonist activity. Drospirenone, a progestin with

mineralocorticoid antagonist activity, lowers blood pressure in hypertensive Post-MW when combined with E2 [179]. This therapeutic regimen may be useful for patients with postmenopausal HTN who require progestin therapy to protect against uterine cancer during E2 therapy for menopausal symptoms [180].

Testosterone

In cross sectional studies involving Post-MW women, serum testosterone decreases in early menopause but increases afterwards, reaching Pre-MW testosterone levels at 70–79 years of age. In women who had undergone surgical menopause, serum testosterone levels did not increase with age, and were 40–50% lower than the levels of testosterone in women with natural menopause [181]. Increases in free and total testosterone may cause hormonal imbalance and interfere with the vascular effects of estrogen. In women, the relationship between circulating levels of free E2, free testosterone and SHBG may be more predictive of changes in carotid intimal thickening than the levels of any of these hormones alone. Data from the Estrogen in the Prevention of Atherosclerosis Trial (EPAT) have shown that the most beneficial hormone profile against the development of carotid artery intima-media thickness and atherosclerosis is increased free E2 and SHBG with concomitant decrease in free testosterone [174].

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by oligo- or anovulation due to feedback suppression of FSH release, and presents with features of the metabolic syndrome including obesity, insulin resistance and dyslipidemia, predisposing the individuals to the development of type-2 diabetes and CVD [182]. In PCOS, there is stimulation of androgen production which leads to increased E1 and E2 levels through peripheral aromatization, particularly in adipose tissue. The biologically available E2 levels also increase because of decreased SHBG [3].

In women, the bulk of testosterone formed is converted to E2 by aromatase. The aromatase gene CYP19A1 is mainly expressed in the ovarian follicles in Pre-MW, and the produced estrogen functions as the circulating hormone on distal target tissues with prominent actions on the reproductive tissues [183]. Conversely, local estrogen synthesis in extragonadal tissues such as adipose tissue plays a role in men and Post-MW [184,185]. This may lead to greater extragonadal effects of estrogen in Post-MW, increasing its levels in vascular tissue. In addition to aromatase, its estrogenic 17 β -HSD partner was recently identified in adipose tissue. A recent study revealed a low conversion of E1 into E2 in pre-adipocytes, and a 5-fold increase in this activity in differentiated adipocytes. The increased estrogenic 17 β -HSD activity is associated with increased protein expression of 17 β -HSD [186].

27-Hydroxycholesterol

27-OH-cholesterol is an abundant cholesterol metabolite that is elevated with hypercholesterolemia and found in atherosclerotic lesions and is a competitive antagonist of estrogen/ER actions in the vasculature. It inhibits both the transcription and non-transcription-dependent estrogen-mediated production of NO by vascular cells, resulting in reduced estrogen-induced vasodilation of rat aorta. Also, increasing 27-OH-cholesterol levels in mice decreases estrogen-dependent expression of vascular NOS and represses carotid artery reendothelialization [187]. Further studies are needed to determine the levels and the role of 27-OH-cholesterol in elderly Post-MW.

VASCULAR BENEFITS VERSUS RISKS of MHT

Meta-analysis of observational studies has shown that MHT is associated with ~33% less fatal CVD among MHT users compared to nonusers [6]. In pre-MW, estrogen causes vasodilation and protects against HTN. The lack of estrogens after menopause and the

resulting reduced arterial elasticity and compliance is likely to contribute to age-related progressive increase in systolic blood pressure and HTN [188]. Impaired endothelial function might precede the development of HTN [188]. Menopause is also associated with hyperactivity of the sympathetic nervous system [52] and activation of the renin-angiotensin-aldosterone system, and together with other factors such as obesity and oxidative stress, they can contribute to the high prevalence of HTN in late Post-MW [189,190]. Studies have suggested that oral estrogens induce an increase in hepatic production of angiotensinogen (the renin substrate), with a subsequent rise in plasma angiotensin I and II, and the development of HTN [191,192]. In contrast, transdermal estrogen either does not alter or could slightly reduce BP in normotensive Post-MW [192–194]. Selective ER modulators such as raloxifene may function as agonists for the novel vasodilating GPER [195] and could also be useful in the management of postmenopausal HTN. Thus, by avoiding certain MHT regimens and adopting more beneficial strategies, the vascular benefits of MHT could be enhanced [180] (Table 4).

Oral MHT increases the risk of venous thrombosis in predisposed women, particularly in the first year [196]. Oral MHT may also facilitate the development of arterial thrombosis, and the rupture of unstable plaques. This might explain the elevated CHD risk during the first year of MHT [13]. Contrary to reports that early menopause increases risk for arterial CVD [197], data from a hospital based case-control study suggests that the risk of venous thromboembolism decreases with early menopause [198]. That is the shorter the exposure to endogenous estrogen is (as in early menopause), the less the risk of a venous thrombotic event. The cause of these apparent contradictory findings is unclear, underscoring the need for further basic research into how sex hormones affect the venous wall. Oral estrogens may also cause an increase in circulating levels of coagulation factors 2, 7, 9, 10 and plasminogen, and increased platelet adhesiveness, and decreased anti-thrombin levels, but these procoagulation effects are less common with transdermal estrogens [199]. It has been shown that basal release of thromboxane from platelets is greater in raloxifene- compared to E2-treated ovariectomized pigs. Raloxifene treatment, compared to E2, increased the production of contractile and pro-aggregatory prostanoids from venous endothelium and platelets [200]. These differences, if found in humans, may increase the thrombotic risk with SERMs compared to natural estrogen.

CLINICAL TRIALS: PAST AND PRESENT

The efficacy of MHT in Post-MW has been examined in several RCTs including HERS, WHI and Raloxifene Use for The Heart (RUTH). However, the outcomes of the MHT RCTs were not as predicted. The purpose of HERS was to determine whether MHT in Post-MW with established CAD prevents future heart attacks or death from CHD, but it failed to demonstrate any cardioprotective benefits [175]. Almost 3,000 women with proven CHD were randomly assigned to MHT containing CEE and MPA or placebo. After 4 years, the frequency of the primary outcome, i.e. fatal and non-fatal CHD combined, did not differ between the two groups. There was also a 50% increase in coronary events in the first year in the MHT group [201]. The WHI was designed to determine fatal and nonfatal heart disease, cancer, and osteoporotic fractures as the primary outcome. In the estrogen-progestin arm, MHT increased risk of heart attacks and strokes. CEE alone increased risk of stroke and deep vein thrombosis [202].

In both HERS and WHI, however, the participants were older (50 to 79 years), with only 10% of the participants between 50 and 54 years old and 20% between 54 and 59 years old. In women assigned MHT, 36% had HTN, 49% were current or past smokers, and 34% were obese. Hence, although subjects were designated as healthy, the process of atherosclerosis was likely advanced in the participants. Also, HERS and WHI used CEE, in which the

primary active ingredients are E1-sulfate, equilin sulfate and 17 α -dihydroequilenin. After menopause, women lose E2, whereas the levels of E1 (largely produced in peripheral tissue) remain unchanged. CEE does not replace E2, and it is possible that the differences in the outcomes between CEE and E2 are caused, in part, by the route of administration; CEE is given orally, whereas E2 is often administered transdermally. The negative findings of HERS and one arm of WHI may have also been caused by concomitant use of MPA [1].

The RUTH RCT in Post-MW with CVD or at increased risk of CVD demonstrated that raloxifene treatment did not change the incidence of coronary events, but increased the risk of fatal stroke and venous thromboembolism [203,204]. While the participants' advanced age could have affected the estrogenic response, this unexpected effect could have been caused by the simultaneous activation and inhibition of some or other ERs by SERMs. Also, even if a given SERM may act as an ER agonist, it may not have the same activity as estrogen.

Recently, the factors thought to be responsible for the conflicting results of MHT RCTs were taken into consideration, potential negative practices were identified, and beneficial strategies were suggested. When treating younger perimenopausal women the benefits of MHT should always be weighed against the potential risks, and the type of MHT should be carefully chosen. Medical evidence-based guidelines and the FDA recommendations are that MHT may be used for the relief of moderate to severe postmenopausal symptoms, but not for the routine prevention of chronic disease conditions and primary or secondary CHD, with the exception that MHT may be used conditionally for the prevention of osteoporosis when other interventions have been considered and are deemed inappropriate [205,206].

New studies have taken the pros and cons of the previous MHT RCTs into account. The Early versus Late Intervention Trial with Estradiol (ELITE) will test the effect of timing of estrogen intervention on the progression of carotid artery intima-media thickness (CIMT), and compare the effect of oral E2 in women less than six years and those more than 10 years past menopause [8]. Also, the Kronos Early Estrogen Prevention Study (KEEPS) will evaluate the effects of low-dose oral CEE and transdermal E2 on both progression of CIMT and coronary calcification in women who are within three years of menopause, and will examine surrogate end points and risk factors of atherosclerosis [8]. The results from ELITE and KEEPS RCTs will allow evaluation of new MHT formulations on the progression of CIMT in an age spectrum spanning two decades of menopause, and will provide valuable data regarding the effects of these MHT formulations on the risk of ischemic stroke [8].

We should note that studies have also tested the effects of estrogen on cardiovascular health of Pre-MW. A study was set out to determine whether Pre-MW would be at a greater risk of developing an acute coronary event when plasma E2 levels are low during the menstrual cycle. Results showed that all 47 acute coronary events in Pre-MW admitted with unstable angina occurred during the first 14 days of the women's menstrual cycles. Two thirds of those events occurred during the first six days. Contrary to the initial hypothesis, no events occurred during the luteal phase when estrogen levels are lowest. This necessitates further investigation of the vascular effects of estrogen in Pre-MW as compared to Post-MW [207].

CONCLUSION

Estrogen and other estrogenic compounds have significant vasodilator and other beneficial vascular effects. Many of the effects of estrogen are mediated via vascular ER. The lack of vascular benefits of MHT in postmenopausal CVD could be caused by factors related to the estrogenic compound used, interactions with other sex hormones, and age-related changes in sex hormone metabolism, the vascular ER, or the vascular architecture. The vascular

benefits of MHT can be enhanced by adjusting the timing and dose of MHT and by using the most effective estrogen preparation and route of administration. MHT may also need to be individualized depending on the endogenous hormone environment, metabolic pathways, dietary phytoestrogens and exposure to xenoestrogens. Genetic and pharmacological manipulations of the age-related changes in vascular ERs could enhance their responsiveness to MHT. These strategies in addition to consideration of the patient's preexisting cardiovascular condition should enhance the effectiveness of MHT in postmenopausal CVD.

Acknowledgments

This work was supported by grants from National Heart, Lung, and Blood Institute (HL-65998, HL-70659, HL-98724) and The Eunice Kennedy Shriver National Institute of Child Health and Human Development (HD-60702). We would like to thank Ms. Kimberly L. Steen and Ms. Jessica A. Haley for their assistance in revising and proof-reading the manuscript.

List of Abbreviations

ACE	angiotensin-converting enzyme
CAD	coronary artery disease
CHD	coronary heart disease
COMT	catechol-O-methyltransferase
CVD	cardiovascular disease
E2	17 β -estradiol
EC	endothelial cell
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor
eNOS	endothelial NO synthase
ER	estrogen receptor
ET-1	endothelin-1
GPR30 (GPER)	G protein-coupled receptor 30
HERS	Heart and Estrogen/progestin Replacement Study
HSD	hydroxysteroid dehydrogenase
HTN	hypertension
KEEPS	Kronos Early Estrogen Prevention Study
MAPK	mitogen-activated protein kinase
MHT	menopausal hormone therapy
MMP	matrix metalloproteinase
MPA	medroxyprogesterone acetate
NO	nitric oxide
Post-MW	postmenopausal women
Pre-MW	premenopausal women
ROS	reactive oxygen species

RUTH	Raloxifene Use for The Heart
SHR	spontaneously hypertensive rat
VSM	vascular smooth muscle
WHI	Women's Health Initiative

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Fig. 1.
Common nucleus of sex steroids. Estrogens, progestins, and androgens share a common cyclopentanoperhydrophenanthrene nucleus, but differ in their side groups.

Fig. 2.

Estrogen synthesis. Cholesterol is transported into the mitochondria by steroidogenic acute regulatory protein (StAR), then converted into pregnenolone by P450ScC (20,22 desmolase). Pregnenolone is released in the cytosol where it is converted into progesterone by 3 β -HSD Δ 4,5 isomerase. Pregnenolone is alternatively converted by various reactions in the testis into androstenediol, which is then converted to testosterone by 3 β -HSD Δ 4,5 isomerase. Progesterone may be converted via sequential pathways into androstenedione then testosterone, which are then converted by aromatase into E1 and 17 β -E2, respectively. 17 β -E2 is transformed into E1 by 17 β -HSD-2, and reversibly from E1 to E2 by 17 β -HSD-1.

Fig. 3.

Estrogen biosynthesis in the fetoplacental system. Cholesterol is taken by the placenta from LDL present in the maternal circulation, then transformed by P450-Scc into pregnenolone, which is sulfated and taken up by fetal adrenal glands to produce dehydroepiandrosterone sulfate (S-DHEA), then hydroxylated to 16 α -OH-S-DHEA in the fetal liver. 16 α -OH-S-DHEA is desulfated in the placenta to produce 16 α -OH-DHEA which then is taken up by the syncytiotrophoblasts for aromatization into E3. Desulfated DHEA may alternatively be converted into androstenedione which is aromatized into E1 or first transformed into testosterone then aromatized to E2. Alternatively, placental pregnenolone may be converted to progesterone which returns to the maternal circulation.

Fig. 4.

Estrogen metabolism. E2 may undergo conjugation by glucuronidation or sulfation for excretion. E2 may also be hydroxylated in ring A (2-OH, 4-OH), ring B (6β-OH), and ring D (16α-OH) and then conjugated for excretion. Also, the 2-OH and 4-OH metabolites may be methylated by COMT, forming 2- and 4-methoxy-E2. Alternatively, 2-OH and 4-OH metabolites may be subject to peroxidases then reductases forming E2-quinones, with simultaneous generation of superoxides leading to oxidative stress. E2 is transformed into E1 by 17β-HSD-2, and reversibly from E1 to E2 by 17β-HSD-1. E1, in turn, may be sulfated or glucuronide conjugated for excretion. E1 may also be hydroxylated in ring D (16α-OH) and ring A (2-OH, 4-OH). 16α-OH-E1 may be converted to E3 by 17β-HSD. 2-OH-E1 and 4-OH-E1 may be subject to methylation by COMT, forming 2- and 4-methoxy-E1 which are then sulfated for excretion. 2-OH-E1 and 4-OH-E1 can also be sulfated or glucuronide conjugated for excretion. The compounds enclosed in boxes are the major estrogen metabolites excreted in urine.

Fig. 5.

Examples of other estrogenic compounds. Phytoestrogens, plant-derived estrogens, include three main groups: flavonoids, stilbenoids, and lignans. Selective estrogen receptor modulators (SERMs) are synthetic drugs that bind ERs with both agonistic and antagonistic effects. Selective ER agonists act specifically on the ER subtype, ER α , ER β or GPER.

Fig. 6.

Estrogen receptor structure. Similar to other members of the nuclear receptor superfamily, ER α and ER β share a common structure with five functional domains termed A/B, C, D, E and F. ER also has phosphorylation sites which affect its function. The A/B domain contains the activation function-1 (AF-1). Domain C is the DNA binding domain which contains zinc (Zn) fingers. Domain D is the hinge domain, linking domain C and E. The ligand binding cavity and activation function-2 (AF-2) are located in domain E. Domain F includes co-factor recruitment regions. GPER is a G protein-coupled receptor that has estrogenic activity and shares little homology with the classical ERs. GPER consists of 3 exo-loops, 4 cyto-loops and a 7- transmembrane domain.

Fig. 7.

Genomic and mitochondrial effects of estrogen. In endothelial cells, estrogen binds to cytosolic ER and dissociates it from heat shock protein 90 (HSP-90). The estrogen-ER complex translocates to the mitochondria where it decreases the production of ROS and H₂O₂, reducing oxidative stress and increases oxidative phosphorylation. The estrogen-ER complex also translocates into the nucleus where it increases the expression of nuclear respiratory factor-1 (NRF-1), and in turn increases the level of nuclear encoded mitochondrial gene products. Estrogen/ER also increases the expression of fos, myc, and jun which in turn promote cell growth and proliferation. Estrogen/ER also stimulates Egr-1, which is upregulated in response to acute and chronic injury. Estrogen could also increase the gene transcription of eNOS and COX2, leading to increased production of NO and PGI₂ with consequent stimulation of vasodilation and angiogenesis.

Fig. 8. Non-genomic effects of estrogen on ECs and VSM. Estrogen binds to ERs in EC membrane and activates phospholipase C (PLC), leading to the generation of IP_3 and Ca^{2+} release from the intracellular stores. Ca^{2+} initially activates eNOS, which dissociates from caveolin-1. Estrogen also initiates the transformation of phosphatidylinositol-4, 5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which activates AKT and phosphorylates cytosolic eNOS which translocates back to the cell membrane where it undergoes myristoylation and palmitoylation. Estrogen may also increase the phosphorylation of eNOS by activating mitogen-activated protein kinase (MAPK) and cAMP-dependent protein kinase (PKA). Activated eNOS converts L-arginine into L-citrulline and leads to increased NO production. The release of NO from ECs activates cGMP-dependent protein kinase (PKG) in VSM which in turn decreases $[Ca^{2+}]_i$, by stimulating the Ca^{2+} extrusion and uptake pumps. Estrogen also activates COX-2 with consequent increase in PGI_2 . PGI_2 increases cAMP in VSM which causes a decrease in

$[Ca^{2+}]_i$ by mechanisms similar to those of cGMP. Estrogen also increases the release of EDHF leading to VSM hyperpolarization and relaxation. Estrogen may also inhibit Ca^{2+} entry, PKC, Rho-kinase, MAPK and other pathways of VSM contraction. Dashed arrows indicate inhibition.

Table 1

Enzymes involved in estrogen biosynthesis, their subcellular location, and consequences of single nucleotide polymorphisms in their genes.

Steroidogenic Enzymes	Subcellular Location	Single Nucleotide Polymorphism	Consequence
Cytochrome P450 P450 scc (CYP11A1:cholesterol side-chain cleavage enzyme)	Mitochondria	TBD	TBD
P450 c17 (17 α -hydroxylase;17,20-lyase)	Smooth Endoplasmic Reticulum	rs743572	TBD
P450 arom (CYP19A:aromatase)	Smooth Endoplasmic Reticulum	rs2414096 rs2446405	Diabetes Mellitus Insulin Sensitivity
Oxidoreductases 3 β -hydroxysteroid dehydrogenase	Smooth Endoplasmic Reticulum	TBD	TBD
17 β -hydroxysteroid dehydrogenase	Smooth Endoplasmic Reticulum	rs2830 rs592389 rs615942	Vasomotor Symptoms

TBD: To Be Determined

Table 2

Comparison of Serum Levels of Estrogens in Pre-MW and Post-MW

Estrogen	Non-pregnant Pre-MW	Post-MW	Reference
Estradiol (E2) (pg/mL)	30–80 (early follicular phase) 150–350 (late follicular preovulatory phase) 100–210 (luteal phase)	< 20	[13]
Estrone (E1) (pg/mL)	43.4 (follicular phase)	9.7–17.6	[19,20]
Estrone sulfate (pg/mL)	389 (follicular phase)	735.1–2298.5	[19,20]

Table 3

US FDA-Approved Estrogen and Estrogen-Progestin Preparations for MHT

Route of Administration	Estrogenic Compound	Commercial Name
Oral	Estradiol - drospirenone	Angeliq® Tablets
	Synthetic conjugated estrogens	Enjuvia™
	Norethindrone acetate/ethinyl estradiol	FemHRT
	Estradiol/norgestimate	Prefest™
	CEE	Premarin®
	CEE/MPA	Prempro™
Transdermal Patches	Estradiol	Alora® Estraderm® Menostar® Vivelle®, Vivelle-Dot®
	Estradiol/Levonorgestrel	Climara Pro™
	Estradiol/Norethindrone acetate	CombiPatch®
Injection	Estradiol valerate	Delestrogen®
	CEE	Premarin®
Topical Emulsion	Estradiol	Estrasorb™
Vaginal	Estradiol acetate	Femring®
	CEE	Premarin®

Table 4

Potential Negative Practices and Suggested Beneficial Approaches for MHT in Postmenopausal CVD.

Potentially Negative	Potentially Beneficial
MHT using CEE	MHT using 17 β -estradiol
Oral administration of MHT	Transdermal administration of MHT
Beginning of MHT late after menopause	Beginning of MHT early after menopause
High dose of MHT	Low dose of MHT
Progestins with adverse effects (MPA)	Cyclic administration of MHT Drospirenone Administration