HHS Public Access

Author manuscript

Clin Sci (Lond). Author manuscript; available in PMC 2017 February 19.

Published in final edited form as:

Clin Sci (Lond). 2016 July 01; 130(13): 1065–1074. doi:10.1042/CS20160042.

Sex hormone therapy and progression of cardiovascular disease in menopausal women

Rabe E. Alhurani[*,†], **C. Anwar A. Chahal**[‡,*], **Ahmed T. Ahmed**[*,§], **Essa A. Mohamed**[*], and **Virginia M. Miller**[‖,¶]

[*]Mayo Clinic Graduate School of Medicine, Rochester, MN 55905, U.S.A

[†]Department of Neurology, Mayo Clinic, Rochester, MN 55905, U.S.A

[‡]Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN 55905, U.S.A

[§]Division of Preventive, Occupational and Aerospace Medicine, Mayo Clinic, Rochester, MN 55905, U.S.A

[‖]Department of Surgery, Mayo Clinic, Rochester, MN, U.S.A

[¶]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN 55905, U.S.A

Abstract

One of the most controversial health decisions facing women is deciding upon the use of hormonal treatments for symptoms of menopause. This brief review focuses on the historical context of use of menopausal hormone treatments (MHT), summarizes results of major observational, primary and secondary prevention studies of MHT and cardiovascular (CV) outcomes, provides evidence for how sex steroids modulate CV function and identifies challenges for future research. As medicine enters an era of personalization of treatment options, additional research into sex differences in the aetiology of CV diseases will lead to better risk identification for CV disease in women and identify whether a woman might receive CV benefit from specific formulations and doses of MHT.

Keywords

17 β-oestradiol; atherosclerosis; carotid intima-medial thickness; conjugated equine oestrogens; coronary artery calcification

INTRODUCTION

Over the last decade, perhaps one of the most controversial medical decision facing women and their physicians is the use of sex hormones during menopause and the impact of their use on cardiovascular (CV) health. This controversy began following the results of the Women's Health Initiative (WHI) Trial in 2002 [2] which contradicted results from large epidemiology and observational studies which reported that the use of hormone treatments

Correspondence: Virginia M. Miller (miller.virginia@mayo.edu).

in menopause would reduce CV diseases (for review, see [3]). Attempts to resolve this controversy included subanalyses of WHI results [4,5]; additional randomized clinical trials [6,7]; statements from professional societies [8,9]; and systematic reviews [10,11].

Collectively, these investigations provided the basis for two important changes in clinical practice: (1) sex hormone therapy should be used for the relief of menopausal symptoms and not to modify CV disease risk [9] and (2) the use of the lowest dose of menopausal hormone treatments (MHT) for the shortest period of time [12]. In addition, a conclusion from the 2015 Cochrane review [11] was that ‘Our review findings provide strong evidence that treatment with hormone therapy in post-menopausal women overall, for either primary or secondary prevention of CV disease events has little if any benefit and causes an increase in the risk of stroke and venous thromboembolic events’.

However, many questions remain. How do these broad brush conclusions apply to individual woman in the era of precision medicine which promotes the right drug at the right dose to the right person at the right time? Do the conclusions apply to all types of steroid hormone therapy, formulations and doses? Will there be sufficient clinical trial data to ever resolve the controversy regarding use of sex hormone treatments and CV health during menopause? This review will consider the physiological outcomes from several large clinical studies with consideration of formulations of hormones, doses, patients’ characteristics and pharmacogenomics in the context of data from basic science studies.

HISTORICAL PERSPECTIVE

As a result of the women’s health movement during the 1980s, in the United States, the National Institutes of Health (NIH) established the Office of Research on Women’s Health in 1990 and Congress passed the NIH Revitalization Act obligating the inclusion of women in NIH-funded research in 1993 [13]. Similar attempts to address the absence of female animals and/or in basic science studies were addressed by policy changes initiated by the NIH in 2015 [14]. In Canada [15] and the European Union [16], policies to address sex and gender disparities in health conditions and research were established also.

Consequences of the lack of attention to understanding sex differences in the aetiology, manifestation, and treatment of CV diseases is that CV diseases remain the major cause of death in women and men. However, although all-cause mortality from CV diseases has declined for both men and women over the last decade, deaths from CV diseases in women still exceed those of men and the mortality has not declined for young women [17]. Therefore, new and focused research [18] into differences in CV regulation and risk factors between women and men across the life-span is needed.

CLINICAL STUDIES OF SEX HORMONES

There are three major life stages that are defined by shifts in the hormonal status of women across their life span: puberty, pregnancy and menopause. These hormonal shifts represent times of vulnerabilities for expression of CV diseases in women that differ from those of men. In spite of various experimental approaches, the question remains as to whether replacing or treating women at menopause with exogenous sex hormones reduces

vulnerabilities for CV diseases. Studies of sex hormones treatments/replacements can be divided into observational, primary prevention and secondary prevention. We will discuss each type of these studies here then the relevant data will be summarized in Table 1 for the major primary and secondary prevention studies.

Some of the controversy regarding CV risk with sex hormones treatments in earlier studies, including the WHI, stems from the mixed inclusion of women with natural menopause and women with hysterectomies with unilateral or bilateral oophorectomy. Data indicate that timing of oophorectomy impacts overall health such that oophorectomy prior to the age of natural menopause increases the risk of CV diseases, the risk of which is reduced by sex hormone replacement [19]. Furthermore, the impact of unilateral and bilateral oophorectomy is not the same [20]. Consensus is growing to differentiate the use of the terms ‘hormone replacement’ for women with oophorectomy prior to the age of natural menopause (50–54 years), and ‘hormone treatments or therapy’ for women after the age of natural menopause. This review will adopt this terminology and focus on MHT, although it must be acknowledged that some of the studies of menopausal women include women with prior oophorectomies.

Observational and primary prevention studies

Multiple, large, well-designed epidemiological studies performed over the past four decades found that use of MHT to reduce menopausal symptoms, also reduced the incidence of CV diseases and reduced all-cause mortality by almost 50% in postmenopausal women [3].

One of the earliest studies by Bush et al. published in 1983 [21] reported that: ‘The relative risk of all-cause death in oestrogen users compared with nonusers was 0.54 in gynaecologically intact women, 0.34 in hysterectomized women, and 0.12 in bilaterally oophorectomized women. The risk of all-cause death in oestrogen users, irrespective of hysterectomy status, was 0.37 times that in nonusers (3.4/1,000 compared with 9.3/1,000)’ for a 40–69 year-old women cohort from the Lipid Research Clinics Program Follow-up Study ‘who had been followed up for an average of 5.6 years’. When this cohort was followed for an average 8.5 years by the same group [22], they found only six deaths due to CV diseases in 593 oestrogen users compared with 44 deaths in 1677 nonusers {age-adjusted relative risk (RR) 0.34 [95% confidence interval (CI) 0.12–0.81] in users of MHT compared with nonusers}. Also a slightly higher prevalence of CV diseases in MHT users than non-users at baseline was detected upon analyses for confounders. Exclusion of women with CV diseases at baseline, i.e. focusing on primary prevention, also showed a trend of protective effect on CV mortality (RR 0.42, 95% CI 0.13–1.10). The mortality benefits were thought to be largely due to increased levels of high density lipoprotein (HDL) in oestrogen users. Importantly these women were prescribed MHT by their clinicians, independent of the research study, thereby reflecting real-world use. Validation of the MHT was performed during the study by investigators recording medication details from the physical MHT packets provided by study participants. Any discrepancies were addressed by contacting physicians or pharmacists. Over two-thirds took oral conjugated equine oestrogens (oCEE), and only 1% took also progestins.

The Nurses' Health Study (NHS) [23] was a prospective cohort questionnaire-based study which followed 70,533 postmenopausal women. CV diseases were confirmed by reviewing the medical records. Similar to the previous studies, participants used various types and doses of MHT reflecting clinical practice. Risk of major coronary events was lower among oestrogen users (short and long duration) compared with never users. The risk reduction was similar in women taking 0.3 mg compared with the higher 0.625 mg oCEE daily. However, the risk of stroke was increased in the higher dose group, as well as in those taking combined oCEE with progestin [oral medroxyprogesterone acetate (oMPA)].

The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial [24] prospectively compared the use of placebo, oCEE monotherapy and three combinations of oCEE with different progestins in 875 healthy postmenopausal women aged 45–64 years. Like observations from the Lipid Research Clinics Program Follow-up Study, oCEE increased HDL cholesterol, and decreased low density lipoprotein (LDL) cholesterol. The study was powered to detect these differences in cholesterol specifically and not whether this translated into improved outcomes. Though the beneficial effects of oestrogen therapy were unlikely to be due to the effects on lipid levels alone, other effects were largely unknown.

The Estrogen in the Prevention of Atherosclerosis Trial (EPAT) [25] was a randomized, double-blind, placebo-controlled study 'to test whether unopposed micronized 17 β-oestradiol (1 mg/day) reduces progression of subclinical atherosclerosis in healthy postmenopausal women (n = 97; mean age = 60.9 years) without preexisting CV disease' with follow-up of 2 years. The progression of subclinical atherosclerosis was less in healthy postmenopausal women who received 17 β-oestradiol compared with those who received placebo.

Subsequent to these studies, WHI Trial was designed to test whether MHT would provide benefit for primary prevention of CV diseases. Hormonal treatments selected were based on prescribing habits in the United States: oCEE (0.625 mg/day) monotherapy in women with hysterectomy, or oCEE (0.625 mg/day) plus oMPA (2.5 mg/day) in women with uterus, due to the known risk of endometrial cancer with unopposed oestrogen therapy [26].

Due to the proposed duration of WHI Trial and to achieve its statistical power to detect significant differences in CV event rates, women from a wide spectrum of ages were recruited (50–79 years, with the vast majority being >60 years). To reduce dropout rates of the placebo arm and to blind the investigators; women with severe menopausal symptoms who could not tolerate a 3-month washout period or current hormone use or might not tolerate placebo were excluded. Hence, participants in the WHI were unlike women from the observational studies, who were patients from clinical practice, where MHT was often initiated early in order to treat menopausal symptoms, and rarely in the late postmenopausal stage. Women with a prior history of CV diseases were excluded from WHI, but this was based on self-report and was not verified through other means. At the time the trial was published, this was accepted as *bona fide*, only to be later realized that many had substantial risk or a prior history of CV diseases.

WHI was terminated early in 2002 by the data and safety monitoring board (DSMB) as data accumulated showing a trend towards increased breast cancer in the MHT group. There was a notable age-adjusted increase in the incidence of breast cancer by approximately 20%, but a paradoxical decrease in mortality associated with breast cancer by 20%. At the time the study concluded, there was an increased risk for coronary artery events, stroke and thromboembolism in the oCEE plus oMPA group. It should be noted the dosing in WHI was shown in NHS to be associated with an increased risk of stroke. However, in the WHI there were benefits beyond the CV system: reducing osteoporosis-related fractures and colon cancer. Despite these benefits, the overall results changed medical practice toward use of low doses of MHT for shortest period of time and only for relief of menopausal symptoms (hot flushes, mood lability/depression, vaginal atrophy and sleep disturbances). Subsequent analyses of data from the WHI suggested that in women using oCEE alone, coronary artery calcification (CAC) was reduced and that women who had a modest CV risk profile benefitted the most [27–30].

Critics of the WHI questioned the higher dosing regimen and the age range of participants, many of whom had been postmenopausal for over 10 years. Data from the basic science literature suggested that there was a therapeutic window (timing hypothesis) in which initiation of use of menopausal treatments would slow progression of atherosclerosis [31]. The Kronos Early Estrogen Prevention Study (KEEPS), funded by a private foundation, The Kronos Longevity Research Institute, was designed based on this hypothesis and women were enrolled who were between 6 and 36 months of their last menses [32,33]. Also based on current clinical recommendations and outcomes of the EStrogen and THromboEmbolism Risk (ESTHER) study [34] that examined risk of thromboembolism with oral compared with transdermal MHT, women in KEEPS were randomized to either: oCEE (Premarin, 0.45 mg/day), transdermal 17 β-oestradiol (tE2) (Climera, 50 μg/day), placebo pills, or placebo patch. Women randomized to active treatments also received oral progesterone (Prometrium; micronized progesterone, 200 mg/day) for 12 days each month. Inclusion criteria were rigorous, including women with conventional CV risk factors (body mass index, blood pressure, lipid profile and fasting glucose) within normative ranges. Women with CAC scores >50 were excluded and the majority of participants were non-smokers. The main outcomes of KEEPS were progression of atherosclerosis defined by increases in carotid intimamedial thickness (CIMT) and CAC. After 4 years of treatment, there were no significance differences among treatment groups in either parameter which most likely reflected the baseline age and good health of the participants, the doses of MHT, and the duration of the study [6].

The timing hypothesis was tested directly in the Early versus Late Intervention Trial with Estradiol (ELITE) which enrolled postmenopausal women with and without prior hysterectomy [35]. About half of the participants were close to the onset of menopause, less than 6 years (mean age 55 years), and about half were more distant from menopause, at least 10 years (mean age 65 years). These women were randomized to either oral 17 β-oestradiol (1 mg/day) or placebo. Also, women who had an intact uterus received 10 days of micronized progesterone gel vaginally if they were in the oestrogen group. As with KEEPS, the outcome measure was the CIMT measured by ultrasound. As reported at the 2015 meeting of the American Heart Association, over a period of up to 6 years, the women who

were randomized to oestradiol, in early menopause but not those later in menopause, had slower progression of CIMT than the women randomized to placebo [35a].

The KEEPS and ELITE Trials used formulations and doses of MHT that are used clinically following the WHI. In addition, review of a Finnish registry that accounts for types of MHT other than oCEE, confirmed decreases in mortality from coronary artery disease and stroke in women using MHT for longer than 10 years and an increase in mortality in women who discontinue use [36,37]. Additional studies need to confirm these observations in other ethnic populations of women. In addition, prospective studies are needed to examine CV effects of new formulations of MHT and of selective estrogen receptor modulators (SERMS) that are being developed for use in treatment of menopausal symptoms.

Secondary prevention studies

Based on the epidemiological and observational studies of the 1980–early 1990s, the Heart and Estrogen/progestin Replacement Study (HERS) [38] started in 1993 as a randomized, blinded, placebo-controlled prevention trial assessing the benefits of MHT in secondary prevention of CV diseases. CV disease in participants included 1 or more of these confirmed diagnosis: ‘myocardial infarction (MI), coronary artery bypass graft surgery, percutaneous coronary revascularization or angiographic evidence of at least a 50% occlusion of 1 or more major coronary arteries’. Women were on average approximately 67 years of age and were randomized to oCEE (0.625 mg/day) plus oMPA (2.5 mg/day). In the first year of the study, there was an increase in coronary heart disease (CHD) in the hormone arm compared with the placebo arm with an estimated relative hazard of 1.52; then there was a decline in relative hazard: 1.00 in year 2, 0.87 in year 3 and 0.67 in years 4 and 5 ($P = 0.009$ for trend in log relative hazard).

The Estrogen Replacement and Atherosclerosis (ERA) Trial [39] was a randomized trial ($n = 309$) comparing oCEE monotherapy (0.625 mg/day), combined therapy of oCEE (0.625 mg/day) plus oMPA (2.5 mg/day) and placebo in postmenopausal women with coronary artery disease confirmed by invasive coronary angiography. The mean age of these women was 65.8 years with a long time since menopause. And consistent with the timing hypothesis, no significant reduction in stenoses or deaths was observed in either group after a mean follow-up of 3.2 years.

A retrospective analysis of the Coumadin Aspirin Reinfarction Study (CARS) [40], identified 1857 postmenopausal women, categorized into never users, prior/current users (taking MHT prior to the MI) and new users (those who initiated MHT around the time of the MI). ‘The three groups were fairly evenly matched in terms of their cardiac history, with the exception of less congestive heart failure among the new users’. New users were at increased risk of further CV events (mainly unstable angina) compared with never users and prior/current users adding a further important insight into the timing of initiation of such treatments.

The Women’s Angiographic Vitamin and Estrogen (WAVE) Trial [41], in ‘a 2 × 2 factorial design’, compared oCEE (0.625 mg/day), oCEE (0.625 mg/day) plus oMPA (2.5 mg/day), or placebo, and vitamin E (400 IU twice/day) with vitamin C (500 mg twice/day), or

placebo. This study included postmenopausal women (n = 423), with angiographically confirmed 15–75% stenosis in at least one coronary artery, with a mean follow-up of 2.8 years. The mean age for the MHT group was 65 years compared with 66 years in the placebo arm. The trial showed no improvement in coronary artery stenoses and suggested an increased incidence of CV events in both those treated with MHT alone, or vitamins E and C alone.

The EStrogen therapy for Prevention of ReInfarction Trial (ESPRIT) [42] was a multicentre randomized placebo controlled trial comparing oral oestradiol valerate (2 mg/day) to placebo in 1017 postmenopausal women with a prior MI (50–69 years old). There was no difference between oestradiol group and placebo group in the primary outcomes of re-infarction, cardiac death, and all-cause mortality at 2 years follow-up. The trial did not extend beyond this time because of the increased risk of endometrial cancer with the unopposed oestrogen use. Importantly, this trial did not show any adverse events and used oral oestradiol valerate, similar to that used by the EPAT primary prevention study [25]. Both ESPRIT and EPAT studies suggested that type and dose of oestrogen may contribute to adverse events in the secondary prevention studies. However, collectively, the results led to the conclusion that MHT should not be used for either primary or secondary prevention of CV diseases in postmenopausal women.

MECHANISMS OF SEX HORMONES ON CV RISK FACTORS DURING MENOPAUSE

In the 1990s, parallel to the above mentioned observational and clinical trials, basic research into the cellular and molecular aspects of the sex steroids was exploding. During that time period, oestrogen receptors α and β were differentiated and animals deficient in the receptors were generated. Cellular mechanisms focused on the antioxidant effects of oestrogen and on the production of various endothelium-derived factors, the most notable of which was nitric oxide, modulation of energy metabolism including mitochondrial function and regulation of cell proliferation and apoptosis [43,44].

Given the plethora of cellular mechanisms and pathways regulated by oestrogen (and other sex steroids), questions remain regarding potential CV benefits of MHT and the reconciliation of the results from the various observational, epidemiological and randomized clinical trials. Is there evidence that MHT modulates CV risk factors for women at menopause? Conventional CV diseases risk calculators consider the following variables: age, sex, body mass index, systolic blood pressure, HDL, use of antihypertensive medication and smoking status. Family history is included in the Reynold's CV risk calculation. However, these risk calculators typically underestimate risk of CV diseases in women, and it is now recommended that pregnancy history, in particular, a history of pregnancy complications of hypertensive pregnancy disorders and gestational diabetes [45] be added to accurately estimate the CV risk in women.

Elevations in cholesterol and LDL are risk factors for atherosclerosis. The clinical guidelines for managing these levels with lipid lowering medications are based on male pattern of occlusive coronary artery disease. As discussed above, oral oestrogens, due to the absorption

of the products into the entero-hepatic circulation (i.e. ‘first pass effect’) in the form of oCEE and oral 17 β-oestradiol, decrease LDL and increase HDL in recently menopausal women not using statins [18,20,21]. The relationship of the lipid profile to development of non-occlusive ischaemic disease in women is unclear and the specific effects of MHT on the incidence and prevalence of the manifestation of this condition has not been investigated.

Body mass index >30 kg/m^2 is a risk factor for CV diseases. This condition is usually accompanied by dyslipidaemia, chronic elevation in inflammatory cytokines and insulin resistance. These risk factors should be the primary target for risk reduction and management in women as data from the WHI suggest that MHT would be least effective in these individuals [34]. Effects of MHT on measures of insulin resistance in low risk women have not been studied directly but observations from KEEPS suggest that tE2 may reduce measures of insulin resistance when compared with oCEE in recently menopausal women [17].

The first observation that oestrogen via oestrogen receptors modulated endothelium-dependent flow mediated vasodilatation in humans was made in a man with genetic variants in oestrogen receptors. This individual, among other conditions, had accelerated atherosclerosis and also reactive hyperaemia was absent [46,47]. Endothelial dysfunction is considered an initial step of the pathogenesis of atherosclerosis. Oestrogen increases production and bioavailability of endothelium-derived nitric oxide through increases in gene transcription and antioxidant effects respectively. However, these actions may be antagonized by certain progestogens [43]. In addition, the relationship of genetic variants in oestrogen receptors and development of CV disease in women has not been established [48,49].

Although examination in genetic variants of oestrogen receptors has not yielded insight into CV risk with MHT in women, targeted genetic analysis of genes associated with innate immunity and coagulation pathways provide evidence of a significant pharmacogenomics interaction of genetic variants influencing effects of MHT. That is, genetic variants in genes associated with innate immunity influenced whether women using either oCEE or tE2 showed increases, decreases or no change in CIMT in the KEEPS Trial [33]. Although this study focused on the pharmacogenomics of MHT on CV outcome, metabolism of sex steroids, in particular oestrogen, involves multiple enzymes for sulfonation and oxidation. The area of pharmacogenomics of oestrogen metabolism is only beginning which may provide a more personalized approach to MHT in the future.

The fact that MHT reduces menopausal symptoms of sleep disturbances, vasomotor instability (hot flushes) and night sweats provides evidence that these hormones modulate responses mediated by the central and autonomic nervous system. Indeed, oestrogen affects uptake and synthesis on adrenergic transmitters, disposition of transmitters and sensitivity of adrenergic receptors [44,50]. Effects of MHT on regulation of blood pressure depends upon the age and type of hormone formulation, as in the WHI oCEE plus oMPA increased blood pressure but in KEEPS neither oCEE nor tE2 caused a significant change in blood pressure in recently menopausal women [2,20]. Also the increased activity of the renin angiotensin system contributes to the development of hypertension, and in a recent study, oestrogen was

found to protect against this process by down-regulating the angiotensin receptors [51]. Additional research is needed into aetiology of hypertension in women as most basic science studies of hypertension have used male animals, and studies of CV regulation in humans do not often take into account age and hormonal status. Furthermore, it is becoming clear that histories of hypertensive pregnancy disorders and even vasomotor instability at menopause may define an underlying phenotype that predisposes women to accelerated development of CV diseases [52,53]. A challenge for future research will be to develop convenient and inexpensive tests that could identify individuals early on a trajectory for developing CV disease to maximize efficient and cost effective intervention strategies.

CONCLUSIONS AND PERSPECTIVE

MHT is a cost effective approach to managing menopausal symptoms in women in the peri-menopausal and immediate menopausal period. Although large epidemiological and observational studies from the 1980–1990s supported that use of MHT reduced the incidence and risk of CV disease in menopausal women, several prospective studies, with designs that did not include women of comparable clinical status to those of the observational studies, provided important information that changed medical practice in regard to the timing of initiation of the treatment, formulations and doses. Furthermore, use of MHT is counter indicated for secondary prevention of CV disease. Although MHT is not recommended to be prescribed specifically for primary prevention of CV disease, some women who initiate MHT for relief of menopausal symptoms may have reduced risk for development of CV disease. Based on subsequent analysis of results from the WHI and other studies, women who might receive CV benefit from menopausal hormone use would be those who undergo bilateral oophorectomy before the age of 45 years, women who experienced natural menopause who initiate treatment within 5 years of menopause, are not obese and with modestly elevated lipid profiles. Because the formulations and doses of oestrogenic products have changed since the publication of results from the WHI, prescribing practices have become more individualized for the patient desired outcome. Therefore, future attempts to evaluate effects of MHT on CV health from observational studies will be challenging. Furthermore, much remains to be learned about the genomics of oestrogen metabolism and pharmacogenomics of existing genetic variants in response to MHT [33]. Therefore, future investigations that are designed to understand CV function throughout the hormonal transitions of a woman’s life including use of MHT must take an integrated approach to consider genetics, metabolism, vascular components (e.g. the endothelium), autonomic control mechanisms as well as accounting for comorbidities, medications and other environmental factors.

Acknowledgments

This manuscript was written in partial fulfilment of requirements for the Mayo Clinic Graduate School of Medicine Biomedical Engineering (BME) 6855 Tutorial in Cardiovascular Physiology course.

FUNDING

This work was supported by the Mayo Clinic Graduate School of Medicine; NIH grant AG44170 (to V.M.M.); the Clinical and Translational Science Award [grant number UL1 TR000135], supporting the Mayo Clinic Center for

Clinical and Translational Science (CCaTS); and the National Center for Advancing Translational Sciences (NCATS), a component of NIH.

Abbreviations

CAC	coronary artery calcification
CHD	coronary heart disease
CIMT	carotid intima-medial thickness
CV	cardiovascular
ELITE	Early versus Late Intervention Trial with Estradiol
EPAT	Estrogen in the Prevention of Atherosclerosis Trial
ESPRIT	EStrogen therapy for Prevention of ReInfarction Trial
HDL	high density lipoprotein
KEEPS	Kronos Early Estrogen Prevention Study
LDL	low density lipoprotein
MHT	menopausal hormone treatments
MI	myocardial infarction
NHS	Nurses' Health Study
NIH	National Institutes of Health
oCEE	oral conjugated equine oestrogen
oMPA	oral medroxyprogesterone acetate
tE2	transdermal 17 β-oestradiol
WHI	Women's Health Initiative

References

1. Kaunitz AM, Manson JE. Management of menopausal symptoms. Obstet Gynecol. 2015; 126:859–876. [PubMed: 26348174]
2. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002; 288:321–333. [PubMed: 12117397]
3. Miller VM, Black DM, Brinton EA, Budoff MJ, Cedars MI, Hodis HN, Lobo RA, Manson JE, Merriam GR, Naftolin F, et al. Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). J Cardiovasc Transl Res. 2009; 2:228–239. [PubMed: 19668346]
4. Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, Anderson G, Howard BV, Thomson CA, LaCroix AZ, et al. Menopausal hormone therapy and health outcomes

during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. JAMA. 2013; 310:1353–1368. [PubMed: 24084921]

5. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA. 2004; 291:1701–1712. [PubMed: 15082697]
6. Harman SM, Black DM, Naftolin F, Brinton EA, Budoff MJ, Cedars MI, Hopkins PN, Lobo RA, Manson JE, Merriam GR, et al. Arterial imaging outcomes and cardiovascular risk factors in recently menopausal women: a randomized trial. Ann Intern Med. 2014; 161:249–260. [PubMed: 25069991]
7. Schierbeck LL, Rejnmark L, Tofteng CL, Stilgren L, Eiken P, Mosekilde L, Kober L, Jensen JE. Effect of hormone replacement therapy on cardiovascular events in recently postmenopausal women: randomised trial. BMJ. 2012; 345:e6409. [PubMed: 23048011]
8. Moyer VA. Menopausal hormone therapy for the primary prevention of chronic conditions: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2013; 158:47–54. [PubMed: 23090711]
9. Shifren JL, Gass ML. The North American Menopause Society recommendations for clinical care of midlife women. Menopause. 2014; 21:1038–1062. [PubMed: 25225714]
10. Gabriel SR, Carmona L, Roque M, Sanchez GL, Bonfill X. Hormone replacement therapy for preventing cardiovascular disease in post-menopausal women. Cochrane Database Syst Rev. 2005:CD002229. [PubMed: 15846631]
11. Boardman HM, Hartley L, Eisinga A, Main C, Roque i Figuls M, Bonfill Cosp X, Gabriel Sanchez R, Knight B. Hormone therapy for preventing cardiovascular disease in post-menopausal women. Cochrane Database Syst Rev. 2015; 3:CD002229.
12. Utian WH, Archer DF, Bachmann GA, Gallaher JC, Grodstein F, Heiman JR, Henderson VW, Hodis HN, Karas RH, Lobo RA, et al. Estrogen and progestogen use in postmenopausal women: July 2008 position statement of The North American Menopause Society. Menopause. 2008; 15:584–602. [PubMed: 18580541]
13. Nichols FH. History of the Women's Health Movement in the 20th century. J Obstet Gynecol Neonatal Nurs. 2000; 29:56–64.
14. Sandberg K, Umans JG. Recommendations concerning the new U.S. National Institutes of Health initiative to balance the sex of cells and animals in preclinical research. FASEB J. 2015; 29:1646–1652. [PubMed: 25713032]
15. Clow, B., Pederson, A., Haworth-Brockman, M., Bernier, J. Gender-based Analysis and Health Planning, Policy and Research in Canada. 2009. http://www.dal.ca/content/dam/dalhousie/pdf/ace-women-health/ACEWH_rising_to_the_challenge_SGBA.pdf
16. Maas AH, van der Schouw YT, Regitz-Zagrosek V, Swahn E, Appelman YE, Pasterkamp G, Ten Cate H, Nilsson PM, Huisman MV, Stam HC, et al. Red alert for women's heart: the urgent need for more research and knowledge on cardiovascular disease in women: proceedings of the workshop held in Brussels on gender differences in cardiovascular disease, 29 September 2010. Eur Heart J. 2011; 32:1362–1368. [PubMed: 21406440]
17. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, et al. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. Circulation. 2015; 131:e29–e322. [PubMed: 25520374]
18. Kohn, L. Better Oversight Needed to Help Ensure Continued Progress Including Women in Health Research. 2015. http://www.gao.gov/assets/680/673276.pdf
19. Archer DF. Premature menopause increases cardiovascular risk. Climacteric. 2009; 12(Suppl 1): 26–31.
20. Rivera CM, Grossardt BR, Rhodes DJ, Brown RD Jr, Roger VL, Melton LJ 3rd, Rocca WA. Increased cardiovascular mortality after early bilateral oophorectomy. Menopause. 2009; 16:15–23. [PubMed: 19034050]
21. Bush TL, Cowan LD, Barrettconnor E, Criqui MH, Karon JM, Wallace RB, Tyroler HA, Rifkind BM. Estrogen use and all-cause mortality. Preliminary results from the Lipid Research Clinics Program Follow-Up-Study. J Am Med Assoc. 1983; 249:903–906.

22. Bush TL, Barrett-Connor E, Cowan LD, Criqui MH, Wallace RB, Suchindran CM, Tyroler HA, Rifkind BM. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. Circulation. 1987; 75:1102–1109. [PubMed: 3568321]

23. Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. Ann Intern Med. 2000; 133:933–941. [PubMed: 11119394]

24. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial JAMA. 1995; 273:199–208.

25. Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, Selzer RH, Liu Cr CR, Liu Ch CH, Azen SP. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2001; 135:939–953. [PubMed: 11730394]

26. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group Control Clin. Trials. 1998; 19:61–109.

27. Manson JE, Allison MA, Rossouw JE, Carr JJ, Langer RD, Hsia J, Kuller LH, Cochrane BB, Hunt JR, Ludlam SE, et al. Estrogen therapy and coronary-artery calcification. N Engl J Med. 2007; 356:2591–2602. [PubMed: 17582069]

28. Miller VM, Manson JE. Women's health initiative hormone therapy trials: new insights on cardiovascular disease from additional years of follow up. Curr Cardiovasc Risk Rep. 2013; 7:196–202. [PubMed: 23682305]

29. Toh S, Hernandez-Diaz S, Logan R, Robins JM, Hernan MA. Estimating absolute risks in the presence of nonadherence: an application to a follow-up study with baseline randomization. Epidemiology. 2010; 21:528–539. [PubMed: 20526200]

30. Hernan MA, Alonso A, Logan R, Grodstein F, Michels KB, Willett WC, Manson JE, Robins JM. Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease. Epidemiology. 2008; 19:766–779. [PubMed: 18854702]

31. Clarkson TB, Appt SE. Coronary artery disease and postmenopausal hormone therapy: is there a time window for prevention? Gynaecol Forum. 2004; 9:11–14.

32. Harman SM, Brinton EA, Cedars M, Lobo R, Manson JE, Merriam GR, Miller VM, Naftolin F, Santoro N. KEEPS: the Kronos Early Estrogen Prevention Study. Climacteric. 2005; 8:3–12.

33. Miller VM, Jenkins GD, Biernacka JM, Heit JA, Huggins GS, Hodis HN, Budoff MJ, Lobo RA, Taylor HS, Manson JE, et al. Pharmacogenomics of estrogens on changes in carotid artery intima-medial thickness and coronary arterial calcification – Kronos Early Estrogen Prevention Study. Physiol Genomics. 2016; 48:33–41. [PubMed: 26508701]

34. Canonico M, Oger E, Plu-Bureau G, Conard J, Meyer G, Levesque H, Trillot N, Barrellier MT, Wahl D, Emmerich J, Scarabin PY. Hormone therapy and venous thromboembolism among postmenopausal women – impact of the route of estrogen administration and progestogens: The ESTHER study. Circulation. 2007; 115:840–845. [PubMed: 17309934]

35. Hodis HN, Mack WJ, Shoupe D, Azen SP, Stanczyk FZ, Hwang-Levine J, Budoff MJ, Henderson VW. Methods and baseline cardiovascular data from the early versus late intervention trial with estradiol testing the menopausal hormone timing hypothesis. Menopause. 2015; 22:391–401. [PubMed: 25380275]

35a. Hodis HN, Mack WJ, Henderson VW, Shoupe D, Budopp MJ, Hwang-Levine J, Li Y, Feng M, Dustin L, Kono N, et al. Vascular effects of early versus late postmenopausal treatment with estradiol. New Eng J Med. 2016; 374:1221–1231. [PubMed: 27028912]

36. Mikkola TS, Tuomikoski P, Lyytinen H, Korhonen P, Hoti F, Vattulainen P, Gissler M, Ylikorkala O. Increased cardiovascular mortality risk in women discontinuing postmenopausal hormone therapy. J Clin Endocrinol Metab. 2015; 100:4588–4594. [PubMed: 26414962]

37. Mikkola TS, Tuomikoski P, Lyytinen H, Korhonen P, Hoti F, Vattulainen P, Gissler M, Ylikorkala O. Estradiol-based postmenopausal hormone therapy and risk of cardiovascular and all-cause mortality. Menopause. 2015; 22:976–983. [PubMed: 25803671]

38. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. JAMA. 1998; 280:605–613. [PubMed: 9718051]
39. Herrington DM, Reboussin DM, Brosnihan KB, Sharp PC, Shumaker SA, Snyder TE, Furberg CD, Kowalchuk GJ, Stuckey TD, Rogers WJ, et al. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. N Engl J Med. 2000; 343:522–529. [PubMed: 10954759]
40. Alexander KP, Newby LK, Hellkamp AS, Harrington RA, Peterson ED, Kopecky S, Langer A, O'Gara P, O'Connor CM, Daly RN, et al. Initiation of hormone replacement therapy after acute myocardial infarction is associated with more cardiac events during follow-up. J Am Coll Cardiol. 2001; 38:1–7. [PubMed: 11451256]
41. Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, Ouyang P, Thompson P, Tardif JC, Higginson L, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. JAMA. 2002; 288:2432–2440. [PubMed: 12435256]
42. Cherry N, Gilmour K, Hannaford P, Heagerty A, Khan MA, Kitchener H, McNamee R, Elstein M, Kay C, Seif M, Buckley H. Oestrogen therapy for prevention of reinfarction in postmenopausal women: a randomised placebo controlled trial. Lancet. 2002; 360:2001–2008. [PubMed: 12504395]
43. Duckles SP, Miller VM. Hormonal modulation of endothelial NO production. Pflugers Arch. 2010; 459:841–851. [PubMed: 20213497]
44. Miller VM, Duckles SP. Vascular actions of estrogens: functional implications. Pharmacol Rev. 2008; 60:210–241. [PubMed: 18579753]
45. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women – 2011 update a guideline from the American Heart Association. J Am Coll Cardiol. 2011; 57:1404–1423. [PubMed: 21388771]
46. Sudhir K, Chou TM, Chatterjee K, Smith EP, Williams TC, Kane JP, Malloy MJ, Korach KS, Rubanyi GM. Premature coronary artery disease associated with a disruptive mutation in the estrogen receptor gene in a man. Circulation. 1997; 96:3774–3777. [PubMed: 9396482]
47. Sudhir K, Elser MD, Jennings GL, Komesaroff PA. Estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women. Hypertension. 1997; 30:1538–1543. [PubMed: 9403579]
48. Rossouw J, Bray P, Liu J, Kooperberg C, Hsia J, Lewis C, Cushman M, Bonds D, Hendrix S, Papanicolaou G, et al. Estrogen receptor polymorphisms and the vascular effects of hormone therapy. Arterioscler Thromb Vasc Biol. 2011; 31:464–469. [PubMed: 21106950]
49. Miller VM, Petterson TM, Jeavons EN, Lnu AS, Rider DN, Heit JA, Cunningham JM, Huggins GS, Hodis HN, Budoff MJ, et al. Genetic polymorphisms associated with carotid artery intima-media thickness and coronary artery calcification in women of the Kronos Early Estrogen Prevention Study. Physiol Genomics. 2013; 45:79–88. [PubMed: 23188791]
50. Joyner MJ, Barnes JN, Hart EC, Wallin BG, Charkoudian N. Neural control of the circulation: how sex and age differences interact in humans. Compr Physiol. 2015; 5:193–215. [PubMed: 25589269]
51. Hinojosa-Laborde C, Craig T, Zheng W, Ji H, Haywood JR, Sandberg K. Ovariectomy augments hypertension in aging female Dahl salt-sensitive rats. Hypertension. 2004; 44:405–409. [PubMed: 15337736]
52. Hautamaki H, Piirila P, Haapalahti P, Tuomikoski P, Sovijarvi ARA, Ylikorkala O, Mikkola TS. Cardiovascular autonomic responsiveness in postmenopausal women with and without hot flushes. Maturitas. 2011; 68:368–373. [PubMed: 21310559]
53. Lantto H, Mikkola TS, Tuomikoski P, Viitasalo M, Vaananen HL, Sovijarvi ARA, Haapalahti P. Cardiac repolarization in recently postmenopausal women with or without hot flushes. Menopause. 2016; 23:528–534. [PubMed: 26599687]

Table 1

The effects of the use of sex hormones on cardiovascular diseases in menopausal women

	Trial (publication year) [reference]	Age*	Formulations, doses and duration of sex hormones' use	Duration* of follow-up	CV outcomes**
Primary prevention	The Postmenopausal Estrogen/Progestin Interventions Trial (1995) [24]	45–64	oCEE, 0.625 mg/day; oCEE, 0.625 mg/day, plus cyclic oMPA, 10 mg/day, for 12 days/month; oCEE, 0.625 mg/day, plus consecutive oMPA, 2.5 mg/day; or oCEE, 0.625 mg/day, plus cyclic micronized progesterone, 200 mg/day for 12 days/month	3	• Oestrogen alone or in combination with a progestin improves lipoproteins and lowers fibrinogen levels without detectable effects on post-challenge insulin or blood pressure. • Unopposed oestrogen is the optimal regimen for elevation of HDL, but the high rate of endometrial hyperplasia restricts use to women without a uterus. In women with a uterus, oCEE with cyclic micronized progesterone has the most favourable effect on HDL and no excess risk of endometrial hyperplasia.
	The Estrogen in the Prevention of Atherosclerosis Trial (2001) [25]	60.9 (6.7)	Unopposed micronized 17 β-oestradiol, 1 mg/day	2	• Overall, the average rate of progression of subclinical atherosclerosis was slower in healthy postmenopausal women taking 17 β-oestradiol than in women taking placebo. • Reduction in the progression of subclinical atherosclerosis was seen in women who did not take lipid-lowering medication but not in those who took these medications.
	The Women's Health Initiative Randomized Controlled Trial of Estrogen plus Progestin (2002) [2]	63.2 (7.1)	oCEE, 0.625 mg/day, plus oMPA, 2.5 mg/day	5.2	• Overall health risks exceeded benefits from use of combined oestrogen plus progestin for an average 5.2-year follow-up among healthy postmenopausal US women. • All-cause mortality was not affected during the trial. • The risk-benefit profile found in this trial is not consistent with the requirements for a viable intervention for primary prevention of chronic diseases, and the results indicate that this regimen should not be initiated or continued for prevention of CHD.
	The Kronos Early Estrogen Prevention Study (2014) [6]	42–58	oCEE (Premarin, 0.45 mg/day) plus oral progesterone (Prometrium; micronized progesterone, 200 mg/day) for 12 days/month; tE2 (Climera, 50 μg/day) plus oral progesterone (Prometrium; micronized	4	• Four years of early MHT did not affect progression of atherosclerosis as measured by changes in CIMT despite improving some markers of CV disease risk.

	Trial (publication year) [reference]	Age*	Formulations, doses and duration of sex hormones' use	Duration* of follow-up	CV outcomes**
			progesterone, 200 mg/day) for 12 days/month		
	The Early versus Late Intervention Trial with Estradiol (2015) [35]	55.4, median time since menopause was 3.5 'Early' 65.4, median time since menopause was 14.3 'Late'	Oral micronized 17 β-oestradiol, 1 mg/day, with 4% vaginal micronized progesterone gel, 45 mg/day, for 10 days/month for women with intact uterus oral micronized 17 β-oestradiol, 1 mg/day, alone for women without intact uterus	16 '10 of randomized treatment and 6 post-intervention'	• Reduced incidence of CHD and total mortality in young postmenopausal women 'younger than 60 years' who initiate MHT near menopause '<10 years since menopause'.
Secondary prevention	The Heart and Estrogen/progestin Replacement Study (1998) [38]	67	oCEE, 0.625 mg/day, plus oMPA, 2.5 mg/day	4.1	• During an average follow-up of 4.1 years, treatment with oCEE plus oMPA did not reduce the overall rate of CHD events in postmenopausal women with established coronary disease. • The treatment did increase the rate of thromboembolic events and gallbladder disease. • Based on the finding of no overall CV benefit and a pattern of early increase in risk of CHD events, we do not recommend starting this treatment for the purpose of secondary prevention of CHD. • However, given the favourable pattern of CHD events after several years of therapy, it could be appropriate for women already receiving this treatment to continue.
	The Estrogen Replacement and Atherosclerosis Trial (2000) [39]	65.8	oCEE, 0.625 mg/day, alone; oCEE, 0.625 mg/day, plus oMPA, 2.5 mg/day	3.2	• Neither oCEE alone nor oCEE plus oMPA affected the progression of coronary atherosclerosis in women with established CV disease. • These results suggest that such women should not use oestrogen replacement with an expectation of CV benefit.
	The Coumadin Aspirin Reinfarction Study (2001) [40]	67 (60–73), never users of MHT 59 (52–66), prior/current users of MHT 58 (51–65), new users of MHT	Different MHT were used by prior/current users and new users	1	• Postmenopausal women who initiated MHT after a recent MI had an increased risk of CV events largely due to excess unstable angina during follow-up.
	The Women's Angiographic Vitamin and Estrogen Trial (2002) [41]	65, MHT group	oCEE, 0.625 mg/day, alone; oCEE, 0.625 mg/day, plus oMPA, 2.5 mg/day	2.8	• In postmenopausal women with coronary disease, neither MHT nor antioxidant vitamin supplements provide CV benefit.

Clin Sci (Lond). Author manuscript; available in PMC 2017 February 19.

Trial (publication year) [reference]	Age*	Formulations, doses and duration of sex hormones' use	Duration* of follow-up	CV outcomes**
				• Instead, a potential for harm was suggested with each treatment.
EStrogen therapy for Prevention of ReInfarction Trial (2002) [42]	50–69	Oral oestradiol valerate, 2 mg/day	2	• Oestradiol valerate does not reduce the overall risk of further cardiac events in postmenopausal women who have survived a MI.

A comparison between main studies that evaluated the effects of the use of sex hormones for primary and secondary prevention of cardiovascular diseases in menopausal women.

*Reported in years – except if otherwise mentioned – as mean with or without range or S.D., or median with or without interquartile range [IQR], or range, or average.

**Published conclusions of each study.