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Postmenopausal hot flushes, vascular health and hormone therapy

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Academic dissertation

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ABSTRACT

Vasomotor hot flushes are complained of by approximately three out of four postmenopausal women, but their frequency and severity show great individual variation. Estrogen withdrawal is a key factor of hot flushes, but the autonomic nervous system, regulating cardiovascular function, may also be involved. Hot flushes have been present in women attending observational studies, but they have been absent (or very mild) in randomized trials. Therefore, if hot flushes are a factor connected with vascular health, they could perhaps be one explanation for the divergence of cardiovascular data in observational versus randomized studies.

The present study was designed to determine the possible impact of vasomotor symptoms on vascular health. One hundred and fifty healthy, recently postmenopausal women showing a large variation in hot flushes were studied in regard to cardiovascular health by way of pulse wave analysis, ambulatory blood pressure and several biochemical vascular markers. In addition, the responses of these variables to hormone therapy were studied. The severity of hot flushes was assessed by using a two-week hot flush diary.

After the nitroglycerin challenge in pulse wave analysis, the time to the onset of the reflected wave (dependent on pulse wave velocity) was 9.2% shorter (p=0.014), and the time to the first systolic peak (dependent on the rapid phase of ventricular ejection) was 12.3% shorter (p=0.025) in asymptomatic women compared with women with severe hot flushes. This can be seen as evidence of vasodilatory reactivity associated with severe hot flushes, and thus, as a hot flush-related vascular benefit. Severe night-time hot flushes were accompanied by transient increases in ambulatory systolic (4.1±10.5 mmHg, p=0.061) and diastolic (3.1±6.8 mmHg, p=0.032) blood pressure and heart rate (3.0±7.2 beats/minute, p=0.043), but the diurnal blood pressure profiles showed no significant difference between women without and with hot flushes of different severity. Vascular biomarkers were unaffected by hot flushes.

In the 6-month hormone therapy trial the women were classified as having either tolerable (≤3 mild episodes/day, not likely to initiate hormone therapy in routine clinical practice) or intolerable (≥7 moderate/severe episodes/day, likely to initiate hormone therapy) hot flushes. These groups were treated in a randomized order with transdermal estradiol hemihydrate gel (1 mg/day), oral estradiol valerate (2 mg/day) alone or in combination with medroxyprogesterone acetate (5 mg/day), or with placebo.

In women with tolerable hot flushes, oral estradiol led to decreases in the time to the first systolic peak (13.2%, p=0.028) and the time to the reflected wave (8.4%, p=0.018) after the nitroglycerin challenge. This potentially unfavorable vasoconstrictive effect was not seen in women with intolerable hot flushes or with the other treatment regimens. Additionally, 24-hour systolic (3.7±1.2 mmHg, p=0.010) and diastolic (1.8±0.8 mmHg, p=0.003) blood pressures rose in women with tolerable hot flushes receiving oral estradiol, whereas decreases (-1.2±1.2 mmHg and -2.1±0.8 mmHg, respectively) were detected in women with intolerable hot flushes. Daytime systolic and diastolic blood pressures also increased (3.0±1.3 mmHg, p=0.017 and 1.8±0.9 mmHg, p=0.003, respectively) in women with tolerable hot flushes with oral estradiol treatment, but the same therapy led to falls (-1.9±1.3 mmHg and 2.4±0.9 mmHg, respectively) in women with intolerable hot flushes. No such effects were observed as regards transdermal estradiol or oral estradiol accompanied by medroxyprogesterone acetate. The responses of lipids, lipoproteins, C-reactive protein and sex hormone-binding globulin to hormone therapy showed no association with hot flush status.

In conclusion, hot flushes may be associated with an increased vasodilatory, and thus a beneficial vascular status. Furthermore, oral estradiol leads to vasoconstrictive changes, and thus to possible vascular harm, but only in women whose hot flushes are so mild that they would probably not lead to the initiation of hormone therapy in clinical practice. Such potential vascular harm of hormone therapy was not seen in women with intolerable hot flushes, or with the other treatment regimens.

Taken as a whole, hot flush status contributes to cardiovascular health before and during hormone therapy. Severe hot flushes associate with enhanced vascular reactivity. Potentially unfavorable vascular effects of oral hormone therapy are seen mainly in women without troublesome hot flushes. Thus, if estrogen is prescribed for indications other than for the control of hot flushes, transdermal route of administration should be favored.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I Evidence for a role of hot flushes in vascular function in recently postmenopausal women. Tuomikoski P, Ebert P, Haapalahti P, Hautamäki H, Rönnback M, Ylikorkala O, Mikkola TS. Obstetrics and Gynecology 2009;113:902–8.

II Vasomotor hot flushes and 24-hour ambulatory blood pressure in recently postmenopausal women. Tuomikoski P, Haapalahti P, Ylikorkala O, Mikkola TS. Annals of Medicine: in press.

III Biochemical markers for cardiovascular disease in recently postmenopausal women with or without hot flushes. Tuomikoski P, Mikkola TS, Hämäläinen E, Tikkanen MJ, Turpeinen U, Ylikorkala O. Menopause 2010;17:145–51.

IV Effect of hot flushes on vascular function: A randomized, controlled trial. Tuomikoski P, Ebert P, Groop P-H, Haapalahti P, Hautamäki H, Rönnback M, Ylikorkala O, Mikkola TS. Obstetrics and Gynecology 2009;114:777–85.

V Impact of hot flushes on 24-hour ambulatory blood pressure in normotensive women: A placebo-controlled trial on postmenopausal hormone therapy. Tuomikoski P, Haapalahti P, Sarna S, Ylikorkala O, Mikkola TS. Annals of Medicine: in press.

VI Hot flushes and biochemical markers for cardiovascular disease: A randomized trial on hormone therapy. Tuomikoski P, Mikkola TS, Tikkanen MJ, Ylikorkala O. Climacteric: in press.

ABBREVIATIONS

AIx	Augmentation index
apoA-I	Apolipoprotein A-I
apoB	Apolipoprotein B
BMI	Body mass index
BP	Blood pressure
CEE	Conjugated equine estrogens
CHD	Coronary heart disease
CI	Confidence interval
CVD	Cardiovascular disease
EFI	Endothelial function index
EPT	Estrogen-progestagen therapy
ET	Estrogen-only therapy
FEI	Free estradiol index
FMD	Flow-mediated dilation
FSH	Follicle-stimulating hormone
HDL	High-density lipoprotein cholesterol
HERS	Heart and Estrogen/progestin Replacement Study
HFWWS	Hot flush weekly weighted score
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
HT	Hormone therapy
LDL	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
Lp (a)	Lipoprotein (a)
MPA	Medroxyprogesterone acetate
OR	Odds ratio
PWA	Pulse wave analysis
SD	Standard deviation
SEM	Standard error of mean
SHBG	Sex hormone-binding globulin
RR	Risk ratio
VLDL	Very low-density lipoprotein cholesterol
WHI	Women's Health Initiative

INTRODUCTION

Hormone therapy (HT) has long been used for the treatment of menopausal symptoms, such as hot flushes. Results from an abundance of observational studies showed a 30–50% reduction in the risk of cardiovascular disease (CVD) in current users of HT. Thus, over a decade ago HT was commonly recommended for the prevention of CVD (*Mikkola and Clarkson 2002, Mikkola and Ylikorkala 2005*). However, results from randomized, placebo-controlled trials, such as the Heart and Estrogen/progestin Replacement Study (HERS) (*Hulley et al. 1998, Grady et al. 2002*) and the Women's Health Initiative (WHI) (*Rossouw et al. 2002, Manson et al. 2003*) indicated an increased risk of CVD in HT users (*Hodis and Mack 2008*). These findings, widely debated in both the medical and lay press, led to major changes in clinical practice when millions of women around the world discontinued use of HT (*Utian 2007*).

Numerous plausible explanations for these divergent data have been suggested, including the "healthy woman" bias in observational studies, and possible differences in treatment regimens and study populations between observational and randomized studies (*Mikkola and Clarkson 2002*, *Grodstein et al. 2003*). The presence of vasomotor hot flushes has also emerged as one possible explanation (*Mikkola and Ylikorkala 2005*, *van der Schouw and Grobbee 2005*). Hypoestrogenism certainly contributes to the onset of hot flushes, but the autonomic nervous system, which regulates the vascular bed, among other things, may be involved (*Freedman 1998*). In clinical practice women with severe hot flushes seek HT treatment, which is the most effective treatment against for them, and thus, such women have been included in HT groups in observational studies. In contrast, hot flushes have been absent, or very mild, in women taking part in randomized clinical trials, where women with severe hot flushes have been excluded from participation.

Differences also exist in treatment regimens, e.g. in United States the use of conjugated equine estrogens (CEE) is favored, whereas in Europe estradiol-based regimens dominate (*Mikkola and Clarkson 2002*, *Grodstein et al. 2003*, *van der Schouw and Grobbee 2005*). It is also possible that progestagens possess direct or indirect vascular effects (*Koh and Sakuma 2004*). In this regard medroxyprogesterone acetate (MPA) has been most widely discussed, because MPA as a continuous complement of CEE was present in both the HERS and WHI trials. However, it is still unsettled whether the vascular effect of MPA is beneficial or harmful (*Clarkson and Appt 2003*).

Moreover, oral and transdermal administration of estrogen leads to different estrogenic milieus (*Kuhl 2005*) and thus, the cardiovascular effects of orally and transdermally administered HT may differ (*Godsland 2001*).

The present studies explored the potential impact of hot flushes of different severity on various factors reflecting vascular health before and during six months of oral or transdermal estradiol, the former with and without MPA.

REVIEW OF THE LITERATURE

Menopause

In 2008 in Finland 191 000 women were between 50–54 years of age (*Statistics Finland 2009*), when women typically enter natural menopause (*Greendale 1999*). Menopause is a consequence of the slow deterioration of ovarian function. The primary trigger for ovarian senescence is not known, but genetically controlled apoptosis of the ovarian cells is involved (*Vaskivuo and Tapanainen 2003*). Therefore, it is no surprise that menopausal age shows a significant familial tendency (*de Bruin et al. 2001*, *Murabito et al. 2005*). Menopause is defined as the onset of the last menstruation, followed by 6–12 months of amenorrhea and therefore, this diagnosis can only be made retrospectively. Menopause before the age of 40 is defined as premature, and it is considered a disease-like condition. Some epidemiological factors, such as smoking, low body mass index (BMI), menstrual irregularities and nulliparity are associated with earlier menopause (*Barton et al. 2001*, *Nelson 2008*), whereas data on the influence of age at menarche and on oral contraceptive use are controversial (*Harlow and Signorello 2000*). Furthermore, socioeconomic factors, such as a low degree of education, unemployment or being divorced or widowed are independent risk factors of early menopause. Race and ethnicity are also associated with the timing of natural menopause (*Gold et al. 2001*), and for instance, Latin women experience menopause approximately nine months earlier than non-Latin white women, while Japanese race/ethnicity is associated with later menopause (*Henderson et al. 2008*). Furthermore, hysterectomy may be associated with 1–2 years earlier onset of menopause (*Lobo 2007*). It is also noteworthy that hypercholesterolemia and hypertension are predisposing factors as regards both earlier menopause and CVD (*Kok et al. 2006*).

During the menopausal transition the level of inhibin B originating from the ovaries decreases and the levels of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increase (Figure 1). As the level of FSH rises, numerous small ovarian follicles undergo activation, and this leads to increases in circulating estradiol levels at an early stage of menopausal transition. However, this is only a transient phenomenon and is not seen in all women, and hypoestrogenism is the ultimate outcome of menopause.

With increasing age the levels of FSH and LH continue to rise, but despite this, follicle depletion progresses (*Burger et al. 2007*, *Kase 2009*). Estradiol produced by the ovaries is the main estrogen during the fertile years, whereas after menopause circulating levels of estradiol are low (usually <20 pmol/L), and estrone (usually <300pmol/L), produced by aromatization in fatty tissue, is the dominating estrogen after menopause. After menopause circulating levels of FSH and LH continue to rise for several years, but in later menopause these levels go down. The levels of LH are of importance because they stimulate androgen secretion from the ovarian stroma. The ovaries are not the only source of androgens, and they are also derived from estrogen aromatization in fatty tissue (*Kase 2009*).

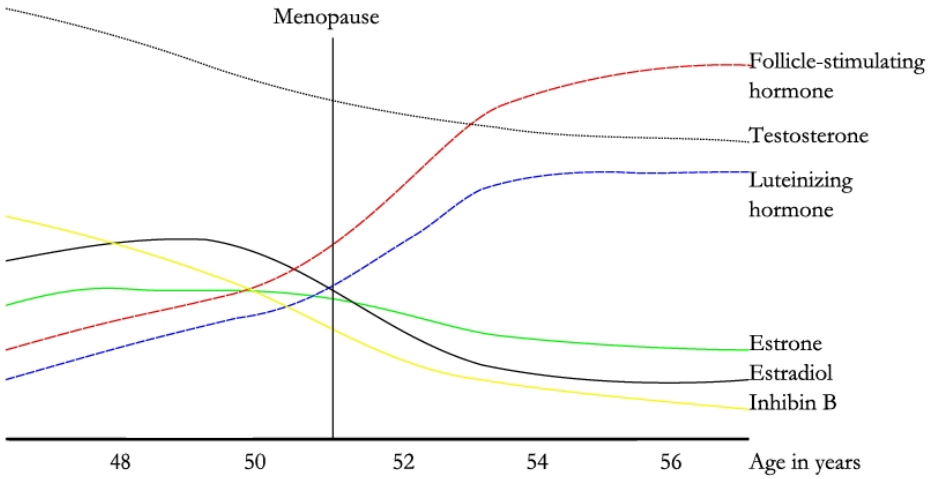

Figure 1. Schematic presentation of the changes in some reproductive hormones and inhibin B during perimenopause *(modified from Strauss and Barbieri 2004).*

Hot flushes

The most typical features of menopause are hot flushes, also referred to as hot flashes or vasomotor symptoms, which are present in up to 75–80% of all women in menopausal age groups (*Dennerstein et al. 2000*, *Stearns et al. 2002*, *Deecher and Dorries 2007*). The frequency of hot flushes usually peaks within the year of the last menstruation (*Nelson 2008*, *Santoro 2008*). In most women hot flushes subside with advancing age, but approximately 20% of women continue to have hot flushes several years after menopause, and in many women symptoms may persist well into their seventies (*Santoro 2008*).

Hot flushes are characterized by rapid episodes of flushing/reddening of the skin and sensations of heat and sweating. They usually start from the chest and rise up to the neck and face, and hot flushes may sometimes be followed by chills (*Deecher and Dorries 2007*, *Sturdee 2008*). Hot flushes are often accompanied by palpitations, anxiousness and broken sleep. There is great individual variation in the frequency and severity of flushes, ranging from sensations lasting from a few seconds to up to an hour, and reoccurring only seldom or almost instantaneously (*Nelson 2008*). Approximately 20% of women regard hot flushes as intolerable (*Hickey et al. 2005*, *Skurnick et al. 2009*), but in almost all women hot flushes may to some extent endanger adequate sleep and lead to a number of other symptoms, such as nervousness or depressed mood, which are more or less secondary to hot flushes. As a net consequence of hot flushes the overall quality of life significantly decreases (*Polo-Kantola et al. 2001*, *Utian 2005*, *Rapkin 2007*, *Ensrud et al. 2009*, *Luoto 2009*).

The etiology of hot flushes is not known. They seem to be preceded by a rise in core body temperature (*Freedman 1998*) (Figure 2). Several factors may trigger this thermoregulatory dysfunction; for instance changes in the circulating concentrations of LH, FSH, estrogens, serotonin, norepinephrine, calcitonin gene-related peptide and neuropeptide Y may be involved (*Barton et al. 2001*, *Shanafelt et al. 2002*, *Whiteman et al. 2003*, *Rapkin 2007*). Fluctuations in estrogen levels that occur during the menopausal transition appear as one key factor of the disturbed thermoregulation (Figure 2). Although the impact of hypoestrogenism on hot flushes is indisputable, no clear associations between the levels of endogenous sex hormones and the presence or severity of hot flushes have been found (*Øverlie et al. 2002*, *Whiteman et al. 2003*, *Randolph et al. 2005*, *Götmar et al. 2008*, *Santoro 2008*). In contrast, FSH elevations, which reflect the degree of hypoestrogenism, show clear correlations to hot flushes (*Randolph et al. 2005*).

The exact details of this thermoregulatory dysfunction in the hypothalamus are not understood, but a rise in core body temperature is somehow registered by the narrowed thermoregulatory zone in the hypothalamus. This launches heat-dispersal mechanisms, partly through changes in the autonomic nervous system, and results in cutaneous vasodilatation, flushing and sweating. These compensatory mechanisms may sometimes overreact, and chills ensue after hot flushes (Figure 2) (*Freedman 2005*, *Deecher and Dorries 2007*, *Sturdee 2008*).

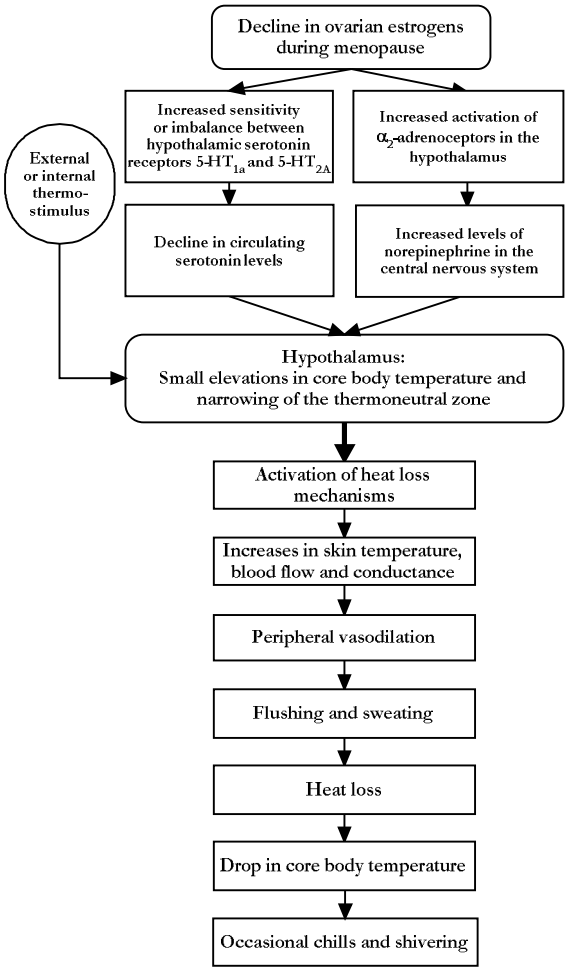

Figure 2. Possible physiological mechanisms for the initiation of hot flushes (*modified from Freedman 1998*, *Stearns et al. 2002*, *Freedman 2005*, *Deecher and Dorries 2007*).

Vasomotor symptoms can be reliably evaluated prospectively by using questionnaires in which both the frequency and severity of symptoms can be assessed (Table 1). One of the most established tests is the Hot Flush Weekly Weighted Score (HFWWS), in which mild symptoms are scored with 1, moderate symptoms are score 2, and severe symptoms are scored 3 (*Sloan et al. 2001*, *Loprinzi and Barton 2009*). With such a system it is possible to estimate the overall burden of hot flushes, because both their frequency and severity are accounted for. Recording hot flushes via such questionnaires mimics a clinical situation where a women herself must subjectively approximate the overall impact of hot flushes and decide on the possible initiation of HT. For pure research purposes, hot flushes have also been objectively quantified by measuring dermal temperature increase and/or changes in skin conductance (*Sievert 2007*). However, such tests are not very sensitive and are prone to many errors. Therefore, in clinical research, hot flushes are most commonly assessed by means of a subjective scale. This must be completed over a reasonably long period, usually for a minimum of 1–2 weeks, because hot flushes show relatively great day-to-day variation (*Sloan et al. 2001*).

Hot flushes	**Definitions**
Absent	No hot flushes
Mild	A transient sensation of heat without sweating
Moderate	Sensation of heat accompanied with sweating, but able to continue activities
Severe	Sensation of heat with sweating that causes cessation of activity

Table 1. Hot flushes defined by the United States Food and Drug Administration (*US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research*).

There are ethnic differences in hot flushes, and, for example, Asian women show a lower incidence of hot flushes (~16%) than Latin, European or African women (~47–57%) (*Green and Santoro 2009, Simpkins et al 2009*). Cultural differences have been presented as one explanation; it is possible that some cultures and languages lack definitions for hot flushes, or that women are reluctant to express their vasomotor symptoms (*Whiteman et al. 2003*). Furthermore, hot flushes also seem more prevalent in regions with large seasonal temperature changes (*Freeman and Sherif 2007*, *Sturdee 2008*).

Surgical menopause is defined as an oophorectomy in a fertile woman. This results in an abrupt decline in endogenous estrogen levels and often in severe hot flushes soon after surgery. Although endogenous estrogen levels show no consistent association with vasomotor symptoms (*Santoro 2008*), the presence of hot flushes has been linked with polymorphisms in estrogen-metabolizing cytochrome P450 enzymes (*Visvanathan et al. 2005*, *Crandall et al. 2006a*, *Woods et al. 2006*, *Schilling et al. 2007*), estrogen receptor alpha (ER-α) (*Malacara et al. 2004*) and beta (ER-ß) (*Takeo et al. 2005*), and genes that regulate angiogenesis (*Schneider et al. 2009*).

Hot flushes also accompany the use of antiestrogens such as tamoxifen, which is commonly used for the adjuvant treatment of breast cancer (*Deecher and Dorries 2007*, *Santoro 2008*). Smoking and alcohol use may predispose women to hot flushes (*Barton et al. 2001*, *Whiteman et al. 2003*, *Santoro 2008*). It has been thought that high BMI predisposes women to hot flushes as a result of the insulating effect of excess body fat (*Freeman and Sherif 2007*). On the other hand, the conversion of extraglandular androgens to estrogens in fatty tissues has been proposed to reduce hot flushes. However, BMI is not a clear-cut determinant of hot flushes, since both high and low BMI have been linked with hot flushes.

As evidenced above, menopausal hot flushes, deriving primarily from the central nervous system, affect many organs and significantly reduce the quality of life for several years in many postmenopausal women. Therefore, reduction of hot flushes has become the primary goal for modern treatment of menopausal symptoms.

Cardiovascular disease

Cardiovascular disease is a major cause of morbidity and mortality in both men and women worldwide (*The World Health Organization 2007*, *Shaw et al 2009*, *van der Schouw 2009*). These diseases may manifest as angina, coronary heart disease (CHD), myocardial infarction, transient cerebral ischemic attacks and stroke (*The World Health Organization 2007*). Men and women differ in their cardiovascular risk profile, and women present with clinically significant disease approximately 10 years later than men (*Knopp 2002*, *Pepine et al. 2006*). However, over the past 20 years the overall cardiovascular risk profile in women has worsened (*Towfighi et al. 2009*), and nowadays the mortality of women as a result of CVD exceeds that of men (*Shaw et al. 2009*).

It has been known for many decades that menopause is associated with an increase in the incidence of CVD (*Kannel et al. 1976*, *Witteman et al. 1989*, *Kannel and Wilson 1995*, *Dubey et al. 2005*, *Collins et al. 2007*, *Shaw et al. 2009*). There are many explanations for this phenomenon, but menopause-induced hypoestrogenism is the most established one (*Mendelsohn and Karas 1999*, *Collins 2001*, *Mendelsohn and Karas 2005*). Hypoestrogenism may lead to vascular inflammation, endothelial dysfunction and increased arterial stiffness, which all endanger vascular health (*Kingwell et al. 2001*, *Staessen et al. 2001*, *Takahashi et al. 2005*, *Zaydun et al. 2006*). It is also known that blood pressure (BP) increases in many women after menopause (*Zanchetti et al. 2005*, *Barton and Meyer 2009*), but the mechanisms behind this change are poorly understood (*Casiglia et al. 2008*, *Cifkova et al. 2008*, *Coylewright et al. 2008*); menopause-associated alterations in the function of the autonomic nervous system may in part contribute to the increase in BP (*Vongpatanasin 2009*). In addition, patterns of the lipid profile deviate in an atherogenic direction in many women during and after menopause (*Tikkanen 1996b*, *Rosano et al. 2007*). Furthermore, vasoactive prostacyclin, thromboxane and endothelins have been linked to the etiology of atherosclerosis alone or through effects on lipoproteins (*Ylikorkala et al. 1984*, *Ylikorkala et al. 1987*, *Mikkola et al. 1995*, *Ylikorkala et al. 1995*, *Mikkola et al. 1996*). These biochemical factors and hypoestrogenism-induced changes in them may predispose women to the development of CVD. It is further known that menopause is accompanied by android fat distribution and reduced glucose tolerance, in other words, features of metabolic syndrome; such changes are seen in up to 40–50% of postmenopausal Western women (*Kuh et al. 2005*, *Feng et al. 2008*, *Janssen et al. 2008*, *Lobo 2008*). The role of hypoestrogenism in CVD initiation gains further support from the data showing that premature menopause and consequent prolonged hypoestrogenism are associated with a particularly high risk of CVD (*Atsma et al. 2006*, *Archer 2009*).

Additional menopausal features

Further symptoms attributed to the menopause include, for example, sleep disturbances, head and joint ache, palpitations, mood changes, oral discomfort and decreased libido (*Polo-Kantola et al. 2001*, *Utian 2005*, *Nelson 2008*, *Ensrud et al. 2009*, *Meurman et al. 2009*, *Nappi and Lachowsky 2009*). Many of these symptoms, such as broken sleep, tiredness or depressive mood may be secondary to hot flushes. It should also be mentioned that some women may be totally asymptomatic, but nevertheless, up 95% of women aged 52–56 years report at least one climacteric symptom (*Jokinen et al. 2003*). Long-term consequences of menopause include atrophy of the vaginal epithelium (*Greendale 1999*, *Santoro and Komi 2009*), whereas data on the correlation between decreasing estrogen levels and cognitive decline are controversial (*Barrett-Connor and Laughlin 2009*). The association between low estrogen levels and bone loss, however, is an established fact (*Waugh et al. 2009*). It has been calculated that a women at 50 years of age is characterized by a 30–40% risk of bone fracture in her subsequent life; in absolute terms, increased bone loss and fracture risk after menopause are very significant determinants of overall quality and even length of life (*Huopio et al. 2000*, *Sirola et al. 2003*, *Rutanen and Ylikorkala 2004, The Finnish Medical Society and the Academy of Finland 2004*).

Postmenopausal hormone therapy

Hormone therapy has been used for approximately 80 years to alleviate hot flushes (*Stefanick 2005*). The corner stone in HT is estrogen, which can be administered orally or transdermally. Estrogen alone (estrogen-only therapy; ET) can be prescribed to hysterectomized women. If a woman has an intact uterus, a progestagen must be added to oral or transdermal estrogen treatment (estrogen-progestagen therapy; EPT) to protect against endometrial hyperplasia (*Farquhar et al. 2009*). In Finland in 2008 various forms of HT were used by a total of 352 600 postmenopausal women (*Paakkari et al. 2008*). Contraindications for HT include a history of breast cancer or other hormone-dependent cancer, undiagnosed gynecological bleeding, thromboembolic disease, myocardial infarction, stroke, and uncontrolled hypertension (*The Finnish Medical Society and the Academy of Finland 2004*). Most liver diseases are cured successfully, and therefore, a sole history of liver disease is no contraindication for the use of HT. Thus, women with a history of intrahepatic cholestasis of pregnancy, for example, tolerate well the use of both oral and transdermal HT (*Ropponen et al. 2005a*, *Ropponen et al. 2005b*, *Tuomikoski et al. 2008*).

Estrogens

Natural human estrogens are (17ß-)estradiol, estrone and estriol; estradiol is the most potent of these. It binds to estrogen receptors α and ß, but it may also act directly via non-genomic mechanisms through cell membrane receptors (*Kuhl 2005*). In addition to the reproductive organs, estrogen receptors can be found in several tissues, e.g. in heart, blood vessels, bone, cartilage, skin and the central nervous system. Estrogens are carried in the blood stream bound to either sex hormone-binding globulin (SHBG) or albumin, and only 2% of circulating estrogens appear free. As a result of mutual conversion between estradiol and estrone, the concentrations of these estrogens in the blood are in equilibrium, and both are converted in the liver and gastrointestinal system to estriol. Various estrogens differ in their binding properties to estrogen receptors, and estrone has only about 4% of the estrogenic activity of estradiol (*Kuhl 2005*).

Estradiol is the drug of choice for menopausal complaints in Europe, whereas in the United States CEE is most commonly used. Conjugated equine estrogens, derived from the urine of pregnant mares, are a mixture of several estrogens, such as 17α- and 17ß-estradiol, estrone, equilin and equilenin. Therefore, the composition and even the biological effects of CEE may vary (*Kuhl 2005*).

Estetrol is a new steroid currently being investigated for the management of vasomotor symptoms. It was originally isolated from the fetal liver, and in rat models it shows both estrogen agonist and antagonist properties (*Coelingh Bennink et al 2008*, *Visser and Coelingh Bennink 2009*). So far there are no human data on the use of estetrol for the treatment of menopausal symptoms, but it may hold promise for this purpose.

Progestagens

The use of unopposed estrogen in women with an intact uterus is associated with an increased risk of endometrial cancer (*Stefanick 2005*, *Furness et al. 2009*). Therefore, either a sequential progestagen at 10- to 14-day courses each month, or at three-month intervals (sequential EPT), or daily (continuous EPT) should be used in combination with estrogen (*Hickey et al. 2005*). Progestagens can be administered orally, transdermally, or through an intrauterine device (*Kuhl 2005*). A levonorgestrel-containing intrauterine device (*Nilsson et al 1981*, *Luukkainen et al. 1986*) has proved to be an excellent treatment for menorrhagia (*Lähteenmäki et al. 1998*, *Hurskainen et al. 2001*), and it can also be used in postmenopausal women in combination with ET to ensure endometrial protection (*Peled et al 2007*, *Sitruk-Ware 2007a*). Progestagens differ in their affinity for steroid receptors and their biological activity also depends on their concentration in tissues (*Kuhl 2005*) (Table 2).

Progestagens	Estro-genic	Anti-estro-genic	Andro-genic	Anti-andro-genic	Gluco-corticoid	Anti-mineralo-corticoid
Progesterone	-	+	-	±	+	+
Dydrogesterone	-	+	-	-	-	-
Progesterone-derivative						
Medroxyprogesterone acetate	-	+	±	-	±	-
Megestrol acetate	-	+	±	+	+	-
Testosterone-derivatives						
Norethisterone	-	+	+	-	-	-
Levonorgestrel	-	+	+	-	±	-
Dienogest	±	±	-	+	-	-
Spironolactone-derivative						
Drospirenone	-	+	-	+	-	++

Table 2. Biological activity of various progestagens; (++) strong, (+) moderate, (±) weak, (-) none (*modified from Schindler et al. 2003, Kuhl 2005, Nath and Sitruk-Ware 2009*).

Route of administration

Orally administered estrogens are subject to first-pass metabolism in the liver, unlike transdermally administered estradiol. Commonly, estradiol is given in valerate form in oral use and as hemihydrate in transdermal use; these agents may slightly affect the estrogenic bioprofiles (*Kuhl 2005*). Estradiol is given transdermally as a patch or as a gel; the latter is also called percutaneous form of administration. Oral and transdermal administration results in different estrogenic milieus; oral estradiol administration causes significantly higher circulating levels of estrone than does transdermal use of estradiol. The biological responses to oral and transdermal estradiol also differ, especially as regards liver-mediated responses (*Godsland 2001*, *Strandberg et al. 2003*, *Modena et al. 2005*) (Table 3). For instance, oral estrogen causes marked beneficial changes in lipids and lipoproteins, whereas lesser changes are seen in connection with transdermal estradiol treatment (with the exception of triglycerides) (*Tikkanen 1996b*). Markers of inflammation, such as C-reactive protein and adhesion molecules, and metalloproteinases, which affect atherosclerotic plaque stability, may be stimulated by oral estradiol, but not transdermal estradiol (Table 3) (*Kuhl 2005*, *Mueck and Seeger 2006*, *Ridker et al. 2008*). Use of oral estradiol may also increase the production of renin substrate from the liver leading to a consequent release of angiotensin-II with potential elevation of BP (*Mueck and Seeger 2004*) (Table 3). Furthermore, individual responses to oral and transdermal estradiol vary, both in terms of relief of vasomotor symptoms and increases in the circulating levels of estradiol (*Tuimala and Vihtamäki 1996*, *Vihtamäki and Tuimala 1998*, *Vihtamäki et al. 2004*). Thus, a clinician considering the most suitable form of HT for a given postmenopausal woman must be aware of these effects as well as the patient's disease history.

	Oral estradiol	Transdermal estradiol
Low-density lipoprotein cholesterol	↓↓	↓
Very low-density lipoprotein cholesterol	↑	↑
Apolipoprotein B	↓↓	↓
Lipoprotein (a)	↓↓	↓
High-density lipoprotein cholesterol	↑↑	↑
Apolipoprotein A	↑↑	↑
Triglycerides	↑↑	↔
Sex hormone-binding globulin	↑↑	↔
C-reactive protein	↑	↔
Adhesion molecules	↓	↔
Metalloproteinase activity	↑	↔
Renin substrate	↑↑	↔

Table 3. Differential effects of oral and transdermal estrogen on various biochemical markers; (↓) decrease, (↓↓) substantial decrease, (↑) increase, (↑↑) substantial increase, (↔) no effect *(modified from Kuhl 2005).*

Effects of hormone therapy

Hot flushes

Estradiol treatment, with or without a progestagen, is the most effective way to alleviate hot flushes. Oral and transdermal HT result in an approximate 75–95% reduction in hot flushes in a dose-dependent manner within 2–6 weeks, as demonstrated in several placebo-controlled studies, and the addition of daily or sequential progestagen does not affect this efficacy (*Maclennan et al. 2004*, *Nelson 2004*, *Grady 2006*). Recently, the focus has been on low-dose regimens; oral doses as low as 0.1–0.5 mg, or transdermal estradiol doses of 0.014 mg, can reduce hot flushes (*Panay et al. 2007*, *van de Weijer et al. 2007*, *Langer 2009*, *Panay 2009*). Progestagens, such as MPA, norethisterone and megestrol, when given without estrogen, may slightly alleviate hot flushes, but alone they are usually not used for the treatment of vasomotor symptoms (*Hickey et al. 2005*).

Hormone therapy at the lowest effective dose for healthy, recently postmenopausal women with disturbing moderate to severe hot flushes is an established treatment recommended by a number of expert organizations (*Rutanen and Ylikorkala, 2004, The Finnish Medical Society and the Academy of Finland 2004, Utian et al. 2008, International Menopause Society Consensus Statement 2009,*). Cessation of HT may lead to reoccurrence of vasomotor symptoms in up to 50% of women, especially if hot flushes were moderate or severe at baseline, although the symptoms may be milder (*Ockene et al. 2005*, *Ness et al. 2006*, *Lindh-Åstrand et al. 2009*, *Lindh-Åstrand et al. 2010*). It is often recommended that the use of HT is stopped for 1–2 months every 2–3 years to evaluate whether hot flushes still occur, and if they do, HT can be continued (*The Finnish Medical Society and the Academy of Finland 2004*). The use of HT can be continued as long as it improves the quality of life, and therefore, there is no upper limit for the duration of use or the age of the woman (*The Finnish Medical Society and the Academy of Finland 2004*).

Cardiovascular system

Because HT has been used for approximately 80 years, abundant clinical data on its effects on CVD have accumulated. These data can be considered as being in two categories; observational studies and randomized trials on HT. Approximately 40 observational or case-controlled studies show a 30–50% lower risk of CVD in users of HT, and therefore, until the late 1990s HT was commonly recommended for the prevention of CVD (*Colditz et al. 1987*, *Stampfer and Colditz 1991*, *Grady et al. 1992*, *Grodstein and Stampfer 1995*, *Barrett-Connor 1996*, *Hu and Grodstein 2002*) (Table 4).

	Population Follow-up	Main outcome measure	Results
Sourander et al. (*1998*)	n=7944 8 years	Cardiac death n=258	EPT: RR 0.21 (95% CI 0.08–0.59)
		Death due to stroke n=51	EPT: RR 0.16 (95% CI 0.02–1.18)
Swedish cohort (*Grodstein et al. 1999*)	n=9236 8 years	Myocardial infarction n=213	ET: RR 0.75 (95% CI 0.56–0.99) EPT: RR 0.69 (95% CI 0.45–0.90)
		Stroke n=289	ET: RR 0.91 (95% CI 0.71–1.17) EPT: RR 0.81 (95% CI 0.61–1.10)
Varas-Lorenzo et al. (*2000*)	n=164 769 5 years	Myocardial infarction n=1242	ET: OR 0.52 (95% CI 0.35–0.78) EPT: OR 0.79 (95% CI 0.59–1.08)
Nurses' Health Study (*Grodstein et al 2000*)	n=70 533 20 years	Coronary heart disease n=1253	ET: RR 0.55 (95% CI 0.45–0.68) EPT: RR 0.64 (95% CI 0.49–0.85)
		Stroke n=767	ET: RR 1.18 (95%CI 0.95–1.46) EPT: RR 1.45 (95% CI 1.10–1.92)
WHI Observatio-nal study (*Prentice et al. 2005*)	n=53 054 5 years	Coronary heart disease n=158	EPT: HR 0.87 (95% CI 0.72–1.05)
		Stroke n=123	EPT: HR 0.86 (95% CI 0.70–1.07)

Table 4. Outcomes of some of the largest observational or case-control studies done after 1995 in which the effects of long-term hormone therapy have been assessed. Abbreviations: CI: 95% confidence interval, ET: estrogen-only therapy, EPT: estrogen-progestagen therapy, HR: hazard ratio, OR: odds ratio, RR: risk ratio, WHI: Women's Health Initiative.

The largest observational study was the Nurses' Health Study (*Grodstein et al. 2000*), which started in 1976. In this study 70 533 postmenopausal women were followed for 20 years and data were updated biannually. The data showed that current users of ET (current at the time) had a 45% reduction in the risk of CHD, which was similar as regards CEE at 0.3 mg (risk ratio [RR] 0.58, 95% confidence interval [CI] 0.37–0.92) and CEE at 0.625 mg (RR 0.54, 95% CI 0.44–0.67), and EPT (Table 4). However, the risk of stroke was increased by 45% in current users of EPT, and by 35% (RR 1.35, 95% CI 1.08–1.68) in ET users at a dose of 0.625 mg, whereas CEE at 0.3 mg did not confer any increase in the risk of stroke (RR 0.54, 95% CI 0.28–1.06) (*Grodstein et al. 2000*) (Table 4). The WHI trial also included an observational study arm consisting of 53 054 women of whom 17 503 (33%) were current EPT users (*Prentice et al. 2005*). During an average follow-up of 5.5 years (maximum 8.4 years) a non-significant protective effect was associated with current HT use (Table 4).

The protective effect of HT against CVD seen in observational studies has in part been attributed to the "healthy woman effect", i.e. women who chose HT were in general healthier than women who did not use HT. Therefore, randomized, placebo-controlled trials, such as HERS (*Hulley et al. 1998*, *Grady et al. 2002*) and the WHI trial (*Rossouw et al. 2002*, *Manson et al. 2003*, *Anderson et al. 2004*) were undertaken. The benefits seen in observational studies were, however, not confirmed in these secondary or primary prevention trials, showing rather an increased risk of CVD (*Herrington and Klein 2003, Ylikorkala 2004*) (Table 5, *a* and *b*).

In the secondary prevention trial HERS the use of CEE at 0.625 mg and MPA at 2.5 mg for ~4.1 years in older women with established CHD was not associated with increased risk of coronary events (RR 0.99, 95% CI 0.80–1.22) or total mortality (RR 1.08, 95% CI 0.84–1.38). There was, however, a significant trend over time for more coronary events in HT users during the first years of use (relative hazard year I: 1.52; year II: 1.00; year III: 0.87; year IV: 0.67, p=0.009 for trend) (*Hulley et al. 1998*). Follow-up after approximately 6.8 years showed that the lower rates of coronary events associated with prolonged HT use did not persist (RR 0.84, 95% CI 0.84–1.17) (*Grady et al. 2002*) (Table 5a).

Secondary prevention trials				
	Population	**Treatment**	**Main outcome**	**Results**
HERS 1998, 2002	n=2763–2321 mean age: 68 established CHD	4.1 to 6.8 years CEE 0.625 mg + MPA 2.5 mg	MI or death	No benefit or harm
ERA 2000	n=309 mean age: 66 established CHD	3.2 years CEE 0.625 mg ± MPA 2.5 mg	Angiography: change in coronary lumen diameter	No benefit or harm
PHOREA 2001	n=321 mean age: 59 CIMT ≥1mm	11 months oral estradiol 1 mg ± gestodene 0.025 mg	Ultrasound: change in CIMT	No benefit or harm
PHASE 2002	n=255 mean age: 66 established CHD	30.8 months transdermal estradiol 2mg ± 4mg cyclic NETA	Unstable angina, proven MI or cardiac death	No benefit or harm
WAVE 2002	n=423 mean age: 65 established CHD	2.8 years CEE 0.625 mg ± MPA 2.5 mg, and/ or vitamin C or E	Angiography: change in coronary lumen diameter	No benefit or harm
ESPRIT 2002	n=1 017 mean age: 62 previous MI	2 years oral estradiol 2mg	MI or death	No benefit or harm
WELL-HART 2003	n=226 mean age: 64 established CHD	3.3 years oral estradiol 1mg ± MPA 2.5 mg	Angiography: change in coronary stenosis	No benefit or harm
WHISP 2006	n=100 mean age: 69 acute coronary event <28 days	1 year oral estradiol 1 mg + NETA 0.5 mg	Death, MI, stroke, CVD admissions	Non-significant reduction in CVD events

Table 5a. Secondary prevention studies on hormone therapy. Abbreviations: CEE: conjugated equine estrogens, CHD: coronary heart disease, CVD: cardiovascular disease, CIMT: carotid intima-media thickness, MI: myocardial infarction, MPA: medroxy-progesterone acetate, NETA: norethindrone acetate, ±: with or without, +: with.

PHASE: Papworth HRT Atherosclerosis Study (*Clarke et al. 2002*), HERS I: Heart and Estrogen/progestin Study (*Hulley et al. 1998*), HERS II: Heart and Estrogen/progestin Study (*Grady et al. 2002*), ERA: Estrogen Replacement Atherosclerosis Trial (*Lakoski et al. 2005*), PHOREA: Postmenopausal Hormone Replacement against Atherosclerosis Trial (*Angerer et al. 2001*), WAVE: Women's Angiographic Vitamin and Estrogen Trial (*Waters et al. 2002*), ESPRIT: Estrogen in the Prevention of Reinfarction Trial (*Cherry et al. 2002*), WELL-HART: Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial (*Hodis et al. 2003*), WHISP: Women's Hormone Intervention Secondary Prevention Study (*Collins et al. 2006*).

The WHI (CEE 0.625 mg with or without MPA) was designed as a primary prevention trial of CHD, breast and colorectal cancer and fractures in healthy, postmenopausal women between the ages 50–79. The trial was planned to continue for 8.5 years, but it was prematurely stopped at 5.2 years because of an increased risk for invasive breast cancer (7 extra cases/10 000 person-years), and the risks of CHD (7 extra cases/10 000 person-years) and stroke (8 extra cases/ 10 000 person-years) were also elevated (*Rossouw et al. 2002*). Interestingly, the use of ET in hysterectomized women was not associated with an increased risk of CHD (HR 0.91, 95% nominal CI 0.75–1.12) or breast cancer (HR 0.77, 95% nominal CI 0.59–1.01), whereas the risk of stroke was elevated (12 more cases/10 000 person-years) (*Anderson et al. 2004)* (Table 5b).

Primary prevention trials				
	Population	**Treatment**	**Main outcome**	**Results**
EPAT 2001	n=222 mean age: 62 LDL cholesterol >3.37mmol/L	2 years oral E2 1 mg ± statin	Ultrasound: change in CIMT	Similar decrease in IMT with ET ± statin treatment
WHI-EP 2002	n=16 608 mean age: 63	5.2 years CEE 0.625 mg + MPA 2.5 mg	CHD events	Hazard ratio 1.29 (nominal 95% CI 1.02–1.63)
			Stroke	Hazard ratio 1.41 (nominal 95% CI 1.07–1.85)
WHI-E 2004	n=10 739 mean age: 64	6.7 years CEE 0.625 mg	CHD events	Hazard ratio 0.91 (nominal 95% CI 0.75–1.12)
			Stroke	Hazard ratio 1.39 (nominal 95% CI 1.10–1.77)

Table 5b. Primary prevention studies on hormone therapy. Abbreviations: CEE: conjugated equine estrogens, CHD: coronary heart disease, CI: 95% confidence interval, E2: estradiol, ET: estrogen only; CIMT: carotid intima-media thickness, LDL: low-density lipoprotein cholesterol, MPA: medroxyprogesterone acetate, ±: with or without, +: with.

EPAT: Estrogen in the Prevention of Atherosclerosis Trial (*Hodis et al. 2001*). WHI-EP: Women's Health Initiative [Estrogen and Progestin] (*Rossouw et al. 2002, Manson et al. 2003*). WHI-E: Women's Health Initiative [Estrogen only] (*Anderson et al. 2004*).

The WHI trial has received much criticism. For instance, the mean age of the participants in the EPT arm was 63 years, and 4.4% reported previous CHD, stroke or transient cerebral ischemia. In other words, they were hardly eligible subjects for a primary prevention trial. Moreover, hypertension (36%), smoking (10.5%), hypercholesterolemia (13%) and diabetes (4.4%) were common among the study subjects. In addition, 25% had previously used HT (*Manson et al. 2003*, *Anderson et al. 2004*). Thus, the primary prevention nature of the WHI trial may be disputed (*Grodstein et al. 2003*, *Mikkola et al. 2004*, *Mikkola and Ylikorkala 2005*).

Several plausible explanations have been suggested for the divergent findings between observational studies and randomized clinical trials regarding the cardiovascular benefit of HT. For instance, women who adhere to the given treatment, whether it is active or placebo, tend to have a more beneficial outcome than those with lower compliance to treatment. Differences in treatment regimens, ET versus EPT and the possible adverse effects of MPA, have also been discussed. Furthermore, the length of follow-up has been criticized; in observational studies this has been up to 10–20 years, whereas in randomized trials it has been only 5–7 years. On the other hand, observational studies can be criticized as regards failure to record carefully early clinical events. Time since menopause and a woman's age at the time of HT initiation have been highlighted as key explanatory factors for the discrepancy between results from observational studies and randomized trials. Advanced age of study subjects in randomized trials may well have been associated with subclinical atherosclerosis, which manifested during the first years of HT use, whereas the arteries in women in observational studies were considerably healthier and were additionally protected against atherosclerotic changes during HT use (*Mikkola and Clarkson 2002*, *Waters et al. 2002*, *Grodstein et al. 2003*, *Herrington and Klein 2003*, *Mikkola et al. 2004*, *Mikkola and Ylikorkala 2005*, *van der Schouw and Grobbee 2005*, *Mikkola and Clarkson 2006*).

Other organ systems

Systemic HT affects the whole body, and therefore, all organs possessing estrogen receptors are affected by the treatment. The use of estrogen sustains bone structure and restores bone mineral density both of which deteriorate after the onset of menopause; the use of estrogen results in a reduction of osteoporotic fractures of up to 40–59% (*Wells et al. 2002*, *Farquhar et al. 2009*). It is also well established that estrogen use is associated with a reduction in the risk of colorectal cancer (*Hickey et al. 2005*, *Lethaby et al. 2008*, *Farquhar et al. 2009*). Most observational studies have shown a significant reduction (up to 40%) in the risk of Alzheimer's disease in the users of HT, but the randomized trials have failed to confirm this and in contrast showed an increase of the risk of (vascular) dementia (*Barrett-Connor and Laughlin 2009*). A two- to three-fold risk of venous thromboembolic events is associated with oral HT use (*Rachon and Teede 2008*, *Canonico and Scarabin 2009*, *Farquhar et al. 2009*), but this risk seems to be lower or even nonexistent as regards transdermal treatment (*Rachon and Teede 2008*, *van de Weijer 2008*, *Farquhar et al. 2009*).

Breast cancer is probably the most feared side effect of HT. The use of HT for over five years is associated with a 1.2- to 2.0-fold risk of breast cancer, being higher as regards EPT versus ET (*Lyytinen et al. 2006*, *Lyytinen et al. 2009a*), with a higher risk for continuous than sequential EPT (*Hickey et al. 2005*, *Lyytinen et al. 2009a*, *Lyytinen et al. 2009b*). Similarly, the use of estradiol and intrauterine progestagen administration has been linked to an elevated risk of breast cancer in a non-randomized follow-up study, but this risk elevation may be in part a result of selection bias; women with a higher risk of breast cancer may have been treated with an intrauterine progestagen-releasing device (*Lyytinen et al. 2010*). However, continuous EPT is associated with a 76% decrease in the risk for endometrial cancer (*Jaakkola et al. 2009*).

Other treatment regimens

Although HT is the most effective treatment for hot flushes (*Nelson et al. 2006*), the increased risk of breast cancer associated with HT use has led to a search for alternative treatments.

Tibolone is a synthetic steroid, metabolized to isomers with estrogenic (3α- and 3ß-hydroxy-tibolone), progestagenic and androgenic (Δ4-tibolone) effects (*Kuhl 2005*, *Notelovitz 2007*). Tibolone treatment is as effective as HT in reducing hot flushes, but it does not stimulate endometrial growth or increase mammographic breast density (*Kuhl 2005*, *Notelovitz 2007*). However, the use of tibolone is accompanied by an increased risk of breast cancer (RR 1.36, CI 1.15–1.96, in Finnish women, for example) (*Lyytinen et al. 2010*). This elevation is of the same magnitude as that connected with ET. The androgenic properties of tibolone may enhance sexual desire, but it also reduces the level of high-density lipoprotein (HDL) cholesterol up to 20–30%, which has caused a concern regarding possible vascular risks associated with its use. At this stage it can only be stated that the vascular risk profile of tibolone is not fully explored (*Notelovitz 2007*, *Garefalakis and Hickey 2008*, *Jernman 2008*).

Selective estrogen receptor modulators (SERMs) have either agonistic or antagonistic estrogen effects in different tissues. In general, SERMs act as estrogen agonists on bone and the cardiovascular system, whereas they act antiestrogenically in the breast (*de Villiers 2009*). However, SERMs do not alleviate hot flushes, but instead may even aggravate or cause them (*Cano et al 2007*, *Shen and Stearns 2009*). There are a number of new SERMs under development, such as ospemifene and bazedoxifene (*Rutanen et al. 2003*, *Ylikorkala et al. 2003*, *Komi et al. 2005*, *Lobo et al. 2009*, *Pinkerton et al. 2009*). These compounds show curious organ specificity, and, for example, ospemifene strengthens the vaginal epithelium without having any effect on the endometrium or the breast (*Rutanen et al. 2003*, *Komi et al. 2005*). Thus, in future these compounds may be used as specific targeted therapies against a given menopausal symptom, such as atrophy of vaginal epithelium, but none of them have been approved for clinical use.

Nonsteroidal treatments include clonidine, selective serotonin and noradrenalin reuptake inhibitors, and gabapentin. They are all clearly less effective than estrogen in the treatment of hot flushes and also cause a number of side effects (*Suvanto-Luukkonen et al. 2005*, *Grady 2006*, *Nelson et al. 2006*, *Sturdee 2008*, *Shen and Stearns 2009*). Phytoestrogens may possess both estrogenic and antiestrogenic properties, and in some trials significant relief of vasomotor symptoms has been reported during their use (*Lethaby et al. 2007*, *Henderson et al.*

2008). However, placebo-controlled trials have failed to show the efficacy of phytoestrogens in reducing hot flushes (*Nikander et al. 2003*, *Lethaby et al. 2007*, *Henderson et al. 2008*). Herbal preparations, such as ginseng, evening primrose, dong quai, black cohosh, and vitamin E, have shown no or very modest efficacy against hot flushes (*Cheema et al. 2007*, *Shen and Stearns 2009*).

Acupuncture and yoga have been tested, but they show no consistent effect in alleviating vasomotor symptoms (*Cho and Whang 2009*, *Lee et al. 2009*). Physical exercise may slightly ameliorate hot flushes, perhaps by increasing hypothalamic and peripheral ß-endorphin levels (*Daley et al. 2009*). In addition, life-style changes such as smoking cessation and reducing alcohol consumption, the use of fans, layered clothing and diet changes may subjectively somewhat alleviate hot flushes (*Barton et al. 2001*, *Santoro 2008*, *Shen and Stearns 2009*). Some data imply that certain relaxation techniques and paced respiration could also be helpful in the management of hot flushes (*Hickey et al. 2005*, *Santoro 2008*, *Shen and Stearns 2009*).

In summary, despite extensive research, there exists no truly effective non-steroidal treatment or non-pharmacological intervention to alleviate hot flushes.

Assessment of cardiovascular health

It has been recognized that in women CVD is often not diagnosed on time, and even if it is, treatment is neglected (*Collins et al. 2007*, *Mikkola 2009*). Therefore, several guidelines for earlier diagnosis and for more active management of CVD in women have been constructed (*D'Agostino et al. 2001*, *Conroy et al. 2003*, *Collins et al. 2007*, *Mosca et al. 2007*). The essential feature in these guidelines is active assessment of CVD risk before the condition becomes clinically manifest. The risk of CVD is modified by several factors, all of which aid the identification of high-risk individuals; such women are targets of primary prevention of CVD (Table 6). Known risk factors alone may not be adequate tools for evaluating low-risk populations and more sensitive tools are needed, for instance, measurements of arterial stiffness and endothelial function. Recently, a multimarker approach that includes the evaluation of traditional risk factors in combination with assessment of vascular properties and biomarkers of CVD has been suggested as a useful method to assess the overall cardiovascular risk profile (*Hamilton et al. 2007*, *Ikonomidis et al. 2008*).

Factors that confer a high risk of cardiovascular disease in women	
Presence of ≥1 Subclinical target organ damage:	**Presence of ≥3 cardiovascular risk factors:**
Increased arterial stiffness, ankle-brachial index or carotid intima-media thickness	Hypercholesterolemia or hypertriglyceridemia
Impaired renal function	Smoking
Left ventricular hypertrophy	Elevated blood pressure
	Impaired glucose tolerance
	Abdominal obesity
Independent risk factors:	Age >65 years
Hypertension, renal disease, diabetes, metabolic syndrome	Family history of cardiovascular disease: women at age <65 years men at age <55 years
Additional risk factors: unhealthy diet, physical inactivity, excessive alcohol consumption, low socioeconomic status, stress, depression	

Table 6. Risk factors of cardiovascular disease in women *(modified from Mancia et al. 2007*, *Mosca et al. 2007*, *Ritz 2007*, *The World Health Organization 2007*, *Evangelista and McLaughlin 2009*, *Schenck-Gustafsson 2009*, *Shaw et al. 2009).*

Vascular function

The endothelium consists of the inner layer of cells on the blood vessel wall, which secretes several substances that regulate vascular tone and growth, and modulate inflammation, platelet aggregation and coagulation (*Koh 2002*, *Munzel et al. 2008*). Vascular tone may be in part controlled by the balance between vasodilatory nitric oxide and the vasoconstrictor endothelin-1 secreted by the endothelium (*Ylikorkala et al. 1998*, *Koh 2002*, *Lane et al. 2006*, *Patel and Arora 2008*). Hypertension, hypercholesterolemia and hypoestrogenism impair endothelial function thus leading to enhanced inflammatory reactions, oxidation of lipoproteins, proliferation of vascular smooth muscle cells, changes in extracellular matrix constitution, accumulation of lipid-rich material and prothrombotic changes (*Koh 2002*); all these are early steps in the development of atherosclerosis. Endothelial dysfunction may also increase arterial stiffness and BP by inducing vasoconstriction (*Lane et al. 2006*, *Wang et al. 2008*). Thus, endothelial dysfunction is a predisposing factor as regards CVD and it also contributes to the clinical progression of these diseases (*Lane et al. 2006*, *Munzel et al. 2008*, *Rachon and Teede 2008*, *Wang et al. 2008*) (Figure 3).

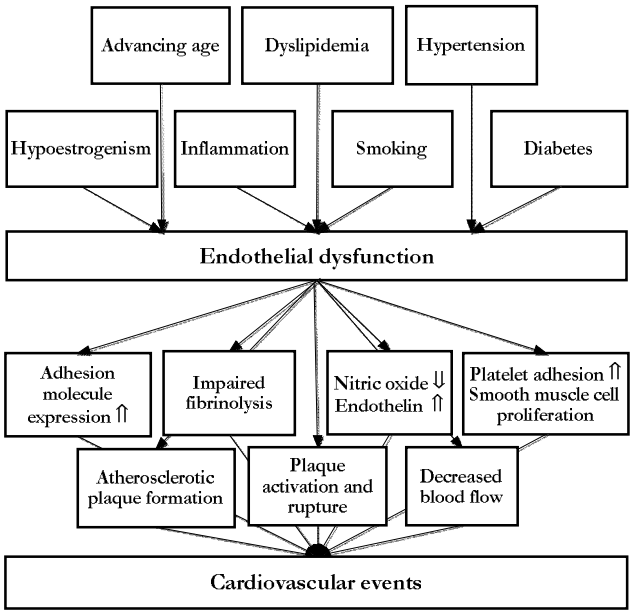

Figure 3. Schematic presentation of factors that cause endothelial dysfunction, and its consequences *(modified from Strandberg and Tikkanen 1996, Ylikorkala et al. 1998, Mikkola and Clarkson 2002).*

Endothelial function can be assessed, for example, by evaluating the ability of a given blood vessel to respond to vasodilatory substances, such as salbutamol and acetylcholine, or to reactive hyperemia, all of which cause release of nitric oxide from the endothelium (*Patel and Arora 2008*). This endothelium-dependent vasodilation can be compared with endothelium-independent vasodilation, which can be triggered by the administration of nitroglycerin, which mimics the action of nitric oxide (*Sarabi et al. 2001*, *Hayward et al. 2002*, *Wilkinson et al. 2002a*, *McEniery et al. 2006*). The dilatory capacity of a vessel can be measured, for instance, by applanation tonometry (pulse wave analysis; PWA) and ultrasonography (flow-mediated dilation; FMD) of a distal artery, and finger-pulse plethysmography (*Conroy et al. 2003*, *Kullo and Malik 2007*). Intracoronary infusion of vasodilatory substances and subsequent quantitative coronary angiography is the gold standard of endothelial testing; this technique, however, is both invasive and expensive (*Patel and Arora 2008*). Both PWA and FMD are noninvasive, but with PWA technology the validity of measurements is controlled by a quality index calculated by the software. Additional ways to assess endothelial function include measuring biomarkers reflecting vascular injury, such as cytokines, inflammation markers, circulating endothelial cells and microparticles, and markers of oxidative stress (*Lane et al. 2006*, *Munzel et al. 2008*).

In addition to endothelial function, blood flow in the arteries is controlled by the stiffness of the vessels, which contributes both to the velocity of the pulse wave and that of the waves reflected back from the periphery towards the heart. The loss of vascular elasticity due to aging, arteriosclerosis, hypertension or other vascular debilitating factor enhances arterial stiffness. This results in an increase in the speed of the forward pressure wave, and amplification of the pulse pressure by the reflected wave (*Mitchell et al. 2004, Zoungas and Asmar 2007*, *Wang et al. 2008*). Increased arterial stiffness may be associated with endothelial dysfunction, alterations in vascular wall matrix proteins, proliferation of smooth muscle cells, inflammation, and genetic determinants may also be involved (*Laurent and Boutouyrie 2007*, *Wang et al. 2008*). Arterial stiffness can be assessed, for example, by measuring pulse transit time (pulse wave velocity), changes in vessel diameter (distensibility), or amplification of the pulse pressure caused by the reflected waveform, for instance PWA. Measurement of arterial stiffness, either as a surrogate end-point or therapeutic target, has been used in both epidemiological studies and clinical trials (*Davies and Struthers 2003*, *Hamilton et al. 2007*, *Zoungas and Asmar 2007*, *Safar 2008*, *Wang et al. 2008*), and it is a valuable tool for the assessment of vascular health in individuals with little or no end-organ disease (*Zoungas and Asmar 2007*).

Blood pressure

Elevated BP is one of the most important cardiovascular risk factors in both men and women (*Ritz 2007*, *Barton and Meyer 2009*, *Evangelista and McLaughlin 2009*). Blood pressure is considered normal if it is <120/80 mmHg, and hypertensive if it is ≥140/90 mmHg (*Mancia et al 2007*). However, it is conspicuous that BP without a threshold, for instance, high normal (130-139/85-89 mmHg), confers an increased risk of CVD (*Vasan et al. 2001*, *Lewington et al. 2002*, *Svetkey 2005*, *Kshirsagar et al. 2006*, *Conen et al. 2007*).

Blood pressure is normally measured manually with a mercury sphygmomanometer. This is prone to many errors of which the so-called "white coat hypertension" effect is the most common. Therefore, for research purposes BP should be measured by means of ambulatory monitoring over 24 hours. Ambulatory BP registration enables the assessment of diurnal changes and variability in BP that are undetectable with standard BP registrations (*Pickering et al. 2006*, *Mancia et al. 2007*). Moreover, ambulatory BP can reveal blunted nocturnal falls in BP, which are known cardiovascular risk factors (*Verdecchia et al. 2007*). In clinical trials ambulatory BP is considered the gold standard of assessing BP, since this technique enables an accurate estimation of 24-hour BPs, and separate analyses of both day- and night time BPs (*Pickering et al. 2006*).

Biochemical markers

The levels of sex hormones have a profound impact on vascular health, as reviewed earlier. This concerns not only estrogens, but also progestagens and androgens, although their impact on cardiovascular health is less clear (*Schindler et al. 2003*, *Dubey et al. 2005, Karim et al. 2008, Ouyang et al. 2009*). A large body of data shows that low levels of SHBG may indicate an elevated risk of CVD in women (*Davison and Davis 2003*, *Rexrode et al. 2003*, *Karim et al. 2008, Sowers et al. 2008*). This association may be related to differences in endogenous estrogen or androgen levels, or insulin resistance (*Davison and Davis 2003*, *Sutton-Tyrrell et al. 2005*, *Crandall et al. 2006b*, *Joffe et al. 2006*, *Castelao and Gago-Dominguez 2008*), since the SHBG molecule itself is hardly a determinant of vascular health.

In addition to impaired glucose tolerance (*Evangelista and McLaughlin 2009*), proatherogenic changes, such as elevations in total cholesterol, low-density lipoprotein cholesterol (LDL) or triglycerides, or a reduction in HDL can predict the risk of CVD in women (*Ridker et al. 2000*, *Knopp 2002*). Further lipid-based CVD markers include apolipoprotein B (apoB) and A-I (apoA-I), and their ratio, as well as lipoprotein a [Lp (a)] (*Dahlen 1994*, *Davison and Davis 2003*, *Carmena et al. 2004*, *Chan and Watts 2006*, *Walldius and Jungner 2006*, *Suk Danik et al. 2008*). Apolipoprotein B molecules are present in very low-density lipoprotein cholesterol (VLDL), LDL and Lp (a) and thus the level of apoB indirectly reflects the total load of circulating atherogenic lipoproteins. In contrast, apoA-I is present only in HDL particles, thus representing cardioprotective lipoproteins (*Davison and Davis 2003*, *Chan and Watts 2006*).

Inflammation is a more recently discovered determinant of atherosclerosis and an elevated risk of CVD (*Lind 2003*). It can be detected, for example, by measuring high-sensitivity C-reactive protein (hs-CRP), fibrinogen, interleukine-6, monocyte-attracting protein-1 and adhesion molecules. Of these, hs-CRP is the most established marker (*Ridker et al. 2000*, *Davison and Davis 2003*, *Lind 2003*, *Mueck and Seeger 2006*, *Wang et al. 2006, Musunuru et al. 2008*, *Packard and Libby 2008*). Plasminogen-activator inhibitor-1 and matrix metalloproteinase-1, in turn, affect atherosclerotic plaque stability and may predict occlusive vascular events (*Mueck and Seeger 2006*).

AIMS OF THE STUDY

The present study was designed to assess the impact of hot flushes on vascular health variables in recently postmenopausal women before and during various forms of HT.

The specific aims were to compare:

- Vascular function (Study I), 24-hour ambulatory BP (Study II) and vascular biomarkers (Study III) in women with no, mild, moderate or severe hot flushes

- Responses of the above-mentioned variables (Studies IV–VI) to oral and transdermal HT in women with tolerable or intolerable hot flushes

SUBJECTS AND STUDY DESIGN

This study was approved by Helsinki University Women's Hospital Ethics Committee (no. 329/E8/03) and registered with the National Agency for Medicine (EudraCT 2004–005091–16) and the U.S. National Institutes of Health Clinical Research Registry (no. NCT00668603). The study was conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki, and written informed consent was obtained from all participants.

The volunteers were recruited via local newspapers between May 2005 and May 2007. Nearly 1500 responding women were first interviewed over the telephone by a trained research nurse. Four hundred women who met our inclusion criteria (age between 48–55 years, last menstrual period within the preceding 6–36 months, no previous HT use) were invited to record their vasomotor symptoms for two weeks on a structured questionnaire that rated the symptoms according to their number and severity (*Panay et al. 2007*).

Hot flushes were defined as mild if there was only a slight sensation of heat which did not disturb daily life. A moderate hot flush was a defined as more intense sensation of heat while awake with some perspiration. A severe hot flush was defined as being more intense with profuse sweating that clearly interfered with daily life or with sleep (*Panay et al. 2007*). Hot flushes were then rated by using the HFWWS, which scores mild symptoms as 1, moderate symptoms as 2 and severe symptoms as 3 (*Notelovitz et al. 2000*, *Sloan et al. 2001*, *Panay et al 2007*) (Table 7).

Hot flushes	Definitions	Score	HFWWS
Absent	No hot flushes		0
Mild	A slight sensation of heat which did not disturb daily life	1	0.5–9.5
Moderate	Intense sensation of heat while awake accompanied by some perspiration	2	10.0–99.5
Severe	Intense sensation of heat with profuse sweating that clearly interfered with daily life or sleep	3	100.0–452.5

Table 7. Definitions of hot flushes of different degrees of severity.
HFWWS=Hot Flush Weekly Weighted Score.

To be able to accurately distinguish the possible impact of hot flushes on vascular health we chose to exclude women with only "intermediate" hot flushes, i.e. ≥3 mild hot flushes to ≤7 moderate to severe hot flushes/day. Intermediate hot flushes, smoking, chronic illness (such as diabetes or hypertension [BP >140/90 mmHg]) or use of prescription drugs (including current or previous use of HT) were the main causes for the exclusion of 1100 subjects. Further exclusion criteria were a level of FSH <30 U/L, BMI >30 kg/m^2, hysterectomy, ovariectomy, and unwillingness or inability to comply with the study plan. Thus, 150 women were eligible for this study (Figure 4).

For Studies I, II and III the women were classified (based on the HFWWS) as having mild, moderate or severe hot flushes, or as being totally asymptomatic (Table 7, Figure 4). At baseline 143 women were available for PWA (Study I). Due to hypertensive readings (BP >140/90 mmHg) in the ambulatory BP recordings, three women out of 150 were excluded from further analysis of ambulatory BP and thus, this study group consisted of 147 women (Study II). One hundred and fifty women were eligible for the analysis of vascular biomarkers for CVD (Study III).

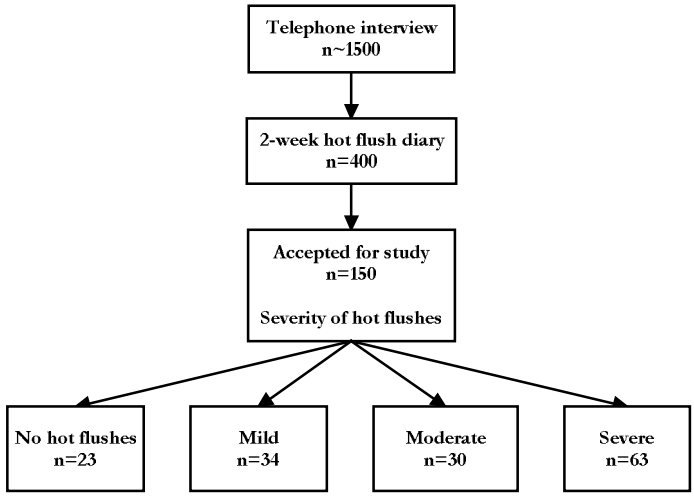

Figure 4. Gathering of the population for studies on the impact of hot flushes on various cardiovascular risk factors (Studies I, II and III).

For the randomized controlled trials on the impact of hot flushes on the responses of CVD risk factors to HT (Studies IV, V and VI) the women were classified as having either intolerable (≥7 moderate/severe symptoms per day) or tolerable hot flushes (≤hot 3 mild symptoms per day). The women were randomized in blocks of four with regard to hot flush status (intolerable or tolerable hot flushes) to receive, in a double blind setting, either 1 mg of transdermal estradiol hemihydrate gel, 2 mg of oral estradiol valerate with or without daily MPA at 5 mg, or placebo for six months; these regimens were provided by Orion Pharma (Espoo, Finland) (Figure 5). All the women used both tablets (active or placebo) and gels (placebo or active) to guarantee the double-blind character of the trial. Compliance was assessed by counting the unused packages. As a safety precaution, each woman showing endometrial thickness of ≥9 mm at six months received MPA (5 mg/day) for two weeks to initiate endometrial shedding; this was started after collection of blood samples and all relevant clinical data. In this setting the impact of hot flushes on the responses of arterial stiffness and endothelial function (Study IV), ambulatory BP (Study V) and biochemical markers for CVD (Study VI) to various forms of HT was studied.

On the whole, a very limited number of participants discontinued the trial. Depending on the study a total of 6 to 9 women discontinued for the following reasons: withdrawal of consent, becoming lost during follow-up, or because of spotting.

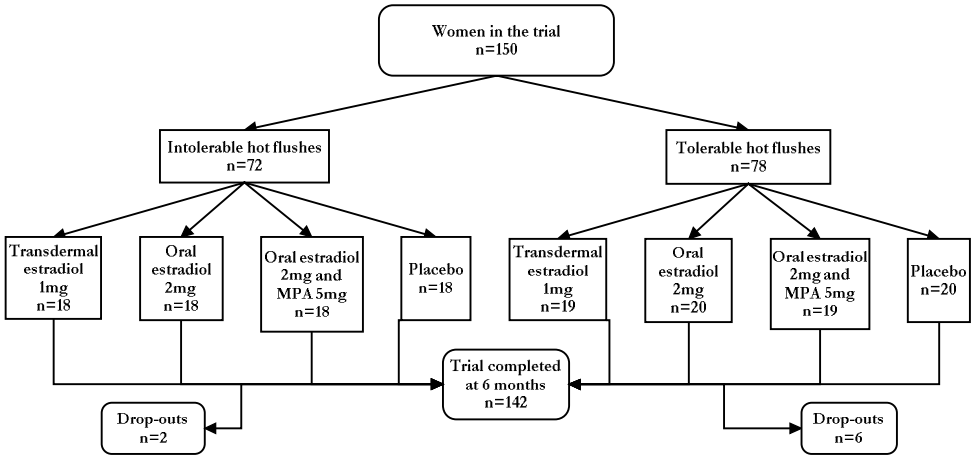

Figure 5. Study protocol for the randomized, placebo-controlled HT Studies IV, V and VI.

METHODS

All participants underwent a thorough physical examination, and findings in pelvic and vaginal ultrasonographic examination, Papanicolau smears and endometrial biopsy samples were normal.

Pulse wave analysis and endothelial testing

For Studies I and VI we used PWA (SphygmoCor® 7.0, AtCor Medical, Sydney, Australia). In PWA central aortic waveforms are derived from a series of peripheral pulse waves that are recorded at the radial artery with a tonometer. The central aortic pulse wave is then calculated using a generalized transfer function, based on the combination of the forward-moving pressure wave generated by left ventricular systole, and waves reflected back to the aorta from points of greater impedance along the arterial tree, such as bifurcations and arterioles (*O'Rourke et al. 2001*, *Mackenzie et al. 2002*, *Wilkinson et al. 2002a*, *O'Rourke and Adji 2005*).

Systemic arterial stiffness was assessed with the augmentation index (AIx), defined as the difference between the second and the first systolic peaks expressed as a percentage of pulse pressure (*O'Rourke et al. 2001*, *Mackenzie et al. 2002*, *Wilkinson et al. 2002a*, *O'Rourke and Adji 2005*). Aortic stiffness was measured from the inflection point after the first systolic peak, which marks the time to the return of the reflected wave, dependent on pulse wave velocity, and it can therefore be used as a marker of aortic stiffness (*Murgo et al. 1980*, *London et al. 1992*, *Marchais et al. 1993*, *Wilkinson et al. 2001*). Furthermore, left ventricular properties were estimated from the aortic pulse wave, by measuring the amplitude and timing of the first systolic peak, which are dependent on stroke volume and the rapid phase of ventricular ejection, respectively (*Wilkinson et al. 2002b*) (Figure 6, Table 8).

In the case of increased arterial stiffness, a rise in AIx and a decrease in time to the reflected waveform are seen, indicating increased augmentation and a faster return of the reflected wave, respectively (*Zoungas and Asmar 2007*). Since AIx is influenced by heart rate, it was normalized to a heart rate of 75beats/min for comparisons (*Wilkinson et al. 2000*, *Wilkinson et al. 2002b*). For the final analyses the lowest AIx value of three consecutive measurements was selected for each patient (*Sarabi et al. 2001*, *McEniery et al. 2006*, *Törmälä et al. 2008*).

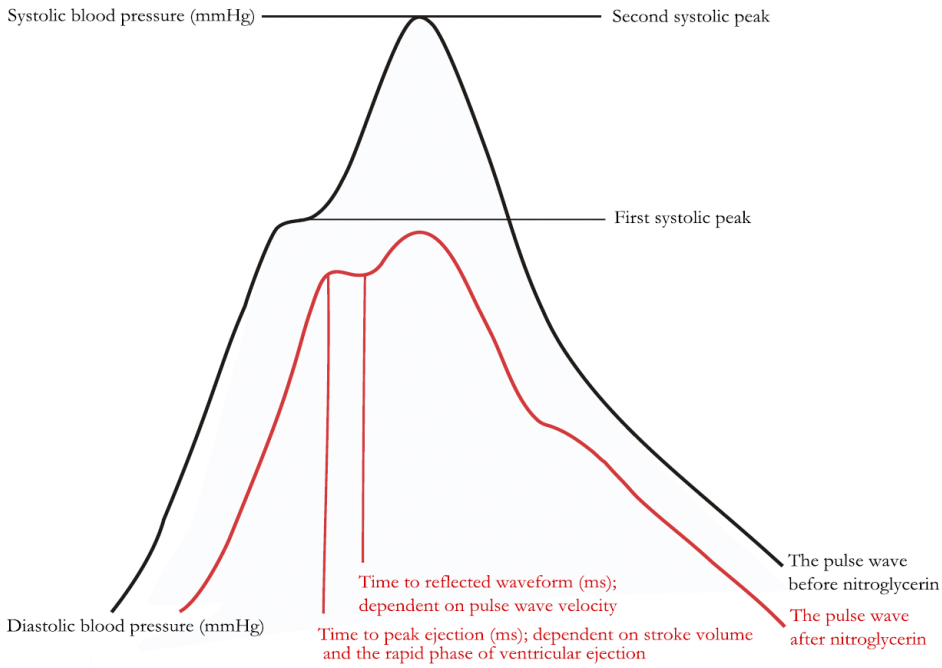

Figure 6. Schematic presentation of the pulse wave before and after nitroglycerin challenge (Study I). Reprinted and slightly modified with permission from Wolters Kluwer Health.

Variables in pulse wave analysis	Definitions:
Augmentation Index (AIx)	$AIx = \frac{P2 - P1}{SBP - DBP} * 100\%$ The difference between the 2nd and the 1st systolic peaks expressed as a percentage of pulse pressure (systolic BP – diastolic BP)
Endothelial Function Index (EFI)	$EFI = \frac{AIx \text{ before Salbutamol} - AIx \text{ after Salbutamol}}{AIx \text{ before Nitroglycerin} - AIx \text{ after Nitroglycerin}} * 100\%$ Endothelium-dependent vasodilation expressed as a percentage of endothelium-independent vasodilation
Time to peak ejection in milliseconds (T_e)	Timing of the first systolic peak; dependent on stroke volume and the rapid phase of ventricular ejection
Time to reflected waveform in milliseconds (TR)	The time to the return of the reflected wave; dependent on pulse wave velocity

Table 8. Definitions of variables derived from pulse wave analysis.

Endothelial function was assessed by complementing PWA with nitroglycerin (causes endothelium-independent vasodilation) and salbutamol (causes endothelium-dependent dilation) challenges, and calculating the endothelial function index (EFI) (Figure 6, Table 8). The AIx measurements were carried out before and 3, 5, 10, 15 and 20 minutes after the administration of 0.5 mg of sublingual glyceryl trinitrate (Nitro®, Orion Pharma, Espoo, Finland). After a 60-minute washout period and a second baseline assessment, the measurements were repeated 5, 10, 15 and 20 minutes after inhalation of 0.4 mg salbutamol (Ventoline® Evohaler, GlaxoSmithKline, Ware, UK) (*Sarabi et al. 2001*, *Hayward et al. 2002*, *Wilkinson et al. 2002a*, *McEniery et al. 2006*, *Rönnback et al. 2007*, *Törmälä et al. 2008*).

Ambulatory blood pressure

The participants wore calibrated oscillometric ambulatory BP monitors (SpaceLabs 90217, SpaceLabs Healthcare, Washington, U.S.A.) on their non-dominant arm for 24 hours (Studies II and V). The monitor was programmed to take readings at 20-minute intervals during periods of wakefulness and at 30-minute intervals during sleep according to each participant's estimated bedtime. The women were instructed to maintain their normal daily activities during the ambulatory BP recordings, and to record their activities and vasomotor symptoms in a diary. States of wakefulness and sleep state were defined according to diary entries.

All successful measurements of systolic and diastolic BP, together with simultaneous heart rate and the exact time of the measurement were transferred from the ambulatory BP system to a spreadsheet for further analysis of 24-h BP and heart rate, and separate analyses for daytime and night-time. Pulse pressure was defined as systolic minus diastolic BP, and absolute dipping was defined as the difference between average systolic BPs awake and asleep. Relative dipping was calculated as the percentage of absolute dipping of systolic BP when awake, and relative dipping of at least 10% was considered a normal dipping pattern (*Pickering et al. 2006*). The acute effects of hot flushes on BP were analyzed from night-time measurements in women with severe hot flushes. Diaries were used to identify the exact time of the hot flush. A recording occurring within 15 minutes after a hot flush was defined as representing the BP and heart rate during and after a hot flush, and the data were compared with the average BP and heart rate at night-time. We were able to identify 44 such hot flush episodes among 26 women, and in case of multiple hot flush experiences the mean value per subject was included.

Assays for biochemical markers for cardiovascular disease

Fasting blood samples were collected and serum was separated with centrifugation. Samples at 6 months were scheduled to be collected approximately two hours after the intake of the study medication. Serum samples, kept frozen in -80ºC, were assayed for total cholesterol, HDL, LDL, VLDL and triglycerides using standard methods (*Havel et al. 1955*). Commercially available kits were employed for apoB, apoA-I and Lp (a), SHBG and hs-CRP and estradiol. The level of estrone was assayed by liquid chromatography-tandem mass spectrometry based on the method of Nelson et al (*2004*) with some modifications (Study III). The free estradiol index (FEI) was calculated as the ratio: estradiol (nmol/L) x100/SHBG (nmol/L) (Table 9). Thyroid-stimulating hormone was tested using chemiluminescent microparticle immunoassay (Abbott Diagnostics, Abbott Park, IL) and FSH was analyzed by time resolved immunofluorometric assay method (AutoDelfia™, Wallac, Turku, Finland) according to the manufacturer's instructions.

Analyte	Source	Principle of assay	Coefficient of variation		Detection limit
			Intra-assay	Interassay	
Total cholesterol	Thermo Electron Co Konelab™ Vantaa, Finland	Enzymatic colorimetry	0.9–1.1%*	0.9–2.0%*	0.1 mmol/L
High-density, low-density, and very low-density lipo-protein cholesterols		Ultracentrifugation	0.42–1.3%*	1.5–2.2%*	0.084 mmol/L
Triglycerides	Thermo Electron Co Konelab™ Vantaa, Finland	Enzymatic colorimetry	1.0–1.9%*	2.0–2.5%*	0.02 mmol/L
Lipoprotein (a)	Thermo Electron Co Konelab™ Vantaa, Finland	Immunoprecipitation	1.3–2.6%*	2.7–3.5%*	30 mg/L
Apolipoprotein B	Thermo Electron Co Konelab™ Vantaa, Finland	Immunoprecipitation	1.6–1.7%*	2.8– 3.4%*	50 mg/L
Apolipoprotein A-I	Thermo Electron Co Konelab™ Vantaa, Finland	Immunoprecipitation	2.7%	5.5%	100 mg/L
Sex hormone-binding globulin	AutoDELFIAT, Wallac, Turku, Finland	Immunofluorometry	1.4–1.8%*	8.2–10.1%*	0.5 nmol/L
High-sensitivity C-reactive protein	Orion Diagnostica Oy Espoo, Finland	Immunoturbidimetry	0.3–6.6%*	2.1–11.9%*	0.25 mg/L
Estrone	CDN Isotopes Inc. Pointe-Claire, Canada	Liquid chromatography-tandem mass spectrometry	<7.8–12.0%*	<7.8–12.0%*	10 pmol/L
Estradiol	Diasorin Inc. Stillwater, MN, USA	Radioimmunoassay	<6.1%	<6.1%	19 pmol/L

Table 9. Characteristics of the assays used for the measurement of biochemical markers of cardiovascular disease. *=for low and high concentrations, respectively.

Statistical analyses

Power analysis was made with the anticipated mean change in PWA in response to HT estimated to be 6.6% with a standard deviation (SD) of 1. The comparisons were calculated to achieve 80% power to detect a 15% difference in the responses to HT between women with intolerable and tolerable hot flushes at a conventional alpha-level of 0.05. The responses in biochemical markers and ambulatory BP were secondary endpoints of this trial.

Normality was assessed with the Shapiro–Wilk test. Logarithmic transformations and inverse transformation were made to achieve normal distribution, or the data was converted to ranked cases. Other statistical methods used are presented in Table 10.

One-way between-groups comparisons	**Within-groups comparisons**	**Test for trend**	**Association between variables**	**Two-way between-groups comparisons**
Parametric tests for normally distributed variables				
One-way analysis of covariance Linear regression analysis	Analysis of variance for repeated measures		Pearson's product-moment correlation coefficient	Two-way between groups analysis of covariance with Tukey HSD and Games–Howell *post hoc* comparisons
Non-parametric tests for variables with non-normal distribution				
Kruskall–Wallis test followed by Mann–Whitney U test with Bonferroni corrections	Wilcoxon´s signed ranks test	Jonckheere–Terpstra test for trend	Spearman's nonparametric correlation coefficient Chi-square test	

Table 10. Statistical methods used in the data analyses.

When appropriate, analyses were conducted adjusting for the time since menopause and the levels of estrone, estradiol and the FEI. Owing to multicollinearity between variables, a univariable instead of a multivariable approach was used (Studies IV and V) and partial eta^2 (η^2) was calculated to describe the proportion of variance in the variables explained by treatment. Tukey HSD and Games–Howell *post hoc* comparisons were used for variables with equal and unequal variances, respectively. Due to significant interaction between hot flush status and the effect of treatment in Studies IV and V separate analyses were carried out for women with intolerable and tolerable hot flushes (Study IV), and the different treatment groups (Study V). A p-value <0.05 was considered statistically significant. All data are presented as mean±SD or mean±standard error of the mean (SEM). All analyses were carried out with SPSS software for Windows version 14.0, 2005 (SPSS Inc. Chicago, IL, USA).

RESULTS

Detailed results are given in the original publications and only the main results are presented here.

Impact of hot flushes on vascular health

Women with no, mild, moderate, or severe hot flushes were comparable in age, weight, height, BMI, and the level of thyroid-stimulating hormone. Importantly, there were no significant differences in the serum levels of estrone, estradiol, or the FEI. A significant trend was observed for the time since the last menstruation to be shorter with increasing severity of hot flushes, being approximately 6 months between asymptomatic women and those with severe hot flushes (Study I p=0.006; Study II p=0.004; Study III p=0.006 [p-for-trend]).

Vascular function (Study I)

In PWA after the challenge with nitroglycerin, time to peak ejection was 12.3% (p=0.025) longer and time to reflected waveform was 9.2% (p=0.014) longer in women with severe hot flushes as compared with asymptomatic women. A significant trend across the groups was also detected; both time to peak ejection and time to reflected waveform after nitroglycerin were longer with increasing severity of hot flushes (p=0.001 and p=0.002, respectively). No effect of salbutamol was seen in any of the study groups. Hot flush status did not influence arterial (AIx) or aortic stiffness (time to reflected waveform), or endothelial function (EFI) (Table 11).

Hot flushes	None	Mild	Moderate	Severe	*p*-value
Augmentation index	23.7±1.7	22.6±1.4	22.0±1.3	22.1±1.1	ns
Endothelial function index	21.3±11.2	37.7±16.1	24.7±6.2	26.7±5.2	ns
Time to peak ejection at baseline	106.5±2.1	110.1±2.2	113.0±2.2	107.7±2.0	ns
Time to peak ejection after nitroglycerin	115.5±3.5	120.2±3.7	127.6±4.6	131.5±3.4	0.025 †; 0.001 ‡
Time to peak ejection after salbutamol	109.1±3.0	106.9±1.7	112.8±2.7	110.7±1.8	ns
Time to reflected waveform at baseline	138.9±2.2	143.2±2.2	145.3±1.7	141.4±2.1	ns
Time to reflected waveform after nitroglycerin	149.8±2.2	157.6±3.2	159.4±3.3	164.0±2.6	0.014 †; 0.002 ‡
Time to reflected waveform after salbutamol	143.9±2.4	142.5±1.7	147.6±2.5	145.6±1.7	ns

Table 11. Variables in pulse wave analysis before and after challenges with nitroglycerin and salbutamol. ns=non-significant, †=*p* for the difference between asymptomatic women and women with severe hot flushes, ‡=*p* for trend across the groups. Data presented as mean ± SEM.

Ambulatory blood pressure (Study II)

Women with different severity of hot flushes were fully comparable in their 24-hour BP and heart rate profiles (Figure 7).

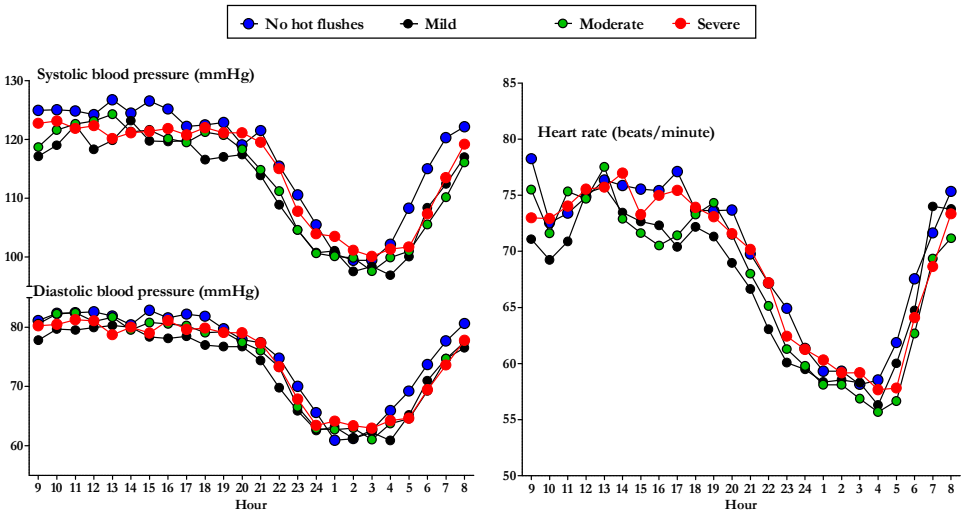

Figure 7. Mean contours of blood pressure and heart rate during 24 hours in women with no, mild moderate or severe hot flushes. Reprinted and slightly modified with permission from Informa UK Ltd.

However, severe night-time hot flushes were accompanied by transient increases in systolic (4.1±10.5 mmHg, p=0.061) and diastolic BP (3.1±6.8 mmHg, p=0.032), and heart rate (3.0±7.2 beats/minute, p=0.043) (Figure 8).

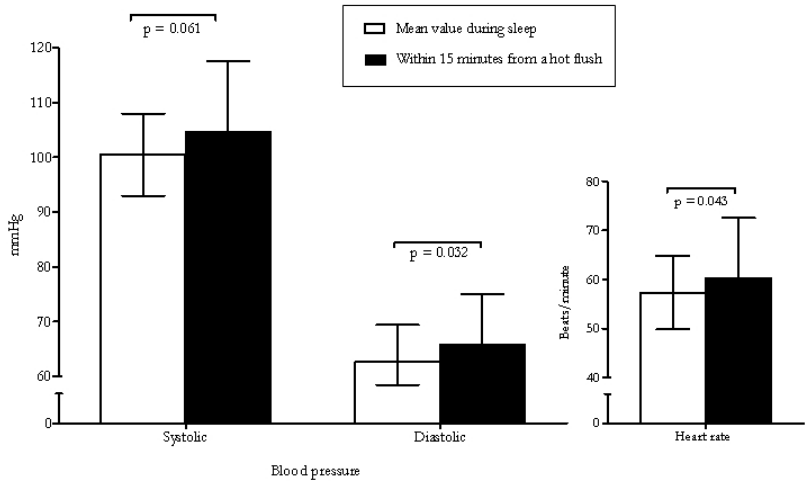

Figure 8. Impact of severe night-time hot flushes on ambulatory blood pressure and heart rate during 44 hot flush episodes in 26 women. Data are presented as mean±SD. A p-value <0.05 was considered statistically significant. Reprinted and slightly modified with permission from Informa UK Ltd.

Biochemical markers for cardiovascular disease (Study III)

Hot flush status was not a determinant for the levels of lipids, lipoproteins, apolipoproteins or the ApoB/ApoA-1 ratio. Moreover, the levels of SHBG and hs-CRP were similar in women with no, mild, moderate or severe hot flushes. Estrogenic variables were also comparable between the study groups (Figure 9).

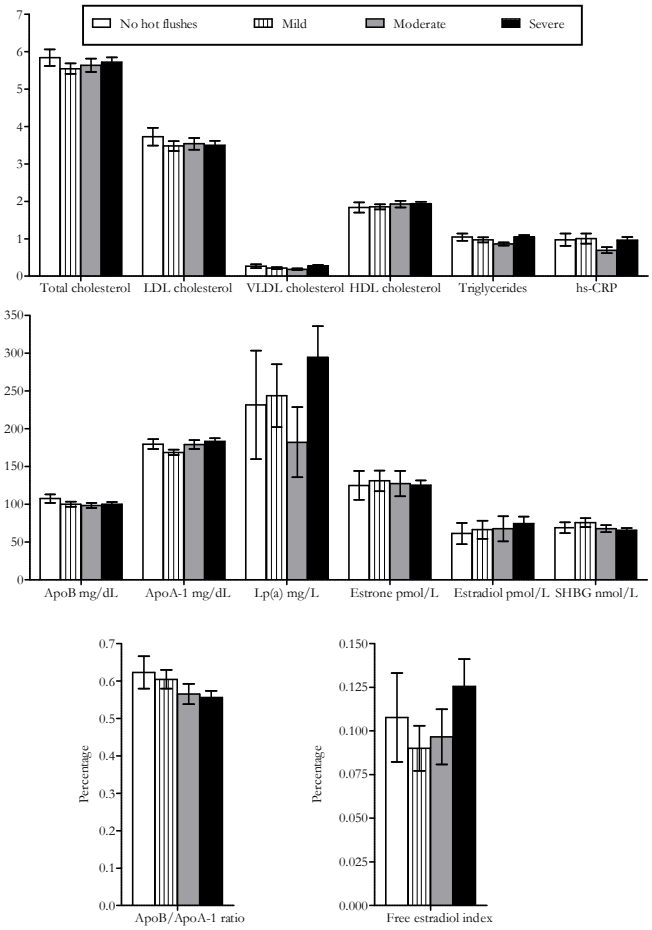

Figure 9. Levels of estrogens and biochemical markers of cardiovascular disease in postmenopausal women with hot flushes of different severities. Data presented as mean ± SEM.

Randomized trial on hormone therapy

All active treatments were equally effective in reducing vasomotor symptoms in women with intolerable hot flushes, whereas no effect was seen in women with tolerable hot flushes. All treatments irrespective of hot flush status caused significant rises in estrone and estradiol levels compared with placebo ($p<0.001$ for all). Transdermal estradiol caused an approximate 3-fold rise in estrone levels, which was smaller than the rises resulting from oral estradiol treatment with or without MPA (14-fold and 20-fold, respectively, $p<0.001$ for both). The rises in estradiol levels (approximately 3-4-fold) were comparable between all three active treatments.

Responses of

Vascular function (Study IV)

In women with tolerable hot flushes, oral estradiol caused significant ($p<0.05$) reductions in both time to peak ejection (~13%) and time to reflected waveform (~8%) after nitroglycerin as compared with the increases caused by transdermal estradiol (~11 and ~6%, respectively), oral estradiol complemented with MPA (~11% and ~7%, respectively), or placebo treatment (~8% and ~6%, respectively) (Figure 10). These changes were independent of time since menopause and changes in the levels of estrogens. In women with intolerable hot flushes both time to peak ejection and time to reflected waveform after nitroglycerin remained unaffected in all treatment groups. Overall, arterial stiffness (AIx) and endothelial function (EFI) were unaffected by the different treatment regimens or hot flush status.

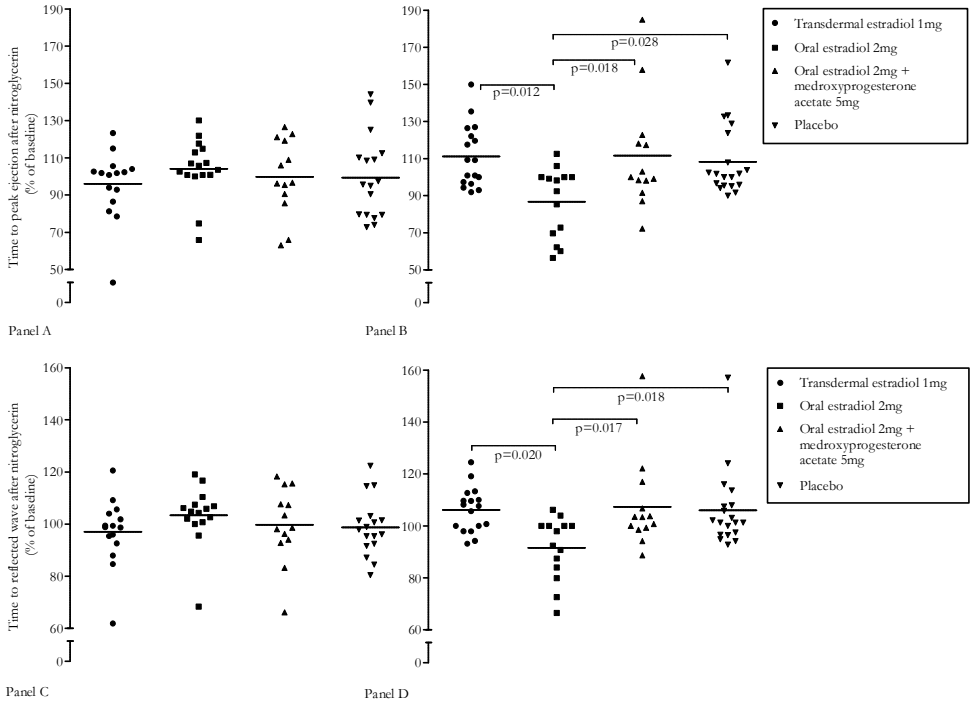

Figure 10. Vascular responses to different hormone therapy regimens and placebo. Time to peak ejection after nitroglycerin in women with intolerable (Panel A) and tolerable hot flushes (Panel B). Time to reflected waveform after nitroglycerin in women with intolerable (Panel C) and tolerable hot flushes (Panel D). Data presented as percentage of baseline with line at mean. A two-tailed *p*-value <0.05 was considered statistically significant. Reprinted and slightly modified with permission from Wolters Kluwer Health.

Ambulatory blood pressure (Study V)

Hot flush status was a significant determinant of the responses to HT of both 24-hour and daytime systolic and diastolic BPs. In women with tolerable hot flushes taking oral estradiol 24-hour systolic and diastolic BPs rose (3.7±1.2 mmHg and 1.8±0.8 mmHg, respectively), whereas decreases (-1.2±1.2 mmHg and -2.1±0.8 mmHg, respectively) were detected in women with intolerable hot flushes (p=0.010 and p=0.003, respectively (Figure 11). Similarly, daytime systolic and diastolic BPs increased (3.0±1.3 mmHg and 1.8±0.9 mmHg, respectively) in women with tolerable hot flushes receiving oral estradiol, but the same therapy led to falls (-1.9±1.3 mmHg and -2.4±0.9 mmHg, respectively) in women with intolerable hot flushes (p=0.017 and p=0.003, respectively) (Figure 11). A tendency towards similar responses was observed for night-time systolic and diastolic BPs, but these did not reach statistical significance. Transdermal estradiol and oral estradiol complemented with MPA caused comparable responses in 24-hour, daytime and night-time BPs. Furthermore, in women with tolerable hot flushes the use of transdermal estradiol led to significant decreases in 24-h systolic and diastolic BPs compared with the increases caused by oral estradiol (p=0.001 and p=0.041, respectively). Hot flush status was not a determinant of the responses in heart rate or nocturnal dipping to the different treatment regimens. The baseline levels were significant determinants of the changes in BPs and heart rate, but no dependence on time since menopause or changes in levels of estrone, estradiol, or the FEI was found.

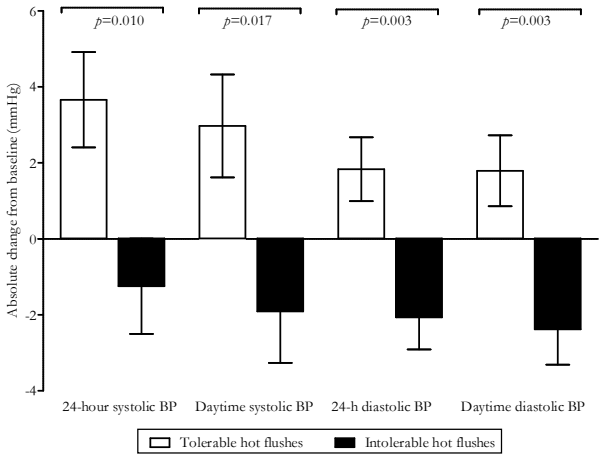

Figure 11. Effect of oral estradiol on 24-hour and daytime systolic and diastolic BPs in women with tolerable and intolerable hot flushes. Data presented as mean ± SEM adjusted for the respective baseline levels. A p-value <0.05 was considered statistically significant.

Biochemical markers for cardiovascular disease (Study VI)

Hot flush status did not affect the responses of biochemical markers for CVD to various forms of HT. When all active treatments (estradiol administered either transdermally, or orally; alone or together with MPA) were assessed together, 7 out of 10 markers, i.e. total cholesterol, LDL, VLDL and HDL cholesterols, apoB, SHBG and hs-CRP, showed more beneficial changes during estradiol use in women with intolerable versus tolerable hot flushes, whereas the changes in triglycerides, Lp (a) and apoA-I were more favorable in women with tolerable hot flushes. Furthermore, in women with intolerable hot flushes treated with any form of estradiol, the rises in serum SHBG concentrations (~73%, n=45, Spearman's rho -0.570, $p<0.001$) were related to falls in HFWWS, but no other statistically significant relations emerged (Figure 12).

On the whole, oral estradiol caused significant increases in HDL (~13.5%), apoA-1 (~10.5%), SHBG (~116.7%), and hs-CRP (~110.3%) as compared with placebo. Adding MPA to oral estradiol treatment significantly blunted the rises of both HDL (~6.4%) and SHBG levels (~50.3%), and caused a significant reduction (~-7.9%) in total cholesterol levels. Compared with placebo transdermal estradiol was effective only in decreasing levels of apoB (-7.8%). Analyses were controlled for the influence of time since menopause and changes in the levels of estrogens, and these were not determinants of the responses of biomarkers.

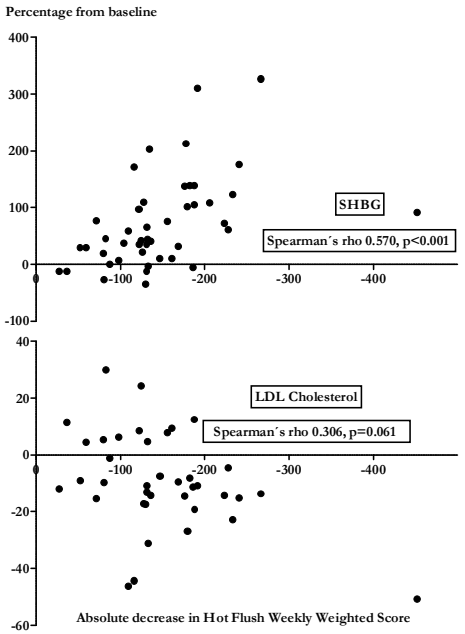

Figure 12.
Correlations between the decreases in the HFWWS and changes in SHBG and LDL cholesterol levels in women with intolerable hot flushes at baseline after receiving six months of any hormone therapy. A two-tailed *p*-value <0.05 was considered statistically significant.

DISCUSSION

Several experimental and human data imply that hypoestrogenism is associated with accelerated atherosclerosis (*Mikkola and Clarkson 2002*). This, at least in part, may explain the increase in the risk of CVD in postmenopausal women (*Shaw et al. 2009*), and also the fact data that postmenopausal HT has been accompanied with a 40–50% reduction in the incidence of CVD in observational studies (*Stampfer and Colditz 1991*, *Grady et al. 1992*, *Grodstein et al. 2000*). These findings, however, were not confirmed in randomized, placebo-controlled trials, which showed not only a lack of benefits, but rather cardiovascular harm in the early phase of HT use (*Hulley et al. 1998*, *Grady et al. 2002*, *Rossouw et al. 2002*, *Manson et al. 2003*, *Anderson et al. 2004*). Exploring the possible explanations for these divergent data was one primary goal of my research, and thus, I explored the role of postmenopausal hot flushes in connection with cardiovascular health.

After the initiation of my study, data have accumulated to show that a woman's age and the time since the last menstruation may significantly alter the cardiovascular effects of HT with potential harm accumulating in the more elderly women (*Grodstein et al. 2006*, *Hsia et al. 2006*, *Ouyang et al. 2006*, *Lobo 2007, Manson et al. 2007*, *Rossouw et al. 2007*, *Hernan et al. 2008*, *Vitale et al. 2008*). This may be related to the decrease of estrogen receptors in vascular walls with advancing age (*Kuhl 2005*). Moreover, it is possible that early atherosclerotic changes, which unavoidably develop in each individual, enhance the risk of vascular harm associated with HT. In other words, the effects of HT may be deleterious in diseased blood vessels even though they are beneficial in healthy blood vessels (*Mikkola and Clarkson 2002*). Therefore, there may be a window (age or time since menopause) for the opportunity to prevent CVD by HT; early initiation of HT may be protective, whereas late initiation may be detrimental (*Mikkola and Clarkson 2002*, *Mikkola and Ylikorkala 2005*, *Mikkola and Clarkson 2006*). This theory is also supported by later analyses of WHI data (*Hsia et al. 2006*, *Manson et al. 2007*, *Rossouw et al. 2007*), although not all experts seem to share this view (*Prentice et al. 2009*). To conclude, the current understanding is that the use of HT is protective against CVD in healthy women between 50–59 years, but not protective and even harmful if initiated after 60 years of age (*Koledova and Khalil 2007*, *van de Weijer 2008, Lobo 2009*).

The presence of vasomotor hot flushes has emerged as one possible contributing factor for the divergent results (*Mikkola and Ylikorkala 2005*, *van der Schouw and Grobbee 2005*, *Mendelsohn and Karas 2007*, *Allison and Manson 2009*, *Andrikoula et al. 2009*, *Pinkerton and Stovall 2009*). In clinical practice, women with troublesome hot flushes seek HT treatment, and thus, such women were included into HT users in observational studies (*Mikkola and Ylikorkala 2005)*. In contrast, hot flushes were mild or absent in the majority of women taking part in the WHI trial, for example, in part as a result of the fact that women with severe vasomotor symptoms would not comply with possibly being randomized to a placebo group (*The Women's Health Initiative Study Group 1998*).

Differences in the cardiovascular effects of HT have also in part been attributed to varying contents of hormone regimens, i.e. the relative high dose of CEE 0.625mg and the use of continuous MPA, which were the compounds used both in the HERS and the WHI trials (*Grodstein et al. 2003*, *van der Schouw and Grobbee 2005*, *Kuhl and Stevenson 2006*). The impact of MPA on the vascular effects of HT has been particularly widely debated (*Clarkson and Appt 2003*, *Koh and Sakuma 2004*, *Kuhl and Stevenson 2006*). This progestagen has antiestrogenic, androgenic and glucocorticoid properties which may counteract the beneficial effects of estrogen (*Kuhl 2005*, *Sitruk-Ware 2007b*). Thus, it is possible that the vascular responses to estrogen with and without MPA differ (*Adams et al. 1997*, *Sitruk-Ware 2000*, *Kawano et al. 2001*), although this is not supported by the results of all clinical studies (*Clarkson and Appt 2003*, *Yeboah et al. 2007*). It is also known that adding MPA to the regimen may prevent estrogen-induced rises in biomarkers (*Godsland 2001*, *Shulman 2002*). For instance, oral estrogen alone or in combination with low-dose MPA (2.5 mg/day) increases the levels of C-reactive protein, but not if oral estrogen is accompanied by a higher dose (5.0 mg/day) of MPA (*Wakatsuki et al. 2002*). Furthermore, it is known that the addition of MPA to estradiol for two weeks does not affect the responses of SHBG or hs-CRP to oral estradiol alone (*Ropponen et al. 2005a, Ropponen et al. 2005b*). Since the data regarding MPA are diverse, it was of special interest to investigate the effects of this progestagen in my study. As regards estrogen, CEE contains a variable mixture of at least ten estrogen sulfates, only three of which are produced in humans, and may therefore yield different clinical effects (*Kuhl 2005*). Thus, to be able to adequately determine the specific effects of estrogen, estradiol was chosen as the estrogen component for my study.

To assess the impact of hot flushes, the study population was carefully chosen to represent healthy, postmenopausal women with no known cardiovascular risk factors. Additional strengths of my study include the use of prospective recording and validated scoring of hot flushes, which enables accurate assessment of vasomotor symptoms (*Sloan et al. 2001*). Vascular health was evaluated using a multimarker approach, which has been suggested to

be the most representative tool to assess the overall cardiovascular health (*Ikonomidis et al. 2008*). The following points should be acknowledged. Firstly, PWA provides assessment of both structural and functional properties of arteries, especially if combined with endothelial testing (*Davies and Struthers 2003, Hirata et al. 2006, Kullo and Malik 2007, Wittrock et al. 2008*). Secondly, BP was measured over 24 hours with ambulatory BP registration, which is the gold standard of BP assessment in clinical research (*Pickering et al. 2006*). Thirdly, the atherosclerotic and inflammatory burdens of the vascular bed were evaluated with established biochemical markers for CVD (*Ridker et al. 2000*, *Knopp 2002*, *Carmena et al. 2004*, *Chan and Watts 2006*).

After the baseline assessments the study subjects were randomized to oral and transdermal estradiol at equipotent doses (*Kuhl 2005*, *Panay 2009*), which enabled comparison of the impact of different routes of estradiol administration. It was already known before the design of the present study that the cardiovascular effects of orally and transdermally administered HT may differ (*Godsland 2001*), and this evidence became stronger during the study (*Khalil 2005*, *Modena et al. 2005*, *Clarkson and Karas 2007*, *Hemelaar et al. 2008*, *L'Hermite et al. 2008*).

As limitations of the study I acknowledge that the study population consisted of lean, white women and thus my results are not be generalizable to obese women or women belonging to other ethnic groups. Moreover, I studied only normotensive women, and the effect of HT in hypertensive women may be different. This reservation is significant; many postmenopausal women show slightly elevated BP, perhaps as a consequence of hot flushes (*Vongpatanasin 2009*) and, in theory, the effect of HT might be most conspicuous in women with hot flushes and mildly elevated BP. Furthermore, my study subjects had entered menopause within the preceding 0.5 to 3 years, so my data may not hold for older women with longer postmenopausal age accumulation. In addition, HT was given only for six months and a longer treatment period might have yielded additional differences. However, 2- to 6-month HT treatments have commonly been used in HT studies (*Mueck and Seeger 2004*, *Teede 2007*). In addition, estradiol was given at conventional doses, and modern low doses may show different effects (*Langer 2009*). Moreover, my data may not be applicable for other estrogen/progestagen combinations. Finally, I studied a limited number of women and therefore, it possible that some changes remained undetected due to the small sample sizes. However, the power of the study was calculated to detect the impact of hot flushes on the outcomes of PWA.

Vascular function

Menopause may increase arterial stiffness, perhaps as a consequence of hypoestrogenism (*Takahashi et al. 2005*, *Zaydun et al. 2006*). Older data have shown increased venous blood flow in the upper extremities of flushing women (*Ginsburg et al. 1981*, *Ginsburg et al. 1989*). My data demonstrate that increasing severity of hot flushes is accompanied by an arterial vasodilatory feature, as evidenced by prolongations of the times to peak ejection and reflected waveform after the nitroglycerin challenge. This may indicate a favorable vascular feature in women with hot flushes of different severities. The presence of hot flushes was not associated with any changes in arterial stiffness or endothelial function (Study I).

During the course of my study some data have emerged that suggest lower FMD in women with hot flushes (*Thurston et al. 2008a*). There are marked differences between my study and that of Thurston and coworkers. First, the women studied by Thurston and colleagues were obese (mean BMI 29 kg/m^2), whereas participants in my study were lean (mean BMI 23 kg/m^2) (Study I). Second, approximately one third of the participants in Thurston's study were African-American women who are known to have in general a less favorable cardiovascular risk profile (*Winkleby et al. 1998*), whereas I studied Caucasian women. Third, the population in the study by Thurston et al. included also women using HT, and thus they cannot be compared with the HT-naïve women in my study. Finally, and perhaps most importantly, hot flushes were retrospectively recalled and the estradiol levels were almost twice as high in flushing women as in women without hot flushes (*Thurston et al. 2008a*), whereas hot flushes in my study were prospectively recorded and the study groups did not differ in estradiol levels (Studies I, II and III).

Besides studying the impact of hot flushes on vascular variables in a cross-sectional setting, the present study evaluated the impact of hot flushes on outcomes of HT in a prospective, randomized trial. Previous data on the impact of HT on vascular function are inconclusive due to the diversity in study methodologies (*Ganz 2002, Koh 2002*, *Teede 2007*, *Bechlioulis et al. 2009*), but in some studies HT has been shown to reduce arterial and aortic stiffness and improve endothelial function (*Hayward et al. 1997*, *Miura et al. 2003*, *Sztejnsznajd et al. 2006*, *Vitale et al. 2008*). In my study the use of oral estradiol was associated with significant reductions in times to both peak ejection and reflected waveform after nitroglycerin, but only in women with tolerable hot flushes (Study IV). This can be seen as a vasoconstrictive response to oral estradiol in these women. Interestingly, in women with intolerable hot flushes, the responses to transdermal estradiol or oral estradiol with or without concomitant MPA were neutral in terms of vasoconstriction or vasodilation. Arterial stiffness and endothelial function remained unaffected in all the study groups (Study IV).

Thus, taken as a whole, the route of estrogen appears to be a critical factor; the transdermal route compares favorably with the oral one, at least in women without troublesome hot flushes.

Ambulatory blood pressure

Elevations in sympathetic nervous activity due to hypoestrogenism are found in women with vasomotor symptoms (*Freedman 2005*, *Deecher and Dorries 2007*, *Sturdee 2008*), and this could result in elevated BP (*Coylewright et al. 2008*, *Vongpatanasin 2009*). Prior to my study some data implied that hot flushes were associated with an increase in either office (*Beljic et al. 1999*) or ambulatory BP (*Brown et al. 2001*, *James et al. 2004*). During the course of my study additional data from cross-sectional studies have shown an association between hot flushes and increases in office (*Gast et al. 2008*, *Thurston et al. 2008a, Gallicchio et al. 2009)* or ambulatory BP (*Gerber et al. 2007*). However, none of these studies utilized the recommended procedure of prospective hot flush recording, but rather a retrospective recall, which certainly is inaccurate (*Pinkerton and Stovall 2009*). Furthermore, these studies included smokers (*Brown et al. 2001*, *James et al. 2004*, *Gerber et al. 2007*, *Gast et al. 2008*, *Thurston et al. 2008a, Gallicchio et al. 2009*) and hypertensive women (*Gerber et al. 2007*, *Gast et al. 2008, Gallicchio et al. 2009*) and thus these data are not generalizable to a healthy and HT-naïve population as I studied; my study population represents the majority of women considering the use of HT in routine clinical practice. Hot flushes have been shown to associate with simultaneous increases (*Freedman and Dinsay 2000*, *Low et al. 2008*) or decreases in BP (*Nelesen et al. 2004*). My data show that severe hot flushes are associated with increases in night-time diastolic BP and heart rate. However, these increases were so short that on the whole, the overall effect of hot flushes on diurnal BP was negligible (Study II). However, heart rate variability may be a more sensitive marker of cardiovascular function than sole heart rate or BP, and this variability shows an interesting association with severe hot flushes (*Hoikkala et al. 2010*).

Hormone therapy modifies a number of vasoregulators, and therefore, HT may affect BP (*Luotola 1983, Khalil 2005*, *Koledova and Khalil 2007*). The progestagen component in HT may also modify BP. For instance, drospirenone, a recently introduced progestagen with antimineralocorticoid activity, present both in oral contraceptive pills and menopausal EPT regimens (*Anttila et al. 2009*), may lower BP (*Nath and Sitruk-Ware 2009*, *Preston 2009*). Decreases in BP have been associated with transdermal estradiol use (*Cacciatore et al. 2001*, *Mueck and Seeger 2004*, *L'Hermite et al. 2008*). On the other hand, oral estradiol may increase BP, perhaps by stimulating the production of renin substrate in the liver; this results in the

release of angiotensin-II, one of the most potent hypertensive agents (*Miller and Duckles 2008*). My data show that the presence of hot flushes affects BP responses according to the type of HT (Study V). In women with tolerable hot flushes oral estradiol was accompanied by increases in both 24-h and daytime BPs, whereas no such effect was seen in women with intolerable hot flushes. This is clinically significant; the former group is usually not a candidate for hormone use, whereas the latter women typically are. Deterioration in the diurnal variation of BP may occur in menopause and HT may improve this by increasing nocturnal BP dipping (*Butkevich et al. 2000*, *Wong et al. 2005*). My data, however, did not show such an effect of HT, perhaps because all participants already had a normal dipping pattern at baseline. The situation might have been different if older women or women with mildly elevated BP were to have been included.

The message for the clinician reads; if a symptomless woman needs estrogen for a reason other than for the control of hot flushes, favor transdermal administration, because this may provide some vascular benefits.

Vascular biochemical markers

When this study was planned, only a reduced antioxidant status was known to be related to hot flushes (*Leal et al. 2000*). During my study, data were published linking hot flushes to risk factors of CVD, such as increased abdominal adiposity (*Thurston et al. 2008b*) and elevated cholesterol levels (*Gast et al. 2008*, *Thurston et al. 2008a*). However, the majority of the studies were cross-sectional studies that included pre-, peri- and postmenopausal women, and women on HT or tobacco users, and women with hypertension and therefore, the results may not truly reflect the causality between hot flushes and various markers for CVD. My data, concerning in healthy, recently postmenopausal women, show no association between baseline hot flush status and biochemical markers for CVD (Study III).

There are no previous data on the impact of baseline hot flush status on the responses of biochemical CVD surrogate markers to HT. My data demonstrate that hot flush status at baseline did not significantly affect the responses of various biochemical markers for CVD to HT, although the majority of markers (70%) showed a tendency towards more beneficial vascular responses in women with intolerable hot flushes (Study VI). As regards the overall effect of various forms of HT, my data are in line with most previous data; oral estradiol is accompanied by more pronounced beneficial changes in lipid and lipoprotein levels than transdermal estradiol with the exception of triglycerides, which do not substantially increase

with transdermal treatment (*Tikkanen 1996a*, *Tikkanen 1996b*, *Godsland 2001*, *Kuhl 2005*, *Hemelaar et al. 2008*). In my study the most pronounced decreases in the levels of total cholesterol were detected in connection with transdermal and oral estradiol, an outcome, however, which may be a result of the small sample size. The addition of MPA to oral estradiol may prevent some of the estradiol-induced changes in biomarkers due to its antiestrogenic properties (*Sitruk-Ware 2004*, *Hermsmeyer et al. 2008*); such an effect of MPA was also seen in my study and most conspicuously in HDL, apoA-I and SHBG (Study VI).

In recent years several re-analyses of HT trials have been made to evaluate the effect of hot flushes on the outcomes of HT. Re-analysis of the WHI data showed that the presence of moderate hot flushes at baseline was associated with a non-significantly lower risk of CVD in women aged 50–59 years (HR 0.86, 95% CI 0.44–1.65) (*Rossouw et al. 2007*). Interestingly, data regarding the effects of HT in older women with hot flushes are more divergent. In the WHI study women aged 70–79 years with moderate to severe hot flushes at the initiation of HT, had an increased risk of CHD associated with HT use (HR 5.08, 95% CI 2.08–12.40) (*Rossouw et al 2007*). Furthermore, re-analyses of the HERS (*Huang et al. 2009*) and Rancho Bernardo (*Svartberg et al. 2009*) studies showed that in women over 65 years of age, hot flushes at baseline seem to be associated either with an increased risk of CHD during the first year of HT use (HERS; HR 9.01, 95% CI 1.15–70.35) or a lower all-cause mortality rate (Rancho Bernardo; HR 0.72, 95% CI 0.55–0.94), respectively. This diversity in results is interesting and warrants further research, since these trials were initially not designed to assess the impact of hot flushes, and the analyses were made retrospectively, when data on the influence of hot flushes had accumulated.

Future directions for research include a need to assess the impact of hot flushes on glucose-insulin balance. Large, prospective studies are needed to confirm the impact of hot flushes on outcomes of HT, and in future trials hot flush status should be considered a potential confounding factor. Furthermore, research should be directed to women with established cardiovascular risk factors at baseline, such as mild hypertension, smoking or hypercholesterolemia. Research should also be focused on older women in their 60s and 70s who still have hot flushes.

In conclusion, HT is the most effective treatment for hot flushes. Healthy, recently postmenopausal women with moderate to severe hot flushes should be given the opportunity to use estrogen alone (if hysterectomized) or in combination with an appropriate progestagen (if with intact uterus) to alleviate hot flushes and other symptoms. Possible contraindications for HT should be noted and cardiovascular risk factors should be evaluated and, if detected, treated accordingly; this particularly concerns elevated BP (*Collins et al. 2007*, *Utian et al. 2008*, *International Menopause Society Consensus Statement 2009*). My data show that the use of oral estradiol may present a risk of vasoconstriction and an increase of BP, but only in women with tolerable hot flushes. Luckily, in clinical practice today such women are seldom prescribed HT. However, if HT is needed for women with no or only mild hot flushes for other indications such as bone protection, transdermal HT should be favored.

SUMMARY

The present studies conducted on healthy, recently postmenopausal women allow the following conclusions:

I Vasodilatory reactivity in women with severe hot flushes is greater than that in women with no, mild, or moderate hot flushes. Thus, vasomotor hot flushes could serve as marker of good vascular function.

II Hot flushes of different degrees of severity are not associated with BP or heart rate except at night, when severe hot flush episodes are followed by transient rises in BP and heart rate.

III Hot flushes of different degrees of severity are not associated with variation in the circulating concentrations of lipids, lipoproteins, SHBG or hs-CRP.

IV The use of oral estradiol alone was accompanied by a vasoconstrictive response in women with tolerable hot flushes, whereas no such effect was detected in women with intolerable hot flushes or with the other treatment regimens.

V Women with tolerable hot flushes responded to oral estradiol with elevations in both 24-hour and daytime systolic and diastolic BP. In contrast, use of transdermal estradiol resulted in decreases n 24-h and daytime BPs. In women with intolerable hot flushes decreases in BP were seen in connection with all treatment regimens.

VI The responses of circulating concentrations of lipids, lipoproteins, SHBG and hs-CRP to oral and transdermal estradiol, the former with or without MPA, were comparable in women with tolerable and intolerable hot flushes. However, women with intolerable hot flushes responded with beneficial changes in 70% of the markers.

The data demonstrate that hot flushes may affect various aspects of vascular function differently. Furthermore, they may clarify in part the conflict between the results of observational and randomized hormone studies. If HT is used for indications other than the control of hot flushes, a transdermal route of estrogen use should be favored.

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REFERENCES

Adams MR, Register TC, Golden DL, Wagner JD, and Williams JK. Medroxyprogesterone acetate antagonizes inhibitory effects of conjugated equine estrogens on coronary artery atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:217-21.

Allison MA and Manson JE. The complex interplay of vasomotor symptoms, hormone therapy, and cardiovascular risk. Menopause 2009;16:619-20.

Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, and Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701-12.

Andrikoula M, Hardiman P, and Prelevic, G. Menopausal hot flush: Is it only a nuisance or also a marker of cardiovascular risk? Gynecol Endocrinol 2009;27:450-4.

Angerer P, Stork S, Kothny W, Schmitt P, and von Schacky C. Effect of oral postmenopausal hormone replacement on progression of atherosclerosis : a randomized, controlled trial. Arterioscler Thromb Vasc Biol 2001;21:262-8.

Anttila L, Kunz M, and Marr J. Bleeding pattern with drospirenone 3 mg+ethinyl estradiol 20 mcg 24/4 combined oral contraceptive compared with desogestrel 150 mcg+ethinyl estradiol 20 mcg 21/7 combined oral contraceptive. Contraception 2009;80:445-51.

Archer DF. Premature menopause increases cardiovascular risk. Climacteric 2009;12 Suppl 1:26-31.

Atsma F, Bartelink ML, Grobbee DE, and van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause 2006;13:265-79.

Barrett-Connor E. The menopause, hormone replacement, and cardiovascular disease: the epidemiologic evidence. Maturitas 1996;23:227-34.

Barrett-Connor E and Laughlin GA. Endogenous and exogenous estrogen, cognitive function, and dementia in postmenopausal women: evidence from epidemiologic studies and clinical trials. Semin Reprod Med 2009;27:275-82.
Barton D, Loprinzi C, and Wahner-Roedler D. Hot flashes: aetiology and management. Drugs Aging 2001;18:597-606.

Barton M and Meyer MR. Postmenopausal hypertension: mechanisms and therapy. Hypertension 2009;54:11-8.

Bechlioulis A, Naka KK, Papanikolaou O, Kontostolis E, Kalantaridou SN, and Michalis LK. Menopause and hormone therapy: from vascular endothelial function to cardiovascular disease. Hellenic J Cardiol 2009;50:303-15.

Beljic T, Babic D, Marinkovic J, and Prelevic, GM. Effect of estrogen replacement therapy on cardiac function in postmenopausal women with and without flushes. Gynecol. Endocrinol 1999;13:104-12.

Brown DE, Sievert LL, Aki SL, Mills PS, Etrata MB, Paopao RN, et al. Effects of age, ethnicity and menopause on ambulatory blood pressure: Japanese-American and Caucasian school teachers in Hawaii. Am J Hum Biol 2001;13:486-93.

Burger HG, Hale GE, Robertson DM, and Dennerstein L. A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. Hum Reprod Update 2007;13:559-65.

Butkevich A, Abraham C, and Phillips RA. Hormone replacement therapy and 24-hour blood pressure profile of postmenopausal women. Am J Hypertens 2000;13:1039-41.

Cacciatore B, Paakkari I, Hasselblatt R, Nieminen MS, Toivonen J, Tikkanen MJ, et al. Randomized comparison between orally and transdermally administered hormone replacement therapy regimens of long-term effects on 24-hour ambulatory blood pressure in postmenopausal women. Am J Obstet Gynecol 2001;184:904-9.

Cano A, Hermenegildo C, Oviedo P, and Tarin JJ. Selective estrogen receptor modulators and risk for coronary heart disease. Climacteric 2007;10:97-111.

Canonico M and Scarabin PY. Hormone therapy and risk of venous thromboembolism among postmenopausal women. Climacteric 2009;12 Suppl 1:76-80.

Carmena R, Duriez P, and Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis. Circulation 2004;109:III2-7.

Casiglia E, Tikhonoff V, Caffi S, Bascelli A, SchiavonL, Guidotti F, et al. Menopause does not affect blood pressure and risk profile, and menopausal women do not become similar to men. J Hypertens 2008;26:1983-92.

Castelao JE and Gago-Dominguez M. Risk factors for cardiovascular disease in women: relationship to lipid peroxidation and oxidative stress. Med Hypotheses 2008;71:39-44.

Chan DC and Watts GF. Apolipoproteins as markers and managers of coronary risk. QJM 2006;99:277-87.

Cheema D, Coomarasamy A, and El-Toukhy T. Non-hormonal therapy of post-menopausal vasomotor symptoms: a structured evidence-based review. Arch Gynecol Obstet 2007;276:463-9.

Cherry N, Gilmour K, Hannaford P, Heagerty A, Khan MA, and the ESPRIT team. Oestrogen therapy for prevention of reinfarction in postmenopausal women: a randomised placebo controlled trial. Lancet 2002;360:2001-8.

Cho SH and Whang WW. Acupuncture for vasomotor menopausal symptoms: a systematic review. Menopause 2009;16:1065-73.

Cifkova R, Pitha J, Lejskova M, Lanska V, and Zecova S. Blood pressure around the menopause: a population study. J Hypertens 2008;26:1976-82.

Clarke SC, Kelleher J, Lloyd-Jones H, Slack M, and Schofiel PM. A study of hormone replacement therapy in postmenopausal women with ischaemic heart disease: the Papworth HRT atherosclerosis study. BJOG 2002;109:1056-62.

Clarkson TB and Appt SE. MPA and postmenopausal coronary artery atherosclerosis revisited. Steroids 2003;68:941-51.

Clarkson TB and Karas RH. Do the cardiovascular disease risks and benefits of oral versus transdermal estrogen therapy differ between perimenopausal and postmenopausal women? Menopause 2007;14:963-7.

Coelingh Bennink HJ, Holinka CF, and Diczfalusy E. Estetrol review: profile and potential clinical applications. Climacteric 2008;11 Suppl 1:47-58.

Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, and Hennekens CH. Menopause and the risk of coronary heart disease in women. N Engl J Med 1987;316:1105-10.

Collins P. Vascular effects of hormones. Maturitas 2001;38:45,50; discussion 50-1.

Collins P, Flather M, Lees B, Mister R, Proudler AJ, and WHISP (Women's Hormone Intervention Secondary Prevention Study) Pilot Study Investigators. Randomized trial of effects of continuous combined HRT on markers of lipids and coagulation in women with acute coronary syndromes: WHISP Pilot Study. Eur Heart J 2006;27:2046-53.

Collins P, Rosano G, Casey C, Daly C, Gambacciani M, Hadji P, et al. Management of cardiovascular risk in the peri-menopausal woman: a consensus statement of European cardiologists and gynaecologists. Eur Heart J 2007;28:2028-40.

Conen D, Ridker PM, Buring JE, and Glynn RJ. Risk of cardiovascular events among women with high normal blood pressure or blood pressure progression: prospective cohort study. BMJ 2007;335:432.

Conroy RM, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, and SCORE project group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003;24:987-1003.

Coylewright M, Reckelhoff JF and Ouyang P. Menopause and hypertension: an age-old debate. Hypertension 2008;51:952-9.

Crandall CJ, Crawford SL, and Gold EB. Vasomotor symptom prevalence is associated with polymorphisms in sex steroid-metabolizing enzymes and receptors. Am J Med 2006a;119:S52-60.

Crandall C, Palla S, Reboussin B, Hu P, Barrett-Connor E, Reuben D, et al. Cross-sectional association between markers of inflammation and serum sex steroid levels in the postmenopausal estrogen/progestin interventions trial. J Women's Health (Larchmt) 2006b;15:14-23.

D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P, and CHD Risk Prediction Group. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA 2001;286:180-7.

Dahlén GH. Lp(a) lipoprotein in cardiovascular disease. Atherosclerosis 1994;108:111-26.
Daley AJ, Stokes-Lampard HJ, and Macarthur C. Exercise to reduce vasomotor and other menopausal symptoms: a review. Maturitas 2009;63:176-80.

Davies JI and Struthers AD. Pulse wave analysis and pulse wave velocity: a critical review of their strengths and weaknesses. J Hypertens 2003;21:463-72.

Davison S and Davis SR. New markers for cardiovascular disease risk in women: impact of endogenous estrogen status and exogenous postmenopausal hormone therapy. J Clin Endocrinol Metab 2003;88:2470-8.

de Bruin JP, Bovenhuis H, van Noord PA, Pearson PL, van Arendonk JA, te Velde ER, et al. The role of genetic factors in age at natural menopause. Hum Reprod 2001;16:2014-8.

de Villiers TJ. Clinical issues regarding cardiovascular disease and selective estrogen receptor modulators in postmenopausal women. Climacteric 2009;12 Suppl 1:108-11.

Deecher DC and Dorries K. Understanding the pathophysiology of vasomotor symptoms (hot flushes and night sweats) that occur in perimenopause, menopause, and postmenopause life stages. Arch Women's Ment Health 2007;10:247-57.

Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, and Burger HG. A prospective population-based study of menopausal symptoms. Obstet Gynecol 2000;96:351-8.

Dubey RK, Imthurn B, Barton M, and Jackson EK. Vascular consequences of menopause and hormone therapy: importance of timing of treatment and type of estrogen. Cardiovasc Res 2005;66:295-306.

Ensrud KE, Stone KL, Blackwell TL, Sawaya GF, Tagliaferri M, Diem SJ, et al. Frequency and severity of hot flashes and sleep disturbance in postmenopausal women with hot flashes. Menopause 2009;16:286-92.

Evangelista O and McLaughlin MA. Review of cardiovascular risk factors in women. Gend Med 2009;6:17-36.

Farquhar C, Marjoribanks J, Lethaby A, Suckling JA, and Lamberts Q. Long term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev 2009;(2):CD004143.

Feng Y, Hong X, Wilker E, Li Z, Zhang W, Jin D, et al. Effects of age at menarche, reproductive years, and menopause on metabolic risk factors for cardiovascular diseases. Atherosclerosis 2008;196:590-7.

Freedman RR. Biochemical, metabolic, and vascular mechanisms in menopausal hot flashes. Fertil Steril 1998;70:332-7.

Freedman RR and Dinsay R. Clonidine raises the sweating threshold in symptomatic but not in asymptomatic postmenopausal women. Fertil Steril 2000;74:20-3.

Freedman RR. Pathophysiology and treatment of menopausal hot flashes. Semin Reprod Med 2005;23:117-25.

Freeman EW and Sherif K. Prevalence of hot flushes and night sweats around the world: a systematic review. Climacteric 2007;10:197-214.

Furness S, Roberts H, Marjoribanks J, Lethaby A, Hickey M, and Farquhar C. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. Cochrane Database Syst Rev 2009;(2):CD000402.

Gallicchio L, Miller SR, Zacur H, and Flaws JA. Hot flushes and blood pressure in midlife women. Maturitas 2010;65:69-74.

Ganz P. Vasomotor and vascular effects of hormone replacement therapy. Am J Cardiol 2002;90:11F-6F.

Garefalakis M and Hickey. M. Role of androgens, progestins and tibolone in the treatment of menopausal symptoms: a review of the clinical evidence. Clin Interv Aging 2008;3:1-8.

Gast GC, Grobbee DE, Pop VJ, Keyzer JJ, Wijnands-van Gent CJ, Samsioe GN, et al. Menopausal complaints are associated with cardiovascular risk factors. Hypertension 2008;51:1492-8.

Gerber LM, Sievert LL, Warren K, Pickering TG, and Schwartz JE. Hot flashes are associated with increased ambulatory systolic blood pressure. Menopause 2007;14:308-15.

Ginsburg J, Swinhoe J, and O'Reilly B. Cardiovascular responses during the menopausal hot flush. Br J Obstet Gynaecol 1981;88:925-30.

Ginsburg J, Hardiman P, and O'Reilly B. Peripheral blood flow in menopausal women who have hot flushes and in those who do not. BMJ 1989;298:1488-90.

Godsland IF. Effects of postmenopausal hormone replacement therapy on lipid, lipoprotein, and apolipoprotein (a) concentrations: analysis of studies published from 1974-2000. Fertil Steril 2001;75:898-915.

Gold EB, Bromberger J, Crawford S, Samuels S, Greendale GA, Harlow SD, et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. Am J Epidemiol 2001;153:865-74.

Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992;117:1016-37.

Grady D, Herrington D, Bittner V, Blumenthal R, Davidson M, et al. and HERS Research Group. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA 2002;288:49-57.

Grady D. Clinical practice. Management of menopausal symptoms. N Engl J Med 2006;355:2338-47.

Green R and Santoro N. Menopausal symptoms and ethnicity: the Study of Women's Health Across the Nation. Women's Health (Lond.Engl) 2009;5:127-33.

Greendale GA. The menopause. Lancet 1999;353:571-80.

Grodstein F and Stampfer M. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. Prog Cardiovasc Dis 1995;38:199-210.

Grodstein F, Stampfer MJ, Falkeborn M, Naessen T, and Persson I. Postmenopausal hormone therapy and risk of cardiovascular disease and hip fracture in a cohort of Swedish women. Epidemiology 1999;10:476-80.

Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, and Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. Ann Intern Med 2000;133:933-41.

Grodstein F, Clarkson, TB and Manson, JE. Understanding the divergent data on postmenopausal hormone therapy. N.Engl.J.Med. 2003;348:645-50.

Grodstein F, Manson JE, and Stampfer MJ. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. J Women's Health (Larchmt) 2006;15:35-44.

Götmar A, Hammar M, Fredrikson M, Samsioe G, Nerbrand C, Lidfeldt J, et al. Symptoms in peri- and postmenopausal women in relation to testosterone concentrations: data from The Women's Health in the Lund Area (WHILA) study. Climacteric 2008;11:304-14.

Hamilton PK, Lockhart CJ, Quinn CE, and McVeigh GE. Arterial stiffness: clinical relevance, measurement and treatment. Clin.Sci (Lond) 2007;113:157-70.

Harlow BL and Signorello LB. Factors associated with early menopause. Maturitas 2000;35:3-9.

Havel RJ, Eder HA and Bragdon J. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345-53.

Hayward CS, Knight DC, Wren BG, and Kelly RP. Effect of hormone replacement therapy on non-invasive cardiovascular haemodynamics. J Hypertens 1997;15:987-93.

Hayward CS, Kraidly M, Webb CM, and Collins P. Assessment of endothelial function using peripheral waveform analysis: a clinical application. J Am Coll Cardiol 2002;40:521-8.

Hemelaar M, van der Mooren MJ, Rad M, Kluft C, and Kenemans P. Effects of non-oral postmenopausal hormone therapy on markers of cardiovascular risk: a systematic review. Fertil Steril 2008;90:642-72.

Henderson KD, Bernstein L, Henderson B, Kolonel L, and Pike MC. Predictors of the timing of natural menopause in the Multiethnic Cohort Study. Am J Epidemiol 2008;167:1287-94.

Hermsmeyer RK, Thompson TL, Pohost GM, and Kaski JC. Cardiovascular effects of medroxyprogesterone acetate and progesterone: a case of mistaken identity? Nat Clin Pract Cardiovasc Med 2008;5:387-95.

Hernan MA, Alonso A, Logan R, Grodstein F, Michels KB, Willett WC, et al. Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease. Epidemiology 2008;19:766-79.

Herrington DM and Klein KP. Randomized clinical trials of hormone replacement therapy for treatment or prevention of cardiovascular disease: a review of the findings. Atherosclerosis 2003;166:203-12.

Hickey M, Davis SR, and Sturdee DW. Treatment of menopausal symptoms: what shall we do now? Lancet 2005;366:409-21.

Hirata K, Kawakami M, and O'Rourke MF. Pulse wave analysis and pulse wave velocity. A review of blood pressure interpretation 100 years after Korotkov. Circ J 2006;70:1231-39.

Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, et al. and Estrogen in the Prevention of Atherosclerosis Trial Research Group. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2001;135:939-53.

Hodis HN, Mack WJ, Azen SP, Lobo RA, Shoupe D, et al. and Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial Research Group. Hormone therapy and the progression of coronary-artery atherosclerosis in postmenopausal women. N Engl J Med 2003;349:535-45.

Hodis HN and Mack WJ. Postmenopausal hormone therapy and cardiovascular disease in perspective. Clin Obstet Gynecol 2008;51:564-80.

Hoikkala H, Haapalahti P, Viitasalo M, Väänänen H, Sovijärvi RA, Ylikorkala O, et al. Association between vasomotor hot flashes and heart rate variability in recently postmenopausal women. Menopause 2009; Dec 12th e-pub ahead of print, doi: 10.1097/gme.0b013e3181c2bb6d.

Hsia J, Langer RD, Manson JE, Kuller L, Johnson KC, et al. and Women's Health Initiative Investigators. Conjugated equine estrogens and coronary heart disease: the Women's Health Initiative. Arch Intern Med 2006;166:357-65.

Hu FB and Grodstein F. Postmenopausal hormone therapy and the risk of cardiovascular disease: the epidemiologic evidence. Am J Cardiol 2002;90:26F-9F.

Huang AJ, Sawaya GF, Vittinghoff E, Lin F, and Grady D. Hot flushes, coronary heart disease, and hormone therapy in postmenopausal women. Menopause 2009;16:639-43.

Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, et al. 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;280:605-13.

Huopio J, Kröger H, Honkanen R, Saarikoski S, and Alhava E. Risk factors for perimenopausal fractures: a prospective study. Osteoporos Int 2000;11:219-27.

Hurskainen R, Teperi J, Rissanen P, Aalto AM, Grenman S, Kivelä A, et al. Quality of life and cost-effectiveness of levonorgestrel-releasing intrauterine system versus hysterectomy for treatment of menorrhagia: a randomised trial. Lancet 2001;357:273-7.

Ikonomidis I, Stamatelopoulos K, Lekakis J, Vamvakou GD, and Kremastinos DT. Inflammatory and non-invasive vascular markers: the multimarker approach for risk stratification in coronary artery disease. Atherosclerosis 2008;199:3-11.

International Menopause Society Consensus Statement. The Writing Group on behalf of the Workshop Consensus Group. Aging, menopause, cardiovascular disease and HRT International Menopause Society Consensus Statement. Climacteric 2009;12:368-77.

Jaakkola S, Lyytinen H, Pukkala E, and Ylikorkala O. Endometrial cancer in postmenopausal women using estradiol–progestin therapy. Obstet Gynecol 2009;114:1197-204.

James GD, Sievert LL, and Flanagan E. Ambulatory blood pressure and heart rate in relation to hot flash experience among women of menopausal age. Ann Hum Biol 2004;31:49-58.

Janssen I, Powell LH, Crawford S, Lasley B, and Sutton-Tyrrell K. Menopause and the metabolic syndrome: the Study of Women's Health Across the Nation. Arch Intern Med 2008;168:1568-75.

Jernman R. Soy supplementation and role of equol production capability in postmenopausal women using tibolone: effects on cardiovascular risk markers. Academic dissertation. University of Helsinki, Faculty of Medicine. Helsinki 2008.

Joffe HV, Ridker PM, Manson JE, Cook NR, Buring JE, and Rexrode KM. Sex hormone-binding globulin and serum testosterone are inversely associated with C-reactive protein levels in postmenopausal women at high risk for cardiovascular disease. Ann Epidemiol 2006;16:105-12.

Jokinen K, Rautava P, Mäkinen J, Ojanlatva A, Sundell J, and Helenius H. Experience of climacteric symptoms among 42-46 and 52-56-year-old women. Maturitas 2003;46:199-205.

Kannel WB, Hjortland MC, McNamara PM, and Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med 1976;85:447-52.

Kannel WB and Wilson PW. Risk factors that attenuate the female coronary disease advantage. Arch Intern Med 1995;155:57-61.

Kase NG. Impact of hormone therapy for women aged 35 to 65 years, from contraception to hormone replacement. Gend Med 2009;6 Suppl 1:37-59.

Karim R, Hodis HN, Stanczyk FZ, Lobo RA, and Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab 2008;93:131-8.

Kawano H, Motoyama T, Hirai N, Yoshimura T, Kugiyama K, Ogawa H, et al. Effect of medroxyprogesterone acetate plus estradiol on endothelium-dependent vasodilation in postmenopausal women. Am J Cardiol 2001;87:238,40, A9.

Khalil RA. Sex hormones as potential modulators of vascular function in hypertension. Hypertension 2005;46:249-54.

Kingwell BA, Medley TL, Waddell TK, Cole TJ, Dart AM, and Jennings GL. Large artery stiffness: structural and genetic aspects. Clin Exp Pharmacol Physiol 2001;28:1040-3.

Knopp RH. Risk factors for coronary artery disease in women. Am J Cardiol 2002;89:28E-34E.

Koh KK. Effects of estrogen on the vascular wall: vasomotor function and inflammation. Cardiovasc Res 2002;55:714-26.

Koh KK and Sakuma I. Should progestins be blamed for the failure of hormone replacement therapy to reduce cardiovascular events in randomized controlled trials? Arterioscler Thromb Vasc Biol 2004;24:1171-9.

Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol 2006;47:1976-83.

Koledova VV and Khalil RA. Sex hormone replacement therapy and modulation of vascular function in cardiovascular disease. Expert Rev Cardiovasc Ther 2007;5:777-89.

Komi J, Lankinen KS, Härkönen P, DeGregorio MW, Voipio S, Kivinen S, et al. Effects of ospemifene and raloxifene on hormonal status, lipids, genital tract, and tolerability in postmenopausal women. Menopause 2005;12:202-9.

Kshirsagar AV, Carpenter M, Bang H, Wyatt SB, and Colindres RE. Blood pressure usually considered normal is associated with an elevated risk of cardiovascular disease. Am J Med 2006;119:133-41.

Kuh D, Langenberg C, Hardy R, Kok H, Cooper R, Butterworth S, et al. Cardiovascular risk at age 53 years in relation to the menopause transition and use of hormone replacement therapy: a prospective British birth cohort study. BJOG 2005;112:476-85.

Kuhl H. Pharmacology of estrogens and progestogens: influence of different routes of administration. Climacteric 2005;8 Suppl 1:3-63.

Kuhl H and Stevenson J. The effect of medroxyprogesterone acetate on estrogen-dependent risks and benefits--an attempt to interpret the Women's Health Initiative results. Gynecol Endocrinol 006;22:303-17.

Kullo IJ and Malik AR. Arterial ultrasonography and tonometry as adjuncts to cardiovascular risk stratification. J Am Coll Cardiol 2007;49:1413-26.

Lakoski SG, Brosnihan B, and Herrington DM. Hormone therapy, C-reactive protein, and progression of atherosclerosis: data from the Estrogen Replacement on Progression of Coronary Artery Atherosclerosis (ERA) trial. Am Heart J 2005;150:907-11.

Lane HA, Smith JC, and Davies JS. Noninvasive assessment of preclinical atherosclerosis. Vasc Health Risk Manag 2006;2:19-30.

Langer RD. Efficacy, safety, and tolerability of low-dose hormone therapy in managing menopausal symptoms. J Am Board Fam Med 2009;22:563-73.

Laurent S and Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension 2007;49:1202-6.

Leal M, Diaz J, Serrano E, Abellan J, and Carbonell LF. Hormone replacement therapy for oxidative stress in postmenopausal women with hot flushes. Obstet Gynecol 2000;95:804-9.

Lee MS, Kim JI, Ha JY, Boddy K, and Ernst E. Yoga for menopausal symptoms: a systematic review. Menopause 2009;16:602-8.

Lethaby A, Hogervorst, E, Richards, M, Yesufu, A, and Yaffe, K. Hormone replacement therapy for cognitive function in postmenopausal women. Cochrane Database Syst Rev 2008;(1):CD003122.

Lethaby AE, Brown J, Marjoribanks J, Kronenberg F, Roberts H, and Eden J. Phytoestrogens for vasomotor menopausal symptoms. Cochrane Database Syst Rev 2007;(4):CD001395.

Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, and Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;360:1903-13.

L'Hermite M, Simoncini T, Fuller S, and Genazzani AR. Could transdermal estradiol + progesterone be a safer postmenopausal HRT? A review. Maturitas 2008;60:185-201.

Lind L. Circulating markers of inflammation and atherosclerosis. Atherosclerosis 2003;169:203-14.

Lindh-Åstrand L, Brynhildsen J, Hoffman M, and Hammar, M. Vasomotor symptoms usually reappear after cessation of postmenopausal hormone therapy: a Swedish population-based study. Menopause 2009;16:1213-7.

Lindh-Åstrand L, Bixo M, Hirschberg AL, Sundström-Poromaa I, and Hammar M. A randomized controlled study of taper-down or abrupt discontinuation of hormone therapy in women treated for vasomotor symptoms. Menopause 2010;17:72-9.

Lobo RA. Surgical menopause and cardiovascular risks. Menopause 2007;14:562-6.

Lobo RA. Metabolic syndrome after menopause and the role of hormones. Maturitas 2008;60:10-8.

Lobo RA, Pinkerton JV, Gass ML, Dorin MH, Ronkin S, Pickar JH, et al. Evaluation of bazedoxifene/conjugated estrogens for the treatment of menopausal symptoms and effects on metabolic parameters and overall safety profile. Fertil Steril 2009;92:1025-38.

Lobo RA. Evaluation of cardiovascular event rates with hormone therapy in healthy, recently postmenopausal women: results from 2 large clinical trials. Arch Intern Med 2009;164:482-4.

London G, Guerin A, Pannier B, Marchais S, Benetos A, and Safar M. Increased systolic pressure in chronic uremia. Role of arterial wave reflections. Hypertension 1992;20:10-9.

Loprinzi CL and Barton DL. On hot flash mechanism, measurement, and treatment. Menopause 2009;16:621-3.

Low DA, Davis SL, Keller DM, Shibasaki M, and Crandall CG. Cutaneous and hemodynamic responses during hot flashes in symptomatic postmenopausal women. Menopause 2008;15:290-5.

Luoto R. Hot flushes and quality of life during menopause. BMC Women's Health 2009;9:13.

Luotola H. Blood pressure and hemodynamics in postmenopausal women during estradiol-17ß substitution. Academic dissertation. University of Helsinki, Faculty of Medicine. Helsinki 1983.

Luukkainen T, Allonen H, Haukkamaa M, Lähteenmäki P, Nilsson CG, and Toivonen J. Five years' experience with levonorgestrel-releasing IUDs. Contraception 1986;33:139-48.

Lyytinen H, Pukkala E, and Ylikorkala O. Breast cancer risk in postmenopausal women using estrogen-only therapy. Obstet.Gynecol. 2006;108:1354-60.

Lyytinen H, Pukkala E, and Ylikorkala O. Breast cancer risk in postmenopausal women using estradiol-progestogen therapy. Obstet Gynecol 2009a;113:65-73.

Lyytinen H, Dyba T, Pukkala E, and Ylikorkala O. Do the dose or route of administration of norethisterone acetate as a part of hormone therapy play a role in risk of breast cancer: Nation-wide case-control study from Finland. Int J Cancer 2009b; Oct 26. [Epub ahead of print]

Lyytinen HK, Dyba T, Ylikorkala O, and Pukkala EI. A case-control study on hormone therapy as a risk factor for breast cancer in Finland: Intrauterine system carries a risk as well. Int J Cancer 2010;126:483-9.

Lähteenmäki P, Haukkamaa M, Puolakka J, Riikonen U, Sainio S, Suvisaari J, et al. Open randomised study of use of levonorgestrel releasing intrauterine system as alternative to hysterectomy. BMJ 1998;316:1122-6.

Mackenzie IS, Wilkinson IB, and Cockcroft JR. Assessment of arterial stiffness in clinical practice. QJM 2002;95:67-74.

Maclennan AH, Broadbent JL, Lester S, and Moore V. Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane Database Syst Rev 2004;(4):CD002978.

Malacara JM, Perez-Luque EL, Martinez-Garza S, and Sanchez-Marin FJ. The relationship of estrogen receptor-alpha polymorphism with symptoms and other characteristics in post-menopausal women. Maturitas 2004;49:163-9.

Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2007;28:1462-536.

Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, et al., and Women's Health Initiative Investigators. Estrogen plus progestin and the risk of coronary heart disease. N Engl J Med 2003;349:523-34.

Manson JE, Allison MA, Rossouw JE, Carr JJ, et al., and WHI and WHI-CACS Investigators. Estrogen therapy and coronary-artery calcification. N Engl J Med 2007;356:2591-602.

Marchais SJ, Guerin AP, Pannier BM, Levy BI, Safar ME, and London GM. Wave reflections and cardiac hypertrophy in chronic uremia. Influence of body size. Hypertension 1993;22:876-83.

McEniery CM, Wallace S, Mackenzie IS, McDonnell B, Yasmin, Newby DE, et al. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. Hypertension 2006;48:602-8.

Mendelsohn ME and Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999;340:1801-11.

Mendelsohn ME and Karas RH. Molecular and cellular basis of cardiovascular gender differences. Science 2005;308:1583-7.

Mendelsohn ME and Karas RH. HRT and the young at heart. N Engl J Med 2007;356:2639-41.

Meurman JH, Tarkkila L, and Tiitinen A. The menopause and oral health. Maturitas 2009;63:56-62.

Mikkola T, Turunen P, Avela K, Orpana A, Viinikka L, and Ylikorkala O. 17 Beta-estradiol stimulates prostacyclin, but not endothelin-1, production in human vascular endothelial cells. J Clin Endocrinol Metab 1995;80:1832-6.

Mikkola T, Ranta V, Orpana A, Viinikka L, and Ylikorkala O. Hormone replacement therapy modifies the capacity of plasma and serum to regulate prostacyclin and endothelin-1 production in human vascular endothelial cells. Fertil Steril 1996;66:389-93.
Mikkola TS and Clarkson TB. Estrogen replacement therapy, atherosclerosis, and vascular function. Cardiovasc Res 2002;53:605-19.

Mikkola TS, Clarkson TB, and Notelovitz M. Postmenopausal hormone therapy before and after the women's health initiative study: what consequences? Ann Med 2004;36:402-13.

Mikkola TS and Ylikorkala O. Hormone therapy and cardiovascular disease--still much to be learnt. Gynecol Endocrinol 2005;20:116-20.

Mikkola TS and Clarkson TB. Coronary heart disease and postmenopausal hormone therapy: conundrum explained by timing? J Women's Health (Larchmt) 2006;15:51-3.

Mikkola TS. Cardiovascular risk assessment in postmenopausal women: the role of the gynecologist. Climacteric 2009;12 Suppl 1:58-61.

Miller VM and Duckles SP. Vascular actions of estrogens: functional implications. Pharmacol Rev 2008;60:210-41.

Miura S, Tanaka E, Mori A, Toya M, Takahashi K, Nakahara K, et al. Hormone replacement therapy improves arterial stiffness in normotensive postmenopausal women. Maturitas 2003;45:293-8.

Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women. The Framingham Heart Study. Hypertension 2004;43:1239-45.

Modena MG, Sismondi P, Mueck AO, Kuttenn F, Lignieres B, et al. and TREAT. New evidence regarding hormone replacement therapies is urgently required transdermal postmenopausal hormone therapy differs from oral hormone therapy in risks and benefits. Maturitas 2005;52:1-10.

Mosca L, Banka CL, Benjamin EJ, Berra K, Bushnell C, Dolor RJ, et al. Expert Panel/Writing Group, American Heart Association, American Academy of Family Physicians, American College of Obstetricians and Gynecologists, American College of Cardiology Foundation, Society of Thoracic Surgeons, American Medical Women's Association, Centers for Disease Control and Prevention, Office of Research on Women's Health, Association of Black Cardiologists, American College of Physicians, World Heart Federation, National Heart, Lung, and Blood Institute and American College of Nurse Practitioners. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. Circulation 2007;115:1481-501.

Mueck AO and Seeger H. Effect of hormone therapy on BP in normotensive and hypertensive postmenopausal women. Maturitas 2004;49:189-203.

Mueck AO and Seeger H. Biochemical markers surrogating on vascular effects of sex steroid hormones. Gynecol Endocrinol 2006;22:163-73.

Munzel T, Sinning C, Post F, Warnholtz A, and Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Ann Med 2008;40:180-96.

Murabito JM, Yang Q, Fox C, Wilson PW, and Cupples LA. Heritability of age at natural menopause in the Framingham Heart Study. J Clin Endocrinol Metab 2005;90:3427-30.

Murgo JP, Westerhof N, Giolma JP, and Altobelli SA. Aortic input impedance in normal man: relationship to pressure wave forms. Circulation 1980;62:105-16.

Musunuru K, Kral BG, Blumenthal RS, Fuster V, Campbell CY, Gluckman TJ, et al. The use of high-sensitivity assays for C-reactive protein in clinical practice. Nat Clin Pract Cardiovasc Med 2008;5:621-35.

Nappi RE and Lachowsky M. Menopause and sexuality: prevalence of symptoms and impact on quality of life. Maturitas 2009;63:138-41.

Nath A and Sitruk-Ware R. Different cardiovascular effects of progestins according to structure and activity. Climacteric 2009;12 Suppl 1:96-101.

Nelesen R, Krohn P, and Dimsdale JE. Hot-flash hypotension. N Engl J Med 2004;351:1577-9.

Nelson HD. Commonly used types of postmenopausal estrogen for treatment of hot flashes: scientific review. JAMA 2004;291:1610-20.

Nelson HD, Vesco KK, Haney E, Fu R, Nedrow A, Miller J, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA 2006;295:2057-71.

Nelson HD. Menopause. Lancet 2008;371:760-70.

Nelson RE, Grebe SK, OKane DJ, and Singh RJ. Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma. Clin Chem 2004;50:373-84.

Ness J, Aronow WS, and Beck G. Menopausal symptoms after cessation of hormone replacement therapy. Maturitas 2006;53:356-61.

Nikander E, Kilkkinen A, Metsä-Heikkilä M, Adlercreutz H, Pietinen P, Tiitinen A, et al. A randomized placebo-controlled crossover trial with phytoestrogens in treatment of menopause in breast cancer patients. Obstet Gynecol 2003;101:1213-20.

Nilsson CG, Luukkainen T, Diaz J, and Allonen H. Intrauterine contraception with levonorgestrel: a comparative randomised clinical performance study. Lancet 1981;1:577-80.

Notelovitz M, Lenihan JP, McDermott M, Kerber IJ, Nanavati N, and Arce J. Initial 17beta-estradiol dose for treating vasomotor symptoms. Obstet Gynecol 2000;95:726-31.

Notelovitz M. Postmenopausal tibolone therapy: biologic principles and applied clinical practice. MedGenMed 2007;9:2. Published online 2007, January 3.

Ockene JK, Barad DH, Cochrane BB, Larson JC, Gass M, Wassertheil-Smoller S, et al. Symptom experience after discontinuing use of estrogen plus progestin. JAMA 2005;294:183-93.

O'Rourke MF, Pauca A, and Jiang XJ. Pulse wave analysis. Br J Clin Pharmacol 2001;51:507-22.

O'Rourke MF and Adji A. An updated clinical primer on large artery mechanics: implications of pulse waveform analysis and arterial tonometry. Curr Opin Cardiol 2005;20:275-81.

Ouyang P, Michos ED, and Karas RH. Hormone replacement therapy and the cardiovascular system lessons learned and unanswered questions. J Am Coll Cardiol 2006;47:1741-53.

Ouyang P, Vaidya D, Dobs A, Golden SH, Szklo M, Heckbert SR, et al. Sex hormone levels and subclinical atherosclerosis in postmenopausal women: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2009;204:255-61.

Øverlie I, Moen MH, Holte A, and Finset A. Androgens and estrogens in relation to hot flushes during the menopausal transition. Maturitas 2002;41:69-77.

Paakkari P, Saastamoinen L, Martikainen J, and Voipio T. Lääkevuosi 2008, Medicines in 2008. www.nam.fi. National Agency for Medicines and the Social Insurance Institution. Accessed 24.11.2009.

Packard RR and Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem 2008;54:24-38.

Panay N, Ylikorkala O, Archer DF, Gut R, and Lang E. Ultra-low-dose estradiol and norethisterone acetate: effective menopausal symptom relief. Climacteric 2007;10:120-31.

Panay N. Estrogen dose: the cardiovascular impact. Climacteric 2009;12 Suppl 1:91-5.

Patel PD and Arora RR. Endothelial dysfunction: a potential tool in gender related cardiovascular disease. Ther Adv Cardiovasc Dis 2008;2:89-100.

Peled Y, Perri T, Pardo Y, and Kaplan B. Levonorgestrel-releasing intrauterine system as an adjunct to estrogen for the treatment of menopausal symptoms--a review. Menopause 2007;14:550-4.

Pepine CJ, Nichols WW, and Pauly DF. Estrogen and different aspects of vascular disease in women and men. Circ Res 2006;99:459-61.

Pickering TG, Shimbo D, and Haas D. Ambulatory blood-pressure monitoring. N Engl J Med 2006;354:2368-74.

Pinkerton JV and Stovall DW. Is there an association between vasomotor symptoms and both low bone density and cardiovascular risk? Menopause 2009;16:219-23.

Pinkerton JV, Utian WH, Constantine GD, Olivier S, and Pickar JH. Relief of vasomotor symptoms with the tissue-selective estrogen complex containing bazedoxifene/conjugated estrogens: a randomized, controlled trial. Menopause 2009;16:1116-24.

Polo-Kantola P, Saaresranta T, and Polo O. Aetiology and treatment of sleep disturbances during perimenopause and postmenopause. CNS Drugs 2001;15:445-52.

Prentice RL, Langer RD, Stefanick ML, Howard BV, Pettinger M, Anderson G, et al. Combined postmenopausal hormone therapy and cardiovascular disease: toward resolving the discrepancy between observational studies and the Women's Health Initiative clinical trial. Am J Epidemiol 2005;162:404-14.

Prentice RL, Manson JE, Langer RD, Anderson GL, Pettinger M, Jackson RD, et al. Benefits and risks of postmenopausal hormone therapy when it is initiated soon after menopause. Am J Epidemiol 2009;170:12-23.

Preston RA. Comparative effects of conventional vs. novel hormone replacement therapy on blood pressure in postmenopausal women. Climacteric 2009;12 Suppl 1:66-70.

Rachon D and Teede H. Postmenopausal hormone therapy and the risk of venous thromboembolism. Climacteric 2008;11:273-9.

Randolph JF Jr, Sowers M, Bondarenko I, Gold EB, Greendale GA, Bromberger JT, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. J Clin Endocrinol Metab 2005;90:6106-12.

Rapkin AJ. Vasomotor symptoms in menopause: physiologic condition and central nervous system approaches to treatment. Am J Obstet Gynecol 2007;196:97-106.

Rexrode KM, Manson JE, Lee IM, Ridker PM, Sluss PM, Cook NR, et al. Sex hormone levels and risk of cardiovascular events in postmenopausal women. Circulation 2003;108:1688-93.

Ridker PM, Hennekens CH, Buring JE, and Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.

Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, et al., and JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195-207.

Ritz E. Total cardiovascular risk management. Am J Cardiol 2007;100:53J-60J.

Ropponen A, Aittomäki K, Tikkanen MJ, and Ylikorkala O. Levels of serum C-reactive protein during oral and transdermal estradiol in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy. J Clin Endocrinol Metab 2005a;90:142-6.

Ropponen A, Aittomäki K, Vihma V, Tikkanen MJ, and Ylikorkala O. Effects of oral and transdermal estradiol administration on levels of sex hormone-binding globulin in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy. J Clin Endocrinol Metab 2005b;90:3431-4.

Rosano GM, Vitale C, Marazzi G, and Volterrani M. Menopause and cardiovascular disease: the evidence. Climacteric 2007;10 Suppl 1:19-24.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, et al., and Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321-33.

Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 2007;297:1465-77.

Rutanen EM and Ylikorkala O. Kapseli 33: Vaihdevuosien hormonihoito. Lääkelaitoksen ja Kelan julkaisuja, 2. painos, Joensuu 2004.

Rutanen EM, Heikkinen J, Halonen K, Komi J, Lammintausta R, and Ylikorkala O. Effects of ospemifene, a novel SERM, on hormones, genital tract, climacteric symptoms, and quality of life in postmenopausal women: a double-blind, randomized trial. Menopause 2003;10:433-9.

Rönnback M, Hernelahti M, Hämäläinen E, Groop PH, and Tikkanen H. Effect of physical activity and muscle morphology on endothelial function and arterial stiffness. Scand J Med Sci Sports 2007;17:573-9.

Safar ME. Pulse pressure, arterial stiffness and wave reflections (augmentation index) as cardiovascular risk factors in hypertension. Ther Adv Cardiovasc Dis 2008;2:13-24.

Santoro N. Symptoms of menopause: hot flushes. Clin Obstet Gynecol 2008;51:539-48.

Santoro N and Komi J. Prevalence and impact of vaginal symptoms among postmenopausal women. J Sex Med 2009;6:2133-42.

Sarabi M, Vessby B, Millgard J, and Lind L. Endothelium-dependent vasodilation is related to the fatty acid composition of serum lipids in healthy subjects. Atherosclerosis 2001;156:349-55.

Schenck-Gustafsson K. Risk factors for cardiovascular disease in women. Maturitas 2009;63:186-90.

Schilling C, Gallicchio L, Miller SR, Langenberg P, Zacur H, and Flaws JA. Genetic polymorphisms, hormone levels, and hot flashes in midlife women. Maturitas 2007;57:120-31.

Schindler AE, Campagnoli C, Druckmann R, Huber J, Pasqualini JR, Schweppe KW, et al. Classification and pharmacology of progestins. Maturitas 2003;46 Suppl 1:S7-S16.

Schneider BP, Radovich M, Flockhart DA, Carpenter JS, Li L, Robarge JD, et al. Exploratory study evaluating the association of polymorphisms of angiogenesis genes with hot flashes. Breast Cancer Res Treat 2009;116:543-9.

Shanafelt TD, Barton DL, Adjei AA, and Loprinzi CL. Pathophysiology and treatment of hot flashes. Mayo Clin Proc 2002;77:1207-18.

Shaw LJ, Bugiardini R, and Merz CN. Women and ischemic heart disease: evolving knowledge. J Am Coll Cardiol 2009;54:1561-75.

Shen W and Stearns V. Treatment strategies for hot flushes. Expert Opin Pharmacother 2009;10:1133-44.

Shulman LP. Effects of progestins in different hormone replacement therapy formulations on estrogen-induced lipid changes in postmenopausal women. Am J Cardiol 2002;89:47E,54E; discussion 54E-5E.

Sievert LL. Variation in sweating patterns: implications for studies of hot flashes through skin conductance. Menopause 2007;14:742-51.

Simpkins JW, Brown K, Bae S, and Ratka A. Role of ethnicity in the expression of features of hot flashes. Maturitas 2009;63:341-6.

Sirola J, Kröger H, Honkanen R, Jurvelin JS, Sandini L, et al., and OSTPRE Study Group. Factors affecting bone loss around menopause in women without HRT: a prospective study. Maturitas 2003;45:159-67.

Sitruk-Ware R. Progestins and cardiovascular risk markers. Steroids 2000;65:651-8.

Sitruk-Ware R. Pharmacological profile of progestins. Maturitas 2004;47:277-83.

Sitruk-Ware R. The levonorgestrel intrauterine system for use in peri- and postmenopausal women. Contraception 2007a;75:S155-60.

Sitruk-Ware R. New hormonal therapies and regimens in the postmenopause: routes of administration and timing of initiation. Climacteric 2007b;10:358-70.

Skurnick JH, Weiss G, Goldsmith LT, Santoro N, and Crawford S. Longitudinal changes in hypothalamic and ovarian function in perimenopausal women with anovulatory cycles: relationship with vasomotor symptoms. Fertil Steril 2009;91:1127-34.

Sloan JA, Loprinzi CL, Novotny PJ, Barton DL, Lavasseur BI, and Windschitl H. Methodologic lessons learned from hot flash studies. J Clin Oncol 2001;19:4280-90.

Sourander L, Rajala T, Räihä I, Mäkinen J, Erkkola R, and Helenius H. Cardiovascular and cancer morbidity and mortality and sudden cardiac death in postmenopausal women on oestrogen replacement therapy (ERT). Lancet 1998;352:1965-9.

Sowers MR, Randolph J Jr, Jannausch M, Lasley B, Jackson E, and McConnell D. Levels of sex steroid and cardiovascular disease measures in premenopausal and hormone-treated women at midlife: implications for the "timing hypothesis". Arch Intern Med 2008;168:2146-53.

Staessen JA, van der Heijden-Spek JJ, Safar ME, Den Hond E, Gasowski J, Fagard RH, et al. Menopause and the characteristics of the large arteries in a population study. J Hum Hypertens 2001;15:511-8.

Stampfer MJ and Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. Prev Med 1991;20:47-63.

Statistics Finland. Tilastokeskus; Väestö iän mukaan 2008 lopussa. http://www.stat.fi/tup/suoluk/suoluk_vaesto.html. Accessed December, 15th, 2009.

Stearns V, Ullmer L, Lopéz J, Smith Y, Isaacs C, and Hayes D. Hot flushes. Lancet 2002;360:1851-61.

Stefanick ML. Estrogens and progestins: background and history, trends in use, and guidelines and regimens approved by the US Food and Drug Administration. Am J Med 2005;118 Suppl 12B:64-73.

Strandberg T and Tikkanen MJ. Lowered cholesterol beneficial for vascular endothelium. Nord Med 1996;111:10-2.

Strandberg TE, Ylikorkala O, and Tikkanen MJ. Differing effects of oral and transdermal hormone replacement therapy on cardiovascular risk factors in healthy postmenopausal women. Am J Cardiol 2003;92:212-4.

Sturdee DW. The menopausal hot flush--anything new? Maturitas 2008;60:42-9.

Suk Danik J, Rifai N, Buring JE, and Ridker PM. Lipoprotein(a), hormone replacement therapy, and risk of future cardiovascular events. J Am Coll Cardiol 2008;52:124-31.

Sutton-Tyrrell K, Wildman RP, Matthews KA, Chae C, Lasley BL, et al., and SWAN Investigators. Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). Circulation 2005;111:1242-9.

Suvanto-Luukkonen E, Koivunen R, Sundström H, Bloigu R, Karjalainen E, Häivä-Mällinen L, et al. Citalopram and fluoxetine in the treatment of postmenopausal symptoms: a prospective, randomized, 9-month, placebo-controlled, double-blind study. Menopause 2005;12:18-26.

Svartberg J, von Muhlen D, Kritz-Silverstein D, and Barrett-Connor E. Vasomotor symptoms and mortality: the Rancho Bernardo Study. Menopause 2009;16:888-91.

Svetkey LP. Management of prehypertension. Hypertension 2005;45:1056-61.

Sztejnsznajd C, Silva ME, Nussbacher A, Gebara OE, D'Amico EA, Rocha DM, et al. Estrogen treatment improves arterial distensibility, fibrinolysis, and metabolic profile in postmenopausal women with type 2 diabetes mellitus. Metabolism 2006;55:953-9.

Takahashi K, Miura S, Mori-Abe A, Kawagoe J, Takata K, Ohmichi M, et al. Impact of menopause on the augmentation of arterial stiffness with aging. Gynecol Obstet Invest 2005;60:162-6.

Takeo C, Negishi E, Nakajima A, Ueno K, Tatsuno I, Saito Y, et al. Association of cytosine-adenine repeat polymorphism of the estrogen receptor-beta gene with menopausal symptoms. Gend Med 2005;2:96-105.

Teede HJ. Sex hormones and the cardiovascular system: effects on arterial function in women. Clin Exp Pharmacol Physiol 2007;34:672-6.

The Finnish Medical Society and the Academy of Finland 2004. Konsensuskokous 2004: Vaihdevuosien hormonihoito. Vammalan Kirjapaino Oy, Vammala 2004.

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

The World Health Organization. Prevention of cardiovascular disease: guidelines for assessment and management of total cardiovascular risk. 2007;1-85. WHO Press, World Health Organization, Geneva, Switzerland.

Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, Hess R, and Matthews KA. Hot flashes and subclinical cardiovascular disease: findings from the Study of Women's Health Across the Nation Heart Study. Circulation 2008a;118:1234-40.

Thurston RC, Sowers MR, Sutton-Tyrrell K, Everson-Rose SA, Lewis TT, Edmundowicz D, et al. Abdominal adiposity and hot flashes among midlife women. Menopause 2008b;15:429-34.

Tikkanen MJ. Estrogens, progestins and lipid metabolism. Maturitas 1996a;23 Suppl:S51-5.

Tikkanen MJ. The menopause and hormone replacement therapy: lipids, lipoproteins, coagulation and fibrinolytic factors. Maturitas 1996b;23:209-16.

Towfighi A, Zheng L, and Ovbiagele B. Sex-specific trends in midlife coronary heart disease risk and prevalence. Arch Intern Md 2009;169:1762-6.

Tuimala RJ and Vihtamäki T. Individual hormone replacement therapy. Maturitas 1996;23 Suppl:S87-90.

Tuomikoski P, Aittomäki K, Mikkola TS, Ropponen A, and Ylikorkala O. Effect of oral and transdermal hormone therapy on hyaluronic acid in women with and without a history of intrahepatic cholestasis of pregnancy. Am J Obstet Gynecol 2008;198:375.e1,375.e5.

Törmälä R, Appt S, Clarkson TB, Groop PH, Rönnback M, Ylikorkala O, et al. Equol production capability is associated with favorable vascular function in postmenopausal women using tibolone; no effect with soy supplementation. Atherosclerosis 2008;198:174-8.

US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for industry: labeling guidance for noncontraceptive estrogen drug products for the treatment of vasomotor symptoms and vulvar and vaginal atrophy symptoms .Prescribing information for health care providers and patient labeling [revision 1]. Rockville, MD: Division of Drug Information, Center for Drug Evaluation and Research (CDER), 2004;1-2.

Utian WH. Psychosocial and socioeconomic burden of vasomotor symptoms in menopause: a comprehensive review. Health Qual Life Outcomes 2005;3:47.

Utian WH. NIH and WHI: time for a mea culpa and steps beyond. Menopause 2007;14:1056-9.

Utian WH, Archer DF, Bachmann GA, Gallagher C, Grodstein F, Heiman JR, et al. and North American Menopause Society. Estrogen and progestogen use in postmenopausal women: July 2008 position statement of The North American Menopause Society. Menopause 2008;15:584-602.

van de Weijer PH, Mattsson LA and Ylikorkala O. Benefits and risks of long-term low-dose oral continuous combined hormone therapy. Maturitas 2007;56:231-48.

van de Weijer PH. Risks of hormone therapy in the 50-59 year age group. Maturitas 2008;60:59-64.

van der Schouw YT and Grobbee DE. Menopausal complaints, oestrogens, and heart disease risk: an explanation for discrepant findings on the benefits of post-menopausal hormone therapy. Eur Heart J 2005;26:1358-61.

van der Schouw YT. Incidence and mortality of cardiovascular disease in postmenopausal women world-wide and relevance for preventive strategies. Climacteric 2009;12 Suppl 1:1-5.

Varas-Lorenzo C, Garcia-Rodriguez LA, Perez-Gutthann S, and Duque-Oliart A. Hormone replacement therapy and incidence of acute myocardial infarction. A population-based nested case-control study. Circulation 2000;101:2572-8.

Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease. N Engl J Med 2001;345:1291-7.

Vaskivuo TE and Tapanainen JS. Apoptosis in the human ovary. Reprod Biomed Online 2003;6:24-35.

Verdecchia P, Angeli F and Cavallini C. Ambulatory blood pressure for cardiovascular risk stratification. Circulation 2007;115:2091-3.

Vihtamäki T and Tuimala R. Can climacteric women self-adjust therapeutic estrogen doses using symptoms as markers? Maturitas 1998;28:199-203.

Vihtamäki T, Luukkaala T and Tuimala R. Skin contamination by oestradiol gel--a remarkable source of error in plasma oestradiol measurements during percutaneous hormone replacement therapy. Maturitas 2004;48:347-53.

Visser M and Coelingh Bennink HJ. Clinical applications for estetrol. J Steroid Biochem Mol Biol 2009;114:85-9.

Visvanathan K, Gallicchio L, Schilling C, Babus JK, Lewis LM, Miller SR, et al. Cytochrome gene polymorphisms, serum estrogens, and hot flushes in midlife women. Obstet Gynecol 2005;106:1372-81.

Vitale C, Mercuro G, Cerquetani E, Marazzi G, Patrizi R, Pelliccia F, et al. Time since menopause influences the acute and chronic effect of estrogens on endothelial function. Arterioscler Thromb Vasc Biol 2008;28:348-52.

Vongpatanasin W. Autonomic regulation of blood pressure in menopause. Semin Reprod Med 2009;27:338-45.

Wakatsuki A, Okatani Y, Ikenoue N, and Fukaya T. Effect of medroxyprogesterone acetate on vascular inflammatory markers in postmenopausal women receiving estrogen. Circulation 2002;105:1436-9.

Walldius G and Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy--a review of the evidence. J Intern Med 2006;259:493-519.

Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006;355;2631-9.

Wang X, Keith JC Jr, Struthers AD, and Feuerstein GZ. Assessment of arterial stiffness, a translational medicine biomarker system for evaluation of vascular risk. Cardiovasc Ther 2008;26:214-23.

Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. JAMA 2002;288:2432-40.

Waugh EJ, Lam MA, Hawker GA, McGowan J, Papaioannou A, Cheung AM, et al. Perimenopause BMD Guidelines Subcommittee of Osteoporosis Canada. Risk factors for low bone mass in healthy 40-60 year old women: a systematic review of the literature. Osteoporos Int 2009;20:1-21.

Wells G, Tugwell P, Shea B, Guyatt G, Peterson J, et al., and Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. Endocr Rev 2002;23:529-39.

Whiteman MK, Staropoli CA, Benedict JC, Borgeest C, and Flaws JA. Risk factors for hot flashes in midlife women. J Women's Health (Larchmt) 2003;12:459-72.

Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, and Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol 2000;525 Pt 1:263-70.

Wilkinson IB, MacCallum H, Hupperetz PC, van Thoor CJ, Cockcroft JR, and Webb DJ. Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man. J Physiol 2001;530:541-50.

Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler Thromb Vasc Biol 2002a;22:147-52.

Wilkinson IB, Mohammad NH, Tyrrell S, Hall IR, Webb DJ, Paul VE, et al. Heart rate dependency of pulse pressure amplification and arterial stiffness. Am J Hypertens 2002b;15:24-30.

Winkleby MA, Kraemer HC, Ahn DK, and Varady AN. Ethnic and socioeconomic differences in cardiovascular disease risk factors: findings for women from the Third National Health and Nutrition Examination Survey, 1988-1994. JAMA 1998;280:356-62.

Witteman JC, Grobbee DE, Kok FJ, Hofman A, and Valkenburg HA. Increased risk of atherosclerosis in women after the menopause. BMJ 1989;298:642-4.

Wittrock M, Scholze A, Compton F, Schaefer J-H, Zidek W, and Tepel M. Noninvasive pulse wave analysis for the determination of central artery sitffness. Microvasc Res 2009;77:109-22.

Wong J, Wong S, Handa P, and Abbott C. Hormone replacement use, arterial distensibility, cardiac structure and circadian blood pressure profile in menopausal women. Blood Press 2005;14:12-20.

Woods NF, Mitchell ES, Tao Y, Viernes HM, Stapleton PL, and Farin FM. Polymorphisms in the estrogen synthesis and metabolism pathways and symptoms during the menopausal transition: observations from the Seattle Midlife Women's Health Study. Menopause 2006;13:902-10.

Yeboah J, Reboussin DM, Waters D, Kowalchuk G, and Herrington DM. Effects of estrogen replacement with and without medroxyprogesterone acetate on brachial flow-mediated vasodilator responses in postmenopausal women with coronary artery disease. Am Heart J 2007;153:439-44.

Strauss, JF III and Barbieri, RL (edit.). Yen and Jaffe's Reproductive Endocrinology. Physiology, Pathophysiology, and Clinical Management. 2004;423-30. Elsevier Saunders, Philadelphia, PN, USA.

Ylikorkala O, Puolakka J, and Viinikka L. Vasoconstrictory thromboxane A2 and vasodilatory prostacyclin in climacteric women: effect of oestrogen-progestogen therapy. Maturitas 1984;5:201-5.

Ylikorkala O, Kuusi T, Tikkanen MJ, and Viinikka L. Desogestrel- and levonorgestrel-containing oral contraceptives have different effects on urinary excretion of prostacyclin metabolites and serum high density lipoproteins. J Clin Endocrinol Metab 1987;65:1238-42.

Ylikorkala O, Orpana A, Puolakka J, Pyorälä T, and Viinikka L. Postmenopausal hormonal replacement decreases plasma levels of endothelin-1. J Clin Endocrinol Metab 1995;80:3384-7.

Ylikorkala O, Cacciatore B, Paakkari I, Tikkanen MJ, Viinikka L, and Toivonen J. The long-term effects of oral and transdermal postmenopausal hormone replacement therapy on nitric oxide, endothelin-1, prostacyclin, and thromboxane. Fertil Steril 1998;69:883-8.

Ylikorkala O, Cacciatore B, Halonen K, Lassila R, Lammintausta R, Rutanen EM, et al. Effects of ospemifene, a novel SERM, on vascular markers and function in healthy, postmenopausal women. Menopause 2003;10:440-7.

Ylikorkala O. HRT as secondary prevention of cardiovascular disease. Maturitas 2004;47:315-8.

Zanchetti A, Facchetti R, Cesana GC, Modena MG, Pirrelli A, et al., and SIMONA participants. Menopause-related blood pressure increase and its relationship to age and body mass index: the SIMONA epidemiological study. J Hypertens 2005;23:2269-76.

Zaydun G, Tomiyama H, Hashimoto H, Arai T, Koji Y, Yambe M, et al. Menopause is an independent factor augmenting the age-related increase in arterial stiffness in the early postmenopausal phase. Atherosclerosis 2006;184:137-42.

Zoungas S and Asmar RP. Arterial stiffness and cardiovascular outcome. Clin Exp Pharmacol Physiol 2007;34:647-51.