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Effects of postmenopausal hot flushes and hormone therapy on quality of life and cardiovascular autonomic function

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ACADEMIC DISSERTATION

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To my loved ones

ABSTRACT

Hot flushes, the most characteristic symptoms in menopause, are encountered by c.a. 80% of women. Hot flushes and other menopausal complaints can significantly impair a woman's quality of life. It is, however, unclear why some women experience intolerable hot flushes while others remain completely asymptomatic. Hot flushes are characterised by cardiovascular reactions such as rapid episodes of reddening of skin and palpitations. Thus, women with or without hot flushes may differ in their cardiovascular reactivity regulated by the autonomic nervous system and responses to hormone therapy. Moreover, hot flushes have been discussed as one explanation for the differing results of hormone therapy's cardiovascular health effects.

The present study was designed to investigate the impact of hot flushes and different forms of hormone therapy on health-related quality of life. The relationship between a history of premenstrual symptoms and the postmenopausal quality of life and hot flushes was also assessed. The aim of this research was to explore the effects of hot flushes on the cardiovascular autonomic function before and during hormone therapy.

The cardiovascular autonomic function was studied in 150 healthy, recently postmenopausal women with a standardised test series in controlled laboratory settings. Women showed a large variation in hot flushes, which were evaluated prospectively with a two-week hot flush diary.

Hot flushes impaired health-related quality of life in menopause, but had little effect on the women's sexual wellbeing. A history of premenstrual symptoms did not predict the severity of postmenopausal hot flushes, but was associated with poor sleep, depressive feelings, and impaired memory and concentration in menopause. Women with hot flushes had non-significantly lower increases in blood pressure in response to isometric muscle contraction than women without hot flushes. Women with hot flushes reacted with more tachycardia during the Valsalva manoeuvre and with slightly blunted parasympathetic activity in heart rate responses to active orthostatic testing compared with asymptomatic women.

In the six-month hormone therapy trial, women with or without hot flushes were treated in a double-blind randomised setting with transdermal estradiol hemihydrate gel (1mg/day), oral estradiol valerate (2 mg/day) alone or in combination with medroxyprogesterone acetate (5 mg/day), or with a placebo.

All hormone therapy regimens alleviated hot flushes and other menopausal symptoms equally effectively, but did not affect sexual wellbeing. In women with pre-treatment hot flushes, hormone therapy improved the health-related quality of life in terms of sleep, anxiety and fears, memory and concentration, and general health compared with those

receiving a placebo. Hot flushes were accompanied with lowered resting blood pressures but increases in blood pressure responses to isometric muscle contraction during all hormone therapy regimens. Resting diastolic blood pressure was lower during estradiol treatment (oral or transdermal) in women with pre-treatment hot flushes compared with non-flushing women. In women with pre-treatment hot flushes estradiol treatment reduced the resting heart rate compared with placebo treatment. This effect was attenuated by medroxyprogesterone acetate in treatment. Hot flushes associated with reduced maximal heart rate in response to isometric muscle contraction during estradiol treatment, yet the addition of medroxyprogesterone acetate also eliminated this effect. In women with hot flushes, hormone therapy reduced very low frequency power during controlled breathing compared with baseline level and with non-flushing women. Otherwise, hormone therapy did not affect heart rate variability.

In conclusion, premenstrual symptoms do not predict troublesome hot flushes, but do associate with impaired quality of life in menopause. Hot flushes impair the health-related quality of life, but can be effectively alleviated with hormone therapy. Hot flushes seem to associate with slightly pronounced sympathetic responses in autonomic regulation of heart rate and blood pressure. This potentially unfavourable activity can be reduced with estradiol treatment in women with hot flushes, who initiate hormone therapy in clinical practice. Progestin-containing hormone therapy blunted or even converted the positive effects of estradiol on heart rate regulation. Thus, the hot flush status contributes markedly to the quality of life and cardiovascular autonomic function before and during hormone therapy. Particularly women with hot flushes appear to benefit most from the positive effects of hormone therapy on the health-related quality of life and cardiovascular regulation.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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

I Savolainen-Peltonen H, Hautamäki H, Tuomikoski P, Ylikorkala O, Mikkola TS. Health-related quality of life in women with or without hot flashes: a randomized placebo-controlled trial with hormone therapy. Menopause 2014;21:732-9.

II Hautamäki H, Haapalahti P, Savolainen-Peltonen H, Tuomikoski P, Ylikorkala O, Mikkola TS. Premenstrual symptoms in fertile age are associated with impaired quality of life, but not hot flashes, in recently postmenopausal women. Menopause December 2014: in press, Epub ahead of print May 2014

III Hautamäki H, Piirilä P, Haapalahti P, Tuomikoski P, Sovijärvi ARA, Ylikorkala O, Mikkola TS. Cardiovascular autonomic responsiveness in postmenopausal women with and without hot flushes. Maturitas 2011;68:368-73.

IV Hautamäki H, Haapalahti P, Piirilä P, Tuomikoski P, Sovijärvi ARA, Ylikorkala O, Mikkola TS. Effect of hot flushes on cardiovascular autonomic responsiveness: A randomized controlled trial on hormone therapy. Maturitas 2012;72:243-8.

V Hautamäki H, Mikkola TS, Sovijärvi ARA, Piirilä P, Haapalahti P. Menopausal hot flushes do not associate with changes in heart rate variability in controlled testing: a randomized trial on hormone therapy. Acta Obstetricia et Gynecologica Scandinavica 2013;92:902-8.

ABBREVIATIONS

CEE	Conjugated equine estrogens
CES-D	Centre for Epidemiological Studies depression scale
CoV	Coefficient of variation
CVD	Cardiovascular disease
E_2	Estradiol
EPT	Estrogen-progestogen therapy
ET	Estrogen therapy
EuroQOL EQ-5D	European Quality of Life Instrument
FSH	Follicle stimulating hormone
HERS	Heart and Estrogen/Progestin Replacement study
HF	High frequency band
HFWWS	Hot flush weekly weighted symptom score
HR	Heart rate
HRQL	Health-related quality of life
HRV	Heart rate variability
HT	Hormone therapy
LF	Low frequency band
MFSQ	McCoy Female Sexuality Questionnaire
MPA	Medroxyprogesterone acetate
NETA	Norethisterone acetate
PMS	Premenstrual syndrome
PMDD	Premenstrual dysphoric disorder
PSST	Premenstrual symptoms screening tool
Rand-36	Rand 36-item Health Survey
RMSSD	Square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals
SF-36	Medical Outcome Study 36-item Short Form General Health Survey
VAS	Visual analogue scale
SD	Standard deviation
SEM	Standard error of mean
TSEC	Tissue-selective estrogen complex
VLF	Very low frequency band
WHI	Women's health initiative study
WHQ	Women's health questionnaire
15D	15-dimensional generic health-related quality of life instrument

INTRODUCTION

Women reach menopause at the mean age of 51. Approximately every three out of four women experience menopausal symptoms, which can significantly deteriorate the quality of life. Typical menopausal symptoms include vasomotor hot flushes, night sweats, broken sleep, mood swings, muscle and joint pain, and impaired memory and concentration *(Nelson 2008)*. Many of the menopausal symptoms resemble premenstrual symptoms, which are complained about by up to 80% of women in fertile age *(Deecher & Dorries 2007)*. Thus, menopausal and premenstrual symptoms might share similar underlying attributes. In clinical practice, women with premenstrual symptoms may often worry whether they also have an increased risk for troublesome menopausal hot flushes.

Despite substantial investigation, the exact etiology of hot flushes, the most characteristic symptoms in menopause, has remained unclear. One plausible mechanism is thermoregulatory imbalance at the hypothalamic level arising from menopausal hypoestrogenism (*Freedman 2005*). Since the autonomic nervous system also regulates the thermal balance of the body, the role of the autonomic function changes in hot flush physiology is under active research *(Hoikkala et al. 2010, Thurston et al. 2010a, Freedman et al. 2011, Thurston et al. 2012, de Zambotti et al. 2013)*. Hot flushes have been associated with a worsened cardiovascular disease (CVD) risk profile in some (*Gast et al. 2008*, *Thurston et al. 2008*, *Thurston et al. 2010b, Thurston et al. 2011a*), but not all (*Tuomikoski et al. 2009a, Hitchcock et al. 2012, Wolff et al. 2013)* studies. The autonomic nervous system is the main regulator of the cardiovascular system. The sympathetic and parasympathetic branches reciprocally control blood pressure, heart rate, cardiac function, and the vascular bed maintaining homeostasis in the body. The cardiovascular autonomic regulation is altered along aging and at menopause; sympathetic activity increases and parasympathetic decreases *(Brockbank et al. 2000, Lavi et al. 2007, Vongpatanasin 2009)*. Whether these changes are associated with menopause itself or menopausal symptoms is not known.

Hot flushes and other menopausal symptoms have been treated with estrogen therapy (ET) for over 70 years. About a decade ago, the differing findings between former observational (*Grady et al. 1992*, *Grodstein 1996*, *Grodstein et al. 2000*, *Stram et al. 2011*) and randomised controlled studies (*Hulley et al. 1998*, *Cherry et al. 2002*, *Grady et al. 2002a*, *Rossouw et al. 2002*) led to a debate concerning the cardiovascular safety of hormone therapy (HT). The randomised controlled studies in women without hot flushes did not confirm the beneficial effects of HT on CVD risk seen in the previous observational studies. Later on, these differing results have been widely discussed and the timing of HT, various HT formulations, the role of progestogen versus estrogen only therapy, and different administration

routes have been suggested as possible explanations for the divergent results *(Mikkola & Ylikorkala 2005, Clarkson et al. 2013, Harman 2014, Tuomikoski & Mikkola 2014)*. The importance of hot flushes as one possible explanation has arisen (*Tuomikoski et al. 2011*).

The present studies were designed to explore the association of premenstrual and menopausal complaints and the potential impact of hot flushes on cardiovascular autonomic function and quality of life before and after six months of different types of postmenopausal HT.

REVIEW OF THE LITERATURE

General aspects of menopause

Menopause, defined as the final menstrual period, is the natural ending of a woman's reproductive life. Natural menopause is defined as spontaneous cessation of menstruation for twelve consecutive months (*McKinlay et al. 1992*). Normally, menopause occurs between ages 45 to 55, and the menopausal transition can last for several years (*Soules et al. 2001, Butler & Santoro 2011*). The time period preceding menopause is characterised by low or lacking concentration of progesterone and rising levels of the follicle stimulating hormone (FSH), often leading to cycle irregularities (*Kase 2009*). Along with FSH, secretion of the luteinizing hormone rises and the estradiol (E_2) level slowly declines, finally reaching the hypoestrogenic state of postmenopause (*Anttila & Salmi 2004*). In clinical practice, an FSH level over 30 IU/l is considered postmenopausal, but it is known to fluctuate around the menopause (*Anttila et al. 1991*). The circulating levels of the FSH and the luteinizing hormone remain high for several years after menopause and the latter also stimulates the androgen production from the ovaries.

Due to fluctuating estrogen levels, most perimenopausal women encounter typical symptoms such as hot flushes, night sweats, mood swings, palpitations, joint and muscle aches, poor sleep, and impaired memory or concentration (*Freeman et al. 2007, Nelson 2008*) (Table 1). Some of these symptoms, such as poor sleep, may be secondary to hot flushes (*Freedman 2014*). Long-term changes after menopause include, e.g., atrophy of the vaginal epithelium (*Santoro & Komi 2009, Kingsberg et al. 2013*), degradation of connective tissues (*Calleja-Agius & Brincat 2012*) and bone loss (*Waugh et al. 2009*). Hot flushes and other menopausal symptoms are bothersome and thus, up to 75% of women seek medical advice from health care professionals (*Carpenter et al. 2011*).

Table 1. *Typical menopausal symptoms.*

Subjective	Objective
Hot flushes	Vaginal atrophy
Night sweats	Osteopenia
Palpitations	Osteoporosis
Poor sleep	Pelvic floor defects
Depressive symptoms	Urine control problems
Headaches	Degradation of connective tissues and skin
Difficulty concentrating	
Poor memory	
Joint aches	
Irritability	
Nervousness	
Anxiety	

Despite the longer life expectancy the mean age at menopause has remained the same (*Gold et al. 2001*, *Kase 2009*). In Finland women reach menopause at 51 years of age on average and in 2013 there were over 1.1 million Finnish women in the age group of 51 years or older (*Tilastokeskus 2014*). The number of postmenopausal women is increasing in Finland and other western countries as the population ages. Smoking (*Gold et al. 2001*, *Parente et al. 2008*), low socioeconomic status (*Gold et al. 2006*), and hysterectomy (*Farquhar et al. 2005*) have been linked with earlier menopause. Association between menopausal age and nulliparity, low number of pregnancies, use of oral contraceptives, or low body mass index have remained inconclusive (*Gold et al. 2001*). Furthermore, some studies have reported ethnical differences in age of natural menopause: Far-Eastern and Japanese women reach their menopause later and Afro-American women earlier than Caucasian women (*Bromberger et al. 1997*, *Gold et al. 2001*, *Richard-Davis & Wellons 2013*). This could have important clinical implications, since younger age at menopause associates with an elevated risk of CVD (*Archer 2009*, *Stuenkel 2012*, *Wellons et al. 2012*), osteoporosis (*Gallagher 2007*, *Shuster et al. 2010*), and shorter life expectancy (*Cooper & Sandler 1998*).

Health-related quality of life

Health-related quality of life (HRQL) is defined as a person's perception of one's physical, cognitive, and mental health (*Utian & Woods 2013*). During the menopausal transition, HRQL is generally decreased, mainly due to menopausal complaints such as hot flushes, mood alterations, poor sleep, impaired memory, and sexual dysfunction. Furthermore, the risk of developing depressive symptoms in perimenopause is increased (*Llaneza et al. 2012*), but association between hot flushes and depressive symptoms is unclear.

Postmenopausal women often complain of impaired memory and concentration, which may decrease HRQL (*Weber et al. 2013a*). Menopausal transition seems to have a temporary negative impact on cognition, but no clear long term effects (*Greendale et al. 2009*, *Henderson 2011*, *North American Menopause Society 2012*). Data on the association between deterioration of memory and hot flushes are controversial (*LeBlanc et al. 2007*, *Maki et al. 2008*, *Greendale et al. 2010*, *Schaafsma et al. 2010*, *Mitchell & Woods 2011*). Since estrogen has direct effects on the brain (*Resnick et al. 2006*), cognitive decline in the perimenopause may be independent from hot flushes, sleep disturbances or depressive symptoms (*Weber et al. 2013b*). Still, the decline of HRQL during menopause and the role of hot flushes in this have remained unclear.

Sexual dysfunction due to troubling symptoms, such as vaginal and vulvar atrophy, decreased vaginal lubrication, and possibly diminished libido, may impair HRQL in menopause. Additionally, psychological and relationship factors determine the individual experience of the menopause, which all affect the sexual well-being and HRQL of women (*Bachmann & Leiblum 2004*, *Nappi et al. 2010*).

Multiple validated questionnaires should be used in studies on HRQL (*Nachtigall 2009*). The HRQL questionnaires are often divided into two types: generic and specific. Generic HRQL questionnaires (e.g. SF-36, Rand-36, EuroQOL EQ-5D, 15D) are applicable across a wide range of population and interventions, whereas specific questionnaires are designed for particular subpopulations or interventions (*Coons et al. 2000*). Furthermore, menopause-specific HRQL questionnaires are recommended for midlife women. For example, the Women's Health questionnaire (WHQ) (*Hunter 1992*) is age-specific (45 to 65 years) and validated reflecting the effects of menopausal symptoms on HRQL (*Hunter 2000*) (Appendix A). Additionally, there are other menopause-specific HRQL questionnaires, such as the Greene Climacteric scale (*Greene 1976*) and the Menopause Rating Scale (*Schneider et al. 2000*). Although both of these scales (Greene Climacteric scale, Menopause Rating Scale) are validated (*Utian & Woods 2013*), they are not widely used in HRQL studies.

The Kupperman Index is an example of a traditional menopause-related symptom list. It was originally developed to investigate the efficacy of

different hormonal preparations (*Kupperman et al. 1953*), but has been modified over the decades. It measures symptoms as a menopausal index, a sum of symptom points weighted according to their prevalence (the higher the score, the worse the menopausal symptoms) (Appendix B). In addition, subjective perception of one's general health and well-being can be evaluated with a visual analogue scale (VAS), ranging from the worst imaginable state to the best imaginable state (scale from 0 to 100) (*Welton et al. 2008*) (Appendix C). This VAS is a part of the validated European Quality of Life questionnaire (Euro QOL) (*Jenkinson et al. 1997*). A specific question of general health compared with one year ago also describes subjective perception of the current HRQL (Appendix C). It is included in the Rand 36-Item Health Survey (Rand-36) and has been used separately, for example, in the WHI study (*Hays et al. 2003*, *Brunner et al. 2005*).

Although some menopause-specific HRQL questionnaires also address sexual function matters, many instruments have been developed specifically for evaluating sexuality. Three types of self-report measures are available: self-administered questionnaires, diaries, and structured interviews (*Rosen 2001*). Typically, female sexual functioning is assessed in the areas of desire, arousal, orgasm, partner factors, and sexual pain (*Bachmann & Leiblum 2004*). These areas are thoroughly covered in the McCoy Female Sexuality Questionnaire (MFSQ) (Appendix D), which is designed and validated to measure aspects of female sexuality during menopausal transition (*McCoy & Davidson 1985*). Other questionnaires are also available, such as the Female Sexual Function Index (*Rosen et al. 2000*), the Female Sexual Function Inventory (*Berman et al. 1999*), and the Brief Index of Sexual Functioning for Women (*Mazer et al. 2000*).

Hot flushes

Hot flushes are the most characteristic symptoms during the menopause, also often referred to as vasomotor symptoms or hot flashes in American literature. Up to 80% of women report hot flushes of different severity (*Nelson 2008*). The flushing usually peaks at one year after final menstruation and subsides with increasing age, but it can last for several years. Sometimes the symptoms can re-start after treatment cessation (*Ockene et al. 2005*). Some women start or continue to have vasomotor symptoms later on after menopause in their 60s or even 70s (*Barnabei et al. 2002*, *Hunter et al. 2012*).

Women show great variation in hot flushes (none to severe), and a familial tendency is apparent (*Staropoli et al. 1998, Murabito et al. 2005*), which has led to several suggestions for the background mechanisms. One suggested explanation is the diversity in genes guiding estrogen metabolism or estrogen receptors (*Miller et al. 2008*). Furthermore, many social and cultural factors, such as socioeconomic status, marital status, diet, and

attitude affect women's coping styles. Even ethnic differences in menopausal symptoms are seen; Asian women experience less symptoms than other ethnic groups and African Americans have more vasomotor symptoms than Caucasian women (*Gold et al. 2006*, *Miller et al. 2006*, *Freeman & Sherif 2007*, *Richard-Davis & Wellons 2013*). The role of body mass in the subjective experience of hot flushes has remained controversial *(Thurston et al. 2011b, Thurston & Joffe 2011)*. Latest results conclude that obese women encounter more hot flushes, cycle irregularities and heavy bleeding in the premenopausal stage, but after the final menstrual period, obese women are less likely to experience hot flushes than their lean counterparts (*Butler & Santoro 2011*).

The assessment of hot flushes varies greatly, and in many studies women answer only one or a few questions whether they have experienced hot flushes during the past months or years. This retrospective method is subject to recall bias. Hot flushes should be registered prospectively for one to two weeks to obtain reliable information and both the severity and frequency should be rated (*Loprinzi et al. 2009*), because hot flushes show considerable day-to-day variation (*Sloan et al. 2001*). There are several hot flush rating scales for scientific and clinical purposes, of which the Hot Flush Weekly Weighted Symptom score (HFWWS) is an established and validated questionnaire (*Sloan et al. 2001*)(Table 9, p. 45). For research purposes, it has been proposed that sternal skin conductance measurement should be used for quantifying hot flushes in clinical trials (*Carpenter et al. 2004*). This method, however, is prone to errors, because other sweating or sympathetic activation can be misinterpreted as hot flushes. Therefore, current opinion recommends the use of prospective hot flush diaries that are based on a woman's subjective evaluation of her hot flushes, as in clinical practice when initiation of HT is considered (*Loprinzi & Barton 2009*).

A hot flush can vary from a mild sensation of warmth to a strong sensation of heat throughout the body with extensive perspiration, reddening of the skin, palpitation, and anxiety. One hot flush usually lasts less than five minutes (*Nelson 2008*). During a hot flush, the sensation of heat starts typically in the chest area or upper trunk and spreads upwards. The frequency of flushing varies individually, ranging from a few per month to several flushes per hour. Hot flushes associate with typical physiological changes, such as increased skin blood flow and heart rate (*Sturdee 2008*).

Etiology of hot flushes has remained unknown. Hot flushes are related to changes in the hypothalamic thermoregulation and associate with a narrowed thermoneutral zone in regulation of the core body temperature *(Freedman 2005)*. The lowering levels of estrogens are followed by decreased endorphin concentrations in the hypothalamus, which increases the release of serotonin and noradrenalin. These neurotransmitters lower the set point in the thermoregulatory nucleus, which causes heat loss during hot flushes (*Freedman 2005*, *Archer et al. 2011*). Heat loss from the skin is regulated by the autonomic nervous system. As part of the thermoregulation of the body, the

sympathetic nervous system controls cutaneous vasomotor activity and sweating. In studies concerning the mechanisms of vasomotor symptoms, skin blood flow and sympathetic nerve activity have increased during hot flushes (*Low et al. 2008*), and women with vasomotor symptoms have shown elevations in sympathetic activity (*Deecher & Dorries 2007*, *Sturdee 2008*, *Freedman et al. 2011*). Since the autonomic nervous system is the main regulator of the peripheral vasculature, it may contribute to the mechanisms behind postmenopausal hot flushes. Interestingly, altered function of the autonomic nervous system has also been suggested as a possible underlying mechanism behind premenstrual symptoms (*Palmero & Choliz M 1991*, *Girdler et al. 1998*).

Resemblance of menopausal symptoms with premenstrual symptoms

Premenstrual symptoms of variable severity have been reported in up to 80% of women (*Halbreich 2003*). These symptoms are most common in women in their thirties and forties but they can affect women's HRQL from the teen age years through to the menopause. Typical premenstrual symptoms include irritability, anxiety or depressive mood, tiredness, sleeping problems, overeating, headache, breast tenderness and bloating (*Halbreich 2004*) (Table 2). Women may have only a single symptom or a cluster of related symptoms. Women with significantly impairing symptoms are diagnosed with premenstrual syndrome (PMS) (*Halbreich et al. 2007*).

The pattern of symptom manifestation is essential for PMS diagnosis. Patients typically experience symptoms in the luteal phase of the menstrual cycle, and once menstruation begins they disappear. According to diagnostic criteria, these symptoms should also cause significant impairment to daily life. Premenstrual syndrome affects 30-40% of the reproductive female population (*Baker & O'Brien 2012, Direkvand-Moghadam et al. 2014*). A particularly severe form of PMS is premenstrual dysphoric disorder (PMDD) with an emphasis on the affective symptoms. It is diagnosed in approximately 1-8% of women (*Halbreich 2003*, *Gehlert et al. 2009*, *Biggs & Demuth 2011*).

The etiology of premenstrual symptoms is not clearly understood (*Biggs & Demuth 2011*), and it is most probably multifactorial (*Halbreich 2003*, *Yonkers et al. 2008*). The proposed underlying mechanisms are various, such as, fluctuation in gonadal hormones, their metabolites and interactions with neurotransmitters (*Halbreich 2003*). Possibly, the normal gonadal hormone fluctuations during the menstrual cycle trigger an abnormal serotonergic response in the 'vulnerable' women. Autonomic regulation has also differed in women with severe premenstrual symptoms that are seen as decreased parasympathetic activity compared with asymptomatic women (*Matsumoto et al. 2006*, *Matsumoto et al. 2007*). Moreover, autonomic activity seems to vary

across the normal menstrual cycle. Some studies show increased sympathetic activity (*Sato et al. 1995*, *Guasti et al. 1999*, *Yildirir et al. 2002*), whereas others show increased parasympathetic activity (*Fuenmayor et al. 2000*, *Princi et al. 2005*) in the luteal phase compared with the follicular phase. Other studies have not found differences in autonomic activity during the phases of the menstrual cycle (*Leicht et al. 2003*, *Nakagawa et al. 2005*). Thus, the autonomic regulation in the etiology of premenstrual symptoms remains unclear.

Table 2. *Typical premenstrual symptoms.*

Psychological	**Physical**
Anger/Irritability	Breast tenderness/swelling
Depression	Weight gain
Anxiety	Bloating
Mood swings	Headache
Anhedonia	Joint pain
Poor sleep	Muscle pain
Decreased interest in home/social/work activities	
Lethargy	
Concentration difficulties	
Overeating/Cravings	

The fact that both premenstrual and postmenopausal symptoms share similar features, such as mood swings, sleeping problems and muscle and joint pain, has initiated research on the possible association between these symptoms. Premenstrual symptoms and a more troublesome perimenopause have associated in some (*Collins & Landgren 1994*, *Morse et al. 1998*, *Freeman et al. 2004*), but not in all studies (*Guthrie et al. 1996*) (Table 3). Premenstrual symptoms have been associated especially with psychological distress in postmenopause (*Stewart & Boydell 1993*, *Morse et al. 1998*). The possible association between premenstrual symptoms and hot flushes, however, remain unclear.

Many validated prospective screening questionnaires and charts of premenstrual symptoms exist, for example the Daily Record of Severity of Problems chart (*Endicott et al. 2006*). In retrospective assessment of premenstrual symptoms, the International Society for Premenstrual Disorders Montreal consensus statement (*O'Brien et al. 2011*) recommends the Premenstrual Symptom Screening Tool (PSST) (*Steiner et al. 2003*). This consists of a comprehensive premenstrual symptom list and questions about how premenstrual symptoms impair working capacity, social activities, home responsibilities or personal relationships rated on a severity scale (Appendix E).

Table 3. *Previous studies on associations between premenstrual symptoms and menopausal complaints.*

Research/author	Methods		Association to previous PMS (+/-)
	Premenstrual symptoms	**Menopausal symptoms**	+ = association - = no association
Stewart & Boydell 1993 *n*=86	Self-report questionnaire of previous PMS and other diagnoses	-Psychological distress -Brief Symptom Inventory	psychological distress in menopause +
Collins & Landgren 1994 *n*=1324	Self-report questions about premenstrual symptoms	Menopause Symptom Inventory	**vasomotor symptoms +**
Guthrie et al. 1996 *n*=438	1 question retrospectively	2 questions (past 2 weeks)	**hot flushes –** E_2 – FSH – (in the post-menopausal group)
Morse et al. 1998 *n*=291	Womens' own list of complaints fitted to MDQ=Menstrual Distress Questionnaire	Menopause-related symptoms (past 2 weeks)	**hot flushes –** dysphoria + skeletal + digestive + respiratory +
Freeman et al. 2004 *n*=320	2 questions (with severity rating)	-hot flushes (frequency and severity, past 1 month) -depressive symptoms (20-item inventory) -sleep -libido	**hot flushes +** depressed mood + decreased libido + poor sleep +

E_2 = Estradiol, FSH = Follicle stimulating hormone, PMS = Premenstrual syndrome

Premenstrual symptoms can be treated with psychotropic, hormonal, and even with surgical methods (*Baker & O'Brien 2012*). Selective serotonin reuptake inhibitors are the drug of choice for severe PMS and PMDD, since improvement of both psychological and somatic symptoms have been demonstrated in several studies (*Dimmock et al. 2000*, *Freeman et al. 2001, Bethea et al. 2002*).

The hormonal treatment of premenstrual symptoms is based on ovulation suppression, and for this oral combined contraceptive pills are commonly used (*Rapkin 2003*). Oral contraceptives containing drospirenone have shown an advantage over other oral contraceptives due to drospirenone's antiandrogenic and antialdosteronic properties (*Pearlstein et al. 2005, Anttila et al. 2011*). Near menopause, a combination of transdermal estrogen and a levonorgestrel releasing intrauterine device is recommended for treatment of PMS (*Baker & O'Brien 2012*).

Risk for cardiovascular disease

Cardiovascular disease, which is the leading cause of morbidity and mortality in both women and men in the Western world (*Mosca et al. 2011*, *Mikkola et al. 2013*), can manifest as coronary heart disease, myocardial infarction, transient ischemic cerebral attacks or stroke. Over the past decades, women's cardiovascular risk profile has worsened (*Towfighi et al. 2009*), and thus, mortality for CVD causes in women is higher than in men (*Collins et al. 2007*, *Shaw et al. 2009*). A large body of epidemiological evidence demonstrates that women's risk for CVD elevates after the menopause compared with age-matched men (*Go et al. 2013*, *Miller et al. 2013*), but the data are not completely uniform (*Barrett-Connor 1997*, *Vaidya et al. 2011*). Recent findings of a large Finnish population study show that CVD mortality in men accelerates at a relatively young age, but in women, the risk shows a steep increase around 60 years of age (*Mikkola et al. 2013*). Thus, it is highly important to identify and improve CVD risk factors in women at their mid-life years (*Puurunen et al. 2011*).

The menopause-induced hypoestrogenism is the most plausible explanation for this sex-specific change in the CVD incidence (*Collins 2001*, *Mendelsohn & Karas 2005*, *Vitale et al. 2009*, *Mikkola et al. 2013*). This theory gains support from studies associating premature or early menopause and the subsequent prolonged hypoestrogenism with an elevation in age-adjusted risk for CVD (*Atsma et al. 2006*, *Archer 2009*, *Shuster et al. 2010*). Hypoestrogenism may promote vascular inflammation, endothelial dysfunction (*Ylikorkala et al. 1998*, *Novella et al. 2012*) and development of an atherogenic lipid profile (*Tikkanen 1996*, *Rosano et al. 2007*).

Postmenopausal hormone therapy

Menopausal symptoms have been treated with ET for over seven decades (*Stefanick 2005*). Current guidelines recommend estrogen as the most effective treatment to alleviate vasomotor symptoms and other menopausal complaints (*Duodecim konsensuslausuma 2005*, *Skouby et al. 2005*, *Santen et al. 2010*, *North American Menopause Society 2012*). Estrogen is also used for the prevention of osteoporosis (*Santen et al. 2010*, *Tuppurainen et al. 2010*, *Sturdee et al. 2011*, *Osteoporoosi: Käypä hoito - suositus 2014*), as estrogen deprivation leads to the deterioration of both bone structure and bone mineral density after menopause. In the USA, conjugated equine estrogens (CEE) are commonly used in HT, whereas in Europe, mainly 17β-estradiol is used, and this is also the only available systemic estrogen in Finland. Since the use of unopposed ET is associated with an increased risk of endometrial cancer, combination therapy with progestin (EPT) is required for endometrial protection if a woman has an intact uterus. In the USA CEE is most often combined with medroxyprogesterone acetate (MPA), whereas in Europe, a large variety of progestins are available for EPT.

The primary indication for HT is alleviation of moderate or severe hot flushes. Initiation of HT is always an individual's decision after weighing the risks and benefits with her doctor. Generally accepted contraindications of HT are a history of breast cancer, venous thromboembolism, untreated hypertension, heart failure, severe liver disease, systemic lupus erythematosus, and vaginal bleeding of unknown origin. The recommendations for HT suggest treatment with the lowest effective dose for the shortest possible time (*Duodecim konsensuslausuma 2005*, *Skouby et al. 2005*, *Santen et al. 2010*). Sole ET has a more favorable risk-benefit profile (*Skouby et al. 2005*, *Santen et al. 2010*, *Sturdee et al. 2011*, *North American Menopause Society 2012*) (Table 4). Current guidelines suggest an individual evaluation of the menopausal symptoms every 2 to 3 years and consideration of HT continuation.

Table 4. *Risks and benefits of postmenopausal hormone therapy.*

	Postmenopausal hormone therapy (Number of cases/10 000 person years)		
Benefits		**Risks**	
Bone fracture	ET: -56 EPT: -46	Breast cancer	ET: -8 EPT: +8
Coronary heart events	ET: -3 EPT: +6	Stroke (> 60 years) #	ET: +11 EPT: +9
Diabetes	EPT: -15	Venous thrombosis #	ET: +7 EPT: +12
Colon cancer	EPT: -6	Gallbladder disease	ET: +33 EPT: +20
		Urinary incontinence	ET: +1271 EPT: +872

not with transdermal administration

Based on (*Mikkola 2012*) and (*Nelson et al. 2012*) review including 9 trials, most of the results reported from the WHI trial

ET=Estrogen therapy

EPT= Estrogen-progestogen therapy

Estrogens

The three natural estrogens of the human body are E_2, estrone, and estriol, of which E_2 is the most potent one. Estrone carries approximately 4% of the estrogenic activity of E_2. Estradiol is produced mainly by the growing ovarian follicles and the corpus luteum, the placenta, and adrenals, but also in the liver, endometrium, brain, muscle, and adipose tissue. There is conversion between the hormonally active 17β-estradiol and the weak estrone and their sulfates. In the postmenopause, estrone is the main estrogen of the body produced by aromatization in the adipose tissue. Only 2% of the circulating estrogens are free and active, whereas the majority are bound to the serum proteins, such as the sex hormone binding globulin and albumin *(Kuhl 2005).*

Estrogen receptors are found throughout the body, e.g., the reproductive organs, breast, muscle, and bone tissues, the brain, blood vessels (vascular smooth muscle and endothelial cells), the heart, and also in the coronary arteries (*Kuhl 2005*, *Turgeon et al. 2006*, *Ling et al. 2006*). There are at least

three receptors: the nuclear ERα and ERβ are based on genomic mechanisms, and the third receptor in the cell membrane acts by rapid non-genomic mechanisms (*Miller et al. 2008*).

The hormonal potency of estrogens is measured by their affinity to receptors and the intracellular concentration of estrogen. This potency is dependent on the free fraction of the circulating estrogen. The CEE is a natural mixture of estrogen sulfates extracted from the urine of pregnant mares, and therefore the composition and potency may vary. The most potent estrogen in the CEE is equilin, but it contains both E_2 and estrone, which humans also produce. The estrogenic potency of CEE is considerably higher compared with E_2 (*Kuhl 2005*), and approximately 0.625 mg of oral CEE is equivalent to 2 mg of oral E_2.

Progestogens

Natural progesterone is produced mainly in the corpus luteum and the placenta. Albumin binds 80% of the circulating progesterone with low affinity, and 17% is bound to corticosteroid-binding globulin with high affinity, while only 3% remains free. Synthetic progestins applied in HT are derivatives of progesterone, 19-norprogesterone, 19-nortestosterone (testosterone), or spironolactone (*Sitruk-Ware 2008*). They differ widely in their hormonal pattern with estrogenic, androgenic or antiandrogenic, glucocorticoid, and antimineralocorticoid actions (Table 5) *(Kuhl 2005, Schindler et al. 2003, Nath et al. 2009)*.

In women with an intact uterus, progestogens are required in HT to inhibit the estrogen-induced proliferation of the endometrium. This antiestrogenic effect is characterised with the 'transformation dose' reflecting the dose needed to cause full secretory transformation of the proliferated endometrium. The biological effects of progestogens are generally dependent on the presence of estrogens. Progesterone has two main types of receptors, PRA and PRB, but it additionally acts by rapid non-genomic interactions with membrane binding sites (*Kuhl 2005*). The receptors are found throughout the body, including the cardiovascular and central nervous systems.

Medroxyprogesterone acetate is a 17-OH-progesterone derivative, which has a 100% bioavailability after oral administration, as it does not undergo inactivation during the first-pass metabolism. Most of MPA (88%) is bound to albumin in the circulation, and it is partly stored in the adipose tissue. Common doses administered in postmenopausal HT are 5-10 mg daily during sequential or cyclic therapy and 2,5 mg during continuous combined therapy. Medroxyprogesterone acetate possesses weak androgenic properties and considerable glucocorticoid effects (*Herkert et al. 2001*). It has been the progestin component in many HT studies.

Table 5. *Biological activity of progestogens available for hormone therapy in Finland. (+ effective, ± weakly effective, - none)*

Progestogen	Estogenic	Anti-estrogenic	Andro-genic	Anti-androgenic	Anti-mineralo-corticoid	Gluco-corticoid
Progesterone	-	+	-	±	+	+
Dydrogesterone	-	+	-	-	±	-
Progesterone derivatives						
Medroxyprogesterone acetate	-	+	±	-	-	+
Testosterone derivatives						
Norethisterone acetate	+	+	+	-	-	-
Levonorgestrel	-	+	+	-	-	-
Lynestrenol	+	+	+	-	-	-
Spironolactone derivatives						
Drospirenone	-	+	-	+	+	-

Route of administration

Systemic HT can be administered through oral, transdermal, and vaginal routes. In addition, a levonorgestrel-containing intrauterine device enables intrauterine endometrial protection during combination therapy (*Suvanto-Luukkonen et al. 1998*). In Finland, tablets for oral and patches and gel for transdermal treatments are available. A common daily dose of oral and transdermal gel E_2 is 1-2 mg and 50-75 µg of transdermal patch E_2. The vaginal route is used in Finland only for topical treatment of the mucosa and for that purpose tablets, rings, and creams are available.

Orally administered estrogens are exposed to first-pass metabolism in the liver, unlike transdermally administered. Thus, orally administered estrogens increase sex hormone-binding globulin, corticosteroid-binding globulin, thyroxin-binding globulin, and angiotensinogen synthesis in the liver more effectively than transdermal estrogens (*Kuhl 2005*). Furthermore, orally and transdermally administered estrogens have showed different effects on some cardiovascular markers. Oral estrogen has positive effects on lipids increasing high-density lipoprotein and decreasing low-density lipoprotein, but negative effects in hemostasis, triglycerides, and inflammatory markers involved in atherosclerotic plaque (*Barton 2013*), whereas transdermal estrogen's effect is neutral. Transdermal estrogen does not increase the risk of venous thromboembolism in contrast to oral estrogens (*Olie et al. 2011*). Knowledge of differences between progestogens administration routes is sparse.

Effects of postmenopausal hormone therapy

Health-related quality of life

Several major studies have been published regarding effects of HT on women's HRQL, (*Hlatky et al. 2002*, *Hays et al. 2003*, *Archer et al. 2005*, *Brunner et al. 2005*, *Welton et al. 2008*) (Table 6). The results are inconclusive, and many of them lack an evaluation of hot flushes. For example, the Women's Health Initiative trial (WHI) found no benefit of ET (*Brunner et al. 2005*) or of EPT (*Hays et al. 2003*) on HRQL. On the contrary, results of the Heart and Estrogen/Progestin Replacement Study (HERS) trial showed improvement in emotional measures of HRQL in women with vasomotor symptoms (*Hlatky et al. 2002*). The effects of HT on HRQL in women without hot flushes is controversial (*Hlatky et al. 2002*, *Hays et al. 2003*, *Brunner et al. 2005*, *Welton et al. 2008*), and only one study showed some beneficial changes in sleep and sexual functioning after HT independent of the baseline hot flushes (*Welton et al. 2008*).

Hormone therapy has improved women's sexual function and satisfaction (*Welton et al. 2008*, *Gast et al. 2009*) especially in symptomatic women within five years from menopause (*Nastri et al. 2013*). However, the WHI trial shows no benefit of HT on sexual functioning (*Hays et al. 2003*), although this finding is criticised because they addressed sexual functioning only with one question. Local ET improves sexual satisfaction and reduces vaginal dryness by improving the vaginal mucosa condition and thickness, lubrication, and sensation in vaginal tissues as the blood flow increases (*Cayan et al. 2008*).

Table 6. *Impact of hormone therapy on Health related quality of life (HRQL) in previous studies.*

Study	Study population	Treatment	Outcome measures	Results
Hlatky et al. 2002 HERS	*n*=2763 with CHD 67 y	3 years EPT (CEE 0.625 mg +MPA 2.5 mg) Placebo	Rand-36 scales, Burnam scale	-All women: physical functioning, mental health, energy ↓ -Women with hot flushes: mental health ↑, depression scores ↓
Gambacciani et al. 2003	*n*=50 54 y	12 weeks EPT (E_2 1mg + NETA 0.5 mg) Controls: calcium	WHQ Impact of hot flushes -	Vasomotor and somatic symptoms,anxiety/fear, depressed mood, and poor sleep ↑
Hays et al. 2003 WHI	*n*=1511 63 y	3 years EPT (CEE 0.625 mg +MPA 2.5 mg) Placebo	Rand-36, WHI Insomnia Rating Scale, Burnam scale	-at 1 year: physical function, bodily pain, sleep slightly ↑ -at 3 years: ↔ -In 50-54 y group with hot flushes: vasomotor symptoms and sleep ↑
Brunner et al. 2005 WHI	*n*=1189 63 y	3 years ET (CEE 0.625 mg) Placebo	Rand-36 WHI Insomnia Rating Scale Burnam scale	-at 1 year: sleep ↑ -at 3 years ↔ -In 50-54 y group with hot flushes HRQL ↔
Archer et al. 2005	*n*=845 56 y	13 months ET(E_2 1 mg) EPT(E_2+drospirenone 1/2/3 mg)	SF-36, WHQ Impact of hot flushes -	-WHQ vasomotor symptoms and sleep ↑ -SF-36 scores ↔
Ylikangas et al. 2005	*n*=208 56 y	9 years EPT (E_2 2 mg + MPA 5 mg) Controls (n=771)	15D Impact of hot flushes -	HRQL ↑ after 6 and 9 years
Welton et al. 2008	*n*=2130 64 y	1 year EPT (CEE 0.625 mg +MPA 2.5/5 mg) Placebo	WHQ EuroQOL EQ-5D CES-D	-WHQ vasomotor symptoms, sleep and sexual functioning ↑ -EuroQOL ↔ -sleep and sexual function ↑ in asymptomatic women
Moriyama et al. 2008	*n*=44 54 y	6 months -ET(E_2 1 mg)/placebo -ET/placebo +physical exercise	SF-36 Kupperman Index	-HRQL ↔ -vasomotor symptoms ↑ -SF-36 ↑ with physical exercise

↑ = improvement, ↓= decline, or ↔ = neutral effect after hormone therapy

Burnam scale = screening of depressive symptoms and disorders, CES-D = Centre for Epidemiological Studies depression scale, EuroQOL EQ-5D = European Quality of Life Instrument, Rand-36 = Rand 36-item Health Survey, SF-36 = Medical Outcome Study 36-item Short Form General Health Survey, 15D = 15-dimensional generic HRQL instrument, MPA = medroxyprogesterone acetate, NETA = norethisterone acetate, CHD = coronary heart disease, WHQ = Women's Health Questionnaire

Cardiovascular disease risk

Estrogen has direct beneficial effects on the endothelial function. Estrogen vasodilates via endothelial nitric-oxide causing relaxation of smooth muscle in the vascular wall (*Miller & Mulvagh 2007*), most likely through ERα (*Miller et al. 2008*). Estrogen also promotes vasodilatation via prostacyclin (*Ling et al. 2006*), while estrogens effect on the vasoconstrictive and proaggregatory endothelin-1 secretion is neutral or even decreasing (*Mikkola et al. 1998*). However, EPT has shown contrary effects on the cardiovascular function (*Koudy et al. 1994*, *Kuhl & Stevenson 2006*, *Sitruk-Ware 2008*); e.g., MPA stimulates coagulation and vasoconstriction in the vascular wall (*Scarabin et al. 2011*). These MPA effects have been suggested to contribute to the unfavorable cardiovascular effects of EPT (*Kuhl 2005*, *Morin-Papunen et al. 2008*).

A large number of observational and case-control studies (*Grady et al. 1992*, *Grodstein & Stampfer 1995*, *Grodstein et al. 2000*) show approximately a 30-50% lower risk of CVD in women using HT. The largest observational and still ongoing study is the Nurse's Health Study (*Grodstein 1996*). This study, initiated in 1976, followed 70 533 women's HT use, and by 2000 the data showed a 45% reduction of coronary heart disease risk in women using ET and a 36% reduction of risk in women using EPT. On the contrary, the risk of stroke was increased by 35% with ET and by 45% with EPT (*Grodstein et al. 2000*).

The observational studies have been criticised due to the "healthy woman effect", meaning, for example, that women who originally chose to use HT might have been healthier than women who did not use HT. Thus, placebo-controlled prevention studies were initiated. The first randomised controlled study on HT was a secondary prevention trial of coronary heart disease, the HERS, in older women with established coronary heart disease. This study associated EPT with more coronary events, particularly during the first months of the treatment (*Hulley et al. 1998*, *Grady et al. 2002a*). Later, the ESPRIT (*Cherry et al. 2002*) trial studying ET, and several other studies of EPT on secondary prevention (*Waters et al. 2002*, *Hodis et al. 2003*, *Lakoski et al. 2005*, *Collins et al. 2006*) failed to demonstrate HT's cardioprotection.

Due to the previous controversial results, a primary prevention trial, the WHI, was initiated in 1992 to assess the risk-benefit profile of HT (ET and EPT) on the risk of chronic diseases. In 2002 after an average of 5.2 years follow-up, the EPT arm was discontinued due to an increase in coronary events with active treatment (*Rossouw et al. 2002*). In 2004, the ET arm was also terminated one year earlier than planned. It showed increased risks for stroke and venous thromboembolism, while the effect on coronary heart disease was neutral (*Anderson et al. 2004*). Re-analyses of the WHI-data according to age groups showed that ET reduced the risk of coronary heart events in the age group 50 to 59 years, and the effect was neutral in older age groups (*Hsia et al. 2006*) as well as in all age groups with EPT (*Rossouw et al.*

2007, *Rossouw et al. 2013*). The latest analyses of WHI's results including an extended post-intervention follow-up (median 8.2 years) conclude that the overall risk of coronary heart disease was not significantly increased after EPT and slightly decreased after ET (*Manson et al. 2014*).

The primary prevention nature of the WHI trial can be criticised (*Mikkola & Ylikorkala 2005*, *Tuomikoski & Mikkola 2014*), since women with a history of a CVD event, such as myocardial infarction, stroke, or transient ischemic attack, were not excluded from the trial. Moreover, the participants' mean age was 63 years, and hypertension, smoking, hypercholesterolemia, and diabetes were common in the study population. A woman's age and time since menopause are likely to influence the outcomes of HT (*Bassuk & Manson 2014, Gurney et al. 2014*). This supports the "timing hypothesis", which suggests that the cardiovascular effects of HT are dependent on the individual's vascular health at the time of initiation (*Mikkola & Clarkson 2002*, *Clarkson et al. 2013*). Therefore, new data about HT effects in healthy, recently menopausal women is required.

The length of follow-up, different treatment regimens in terms of ET versus EPT, and moreover possible adverse effects of MPA have been suggested to explain the divergent results between observational studies and randomised controlled trials (*Harman 2014*). More importantly, women in the WHI were practically asymptomatic regarding hot flushes, whereas participants of the observational studies were younger and entered the studies specifically to treat their hot flushes. Thus, future research on women's cardiovascular health should consider the role of hot flushes (*van der Schouw & Grobbee 2005*, *Tuomikoski et al. 2011*).

Effects on other organs

Estrogen sustains bone structure and restores bone mineral density, thus HT reduces osteoporotic fractures by 40-59% (*Farquhar et al. 2009*, *Santen et al. 2010*). Combination therapy reduces the risk of colorectal cancer (*Marjoribanks et al. 2012*), but the effect of ET remains contradictory and is most likely neutral (*Santen et al. 2010*). Progestins in HT protect the endometrium from estrogen's proliferative effect, thus EPT reduces the risk of endometrial cancer (*Brinton & Felix 2014*). The risk of endometrial cancer is even lower with continuous combined EPT than in women not using HT (*Jaakkola et al. 2009*, *Santen et al. 2010*, *Jaakkola et al. 2011*).

An increased breast cancer risk is the most feared side effect of HT. The current understanding is that EPT increases breast cancer risk (*Lyytinen et al. 2009*, *Rossouw et al. 2013*). Data on ET is not uniform: latest analysis of the WHI trial shows ET to lower breast cancer risk (*Manson et al. 2014*), whereas a large Finnish cohort study (*Lyytinen et al. 2006*) and statements (*Santen et al. 2010*, *Marjoribanks et al. 2012, Santen 2014*) conclude that the risk is increased after 5 years of ET use or even earlier.

It is well demonstrated that oral HT increases the risk of venous thromboembolism ca. 2-fold (*Olie et al. 2011*), the risk being highest during the first year of use (*Miller et al. 2002*). There is some evidence that EPT would increase the risk more than ET (*Smith et al. 2004, Sare et al. 2008*), but the mechanisms are unclear. A recent observational study associated the use of oral CEE with a greater risk of venous thromboembolism than oral E_2 use (*Smith et al. 2014*).

Several observational and follow-up studies indicate that HT reduces the risk of all-cause dementia and Alzheimer's disease (*Barrett-Connor & Laughlin 2009*, *O'Brien et al. 2014*). However, more recent placebo-controlled studies have yielded inconclusive results. Studies on ET show a neutral impact on memory (*Shumaker et al. 2004*, *Resnick et al. 2009*). On the other hand, in postmenopausal women older than 65 years, HT does not appear to improve memory, and EPT may even be harmful (*Binder et al. 2001*, *Grady et al. 2002b*, *Shumaker et al. 2003*, *Resnick et al. 2006*). The "timing hypothesis" of HT initiation might also be important regarding memory and cognition (*Barrett-Connor & Laughlin 2009, Fischer et al. 2014*), especially vascular dementia (*Henderson 2014*). Moreover, hot flushes have not been evaluated in the memory and cognition studies.

Estrogens possess neurotrophic and neuroprotective effects on the central nervous system (*Resnick et al. 2006*, *Henderson 2014*). In contrast, MPA has showed negative impacts and attenuation of estrogen-induced neuroprotection in the brain (*Nilsen & Brinton 2002*, *Liu et al. 2010*, *Irwin et al. 2011*). Estrogen also affects the autonomic nervous system's tone in the brain controlling sleep, heart rate, and body temperature (*Mohamed et al. 1999*, *Saleh & Connell 2007*, *Miller et al. 2008*).

Cardiovascular autonomic nervous system

The cardiovascular autonomic nervous system maintains the circulatory balance of the body. It holds a basal tone and a balance between the sympathetic and parasympathetic divisions and can be additionally stimulated by means of changes in, e.g., position or physical activity. For example, when standing up from a supine position, autonomic reflexes change the blood pressure and heart rate (HR) to maintain sufficient circulation in the brain. The sympathetic nervous system is responsible for energy production and general activity of the body and enables the fight or flight –response when necessary. The parasympathetic nervous system is dominant when immediate reactions are unnecessary, known as rest and digest –response, guiding the balance of the body towards a resting tone. The sympathetic nervous system and the parasympathetic nervous system typically function reciprocally. However, during rapid circulatory adaptations, they rather complement each other to maintain the homeostasis. The hypothalamus is the most important coordinating center of the autonomic regulation, but respiratory, cardiac, and vascular regulation centers are located in the brain stem (pons and medulla) (Figure 1).

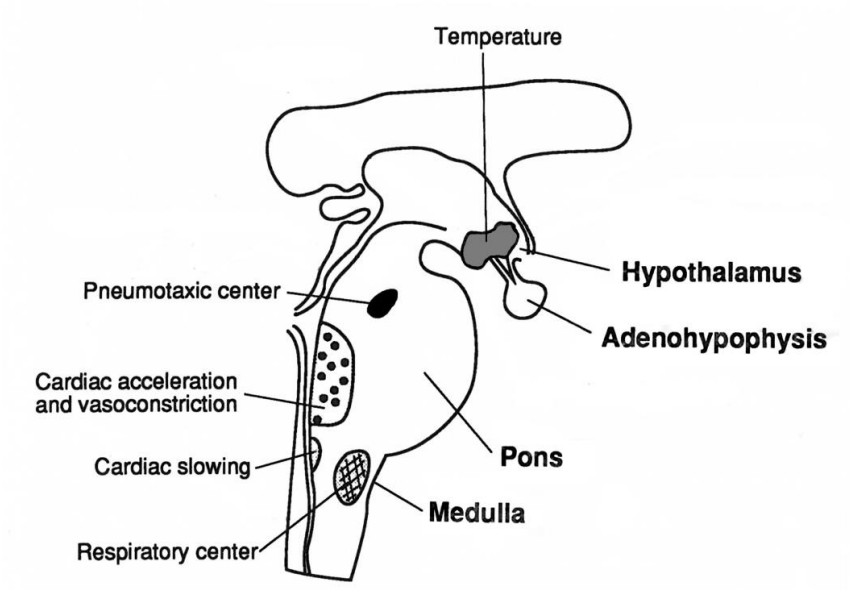

Figure 1. *Cardiac, respiratory, and thermoregulatory control centers located in the brain stem.*

The function and responses of the autonomic nervous system are mainly based on reflex arcs: input from visceral mechano- and chemosensory or thermal receptors travels by afferent pathways to the central nervous system, where the information is modulated and transmitted to the effector organ through the efferent pathways (Figure 2). The efferent autonomic pathways consist of preganglionic and postganglionic neurons with synapses in autonomic ganglia. The postganglionic neurons innervate the effector organs.

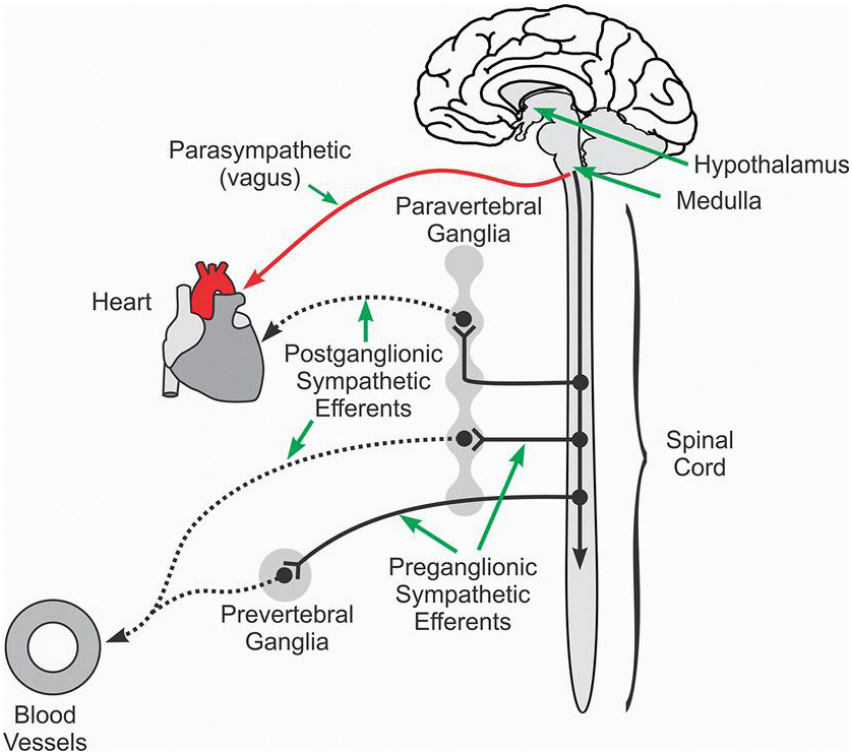

Figure 2. *Efferent autonomic innervation of heart and blood vessels. Printed with permission from Professor Richard Klabunde, PhD, cvpphysiology.com.*

Blood pressure

The sympathetic nervous system regulates the vascular tone of all blood vessels (Figure 2), except small arterioles and venules, mainly via receptors located in the smooth muscle layer of the vascular wall. However, blood vessels lack parasympathetic innervation, except for cranial, visceral and genitourinary vessels. By affecting peripheral resistance, the sympathetic nervous system regulates arterial blood flow and blood pressure of all organs; by vasoconstriction and vasodilatation, it balances the amount of blood between the venous capacitance vessels and active circulation.

Both the sympathetic and parasympathetic nervous systems act as major short-term regulators of the systemic blood pressure maintaining stable mean arterial pressure throughout the body. The baroreceptors, located within the wall of the carotid sinuses and in the wall of the aortic arch, react to stretch in the vessel wall following pressure changes, and this information is mediated to the central nervous system. Elevation of blood pressure leads rapidly to parasympathetic excitation and sympathetic inhibition, which reduces HR, cardiac output, and peripheral resistance lowering the systemic blood pressure. When blood pressure drops suddenly, for example after standing up, opposite changes occur. This is defined as the arterial baroreflex (*Sunagawa et al. 2001*, *Vongpatanasin 2009*) (Figure 3).

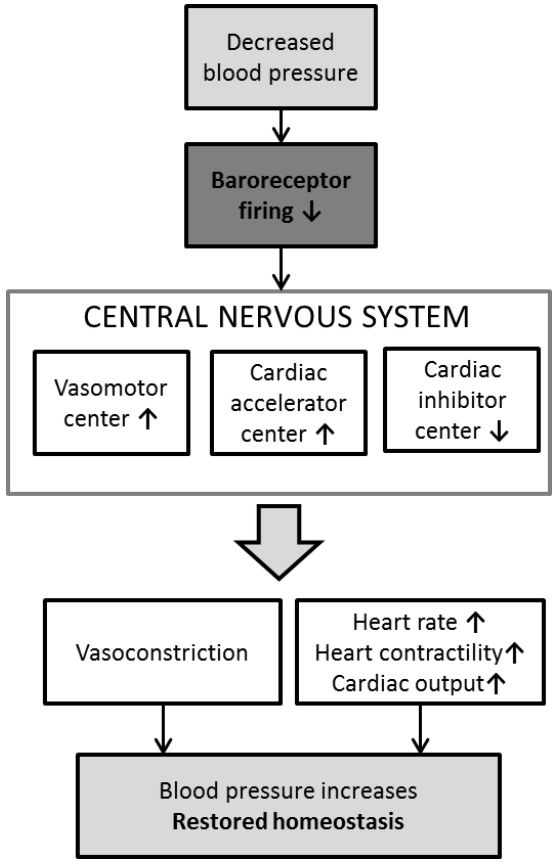

Figure 3. *The arterial baroreceptor reflex.*

Heart rate

The sinus node (sinoatrial node) is located in the right atrium and acts as the heart's pacemaker. Sympathetic stimulation increases the firing rate of the sinus node. Parasympathetic stimulation acts vice versa, but has a stronger influence on the sinus node than sympathetic stimulation. Thus, the resting HR is mainly under parasympathetic control, meaning that a withdrawal of parasympathetic stimulation increases HR (*Haapalahti et al. 2000*). The parasympathetic activity to the heart is mediated by the vagus nerve and therefore is often referred to as "vagal" control of the heart (*Hildreth et al. 2009*) (Figure 2).

The heart rate constantly varies from beat-to-beat and is influenced by respiration, blood pressure and thermal conditions under autonomic regulation (*Perini & Veicsteinas 2003*). This is defined as heart rate variability (HRV). The component of HR that is in phase with the respiration frequency is defined as respiratory sinus arrhythmia (*Sin et al. 2010*). The heart rate accelerates during inspiration and decelerates during expiration. The respiratory sinus arrhythmia is dependent upon both respiratory rate and depth (i.e. tidal volume). The information of respiratory sinus arrhythmia accumulates from arterial baroreceptors, low-pressure receptors in the heart, and stretch receptors in the lungs, the vagus nerve mediates the impulses from the central autonomic nuclei peripherally (*Wheeler & Watkins 1973*). It reflects cardiac parasympathetic control of HR via sinus node *(Grossman & Taylor 2007, Haapalahti et al. 2006).*

Modulators of cardiovascular autonomic function

Tonic parasympathetic activity is higher in women than in men (*Huikuri et al. 1996*, *Sinnreich et al. 1998*), but this difference diminishes along with aging (*Fagard et al. 1999*). Women have a higher resting HR (*Fagard et al. 1999*, *Gerritsen et al. 2003*) and attenuated arterial baroreflex responsiveness (*Huikuri et al. 1996*) compared with men. Sympathetic activity increases with age (*Seals & Esler 2000*), when the cardiovascular autonomic nervous system's regulation capacity declines. Common consequences are elevated blood pressure and orthostatic hypotension; the former associates with increased peripheral vascular resistance due to increased sympathetic activation and the latter with decreased sensitivity of the baroreceptors. Hypertension in the elderly, however, is attributed mainly to increased arterial stiffness and structural narrowing of small arteries. It is well demonstrated that especially HR responses to physiological stimuli (*Baldwa & Ewing 1977*, *Piha 1991*, *Fagard et al. 1999*) and parasympathetic regulation (*Saeki et al. 1998*, *Umetani et al. 1998*, *Fagard et al. 1999*, *Vallejo et al. 2005*) decrease with age.

Sleep is characterised by rapid fluctuations in autonomic activity in a complex manner during the different sleep stages (*Ako et al. 2003*, *Virtanen et*

al. 2008). Parasympathetic activity dominates during non-REM sleep and sympathetic activity dominates during REM sleep (*Versace et al. 2003*). Diminished HRV associates with both low body mass index (<19 kg/m2) (*Cifkova et al. 2008*, *Mazurak et al. 2011*), and obesity (≥30kg/m2) (*Quilliot et al. 2001*, *Cifkova et al. 2008*, *Sztajzel et al. 2009*). However, strong evidence suggests that obesity and metabolic syndrome are generally accompanied with sympathetic overdrive (*Grassi et al. 2004*, *Grassi 2006*, *Lambert et al. 2010*). Stress is also associated with activation of the sympathetic nervous system (*Kajantie & Phillips 2006*).

Physical activity and endurance training enhance cardiovascular autonomic function seen as an increased resting HRV (*Middleton & De Vito 2005*), whereas in several diseases impairment is seen as a decresed HRV: diabetes (*Bagherzadeh et al. 2013*), hypothyroidism (*Celik et al. 2011*), and neurological conditions, e.g., Parkinson's disease and epilepsy, as well as alcohol abuse. Depression also relates to a higher resting HR and lower HRV (*Carney et al. 2005*), which in turn has been linked to increased risk of CVD (*Pizzi et al. 2008*, *Taylor 2010*).

Autonomic function and cardiovascular disease risk

Heart rate and HRV reflect CVD risk and outcome. Greater cardiovascular reactivity and sympathetic activity in response to stress are related to worsened cardiovascular risk (*Chida & Steptoe 2010*, *Chumaeva et al. 2010*, *Fuller-Rowell et al. 2013*). High resting HR relates to all-cause mortality, death from CVD, and sudden cardiac death (*Habib 1999*, *Thayer & Lane 2007*). Reduced HRV in post-myocardial infarction patients is associated with mortality (*Kleiger et al. 1987*). More specifically, lowered vagal function has related with all-cause mortality (*Tsuji et al. 1994*, *Thayer et al. 2010*) and both sudden and non-sudden death after acute myocardial infarction (*Huikuri & Stein 2012*). Decreased HRV has been also associated with the development of myocardial infarction (*Tsuji et al. 1996*), recurrence of myocardial infarction (*Task force 1996*), arterial hypertension (*Singh et al. 1998*, *Schroeder et al. 2003*, *Thayer & Lane 2007*, *Erdogan et al. 2011*), diabetic autonomic neuropathy (*Task force 1996*), progression of atherosclerosis (*Huikuri et al. 1999*), heart failure (*La Rovere et al. 2003*), and ischemic and idiopathic cardiomyopathy (*Rashba et al. 2006*). Depressed baroreflex sensitivity is related to ischemic heart disease and heart failure, hypertension, renal failure (*La Rovere et al. 2011*), and cerebral stroke (*Robinson et al. 2003*). In addition, altered autonomic nervous function is suggested to play a role in hypertension and e.g. angina (*Lucini et al. 2002*, *Christou et al. 2005*, *Joyner et al. 2008*).

Autonomic function and menopause

Findings on the effect of menopause on blood pressure are divergent. Blood pressure, both resting and responsive to activity, is thought to increase more steeply around the menopause (*Vongpatanasin 2009*). Several theories on the menopause-related elevation of blood pressure exist. According to one theory, vasodilatation is dominant in young women, resulting in low blood pressure levels, and with aging, sympathetic nervous activity rises, and the vasodilatory effect is lost (*Hart et al. 2012*). Plasma noradrenalin levels are also elevated in postmenopausal women compared with premenopausal women (*Sherwood et al. 2010*), reflecting vasoconstriction. Another theory suggests that the alteration in central autonomic regulation together with enhanced vascular adrenergic sensitivity cause the elevation of blood pressure in postmenopausal women (*Vongpatanasin 2009*). However, the role of sex steroid hormones and menopausal symptoms in the blood pressure regulation remain unclear.

The balance of the cardiovascular autonomic function shifts towards sympathetic dominance with advancing age, but the role of menopause is unclear (*Lavi et al. 2007*). Some studies have shown signs of decreased HRV (*Monda et al. 2006*) and increased sympathetic activity in postmenopausal women compared with premenopausal women: augmentation of muscle sympathetic nerve activity (*Narkiewicz et al. 2005*), higher resting HR, and more increase in sympathetic activity in response to stress (*Farag et al. 2003*). Yet, the results are not uniform (*Day et al. 2011*) and some clinical and experimental evidence suggests that advancing age rather than menopause itself explains these findings (*Tezini et al. 2013*). Estrogen affects autonomic tone, seen clearly as attenuated HRV after a sudden decrease of estrogen due to surgical menopause (*Mercuro et al. 2000*). The effect of slowly depleting and fluctuating estrogen levels during the physiological menopause remain unclear and demands further research.

Autonomic function and hormone therapy

Estrogen with (*Beljic et al. 1999*, *Gautam et al. 2011*) or without progestogen (*Christ et al. 2002*, *De Meersman et al. 1998*, *Vongpatanasin et al. 2001*) has lowered blood pressure in short-term (*De Meersman et al. 1998*, *Beljic et al. 1999*, *Gautam et al. 2011*) and ambulatory measurements (*Vongpatanasin et al. 2001*, *Christ et al. 2002*). Hormone therapy lowers HR (*Rosano et al. 1997*, *Beljic et al. 1999*) and increases HRV in some (*Yildirir et al. 2001*, *Gautam et al. 2011*), but not all studies (*Lipsitz et al. 1995*, *Hunt et al. 2001*, *Niskanen et al. 2002*, *Carnethon et al. 2003*) (Table 7). The conflicting results could be at least in part explained with varying methodologies and different HT regimens. For example, EPT has attenuated the positive effects of estrogen-only therapy (*Christ et al. 2002*). Of the three placebo-controlled studies, one showed reduced HR response to isometric muscle contraction (handgrip test) but no effect on resting HR after ET (*De Meersman et al. 1998*). Another study detected no differences in HR or blood pressure responses between ET, EPT or placebo groups during a psychologically stressful task (*Farag 2002*). Furthermore, transdermal E_2 has also been shown to decrease sympathetic nerve discharge and resting HR (*Vongpatanasin et al. 2001*). Only one previous study associated flushing with a more prominent decrease in HR and systolic blood pressure after EPT (*Beljic et al. 1999*). Taken together, the previous studies show some positive effects of HT, but data are insufficient regarding HT in relation to cardiovascular autonomic function and pre-treatment hot flush status. Hormone therapy's effects on cardiovascular autonomic responses are especially poorly understood.

Table 7. *Effects of hormone therapy on heart rate (HR) and heart rate variability (HRV) in previous studies.*
↑ = improvement, ↓= decrease, or ↔ = neutral effect after hormone therapy

Study	Population (n, mean age)	Treatment	Methods	Results
Lipsitz et al. 1995	*n*=20 67 y	13±3 years, Controls ET or EPT (different regimens)	short-term HRV 60˚ head-up tilt test	-HRV ↔
Rosano et al. 1997	*n*=30 57 y	4 months, Controls ET (TE 50 µg)	ambulatory HR, HRV	-ET: HR ↓, HRV ↑
De Meersman et al. 1998	*n*=8 52 y	ET (CEE 0.625 mg) crossover (1month,6 wk washout)	short-term HR handgrip and Valsalva	-resting HR ↔ -handgrip: lower HR ↓
Beljic et al. 1999	*n*=30 47 y (flushers) 50 y (non-flushers)	12 months, no controls ET (TE 50 µg) + EPT (TE+MPA 5 mg)	short-term HR	-ET/EPT: HR ↓, more in flushers
Yildirir et al. 2001	*n*=46 48 y ET 46 y EPT	6 months, no placebo ET (CEE 0.625 mg) EPT(CEE+ MPA 2,5 mg)	short-term HR, HRV	-resting HR ↔ -ET/EPT: HRV ↑
Hunt et al. 2001	*n*=11 60 y	6 months, no placebo ET (CEE 0.625 mg) + 1 week of progesterone (10 mg) at 3+6 months	short-term HR	-HR ↔
Christ et al. 2002	*n*=62 57 y ET 59 y EPT	≥6 months use before study ET, EPT (different progestogens) Controls	ambulatory HR, HRV	-EPT: HRV ↓ and HR ↓ vs. ET/controls
Niskanen et al. 2002	*n*=31 61 y	6 months, Controls: clodronate EPT (E_2 2 mg + norethisterone 1 mg)	short-term HRV	-HRV ↔
Farag et al. 2002	*n*=40 58 y ET 53 y EPT	3 months, Placebo ET (E_2 2 mg), EPT (E_2 + MPA 5 mg)	short-term HR, HRV stressful task	-HR ↔ in response to task -EPT: HRV (HF) ↑
Carnethon et al. 2003	*n*=2621 54 y ET 56 y EPT	Observational study, 11 years ET and EPT ever/current users (dose or composition of HT not known)	short-term HR, HRV	-HR and HRV ↔
Gautam et al. 2011	preMP, *n*=30 30-45 y postMP, *n*=30 postMP EPT, *n*=30 45-55 y	3 months, no placebo EPT (CEE 0.625 mg + MPA 2.5 mg)	HRV: deep breathing (E/I ratio) orthostatic test (30/15 ratio)	-EPT: HRV ↑, E/I ↑, 30/15 ratio ↑ vs. postmenopausal women without EPT

ET = Estrogen therapy, EPT = Estrogen-progestogen therapy, CEE = Conjugated equine estrogens, MPA = Medroxyprogesterone acetate,
TE = Transdermal estradiol, MP = Menopause

Assessment of cardiovascular autonomic function

Measurements of cardiovascular autonomic function in controlled laboratory conditions quantify resting levels and responses of blood pressure, HR, and HRV to specific maneuvers challenging the autonomic nervous system. The clinical responses always reflect the balance of sympathetic and parasympathetic systems and their reciprocal reactions. The main features of the cardiovascular autonomic function tests in a short-term setting are presented in Table 8.

Heart rate variability, expressing beat-to-beat fluctuation in sinus rhythm, can be measured as a function of time (time domain), frequency (frequency domain), or with non-linear methods (*Kleiger et al. 2005*, *Lombardi & Stein 2011*), all evaluating the contribution of parasympathetic and sympathetic control (*Task force 1996*). The time domain and non-linear measurements are recommended for ambulatory long-term recordings, whereas frequency domain analyses are recommended for controlled short-term recordings (*Task force 1996*, *Tahvanainen et al. 2012*).

Time domain indexes of HRV measure the dispersion of individual R-R intervals around their mean (*Zaza & Lombardi 2001*). The square root of the mean of the sum of the squares of differences between adjacent normal-to-normal R-R intervals (RMSSD) and the coefficient of variation of R-R intervals (CoV=SD/mean x 100, proportioning HRV to HR), and the percentage fraction of consecutive R-R intervals that differ by more than 50 milliseconds (pNN50) express the time domain HRV in short-term measurements (*Task force 1996*).

Heart rate variability oscillates at different frequencies. The purpose of the frequency domain analysis of HRV (power spectral density analysis) is to describe the distribution of variance as a function of frequency, thereby enabling recognition of sympathetic and parasympathetic regulation. The HRV power spectrum has three major components; high (HF), low (LF), and very low (VLF) frequency bands, which are quantified by measuring the areas under the curve (VLF ≤0.04 Hz, LF 0.04–0.15 Hz, HF 0.15–0.40 Hz) (Figure 5). The LF power associates with oscillations of arterial pressure (considered mainly as a marker of sympathetic modulation), and HF power is dependent on respiratory sinus arrhythmia (considered to be a marker of parasympathetic modulation). Both LF and HF can be reported in absolute (power) and normalised (relative to total power as a sum of LF and HF) values nLF and nHF. The LF/HF ratio is an established indicator of sympathetic-parasympathetic balance (*Malliani et al. 1991*, *Lombardi & Stein 2011*). The VLF is not clearly attributed to a specific neural component, but associates rather with thermoregulatory rhythms, the renin-angiotensin (*Taylor et al. 1998*) or with peripheral vasomotor regulation (*Perini & Veicsteinas 2003*). In short-term measurements, HRV should be controlled

for respiration, since they are strongly linked. Therefore, HRV is usually quantified during controlled breathing timed at a certain frequency (Figures 4 and 5).

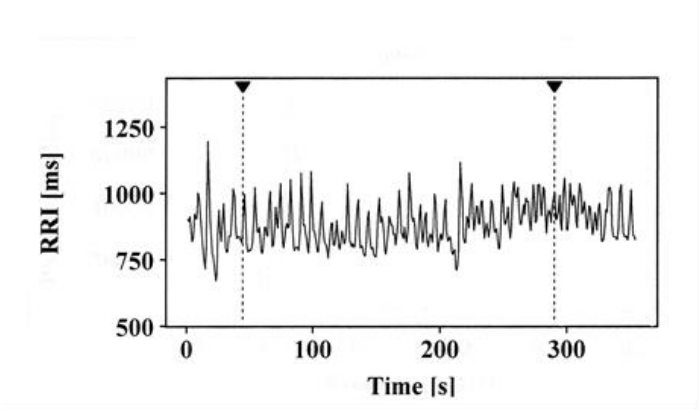

Figure 4. *Tachogram of the controlled breathing test. RRI= R-R interval*

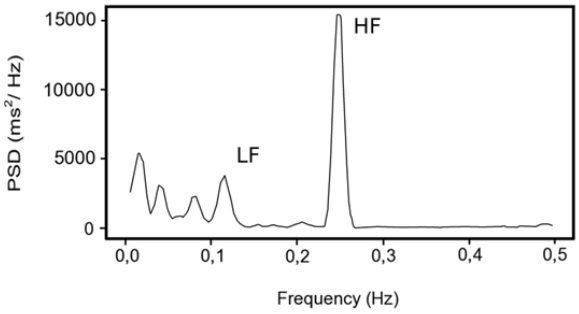

Figure 5. *An example of the power spectrum distribution of heart rate variability during controlled breathing test produced by spectral analysis of the tachogram in Figure 4. The respiration-related and dominant high frequency (HF) component at 0.25 Hz corresponding to the controlled breathing frequency of 15/min is clearly identified.*
LF = power in low frequency band (0.04–0.15 Hz)
HF = power in high frequency band (0.15–0.4 Hz)

Table 8. *Main principles of cardiovascular autonomic function tests in a short-term setting.*

Test	Main features	Component assessed primarily		Measured variables	Normal findings and clinical applications
		Sympathetic	Parasympathetic		
Controlled breathing	Five minutes of breathing timed at 15 breathing cycles/minute	+	+	HR HRV	-HRV with dominant HF component -Depressed HRV: first sign of diabetic neuropathy, risk of mortality and arrhythmias after MI
Deep breathing	Six maximal breathing cycles in one minute	-	++	HR Deep breathing difference	-Decreased deep breathing difference: sign of parasympathetic dysfunction (normal range 8-27 beats/min, strongly age-dependent)
Active orthostatic test	Active change of position from supine to standing for 8 min	+	++	Blood pressure HR HRV 30/15 –ratio	-biphasic HR response and stable blood pressure during standing -Low 30/15-ratio (<1.08):parasympathetic dysfunction -Syncope, hypotension or tachycardic reactions are signs of orthostatic intolerance
Valsalva manoeuvre	Forced expiration 15 s against constant 40 mmHg airway pressure	+	++	HR Valsalva ratio Tachycardia ratio	-HR responses to changes in intra-thoracic pressure -Lower Tachycardia ratio reflects more HR increase during strain, lower Valsalva ratio reflects worse parasympathetic function (<1.2 abnormal)
Handgrip test	Isometric muscle contraction with 30% of maximal handgrip force for 3 minutes	++	-	Blood pressure HR	-Rises in blood pressure and HR during strain -Elevation of diastolic blood pressure <10 mmHg is abnormal

HR = Heart rate, HRV = Heart rate variability, HF = High frequency, MI = Myocardial infarction

Deep breathing

The most specific and most often utilised test of parasympathetic function is the deep breathing test, since respiratory sinus arrhythmia is dependent on the parasympathetic branch of the cardiovascular autonomic nervous system. When the subject breathes cued slowly and deeply using subjective maximal vital capacity, the difference between maximum and minimum HRs is calculated from concomitant electrocardiogram. This is known as "the deep breathing difference" reflecting the magnitude of the respiratory sinus arrhythmia (*Wheeler & Watkins 1973*).

Valsalva manoeuvre

The Valsalva manoeuvre causes dynamic variations of HR and blood pressure due to changes in intra-thoracic pressure. The Valsalva manoeuvre is carried out by blowing against resistance, which elevates the intra-thoracic pressure reducing the venous return to the heart. The responses of HR and blood pressure are mediated by the baroreflex (*Baldwa & Ewing 1977*).

Orthostatic test

Posture change from supine to standing causes blood to pool in the capacitance vessels of the trunk and lower extremities. Baroreceptors react to the loss of central blood volume, and the arterial baroreflex induces a rapid parasympathetic withdrawal and a sympathetic activation within a few seconds *(Thulesius 1976)*. This results in rapid HR acceleration (20-25%), improvement in cardiac contractility and elevation of the peripheral vascular resistance. As a result, systolic blood pressure remains almost unchanged during standing.

Handgrip test

The sympathetic function can specifically be tested with isometric muscle exercise, such as the handgrip test, where isometric muscular contraction causes generalised sympathetic activation to the heart and blood vessels. Usually, a dynamometer is squeezed for 3 minutes, and HR and blood pressure responses are measured during the exercise (*Ewing et al. 1974*). In the beginning, the HR accelerates due to parasympathetic withdrawal and later due to sympathetic activation (*Martin et al. 1974*).

AIMS OF THE STUDY

This thesis was designed to investigate the impact of hot flushes on the health-related quality of life and cardiovascular autonomic nervous function in recently postmenopausal women before and during various forms of hormone therapy. Moreover, it was elucidated whether there is a relation between premenstrual symptoms and menopausal complaints.

The specific aims of the studies were to evaluate:

- Health-related quality of life in women with or without hot flushes before and during postmenopausal hormone therapy (Study I)
- The association between premenstrual symptoms and postmenopausal hot flushes affecting health-related quality of life (Study II)
- Impact of hot flushes on the cardiovascular autonomic responsiveness (Study III)
- The effect of postmenopausal hormone therapy on cardiovascular autonomic responsiveness in women with and without hot flushes (Study IV)
- The effect of hot flushes and postmenopausal hormone therapy on heart rate variability (Study V)

SUBJECTS AND STUDY DESIGN

The women for this study were recruited via local newspaper advertisements in 2005-2006. First, a trained research nurse interviewed all 1500 willing responders over the telephone. Second, the 400 women who met the inclusion criteria (age 48-55 years, time since last menstrual period 6-36 months, no previous HT use) recorded their vasomotor symptoms for two weeks with a structured questionnaire that defined the severity and number of the symptoms (*Panay et al. 2007*). Finally, after further exclusion criteria (chronic illnesses or use of regular medication, smoking, level of FSH <30 IU/l, body mass index >30 kg/m^2, previous hysterectomy or ovariectomy and inability to comply with the study plan) and rating the vasomotor symptoms, a total of 150 women were eligible for this study (Figure 6) (*Tuomikoski et al. 2009a*, *Tuomikoski et al. 2009b*).

In the hot flush diary, vasomotor symptoms were defined as absent if there were no sensations of heat at all, mild if there was only a slight sensation of heat without sweating, moderate if there was an intense sensation of heat and some perspiration while being awake, and severe with profuse sweating and the hot flush clearly interfering with daily life or sleep. To obtain an overall picture of vasomotor hot flushes, mild symptoms were scored 1, moderate symptoms 2 and severe symptoms 3, and furthermore, the HFWWS score was calculated as the sum of all weighted symptoms during one week (*Notelovitz et al. 2000, Sloan et al. 2001*) (Table 9). The participants showed great variation in hot flushes at baseline (0–298 in HFWWS score) and were divided into four subgroups according to the scores: 23 women reported no hot flushes, 34 mild flushes (HFWWS 0.5–9.5), 30 moderate flushes (HFWWS 10–99.5) and 63 severe flushes (HFWWS ≥100). In order to accurately evaluate the significance of hot flushes in our study, we excluded women with intermediate hot flushes, i.e. >3 mild hot flushes to <7 moderate to severe hot flushes/day.

Table 9. *Classification of hot flushes.*

Hot flushes	Definition	Score	HFWWS = amount of scored symptoms during one week
None	No hot flushes	0	0
Mild	Slight sensation of heath without sweating	1	0.5-9.5
Moderate	An intense sensation of heat with some perspiration while being awake	2	10.0-99.5
Severe	An intense sensation of heat with profuse perspiration that interferes with daily life or sleep	3	≥100.0

HFWWS = Hot Flush Weekly Weighted Symptom score
An example: A woman experiences 22 mild, 30 moderate and 6 severe hot flushes during two weeks.
HFWWS = (22x1 + 30x2 + 6x3)/2 = 100/2 = 50

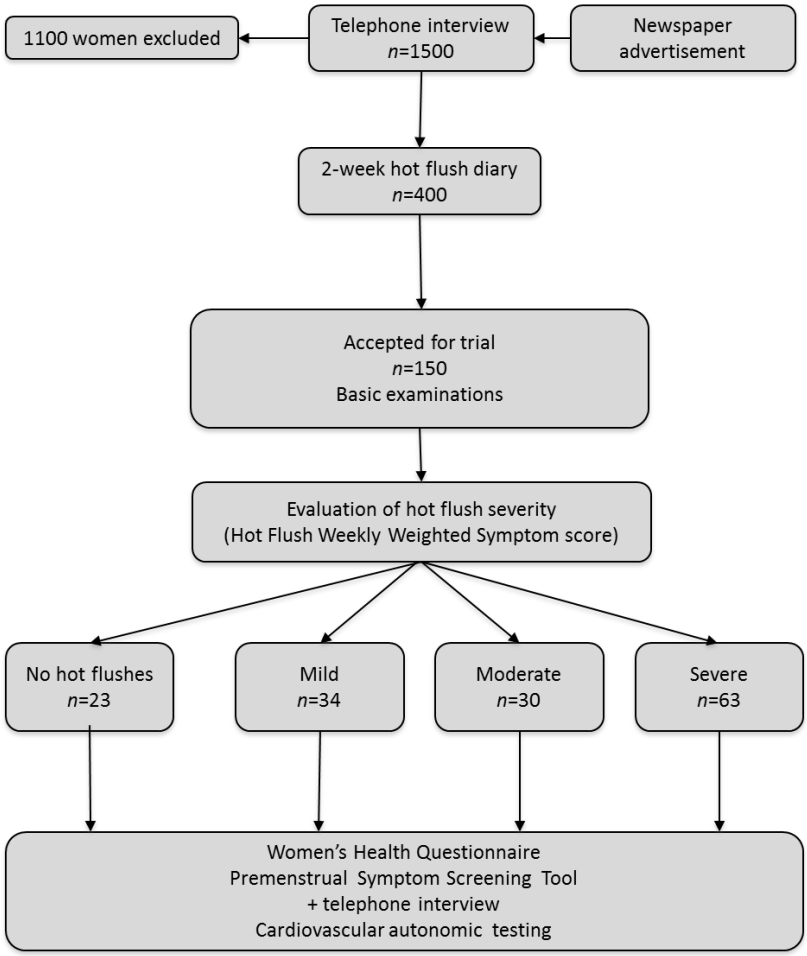

Figure 6. *Study protocol for studies II and III.*

In studies II and III, women were classified as asymptomatic, or having mild, moderate or severe hot flushes according to the HFWWS (Figure 6, Table 9). For the randomised controlled studies I, IV, and V women with ≥7 moderate or severe hot flushes/day were classified as "women with hot flushes" and women reporting only ≤3 mild hot flushes/day or no hot flushes at all were classified as "women without hot flushes".

For the HT trial, the women were randomised in blocks of four according to the hot flush status at baseline. They were treated either with transdermal E_2 hemihydrate gel 1 mg/day, oral E_2 valerate 2 mg/day alone or combined with MPA 5 mg/day or with placebo for six months with a double-blind and double-dummy technique (Figure 7). After a careful comparison of treatment effects, we did not find differences between oral and transdermal E_2. Therefore, to better compare the effects of unopposed E_2 to E_2 with MPA, oral and transdermal groups were combined into a sole E_2 group (ET) in Study IV. In studies I and V all treatments showed comparable effects and were therefore combined into a single HT group for further comparisons.

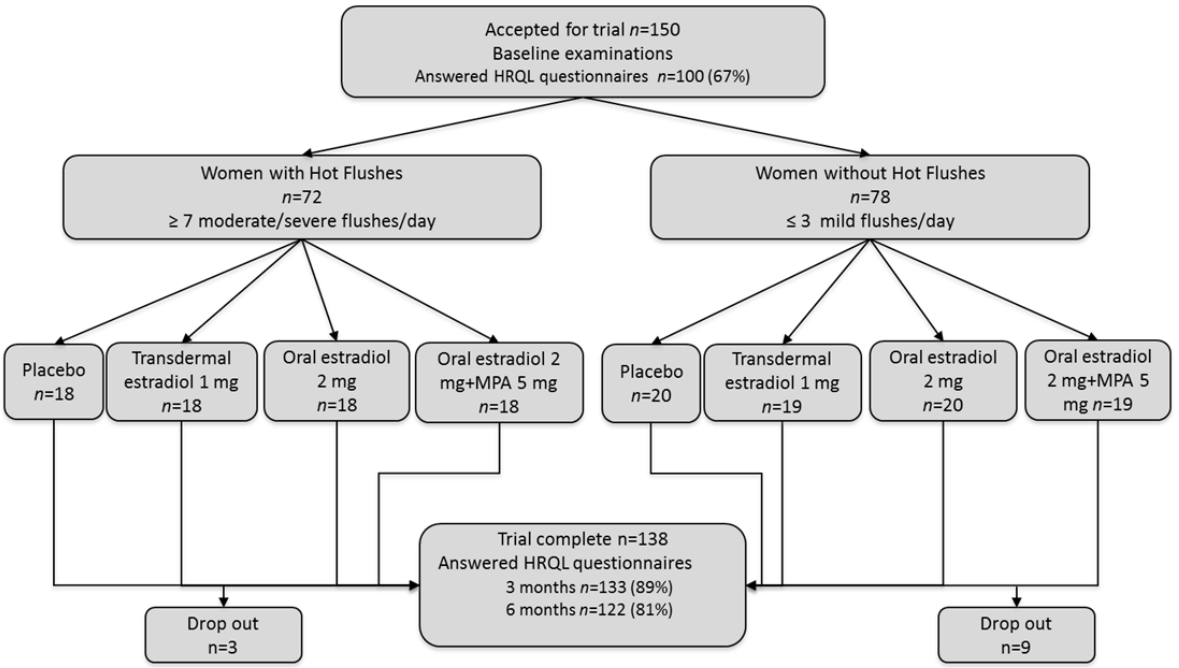

Figure 7. *The study protocol for hormone therapy studies I, IV, and V.*

The treatment regimens were provided by Orion Pharma Oyj (Espoo, Finland). To guarantee the double-dummy technique of the trial, all women used both tablets (active or placebo) and gel (active or placebo) which was packed separately as daily doses. Compliance was evaluated by counting the returned unused packages and tablets. Endometrial thickness was evaluated at baseline and at three and six months' clinical visits. As a precaution women, were treated with MPA 5 mg/day for two weeks after the study protocol if endometrial thickness was ≥ 9 mm.

To further study the relationship between premenstrual symptoms and postmenopausal hot flushes, a questionnaire was sent by mail to the same study population described above in April 2012. This questionnaire included PSST and additional questions about age at possible manifestation of premenstrual symptoms, menstrual cycle and occurrence of depression or other mental disorders. Of the 150 women, 120 (80%) returned the questionnaire and were further interviewed over the telephone by a research nurse to confirm their answers.

Of the 150 women included in the trial, 138 (92%) completed the study protocol and underwent cardiovascular autonomic testing at 6 months (Studies IV and V). Twelve women discontinued the trial (7 withdrew consent, 3 were lost during follow up, 2 discontinued due to spotting), and these women were evenly distributed between the different treatment groups. In addition, in the HRQL part of the trial (Study I), nine women's data collection was incomplete. Hereby, 132 women (68 with hot flushes and 64 without) were eligible for HRQL data analysis. Therefore, the missing data were replaced and analysed according to intention to treat –principle with linear interpolation. In Study II, data from 120 women were available for analyses.

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

Cardiovascular autonomic function in relation to premenstrual and vasomotor symptoms

To further study those women that tend to have strong symptoms in their fertile age and also later on at menopause, we compared the cardiovascular autonomic responses of women having both severe premenstrual and postmenopausal vasomotor symptoms (PSST score ≥15, HFWWS ≥100) with women having only mild or no symptoms at all (PSST score ≤3, HFWWS ≤9.5). Furthermore, to find out whether previous premenstrual symptoms would be an independent determinant in the autonomic regulation in menopause, we compared women having both severe premenstrual and vasomotor symptoms (PSST score ≥15, HFWWS ≥100) with women having severe vasomotor symptoms but only mild premenstrual symptoms (PSST score ≤3, HFWWS ≥100).

METHODS

Women's Health Questionnaire

We measured the HRQL with the WHQ, which is a reliable and well-documented tool to assess a wide range of physical and emotional symptoms and possible health changes in middle-aged women (*Hunter 1992*, *Hunter 2000*, *Zöllner et al. 2005*). It is a self-administered questionnaire composed of 36 items capturing 9 domains of women's health: vasomotor symptoms (two items), somatic symptoms (seven items), anxiety and fears (four items), depression (seven items), sleep problems (three items), sexual behavior (three items), memory and concentration (three items), menstrual cycle-related symptoms (four items) and attractiveness (two items) (Appendix A). Each item is answered on a four-point scale (1-4) and then reduced to a binary scale (1 and 2=0, 3 and 4=1) for scoring. A mean score (between 0 and 1) is calculated for each domain (of the corresponding items), and thus the higher the score, the better the quality of life.

General health and menopausal symptoms

The women evaluated their general health by a VAS (range from 0 to 100), higher score indicating better health. In one question, women compared their current health state with the average of past 12 months (scale 1= worse, 2=about the same, 3=better) (Appendix C). Menopausal symptoms concerning the past two weeks were assessed with a modified Kupperman Index (*Kupperman et al. 1953*) comprised of 19 items answered on a four-point scale regarding the frequency of the symptoms (Appendix B).

Sexual wellbeing

Sexual wellbeing was assessed with a modified MFSQ (*McCoy & Davidson 1985*, *Wiklund et al. 1993*), where the woman's sexual experience during the past four weeks is asked by a self-report questionnaire. The MFSQ provides an overall score and three subscales denoting sexual problems (two items), sexual satisfaction (five items) and satisfaction with the partner (three items). Each item consists of seven-point Likert-scales, higher score indicates better sexual satisfaction, except for the sexual problems subscale (Appendix D).

Premenstrual symptoms screening tool

The prevalence and severity of premenstrual symptoms were evaluated with the PSST consisting of 19 items (Appendix E) (*Steiner et al. 2003*). The first 14 items assess specific premenstrual symptoms, and 5 additional items evaluate the impairment of work efficiency, taking note of home responsibilities, social activities or relationships because of premenstrual symptoms. All items have a severity rating (not at all, mild, moderate and severe). We calculated the PSST score as the sum of all items rated on a four-point scale (0-3 points), reflecting the severity of premenstrual symptoms.

Cardiovascular autonomic function tests

The participants underwent a series of cardiovascular autonomic function tests at baseline (Study III) and after 6 months of HT (studies IV and V) in the following order:

1. **Controlled breathing.** A 5-minute baseline recording while breathing quietly at 15 cycles/minute.
2. **Deep breathing.** Six breathing cycles (inspiration + expiration) at maximal tidal volume in one minute.
3. **Active orthostatic test.** The women stood up quickly from a supine position and remained standing still for 8 minutes. Blood pressure was measured at 0.5, 1, 3, 5 and 8 minutes.
4. **Valsalva manoeuvre.** After maximal inspiration, forced expiration against a resistor with airway pressure of 40 mmHg was maintained for 15 seconds.
5. **Sustained handgrip.** The women squeezed a dynamometer at 30% of predetermined maximal handgrip strength for 3 minutes. Blood pressure was measured at rest and at 1, 2 and 3 minutes.

Experienced staff carried out the measurements in the forenoon in an undisturbed environment. The testing room was specifically designed for clinical cardiovascular experiments: quiet, dim, and a stable temperature at 24 °C.

The women were instructed to refrain from alcohol for 36 hours and caffeinated beverages for 6 hours before testing. Prior to testing, all women had 10 minutes of supine rest. Additionally, a 5-minute minimum rest before each test allowed the stabilisation of the basic physiologic state and was followed by a 5-minute baseline recording. Blood pressure was measured manually with a calibrated sphygmomanometer. The heart rate was monitored continuously with standard 12-lead electrocardiography during all tests, and the signals were digitised at 200 Hz (WinAcq, Absolute Aliens Co., Turku, Finland).

Heart rate responses to the autonomic tests were reviewed blind to the hot flush status with biosignal analysis software (WinCPRS version 1.3, Absolute Aliens Co., Turku, Finland) by an experienced physiologist. Automatic R-wave detection was followed by manual editing of data with linear interpolation to exclude occasional ectopic beats or occasional artefacts. Inadequate Valsalva manoeuvres (expiration pressure less than 40 mmHg, duration less than 15 seconds) and handgrip tests (duration less than 3 minutes) were also excluded from further analyses. Mean, minimum

(corresponding to maximum HR) and maximum (corresponding to minimum HR) R-R intervals during the tests were identified. Maximum changes of HR and blood pressure from baseline were calculated.

The HR responses in the autonomic tests were further quantified by using standard calculated indices (Table 10).

Heart rate variability in the time domain was assessed during controlled and deep breathing and the active orthostatic test by calculating the RMSSD and CoV of R-R intervals.

Heart rate variability in the frequency domain was assessed during controlled breathing and the active orthostatic test by computing the spectral variation of R-R intervals using a fast Fourier transform (*Task force 1996*). Power spectra were further quantified by measuring the power in the VLF, LF, and HF frequency bands and by calculating the LF/HF ratio.

Table 10. *Definitions of variables derived from cardiovascular autonomic tests.*

Variable	Definition
Deep breathing difference[1]: E-I E-I(b)	 maximum R-R interval during expiration – minimum R-R interval during inspiration (ms) minimum heart rate during expiration – maximum heart rate during inspiration (beats/min)
30/15 –ratio[2] (Active orthostatic test)	$= \frac{\text{longest R-R interval between 21st and 45th heart beat}}{\text{shortest R-R interval between 5th and 21st heart beat}}$ after standing up
Valsalva ratio[3] (Valsalva manoeuvre)	$VR = \frac{\text{maximum R-R interval after strain}}{\text{minimum R-R interval during strain}}$
Tachycardia ratio[4] (Valsalva manoeuvre)	$TR = \frac{\text{minimum R-R interval during strain}}{\text{mean R-R interval before strain}}$

[1] (*Wheeler & Watkins 1973*)
[2] (*Ewing & Clarke 1978*)
[3] (*Levin 1966*)
[4] (*Baldwa & Ewing 1977*)

Statistical analyses

Power analysis was made on the primary outcome measure, i.e. differences between women with and without hot flushes in the responses to HT in pulse wave analysis (*Tuomikoski et al. 2009a*), and responses in the HRQL and cardiovascular autonomic function tests were secondary end-points of this trial.

Normality was assessed with the Shapiro-Wilk test. Normally distributed data were compared in all studies by means of the Student's *t* test or one-way analysis of variance. Mann–Whitney *U* or Kruskall–Wallis tests were used for data showing non-Gaussian distributions. Tukey HSD and Games-Howell post hoc comparisons were used for parametric and non-parametric data respectively. Wilcoxon's signed rank test was used for within-groups comparisons and for within group comparisons of treatment effects (Studies IV and V) when appropriate.

To explore the impact of hot flushes on the cardiovascular autonomic nervous testing variables during HT (Studies IV and V), a two-way between groups ANOVA with analysis of covariance was used. A univariable instead of multivariable approach was used due to the multicollinearity between the investigated variables. The effect of treatment (partial eta^2, η^2) was assessed as the absolute change after HT controlling for possible confounding factors (the baseline value of the variable in question, time since the last menstruation, the levels on E_2) expressed as percentages from baseline. Pearson's test was used for correlating normally distributed variables and Spearman's *rho* for correlating non-normally distributed variables (Studies I and II). A Chi-square test for independence, multiple and logistic regression analyses were used to explore the predictive value of premenstrual symptoms and the relation of premenstrual and postmenopausal vasomotor symptoms (Study II).

A *p*-value <0.05 was considered statistically significant. Data are expressed as mean ± standard deviation (SD) (Study I, III) or mean ± standard error of mean (SEM) (Studies II, IV, and V), regardless of the variables' distribution pattern. All analyses were performed by using SPSS 16.0-20.0 software for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The main results are presented here, and more detailed results are found in the original publications attached.

In women with and without hot flushes, the main baseline characteristics were similar, except that the time since last menstruation was shorter in women with hot flushes than in women without hot flushes (16.9±1.2 *vs.* 21.9±1.1 months, p=0.001) (Table 11).

Table 11. *Baseline characteristics of the studied 150 women. Data are presented as mean ± SD.*

	Hot flushes		
	Yes (*n*=72)	**No** (*n*=78)	***p*-value**
Age (years)	52.5±2.4	52.9±2.0	0.246
Body mass index (kg/m^2)	22.8±2.4	23.1±2.2	0.454
Hot flush weekly weighted symptom score	161.2±65.5	7.0±8.4	**<0.001**
Serum follicle stimulating hormone(IU/L)	67.9±22.9	78.1±29.3	0.052
Serum estradiol (pmol/L)	80.8±86.5	58.7±60.2	0.142
Age at menarche (years)	13.2±1.5	13.2±1.5	0.741
Age at menopause (years)	50.5±3.3	50.9±2.6	0.600
Time since last menstruation (months)	16.9±1.2	21.9±1.1	**0.001**
Heart rate at rest (beats/min)	67.4±9.4	68.0±8.3	0.714
Systolic blood pressure at rest (mmHg)	121.1±14.3	119.8±16.2	0.606
Diastolic blood pressure at rest (mmHg)	83.2±8.9	80.6±9.0	0.166
History of hormonal contraception (%)	71.8	66.7	0.496
Deliveries	1.9±0.1	1.8±0.1	0.887
Weekly use of alcohol (%)	60.0	60.3	0.975
Employed (%)	94.4	96.2	0.496
Absence from work (last 6 month) (%)	33.8	35.4	0.838
In relationship (%)	68.6	74.4	0.436
Sleep disturbances (%)	73.2	34.2	**0.0001**

Impact of hot flushes on health-related quality of life (Study I)

Hot flushes impaired HRQL assessed by the WHQ. Poor sleep, impaired memory, concentration, and sexuality, somatic and depressive symptoms, and feeling less attractive, were more common in women with hot flushes. The severity of hot flushes, quantitated with HFWWS, correlated with poor sleep (correlation coefficient r=-0.525, p<0.0001), somatic symptoms (r=-0.348, p<0.0001), menstrual cycle-resembling complaints (r=-0.304, p<0.0001), anxiety and fears (r=-0.283, p< 0.0001), impaired memory and concentration (r=-0.279, p=0.001), and sexuality (r=-0.174, p=0.035). No association emerged between HFWWS and depression or feelings of attractiveness.

The severity of hot flushes also correlated negatively with women's subjective evaluation of their general health status with VAS (correlation coefficient r=-0.227, p=0.005). Thus, more troublesome hot flushes related to a worse perception of general health.

Insomnia and sleep disturbances, irritability, exhaustion, depressive mood, joint pains, palpitation, nausea, and edema were significantly more common in women with than without hot flushes and also correlated to HFWWS. Women with severe hot flushes (HFWWS ≥100) had more sexual problems (pain during intercourse and insufficient lubrication) than asymptomatic women (HFWWS=0) (5.8±0.3 *vs.* 4.3±0.4, p=0.015). Otherwise, hot flushes were no determinants for sexual wellbeing measured with MFSQ.

Association between premenstrual symptoms and postmenopausal hot flushes (Study II)

Of the 120 women responding to the PSST, 107 (89.2%) reported a history of premenstrual symptoms, and in 52 women (43.3%) these symptoms had been moderate or severe. Premenstrual symptoms interfered with work efficiency, relationships at work or at home, home responsibilities or social life activities in 64 women (53.3%). The mean PSST score among all women was 7.9±0.7, ranging from 0 to 38. The PSST score and the HFWWS score lacked significant correlation (r=0.087, p=0.346), indicating that women with a history of severe premenstrual symptoms were not more likely to experience severe hot flushes during their menopause. The PSST scores showed non-significant differences between women with none (7.8±1.4), mild (5.0±1.0), moderate (7.7±1.3), and severe (9.4±1.2) hot flushes (Figure 8).

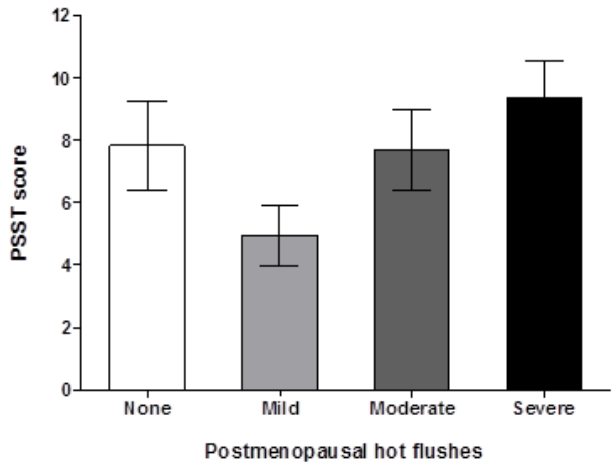

Figure 8. *Scores of Premenstrual Symptoms Screening Tool (PSST) in women with and without postmenopausal hot flushes of different degrees of severity.*
Data are presented as mean ± SEM.

Although premenstrual symptoms and postmenopausal hot flushes lacked clear correlation, the severity of premenstrual symptoms in fertile age associated with a more problematic postmenopause in terms of increased mood symptoms and a decline in memory and concentration capacity, quality of sleep and personal feelings of attractiveness (Table 12).

Table 12. *Correlations between Women's Health Questionnaire (WHQ) domains and Premenstrual Symptoms Screening Tool (PSST) score in recently postmenopausal women (n=120).*

Data are presented as mean ± SEM.

WHQ domain	value	Correlation to PSST score (*r*)	*p*-value
Depression	0.885±0.016	-0.263	**0.023**
Sleep	0.719±0.032	-0.282	**0.011**
Attractiveness	0.755±0.037	-0.260	**0.022**
Memory and concentration	0.726±0.031	-0.448	**<0.001**
Anxiety	0.904±0.018	-0.214	0.056
Vasomotor	0.480±0.047	-0.062	0.587
Somatic	0.750±0.022	-0.167	0.139
Menstrual	0.832±0.023	0.047	0.679
Sexual	0.709±0.037	-0.089	0.493

Impact of hot flushes on cardiovascular autonomic responsiveness (Study III)

Blood pressure

Resting blood pressures were comparable between women with no, mild, moderate, and severe hot flushes (Figure 9). In response to active posture change from supine to upright, the diastolic blood pressure increased (p<0.0001) compared with supine levels (Figure 9, panel a). During the handgrip test, both systolic and diastolic blood pressures increased significantly (Figure 9, panel b). The observed blood pressure responses to active orthostatic and handgrip tests were normal, and no significant differences between the study groups emerged. Women with hot flushes (mild, moderate, and severe groups combined) had slightly lower maximal systolic (140±2 *vs.* 145±3 mmHg, p=0.123) and diastolic (92±1 *vs.* 95±2 mmHg, p=0.081) blood pressures during the handgrip test than asymptomatic women.

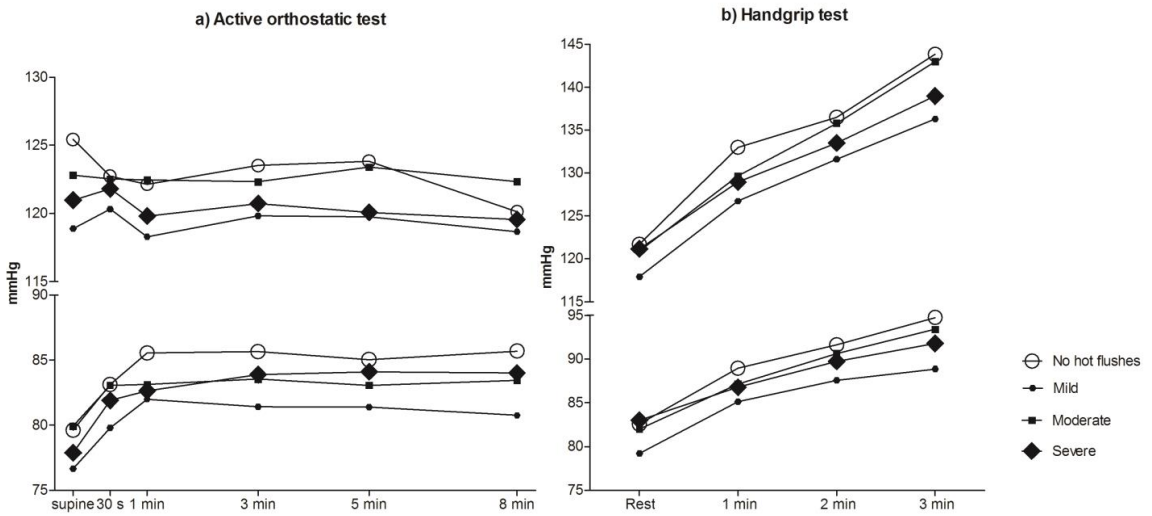

Figure 9. *Responses of systolic and diastolic blood pressure to a) active orthostatic and b) handgrip tests in women with and without hot flushes.*

Heart rate

Resting HRs (no hot flushes 69±10 beats/min, mild 66±7 beats/min, moderate 69±9 beats/min, and severe 67±9 beats/min, *p*=ns between groups) and maximal HRs during active orthostatic, handgrip, or Valsalva tests were equivalent between the study groups. During the Valsalva manoeuvre, the HR increased, and the release of strain was followed by reflex bradycardia. The Tachycardia ratio was lower in women with hot flushes compared with women without hot flushes, reflecting a stronger HR acceleration during Valsalva relative to the resting HR (Figure 10, panel a). This difference became evident when all women with hot flushes of different severities were combined into a single group and compared with asymptomatic women. The Valsalva ratio was slightly higher in women with hot flushes compared with women without hot flushes (Figure 10, panel b). Furthermore, results from the deep breathing and active orthostatic tests support these findings showing a tendency towards a more pronounced HR elevation in women with hot flushes (Figure 10, panels c and d).

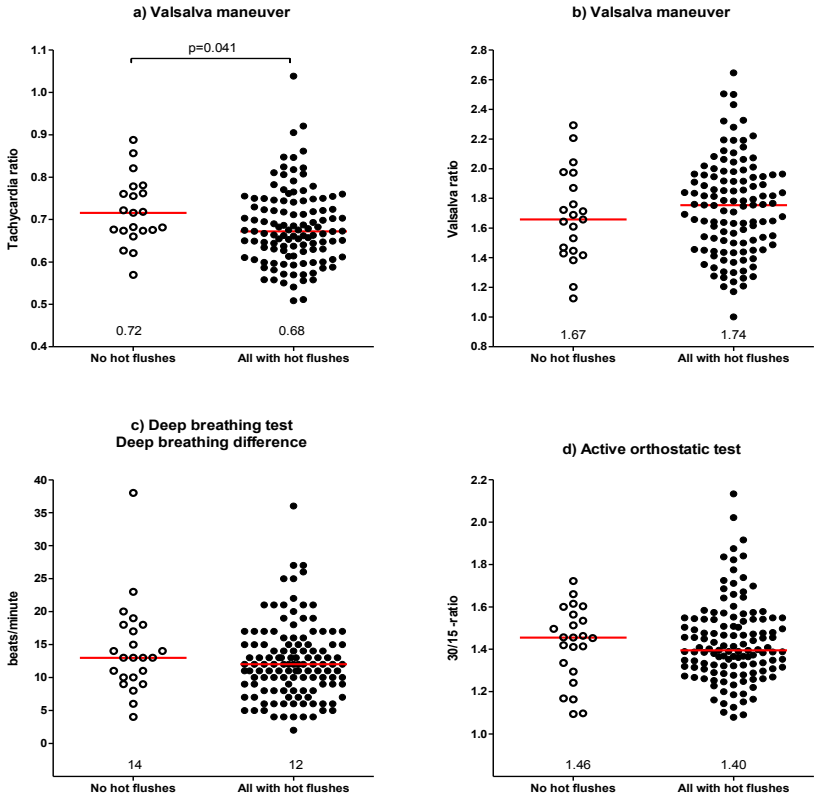

Figure 10. *Responses of heart rate to cardiovascular autonomic tests in women with and without hot flushes. Median/mean values of each group are indicated in numeric form above the x-axis. Line at mean (panels a, b) or median (panels c, d).*

Heart rate variability

The HRV in time (Table 13) or frequency domain measured at rest and during controlled and deep breathing lacked association with the hot flush status. A change of position from supine to standing caused expected increases in LF power and decreases in HF power equally in all study groups (p<0.01 for all), but HRV variables showed no relation to hot flush severity, neither in time (Table 13) nor frequency domain.

Table 13. *Heart rate variability in time domain during controlled and deep breathing and active orthostatic test in 148 women with different hot flush severities.*

	Hot Flushes			
	None (*n*=23)	**Mild** (*n*=33)	**Moderate** (*n*=29)	**Severe** (*n*=63)
Controlled breathing				
RMSSD (ms)	26 (11-77)	23 (12-83)	23 (8-76)	26 (9-196)
CoV (%)	2.4 (1.6-7.2)	2.6 (1.5-7.4)	2.4 (1.1-9.2)	2.7 (1.1-21.3)
Deep breathing				
RMSSD (ms)	43 (12-130)	40 (15-89)	46 (8-125)	41 (11-172)
CoV (%)	5.1 (2.4-16.0)	4.5 (2.2-9.1)	5 (1.3-14.3)	4.5 (2.0-18.1)
Active orthostatic (standing)				
RMSSD (ms)	16 (6-72)	18 (8-46)	18 (7-95)	17 (10-94)
CoV (%)	2 (0.9-6.9)	2.2 (1.1-5.3)	2.1 (0.9-7.8)	2.1 (1.1-11.6)
Active orthostatic (Δ)				
RMSSD (ms)	-13 (-53-9)	-11 (-55-12)	-4 (-39-32)	-10 (-154-33)
CoV (%)	-0.8 (-4.3 -1.0)	-0.8 (-4.0-1.6)	-0.1 (-3.7-3.9)	-0.7 (-15.3-2.7)

Data are presented as median (range), *p*=ns between all study groups

Δ Change from supine to standing position

RMSSD = the square root of the mean of the sum of squares of adjacent normal-to-normal R-R intervals

CoV = Coefficient of variation = HRV in proportion to mean R-R interval

Associations between cardiovascular autonomic function and a history of premenstrual symptoms

(unpublished data)

To evaluate if a history of severe premenstrual symptoms would be an independent factor in the autonomic regulation in menopausal women, we compared women having severe premenstrual and severe vasomotor symptoms (PSST score ≥15, HFWWS ≥100) and women with mild or no such symptoms at all (PSST score ≤3, HFWWS ≤9.5). Women with severe symptoms had a higher Valsalva ratio (1.951±0.056 *vs.* 1.639±0.057, p=0.005) and a more pronounced elevation of HR during strain (37.3±1.1 *vs.* 30.6±2.6 beats/min) than women with mild symptoms. Furthermore, when comparing women having both severe premenstrual and vasomotor symptoms (PSST score ≥15, HFWWS ≥100) with women having severe vasomotor symptoms but only mild premenstrual symptoms (PSST score ≤3, HFWWS ≥100), the above-mentioned differences persisted (Figure 11). In addition, women with both severe premenstrual and vasomotor symptoms had a lower Tachycardia ratio compared with women with mild premenstrual symptoms (Figure 11, panel b).

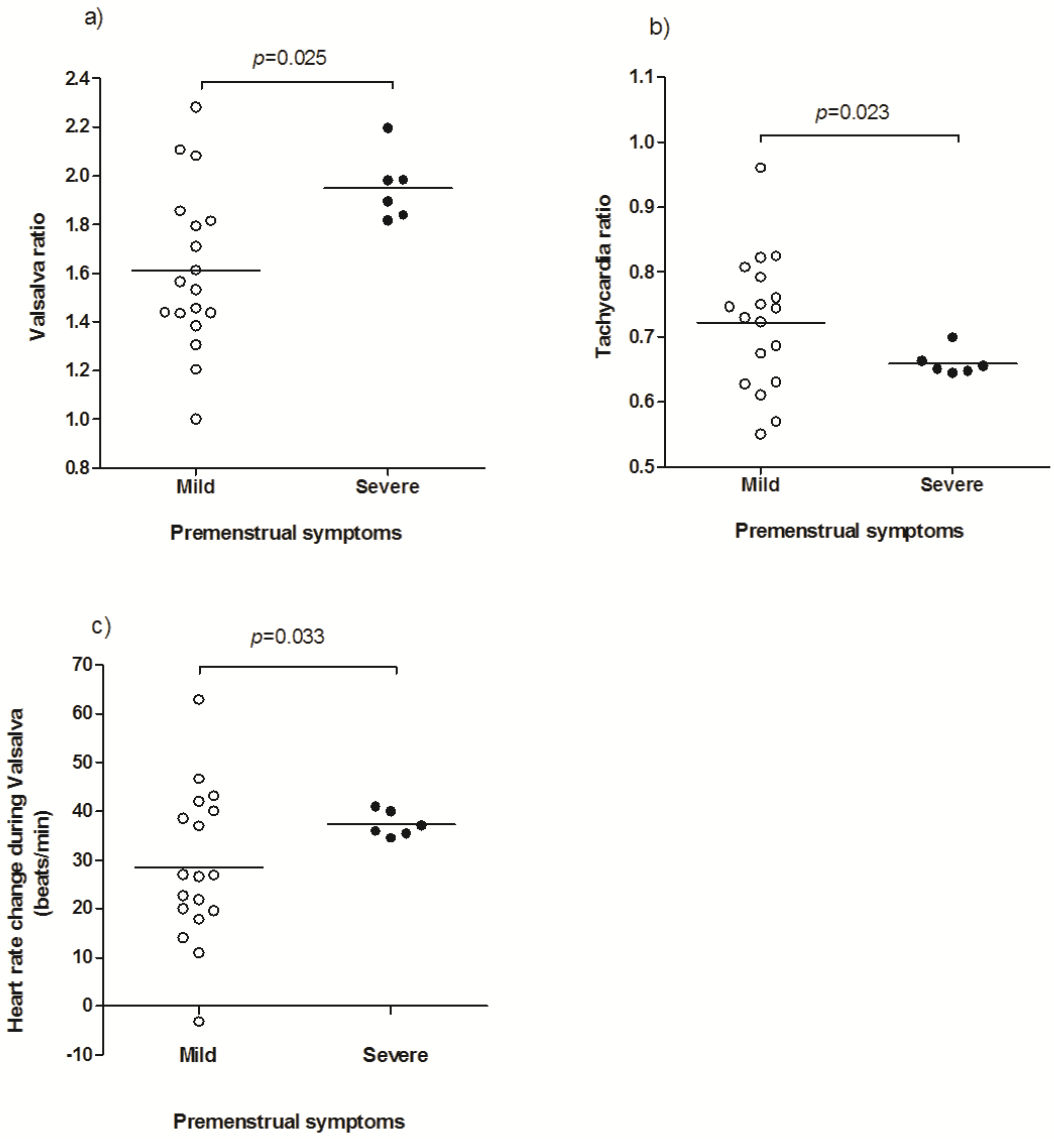

Figure 11. *Heart rate responses during Valsalva manoeuvre in women with severe postmenopausal hot flushes but a history of either mild/none or severe premenstrual symptoms; a) Valsalva ratio, b) Tachycardia ratio, and c) heart rate change during strain. (Lines at mean).*

Effects of hormone therapy on

Hormone levels

All oral and transdermal ET, and ET+ MPA treatments led to significant elevations in serum E_2 levels compared with placebo treatments (p<0.01). All active treatments also reduced hot flushes effectively (p<0.01 for all compared with placebo, p<0.0001 compared with baseline level), but also placebos caused a modest 23% reduction in HFWWS from baseline. (Table 14)

Table 14. *Levels and changes in estradiol (E_2) and hot flush weekly weighted symptom score (HFWWS) after six months of hormone therapy.*
Data are presented as mean ± SEM.
MPA=medroxyprogesterone acetate

	Oral E_2	Transdermal E_2	Oral E_2 and MPA	Placebo
E_2 (pmol/L)	218±18*	204±29*	174±15*	69±17
Change of E_2 (pmol/L)	+157±22*	+125±33*	+106±19*	-3±20
In women with pre-treatment hot flushes:				
HFWWS	6±3*	35±15*	4±2*	113±19
Change of HFWWS (% from baseline)	-96±1*	-80±7*	-97±1*	-23±16

*p <0.0001 compared with placebo

Health-related quality of life (Study I)

In women with pre-treatment hot flushes, HT significantly improved scores of the WHQ domains regarding vasomotor symptoms (0.741±0.410 *vs.* 0.042±0.129, $p<0.0001$), sleep (0.787±0.268 *vs.* 0.556±0.249, $p=0.001$), anxiety and fears (0.942±0.133 *vs.* 0.826±0.193, $p=0.005$), and memory and concentration (0.849±0.228 *vs.* 0.454±0.301, $p<0.0001$) compared with placebo treatment. At three months, placebo treatment also improved some of the WHQ scores compared with baseline levels (vasomotor symptoms +0.208, $p=0.044$, sleep +0.139, $p=0.022$, anxiety and fears +0.125, $p=0.028$), but these positive effects were no longer evident after the six month treatment. In women without hot flushes, HT slightly improved memory and concentration from the baseline level (0.803±0.273 to 0.917±0.178, $p=0.026$) and reduced insomnia (1.635±0.864 to 1.386±0.618, $p=0.012$).

Hormone therapy significantly relieved exhaustion (1.9±0.8 *vs.* 2.5±0.8, $p=0.006$), irritability (1.9±0.7 *vs.* 2.3±0.8, $p=0.011$), edema (1.5±0.8 *vs.* 1.7±0.5, $p=0.041$), joint or muscle pains (1.8±1.0 *vs.* 2.2±0.8, $p=0.013$), and vaginal dryness (1.3±0.6 *vs.* 2.1±1.0, $p=0.002$), compared with placebo recipients in women with pre-treatment hot flushes, whereas HT did not relieve depressive mood (1.3±0.5 *vs.* 1.4±0.6, $p=0.341$) or insomnia (2.0±1.0 *vs.* 2.4±1.0, $p=0.051$). Irritability was more common among non-flushing women receiving HT compared with placebo users (1.7±0.7 *vs.* 1.4±0.8, $p=0.024$). Otherwise, HT effects on menopausal symptoms were neutral in women without hot flushes.

In women with (but not without) pre-treatment hot flushes, HT improved the general health (2.0±0.3 *vs.* 1.8±0.5, $p=0.040$) compared with placebo treatment. However, HT did not affect sexual wellbeing (sexual satisfaction, sexual problems, or satisfaction with the partner) in women with or without flushes.

Blood pressure (Study IV)

In women with pre-treatment hot flushes, HT lowered the systolic and diastolic resting blood pressure (Figure 12, panel a). Estradiol therapy decreased flushing women's diastolic blood pressure compared with a slight elevation in non-flushing women (−4.0±1.2 mmHg in women with hot flushes *vs.* 0.9±1.5 mmHg in women without hot flushes, p=0.045). Placebos also reduced the resting blood pressure in women with hot flushes.

After HT, the systolic and diastolic blood pressure increases in response to the handgrip test were more pronounced in women with pre-treatment hot flushes than in asymptomatic women, particularly with ET (Figure 12, panel b). In contrast, in women without hot flushes, the diastolic blood pressure increased less after ET compared with flushing women (−2.3±1.4 *vs.* 5.1±1.5 mmHg, p=0.003). However, blood pressure responses to the handgrip test between the different treatment groups were comparable (Figure 12, panel b).

Active standing caused similar responses in blood pressure in all study groups, and the responses related neither to hot flush status nor HT regimens.

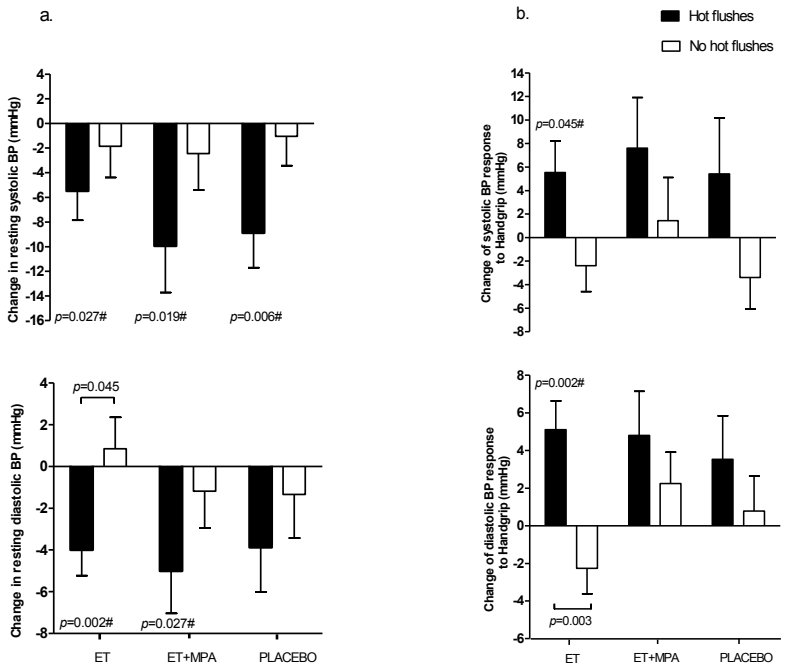

Figure 12. *Change in a) resting blood pressure and b) blood pressure response to handgrip strain in women with and without pre-treatment hot flushes after 6 months hormone therapy. Data are expressed as mean ± SEM. # compared with the level before treatment ET = estradiol therapy, MPA = medroxyprogesterone acetate, BP = blood pressure*

Heart rate (Study IV)

In women with pre-treatment hot flushes, ET reduced the resting HR compared with placebo treatment (-2.2±0.7 *vs.* 1.3±1.1 beats/min, p=0.03). Estradiol + MPA, however, slightly increased the resting HR (+0.7±1.2 beats/min) in women with hot flushes. Women with hot flushes also showed attenuated HR responses during the handgrip test after ET compared with placebo users (-2.2±1.3 *vs.* 2.8±1.5 beats/min, p=0.038), whereas ET + MPA increased the maximal HR during the handgrip compared with ET (3.1±1.4 *vs.* -2.2±1.3 beats/min, p=0.02). Similar, although non-significant, effects of HT on maximal HRs were seen during active orthostatic and Valsalva tests in women with pre-treatment hot flushes. Neither hot flushes nor HT influenced the deep breathing-induced oscillations of the HR, the Valsalva or Tachycardia ratios during Valsalva strain, or the 30/15 -ratio during standing.

Heart rate variability (Study V)

Hormone therapy did not affect HRV in time domain or in frequency domain measurements (HF, LF or LF/HF ratio) in women with or without pre-treatment hot flushes. However, during controlled quiet breathing, HT reduced VLF power in women with hot flushes compared with baseline level (p=0.018, Figure 13) and with non-flushing women (258±28 *vs.* 441±70 ms^2, p=0.025). Women without hot flushes receiving a placebo showed smaller changes of normalised HF and LF after standing up than women in other groups (nHF -0.016±0.050 *vs.* -0.166±0.026, nLF 0.002±0.069 *vs.* 0.173±0.029, $p \leq 0.015$ between women without hot flushes after placebo and women with hot flushes after HT).

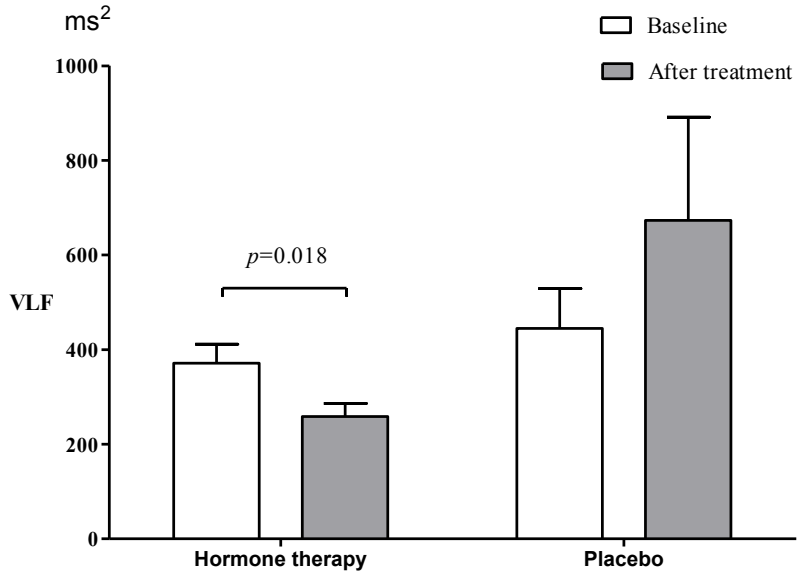

Figure 13. *Very low frequency power (VLF) at baseline and after treatment in women with pre-treatment hot flushes during controlled quiet breathing.*
Data are presented as mean ± SEM.

Table 15. *Subjects, methods and main results of the original studies.*

Study	Study population	Treatment	Outcome measures	Results
I HRQL and sexual function in women with and without hot flushes	*n*=132	6 months -Oral E_2 2mg -Oral E_2 2mg+ MPA 5mg -Transdermal E_2 1mg -Placebo	WHQ MFSQ VAS Kupperman index	-Women with hot flushes had generally worse HRQL than women without hot flushes -HT improved vasomotor and other menopausal symptoms and scores of general health, memory and concentration, anxiety, and sleep
II Association between premenstrual and menopausal symptoms	*n*=120	-	History of premenstrual symptoms, menopausal HRQL PSST, WHQ	-Severity of premenstrual symptoms lacked correlation with hot flushes but associated with deteriorated menopausal HRQL
III Cardiovascular autonomic function in women with hot flushes of different severities	*n*=150	-	Responses of blood pressure, HR and HRV to autonomic testing	-Hot flushes associated with tachycardic HR responses -Comparable HRV -Comparable blood pressure
IV Impact of HT on cardiovascular autonomic function in women with and without hot flushes	*n*=138	6 months -Oral E_2 2mg -Oral E_2 2mg+ MPA 5mg -Transdermal E_2 1mg -Placebo	Responses of blood pressure and HR to autonomic testing	-HT lowered resting blood pressure and augmented blood pressure response to strain in women with hot flushes -E_2 lowered resting HR and HR responses to strain in women with hot flushes, whereas E_2+MPA acted reciprocally
V Impact of HT on HRV in women with and without hot flushes	*n*=138	6 months -Oral E_2 2mg -Oral E_2 2mg+ MPA 5mg -Transdermal E_2 1mg -Placebo	HRV during autonomic testing	-HT did not affect HRV in women with or without hot flushes

HRQL = Health-related quality of life, E_2 = estradiol, MPA = Medroxyprogesterone acetate, HT = hormone therapy, WHQ = Women's health questionnaire, MFSQ = McCoy female sexual questionnaire, VAS = Visual analogue scale, PSST = Premenstrual symptoms screening tool, HR = heart rate, HRV = heart rate variability

DISCUSSION

Quality of life is of vital importance in women's general wellbeing throughout their different life stages. Research on women's HRQL is gaining much interest along with longer life expectancy, and since a worsened quality of life can impair a woman's working capacity, it can even result in absence from work. In particular, hot flushes, mood swings, poor sleep, physical complaints, and disturbances of memory and concentration are generally thought to deteriorate menopausal women's HRQL (*Hess et al. 2012*, *Ayers & Hunter 2013*). However, it has not been concluded whether hot flushes are the major determinant for a worsened HRQL. According to some theories non-flush symptoms could be only secondary phenomena to hot flushes. Hot flushes show great day-to-day variation, and women's capacity to tolerate hot flushes varies considerably (*Freeman et al. 2005*). Therefore, the use of multiple menopause-specific HRQL instruments, careful and prospective assessment of hot flushes and other menopausal symptoms are essential, as done in the present study.

The background mechanisms of hot flushes have remained unclear, although increasing evidence of the cardiovascular autonomic function's involvement has been gained during the course of our study (*Thurston et al. 2010*, *Freedman et al. 2011*, *Thurston et al. 2012*). Interestingly, as menopausal and premenstrual symptoms share many similarities, changes in cardiovascular autonomic function are also seen in women with severe premenstrual symptoms.

Hot flushes and postmenopausal health-related quality of life

The current study shows that hot flushes are major determinants in decrease of HRQL, as they associated with worsened general health perception and with all domains of the WHQ. Previous studies have yielded inconclusive results on the relationship of hot flushes and memory deterioration (*LeBlanc et al. 2007*, *Maki et al. 2008*, *Greendale et al. 2010*, *Schaafsma et al. 2010*, *Mitchell & Woods 2011*), possibly due to many different methods. In our study, women with hot flushes experinced more subjective difficulties with memory and concentration than women without hot flushes, and the difficulties correlated with the severity of hot flushes. Although women with hot flushes reported more depressive symptoms than their asymptomatic counterparts, depressive scores lacked correlation with the severity of hot flushes (HFWWS). This likely implies that depressive mood is an independent symptom apart from hot flushes. Depressive symptoms have preceded the occurrence hot flushes around menopause (*Freeman et al. 2009*),

supporting our results, although women with depressive symptoms in menopause are more likely to report hot flushes (*Freeman 2010*).

Hot flushes were effectively reduced with all HT regimens. Hormone therapy alleviated poor sleep and anxiety, and it enhanced memory and concentration capacity in women with pre-treatment hot flushes. Previous trials, such as WHI and HERS, included mainly asymptomatic women well past the menopause and are therefore likely to underestimate the positive effects of HT on HRQL for women with hot flushes.

The present study shows that in women without pre-treatment hot flushes, HT improved memory and concentration capacity compared with the baseline, although such improvements were not detected in other HRQL areas. These findings may also support the "timing hypothesis" of HT also on memory; HT started in early postmenopause could have protective effects on memory (*Henderson 2014*, *Rocca et al. 2014*), both in women with and without hot flushes. Evidence of estrogen's neuroprotective effect is convincing (*Barrett-Connor & Laughlin 2009, Fischer et al. 2014*), whereas an increasing amount of evidence suggests that MPA could be detrimental to cognitive function (*Nilsen & Brinton 2002*, *Resnick et al. 2006*, *Liu et al. 2010*, *Irwin et al. 2011*). In our study, MPA did not counteract the positive effects of E_2 on HRQL parameters during the six months treatment period. It is possible that MPA effects are different in recently postmenopausal women compared to older women assessed in the previous studies (*Resnick et al. 2006*, *Veerus et al. 2012*).

Hormone therapy did not improve depressive mood significantly in the present study. A recent Finnish registry finding revealed that HT-use is associated with depression (*Toffol et al. 2012*), which could imply that women with depressive symptoms are more likely to use HT. Some other (*Soares et al. 2001*, *Hlatky et al. 2002*), but not all randomised controlled trials (*Morrison et al. 2004, Hays et al. 2003, Welton et al. 2008*) have found positive effects of HT on depressive symptoms in older women. Interestingly, in most HRQL variables, a placebo effect was detected at three months but not anymore after six months treatment. This emphasises the importance of the placebo arm also in HRQL studies, particularly in a short-term assessment.

Evaluation of sexual function in a setting specifically comparing women with or without hot flushes after natural menopause, has not been previously reported. It has been shown that after surgical menopause, women tend to experience more menopausal symptoms, which associate with sexual dysfunction, than after natural menopause (*Topatan et al.2012*). In the present study, severe hot flushes associated with sexual problems, but not with generally poorer sexual function measured with the specific menopausal MFSQ. Neither did the hot flush status influence HT's effect on sexual

function. Some (*Welton et al. 2008*, *Gast et al. 2009*), but not all (*Hays et al. 2003*, *Wierman et al. 2010*) studies have shown beneficial effects of HT on sexual function. Local and systemic HT indisputably alleviates vaginal dryness and concomitant dyspareunia. Since the women in the present study were recently menopausal, vaginal epithelial atrophy might not yet have developed. This could at least in part explain the differing results compared to the previous studies with elderly women (*Welton et al. 2008*, *Gast et al. 2009*).

Premenstrual and postmenopausal symptoms

In the present study, the severity of premenstrual symptoms during fertile years lacked association with hot flushes. Thus, my data indicate that women with premenstrual symptoms are not at increased risk to develop vasomotor symptoms when they reach menopause. Instead, premenstrual symptoms correlated with poor sleep, depressive symptoms, impaired memory and concentration capacity and feelings of less attractiveness recently after menopause. It is possible that the retrospective assessment of premenstrual symptoms could affect the reliability in recalling the symptom severity. In previous studies, premenstrual symptoms and hot flushes had associated in two studies (*Collins & Landgren 1994*, *Freeman et al. 2004*) but lacked association in two other studies (*Guthrie et al. 1996*, *Morse et al. 1998*). Varying methods explain, at least in part, the differing findings of the previous studies and also compared with this study. For example, in the previous follow-up studies, evaluation of hot flushes was retrospective and lacked a severity rating, or the assessment of premenstrual symptoms was limited.

Premenstrual symptoms are characterised by mood swings and depressive feelings. Women with PMS have more diagnosed depression than the general population (*Halbreich 2003*), and a recent review outlined that menopausal women also have an elevated risk for depression (*Llaneza et al. 2012*). These results support our findings, showing that premenstrual symptoms correlate with depressive mood at menopause. It is possible that women with premenstrual symptoms are vulnerable for mood symptoms throughout the different stages of their reproductive life, including the menopause. The finding that the history of premenstrual symptoms associated with depressive mood, poor sleep and memory but not with hot flushes might indicate that these menopausal complaints affect women's HRQL independently, and are not just secondary to hot flushes.

Although the underlying mechanisms of both premenstrual and menopausal symptoms remain unclear, alterations involving the cardiovascular autonomic nervous system have been related to both premenstrual symptoms (*Girdler et al. 1998*, *Matsumoto et al. 2006*, *Matsumoto et al. 2007*) and menopausal transition (*Brockbank et al. 2000*).

Previous data indicates possibly reduced HRV during the luteal phase in PMS patients (*Matsumoto et al. 2006*, *Matsumoto et al. 2007*, *Baker et al. 2008*) and generally reduced HRV in PMDD patients (*Landen et al. 2004*, *Matsumoto et al. 2007*).

To shed light on the suggested role of cardiovascular autonomic function in premenstrual and postmenopausal symptoms, we compared the cardiovascular autonomic responses of women with both severe premenstrual and vasomotor symptoms to those having only mild premenstrual and vasomotor symptoms. Women with the most severe premenstrual symptoms and hot flushes reacted with a more pronounced HR increase during the Valsalva manoeuvre than women with mild premenstrual and vasomotor symptoms. The HR increase during the Valsalva manoeuvre is mediated both by a surge in sympathetic activity and a rapid withdrawal of parasympathetic tone. To further investigate the role of previous premenstrual symptoms possibly affecting cardiovascular autonomic responses in menopause, we compared women with severe premenstrual symptoms and hot flushes to those who had severe hot flushes but only mild or absent premenstrual symptoms. The differences in HR responses to Valsalva were similar to the former comparison groups. This implies that women with previous severe premenstrual symptoms could possess more sympathetic drive, irrespective of hot flushes. This finding suggests that qualities involving the cardiovascular autonomic nervous system could act as background mechanisms of premenstrual symptoms.

Hot flushes and cardiovascular autonomic function

Exploring the impact of hot flushes on cardiovascular autonomic regulation before and during HT was one of the primary goals of this research. We hypothesised that cardiovascular function in women with hot flushes might differ from that of asymptomatic women.

Hot flushes have been linked to various cardiovascular risk factors, such as endothelial dysfunction (*Thurston et al. 2008*, *Bechlioulis et al. 2009*), increased calcification of the aorta (*Thurston et al. 2008*), higher carotid intima media thickness *(Thurston et al. 2011b),* and unfavorable lipid profile (*Gast et al. 2008*). On the contrary, the recent findings of the Kronos Early Estrogen Prevention Study show that vasomotor symptoms do not associate with subclinical atherosclerosis evaluated with coronary artery calcium and carotid intima-media thickness *(Wolff et al. 2013)*. Previous studies on the association between hot flushes and elevated blood pressure have also resulted in inconsistent findings (*Nelesen et al. 2004*, *Gast et al. 2008*, *Gallicchio et al. 2010*, *Gast et al. 2010*). The autonomic nervous system is the main short-term regulator of blood pressure and is also involved in the long-term control (*Joyner et al. 2008*). After menopause and along with aging the increased

sympathetic activity leading to vasoconstriction could promote the development of hypertension (*Barton & Meyer 2009*, *Barnes et al. 2014*, *Hart & Charkoudian 2014*). On the other hand, calcification and atherosclerosis of blood vessels diminishes the vascular reactivity and could thereby deteriorate cardiovascular autonomic regulation. Altogether, endogenous and exogenous estrogens appear to have an inhibitory role on the sympathetic activity (*Mercuro et al. 2000*), and the reduced parasympathetic input in postmenopausal women, possibly due to hypoestrogenism, leaves the cardiovascular system exposed to unopposed sympathetic stimulation.

In this study, women with hot flushes compared to non-flushing women show slightly smaller rises in systolic and diastolic blood pressures during isometric muscle contraction, provoking sympathetic activation. This novel finding could be explained at least in part by functional differences at the vascular level (*Tuomikoski et al. 2009a*). The flushing women might have a smaller vasoconstriction reserve due to the higher underlying sympathetic tone in the vasculature. This could diminish the additional sympathetic response provoked by a handgrip test leading to smaller blood pressure elevations than in asymptomatic women.

After menopause women are thought to have more sympathetic drive and attenuated parasympathetic activity than younger women (*Lavi et al. 2007*, *Yang et al. 2013*). Whether this shift is due to just advancing age or truly to menopause related changes in estrogen metabolism is unclear. Our data showed increased sympathetic activity and slightly blunted parasympathetic HR responses in these recently postmenopausal women, suggesting that the previously seen menopausal change in autonomic activity would result from menopause *per se*, not just aging. Moreover, as this phenomenon related to hot flushes, the hot flush status could be an important determinant.

Hot flushes lacked association with HRV in this controlled short-term setting. This is in line with our earlier findings seen in ambulatory HRV measurements in recently postmenopausal women (*Hoikkala et al. 2010*). One rather small study, using a short-term HRV measurement, associated menopausal symptoms and hot flushes with higher LF/HF-ratio indicating increased sympathetic activity (*Lee et al. 2011*). Heart rate variability is a sensitive attribute of autonomic regulation and has been reduced after menopause (*Brockbank et al. 2000*). Attenuated HRV is seen also in CVDs (*Buccelletti et al. 2009*). In terms of HRV, our results indicate that hot flushes do not associate with impaired autonomic regulation of the HR. During hot flushes, parasympathetic withdrawal has been detected in short-term measurements (*Thurston et al. 2010b*) and in an ambulatory setting during daytime and sleep (*Thurston et al. 2012*). Increases in LF power indicating rises in sympathetic activity (*Freedman et al. 2011*) and decreases in HF power as a sign of vagal withdrawal (*de Zambotti et al. 2013*) were detected during

nighttime ambulatory measurements. These data suggest a significant contribution of autonomic regulation in hot flushes.

In addition to studying the impact of hot flushes on cardiovascular autonomic function in a cross-sectional setting, the present study assessed the impact of hot flushes on outcomes of HT in a prospective, randomised trial. Previous data on the effects of HT on cardiovascular autonomic function are inconclusive, probably due to the diversity in methodologies and HT components. Only one study has compared flushing and non-flushing women, but without any placebo-control (*Beljic et al. 1999*). In our study, hot flushes were accompanied with reductions in resting blood pressures and HRs and in responses of HR to cardiovascular autonomic function tests after ET. Lowered resting blood pressure after ET is in line with our previous results in ambulatory measurements, as E_2 decreased 24-hour and daytime blood pressures in women with hot flushes (*Tuomikoski et al. 2010*). In the present study however, blood pressures elevated more during the handgrip test in women with pre-treatment hot flushes than in women without hot flushes. This might suggest that E_2 restores the vascular reactivity, which is reduced due to the sympathetic overdrive associated with menopause and hot flushes, and further improves the vasculature's reactivity to physical strain. These results indicate that the potentially beneficial effect of estrogen on autonomic nervous function is seen particularly in women with hot flushes. It is not possible to deduce whether these effects are secondary consequences of vanished hot flushes or a direct effect of E_2 on the autonomic regulation of blood pressure and HR.

The present results show neutral effects of HT on HRV in controlled short-term measurements, regardless of the route of HT administration. These results were not affected by the hot flush status. Our study group has earlier observed potentially unfavorable effects of HT on nighttime HRV in ambulatory measurements (*Lantto et al. 2012*). These were seen regardless of the pre-treatment hot flush status. The HT effects seen in the nighttime ambulatory assessment could be explained by indirect autonomic influences of sleep and HRV in its different stages (*Ako et al. 2003*, *Versace et al. 2003*). Previously in the same ambulatory setting, VLF power during nighttime hot flushes was increased (*Hoikkala et al. 2010*). In the present study, HT attenuated VLF in women with pre-treatment hot flushes. The physiological source of VLF has remained unclear, but it has been associated with peripheral vasomotor regulation (*Perini & Veicsteinas 2003*), thermoregulation, and the renin-angiotensin system (*Taylor et al. 1998*). This could relate to alleviation of hot flushes and further to HT's effects on thermoregulation or the vasculature.

The hot flush has emerged as one possible determinant for the conflicting estrogen mediated vascular effects (*van der Schouw & Grobbee 2005*, *Mikkola*

2011, *Tuomikoski et al. 2011*). Some studies have associated hot flushes with an adverse cardiovascular risk profile (*Gast et al. 2008*, *Gast et al. 2010, Huang et al. 2009*), but our study group has shown that in recently menopausal women, hot flushes were accompanied by vasodilatory effects (*Tuomikoski et al. 2009a*) and furthermore, the effect of E_2 on vascular function depends on the pre-treatment hot flush status (*Tuomikoski et al. 2009b*). In our studies, the responses to E_2 with or without concomitant MPA were neutral in terms of vasoconstriction or vasodilatation in women with hot flushes, whereas in women without hot flushes E_2 induced a vasoconstrictive response. These findings also gain support from the re-analysis of the WHI study results showing that vasomotor hot flushes at the onset of menopause, but not later after menopause, were associated with reduced cardiovascular events (*Szmuilowicz et al. 2011*). The mechanisms behind these findings might relate to differences in cardiovascular autonomic regulation.

The progestogen component, particularly MPA, may modify the effects of HT (*Kuhl & Stevenson 2006*, *Sitruk-Ware 2007*, *Scarabin et al. 2011*, *Yang et al. 2013*). In our study, MPA attenuated the potentially beneficial effects of ET on resting HR and on HR response to the handgrip test in women with pre-treatment hot flushes. The large placebo controlled studies (WHI-EPT, HERS) have associated MPA-containing HT with adverse cardiovascular events (*Grodstein et al. 2003*). Medroxyprogesterone acetate has been also suggested to predispose to arrhythmias (*Gökce et al. 2005*, *Lantto et al. 2012*). This progestin has androgenic, antiestrogenic, and glucocorticoid features (*Kuhl 2005*), which may counteract the effects of estrogen.

Strengths and limitations

The study population was carefully selected to represent healthy, recently postmenopausal women without cardiovascular risk factors in order to investigate the impact of hot flushes on HRQL and cardiovascular autonomic function. Other strengths of our study are the prospective and validated assessment of hot flushes and application of multiple instruments in evaluating HRQL and other menopausal complaints. Cardiovascular autonomic function was assessed with a standardised laboratory test series in carefully controlled laboratory settings, expected to detect even slight differences in cardiovascular function and facilitating assessment of the relative contributions of the sympathetic and parasympathetic branches.

For the HT part of our study, 150 participants were randomised to transdermal and oral E_2 or the latter combined with MPA. This enabled comparison of the administration routes, which were suspected to vary knowing the differing estrogenic milieus they create. However, transdermal and oral estrogen affected HRQL and cardiovascular autonomic function

similarly in this study. The MPA was chosen as a progestin because it had been used in most of the previous large HT trials *(Hulley et al. 1998, Rossouw et al. 2002)*.

As limitations of the study, it should be noted that the participants were lean, healthy, and white, and thus, the results may not apply to obese women or those with diseases and medications or of other ethnic populations. Recruitment by a newspaper advertisement could affect the selection process toward healthier women. We studied recently postmenopausal women, and therefore this data may not hold for older women. It can be also speculated that a longer treatment period might have yielded additional differences. Finally, the number of women in our study was limited, and it is therefore possible that some differences remained undetected due to small sample sizes.

Future directions

In the future, research should assess effects of different progestins on cardiovascular autonomic function and memory. Lower doses of oral estrogen and the transdermal route are under active research (*Harman et al. 2005*, *ELITE*). A novel treatment for menopausal symptoms, tissue-selective estrogen complex "TSEC" containing both estrogen and a selective estrogen receptor modulator, is being developed (*Kagan 2012*). Ideally, a TSEC would effectively alleviate hot flushes and prevent bone loss, lacking effects on the cardiovascular system, breast tissue, and endometrium. Larger, prospective studies are needed to confirm the impact of hot flushes on the outcomes of HT and to outweigh the quite large variation of individual autonomic responses. Future trials should always consider the hot flush status as a possible determinant for HT effects.

In conclusion, ET and EPT both alleviated hot flushes and improved HRQL equally well. A history of premenstrual symptoms appears to predispose women to worsened HRQL in menopause, but they are not at an increased risk of developing hot flushes, which is comforting for women having troublesome premenstrual symptoms. Hot flushes associate with increased sympathetic activity in regulation of the HR, which could be seen as an unfavorable factor for cardiovascular health. However, this can be reduced with ET, especially in women with pre-treatment hot flushes. Adding MPA to E_2 seems to blunt some positive effects of ET. Oral and transdermal administration routes of E_2 resulted in similar responses. Thus, the hot flush status contributes clearly to the quality of life and cardiovascular autonomic function before and during HT. Particularly women with hot flushes appear to benefit most from HT's positive effects on the HRQL and cardiovascular regulation.

CONCLUSIONS

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

- Hot flushes are major determinants of menopausal decrease in health-related quality of life, but not of sexual function.

- Estrogen therapy and estrogen-progestogen therapy equally effectively improved health-related quality of life. Different administration routes of estrogen showed similar effects.

- Premenstrual symptoms do not predict hot flushes, but they are in association with worsened health-related quality of life in menopause.

- Hot flushes contribute to cardiovascular autonomic responsiveness.

- Hot flushes were accompanied with beneficial effects of estrogen therapy on cardiovascular autonomic responsiveness, whereas combining medroxyprogesterone acetate with hormone therapy attenuated the potentially beneficial effects of estrogen.

- Hot flushes lacked association with heart rate variability and hormone therapy's effect on heart rate variability.

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APPENDICES

Appendix A. *Questions of the Women's Health Questionnaire answered on a four-point scale. (Yes definitely, Yes sometimes, No not much, No not at all).*

Vasomotor
I have hot flushes
I suffer from night sweats
Somatic
I have headaches
I feel more tired than usual
I have dizzy spells
I suffer from backache or pain in my limbs
I feel sick or nauseous
I often notice 'pins and needles' in my hands and feet
I need to pass urine/water more frequently than usual
Anxiety and fears
I get very frightened or panic feelings for apparently no reason at all
I feel anxious when I go out of the house on my own
I feel tense or 'wound up'
I get palpitations or a sensation of 'butterflies' in my stomach or chest
Depression
I feel miserable and sad
I have lost interest in things
I feel life is not worth living
I am more irritable than usual
I still enjoy the things I used to
I have a good appetite
I have feelings of well-being
Sleep
I am restless and can't keep still
I have difficulty in getting off to sleep
I wake early and then sleep badly for the rest of the night
Sexual
I have lost interest in sexual activity
I am satisfied with my current sexual relationship (please omit if not sexually active)
As a result of vaginal dryness sexual intercourse has become uncomfortable
Memory and concentration
I am more clumsy than usual
I have difficulty in concentrating
My memory is poor
Menstrual
My stomach feels bloated
I have abdominal cramps or discomfort
I have heavy periods (please omit if no periods at all)
My breasts feel tender or uncomfortable
Attractiveness
I feel rather lively and excitable
I feel physically attractive

Appendix B. *Questions of menopausal symptoms modified from the Kupperman Index.*

Night sweats
Hot flushes
Numbness
Insomnia
Irritability, nervousness
Feeling exhausted
Depressive mood
Dizziness
Weakness, Fatigue
Aching joints or muscles
Headaches
Palpitation
Vaginal or genital dryness
Edema
Shortness of breath
Dryness of mouth
A lump-feeling in the throat
Nausea
Trembling

Appendix C. *General health.*

1. Verrattuna keskimääräiseen terveydentilaani viimeisten 12 kuukauden aikana, terveydentilani on tällä hetkellä ...

   1 parempi
   2 suunnilleen sama
   3 huonompi

2. Alla on asteikko, johon pyydämme teitä merkitsemään tämänhetkisen terveydentilanne. Vetäkää viiva siihen kohtaan asteikkoa, joka parhaiten kuvaa terveydentilaanne tänään.
   Parasta terveydentilaa, jonka voitte kuvitella merkitään 100 :lla, huonointa 0 :lla.

0 10 20 30 40 50 60 70 80 90 100

|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|

Huonoin kuviteltavissa oleva terveydentila

Paras kuviteltavissa oleva terveydentila

Appendix D. *Items of the McCoy Female Sexuality Questionnaire answered on a seven-point Likert scale.*

Sexual satisfaction
Enjoyment of sexual activity
Satisfaction with frequency of sexual activity
Frequency of sexual thoughts and fantasies
Excitement/arousal during sexual activity
Frequency of orgasm
Sexual problems
Insufficient lubrication
Painful sexual intercourse
Satisfaction with the partner
Level of sexual interest
Satisfaction with partner as lover
Satisfaction with partner as friend

Appendix E. *Premenstrual symptom screening tool (PSST).*

Tässä osiossa kartoitamme esiintyikö Teillä *Kuukautisia edeltävään oireyhtymään* (engl. Premenstrual syndrome, PMS) liittyviä oireita. Pyydämme Teitä muistelemaan mahdollisimman tarkasti aikaa, jolloin Teillä vielä oli kuukautiset (esim. 30-40-vuotiaana) ja vastaamaan seuraaviin kysymyksiin sen perusteella. Jos mahdollista, niin vastatkaa sellaisen ajanjakson pohjalta, jolloin Teillä oli luonnollinen kuukautiskierto ilman mitään hormonaalista ehkäisyä tai muuta hormonihoitoa.

Merkitse rasti (x) sopivaan ruutuun

Oliko Teillä joitakin seuraavista kuukautisia edeltävistä oireista, jotka alkavat ennen kuukautisvuotoa ja loppuvat ensimmäisten vuotopäivien aikana?

Oire	Ei lainkaan	Lievä	Kohtalainen	Vaikea
1. Vihaisuus/ärtyneisyys				
2. Ahdistuneisuus/jännittyneisyys				
3. Itkuisuus/hylätyksi tulemisen tunne				
4. Masentunut mieliala/toivottomuus				
5. Kiinnostuksen väheneminen työasioihin				
6. Kiinnostuksen väheneminen kotitöihin/kotiasioihin				
7. Kiinnostuksen väheneminen sosiaalisiin aktiviteetteihin				
8. Keskittymisvaikeus				
9. Uupumus/energianpuute				
10. Ylensyöminen/ruuanhimot				
11. Unettomuus				
12. Uneliaisuus (lisääntynyt unen tarve)				
13. Musertuneisuuden tunne/olla poissa tolaltaan				
14. Fyysisiä oireita: rintojen arkuus, päänsärky, nivel- tai lihaskipu, turvotus, painonlisäys				

Ovatko yllä olevassa taulukossa listatut oireesi vaikuttaneet:

	Ei lainkaan	Lievästi	Kohtalaisesti	Vaikeasti
A. Sinun työtehokkuuteesi ja tuotteliaisuuteesi				
B. Sinun suhteisiin työtovereihisi				
C. Sinun suhteisiin perheeseesi				
D. Sinun sosiaalisen elämäsi aktiviteetteihin				
E. Sinun kodin velvoitteiden hoitamiseen				

Ympyröikää kohdallanne sopivin vaihtoehto tai kirjoittakaa vastaus sille varattuun tilaan.

Oliko kuukautiskiertonne pääsääntöisesti säännöllinen (23-35 päivää)?

1. Ei 2. Kyllä

Käytittekö jotain hormonaalista hoitoa tai hormonaalista ehkäisyä kierron säännöllistämiseksi?

1. En 2. Kyllä, mitä valmisteita_____

Jos Teillä oli PMS-oireita, minkä ikäisenä ne alkoivat? _____ vuotiaana

Jos Teillä oli PMS-oireita, minkä ikäisenä ne loppuivat? _____ vuotiaana

Esiintyikö Teillä kuumia aaltoja (vaihdevuosien kuumia aaltoja muistuttavia) kuukautiskierron lopulla (ennen kuukautisvuodon alkua)?

1. Ei 2. Kyllä

Hakeuduitteko lääkärin vastaanotolle mahdollisten PMS-oireidenne takia?

1. En 2. Kyllä

Jos, niin määrättiinkö teille jotain hoitoa PMS-oireisiin?

1. Ei 2. Kyllä, mitä valmisteita_____

Onko teillä todettu masennusta tai muuta psyykkistä sairautta?

1. Ei 2. Kyllä, vuonna_____, mikä_____

Oletteko joskus käyttänyt masennuslääkitystä?

1. En 2. Kyllä, vuonna_____, mitä valmistetta_____

Kiitos vastauksistanne!