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Sleep and Menopause

Hormone Therapy and Sleep Deprivation

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Only as high as I reach can I grow.
Only as far as I seek can I go.
Only as deep as I look can I see.
Only as much as I dream can I be.

- Karen Ravn

To my Family

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SLEEP AND MENOPAUSE ─ *Hormone Therapy and Sleep Deprivation*
Sleep Research Unit, Department of Physiology and Department of Obstetrics and Gynecology, University of Turku, Finland
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ABSTRACT

This study evaluated the effect of menopause, hormone therapy (HT) and aging on sleep. Further, the mechanisms behind these effects were examined by studying the associations between sleep and the nocturnal profiles of sleep-related hormones. Cross-sectional study protocols were used to evaluate sleep in normal conditions and during recovery from sleep deprivation. The effect of initiation of HT on sleep and sleep-related hormones was studied in a prospective controlled trial. Young, premenopausal and postmenopausal women were studied, and the methods included polysomnography, 24-h blood sampling, questionnaires and cognitive tests of attention.

Postmenopausal women were less satisfied with their sleep quality than premenopausal women, but this was not reflected in sleepiness or attention. The objective sleep quality was mainly similar in pre- and postmenopausal women, but differed from young women. The recovery mechanisms from sleep deprivation were relatively well-preserved after menopause. HT offered no advantage to sleep after sleep deprivation or under normal conditions. The decreased growth hormone (GH) and prolactin (PRL) levels after menopause were reversible with HT. Neither menopause nor HT had any effect on cortisol levels. In premenopausal women, HT had only minor effects on PRL and cortisol levels. The temporal link between GH and slow wave sleep (SWS) was weaker after menopause. PRL levels were temporally associated with sleep stages, and higher levels were seen during SWS and lower during rapid-eye-movement (REM) sleep.

Sleep quality after menopause is better determined by age than by menopausal state. Although HT restores the decreased levels of GH and PRL after menopause, it offers no advantage to sleep quality under normal conditions or after sleep deprivation.

Keywords: sleep; menopause; hormone therapy; estrogen; progestin; sleep deprivation; aging; growth hormone; prolactin; cortisol

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***UNI JA VAIHDEVUODET** – Hormonihoito ja Univaje*

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TIIVISTELMÄ

Väitöskirjatyössä selvitettiin vaihdevuosien, hormonihoidon ja iän vaikutuksia uneen. Lisäksi tutkittiin näiden vaikutusten taustalla olevia mekanismeja arvioimalla univaiheiden ja uneen liittyvien hormonien välisiä yhteyksiä. Poikkileikkaustutkimusasetelmaa käytettiin arvioitaessa unta normaaliolosuhteissa ja univajeen jälkeisen toipumisyön aikana. Prospektiivisen tutkimusasetelman avulla selvitettiin hormonihoidon vaikutusta unen laatuun ja unta säätteleviin hormoneihin. Tutkimuksiin osallistui sekä nuoria että pre- ja postmenopausaalisia naisia. Tutkimusmenetelminä käytettiin unirekisteröintiä, 24 tunnin verikeräystä, kyselylomakkeita ja kognitiivisia tarkkaavuustestejä.

Postmenopausaaliset naiset kokivat unen laatunsa heikommaksi kuin premenopausaaliset naiset, mutta tämä ei aiheuttanut uneliaisuuden lisääntymistä tai tarkkaavuuden heikentymistä. Objektiivinen unen laatu oli pääosin samanlaista pre- ja postmenopausaalisilla naisilla, mutta erosi nuorten naisten unen laadusta. Univajeen jälkeiseen toipumiseen tarvittavat mekanismit näyttivät säilyvän kohtalaisen hyvin myös vaihdevuosien jälkeen. Hormonihoito ei parantanut unen laatua univajeen jälkeen eikä normaaliolosuhteissa. Vaihdevuosien jälkeen todetut alhaisemmat kasvuhormonin ja prolaktiinin tasot pystyttiin hormonihoidolla palauttamaan premenopausaalia tasoja vastaaviksi. Sen sijaan vaihdevuosilla, kuten hormonihoidollakaan, ei ollut vaikutusta kortisoli-tasoihin. Hormonihoidolla oli vain vähäisiä vaikutuksia premenopausaalisten naisten prolaktiinin ja kortisolin tasoihin. Kasvuhormonin ja syvän unen välinen ajallinen yhteys oli heikompi postmenopausaalisilla kuin premenopausaalisilla naisilla. Prolaktiini-tasot olivat ajallisessa yhteydessä univaiheisiin ja tasot olivat korkeammat syvän unen kuin vilkeunen aikana.

Väitöskirjatyön perusteella muutokset naisen unen laadussa ovat ennemmin ikääntymisen kuin vaihdevuosien aiheuttamia. Vaikka hormonihoito nostaa kasvuhormonin ja prolaktiinin pitoisuudet vaihdevuosia edeltäneelle tasolle, ei hormonihoito paranna unen laatua normaaliolosuhteissa eikä univajeen jälkeen.

Avainsanat: uni, vaihdevuodet, hormonihoito, estrogeeni, progestiini, univaje, ikääntyminen, kasvuhormoni, prolaktiini, kortisoli

CONTENTS

ABBREVIATIONS

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BDI	Beck Depression Inventory
BMI	body mass index
BNSQ	The Basic Nordic Sleep Questionnaire
CBG	cortisol-binding globulin
CEE	conjugated equine estrogen
CNS	central nervous system
CRH	corticotropin-releasing hormone
CV %	coefficient of variation %
E_2	estradiol (17β-estradiol)
ECG	electrocardiogram
EEG	electroencephalogram
EMG	electromyogram
EOG	electro-oculogram
EPT	estrogen-progestin therapy
EQ-5D	EuroQuality of Life questionnaire
ET	estrogen therapy
GHRH	growth hormone - releasing hormone
HT	hormone therapy
IGF-1	insulin-like growth hormone factor 1
LH	luteinizing hormone
MPA	medroxyprogesterone acetate
n	number of subjects
IU	international unit
FSH	follicle stimulating hormone
GABA	gamma-aminobutyric acid
GH	growth hormone
NETA	norethisterone (acetate)
NREM	non-rapid eye movement
ns	non-significant
OC	oral contraception
PRL	prolactin
PSG	polysomnography
REM	rapid eye movement
S	stage
SCN	suprachiasmatic nuclei
SD	standard deviation
SHBG	sex hormone-binding globulin
SSS	Stanford Sleepiness Scale
SWA	slow wave activity
SWS	slow wave sleep
TRH	thyrotropin-releasing hormone
VAS	visual analog scale
VIP	vasoactive intestinal peptid

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Turku, September 2008

Nea Kalleinen

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals. The original communications have been reproduced with the permission of the copyright holders.

I Kalleinen N, Polo-Kantola P, Himanen S-L, Alhola P, Joutsen A, Urrila AS, Polo O. Sleep and the menopause - Do postmenopausal women experience worse sleep than premenopausal women? *Menopause Int* 2008;14:97-104.

II Kalleinen N, Polo O, Himanen S-L, Joutsen A, Urrila AS, Polo-Kantola P. Sleep deprivation and hormone replacement therapy in postmenopausal women. *Sleep Med* 2006;7:436-47.

III Kalleinen N, Polo O, Himanen S-L, Joutsen A, Polo-Kantola P. The effect of estrogen plus progestin treatment on sleep: a randomized, placebo-controlled, double-blind trial in premenopausal and late postmenopausal women. *Climacteric* 2008;11:233-243.

IV Kalleinen N, Polo-Kantola P, Irjala K, Porkka-Heiskanen T, Vahlberg T, Virkki A, Polo O. 24-h serum levels of growth hormone, prolactin and cortisol in pre- and postmenopausal women. The effect of combined estrogen and progestin treatment. *J Clin Endocrinol Metab* 2008;93:1655-61.

V Kalleinen N, Virkki A, Polo O, Porkka-Heiskanen T, Irjala K, Polo-Kantola P. Associations between sleep stages and nocturnal levels of growth hormone and prolactin in pre- and postmenopausal women. *(Manuscript)*.

1. INTRODUCTION

Aging involves gradual changes in sleep patterns and sleep architecture. The decreased percentage of time spent in slow wave sleep (SWS) is a hallmark of age-related sleep changes (Bliwise 2005). Women are generally more likely to have sleep complaints than men of the same age. This female predisposition is more notable in the elderly, although, for instance, SWS is better preserved in women (Reynolds et al. 1986, Zhang & Wing 2006). The prevalence of sleep complaints in women seems to increase most strikingly during middle age. The increase is frequently attributed to menopause.

The marked reduction in postmenopausal sleep complaints during hormone therapy (HT) suggests that the complaints are, in part, due to the menopause-induced deficiency of female sex hormones (e.g. Erkkola et al. 1991, Polo-Kantola et al. 1998, Sarti et al. 2005). The treatment effect on subjective sleep quality has been achieved with both unopposed estrogen (ET) and a combination of estrogen plus progestin (EPT). However, studies with polysomnography (PSG) have failed to demonstrate consistent objective markers behind the subjective sleep changes (e.g. Purdie et al. 1995, Polo-Kantola et al. 1999a, Saletu-Zyhlarz et al. 2003). Menopausal sleep complaints are often attributed to other climacteric symptoms, such as nocturnal vasomotor symptoms and higher levels of anxiety (Shaver et al. 1991, Shaver & Paulsen 1993). However, the findings on the association between objective measures of sleep and vasomotor symptoms are conflicting.

The duration of climacteric symptoms vary. In some women, the symptoms start as early as in the premenopausal state and persist even for decades after menopause. HT is used to alleviate climacteric symptoms in peri- and postmenopausal women and is also effective in controlling early symptoms in premenopausal women. In addition, HT has other beneficial effects on diverse organs and systems, such as on lipid profile or bone formation. However, the adverse effects of HT, for instance on blood coagulation and breast tissue, limit its usage. The brain can be considered to be an important target organ for estrogen. Estrogen receptors are found in several brain areas involved in sleep regulation (McEwen & Alves 1999). In addition to direct effects of the female sex hormones on their target organs, many of their effects are indirect, mediated through other hormones. Growth hormone (GH), prolactin (PRL) and cortisol are some of the hormones that are affected by aging, important in sleep regulation and influenced by exogenous sex hormones (Ho & Hoffman 1993, Van Cauter et al. 1996, Van Cauter 2005). The present knowledge of the temporal relationship between sleep and GH or PRL is based on studies including only young males. However, there is evidence that the findings may not apply to women, especially to older ones (Steiger 2007).

The aim of this study was to determine possible menopause-induced sleep changes and to investigate the effect of aging and EPT on sleep under normal conditions and, for the first time, during recovery from sleep deprivation. The effect of initiation of

EPT on sleep was studied in pre- and postmenopausal women under normal conditions, whereas the effects of long-term EPT were evaluated during recovery from sleep deprivation. Further, to understand the mechanisms behind the alterations in sleep, the 24-h serum profiles of GH, PRL and cortisol were measured in pre- and postmenopausal women before and after EPT. These profiles were then used to study the temporal relationships between sleep and GH as well as between sleep and PRL.

2. REVIEW OF THE LITERATURE

2.1. Sleep

Sleep state is characterized by reduced awareness and responsiveness, both to internal and external stimuli, as well as by motor inhibition. Although the length of sleep varies considerably between individuals, sleep occupies approximately one-third of each person's lifetime. Sleep is considered important for energy conservation, tissue recovery and cognitive functioning. The study of sleep and its disorders is advancing rapidly but the impact of sleep on health and medical conditions remain largely unrecognized or overlooked.

2.1.1. Sleep architecture

Sleep is conventionally examined with polygraphic sleep recording, PSG, which consists of an electroencephalogram (EEG), electro-oculogram (EOG) and electromyogram (EMG). Electrophysiologically, there are two main states: non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep is further subdivided into four sleep stages (stages, S, 1-4) determined by criteria which were originally standardized in the 1960's (Rechtschaffen & Kales 1968) (Figure 1). REM sleep can be divided into tonic and phasic parts. NREM and REM sleep alternate through the night in approximately 90-110-minute cycles (Carskadon & Dement 2005).

The four NREM stages run parallel to the depth of sleep, with arousal thresholds lowest in S1 and highest in S4. S1 sleep is regarded as a transition phase between wakefulness and sleep, in which EEG shows similarities to both sleeping (low reactivity) and wakefulness (relatively desynchronous EEG). S2 sleep is characterized by the appearance of sleep spindles and K complexes. K complexes are often produced by the same stimulus that produces awakening in S1 sleep. Normally, after falling asleep, a brief episode of S1 precedes S2. As S2 progresses, high-voltage (at least 75 μV) slow wave activity (SWA) gradually appears in the EEG. When SWA accounts from 20% up to 50% of the EEG activity in a 30-second analyzing window (epoch), it is determined as S3, which is transitional to S4 at which stage more than 50 % of high-voltage activity occurs. S3 and S4 are considered to be deep sleep and they are usually combined and referred to as SWS. Spectral analyses can be used to determine slow waves and monitor the SWA. SWS is usually followed by a short period of S2 preceding the first REM episode. REM sleep is characterized by desynchronized EEG frequencies, loss of EMG activity and presence of rapid eye movements (Shneerson 2000, Carskadon & Dement 2005).

As sleep progresses, the REM episodes become prolonged, while NREM sleep is shorter and lighter. Sleep is interrupted by physiological brief awakenings, especially later in the night and near REM sleep transitions. They are typically too fleeting to be remembered in the morning. Furthermore, short EEG arousals occur throughout the night. In a normal young adult S1 generally comprises about 2–5%, S2 45–50%, SWS 13–23% and REM, occurring in four to six discrete episodes, 20–25% of the total sleep

time. Sleep architecture is, however, strongly affected by age (Carskadon & Dement 2005). Subjective sleep quality seems to be associated with depth of sleep (i.e. SWS) and continuity (i.e. total sleep time and amount of wake time) (Keklund & Akerstedt 1997). The described golden standard (Rechtschaffen & Kales 1968) for sleep staging has been criticized for applying poorly to elderly subjects, especially the voltage criteria for SWS, and for the division of sleep into 30-second epochs that do not flexibly reflect the physiology of sleep (Himanen & Hasan 2000).

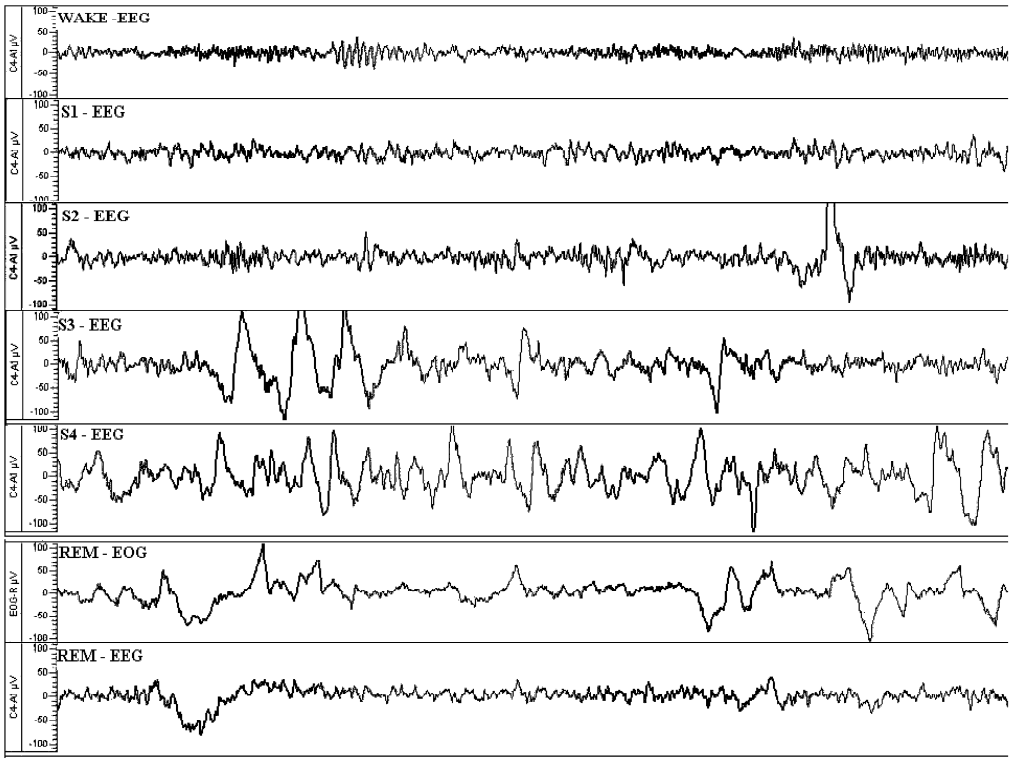

Figure 1. Wake and sleep stages (S1-4 and REM).

2.1.2. Regulation of sleep

Sleep is regulated by two different processes: homeostatic process S and circadian process C (Borbely 1982). In the two-process model, process S rises during the wake state causing increased sleep pressure and declines during sleep. Process S interacts with process C, which is independent of sleep and controls the appropriate timing of sleep. Homeostatic drive for sleep is usually greatest in the first half of the sleep period. The circadian drive becomes greatest in the latter half of the sleep period, thus maintaining sleep as the homeostatic drive declines. NREM sleep, particularly SWA, which is considered the marker of the process S, is determined mainly by homeostatic markers, whereas REM sleep is dependant on both homeostatic and circadian factors. The threshold for entering REM sleep is also under the control of an ultradian rhythm

with an approximately 90-min cycle through both wakefulness and sleep. In wakefulness this may be manifested only by a transient feeling of drowsiness. The propensity for REM sleep is usually greatest just after the core body temperature nadir (Borbely & Achermann 1999, Czeisler et al. 2005).

The suprachiasmatic nuclei (SCN) in the supraoptic region of the anterior hypothalamus (Figure 2) contain cells responsible for the endogenous circadian rhythmicity. The circadian period is slightly greater than 24 hours. Environmental light-dark schedules are the primary synchronizer of this circadian pacemaker, but other stimuli, such as exercise, can shift circadian phase. The circadian pacemaker drives the prominent daily fluctuations in the core body temperature and in the secretion of many hormones, such as cortisol and melatonin. It also interacts with sleep-wake regulatory processes. All elements of the circadian system can be influenced by environmental, social, behavioral, genetic, pharmacological, and age factors (Shneerson 2000, Czeisler et al. 2005).

The most important brain areas involved in sleep regulation include the medulla oblongata, pons, formation reticularis, midbrain, thalamus, hypothalamus, preoptic area, basal forebrain, hippocampus and cerebral cortex (Figure 2). During wakefulness, there is increased sympathetic tone and decreased parasympathetic tone that maintain most organ systems in a state of action or readiness. Wakefulness state is maintained by neurons in the brainstem reticular formation, which in turn excite neurons in the nonspecific thalamocortical projection system, in the posterior hypothalamus and in the basal forebrain. The thalamocortical, hypothalamocortical and basalocortical projections serve in turn to activate the cerebral cortex in a long-lasting and widespread manner stimulating a characteristic pattern of fast desynchronized activity on the EEG, manifesting wakefulness. The ascending reticular activating system provides cholinergic, noradrenergic, and glutaminergic stimulation to the thalamus, hypothalamus and basal forebrain resulting in cholinergic and glutaminergic excitation of the cortex (Jones 2005). In addition, dopamine, histamine, orexin, substance P, vasoactive intestinal peptide, neurotensin, corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) as well as adrenalin and somatostatin are involved in promoting wakefulness (Jones 2005, Miller & O'Callaghan 2006, Steiger 2007). Serotonin acts on multiple receptors and is shown to have a role in both wakefulness and sleep. However, the present consensus supports a stronger role in wakefulness (Miller & O'Callaghan 2006).

In sleep state, a shift from sympathetic to parasympathetic regulation occurs and activating systems are dampened. Parasympathetic control centers, comprised of neurons in the solitary tract nuclei and in the anterior hypothalamus and preoptic area, are important in this process. The noradrenergic projections of the solitary tract nuclei to midbrain and forebrain structures inhibit activity in the ascending reticular activating system, thus resulting in activation of inhibitory gamma-aminobutyric acid-ergic (GABAergic) thalamocortical projections to the cortex. This disfacilitation and hyperpolarization of thalamocortical systems consequently shifts the pattern of discharge from tonic and fast to bursting at a slow rate with intervening pulses reflected as spindles and SWA in the EEG (Jones 2005). In addition to GABA, there

are numerous other neurotransmitters and neuromodulators that are important for sleep state. The concentration of adenosine in the basal forebrain increases during wakefulness, promoting sleep, and falls during NREM sleep (Porkka-Heiskanen et al. 1997). The increase in adenosine concentration is dependant on the rise of nitric oxide concentration created with inducible nitric oxide synthase (Kalinchuk et al. 2006). Melatonin, which is secreted by the pineal gland under the control of SCN, is suppressed by light exposure (Lewy et al. 1980) and plays a role in seasonal timing (Rajaratnam et al. 2003). Growth hormone -releasing hormone (GHRH), ghrelin, galanin and neuropeptide Y (Steiger 2007) as well as interleukin 1 and tumor necrosis factor-alpha, are also shown to promote sleep (Krueger & Majde 2003).

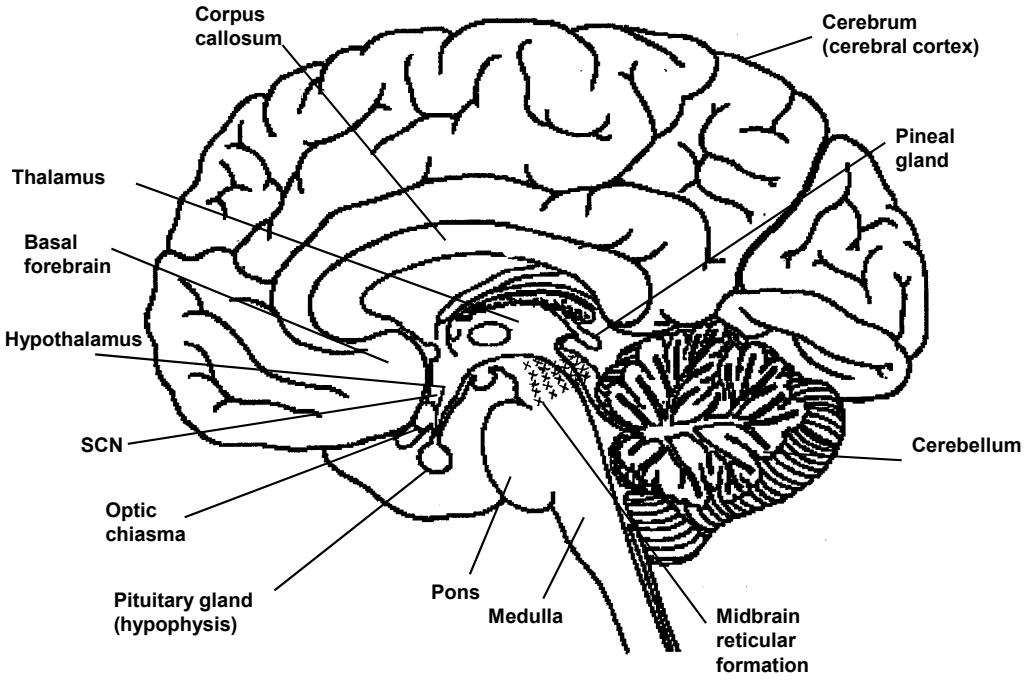

Figure 2. Neuro-anatomy of some of the sleep-related structures.

2.1.3. Sleep, gender and aging

Most of the sleep studies are conducted on men. Only during recent decades has the number of studies on women increased. In addition to the influence of the menstrual cycle and menopause on female sleep (see Chapter 2.2.1), there are some basic gender differences in human sleep. Women have more SWS, both in normal conditions (Manber & Armitage 1999) and after sleep deprivation (Reynolds et al. 1986). In addition, higher sleep spindle density (Gaillard & Blois 1981), more SWA (Dijk et al. 1989) and a slower age-related decline in SWS (Carskadon & Dement 2005) have been suggested. In addition to the decrease in SWS and SWA (Gaudreau et al. 2001a, Bliwise 2005), which is the most obvious sleep change observed across the whole maturational process, aging induces several changes in sleep characteristics and associated events. Older persons generally have shorter sleep and more awakenings

than younger persons (Bliwise 2005). As a consequence, sleep efficiency decreases to approximately 80% and the percentage of S1 increases. Latency to REM sleep also tends to shorten with increasing age. There is also an age-dependent impairment in phase shifting, making young people able to sleep over a wider range of circadian phases than older persons. The circadian phase of older subjects is also set to an earlier hour, making them more morning types. They also display a lower amplitude of body temperature and melatonin rhythms (Bliwise 2005, Czeisler et al. 2005).

Sleep problems, particularly insomnia, are a common health complaint. In the general population in Western countries, the prevalence of reported insomnia of any degree is estimated to be 29–37% (Ancoli-Israel & Roth 1999, Leger et al. 2000, Ohayon & Partinen 2002, Morphy et al. 2007, Kronholm et al. 2008). There seems to be an age-related increase in sleep complaints (Leger et al. 2000, Zhang & Wing 2006), although not all studies support this conclusion (Morphy et al. 2007). There is also evidence of a female predisposition to insomnia with even more significance in the elderly (Zhang & Wing 2006). Leger et al. (2000) reported that in the age group of 18 to 24 years while 17.4% of women suffered from insomnia, only 7.4% of men did so. In the age group of over 65 years, the corresponding figures were 25.1% and 15.5%. However, these differences in sleep complaints are not totally supported by objective sleep studies (Manber & Armitage 1999, Young et al. 2003). The main factor for this inconsistency is presumably the misperception of sleep. There seem to be no gender differences in sleep misperception in young subjects (Baker et al. 1999b). However, the discrepancy between subjective and objective markers of sleep is suspected to be greater in older women than in older men and probable existence of different objective criteria for good sleep in older women has been suggested (Vitiello et al. 2004).

2.1.4. Sleep deprivation

Although people sleep on average 7 to 8.5 h per day, there are considerable individual differences in the length of sleep (Carskadon & Dement 2005, Kronholm et al. 2006). In the Finnish population, self-reported sleep duration has decreased 5.5 min per each 10 years during the last 33 years (Kronholm et al. 2008). The proportion of people sleeping 7 h has increased, but the numbers at the extreme ends of sleep duration distribution have remained unchanged. The 24-h society sets great demands on the normal sleep-wake cycle. In addition to creating sleep loss, night or shift work increases the prevalence of short sleepers in the population (Kronholm et al. 2006). Although people sleep for approximately one third of their life, the functions of sleep are only partly understood. However, a lack of sleep has been proven to be harmful.

The importance of sleep can be investigated with sleep deprivation protocols. In acute sleep deprivation, the subjects are kept awake beyond their habitual bedtime for varying time periods, generally from 24 to 72 h. In chronic sleep deprivation, the duration of sleep is restricted during several consecutive nights. In addition, acute and chronic sleep deprivation can be either total or selective. In total sleep deprivation, sleep as a whole is prevented, whereas in selective deprivation only a certain stage, most often REM sleep, is prevented. Both acute and chronic total sleep deprivation

occur in the normal life of many people. Sleep is considered important for bodily restitution, cognitive functioning and mental health. Sleep loss increases sleepiness and impairs cognitive functioning (Alhola et al. 2005, Boonstra et al. 2007), which in turn can have dramatic consequences, such as accidents (Barger et al. 2005). Sleep deprivation also predisposes people to psychological and physiological health problems. Sleep deprivation is shown to impair mood (Reynolds et al. 1986), decrease glucose tolerance, increase cortisol concentrations and the activity of the sympathetic nervous system (Spiegel et al. 1999) as well as cause a deterioration in immune responses (Redwine et al. 2000).

Sleep deprivation increases sleep propensity as a consequence of the homeostatic regulation of sleep (see Chapter 2.1.2). One important homeostatic factor increasing sleep pressure after prolonged wakefulness is adenosine, the concentration of which increases during wakefulness and decreases while asleep (Porkka-Heiskanen et al. 1997). The recovery sleep after total acute sleep deprivation is characterized by increased sleep time, SWS and SWA, as well as decreased sleep latency, latency to SWS, amount of wakefulness and S1 (Brendel et al. 1990, Gaudreau et al. 2001b, Bonnet 2005). In older subjects, these changes are mainly similar, although not as pronounced as in young subjects. Older healthy subjects usually have a decreased REM latency, whereas an increase is common in young adults (Reynolds et al. 1986, Bonnet 2005). However, this decrease in the REM latency of older normal subjects has not been found in older depressed or demented individuals (Reynolds et al. 1987). Studies using several recovery nights have documented that by the second recovery night sleep architecture approaches baseline, apart from an increase in the REM sleep of young adults (Carskadon & Dement 1985, Reynolds et al. 1987, Brendel et al. 1990, Bonnet 2005). REM rebound effects appear to be related to the amount of lost SWS. Thus the pressure for SWS may be lower in older subjects, whereas the propensity to SWS in the young adults may be so marked that it replaces REM sleep during the first recovery night (Bonnet 2005).

2.1.5. Sleep and cognition

Good sleep is often considered one of the basic sources of better cognitive performance but findings from the effect of sleep architecture on cognitive performance are not unanimous. Some authors associate better attention with a higher representation of S2 sleep (Walker et al. 2002a), whereas others regard SWS (Takashima et al. 2006) and REM (Walker et al. 2002b) sleep as important. There is also evidence that sleep organization, the interplay of sleep stages within an ultradian cycle, may be crucial for the memory in young (Ficca et al. 2000) as well as in older persons (Mazzoni et al. 1999). The discrepancy in study outcomes results partly from the fact that cognition is not a single issue, but refers to a variety of functions.

Concentration, attention and reaction speed are some of the cognitive performances shown to deteriorate as a function of decreased sleep quality, sleep loss or increased age (Wilkinson & Allison 1989, Lezak 1995, Smith et al. 2002, Fontani et al. 2004, Alhola et al. 2005). Suggestions about menopause creating acceleration in the deterioration of cognitive functioning have also been made (Halbreich et al. 1995).

Studies using menopausal hormone therapy (HT) have reported that HT postpones the aging-related decline in concentration ability and visuomotor function (Lokkegaard et al. 2002) as well as improving vigilance and speed of information processing (Smith et al. 2001, Saletu et al. 2002). However, not all studies have been able to repeat these findings (Polo-Kantola et al. 1998, Portin et al. 1999, Alhola et al. 2006). In addition, during acute sleep deprivation, HT gives no benefit in performances in reaction speed or vigilance tests (Karakorpi et al. 2006).

2.2. Endocrine function and sleep

There are several diverse associations between human endocrine function and sleep. The predominant influences on endocrine rhythms are intrinsic circadian rhythmicity and the sleep-wake cycle, which interact to varying degrees to produce the characteristic 24-hour profile of each hormone. In healthy adults, reproducible changes of essentially all hormonal and metabolic variables occur during sleep and around wake-sleep and sleep-wake transitions. In addition, the effect of hormones is dependent on several variables, such as binding proteins as well as receptors in the target organs. In this chapter, associations between endocrine function and sleep are reviewed briefly and only in the context of the present study.

2.2.1. Reproductive endocrinology and sleep in women

Female reproductive hormones, most importantly endogenous estrogen and progesterone, not only exert actions on female reproductive organs and maintain fertility, but also have various implications in the central nervous system (CNS), through which they also influence sleep and circadian rhythms. Estrogen has numerous effects on the brain. The actions in the CNS include modulation of gene regulation and neuronal excitability. Estrogen has an effect on several neurotransmitters, including acetylcholine, serotonin, dopamine, noradrenaline and GABA (Manber & Armitage 1999, McEwen & Alves 1999, Dzaja et al. 2005). The studies concerning estrogen actions on the CNS are mostly animal studies: in humans the effects have been investigated indirectly, usually evaluating the impact of menopausal hormone therapy.

Progesterone acts as a sedative according to both animal and human studies. It is shown to induce benzodiazepine-like agonistic modulation of the $GABA_A$ receptor (Lancel et al. 1996). The sleep EEG changes, such as decrease of wakefulness, appear to be mediated via the conversion of progesterone into its major metabolite allopregnanolone (Lancel et al. 1997, Steiger et al. 1998, Manber & Armitage 1999). During waking hours, luteinizing hormone (LH) is secreted in a high frequency – low amplitude pulse pattern, but during sleep this secretion is changed to a low pulse frequency with a high amplitude (Rossmanith 1998).

2.2.1.1. Menstrual cycle

The menstrual cycle lasts on average 28 days and consists of follicular (before ovulation) and luteal (after ovulation) phases. Day 1 is identified as the first day of bleeding (menstruation) and ovulation occurs around day 14. Estrogen levels peak just

before ovulation, triggering a surge in LH secretion, which in turn induces ovulation leading to a rise in body temperature. Progesterone levels dominate in the luteal phase together with a second, but smaller, increase in estrogen levels. Despite the large changes in hormonal milieu, sleep is remarkably stable across the normal menstrual cycle. Most sleep changes occur together with core body temperature changes, which take place in the luteal phase (Armitage et al. 2005, Dzaja et al. 2005). Some studies have found no differences in sleep between mid-follicular and mid-luteal phases (Baker et al. 2001b), whereas others have found a shorter REM-latency (Lee et al. 1990), less REM sleep (Baker et al. 1999a) or increased sleep spindle frequency activity (Driver et al. 1996) during the luteal phase.

Oral contraceptives (OC) suppress endogenous reproductive hormones and therefore prevent ovulation and normal cyclicity resulting in a stable reproductive hormone status. As a result of OC use, body temperature is raised throughout the entire 24 h period in the active phase of the pills (normally 21 days) as well as during the interval without the pills (normally 7 days) (Baker et al. 2001a). OC use also seems to alter sleep: healthy women using OC have less SWS compared to the luteal phase of the naturally cycling women (Baker et al. 2001a, Baker et al. 2001b, Burdick et al. 2002). They also have less stage 2 sleep in the active phase compared to their interval phase of the contraceptive pack or to naturally cycling women (Baker et al. 2001a, Burdick et al. 2002). In addition, shorter sleep latency, shorter REM latency and more REM sleep has been observed in healthy women with OC use compared with naturally cycling women in a study, which was not controlled for menstrual phase (Burdick et al. 2002).

2.2.1.2. Menopause

Menopause is defined as the permanent cessation of menstruation, which can be declared only after 12 months of amenorrhea. The average age for natural menopause is 51 years (Luoto et al. 1994, Moe 2005). Since the life expectancy for Finnish women is 82.8 years (Suomen Tilastokeskus 2007), the women spend more than one-third of their lives in a postmenopausal state. At present, there are over one million women aged 50 or older in Finland (Suomen Tilastokeskus 2008). Menopause is preceded by perimenopause, beginning from the first signs of declining ovarian function. Postmenopause starts when menopause is diagnosed (World Health Organization Scientific Group 1996). The climacterium comprises both the perimenopause and that part of the postmenopausal period during which climacteric symptoms occur. In literature, the term premenopause often encompasses the time period just before menopause and is thus practically a synonym for perimenopause. However, it can also be regarded as covering all the fertile time before menopause. Perimenopause can be further divided into early and late transitions based on menstrual cycle length changes. Early transition begins when cycle predictability decreases according to the definition of the Study of Women Health Across the Nation (SWAN), or when there is at least one cycle length change (i.e. more than seven days) according to the definition of the Stages of Reproductive Aging Workshop (STRAW). Late transition is defined as 3-11 (SWAN) or 2-11 (STRAW) months of amenorrhea. The most recent classification system is PENN-5 in which late premenopause is characterized as one cycle length

change (i.e. more than seven days) early transition as two or more cycle length changes and late transition as 3-11 months of amenorrhea (Gracia et al. 2005).

The endocrine changes during climacterium occur gradually. The first signs of the approaching menopause are the decline of the number of oocytes and ovulatory cycles, leading to disruption of the regular pattern of the menstrual cycle. The production of follicle stimulating hormone (FSH) and LH increase while the secretion of estrogen and progesterone decrease. However, during the menopausal transition these hormone levels show a great degree of variability with abrupt changes from typical postmenopausal pattern to those characteristic of the reproductive age group (Burger 1996). However, menstrual flow changes alone are rarely combined with hormonal changes (Burger et al. 1995). The depletion of functional ovarian follicles results in a decrease in estradiol and inhibin production. As these hormones form a part of a close-loop feedback system for FSH production, their reduction is followed by a rise in FSH levels. After menopause, FSH levels are about 10-15 times higher (> 30 IU/L) compared to the levels in young women during follicular phase levels (Burger 1996). While estradiol levels decrease even under detectable limits, the main estrogen in postmenopausal women is estrone, derived predominantly from the peripheral aromatization of adrenal androstenedione in muscle and adipose tissue. This explains the strong positive correlation between BMI and estrone levels (Shifren & Schiff 2000). With increasing age, adrenal and ovarian androgen production also decline. This results in reduced testosterone levels. The majority of circulating sex hormones is bound to serum proteins, chiefly to sex hormone-binding globulin (SHBG), when the unbound fraction is mainly biologically active. Androgens decrease and estrogens increase SHBG levels, but menopause induces only a slight decrease in SHBG levels (Burger 1996, Shifren & Schiff 2000)

The deficiency in ovarian function and concomitant decline in estrogen levels brings about various climacteric symptoms, both somatic and mental, which impair the quality of life. In addition, risks for certain diseases, such as osteoporosis, cardiovascular diseases and Alzheimer's disease, increase after menopause (Vitiello et al. 2007). The duration of climacteric symptoms vary and in some women may persist even for decades (Barnabei et al. 2002). Hallmark symptoms during the climacterium comprise hot flashes and sweating – globally termed as 'vasomotor symptoms'. Other common symptoms include palpitation, headache, mood changes, vaginal dryness, sexual dysfunction and sleep disturbances (Erkkola et al. 1991, World Health Organization Scientific Group 1996, Nelson 2008).

Postmenopausal and perimenopausal women suffer more often from disturbed sleep than premenopausal women (Kuh et al. 1997, Leger et al. 2000, Kravitz et al. 2003, Young et al. 2003, Parry et al. 2006). Only in a few studies have the subjective sleep ratings been independent of menopausal state (Shaver & Paulsen 1993, Owens & Matthews 1998, Sharkey et al. 2003). The subjective sleep disturbances in menopause have often been attributed to nocturnal vasomotor symptoms (Shaver et al. 1991, Shaver & Paulsen 1993, Polo-Kantola et al. 1999b, Kravitz et al. 2003, Nelson 2008), higher levels of anxiety and depression (Shaver et al. 1991, Shaver & Paulsen 1993, Owens & Matthews 1998), as well as related to sleep disorders (Freedman & Roehrs

2007). However, the findings on the association between objective measures of sleep and hot flashes are conflicting (Erlik et al. 1981, Freedman & Roehrs 2004). A more recent study has found that in the second half of the night, REM sleep and the concomitant suppression of thermoregulation inhibits hot flashes and associated arousals and awakenings (Freedman & Roehrs 2006). Despite the well-documented deterioration of subjective sleep quality after menopause, the few sleep studies with PSG measures have failed to demonstrate consistent sleep changes behind the complaints. Studies comparing sleep architecture in pre- and postmenopausal women have shown either no difference (Shaver et al. 1988) or, contrary to initial predictions, better sleep, such as less S1 and S2 sleep and more SWS, after menopause (Sharkey et al. 2003, Young et al. 2003).

2.2.1.3. Prolactin

PRL is one of the growth factors that influence sleep. It is both a circulating hormone and a neural protein, having a precise localization within the brain (Roky et al. 1995). PRL secretion from the anterior pituitary is under the control of inhibitory factors released into hypothalamic pituitary portal vessels. The predominant inhibitory factor is dopamine. TRH (Tukel et al. 2000), vasoactive intestinal peptide (VIP) (Kato et al. 1984) and stress are physiologically significant stimulating factors for the release of PRL. In addition, estrogens stimulate PRL gene transcription (Tansey & Schlechte 2001). As a result, women have higher levels of PRL than men and premenopausal PRL levels are higher than postmenopausal levels (Fernandez et al. 1990, Katznelson et al. 1998). During the normal menstrual cycle, PRL levels are higher in the luteal phase than in the follicular phase (Fernandez et al. 1990). Nocturnal PRL levels seem to be higher in postmenopausal women than in men of the same age (Latta et al. 2005). Instead, 24 h mean levels may not differ between elderly men (aged 62-78 years) and women (aged 64-91 years) or between young (aged 19-32 years) and elderly men (Touitou et al. 1981). The primary role of PRL is to prepare breast tissue for lactation, resulting in increasing PRL levels during pregnancy (Tansey & Schlechte 2001).

Under normal conditions PRL concentrations are minimal around noon, rise soon after sleep onset and maximal values are observed in the early morning hours (Sassin et al. 1972, Gronfier & Brandenberger 1998, Van Cauter 2005) (Figure 3). Stimulation of PRL secretion by VIP prolongs NREM and REM sleep periods and decelerates NREM-REM sleep cycles (Roky et al. 1995, Murck et al. 1996). PRL secretion appears to be low at the time of REM sleep onset (Parker et al. 1974, Follenius et al. 1988) and morning awakening coincides with an immediate offset of active secretion (Spiegel et al. 1994). However, some earlier studies argue against the temporal relationship between REM-NREM-cyclicity and plasma PRL (Higuchi et al. 1979, Van Cauter et al. 1982). PRL seems also to be involved in SWS regulation since PRL secretion during sleep is coupled to delta waves, at least in young healthy men (Spiegel et al. 1995). In addition, SWS is shown to increase in patients, both sexes, with hyperprolactinemia (Frieboes et al. 1998) and in lactating women who have higher PRL levels compared with women who bottle feed their infants (Blyton et al. 2002).

Although sleep deprivation results in decreased PRL and daytime naps are associated with a rise in circulating PRL (Baumgartner et al. 1993, Van Cauter 2005) (Figure 3), there is also a sleep-independent circadian component of PRL secretion. The circadian PRL secretion is characterized as a progressive increase during the hours preceding the habitual bedtime, with a greater amplitude in women (Desir et al. 1982, Waldstreicher et al. 1996, Van Cauter 2005). Melatonin is shown to influence prolactin secretion independent of sleep-related factors (Bispink et al. 1990, Okatani & Sagara 1993), but the main control for circadian PRL secretion is proposed to be driven by the SCN (Waldstreicher et al. 1996).

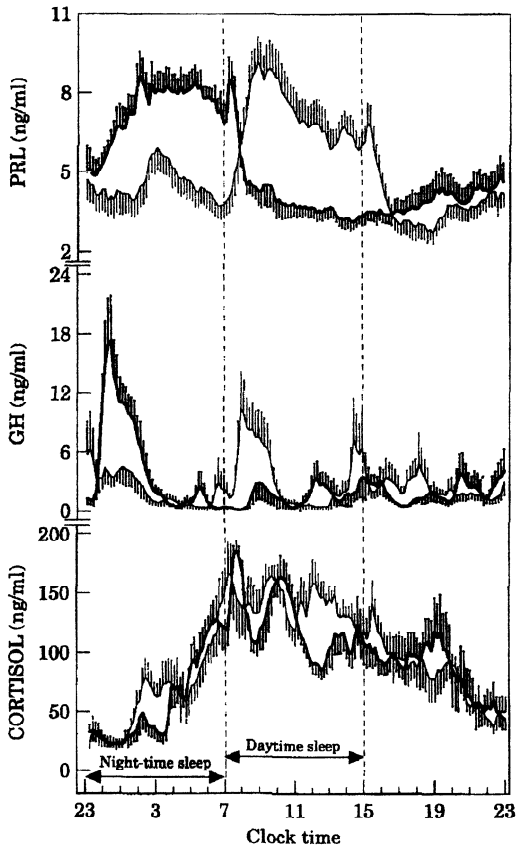

Figure 3. The mean (± standard error) 24-h profiles of plasma PRL, GH and cortisol during a normal 24-h sleep-wake cycle (darker lines), and during a 24-h cycle with an abrupt sleep shift consisting in an 8-h delay in the sleep period. (Reprinted from Gronfier & Brandenberger 1998, with the permission from Elsevier Limited.)

2.2.2. Growth hormone axis

In healthy adults, the 24-hour profile of GH levels consists of stable low levels which are abruptly interrupted by bursts of secretion. Sleep onset elicits a pulse in GH secretion whether sleep is advanced, delayed, or discontinued and reinitiated (Takahashi et al. 1968, Born et al. 1988, Gronfier & Brandenberger 1998, Van Cauter 2005) (Figure 3). Pituitary release of GH is stimulated by hypothalamic GHRH and gastrointestinal ghrelin and inhibited by hypothalamic somatostatin (Steiger 2007). In part, the stimulating effects of GH on tissue growth and protein anabolism are initiated by the hepatic production of insulin-like growth factor I (IGF-1). The increase in IGF-I in turn reduces GH secretion through negative feedback (Obal & Krueger 2004). GH and IGF-1 levels decrease with increasing age in both sexes (Rudman et al. 1981, Ho et

al. 1987, Ho & Hoffman 1993, Van Cauter et al. 2000). Women have higher levels of GH than men of the same age, but this difference disappears after menopause (Ho et al. 1987, Ho & Hoffman 1993, Latta et al. 2005). Diminished GH and especially diminished IGF-1 action in aging may account for some of the undesirable changes in body composition and function, such as decrease in protein synthesis and turnover of bone and increase in fat mass, since GH treatment has had beneficial effects in clinically healthy elderly individuals (Rudman et al. 1981). However, side effects, e.g. insulin resistance, edema and arthralgia, limit the usefulness of GH therapy (Ho & Hoffman 1993).

Sleep-onset GH secretion, coinciding with SWS, seems to be primarily regulated by GHRH stimulation which coincides with somatostatin withdrawal (Van Cauter et al. 1998, Steiger 2007). Repetitive hourly i.v. injections of GHRH, which mimic the pulsatile endogenous release, increase GH and NREM sleep, and decrease awakenings but only in men. In women, opposite sleep-impairing effects are found (Antonijevic et al. 2000a). However, after sleep deprivation, during recovery sleep, GHRH administration increases NREM sleep to the same extent in men and in women (Schussler et al. 2006). Administration of GH decreases NREM sleep due to negative feedback inhibition of GHRH. The sleep-onset GH pulse generally accounts for the majority of the 24-hour GH secretion in men, whereas in women daytime GH pulses are more frequent (Van Cauter et al. 1998, Antonijevic et al. 1999). GH release preceding sleep seems to increase sleep fragmentation and impair GH secretion after sleep onset in women but not in men (Latta et al. 2005). In men, the amount and timing of nocturnal GH secretion is associated with SWS (Golstein et al. 1983, Holl et al. 1991, Van Cauter et al. 1992, Van Cauter et al. 2000). This association is independent of age, whereas both GH secretion and SWS decline with increasing age (Kern et al. 1996, Van Cauter et al. 2000). Nocturnal GH secretion may, however, occur in the absence of SWS and not all SWS periods are associated with detectable GH secretion (Van Cauter et al. 1992). In women, the association between SWS and GH seems to be weaker than in men (Jarrett et al. 1990, Moe et al. 1998, Latta et al. 2005, Schuessler et al. 2005), but the temporal relationships between GH and SWS in pre- and postmenopausal women are largely unknown.

2.2.3. Corticotrophic axis

During the first half of the night when the GH surge is present, cortisol levels reach their nadir. During the second half, cortisol levels increase while GH release is low. The acrophase is reached in the early morning (Figure 3). The hypothalamic release of CRH stimulates the secretion of pituitary adrenocorticotrophic hormone (ACTH) which directly stimulates adrenal cortisol release. Cortisol in turn inhibits CRH release (Van Cauter 2005, Steiger 2007). In circulation, cortisol is predominantly bound to cortisol-binding globulin (CBG) (Levine et al. 2007). Cortisol is essential in response to acute stress, but chronic elevation of cortisol has been implicated in the pathogenesis of several psychiatric and somatic disorders, including depression, obesity, diabetes and cardiovascular disease (Rosmond & Bjorntorp 2000, Costa e Silva 2005).

The overall 24-h profile of cortisol concentrations is not markedly affected by the absence of sleep (Gronfier & Brandenberger 1998) (Figure 3). This circadian secretion pattern is preserved in both sexes as age increases (Ferrari et al. 2001), but mean cortisol levels tend to increase and the timing of the circadian elevation advance with aging (Van Cauter et al. 1996). Premenopausal women have lower cortisol levels than men of similar age, but this difference seems to disappear after menopause (Van Cauter et al. 1996, Deane et al. 2002). Postmenopausal women who are not using menopausal HT seem to have higher cortisol levels than premenopausal women, but the effect of age has not been excluded (Patacchioli et al. 2006). Changes in the GHRH:CRH ratio in favor of CRH appears to contribute to shallow sleep, elevated cortisol levels and decreased GH in aging and in depression. Administration of CRH in men results in a decrease in SWS (Vgontzas et al. 2001, Steiger 2007). The administration of cortisol, in turn, increases SWS (Friess et al. 2004) most likely due to negative feedback inhibition of endogenous CRH.

2.3. Hormone therapy

Climacteric symptoms are effectively treated with HT. HT consists of unopposed estrogen (ET) or, for women with an intact uterus, estrogen combined with progestogen (EPT). There are a number of cyclic (unopposed estrogen followed by progestogen) and continuous (combined estrogen plus progestogen every day) EPT preparations, both oral and transdermal, on the market. Continuous combined preparations are usually prescribed only to women who are clearly postmenopausal. Oral estrogens are available as synthesized estrogens, which are precursor molecules designed to avoid rapid metabolism before reaching the target organ, or as natural estrogens, which are identical to the estradiol produced by the functioning ovary. Ethinyl estradiol, estradiol valerate, esterified estrogens and piperazine estrone sulfate are some of the synthesized estrogens and 17β-estradiol (E_2), estrone and estriol are natural estrogens. Conjugated equine estrogen (CEE) is a mixture of over 100 compounds, which are extracted from the urine of horses, but the principal steroid is estrone sulfate. Progestogens include natural progesterone and synthetic progestins, but only the latter is currently used in HT. Progestins can be derived from progesterone, 17α-hydroxyprogesterone, 19-nortestosterone or from spirolactone (Barlow & Wren 2005, Kuhl 2005). EPT containing norethisterone or norethisterone acetate (NETA) is the most commonly prescribed in Finland (National agency for medicines, Finland 2007).

Common side-effects of HT are bloating, edema, mastalgia, endometrial spotting/bleeding and headache. These may be managed by reducing the dosage, varying the delivery system or changing the delivery schedule or the compound, especially the progestogen component (Barlow & Wren 2005, Kuhl 2005). The most important risks associated with HT are stroke, venous thrombosis and breast cancer. The benefits from HT, other than alleviation of climacteric symptoms, include decline in bone turnover and decrease in the incidence of osteoporosis-related fractures regardless of age at initiation of the therapy. In addition, HT may reduce the risk of colon cancer and when initiated around the time of menopause it seems to be

associated with a reduced risk of cardiovascular morbidity and mortality as well as Alzheimer's disease (World Health Organization Scientific Group 1996, Writing group for the Women's health initiative 2002, Board of the International Menopause Society 2007, Vitiello et al. 2007). However, the main indication for HT is the alleviation of climacteric symptoms (Board of the International Menopause Society et al. 2007).

2.3.1. Hormone therapy and sleep

The higher rate of sleep complaints in postmenopausal women compared to premenopausal women is in part due to aging, but the linkage between the complaints and menopause is strongly supported by the specific treatment response to HT (Erkkola et al. 1991, Wiklund et al. 1993, Polo-Kantola et al. 1998, Sarti et al. 2005). This implies that sleep complaints are part of the climacteric symptom array and as such are an indication of HT (Board of the International Menopause Society et al. 2007). The well-defined improvement in subjective sleep quality is contrasted by the lack of consistent objective findings. All prospective studies conducted with ET have found at least some improvement in objective sleep quality (Thomson & Oswald 1977, Schiff et al. 1979, Erlik et al. 1981, Scharf et al. 1997, Keefe et al. 1999, Polo-Kantola et al. 1999a, Antonijevic et al. 2000b, Saletu-Zyhlarz et al. 2003, Parry et al. 2004). However, three of the six prospective studies with EPT have not been able to find improvements in objective sleep markers (Pickett et al. 1989, Purdie et al. 1995, Saletu-Zyhlarz et al. 2003). In the majority of the studies with positive findings during either ET or EPT, the improvement in objective sleep quality has been observed as increased REM sleep (Thomson & Oswald 1977, Schiff et al. 1979, Keefe et al. 1999, Antonijevic et al. 2000b) or a decrease in awakenings or wake time (Thomson & Oswald 1977, Erlik et al. 1981, Scharf et al. 1997, Keefe et al. 1999, Antonijevic et al. 2000b, Montplaisir et al. 2001, Parry et al. 2004). Other improvements have been shortened sleep latency (Schiff et al. 1979, Saletu-Zyhlarz et al. 2003), increased sleep efficiency (Scharf et al. 1997, Montplaisir et al. 2001, Parry et al. 2004), reduced S1 sleep (Parry et al. 2004) or a reduction in movement arousals (Polo-Kantola et al. 1999a). (Appendix 1)

The lack of consistency between the results of these previous studies has been attributed to the great differences in study protocols based on hormone regimens, the length of the treatment, age or symptomatology of the subjects or the type of menopause (natural of surgical). The majority of improvements in objective sleep gained by HT administration have accompanied a reduction in hot flashes (Thomson & Oswald 1977, Schiff et al. 1979, Erlik et al. 1981, Scharf et al. 1997, Montplaisir et al. 2001) but not all studies have observed the same (Keefe et al. 1999, Antonijevic et al. 2000b). In addition to the prospective studies, there is one large observational epidemiology study on postmenopausal women with various HT regimens, the types of which were not reported (Young et al. 2003). This study is important since it observed decreased SWS, increased S1 sleep and sleep fragmentation in HT-users compared with postmenopausal women without HT. In another cross-sectional study, postmenopausal ET-users had longer sleep latency than women not on ET under baseline conditions, but the impact of stress created with nocturnal blood sampling was

lesser in ET-users (Moe et al. 2001). Middle-aged women may also experience climacteric symptoms, such as vasomotor symptoms, although they are premenopausal in terms of FSH levels and menstrual cyclicity. Their symptoms are often successfully treated with HT, but no previous study has investigated the effects of HT on the sleep quality in these women.

2.3.2. Hormone therapy and prolactin

Although few previous studies have focused on the effects of HT on PRL levels, many studies have reported PRL concentrations as additional findings or when describing the overall hormonal status of the study subjects. A majority, but not all (Lind et al. 1978), of the prospective studies have found a rise in serum PRL concentrations during oral ET in postmenopausal women (Yen et al. 1974, Robyn et al. 1978, Schiff et al. 1980, Chang et al. 1982, Shah et al. 1999, Parry et al. 2004). Despite the increased PRL levels, oral ET has been shown to blunt the nocturnal PRL changes (Schiff et al. 1980) although the sleep/wake ratio seems to be preserved (Chang et al. 1982). In contrast, no studies with transdermal ET have been able to find any change in postmenopausal PRL levels (Cagnacci et al. 1991, Perrone et al. 1994, Bednarek-Tupikowska et al. 2006). Not even the combination of transdermal ET with either transdermal or oral progestogen seems to be effective in increasing PRL concentrations (Cagnacci et al. 1991, Perrone et al. 1994, Castelo-Branco et al. 1995), apart from one exception (Bednarek-Tupikowska et al. 2006). Oral EPT, however, does seem to increase PRL levels rather consistently, similarly to oral ET (Chang et al. 1982, Metka et al. 1994, Schlegel et al. 1999). However, there are prospective (Lind et al. 1978, Helgason et al. 1982, Castelo-Branco et al. 1995) and cross-sectional (Dawood et al. 1986) studies in which increased PRL concentrations have not been observed. In menstruating young women (aged 18–33 years), a very high dose of estrogen is shown to increase PRL (Vekemans & Robyn 1975). The correlations between nocturnal PRL levels and sleep stages in postmenopausal women have been tested in one study, but no association has been found during ET or placebo (Schiff et al. 1980). (Appendix 2)

2.3.3. Hormone therapy and growth hormone

Previous trials have not brought a consensus on the effects of HT on postmenopausal GH levels. Studies administering unopposed oral ET have found a rise in GH levels (Dawson-Hughes et al. 1986, Fröhlander & von Schoultz 1988, Kelly et al. 1993, O'Sullivan & Ho 1995, Bellantoni et al. 1996, Friend et al. 1996, Shah et al. 1999, Bray et al. 2001), whereas in the majority of studies with transdermal ET no effect on GH levels has been found (O'Sullivan & Ho 1995, Bellantoni et al. 1996, Lieman et al. 2001). However, some studies have exceptionally found increased GH secretion also during transdermal treatment (Friend et al. 1996, Genazzani et al. 1997). Oral EPT has been shown either to increase GH levels (Weissberger et al. 1991, Hartmann et al. 1995, Fonseca et al. 1999) or to have no effect on GH levels (Cano et al. 1999, Villa et al. 2008). Again, with transdermal EPT, no change in GH has been found (Bellantoni et al. 1991, Weissberger et al. 1991, Cano et al. 1999). Transdermally delivered estrogen bypasses the first-line hepatic route and thus does not expose the liver to high first-pass estrogen concentrations. This presumably accounts for the lack of change in

IGF-1 (Bellantoni et al. 1996) and GH concentrations (Weissberger et al. 1991) observed in most studies with transdermal administration. (Appendix 3)

Most previous studies on the effects of oral EPT on GH have included various types of estrogen and progestin, which has probably contributed to the conflicting results. Particularly the progestin component may have importance since combining oral estrogen with androgenic testosterone-derived progestin seems to counteract the increase in SHBG and the decrease in IGF-1 synthesis induced by oral estrogens in the liver (Campagnoli et al. 1994, Campagnoli et al. 2003, Nugent et al. 2003). No previous study has evaluated the effect of HT on GH in middle-aged premenopausal women. In addition, there are no previous prospective trials in which the effect of HT on both GH levels and sleep have been evaluated in middle-aged premenopausal or in postmenopausal women. In a cross-sectional study on postmenopausal women with or without long-term ET, the ET-users had higher GH levels, but no difference in objective sleep variables was found between the groups (Moe et al. 1998).

2.3.4. Hormone therapy and cortisol

The study results on the effects of HT on cortisol are conflicting. With oral ET, either an increase (Gudmundsson et al. 1999) or no change (Schiff et al. 1980, Cagnacci et al. 1997, Slayden et al. 1998, Komesaroff et al. 1999, Parry et al. 2004) in cortisol levels has been observed, whereas transdermal ET has decreased (Bernardi et al. 2003, Pluchino et al. 2005) or had no effect (Cucinelli et al. 2002) on cortisol levels. During oral or transdermal EPT, cortisol levels seem to decrease whenever the progestin component is other than NETA (Bernardi et al. 2003, Pluchino et al. 2005). However, there are also EPT studies which argue this finding by showing either no change (Parry et al. 2004, Pripp et al. 2004) or even an increase (Fonseca et al. 2001, Shifren et al. 2007) in cortisol levels during the use of partly the same compounds that have resulted in declined cortisol levels in other studies. The remarkable inconsistency between the studies is probably accounted for by great differences in the study protocols, such as in cortisol sampling (e.g. single blood sample, 24-h collections, nocturnal samples) or duration of the treatment. In a prospective study, in which no change in cortisol levels was seen during ET, no correlation between nocturnal cortisol levels and sleep stages was found either (Schiff et al. 1980). Urinary free cortisol levels have been shown to negatively correlate with REM sleep in postmenopausal women not using HT but not in those using ET (Prinz et al. 2001). Further, during nocturnal blood sampling, urinary free cortisol has been found in negative association with sleep efficiency, SWS, S2 sleep and REM sleep, but again only in postmenopausal women who are not using HT (Prinz et al. 2001). (Appendix 4)

2. AIMS OF THE STUDY

The aim of the study was to investigate the sleep of aging women in the normal state, after sleep deprivation and during HT. The following questions were to be answered:

I Does menopause have an impact on sleep quality? Are the possible effects reflected in attention, sleepiness or mood?

II What are the differences in recovery sleep after sleep deprivation between postmenopausal and young women? Is long-term HT beneficial for recovery sleep?

III Does EPT have an effect on sleep quality in premenopausal or postmenopausal women?

IV Does menopause modify 24-h levels of GH, PRL and cortisol? Does EPT have an effect on these levels in premenopausal or postmenopausal women?

V Is there a temporal association between GH and SWS or between PRL and sleep stages in premenopausal or postmenopausal women? If the associations differ across menopause, does HT have any impact?

4. SUBJECTS AND METHODS

4.1. Subjects

In total, 68 women took part in the five studies (Table 1). The pre- and postmenopausal women were recruited through announcements in local newspapers (Figure 4). Over 400 women called and were first informed about the study protocol and then screened for exclusion criteria in a 10-30 minute telephone interview (P.P-K. and N.K.). Altogether, 150 of them visited the sleep laboratory for a more detailed interview (1-1.5 h) where the exclusion criteria were ensured (Table 2), the study protocol was explained and a physical examination was performed. In addition, a gynecological examination including transvaginal ultrasound was performed on all subjects (P.P-K). Premenopausal status was defined by the serum follicle stimulating hormone (FSH) level (< 23 IU/mL) and ongoing menstrual cycle. Postmenopause was determined by age and amenorrhea. In all studies, the term premenopause referred to the few years before menopause and younger women were referred to separately. The studies were conducted in the Sleep Research Unit of the University of Turku, Finland, and in the department of Obstetrics and Gynecology of Turku University Central Hospital, Finland. All subjects signed an informed consent form after oral and written information. The study protocols had the approval of the Ethical Committees of Turku University Central Hospital (for Studies I-V) and the University of Helsinki (for Studies I and II).

Table 1. Subject characteristics, expressed as group means (standard deviation), for the study subjects.

		N	Age (years)	BMI (kg/m2)	S-FSH (IU/L)	E2 (pmol/L)	EQ-5D	BNSQ insomnia	Vasomotor symptoms
Study I, II	Young	11	23.1 (1.6)	23.1 (3.1)	3.8 (2.8)	95.5 (83.6)	7.4 (0.8)	10.4 (2.3)	2.5 (0.8)
Study I	Premenopausal	20	47.7 (2.3)	23.9 (2.4)	10.6 (4.8)	327.2 (330.2)	7.1 (0.6)	13.9 (3.8)	2.9 (0.9)
Study III, IV, V	EPT	8	48.1 (1.2)	24.1 (2.3)	12.3 (5.4)	442.9 (422.9)	7.0 (0.6)	12.8 (4.2)	2.4 (0.5)
	placebo	8	47.5 (2.2)	24.3 (2.5)	9.9 (3.9)	159.0 (102.1)	7.1 (0.7)	14.1 (2.7)	3.0 (1.0)
Study I	Postmenopausal	28	63.3 (3.6)	27.2 (4.2)	75.2 (29.1)	31.5 (10.5)	7.4 (0.8)	16.2 (3.6)	4.0 (2.3)
Study II	HT-users	9	64.1 (4.7)	24.2 (1.9)	6.0 (7.0)	0.2 (0.1)	7.3 (0.7)	12.8 (4.3)	
	non-HT	10	64.6 (4.6)	26.1 (1.7)	77.1 (19.4)	0.03 (0.005)	7.3 (0.7)	16.1 (2.6)	
Study III, IV, V	EPT	9	62.7 (3.7)	30.2 (5.0)	69.9 (41.0)	31.2 (13.9)	7.4 (1.1)	15.1 (5.3)	5.0 (2.8)
	placebo	9	63.0 (1.9)	25.0 (3.3)	78.9 (29.4)	29.9 (15.0)	7.6 (0.5)	17.5 (1.9)	3.6 (2.3)

BMI = body mass index, FSH = follicle stimulating hormone, E_2 = oestradiol, EQ-5D = EuroQol quality of life questionnaire, BNSQ = Basic Nordic Sleep Questionnaire.

The young women (in Studies I and II) were recruited from Helsinki via announcements at the university and studied in the Sleep Research Unit of Institute of Biomedicine, University of Helsinki, Finland. The Turku Sleep Research Unit served as the supervising and monitoring unit of the study. The strict timetable of procedures was carefully followed and monitored through event-by-event report at both sleep centers. The menstruating women (premenopausal and young women) were studied during the first days of their cycle. In all studies, the subjects kept a sleep diary for

three weeks before and one week after the sleep studies to ensure a regular sleep-wake schedule (10-11 p.m. to 6-7 a.m.). During the sleep studies and one week before, all caffeine intake, the use of alcohol and traveling abroad were prohibited. Coffee drinkers were provided with caffeine-free coffee to consume during that period. The composition of food provided for the subjects was similar during the sleep studies. When allowed to sleep at the sleep laboratory, the subjects spent the time between 11 p.m. and 7 a.m. in bed in a dark room, where only red light was used when needed.


Wanted
45 – 50-YEAR-OLD or OVER 60-YEAR-OLD WOMEN
to take part in a **SLEEP STUDY**.

Requirements for participation:
- no current use of hormone therapy
- no current use of sleep medication

Please, contact
Päivi Polo-Kantola tel: 878 698 or
Nea Kalleinen tel: 787 896

Figure 4. The newspaper announcement for recruitment of the study population.

4.1.1. Study I

Study I was a cross-sectional trial with three study groups: young, premenopausal and postmenopausal women. It examined sleep characteristics in these populations and associations between sleep, menopause, mood and cognitive performance. Eleven young (20-26 years), 21 premenopausal (45-51 years) and 29 postmenopausal (59-71 years) women volunteered. The subjects spent two consecutive nights at the sleep laboratory: adaptation and study nights. Sleep studies were performed from 11 p.m. to 7 a.m. and cognitive tests were carried out in the mornings. Blood samples for serum FSH and E_2 measurement were taken in the morning following the adaptation night.

The pre- and postmenopausal women were not using HT, but the young women were taking OC (ethinyl estradiol 20µg + desogestrel 0.15mg**,** Mercilon: Organon, Oss, the Netherlands). In the premenopausal group, two women had previously used HT and the average time of use was 3.5 months (range 3–4 months). In the postmenopausal group, 21 women had previously used HT, with an average usage time of 68 months (range 2–156 months). The washout period for HT was at least 12 months in

Table 2. Exlusion criteria for all studies.

Ongoing malignancy
Cardiovascular disease (apart from treated hypertension)
Severe migraine
Other neurological disease
Significant loss of consciousness previously
Fibromyalgia
Mental disease
Previously diagnosed and treated nocturnal breathing disorder
Restless legs syndrome
Narcolepsia
Thyroid disease (4.5mU/L < TSH < 0.4mU/L)
Other endocrinological disease (apart from treated hyperlipidemia)
Anemia (Hb < 118 g/L)
B-leucocytes < 3.4 or > 9.0 E9/L
B-thrombocytes < 150 or > 400 E9/L
Current use of any medication with CNS effects
Shift work or irregular sleep-wake-rhythm
Smoking
Excessive consumption of caffeine (> 5 cups of coffee/day)
Abuse of alcohol
Use of narcotics
Positive drug urine screen

TSH = thyroid stimulating hormone, Hb = hemoglobin, CNS = central nervous system

all women, except in one premenopausal woman, in whom it was five months.

All women completed the study. One subject from the premenopausal group and one from the postmenopausal group were excluded because of missing data. In addition, spectral analysis could not be performed for one young subject. The characteristics of the study groups are summarized in Table 1. The three study groups differed as expected regarding age and FSH levels, but they were similar according to experienced sleepiness (Basic Nordic Sleep Questionnaire, BNSQ; Partinen & Gislason 1995, Appendix 5), quality of life and experienced state of health (EQ-5D, Ohinmaa et al. 1996) as well as vasomotor symptoms. Compared with postmenopausal women, the premenopausal and young women had a lower body mass index (BMI) and longer education. The premenopausal women had higher E_2 levels than postmenopausal or young women. In addition, the young women scored lower in insomnia (BNSQ) compared to the pre- or postmenopausal women. None of the women was clinically depressive (Beck Depression Inventory, BDI, Beck et al. 1961).

4.1.2. Study II

Study II was a prospective study of the effects of HT on recovery from acute total sleep deprivation. The duration of the sleep deprivation was 40 h. The subjects included 20 postmenopausal women (aged 58-72 years) and 11 young women (aged 20-26 years) who served as controls. The postmenopausal women were divided into groups according to their current use of HT: 10 HT users (oral, continuous combined, estradiol hemihydrate 2mg + norethisterone acetate 1mg, Kliogest: Novo Nordisk, Bagsværd, Denmark) and 10 women not on HT (non-HT). In the non-HT group, seven women had previously used HT with a minimum time of discontinuance of 24 months. The eleven young women were taking OC (ethinyl estradiol 20µg + desogestrel 0.15mg, Mercilon: Organon, Oss, the Netherlands).

The study design included four consecutive nights at the sleep laboratory: adaptation, baseline, sleep deprivation and recovery nights. A blood sample for serum FSH and E_2 measurements was drawn in the morning following the adaptation night to ensure the appropriate use of HT. The sleep deprivation period began at the conclusion of the baseline night (7 a.m.) and extended to the start of the fourth night (11 p.m.), thus lasting 40 hours. Ambulatory polysomnographic recordings were carried out from 8 p.m. on the third evening to 8 p.m. on the fourth evening. The subjects were constantly accompanied by the study staff at the sleep laboratory to ensure compliance. The subjects also carried out repeated cognitive tests during the course of the study (results published elsewhere, Alhola 2007). During the deprivation day, the subjects were allowed to go out for a short walk if they chose, while wearing the ambulatory device.

All women completed the entire study protocol. In the HT group, one subject was excluded because her sleep data could not be retrieved. Spectral analysis could not be performed for one young subject due to technical problems. Further, one woman from the non-HT group did not complete the morning questionnaire. The characteristics of the study groups are summarized in Table 1. The postmenopausal groups were

comparable in terms of age, BMI, EQ-5D and experienced insomnia and sleepiness (BNSQ). No clinically significant depression (BDI) was observed in any subject. HT users had lower FSH and higher E_2 serum levels than the women in the non-HT group.

4.1.3. Studies III-V

The study design in studies III-V was a randomized, placebo-controlled, double-blind, six-month follow-up trial. The effects of HT on sleep (Studies III and V) and on hormones involved in sleep regulation (Studies IV and V) were examined. In addition, interactions between sleep and GH and PRL were evaluated (Study V). The subjects consisted of 17 premenopausal women (aged 45-51 years) and 18 postmenopausal women (aged 58-70 years). No current use of HT was allowed and the washout period for previous use was at least 12 months. In the premenopausal group, one woman had used HT for three months. In the postmenopausal group, 13 women had used HT previously: the average time of use was 74 months (range 3-156) and the average washout period was 51 months (range 12-147).

The study design is illustrated in the flow chart (Figure 5). The duration of the study was 6 months. At baseline (Sleep Study 1), sleep studies were conducted on three consecutive nights, in which the first night served as an adaptation to the laboratory environment and the equipment. The second night served as the sleep study night for Study III and the third night, blood sampling night, as the study night for Studies IV and V. On the evening prior to the third night (Studies IV, V), at 6.30 p.m. an indwelling catheter was inserted into a forearm vein. The catheter was kept patent with a slow heparinized saline infusion. Beginning at 9 p.m., a 2 ml blood sample was remotely drawn every 20 min for the duration of 24 h. At night (11 p.m. – 7 a.m.), plastic tubing connected to the catheter ran through a soundproof lock into an adjacent room, allowing repeated blood sampling without disturbing the subject's sleep. To ensure successful sampling, the arm of the subject was loosely attached to the side of the bed. The last sample was drawn at 9 p.m. in the following evening. In the analyses, night-time referred to the time period from 11 p.m. to 7 a.m. and daytime to the time period from 7.20 a.m. to 9 p.m.

After the baseline studies, the subjects were randomized for the six-month treatment period in six-person blocks. Premenopausal women received cyclic EPT (2 mg estradiol valerate for 16 days and 2 mg estradiol valerate + 1 mg norethisterone for 12 days, Mericomb: Novartis, Basel Switzerland) or placebo, delivery beginning during day one of their menstrual cycle (Studies III and IV). The postmenopausal women received continuous EPT (2 mg estradiol valerate + 0.7 mg norethisterone, Merigest: Novartis, Basel, Switzerland) or placebo (Studies III-V). Randomization was performed at the pharmacy of the Turku University Central Hospital, where randomization codes were kept until the data analyses were completed. After three months of treatment, the subjects were interviewed to ensure compliance and a blood sample was drawn to control for E_2 and FSH. At the end of the 6-month treatment period, the sleep study (Studies III, V) and blood sampling (Studies IV, V) protocols

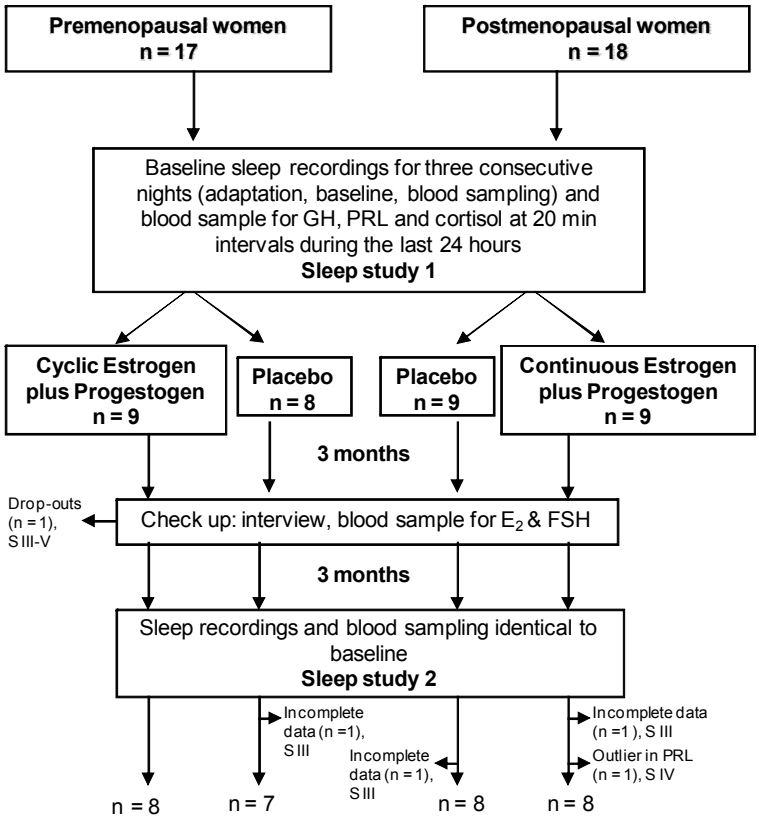

Figure 5. Flow chart for Studies III-V. In Study V, premenopausal women were studied only during sleep study 1. S = study

were repeated identically to baseline (Sleep study 2). All subjects followed regular eating hours and they had similar food composition during all sampling periods.

A total of 34 women completed the entire protocol. At the 3-month check up, one premenopausal (EPT) woman dropped out for personal reasons (Study III and IV). For six postmenopausal women, Sleep Study 2 was performed prior to six months of treatment. From the postmenopausal EPT group, one woman attended the second sleep study after 3 months, two women after 4 months and one woman after 5 months, mainly due to side-effects (bloating, heavy uterine bleeding). One postmenopausal woman from the placebo group attended the second sleep study after 4 months of treatment and another developed a venous thrombosis of the eye, shortening the treatment period to 5 months (Studies III-V). Further, one subject from the postmenopausal EPT group was deleted from the PRL analysis as an outlier due to exceptionally high levels (Study IV) (Figure 1). The characteristics of the study groups are summarized in Table 1. Within pre- and postmenopausal women, the groups receiving EPT or placebo were similar in age, FSH and E_2 levels, mood (BDI), EQ5D, vasomotor symptoms and experienced insomnia and sleepiness. By chance, the BMI

was greater in the postmenopausal EPT group than in the postmenopausal placebo group, but the premenopausal groups did not differ in terms of BMI.

4.2. Methods

4.2.1. Sleep recordings

The all-night PSG recordings consisted of continuous monitoring of two (Study I, II: young women) to four (Studies I-V) EEGs (C3/A2, C4/A1, O1/A2 and O2/A1), two EOGs, a mandibular EMG and an electrocardiogram (ECG) (Embla, Medcare Flaga hf. Medical devices, Reykjavik, Iceland). Sleep stages were scored visually in 30-second epochs by the same scorer (N.K.), who was blinded from the treatment (Studies III, V), according to the conventional criteria (Rechtschaffen & Kales 1968). For quality control, the sleep data was rescored by a senior scorer (P.P-K.). Sleep onset was considered to be the appearance of three consecutive epochs of S1 or the first epoch of any other stage. Sleep latency was the period from lights off to sleep onset (Studies I-III). Sleep stages [S1, S2, SWS, REM and movement time (MT) as well as wake time after sleep onset] were expressed as the percentage of time in bed (from lights off to lights on) (Studies I-III and V). Sleep efficiency was defined as the percentage of total sleep time (S1+S2+SWS+REM+MT) out of time in bed. Awakening was defined as entering into the wake stage from sleep. To score an arousal during sleep, EEG α-activity for at least three seconds was required (The Atlas Task Force 1992) (Studies I-III).

To quantify the SWA (0.75-4 Hz) in NREM sleep episodes, spectral analysis was used (Studies I-III). Sleep cycles were defined according to the rules of Feinberg and Floyd (1979), apart from the requirement for the minimum duration of the first REM episode. Only full cycles were included. The first NREM episode was considered to be the period from sleep onset to the beginning of the first REM episode and the subsequent NREM episodes from the end of each REM episode to the beginning of the next. SWA power was calculated every 30 seconds in each NREM-sleep episode to normalize the time course of SWA. The power spectrum was calculated from the C3–A2/C4–A1 derivation in 4-second epochs using 256 point fast Fourier transform with 50 percent overlapping. EEG artifacts (movements, eye movements) and event triggered slow waves with increased muscle tone were visually identified and omitted (Heinzer et al. 2001) (S.-L.H.). To preserve sleep continuity, power spectrum samples that overlapped manually marked artifacts were considered missing data. Comparisons of SWA were carried out on the first four NREM sleep episodes.

4.2.2. Questionnaires

Subjective sleep quality, sleepiness and mood were evaluated in the mornings immediately after awakening. A morning questionnaire on sleep quality, sleep efficiency, sleep latency, number of awakenings, waking too early in the morning and morning tiredness was used to assess the subjective sleep quality of the preceding night (Studies I, II, Appendix 6). The variables were categorical, with a low number

referring to good sleep or to a low level of sleeping problems. Three questions (sleep efficiency, sleep latency and the number of awakenings) were used to investigate correlations between subjective and objective sleep quality. Mood was evaluated with five visual-analogue scales (VAS, Study I, Appendix 7). For statistical analyses, the answers concerning the levels of depression, sleepiness and irritability were reversed, resulting in a lower number referring to better mood in all questions. Alertness was assessed by the Stanford Sleepiness Scale (SSS) (Study I, Hoddes et al. 1973), according to which the subjects assess their current alertness on a scale from 1 ("feeling active and vital, alert, wide awake") to 7 ("almost in reverie, sleep onset soon, losing the struggle to remain awake"). Climacteric vasomotor symptoms were scored using two questions on the past six months (night sweats and hot flashes). The frequency of the symptoms was determined on the following four-point scale: 1 ("seldom or never"), 2 ("approx. once a month"), 3 ("approx. once a week"), 4 ("almost every day"). The vasomotor symptom score was the sum of the two answers.

4.2.3. Cognition

Cognitive tests (Study I) were performed using the CogniSpeed program (AboaTech LTD, Turku, Finland) (Revonsuo & Portin 1995). Three attentional tests were used: simple reaction time (SRT), two-choice reaction time (2-CRT) and vigilance tests. The tests, apart from the test of vigilance, were carried out on both evenings before bedtime and on both mornings immediately after awakening. Only the tests performed in the second morning were used for the final analysis. A practice session was performed before the first tests. RTs of correct responses were measured in milliseconds (ms). In the 2-CRT, the number of errors was counted as well. Sustained attention was measured with the vigilance test, a visual test of letter cancellation that was carried out in the second morning. Immediately before the test there was a practice session. In the statistical analysis, mean individual RTs were used as a measure of speed, and the omission rate and the number of errors (false positives) as measures of accuracy.

4.2.4. Hormone assays

The blood samples, taken during the studies, were drawn into EDTA tubes and placed in the refrigerator for 20 min. Thereafter, they were centrifuged to separate serum, frozen and kept at -28˚C until the next day and then stored at -70˚C until assayed. Serum E_2, FSH (all studies), GH, PRL and total cortisol levels (Studies IV, V) were measured with the AutoDELFIA assays (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). The FSH, GH and PRL assays were solid phase, two-site fluoroimmunometric assays based on the direct sandwich technique (two monoclonal antibodies directed against two separate antigenic determinants on the hormone molecules). The E_2 assay was a solid phase fluoroimmunoassay based on competition between europium-labeled E_2 and the sample for polyclonal anti-E_2 antibodies. The cortisol assays were solid phase, two-site fluoroimmunometric assays based on the competitive reaction between europium-labeled cortisol and the sample cortisol for a limited number of binding sites on cortisol specific, biotinylated monoclonal antibodies. The analytical sensitivity (zero standard mean + 2 standard deviation, SD)

was 0.05 U/l for FSH, 0.03 mU/l for GH, 0.04 ng/mL for PRL, 0.05 nmol/l for E_2 and 15 nmol/L for cortisol. The interassay coefficient of variation (CV) was 2.3% for FSH at a concentration of 44.8 IU/l, 2.5% for GH at a concentration of 0.43 mU/l, 2.8% for PRL at a concentration of 54.8 ng/ml, 8.5% for E_2 at a concentration of 0.18 nmol/l and 1.9% for cortisol at a concentration of 212 nmol/l.

4.2.5. GH analyses

A simple computer algorithm was designed to ensure reproducible and unambiguous detection of the serum GH peaks (Studies IV, V). The results were validated against an experienced manual scorer's visual judgment. Missing observations were first linearly approximated from the available adjacent observations, and the GH levels were then approximated with cubic splines. GH peaks below 1.0 mU/l or below two times the individual signal baseline were excluded. To exclude false observations caused by signal noise, it was also required that within an 8-minute window before and after each GH peak, the signal amplitude must change at least 0.03 mU/l for the post- and 0.06 mU/l for the premenopausal group.

4.2.6. Nocturnal PRL profiles and sleep stages

The link between the stages of sleep and nocturnal PRL secretion was inspected by using a mathematical model (explained in detail in Study V, Appendix A). The model was inspired by the widely-used deconvolution approaches for estimating the hormonal secretion rates (Johnson & Veldhuis 1995). Although the modeling of the underlying dynamics is identical with the approaches presented in some previous reports (Van Cauter et al. 1992, Keenan et al. 2005), the aim was to characterize the connection between the stages of sleep and PRL secretion directly, while the other existing approaches concentrate on identifying the shape and location of the individual hormone bursts. The model incorporates a term for each individual stage of sleep and for the baseline secretion. The circadian component is modeled through a linear transient between wakefulness and sleep.

4.2.7. Statistical analyses

In Studies I-III, repeated measures analysis of variance (ANOVA) was used to evaluate the differences between the groups at different conditions (baseline and recovery from sleep deprivation, Study I, or on EPT, Study III). In addition, difference in change between the conditions was analyzed. Because of skewed data, non-parametric tests were applied. In studies with more than two study groups (Studies I, II), the differences were first evaluated with the Kruskal-Wallis test. If the results were significant, they were further evaluated with the Mann-Whitney U-test (Studies I-II) and adjusted according to the Bonferroni procedure. All correlations were examined with Spearman's correlation coefficient. In Study I, subjective sleep quality variables from the morning questionnaire were analyzed with Fisher's exact test. In the same study, post-hoc analysis between pre- and postmenopausal groups was carried out with the Kruskal-Wallis test. In Study II, the differences within the groups between baseline and recovery nights were evaluated with Wilcoxon signed rank.

In Study IV, analysis of covariance (ANCOVA) was used in comparisons between the groups and treatments, apart from the BMI, age and the difference between daytime and nighttime concentration analyses, which were carried out with two-sample t-test. Because of group differences, BMI was used as covariate in baseline comparisons. When analyzing the differences after treatment, BMI and baseline levels were used as covariates. In Study V, two-sample t-tests were used in comparisons between the groups for sleep variables. In addition, difference from baseline to after treatment within the postmenopausal groups was analyzed with one-sample T-tests. The goodness of the fit of the PRL secretion rate model was judged by inspecting the mean absolute errors and correlations between the model predictions and the empirical measurements. Deviations in GH and PRL levels with respect to different sleep stages were tested with the Wilcoxon rank sum and signed rank tests, respectively. For all statistical analyses, a p-value of less than 0.05 was considered significant. Statistical analyses were performed using SAS System for Windows (SAS Institute Inc., Cary, NC, release 9.1) in Studies II, III and IV, SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA, release 16.0.1) in Studies I and V and R: A Language and Environment for Statistical Computing 2.6.2 (R Foundation for Statistical Computing, Vienna, Austria, release 2.6.2) in Study V.

5. RESULTS

5.1. Sleep quality in women

5.1.1. Comparisons between young, premenopausal and postmenopausal women (Studies I, II and V)

The means and SD for selected objective sleep variables are presented in Table 3 for all study groups. The postmenopausal women, with or without HT (Studies I, II), and premenopausal women (Study I) had less total sleep time, SWS, lower sleep efficiency and SWA, more time awake and more awakenings than the young controls. The pre- and postmenopausal women (Study I) had also higher insomnia scores in BNSQ (Table 1) compared to the young women, but no difference was found on the VAS of sleepiness and mood, on the sleepiness in SSS (data not shown) or according to the subjective sleep variables of the morning questionnaire. Compared to the young women, the postmenopausal HT-users (Study II) had a shorter latency to REM sleep, more S1 sleep and more reports of waking too early in the morning, whereas the non-HT-users spent less time in REM sleep.

Table 3. Selected sleep variables at baseline, expressed as mean (standard deviation), for the study groups

	Young	Premenopausal			Postmenopausal				
	Studies I, II	Study I	Study III		Study I	Study II		Study III	
			EPT	placebo		HT-users	non-HT	EPT	placebo
	N = 11*	N = 20	N = 8	N = 7	N = 28	N = 9	N = 10	N = 8	N = 8
Total sleep time (min)	448.2 (27.2)	404.9 (44.8)	397.7 (45.0)	419.5 (42.0)	384.7 (54.6)	402.5 (35.6)	372.3 (68.1)	377.7 (47.1)	405.1 (48.1)
Sleep efficiency (%)	93.4 (5.6)	84.3 (9.3)	82.9 (9.4)	87.4 (8.7)	80.2 (11.4)	83.9 (7.4)	77.7 (14.3)	78.7 (9.8)	84.4 (10.0)
Sleep latency (min)	14.1 (9.2)	15.2 (15.8)	16.3 (15.1)	18.4 (21.6)	16.8 (14.4)	16.2 (14.6)	18.6 (14.5)	16.3 (16.2)	17.9 (15.1)
SWS latency (min)	12.8 (6.4)	20.3 (17.3)	25.8 (19.2)	15.0 (6.5)	15.9 (16.3)	14.1 (11.4)	12.5 (5.7)	22.2 (24.2)	15.9 (17.5)
REM latency (min)	107.9 (36.3)	83.5 (31.1)	83.6 (23.5)	62.7 (15.6)	74.3 (25.5)	60.9 (13.3)	80.5 (17.2)	82.3 (37.4)	64.8 (16.9)
S1%	6.4 (2.8)	7.8 (3.2)	7.5 (2.1)	8.3 (4.9)	8.1 (3.3)	11.0 (3.4)	8.7 (3.6)	8.5 (4.0)	7.3 (2.3)
S2%	43.7 (4.1)	43.2 (8.8)	42.7 (8.9)	44.3 (9.5)	39.7 (7.9)	47.3 (6.9)	41.0 (10.6)	36.8 (6.4)	41.2 (5.6)
SWS%	22.1 (3.2)	12.6 (5.8)	11.4 (5.0)	13.0 (7.2)	14.2 (6.5)	8.1 (4.1)	12.6 (5.6)	15.1 (7.4)	15.1 (7.4)
REM%	21.0 (4.2)	20.6 (6.1)	21.2(7.3)	21.7 (6.8)	18.1 (5.3)	17.4 (3.7)	15.6 (4.7)	18.2 (4.1)	20.8 (6.7)
wake%	3.7 (5.3)	12.6 (8.4)	13.7 (8.2)	8.8 (6.1)	16.3 (9.4)	12.9 (6.6)	18.2 (12.0)	17.9 (7.4)	11.9 (8.0)
Sleep stage transitions	143.6 (31.5)	161.6 (35.3)	154.5 (28.3)	170.3 (35.3)	167.2 (42.0)	181.3 (41.1)	155.0 (40.5)	182.9 (31.2)	153.3 (45.2)
Awakenings	6.1 (5.2)	17.1 (6.4)	17.5 (7.1)	17.3 (7.3)	19.9 (8.0)	27.0 (14.1)	17.4 (5.7)	23.5 (10.3)	16.9 (5.3)
Arousals	75.8 (23.0)	97.2 (45.7)	89.8 (40.4)	107.1 (51.4)	123.8 (70.8)	91.7 (39.2)	77.1 (28.2)	161.4 (87.0)	139.5 (67.0)
SWA									
1st episode	0.7 (0.2)	0.2 (0.09)	0.2 (0.09)	0.3 (0.1)	0.2 (0.08)	0.1 (0.03)	0.2 (0.08)	0.2 (0.1)	0.2 (0.08)
2nd episode	0.4 (0.2)	0.2 (0.2)	0.2 (0.09)	0.3 (0.3)	0.2 (0.07)	0.09 (0.03)	0.1 (0.05)	0.2 (0.07)	0.2 (0.07)

N = 10 in the results for SWA

In Study I, there were no differences between pre- and postmenopausal women in the objective sleep variables displayed in Table 3 or in the subjective sleep variables of the corresponding night, but postmenopausal women had higher insomnia scores for the past three months (BNSQ) than premenopausal women (Table 1). When sleep was challenged with an intravenous catheter during the blood sampling protocol in Study V, no difference in sleep architecture between pre- and postmenopausal women was found (Table 4).

Table 4. Selected sleep variables during the blood sampling night, expressed as group means (standard deviation), for the study groups of Study V.

	Premenopausal	Postmenopausal	
	Baseline	Baseline	During EPT
	N = 16	N = 18	N = 9
Total sleep time (min)	361.9 (81.5)	358.3 (67.7)	335.6 (49.5)
S1 %	8.5 (3.2)	9.9 (3.3)	7.4 (4.1)
S2 %	39.3 (11.3)	36.2 (9.6)	39.0 (10.6)
SWS %	10.0 (4.5)	12.2 (5.2)	9.5 (6.5)
REM %	17.6 (8.1)	16.3 (6.0)	14.1 (5.7)
Wake %	20.7 (16.3)	21.8 (13.1)	27.5 (9.2)

5.1.2. Sleep deprivation and hormone therapy in postmenopausal women (Study II)

Irrespective of age or grouping, all study subjects responded to sleep deprivation with an increased total sleep time and percentage of SWS, better objective and subjective sleep efficiency, shorter sleep latency and latency to SWS, a smaller percentage of S1 sleep and wake time as well as fewer arousals (Table 5). Furthermore, the number of awakenings and sleep stage transitions was reduced, the SWA in the second NREM episode increased (less compared to non-HT users or to young controls) and the reports of waking too early reduced in the HT group. In the non-HT group the percentage of REM sleep as well as SWA in all NREM sleep episodes were increased and subjective sleep latency shortened. In the young control group, the number of objective and subjective awakenings was reduced, SWA in the first NREM sleep episode increased (more compared to postmenopausal women) and subjective sleep latency shortened.

Table 5. The effect of sleep deprivation, in Study II, on selected sleep parameters, expressed as group means (standard deviation) of the difference between the baseline and the recovery nights.

	Young	HT	Non-HT
	N = 11*	N = 9	N = 10
Total sleep time (min)	26.4 (28.5)	49.7 (30.7)	56.8 (67.1)
Sleep efficiency (%)	5.5 (5.9)	10.4 (6.3)	11.7 (14.2)
Sleep latency (min)	-10.0 (9.3)	-7.9 (14.9)	-9.6 (13.6)
Latency to SWS	-8.3 (6.5)	-7.7 (11.1)	-9.2 (6.1)
Latency to REM	19.6 (47.9)	-5.8 (13.0)	-2.0 (36.0)
S1 %	-3.1 (2.2)	-4.5 (3.0)	-3.6 (3.3)
S2 %	-0.2 (5.5)	2.5 (8.3)	-2.2 (10.3)
SWS %	11.0 (4.8)	8.8 (4.9)	13.0 (5.1)
REM %	-2.5 (3.6)	3.5 (7.0)	4.6 (5.7)
Wake %	-3.5 (5.4)	-8.8 (5.5)	-9.5 (11.9)
Number of sleep stage transitions	-1.5 (16.8)	-36.3 (33.8)	-5.1 (42.8)
Number of awakenings	-4.9 (4.5)	-14.6 (11.9)	-6.5 (8.3)
Number of arousals	-16.5 (9.8)	-38.7 (32.7)	-28.2 (29.8)
SWA			
1st NREM sleep episode	0.5 (0.5)	0.03 (0.04)	0.1 (0.06)
2nd NREM sleep episode	0.1 (0.3)	0.05 (0.05)	0.1 (0.07)

* N = 10 in the results for SWA.

As a result of these responses to sleep deprivation, the main baseline group differences in objective sleep variables became more apparent during the recovery night. Postmenopausal women still a had shorter total sleep time, lower sleep efficiency and SWA, a greater percentage of wake time and more awakenings compared to young controls. In addition, the HT-users had a shorter latency to REM sleep, more S1 sleep and less SWS than the young controls, similarly to baseline. The HT-users differed from the non-HT-user by having more S2 sleep and lower SWA in the first NREM sleep episode. There were no differences in subjective variables of the recovery night.

5.1.3. Comparisons between subjective and objective sleep quality (Studies I, II)

In Study I, the subjective and objective sleep latency correlated in the premenopausal group and subjective and objective sleep efficiency in the postmenopausal group. When all the women in Study II (postmenopausal HT- and non-HT-users, young controls) were considered as one group, there was no correlation between subjective and objective sleep variables (sleep efficiency, sleep latency, awakenings) at baseline, but on the recovery night all three subjective and corresponding objective measures correlated. The changes in subjective and objective measures from baseline to recovery correlated only in sleep latency. When the three study groups were evaluated separately, there was correlation only between the subjective and objective sleep latency of the young controls on the recovery night. The improvement in subjective and objective sleep latency correlated in the HT-group as well as the subjective and objective decrease in awakenings in the young controls.

5.2. The effect of hormone therapy on sleep and sleep-associated hormones

The long-term use of HT in postmenopausal women was associated with shorter latency to REM sleep compared to the non-HT users (Study II) (Table 3). In addition, the HT-users reported more waking too early in the morning than non-HT users.

5.2.1. The effect of 6-month EPT on sleep (Study III)

There were no baseline differences between the premenopausal EPT and placebo groups or between the postmenopausal EPT and placebo groups in insomnia and sleepiness scores (BNSQ, Table 1). In objective sleep variables (Table 3), the only baseline difference was that SWA during the third and fourth NREM sleep episodes was higher in the premenopausal placebo group than in the premenopausal EPT group. As expected, during EPT serum E_2 levels increased and FSH levels decreased in postmenopausal women. The 6-month EPT had no effect on the insomnia and sleepiness scores of pre- or postmenopausal women. In addition, no effect on premenopausal objective sleep quality was found in the variables shown in Table 3. In postmenopausal women, EPT, unlike placebo, decreased climacteric vasomotor symptoms [EPT: mean -2.4 (SD 2.7) vs. placebo: 0.6 (1.1), baseline-values shown in Table 1] and SWA during the second NREM sleep episode [EPT: mean -0.04 (SD 0.05) vs. placebo: 0.02 (0.03), baseline-values shown in Table 3]. However, there was no difference between the postmenopausal EPT and placebo groups in those variables

during Sleep Study 2 (flow chart of the study design in Figure 5) but postmenopausal women in the EPT group had more awakenings [mean 30.4 (SD 8.4)] compared to the placebo group [mean 20.5 (SD 5.6)].

In the catheter night, during blood sampling, EPT had no effect on the total sleep time, percentages of sleep stages or wake time in postmenopausal women (Table 4).

5.2.2. The effect of EPT on 24-h serum levels of GH, PRL and cortisol (Study IV)

At baseline, postmenopausal women had lower mean 24-hour, night-time and maximum GH and PRL than premenopausal women, but the cortisol values did not differ. After EPT, postmenopausal mean 24-h GH or PRL levels did not differ from premenopausal baseline levels.

There were no baseline differences between the premenopausal EPT and placebo groups in GH, PRL or cortisol levels (Table 6). After EPT, night-time PRL and cortisol as well as the maximum PRL value were higher compared to placebo with no difference in GH levels (Table 6). The mean night-time PRL concentrations were higher compared to daytime in premenopausal EPT and placebo groups at baseline and after treatment, but there was no difference in the corresponding cortisol concentrations. The mean night-time GH concentrations were higher compared to daytime in both groups at baseline but after treatment only in the EPT group.

Table 6. Concentrations of GH, PRL and cortisol (COR) expressed as mean (standard devitation), for the postmenopausal and premenopausal EPT and placebo groups at baseline and during EPT.

		Premenopausal			Postmenopausal		
		Baseline		During	Baseline		During
		EPT	Placebo	EPT	EPT	Placebo	EPT
		N=9	N=8	N=8	N=9*	N=9	N=9
GH 24 h	(mU/l)	1.9 (0.7)	1.7 (0.9)	1.6 (0.5)	0.7 (0.6)	1.2 (0.5)	1.1 (0.8)
GH night		3.4 (1.9)	2.3 (0.9)	2.6 (0.9)	0.8 (0.8)	1.7 (0.8)	1.0 (0.8)
GH day		1.3 (0.5)	1.4 (1.1)	1.1 (0.5)	0.7 (0.7)	1.0 (0.6)	1.1 (0.9)
GH max		18.5 (9.1)	14.1 (4.2)	15.7 (5.7)	5.7 (5.2)	9.9 (5.4)	8.9 (7.1)
GH min		0.1 (0.1)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.04)
PRL 24 h	(ng/ml)	10.6 (3.6)	9.3 (2.1)	10.2 (3.5)	7.3 (2.3)	6.3 (1.3)	9.2 (2.7)
PRL night		17.5 (6.7)	14.5 (2.2)	17.8 (4.5)	10.2 (3.2)	9.7 (2.0)	13.7 (4.4)
PRL day		7.0 (3.0)	6.7 (2.2)	6.4 (3.5)	5.8 (1.9)	4.6 (1.3)	7.1 (2.1)
PRL max		32.1 (14.1)	24.3 (2.3)	39.2 (8.8)	15.1 (4.0)	15.8 (3.7)	25.3 (11.7)
PRL min		3.7 (2.0)	3.8 (2.0)	3.3 (1.4)	3.5 (1.3)	2.4 (0.6)	3.8 (1.6)
COR 24 h	(nmol/l)	203.4 (32.4)	192.5 (35.5)	218.5 (26.3)	206.4 (35.6)	206.6 (44.8)	200.4 (21.8)
COR night		219.0 (31.5)	195.6 (51.7)	226.7 (24.7)	204.5 (35.5)	207.8 (46.1)	202.6 (61.5)
COR day		210.6 (36.6)	206.2 (32.0)	231.5 (31.6)	220.6 (52.0)	218.9 (52.1)	240.7 (82.3)
COR max		521.0 (51.0)	509.2 (62.4)	508.2 (56.1)	518.0 (129.2)	512.2 (97.4)	544.5 (175.8)
COR min		54.8 (23.2)	41.4 (13.5)	55.6 (16.8)	46.9 (25.4)	58.6 (32.1)	67.3 (42.7)

* The results concerning prolactin, n = 8.

The postmenopausal EPT and placebo groups did not differ at baseline in GH, PRL or cortisol levels, apart from higher minimum PRL in the EPT group (Table 6). After

treatment, mean 24-hour, daytime and maximum GH and PRL values, the number of GH peaks during 24-h and during daytime as well as mean night-time PRL were all higher in the postmenopausal EPT group compared to the placebo group. No differences in cortisol levels were found (Table 6). The mean night-time PRL concentrations were higher than the mean daytime levels in EPT and placebo groups at baseline and after treatment, but there was no difference in the corresponding GH concentrations. The mean night-time cortisol concentration was lower than the daytime concentration only after EPT.

5.2.3. Interactions between GH and slow wave sleep

When studied in 20-minute time frames, the mean percentual amount of SWS was greatest between 40 to 20 minutes prior to the first GH peak after sleep onset in pre- and postmenopausal women. The overnight mean amount of SWS did not differ between pre- and postmenopausal women but from 40 to 20 minutes before the first GH peak the amount of SWS was significantly higher in premenopausal than in postmenopausal women (see Study V, Figure 2). This indicated that in postmenopausal women SWS was more scattered throughout the night. Postmenopausal HT did not change this pattern. The number of GH peaks after sleep onset did not differ between premenopausal (mean 2.2) and postmenopausal women (mean 2.1). Postmenopausal HT had no significant effect on the number of peaks. The average time interval from sleep onset to the first GH peak was shorter in premenopausal than in postmenopausal women at baseline (premenopausal mean 73.7 min, postmenopausal mean 120.6 min) and after treatment when postmenopausal women in the HT group were compared to premenopausal women. HT had no significant effect on the time interval.

5.2.4. Interactions between PRL and sleep stages

Based on the observation that the measured nocturnal PRL levels appeared low during REM and higher during SWS in most of the subjects, and assuming that the PRL secretion rate was directly proportional to the sleep stages, the first model of the link function between sleep stages and PRL levels was created. This algorithm systemically overestimated the PRL levels after sleep onset, frequently underestimated the levels at the end of the night, and produced insufficient dynamics for the whole night. The initial model was then improved by including a circadian component (see Study V: Appendix A and Figure 2), which was assumed to manifest itself as a linear transient between wakefulness and sleep. With this improved model, the measured PRL levels could be predicted with high accuracy in both pre- and postmenopausal women [the average correlations and the mean (absolute) errors between the model predictions and the measurements were 0.84 and 2.4 ng/mL for premenopausal and 0.81 and 1.25 ng/mL for postmenopausal women, respectively]. For pre- and postmenopausal women, the mean characteristic level of PRL during REM was significantly lower than the corresponding SWS value (premenopausal: REM 18.2 ng/mL, SWS 28.0 ng/mL; postmenopausal: REM 10.5 ng/mL, SWS 20.0 ng/mL).

6. DISCUSSION

The purposes of the present study were to evaluate whether menopause induces changes in sleep quality and to examine the effects of HT and aging on sleep quality. Further aims were to investigate the impact of HT on GH, PRL and cortisol levels and to determine the temporal associations of GH and PRL with sleep stages. The study subjects included young, middle-aged premenopausal, and postmenopausal women. The studies were conducted under normal conditions, after sleep deprivation or during HT. Based on the results from previous studies of subjective sleep, reduced sleep quality and its marked improvement with HT (e.g. Erkkola et al. 1991, Polo-Kantola et al. 1998, Sarti et al. 2005) has been suggested after menopause. However, studies with objective measurements of sleep quality have not been able to show similar results (e.g. Purdie et al. 1995, Polo-Kantola et al. 1999a, Saletu-Zyhlarz et al. 2003). The relationships between GH and PRL with sleep stages have mainly been studied in young males, although there are gender differences in sleep quality as well as in GH and PRL profiles (Ho et al. 1996, Waldstreicher et al. 1996, Manber & Armitage 1999, Antonijevic et al. 2000a, Steiger 2007).

According to the present study, women are less satisfied with their general sleep quality after menopause, but the objective sleep quality of pre- and postmenopausal women are mainly similar. The recovery response following sleep deprivation is relatively well preserved in postmenopausal women although the sleep quality is worse than in young women. The impact of HT on objective and subjective sleep quality is marginal under normal conditions or after sleep deprivation. In premenopausal women, HT has no effect, and postmenopausal women on HT have even more awakenings than women on placebo under normal conditions. After sleep deprivation, postmenopausal sleep quality on the recovery night is somewhat worsened on HT, mainly seen in the spectral analysis of the EEG. The temporal association between GH and SWS is weaker in postmenopausal women than in premenopausal women. Although the decreased GH levels after menopause are reversible with HT, the temporal link between SWS is not affected. PRL levels are temporally associated with sleep stages as previously described in young males. PRL levels are higher during SWS than during REM sleep. The decreased PRL levels after menopause can also be increased with HT, whereas cortisol concentrations are independent of menopause or HT.

6.1. Methodology

6.1.1. Subjects

The present study evaluated sleep quality and the effect of HT on healthy pre- and postmenopausal women without the interference or interaction of existing systemic diseases. Selecting healthy subjects was essential for a reliable conclusion regarding the studied effects, although the results may not be completely comparable to general populations. To ensure a healthy study sample, the exclusion criteria included previous

severe diseases, and allowing only minor stable diseases, such as treated hypertension or hyperlipidemia. In addition, women with lifestyles affecting the studied parameters or interfering with the protocol design, such as alcohol abuse, smoking, irregular sleeping habits or excessive consumption of caffeine, were excluded. Basic laboratory tests were carried out on all the women. In addition, a physical examination, including a gynecological examination was performed in all but young women.

All pre- and postmenopausal subjects were recruited through newspaper advertisements, which might have favored selection of women who are more concerned about their well-being or more symptomatic than women on average. However, women with evident sleep problems were excluded and the varying climacteric vasomotor symptoms were evenly distributed in the study population. The young subjects (Studies I and II) were recruited through advertisements at the university and they received financial compensation for their participation, which may increase motivation. However, the pre- and postmenopausal women, who were unpaid volunteers, were also all highly motivated and none of the subjects discontinued the demanding sleep deprivation protocol (Study II), and only one premenopausal woman withdrew from the randomized EPT trial (Study III-IV).

Study I was observational because the use of HT was determined according to the individual preference of the subjects. Duration of the substitution in most of the users was long. This may cause the so-called “healthy-user bias”, meaning that women on HT might be healthier in general (Matthews et al. 1996). In addition, information about the prior use of HT was retrospective and based on subject’s recollection, which is prone to errors. The young women (Studies I and II) were using oral contraception, but they were regarded as control subjects. However, in previous studies, no major differences either in sleep variables (Baker et al. 2001b) or in the cognitive variables (Wright & Badia 1999) tested in Study I have been found between OC users who are in the beginning of their ‘cycle’ (not taking pills) and naturally cycling women during their follicular phase. Therefore, in the present study, all menstruating (young and premenopausal) women were studied at the beginning of their cycle (follicular phase). Since the premenopausal women were not estrogen-deficient, which was verified with FSH and E_2 measurements, the use of cyclic HT might disturb their normal menstrual cyclicity (Studies III and IV). This in turn might cause other endocrinological perturbations and affect sleep. However, the evaluation of the effects of HT on middle-aged premenopausal women is important since HT is used to treat these women when presenting early climacteric symptoms.

6.1.2. Questionnaires and cognitive tests

The subjective sleep quality of the past three months was evaluated with BNSQ, which is a validated and widely-used questionnaire (Partinen & Gislason 1995). The morning questionnaire is a standard questionnaire used in our sleep laboratory and in clinical practice to evaluate the subjective sleep quality of the preceding night. It has been shown to effectively separate good sleepers from poor sleepers (Hyyppa & Kronholm 1987, Kronholm et al. 1987). The BDI is a standardized tool for assessing the degree of

depression (Beck et al. 1961) and in the present study, it was used to control for the confounding factor of any incipient depression. Another widely-used instrument for describing and valuing health is the EQ-5D questionnaire (The EuroQol group 1990, Ohinmaa et al. 1996), which in this study was used to describe the characteristics of the groups. No validated questionnaire for climacteric symptoms was used since the emphasis was on vasomotor symptoms, which were addressed only to control for confounding factors. Subjective alertness and mood was assessed with the SSS and VAS. The SSS is a widely-used and well-standardized questionnaire on sleepiness (Hoddes et al. 1973). The VAS method, used here for subjective evaluation of mood, has been shown to be sensitive and clinically valid to evaluate the intensity of symptoms and measure emotional and symptomatic changes (Aitken 1969).

Decreased sleep quality has been shown to be associated with impaired cognitive performance (Smith et al. 2002). Thus, the cognitive tests in Study I were used to add to the sensitivity of the study for detecting menopause-induced physiologically important alterations in sleep. All the tests used have previously been validated and successfully used in cognitive trials (Revonsuo & Portin 1995, Polo-Kantola et al. 1998, Portin 2000, Alhola 2007). To reduce the practice effect, only the tests performed in the morning following the study night were analyzed.

6.1.3. Hormone assays

The blood samples before the study and for FSH and E_2 during the study were taken in the mornings in all subjects to avoid the bias caused by diurnal variation. In Study IV, the intravenous catheter was inserted two and half hours before the 24-h collection of blood samples (GH, PRL and cortisol) was started to avoid iatrogenic disturbance on cortisol concentrations. All samples were analyzed in a standardized laboratory (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). The blood samples were carefully preserved at the proper temperatures before centrifugation and thereafter. Since blood samples were drawn at 20-min intervals, the hormone levels between sample times were not known, and the concentration profiles of GH, PRL and cortisol were based on approximation with cubic splines (Study IV and V). Decreasing the sampling interval in the present study might have produced more GH pulses. However, a previous study on men has shown that these additional pulses have been smaller bursts that composed the large pulse detected with less frequent sampling (Holl et al. 1991). Therefore, it is unlikely that higher frequency sampling would have generated significantly different results in the present study (Study IV and V).

6.1.4. EPT preparations

Oral combined EPT is the most-frequently prescribed HT because it is suitable for women with an intact uterus. According to clinical guidelines, cyclic EPT preparations were used in premenopausal women and continuous EPT in postmenopausal women who were already a few years from their menopause. In Finland, the most common progestin in oral EPT is NETA (National agency for medicines, Finland 2007). The effects of EPT containing NETA are, therefore, clinically important to study, especially since its effects on cortisol concentrations, for instance, seem to differ from other EPT

preparations (see Chapter 2.3.4). Gynecological examinations, including transvaginal ultrasound, were performed at the beginning of the study and when needed, for instance, due to abnormal uterine bleeding. The wash-out period for previous HT use was 12 months at the minimum, apart from one premenopausal woman, in whom it was 5 months. This can be regarded as a sufficient time to avoid any carry-over effect as well as withdrawal symptoms, either of which could create bias in studies with very short wash-out periods (Antonijevic et al. 2000b). All women on EPT had serum E_2 concentrations higher than 100 pmol/L, apart from one postmenopausal woman in Study II. However, her E_2 concentration was higher than those of postmenopausal women not using HT in the same study.

6.1.5. Sleep recordings and analyses

All-night PSG is the state-of-the-art method for studying sleep. The techniques derive directly from the earliest studies following the discovery of REM sleep in the 1950's. The standardized manual for scoring sleep stages was first introduced in the 1960's (Rechtschaffen & Kales 1968): however, it was not until last year that the first modifications and additions to this manual were made (Iber et al. 2007). This new manual contains some new definitions and rule modifications as well as new rules for pediatric visual scoring. The sleep recordings in this study were scored according to the old standards. Scoring of sleep recordings is laborious and time-consuming, since no reliable computerized scoring method is available. In contrast to the used manual scoring, computerized method would yield strictly reproducible and unambiguous results.

To decrease bias originating from subjective evaluations, the recordings of the present studies were all scored by the same scorer (N.K.), then re-scored by a senior scorer (P.P-K.) and further checked by a senior scorer in another sleep center (S-L.H.). During the analyses all scorers were blinded from the treatment. In addition, a quantitative EEG analysis was performed as SWA was analyzed. Previously described inconsistency between subjective and objective sleep quality, "sleep state misperception", (Baker et al. 1999b, Young et al. 2003, Regestein et al. 2004, Vitiello et al. 2004) was also observed in the present studies (Studies I and II). This may, at least partially, originate from the limitations of the standard scoring system, such as epoch lengths of 30 s, which ignores the probable existence of more rapid sleep stage changes (Himanen & Hasan 2000). In addition, cortical electrical events detected with EEG may not be sensitive enough to disclose alterations in deeper brain structures, such as autonomic nervous system centers (Polo 2003).

6.1.6. Study design

In the present studies, both observational design (Studies I and II) and prospective, randomized, placebo-controlled, double-blind protocol (Studies III-V) were used. The latter is considered better for examining treatment effects. However, HT is very potent, and the subject may easily detect whether she is receiving active treatment or placebo. Side-effects also appeared in the present double-blind study, but the researcher principally responsible for scoring and data analyses (N.K.) was not clinically in

charge of the subjects and thus remained blinded. In some subjects, side effects shortened the treatment period below the planned six months. However, the intervention was never shorter than three months, which is comparable to most previous studies and can be considered sufficient in clinical practice to draw conclusions on the treatment efficacy.

In sleep studies, it is important to control for confounding factors affecting sleep-wake state. Adaptation to the sleep laboratory is also considered essential. Several means were applied in trying to ensure sleep-wake control in the present studies. In all studies, the inclusion criteria included a regular sleep-wake schedule (10 – 11 p.m. to 6 – 7 a.m.) and the subjects kept a sleep diary for three weeks before and one week after the sleep studies to confirm the schedule. Traveling abroad was prohibited one week before the sleep studies. During the sleep studies, and for one week before, the use of alcohol and caffeine were prohibited. Coffee and tea drinkers were provided with caffeine-free coffee or tea. To avoid withdrawal symptoms, women with excessive use of caffeine were excluded before the study. In addition, all women slept for one adaptation night in the laboratory before the study night to minimize the adverse effect of unfamiliar surroundings on sleep quality. One could argue against using the data from the second night, during which the decreased sleep quality of the first night may rebound as "better than normal" sleep quality. However, the "first night effects" are shown to rapidly adapt out by the second night of sleep (Agnew et al. 1966).

The common limitation in previous sleep studies has been the small sample size, which is also true for the present study. Few previous randomized, placebo-controlled PSG studies have exceeded our numbers and none have studied premenopausal women. The laborious methodology and study design typically results in a relatively small number of subjects. In the present study, it took over 350 nights in total to collect the entire data. Increasing the sample size might have produced additional statistically significant differences between the pre- and postmenopausal groups and especially between the EPT and placebo groups: however, their clinical significance would be questionable in many variables, since the raw data seemed to be quite similar across the groups. By chance, randomization did not result in similar BMI in the postmenopausal groups (Studies III-V), but this was controlled by statistical corrections. The sleep studies were performed at two locations, in Turku (Studies I-V) and Helsinki (Studies I-II). However, a strict and uniform timetable and performance of procedures were carefully followed at both sleep centers. Therefore bias based on study location is highly unlikely.

6.2. Sleep in women

6.2.1. The effect of menopause and age on sleep quality

In the present study, differences in sleep quality between young (aged 20-26 years), premenopausal (aged 45-51 years) and postmenopausal (with or without HT, aged 58-72 years) women were tested with an observational cross-sectional study design (Studies I and II). The majority of changes in sleep quality were observed only when

comparing the pre- and postmenopausal women to the young women (Study I). The pre- and postmenopausal women had higher insomnia scores in the BNSQ than the young women. The well-documented age-related worsening of the overall sleep architecture and decline in SWS (Reynolds et al. 1986, Gaudreau et al. 2001a, Bliwise 2005) were also observed in the present study between the young women and the older groups. Long-term postmenopausal HT use did not markedly affect the differences in sleep architecture (Study II). Although the pre- and postmenopausal women objectively slept worse than the young women, the subjective sleep qualities of the corresponding night appeared to be similar, partly attributed to sleep misperception (Baker et al. 1999b, Vitiello et al. 2004) (see also Chapter 6.2.2.). The sleep quality of the premenopausal women was closer to that of the postmenopausal women than that of the young women. Although longitudinal studies across menopause could better address this issue, it seems that the major impairment in objective sleep quality occurs before middle age, and the menopausal state per se does not appear to essentially contribute to this.

According to the insomnia scores of the BNSQ, assessing the sleep quality of the preceding three months, the present results confirmed earlier observations that postmenopausal women are less satisfied with their sleep than premenopausal women (Kuh et al. 1997, Leger et al. 2000, Kravitz et al. 2003, Young et al. 2003). Sharkey et al. (2003), in turn, have shown that subjective sleep ratings of the preceding study night (the questionnaire was not identified in the report) are independent of menopausal state. This was also true in the present study, since subjective sleep quality based on the morning questionnaire did not differ between pre- and postmenopausal women. The difference in subjective sleep quality between the results from BNSQ and from the morning questionnaire showed that the two questionnaires investigate different aspects of sleep quality. Trouble with sleeping has been shown to associate with higher levels of depression, tension and stress as well as to increase sleepiness and impair cognitive function (Shaver & Paulsen 1993, Owens & Matthews 1998, Kravitz et al. 2003, Regestein et al. 2004). The higher insomnia scores of the postmenopausal women in the present study certainly indicated some level of disturbance in their sleep compared with the premenopausal women, but the lack of increased daytime sleepiness, worsened mood or impaired cognitive function suggested low clinical impact.

There are only three previous observational studies with PSG measures in pre- and postmenopausal women under normal conditions. The results of the present study (Study I) are in line with those of Shaver and collaborators (1988), who evaluated sleep parameters in 20 premenopausal and 24 postmenopausal women and found no differences based on menopausal state. The more recent studies have shown worse overall sleep architecture in premenopausal women compared with postmenopausal women. Sharkey et al. (2003) observed longer SWS latency and more S1 sleep in premenopausal women (n = 13) than in postmenopausal women (n = 12). In the large study by Young et al. (2003), premenopausal women (n = 493) had lower sleep efficiency, less SWS and more S2 sleep than postmenopausal women (n = 226). The findings in the latter studies were in different sleep variables, which could be partly

explained by variation in sample sizes. In addition, a previous sleep study conducted during over-night blood sampling found no difference in sleep architecture between pre- and postmenopausal women (Lukacs et al. 2004), which is in line with the present results (Study V). Altogether, all the previous PSG studies disagree with the hypothesis drawn from subjective studies that postmenopausal women sleep worse than premenopausal women. The fact that the sleep problems are subjective but not objective does not make them less real but challenges the methodology. It is possible that postmenopausal women may evaluate their sleep quality using other criteria than those that are conventionally considered objective measures of good-quality sleep or can be detected with PSG (Vitiello et al. 2004).

6.2.2. Sleep deprivation and hormone therapy in postmenopausal women

There is no previously published data on the effects of HT on recovery sleep following sleep deprivation. The hypothesis of the present sleep deprivation study (Study II) was that sleep deprivation would make the conventional PSG measurements more sensitive to HT-effects. This hypothesis was partly confirmed since the differences between postmenopausal HT-users and non-users were better found in the recovery night variables than at baseline. Sleep deprivation also amplified the baseline finding of decreased objective sleep quality in postmenopausal women compared to young women. In addition, the perception of the sleep quality of the preceding night was not in agreement with objective measures until sleep propensity was increased by prolonged wakefulness. The recovery mechanisms from sleep deprivation seemed to be relatively well-preserved in postmenopausal women, although the SWS response to sleep deprivation appeared to be weakened after menopause and more so in postmenopausal HT-users.

A discrepancy between subjective and objective sleep quality is common in sleep research, the reasons of which are not fully understood (Baker et al. 1999b, Young et al. 2003, Vitiello et al. 2004). This sleep state misperception makes diagnosis and treatment of sleep problems more difficult for the clinician. In the sleep deprivation study (Study II), the women could estimate their sleep accurately in the morning following recovery sleep but not after the baseline night. Furthermore, the young women had a somewhat better sleep state perception than the older women. Therefore, total sleep deprivation, which increases sleep propensity, could offer a tool for more precise congruence between subjective and objective sleep quality. The subjective variables used (Study I and II) were categorical, and thus exact numeric correlations to objective variables could not be made. In Study I, with only baseline measurements, correlations between subjective and objective sleep variables were scarce. The study groups did not differ in terms of reported vasomotor symptoms or mood symptoms. It is possible that with more symptomatic (climacteric or mental) subjects, objective correlates of subjective symptoms would have become more evident.

Previous sleep deprivation studies have shown that total sleep deprivation results in mainly similar, though not as pronounced, changes in recovery sleep in a comparison between older subjects and young subjects (Carskadon & Dement 1985, Reynolds et al.

1986, Brendel et al. 1990, Gaudreau et al. 2001b). In the present study, sleep efficiency and the percentage of SWS increased and sleep latency, latency to SWS, the percentage of S1 sleep and the number of arousals decreased during recovery sleep compared to baseline sleep in postmenopausal women, similarly to young women. This suggests a relatively well-preserved homeostatic regulation of sleep (see also Chapter 2.1.2.). Power spectrum analysis, however, revealed that SWA increased less in postmenopausal women after sleep deprivation, suggesting a weaker SWS rebound response in comparison with young women. In the older groups, the increase in SWS was reflected as a decline in S1 sleep, whereas in the young controls REM sleep also decreased. Studies using several recovery nights have documented that by the second recovery night sleep architecture approaches baseline, apart from an increase in the REM sleep of young adults (Carskadon et al. 1981, Carskadon & Dement 1985, Bonnet 2005). Because of our study design, with only one recovery night, the evaluation of prolonged REM sleep changes was not possible.

In response to sleep deprivation, the postmenopausal non-HT-users had less S2 sleep during the recovery night compared to postmenopausal HT-users. This seemed to be attributed to a greater amount of SWS, but did not reach statistical significance after Bonferroni correction. In the large observational epidemiological study conducted under normal conditions by Young et al. (2003), a smaller difference in SWS, approximately a little over 6 min, was statistically significant. Thus, the lack of statistical difference in the present study resulted presumably from greater standard deviations and smaller sample sizes. However, the spectral analysis showed greater SWA during the first NREM sleep episode of the recovery night in non-HT-users than in HT-users. This difference was observed also at baseline (although after statistical corrections remained as a trend). During recovery from sleep deprivation, SWA generally increases most during the first NREM sleep episode (Carskadon & Dement 2005), which was also seen in the present study in the young women and in the non-HT-users. In the young women, the SWA had already returned to baseline during the second episode, while in the non-HT users SWA remained higher above baseline throughout the night. It seemed as if the sleep of non-HT-users was less efficient in completing the need for rebound SWA, thus resulting in a prolonged SWA response. In turn, the SWA rebound of HT-users was delayed since they could increase SWA only during the second NREM sleep episode. This increase did not differ from the SWA in non-HT-users during the respective episode, although in HT-users the SWA returned to baseline levels after the one episode of increased SWA, similarly to the young women. This could suggest that recovery processes were less enhanced in HT-users. However, definite answers on the effect of HT can only be drawn from prospective randomized studies in which user bias can be minimized. For example, a woman may choose HT because of already existing poor sleep quality and a reduced ability to respond to prolonged wakefulness. In that case, SWA could be even lower without HT.

6.2.3. The effect of 6-month EPT on sleep quality in pre- and postmenopausal women

Sleep problems are already evident in menstruating premenopausal women approaching menopause and continue until late postmenopause even in healthy women (Leger et al. 2000, Barnabei et al. 2002, Kravitz et al. 2003, Zhang & Wing 2006). These problems may partly be the consequence of low sex hormone levels or aging, or both. Several previous studies also with recently-postmenopausal women have shown that HT in general is beneficial to subjective sleep quality and that it partly improves objective sleep (Polo-Kantola et al. 1998, Montplaisir et al. 2001, Gambacciani et al. 2003, Saletu-Zyhlarz et al. 2003, Sarti et al. 2005). The aim of the present prospective, randomized placebo-controlled study was to evaluate whether HT improves sleep quality in women beyond the typical climacteric era. The findings were marginal and most likely clinically insignificant. It seems that neither the subjective nor objective sleep quality of middle-aged premenopausal or older postmenopausal women is affected by the initiation of HT (Study III).

Middle-aged but still premenopausal women have previously not been investigated in a sleep study similar to the present study (Study III). However, these women are also treated with HT if they are suffering from early climacteric symptoms, and they also report more sleep problems compared both to young women and to their male counterparts (Leger et al. 2000, Zhang & Wing 2006). The present results showed that EPT had hardly any influence on the sleep of premenopausal women, resulting only in more awakenings from S1 sleep than during placebo. Since awakenings from other sleep stages as well as the total number of awakenings did not differ between premenopausal EPT and placebo groups, the clinical relevance of the finding is minimal. In the light of the present results, HT effects on sleep quality need not be considered if HT is justified by other indications in middle-aged premenopausal women.

Sleep complaints of recently postmenopausal women as well as those of older postmenopausal women are reduced during HT (Erkkola et al. 1991, Wiklund et al. 1993, Polo-Kantola et al. 1998, Barnabei et al. 2002, Gambacciani et al. 2003, Sarti et al. 2005), but findings on objectively measured sleep have been inconsistent. All prospective HT studies with unopposed estrogen, either oral or transdermal, have found improved objective sleep quality, although in divergent variables (Thomson & Oswald 1977, Schiff et al. 1979, Erlik et al. 1981, Scharf et al. 1997, Keefe et al. 1999, Polo-Kantola et al. 1999a, Antonijevic et al. 2000b, Saletu-Zyhlarz et al. 2003, Parry et al. 2004). Since ET is suitable only for hysterectomized women, a combination treatment is commonly used, but its effects on objectively measured sleep are inconsistent. CEE combined with micronized progesterone increased sleep efficiency and reduced wake time after sleep onset (Montplaisir et al. 2001) but combination with medroxy-progesterone acetate (MPA) (Pickett et al. 1989) or with norgestrel (Purdie et al. 1995) showed no effects on objective sleep. Instead, E_2 combined with medroxyprogesterone acetate (MPA) increased sleep efficiency and decreased S1 sleep in healthy postmenopausal women (Parry et al. 2004): it also increased REM sleep and

decreased waking episodes in postmenopausal obstructive sleep apnea patients, whose sleep apnea was also reduced (Keefe et al. 1999).

Some of the discrepancy between ET and EPT studies as well as between different EPT studies can be explained by the type of regimen, especially the progestin component, the duration of the treatment and by subject selection. For instance, some have recruited only patients such as postmenopausal insomniacs (Saletu-Zyhlarz et al. 2003) or sleep apnea patients (Keefe et al. 1999). Further, the washout period for previous HT use as well as the length of the treatment was only 2 weeks (Antonijevic et al. 2000b) in one study, whereas a 6-month treatment was used in another (Montplaisir et al. 2001). In the present study, a 6-month treatment of estradiol valerate combined with NETA increased awakenings and decreased SWA during the second NREM sleep period in postmenopausal women under normal conditions (Study III). These minor and rather unfavorable effects on sleep under normal conditions are partly in line with the results of a previous crossover study in which estradiol valerate combined with dienogest increased latency to S1 sleep and decreased total sleep time compared to the previous placebo treatment (Saletu-Zyhlarz et al. 2003). That study was, however, a three-arm trial, in which the effects were not observed in all groupings, and only postmenopausal insomniacs were recruited. In a previous cross-sectional sleep study conducted during frequent blood sampling, the sleep of postmenopausal women on ET was less affected by the intravenous catheter than that of women not using ET (Moe et al. 2001). However, in the present study, no effect of EPT on sleep architecture was observed during the night of blood sampling (Study V).

Sleep disturbances during menopause are often attributed to nocturnal hot flashes or night sweats which can be effectively treated with HT. The alleviation of climacteric vasomotor symptoms could explain the improvements in subjective sleep quality, but PSG studies do not uniformly support a causal relationship between hot flashes and objectively measured sleep disruption (Polo-Kantola et al. 1999a, Freedman & Roehrs 2004, Freedman & Roehrs 2006). Two of the previous sleep studies with EPT administration did not report any vasomotor symptoms (Pickett et al. 1989, Saletu-Zyhlarz et al. 2003), and one investigated only asymptomatic (in terms of climacteric symptoms) sleep apnea patients (Keefe et al. 1999). Those studies that reported a decrease in vasomotor symptoms found either increased sleep efficiency and reduced wake time (Montplaisir et al. 2001) or no improvement in objective sleep (Purdie et al. 1995, Montplaisir et al. 2001) depending on the regimen. In the present study, EPT decreased vasomotor symptoms compared to placebo in postmenopausal women. Nevertheless, subjective sleep quality remained unchanged, and the number of awakenings even increased with EPT. It seems that although the alleviation of vasomotor symptoms is favorable for sleep during the menopausal transition, other factors, especially the effects of aging, are more crucial for older postmenopausal women. For treating sleep problems with HT, menopausal and recently postmenopausal women remain the main target, especially if they have climacteric vasomotor symptoms. Initiation of HT in older postmenopausal women should be regarded with caution since the risks of HT may exceed the benefits (World Health Organization Scientific Group 1996, Writing group for the Women's health initiative

2002, Board of the International Menopause Society 2007, Vitiello et al. 2007). The present study cannot answer the question of whether discontinuation of HT would influence the sleep quality of those older postmenopausal women who have initiated the treatment during menopause.

6.3. Sleep, endocrine function and hormone therapy

6.3.1. The effect of EPT on serum levels of GH, PRL and cortisol

The present study (Study IV) provided further evidence that menopause is associated with decreased 24-h levels of GH and PRL and that EPT returns their levels towards those of the middle-aged premenopausal women. In contrast, the 24-h cortisol production seemed to be independent of menopause, EPT or age. As expected, in middle-aged premenopausal women, EPT had fewer effects, and these were limited to nighttime increases of PRL and cortisol. Although generally the literature on this area is inconsistent, the results of the present study were in line with some of the previous studies on postmenopausal women. In addition to the possible differences generated from varying sample sizes and treatment durations, both the administration route of estrogen and its combination with different progestins affect the outcomes (Weissberger et al. 1991, Campagnoli et al. 2003). In contrast to transdermal administration, oral estrogens pass first through the liver at a high concentration and increase the production of binding proteins, such as SHBG (Campagnoli et al. 2003) and CBG (Qureshi et al. 2007). This may result in no change in active hormone levels despite alterations in total concentrations. Oral estrogens also induce inhibition of hepatic IGF-1 synthesis (Weissberger et al. 1991, Campagnoli et al. 1994). However, combining oral estrogen with androgenic testosterone-derived progestins, such as NETA in the present study, may counteract the estrogen actions on SHBG and IGF-1 synthesis in the liver (Campagnoli et al. 1994, Campagnoli et al. 2003).

6.3.1.1. GH

Previous studies have shown increased GH levels after both short- (10 days) and long term (over 3 years) unopposed oral ET in prospective (Dawson-Hughes et al. 1986, Fröhlander & von Schoultz 1988, Kelly et al. 1993, O'Sullivan & Ho 1995, Bellantoni et al. 1996, Friend et al. 1996, Shah et al. 1999, Bray et al. 2001) and in cross-sectional studies (Moe et al. 1998, Kanaley et al. 2005). In contrast, studies using transdermal ET (O'Sullivan & Ho 1995, Bellantoni et al. 1996, Lieman et al. 2001) or EPT (Bellantoni et al. 1991, Weissberger et al. 1991, Cano et al. 1999) have found no change in GH levels, which has been attributed to the lack of hepatic portal delivery of E_2. Exceptions to this are the studies by Friend et al. (1996) and Genazzani et al. (1997). In the former, increased GH levels with a concomitant decrease in IGF-1 were observed after transdermal administration of E_2 at high doses (two 0.1 mg patches, changed daily or 1 mg orally every 12 h). The authors concluded that the effects seen were most likely related to the inhibited negative feedback from reduced IGF-1 levels and suggested a threshold for E_2 effect on GH release. According to authors, E_2 levels above 600 pmol/L may result in similar degrees of stimulation of GH secretion,

irrespective of the route of administration. In the other study, by Genazzani et al. (1997), the E_2 levels achieved with treatment were not reported, but conventional doses of transdermal E_2 were used. In the studies with no effect on GH during transdermal ET or EPT, the achieved E_2 levels have been lower than 600 pmol/L, but comparable to the levels of premenopausal women.

Previous studies on the effect of oral EPT on GH are fewer and have included various types of estrogen and progestin, but the majority has produced results that are in line with the present study. Two months of cyclic ethinyl-estradiol combined with NETA (Weissberger et al. 1991), three months of cyclic CEE combined with chlormadinone (Fonseca et al. 1999) and ten months of cyclic estradiol valerate combined with dydrogesterone (Hartmann et al. 1995) increased GH levels in postmenopausal women, whereas estradiol valerate combined with MPA (Cano et al. 1999) or estradiol hemihydrate combined with dydrogesterone (Villa et al. 2008) had no effect on GH levels. In the latter study, the subjects were all overweight (mean BMI > 29 kg/m^2). The increased GH levels observed in the previous studies have been comparable to the levels of premenopausal women over 40 years of age (Fonseca et al. 1999) but not those of young women (mean age 27 years) (Hartmann et al. 1995). Since both unopposed estrogen and its combination with NETA increase GH, other factors than modulation of IGF-1 synthesis also have a role in increasing GH during HT. Central mechanisms have also been suggested because estrogen has been shown to increase the peak GH response to GHRH (Dawson-Hughes et al. 1986). The use of oral estrogen combined with oral NETA, which prevents the fall in IGF-1, could protect against a loss of lean body mass induced by oral ET (O'Sullivan & Ho 1995, Nugent et al. 2003). However, androgenic progestogens may also attenuate the favorable effects of oral estrogens on lipid profile (Zegura et al. 2006).

6.3.1.2. PRL

The effect of HT on the PRL levels of middle-aged premenopausal women has not been studied before the present study. Vekemans and Robyn (1975) investigated younger women (aged 18-33 years) and observed an increase in PRL levels after a very high dose of ethinyl estradiol (400 μg). The increase was greater during the day than during the night. In the present study, with a conventional dosage of cyclic EPT, premenopausal PRL increased only during the night. The prospective studies on the effect of oral ET or EPT on postmenopausal PRL levels have produced rather consistent results of increased PRL levels during the treatment (Robyn et al. 1978, Schiff et al. 1979, Chang et al. 1982, Metka et al. 1994, Schlegel et al. 1999, Shah et al. 1999, Parry et al. 2004). Chang et al. (1982) found that PRL levels, both during the night and during the day, were higher after four weeks of cyclic EPT compared to the levels at baseline, which is similar to the results from the present study. However, there are also studies in which no change (Lind et al. 1978) or even a minor decrease (Helgason et al. 1982, Castelo-Branco et al. 1995) in PRL has been observed during oral ET or EPT. The inconsistent results are not explained by treatment regimens, since similar compounds have produced divergent outcomes across studies. The lack of findings in the study by Lind et al. (1978) may be the result of exceptionally small

group sizes. Transdermally administrated ET or EPT seem to have no effect on PRL concentrations according to most of the previous studies (Cagnacci et al. 1991, Perrone et al. 1994, Castelo-Branco et al. 1995), although again the literature is not unanimous (Bednarek-Tupikowska et al. 2006). Restoring the premenopausal PRL concentrations with oral HT in postmenopausal women may not be solely beneficial, since higher circulating PRL may increase the risk for breast cancer (Tworoger et al. 2007).

6.3.1.3. Cortisol

Replacing the low endogenous estrogen levels after menopause has produced conflicting and even confusing outcomes on cortisol levels. In addition to the type of HT, the length of the treatment seems to be one of the major determinants for the divergent results in the prospective studies. Studies with a follow-up of 12 months have reported decreased serum cortisol levels during treatments with various types of estrogen combined with different progestins but no change after a combination of oral estradiol with NETA (Bernardi et al. 2003, Pluchino et al. 2005). The treatment durations might not have been sufficiently long in the other studies to show ET or EPT effects on cortisol (Schiff et al. 1979, Cagnacci et al. 1997, Slayden et al. 1998, Komesaroff et al. 1999, Cucinelli et al. 2002, Parry et al. 2004). However, there are also studies that have even observed increased cortisol concentrations during short-term ET (Gudmundsson et al. 1999) and EPT (Fonseca et al. 2001, Shifren et al. 2007). It is unlikely that the divergent results are due to different blood sampling protocols as both continuous blood sampling for 24 h and single morning blood samples have been used to determine cortisol levels. The results from the present study can be considered reliable since EPT with NETA has not been shown to have any effect on postmenopausal cortisol levels even after 12 months of treatment. The lack of findings with treatment including NETA might originate from the probable inhibitive action of NETA on estrogen-induced CBG synthesis or from the fact that it is not metabolized into progesterone (Kuhl 2005). It has been shown that, when compared to young men, the sleep of middle-aged men is more vulnerable to CRH despite similar elevations of ACTH and cortisol (Vgontzas et al. 2001). Further investigation into the role of CRH in female sleep across menopause and during HT is needed.

6.3.2. The relationship between GH and SWS before and after menopause

The reciprocal interaction of GHRH, dominating during the first half of the night, and CRH, acting during the second half, is suggested to play a major role in sleep regulation (Van Cauter et al. 1998, Steiger 2007). However, in women, GHRH is shown to induce CRH-like effects (Antonijevic et al. 2000a). A close temporal relationship between SWS (Holl et al. 1991, Van Cauter et al. 1992) or SWA (Gronfier & Brandenberger 1998) and GH secretion, with the majority of GH secreted shortly after sleep onset coinciding with SWS (Van Cauter et al. 1998, Van Cauter 2005) has been observed in studies including only men. In the present study, the results of the lack of difference between daytime and nighttime GH levels in postmenopausal women and of HT restoring the decreased postmenopausal GH levels (Study IV) without affecting SWS (Study III) led to the hypothesis that GH and SWS might be

less closely associated after menopause. The present study was able to confirm this (Study V). SWS was more scattered throughout the night after menopause, whereas in the premenopausal women, the majority of SWS was confined to 40-20 minutes before the first nocturnal peak. HT could not improve the association.

A previous study of SWS deprivation in men argued against the close temporal link between SWS and GH and suggested that the timing of nocturnal GH secretion is more dependent on sleep onset (Born et al. 1988). However, the deprivation was not complete but allowed some slow waves and S3 sleep. Based on previous literature, it has been concluded that the strong relationship witnessed between SWS and GH is not obligatory and variations in somatostatinergic tone may account for dissociations (Van Cauter et al. 1998, Steiger 2007). In the present study, postmenopausal SWS was more scattered throughout the night rather than confined close to the first GH peak after sleep onset as in premenopausal women. The first GH peak also occurred later in postmenopausal women, and its timing had more interindividual variation compared to premenopausal women. It seems that other factors than decreased female sex hormone levels underlie the differences found between pre- and postmenopausal women since HT had no effect.

6.3.3. The relationship between PRL and sleep stages in pre- and postmenopausal women

Although estrogen has been shown to increase PRL levels (Vekemans & Robyn 1975, Chang et al. 1982) and the circadian component of PRL secretion has been suggested to be more pronounced in women than in men (Waldstreicher et al. 1996), the previous studies of the association between REM and NREM sleep and the concomitant PRL levels have been conducted in young men. In addition to circadian PRL secretion, the sleep dependent component of secretion has been shown in men as decreasing or unchanged levels during REM sleep onset (Parker et al. 1974, Follenius et al. 1988, Spiegel et al. 1994) and increased levels during SWA (Spiegel et al. 1995) but not with SWS (Van Cauter et al. 1982, Spiegel et al. 1994). The higher nocturnal than daytime levels of PRL as well as the visual inspection of the nocturnal PRL profiles suggested a sleep stage dependent pattern of nocturnal PRL in the pre- and postmenopausal women of the present study. The associations between PRL and sleep stages were studied by means of a new method of creating a computerized mathematical model of link function between nocturnal PRL profiles and sleep stages (Study V). The present study was able to show that nocturnal levels of PRL in the middle-aged premenopausal and older postmenopausal women were partly sleep stage dependent, with lower levels during REM and higher during SWS.

Not all previous studies have supported the finding of lower PRL levels during REM sleep in men (Higuchi et al. 1979, Van Cauter et al. 1982). The development in computer techniques witnessed only during recent years has made it possible to manage more complex models and therefore enabled the use of more sophisticated methods in the present study than in any of the previous studies. However, the present model could still be improved by taking into account the sleep stage history and the

circadian component for the whole night. In addition, only the link function was studied: therefore the underlying physiological mechanisms are not resolved in the present study. The weaker dynamics of the nocturnal PRL profiles in the postmenopausal women compared to the premenopausal women suggested some impact from menopause. However, the model functioned just as well in pre- and postmenopausal women. Therefore, it can be concluded that although the PRL levels were decreased after menopause and could be restored with HT, the association between sleep stages and nocturnal PRL profiles seems not to be dependent on sex hormone levels.

7. SUMMARY AND CONCLUSIONS

In this thesis, the sleep quality of aging women was studied in the normal state, after sleep deprivation and during hormone therapy. In the normal state, sleep qualities of young, premenopausal and postmenopausal women were compared and the effect of HT on premenopausal and postmenopausal sleep quality was studied. After sleep deprivation, the recovery sleep of young and postmenopausal women as well as the effect of HT was studied. Further, the effect of HT on endocrine function and the relationships between endocrine function and sleep were studied. The main outcomes can be summarized as follows:

I Postmenopausal women are less satisfied with their sleep than premenopausal ones. However, neither direct (PSG) nor indirect (cognitive function tests, assessment of sleepiness and mood) measures of sleep disturbances revealed any information regarding possible mechanisms or consequences of the poor sleep perceived by postmenopausal women. The major impairment in objective sleep quality seems to occur even before middle age, and the menopausal state per se does not appear to essentially contribute to this. However, longitudinal studies across menopause could better address this.

II Sleep deprivation amplified the previous finding of decreased objective sleep quality in postmenopausal women compared to young women and decreased sleep misperception. The recovery mechanisms from sleep deprivation seemed to be relatively well preserved in postmenopausal women. HT had disadvantageous but subtle effects on polysomnographically measured recovery sleep, mainly discovered in the spectral analysis of the EEG. In the light of this first study on HT and sleep deprivation, it can be concluded that HT offers no advantage to recovery sleep after prolonged wakefulness.

III HT had only minor and most likely clinically insignificant effects on premenopausal and postmenopausal sleep quality. Neither middle-aged premenopausal nor older postmenopausal women benefit from initiation of estrogen-progestin treatment in terms of their sleep quality.

IV Menopause was associated with decreased levels of GH and PRL. HT returned the levels towards those of the middle-aged premenopausal women. In contrast, the 24-h cortisol production was independent of menopause, EPT or age. As expected, in middle-aged premenopausal women, EPT had fewer effects, which were limited to night-time increases of PRL and cortisol.

V The temporal association between GH and SWS is weakened after menopause and remains weak during HT. In contrast, the sleep stage specific control of PRL secretion, observed with a best-fit mathematical model, seems not to be affected by menopause. PRL levels are lower during REM and higher during SWS in middle-aged premenopausal and older postmenopausal women.

In conclusion, the sleep quality of postmenopausal women is more determined by age than hormonal state. However, the link between GH and SWS is weaker after menopause, though HT has no effect on the association. The temporal association between PRL and sleep stages in pre- and postmenopausal seems to resemble that previously described in young men. The cortisol concentrations seem to be independent of menopause or HT. Although HT restores the decreased levels of GH and PRL after menopause, it offers no advantage to sleep quality in the normal state or after sleep deprivation. Nevertheless, if HT is justified by other indications, its effects on sleep quality need not to be considered in middle-aged premenopausal or older postmenopausal women.

APPENDICES

APPENDIX 1. The effect of HT on sleep. Prospective trials with PSG measurements

HT type	Authors	Study design	Subjects: menopausal state	Subjects: N	Subjects: Age (y) range / mean	Treatment: type and duration	Findings	Comments
ET	Thomson and Oswald 1977	Prospective, placebo-controlled, double-blind	perimenopausal	34	50	Oral piperazine estrone sulphate 1.5mg x2/day or placebo, 8 weeks	Wake time and awakenings ↓ REM sleep ↑	Hot flashes ↓, mood or anxiety ↔ (10 had hot flashes at baseline)
	Schiff et al. 1979	Prospective, placebo-controlled, double-blind, crossover	naturally postmenopausal surgically postmenopausal	8 8	31-65	Oral CEE 0.625 mg/day or placebo 4 weeks then pl/CEE 4 weeks then CEE/pl 4 weeks	Sleep latency ↓ REM sleep ↑	Hot flashes ↓
	Erlik et al. 1981	Case-control	postmenopausal	4	30-55	Oral ethinyl estradiol 50 µg x4/day 4 weeks	Awakenings ↓	Hot flashes ↓ (associated with sleep) No placebo group
	Scharf et al. 1997	Prospective, single-blind, placebo-controlled	postmenopausal	7	45-60	Oral CEE 0.625 mg/day or placebo 4 weeks	Sleep efficiency↑ Cyclic alternating patterns of sleep and awakenings ↓	Hot flashes ↓
	Polo-Kantola et al. 1999	Prospective, randomized, double-blind, crossover	postmenopausal	62	47-65 / 56	Transdermal E_2 50 µg/24h or 2.5 g/day 3 months + 1 month wash-out and cross-over	Movement arousals ↓	Hot flashes ↓ (not associated with objective sleep)
	Antonijevic et al. 2000	Prospective, crossover	postmenopausal	11	46-62	Transdermal E_2 50 µg/24h 2 weeks	REM sleep ↑, wake time ↓ during first two sleep cycles	2-weeks wash-out time for 5 long-term ET users No complaints about hot flashes at baseline
ET + EPT	Saletu-Zyhlarz et al. 2003	Prospective, randomized, double-blind, placebo-controlled, three-arm trial	postmenopausal insomniacs	49	58	Oral estradiol valerate 2mg/day (EV) + dienogest(P)3mg/day or unopposed EV 2 months then open-label phase with EV + dienogest 2mg/day 2 months	Unopposed EV: latency to S2 ↓ EV+P3mg: no change EV+P2mg: latency to S1 ↑ and total sleep time ↓	subjective sleep quality ↑ No measurements for hot flashes
	Parry et al. 2004	Prospective, randomized, placebo-controlled	postmenopausal	14	45-72	Oral E_2 1-2mg or E_2 + MPA 2.5-5mg or placebo 2 months	E_2: WASO ↓ E_2+MPA: sleep efficiency ↑, S1 sleep ↓	PRL amplitude ↑, cortisol ↔ No measurements for hot flashes
EPT	Pickett et al. 1989	Prospective, randomized, placebo-controlled, crossover	postmenopausal	9	46-57 / 50	Oral CEE 1.25 mg/day + MPA 20 mg/day or placebo, 1 week	No improvement in PSG parameters	No measurements for hot flashes Sleep-disoredered breathing ↓
	Purdie et al. 1995	Prospective, randomized single-blind, placebo-controlled	postmenopausal	33	49-60 / 54	Oral CEE 0.625mg/day + norgestrel 0.15mg/day (days 17-28) or placebo 3 months	No improvement in PSG parameters	Hot flashes ↓ Psychological wellbeing ↑
	Keefe et al. 1999	Prospective, crossover	postmenopausal obstructive sleep apnea patients	5	48-62 / 56	E_2 or E_2 + MPA (for 12 days) 3-4 weeks	REM sleep ↑, waking episodes ↓	All free of hot flashes Sleep apnea ↓
	Montplaisir et al. 2001	Prospective, randomized, two group-treatment study	postmenopausal	21	45-65	Oral CEE + either MPA 5mg/day or micronized progesterone 200mg/day 6 months	Sleep efficiency ↑, wake time ↓ during CEE +micronized progesterone	Hot flashes ↓ Subjective sleep quality ↑

HT = hormone therapy, PSG = polysomnography, ET = estrogen therapy, EPT = estrogen-progestin therapy, REM = rapid eye movement sleep, CEE = conjugated equine estrogen, pl = placebo, PRL = prolactin, E_2 = estradiol = 17β-estradiol, S = sleep stage, MPA = medroxyprogesterone acetate, WASO = wake time after sleep onset.

APPENDIX 2. The effect of HT on PRL levels. Prospective trials

HT type	Authors	Study design	Subjects: menopausal state	N	Age (y): range / mean	Treatment type and duration and blood sampling protocol	Findings	Comments
ET	Yen et al. 1974	prospective open-label	naturally postmenopausal surgically postmenopausal	2 2	53-54 37-56	Oral ethinyl estradiol 1ug/kg 4 weeks samples every 15min for 3h at 0,1,2,3,4 weeks	PRL ↑ within the 1st week of treatment plateau was reached in 3-4 weeks	Sampling started at 0800 h or at 1700 h
	Vekemans and Robyn 1975	prospective	premenopausal	5	18-33	Oral ethinyl estradiol 400μg/day from day 5 of cycle samples 15th day of the cycle for 24h at 2h interval at baseline and during treatment (1 cycle)	PRL ↑ increase greater during the day than at night. Circadian pattern was preserved	Very high dose of estradiol
	Robyn et al. 1978	prospective, double-blind cross-over	postmenopausal	7	52-78	Oral ethinyl estradiol 25ug 4 weeks (or moxestrol 5ug) single samples every other day (b/w 1000 and 1600 h) for 1 week before and during	PRL ↑	
	Schiff et al. 1980	Prospective, placebo-controlled, double-blind, crossover	postmenopausal	10	?	Oral CEE 0.625 mg/day or placebo 4 weeks samples every 20min during the night at the end of 28 days	Baseline PRL ↑	No correlation found b/w sleep stages and PRL Sleep latency ↓, REM sleep ↑ Cortisol ↔
	Helgason et al. 1982	prospective, open-label	postmenopausal	14	48-54 / 51	Oral E_2 2mg cyclic, 3 months single samples at 0,1,3 months	PRL ↓	All subjects had typical climacteric complaints
	Shah et al. 1999	prospective, placebo-controlled, crossover	postmenopausal	9	53-71	Oral E_2 2mg or placebo 7-10 days samples every 10min for 24h	PRL ↑	Baseline BMI 23-27 kg/m^2, deconvolution used Wash-out for previous HT was 6 weeks
ET	Chang et al. 1982	prospective, open -label	postmenopausal	5	47-58	Oral ethinyl estradiol 50ug/d 4 weeks (2subj at AM, 2 subj.PM dosing 4wk+4wk:AM↔PM) 3 of the 4 + 1 had combination with MPA 10mg 4 weeks, samples every hour for 24h, 0 and 4 weeks	PRL↑ during the night and day with ET (AM and PM dosing) and EPT	3-10 years from menopause Wash-out for previous HT was 5 months
	Lind et al. 1978	prospective, open-label	postmenopausal	9	?	Oral CEE 1.25mg (n=2) Oral piperazine estrone sulphate 1.5mg (n=2) Oral piperaz. estr. sulp+norethisterone 5mg (n=2) Oral estradiol valerate 2mg+norgestrel 0.5mg (n=3) cyclic, single samples 0,1,2,3,4,5,6 months (day21)	PRL ↔	Women with hysterectomy and and those with current HT were excluded
+	Cagnacci et al. 1991	prospective, open-label	postmenopausal	50	44-55 / 53	Transdermal E_2 50ug, cyclic 6 months then comb.with MPA 5mg cyclic, 12 months. 40 treated, 10 controls.single samples 0,1,3,6,12,24 months	PRL ↔ in ET or EPT	Hot flashes ↓ maximally after 3 months 1-3 years from menopause
	Parry et al. 2004	Prospective, randomized, placebo-controlled	postmenopausal	14	45-72 / 55	Oral E_2 1-2mg or E_2 + MPA 2.5-5mg or placebo 2 months samples every 30min at 1800-1000, 0 and 8 months	E_2: PRL amplitude ↑ E_2+MPA ?	Wash-out for previous HT was 3 months No difference in cortisol. Sleep results in App. 1
EPT	Bednarek-Tupikowska et al. 2006	prospective, open-label	naturally postmenopausal surgically postmenopausal premenopausal controls	54 26 40	51 52 48	→transd.E_2 50μg+oral MPA 5mg (12last days) →transd. E_2 50μg 4 months single sample 0 and 4 months	→ PRL ↑ → PRL ↔	No change in leptin levels (study aim) Postmenop.subj.had climacteric symptoms EPT:mean 20 years from menopause ET: mean 29 years from menopause
EPT	Perrone et al. 1994	Prospective, randomized	postmenopausal	21	40-56	Transd.E_2 50ug cont. + transd.NETA 250ug cyclic (n=11) transd.E2 cont+oral MPA 10mg cyclic (n=10) 6 months single samples 0 and 6 months	PRL ↔	All subjects had climacteric symptoms At least 1 year from menopause
	Castelo-Branco et al. 1995	prospective, randomized	postmenopausal	90	43-57 / 49	Oral CEE 0.625mg cyclic+ MPA 2.5mg cyclic (n=15) transd.E_2 50ug,cyclic+MPA 2.5mg cyclic (n=15) oral CEE 0.625mg cont.+MPA 2.5mg cyclic (n=15) oral CEE 0.625 cont.+ MPA 2.5mg cont. (n=18) control group treatment free (n=22) 12 months samples 0,6,12 months (between days 21-24)	→ PRL ↓ at 6 and 12 months → PRL ↓ at 6 and 12 months → PRL ↓ at 6 and 12 months → PRL ↓ at 12 months	All subjects had climacteric symptoms 1-3 years from menopause
	Metka et al. 1995	prospective, open-label	postmenopausal	371	51	Oral CEE 0.625mg cont+ gestagen medrogestone 5mg cyclic, 6months (n=224), control group (n=147) single samples 0,3,6 months (between days 1-7)	PRL ↑ at 3 and 6 months	
	Schlegel et al. 1999	prospective, open-label	postmenopausal	38	48-62	Oral CEE 0.3mg (n=13) or 0.6mg (n=13) or 1.25mg (n=12) cyclic +medrogestone 5mg cyclic, single samples 0,1,6mo(days 10,21,28)	PRL ↑ with 0.6mg and 1.25mg CEE +medrogestone	LDL, cholesterol, apoB ↓ and E_2, SHBG ↑

HT = hormone therapy, ET = estrogen therapy, EPT = estrogen-progestin therapy, PRL = prolactin, CEE = conjugated equine estrogen, REM = rapid eye movement, E_2 = estradiol = 17β-estradiol, BMI = body mass index, MPA = medroxyprogesterone acetate, NETA = norethisterone, LDL = low density lipoprotein, SHBG = sex hormone binding globulin, ? = not reported.

APPENDIX 3. The effect of HT on GH levels. Prospective trials

HT type	Authors	Study design	Subjects: menopausal state	Subjects: N	Subjects: Age (y) range / mean	Treatment type and durration and blood sampling protocol	Findings	Comments
ET	Dawson-Hughes et al. 1986	prospective, open-label	postmenopausal	17	50-73	Oral ethinyl estradiol 20ug, 15 days samples 0 and 15 days	GH↑	Normal weight women studied separately
	Fröhlander and Schoultz 1988	prospective, open-label	postmenopausal	14	44-62 / 53	Oral ethinyl estradiol 10ug cyclic single samples 0 and 3 months	GH ↑	IGF-1 ↓. All subjects had climacteric symptoms. At least 6 months from menopause. Wash-out for any previous medication was 3 months.
	Kelly et al. 1993	prospective, randomized, cross-over	postmenopausal	6	54-71 / 60	Oral ethinyl estradiol 20ug, CEE 1.25mg or estradiol valerate 2mg 1 month, samples every hour for 24h, 0 and 1 months (MPA after sampling)	mean 24-h GH ↑	GHBP, SHBG↑ IGF-1 ↓ (%-decrease related to %-increase in GH) BMI not mentioned (subjects of normal weight)
	O'Sullivan and Ho 1995	prospective, open-label, randomized, cross-over	postmenopausal	9	62	Transdermal E_2 100µg/day oral CEE 1.25mg, 12 weeks MPA 12 days then cross-over, single samples 0 and 10 weeks	→ GH ↔ → GH ↑	No difference and no change in body weight
	Bellantoni et al. 1996	prospective, randomized, cross-over, placebo-controlled	postmenopausal	16	49-75	Oral CEE 1.25mg transdermal E_2 100µg 6+ 6weeks (8-week treatment free interval). Samples every 20min at 2000-0800. (MPA after the final protocol)	→ GH ↑ → GH ↔	IGF-1 ↓ with oral ET GHRH stimulated GH did not differ GHRH stimulated GH was not affected with either ET Baseline BMI did not differ.
	Friend et al. 1996	prospective, randomized, cross-over	postmenopausal	8	52-80	Oral E_2 1mg twice daily or transd. E_2 100µg 2patches/day 15 days + 15 days (separated by 8 weeks) samples every 5min for 24h	GH ↑ (pulse height, pulse area, incremental pulse area, interpeak valley conc. and valley nadir during both treatments)	Pulse frequency ↔ IGF-1 ↓ Mean BMI did not change during the study
	Genazzani et al. 1997	prospective, randomized	postmenopausal	30	?	transd. E_2 50ug or 100ug or tibolone 2.5mg, 5 weeks (MPA 10mg for 12 days after 5 weeks). Single samples 0 and 5 weeks + 5 subjects in each group: sampling every 10min for 6h	GH ↑ (mean and pulse amplitude) number of GH pulses ↓ in all groups	IGF-1 ↓ E_2 levels during treatment not reported
	Shah et al. 1999	prospective, placebo-controlled, crossover	postmenopausal	9	53-71	Oral E_2 1mg twice daily or placebo 7-10 days samples every 10min for 24h	GH ↑ (24-h mean app.doubled and amplit.↑) basal GH, pulse frequency, -duration ↔	PRL ↑. Baseline BMI mentioned, deconvolution Wash-out for previous HT was 6 weeks
	Lieman et al. 2001	prospective	postmenopausal premature ovarian failure	8 8	51-70 25-40	Transdermal E_2 (50-100µg to achieve E_2 conc.of 367pmol/l) 8 weeks (8 had used longer) samples every 10min for 24h	GH seemed to ↑ but after controlling for BMI difference dissappeared GH greater in the young than in the older	BMI greater in postmenopausal, adjusted for BMI
	Bray et al. 2001	prospective, single-blind, cross-over, placebo-controlled	postmenopausal	13	58	Oral micronized E_2 1mg twice daily or placebo 14 days treatment-free washout 4 weeks. Samples every 10min at 0800-1400	GH ↑ (mean 6-h fasting) compared to placebo	Mean BMI mentioned but no correlations made
EPT	Bellantoni et al. 1991	prospective, randomized, double-blind, placebo	postmenopausal	28	45-71	Transd.E_2 0, 50, 100 and 150ug/day +oral MPA 10mg/day weeks 3-4 and 7-8, 8 weeks, samples 0 and 8 weeks	GH ↔	No baseline BMI differences, (some)correlations made Wash-out for previous HT was 6 weeks
	Weissberger et al. 1991	prospective	postmenopausal premenopausal controls	12 7	53-77 18-28	Oral ethinyl estradiol 20µg (n=7) or transdermal E_2 100µg (n=7) cyclic, both (n=2) +NETA 5mg cyclic (days 15-21), 2 cycles samples every 20min for 24h, 0 and 3rd cycle	GH ↑ (24-h mean, pulse amplitude) with oral GH ↔ with transdermal	IGF-1 ↓ with oral, ↑ with transdermal Postmenopausal GH < premenopausal GH BMI did not differ between groups, not controlled (2 had both treatments separated by 6 months)
	Hartmann et al. 1995	prospective, randomized	postmenopausal premenopausal controls	23 25	39-56 / 52 20-37 / 28	Oral estradiol valerate 2mg cont+dydrogesterone 10mg for 10 d, 10 months, treatment group (n=13), no treatment (n=10) samples every 2h for 24h, 0 and 10 months	GH ↑ (did not reach the premenopausal levels)	IGF-1 ↑ BMI differed b/w young and postm. but not b/w postmenopausal groups, not controlled for BMI
	Fonseca et al. 1999	prospective, randomized	postmenopausal premenopausal controls	50 ?	46-65 40-49	Oral CEE 0.625mg or 1.25mg/day cyclic + chlormadinone acetate 2mg cyclic (5 days), 3 cycles single sample at 0700-0800, 0 and 3rd cycle (day21)	GH ↑ (reached premenopausal levels) pos.correlation between GH&IGF-1 and estradiol in CEE 0.625mg	IGF-1 ↑ and insulin ↓ BMI not mentioned
	Cano et al. 1999	prospective, randomized, cross-over	postmenopausal premenopausal controls	19 17	34-61 / 49 31-46 / 38	Oral estradiol valerate 2mg or transd. E_2 50 or 100µg + MPA 5mg weeks 3-4, 7-8. 8 weeks single samples at 0900, before and last day	GH ↔ with all treatments	No difference between pre- and postmenopausal GH IGF-1 ↑ with oral No difference in BMI b/w the groups, correlations NS
	Villa et al. 2008	prospective, double blind, placebo-controlled	postmenopausal	18	53	hemihydrate E_2 2mg cont + dydrogesterone 10mg cyclic (n=10) or placebo (n=8), 2 months, single samples 0 and 2 months	GH ↔	Ghrelin ↔ BMI high (mean>29, no difference b/w the groups)

HT = hormone therapy, GH = growth hormone, ET = estrogen therapy, EPT = estrogen-progestin therapy, IGF-1 = insulin-like growth factor 1, CEE = conjugated equine estrogen, GHBP = GH binding protein, SHBG = sex hormone binding globulin, BMI = body mass index, E2 = estradiol = 17β-estradiol, MPA = medroxyprogesterone acetate, PRL = prolactin, NETA = norethisterone, NS = non-significant, ? = not reported.

APPENDIX 4. The effect of HT on cortisol levels. Prospective trials

HT type	Authors	Study design	Subjects: menopausal state	Subjects: N	Subjects: Age (y) range / mean	Treatment type and duration and blood sampling protocol	Findings	Comments
ET	Schiff et al. 1980	Prospective, placebo-controlled, double-blind, crossover	postmenopausal	10	?	Oral CEE 0.625 mg/day or placebo 1 month samples every 20 min during the night at the end of 28 days	Baseline cortisol ↔	No correlation b/w sleep stages and cortisol Sleep latency ↓, REM sleep ↑, PRL ↑
	Slayden et al. 1989	prospective, open-label	postmenopausal	14	54-67 / 60	transdermal E_2 50µg/day, 3 months single morning sample 0 and 3 months	Cortisol ↔	
	Cagnacci et al. 1997	prospective, open-label	postmenopausal	7	54-62	Oral CEE 0.625mg, cyclic at least 2 cycles samples every 20min for 48h	Cortisol ↔ 1st nocturnal peak delayed of about 60min	
	Gudmundsson et al. 1999	prospective, open label	postmenopausal	6	54-61	Oral CEE 0.625mg 6-8wks samples every 15min for 24h	Cortisol ↑ (24-h mean, amplitude, peak level, nadir)	1 of the subjects had climacteric symtoms ET lowered the 24-h body temperature
	Komesaroff et al. 1999	prospective, randomized, placebo-controlled, double-blind	perimenopausal	12	48-50	Oral estradiol valerate 2mg or placebo 2 months single samples 0 and 2 months	Cortisol ↔	FSH was elevated in all subjects (cortisol response to stress ↑ during ET)
	Cucinelli et al. 2002	prospective, open-label	postmenopausal controls	20 8	47-63 ?	Transdermal E_2 50µg/day 3 months control patients were on placebo single samples 0 and 3 months	Cortisol ↔	All were seeking treatment for climacteric symptoms Progesterone-induced withdrawal after the study
ET + EPT	Bernardi et al. 2003	prospective, open-label	postmenopausal	186	55	Transdermal E_2, 50µg/day, (TE) (n=40), TE cont.+MPA 10mg cyclic (n=18), TE cont.+ nomegestrol 5mg cyclic (n=16), TE cont.+dihydrogesterone 10mg cyclic (n=19), Oral E_2 2mg cont.+NETA 1mg cyclic (n=6), Oral E_2 2mg cont.+NETA 1mg cont. (n=18), Oral estradiol valerate 2mg+levonorgestrel 0.075mg cyclic (n=9), Oral estr.val.2mg +cyproterone acetate1mg cyclic (n=12), Oral CEE 0.625mg cyclic+MPA 5mg cyclic (n=21) Oral CEE cont.+MPA cont. (n=10) single samples 0, 1, 3, 6, 9, 12 months at 0800-0900 (end of cycle)	Cortisol ↓ progressively in all but NETA groups. Cortisol ↔ in NETA-groups	The type of HT according to individual preference but randomized to the various groups of therapy
	Parry et al. 2004	Prospective, randomized, placebo-controlled	postmenopausal	14	45-72 / 55	Oral E_2 1-2mg or E_2 + MPA 2.5-5mg or placebo 2 months samples every 30min at 1800-1000, 0 and 2 months	Cortisol ↔ with ET or EPT	ET: PRL amplitude ↑ sleep results in Appendix 1
	Pluchino et al. 2005	prospective, open-label	postmenopausal	85	54	Transdermal 50µg (n=40) (hyst.- +ovariectomized) Oral E_2 + NETA 1mg cont. (n=18) Oral CEE 0.625mg + MPA 5mg cont. (n=10) Oral tibolone 2.5mg (n=17) single samples 0,1,3,6,9,12 months at 0800-0900	→ Cortisol ↓ at 6 mo, min. at 12 months → Cortisol ↔ → Cortisol ↓ at 12 months → Cortisol ↔	
EPT	Fonseca et al. 2001	prospective, open-label	postmenopausal	25	47-60 / 52	Oral CEE 0.625mg cyclic +chlormadinone acetate 2mg cyclic single samples 0 and 3 months at 0700-0800	Cortisol ↑	Positive correlation between E_2 and estradiol
EPT	Pripp et al. 2004	prospective, placebo-controlled	postmenopausal coronary heart disease patients	28	63	Oral CEE 0.625mg cont.+MPA 5mg cyclic (n=15) or placebo (n=13) single samples 0, 6 and 12 months at 0800-0900	Cortisol ↔	SHBG and CBG levels ↑
	Shifren et al. 2007	prospective, randomized, open-label, crossover	postmenopausal	27	56	Oral CEE 0.625mg+micronized progesterone 100mg cont., transdermal E_2 50µg/day+oral micr.prog.100mg cont. single samples 0 and 3 months	→ Cortisol ↑ (total) ↔ (free) → Cortisol ↔ (total or free)	CBG ↑ with oral EPT All were current HT-users with 6-wk withdrawal before the study

HT = hormone therapy, ET = estrogen therapy, EPT = estrogen-progestin therapy, CEE = conjugated equine estrogen, REM = rapid eye movement, PRL = prolactin, E_2 = estradiol = 17β-estradiol, FSH = follicle stimulating hormone, MPA = medroxyprogesterone acetate, NETA = norethisterone acetate, SHBG = sex hormone binding globulin, CBG = cortisol binding globulin, ? = not reported.

Appendix 5. The Basic Nordic Sleep Questionnaire (BNSQ): Questions concerning insomnia and sleepiness (Partinen & Gislason, 1995)

Insomnia:

1. How often have you had difficulties falling asleep during the past three months?
    1. Never or less than once per month
    2. Less than once per week
    3. On 1-2 evenings (nights) per week
    4. On 3-5 evenings (nights) per week
    5. Every evening (night) or almost every evening (night)

2. How often have you woken up during the night during the past three months?
    1. Never or less than once per month
    2. Less than once per week
    3. On 1-2 nights per week
    4. On 3-5 nights per week
    5. Every night or almost every night

3. If you usually wake up during the night, how many times did you wake up in the course of the night during the past three months?
    1. Usually I do not wake up at night
    2. Once per night
    3. 2 times per night
    4. 3-4 times per night
    5. At least 5 times per night

4. How often have you woken up too early in the morning and not been able to fall asleep again during the past three months?
    1. Never or less than once per month
    2. Less than once per week
    3. On 1-2 nights per week
    4. On 3-5 nights per week
    5. Every morning or almost every morning

5. How have you slept during the past three months?
    1. Well
    2. Quite well
    3. Not well but not badly either
    4. Quite badly
    5. Badly

Sleepiness:

1. Have you felt disturbingly tired in the mornings during the past three months?
    1. Never or less than once per month
    2. Less than once per week
    3. On 1-2 nights per week

4. On 3-5 nights per week
5. Every morning or almost every morning

2. Did you feel disturbingly tired during the daytime during the past three months?
   1. Never or less than once per month
   2. Less than once a week
   3. On 1-2 days per week
   4. On 3-5 days per week
   5. Daily or almost daily

3. Have you suffered from compulsive falling asleep at work during the past three months?
   1. Never or less than once per month
   2. Less than once per week
   3. On 1-2 days per week
   4. On 3-5 days per week
   5. Daily or almost daily

4. Have you suffered from compulsive falling asleep during your leisure time during the past three months?
   1. Never or less than once per month
   2. Less than once per week
   3. On 1-2 days per week
   4. On 3-5 days per week
   5. Daily or almost daily

5. How often do you take a nap during the daytime?
   1. Never or less than once per month
   2. Less than once per week
   3. On 1-2 days per week
   4. On 3-5 days per week
   5. Daily or almost daily

Appendix 6. Morning questionnaire: Questions concerning the sleep of the preceding night.

1. I fell asleep (sleep latency)
   1. soon
   2. quite soon
   3. after a long time

2. I slept (sleep efficiency)
   1. very well
   2. well
   3. rather well
   4. poorly
   5. very poorly

3. I slept (sleep quality)
   1. better
   2. the same
   3. worse than usually

4. During the night I woke up (awakenings)
   1. not once
   2. once
   3. several times

5. I woke up earlier than usual and could not get back to sleep (morning awakening)
   1. not true
   2. true

6. In the morning I felt (morning tiredness)
   1. fresh
   2. quite fresh
   3. quite tired
   4. tired

Appendix 7. Mood test
Make a mark (x) on each line, indicating how you are feeling at the moment.

1. very depressed ---------- not depressed at all
2. not tired at all ---------- very tired
3. very sleepy ---------- not sleepy at all
4. not tense at all ---------- very tense
5. very irritable ---------- not irritable at all

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