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Nocturnal Transcutaneous Carbon Dioxide and Early Changes in Atherosclerosis in Pre- and Postmenopausal Women

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To Aida, Venla and Tero

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ABSTRACT

The risk of cardiovascular diseases and sleep-disordered breathing increases after menopause. This cross-sectional study focuses on overnight transcutaneous carbon dioxide ($TcCO_2$) measurements and their power to predict changes in the early markers of cardiovascular and metabolic diseases. The endothelial function of the brachial artery, the intima-media thickness of the carotid artery, blood pressure, glycosylated hemoglobin A1C and plasma levels of cholesterols and triglycerides were used as markers of cardiovascular and metabolic diseases.

The study subjects consisted of healthy premenopausal women of 46 years of age and postmenopausal women of 56 years of age.

From wakefulness to sleep, the $TcCO_2$ levels increased more in postmenopausal women than in premenopausal women. In estrogen-users the increase in $TcCO_2$ levels was even more pronounced than in other postmenopausal women. From the dynamic behaviour of the nocturnal $TcCO_2$ signal, several important features were detected. These $TcCO_2$ features had a remarkable role in the prediction of endothelial dysfunction and thickening of the carotid wall in healthy premenopausal women. In addition, these $TcCO_2$ features were linked with blood pressure, lipid profile and glucose balance in postmenopausal women.

The nocturnal $TcCO_2$ profile seems to contain significant information, which is associated with early changes in cardiovascular diseases in middle-aged women. $TcCO_2$ might not only measure the tissue carbon dioxide levels, but the $TcCO_2$ signal variation may also reflect peripheral vasodynamic events caused by increased sympathetic activity during sleep.

Keywords: sleep, oxygen, carbon dioxide, transcutaneous measurement, menopause, cardiovascular diseases

Jenni Aittokallio

Unenaikainen transkutaaninen hiilidioksidimittaus ja sydän- ja verisuonitautien varhaismuutokset pre- ja postmenopausaalisilla naisilla

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TIIVISTELMÄ

Sydän- ja verisuonisairauksien ja unenaikaisten hengityshäiriöiden vaara lisääntyy naisilla vaihdevuosien jälkeen. Väitöskirjatyössä pyrittiin selvittämään, onko vaihdevuosi-iässä tapahtuvilla verenkierron ja hengityksen muutoksilla yhteyttä keskenään. Varhaisia sydän- ja verisuonisairauksien tai metabolisen oireyhtymän muutoksia tutkittiin mittaamalla olkavaltimon endoteelin toimintaa, kaulavaltimon seinämän paksuutta, verenpainetta, glykosyloitunutta hemoglobiini-A1C:ta sekä plasman kolesteroleja ja triglyseridiä. Unenaikaista hengitystä tutkittiin unipolygrafian avulla, jossa aivosähkötoiminnan lisäksi mitattiin ilmavirtausta ja noninvasiivisesti verikaasuja. Erityisen mielenkiinnon kohteena oli yöllinen ihon kautta diffundoituvan hiilidioksidin ($TcCO_2$) jatkuva mittaus. Tutkimuksiin osallistuivat perusterveet 46-vuotiaat premenopausaaliset ja 56-vuotiaat postmenopausaaliset naiset.

$TcCO_2$-pitoisuudet nousivat lähes kaikilla naisilla siirryttäessä valvetilasta uneen, mutta nousu oli postmenopausaalisten ryhmässä suurempi kuin premenopausaalisilla naisilla. Lisäksi estrogeenihoitoa käyttävillä naisilla $TcCO_2$ nousi nukkuessa vielä enemmän kuin muilla postmenopausaalisilla naisilla. $TcCO_2$-mittauksista tutkittiin myös signaalin vaihtelua. Unenaikaisen $TcCO_2$-käyrän dynaamisessa vaihtelussa ilmeni piirteitä, joilla pystyttiin ennustamaan verisuonten endoteelin toiminnan heikkenemistä ja kaulasuonen paksuuntumista jo vielä terveillä premenopausaalisilla naisilla. Lisäksi nämä $TcCO_2$-piirteet olivat yhteydessä verenpaineeseen, veren rasva-arvoihin ja sokeritasapainoon postmenopausaalisilla naisilla.

Väitöskirjatyön perusteella unenaikaiset $TcCO_2$-signaalin piirteet ovat yhteydessä sydän- ja verisuonitautien varhaismuutoksiin keski-ikäisillä naisilla. $TcCO_2$-vaihtelut eivät ehkä mittaa ainoastaan kudoksen CO_2-osapaineen muutoksia, vaan voivat kuvantaa myös ihoverisuoniston sympaattisen hermoston ja ihoverisuonen endoteelin toiminnan aktiivisuutta.

Avainsanat: uni, happi, hiilidioksidi, transkutaaninen mittaus, vaihdevuodet, sydän- ja verisuonisairaudet

CONTENTS

ABBREVIATIONS

AHI	apnea-hypopnea index
Ang-II	angiotensin II
ANOVA	analysis of variance
AT1	angiotensin 1
AUC	area under the ROC curve
BMI	body mass index
BNSQ	The Basic Nordic Sleep Questionnaire
BP	blood pressure
CO_2	carbon dioxide
CPAP	continuous positive airway pressure
CVD	cardiovascular diseases
DBP	diastolic BP
DMII	diabetes mellitus type II (2)
ECG	electrocardiogram
EDS	excessive daytime sleepiness
EEG	electroencephalogram
EMG	electromyogram
EOG	electro-oculogram
ESS	Epworth sleepiness scale
ET	estrogen therapy
ET-1	endothelin-1
$EtCO_2$	end-tidal CO_2
F	female
FEV_1	forced expiratory volume in one second
FMD	flow-mediated dilatation
FSH	follicle stimulating hormone
GHbA1C	glycosylated hemoglobin A1C
HDL	high-density lipoprotein (cholesterol)
HF	high frequency
IMT	intima-media thickness
IRR	increased respiratory resistance IU international unit
LDL	low-density lipoprotein (cholesterol)
LF	low frequency
M	male
n	number of subjects
NMD	nitroglycerin-mediated dilatation
NO	nitric oxide
NREM	non-REM (sleep)
OSA	obstructive sleep apnea
ODI_4	arterial oxyhemoglobin desaturation of 4% units or more per hour
$PaCO_2$	partial pressure of arterial CO_2
PSG	polysomnography
RDI	respiratory disturbance index REM rapid eye movement (sleep)
RERA	respiratory effort-related arousal ROC receiver operating characteristic S1 sleep stage 1
S2	sleep stage 2
S3	sleep stage 3
S4	sleep stage 4
SaO_2	arterial oxyhemoglobin saturation
SBP	systolic BP
SDB	sleep-disordered breathing
SWS	slow-wave sleep
$TcCO_2$	transcutaneous CO_2
XOR	xanthine oxidoreductase

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-IV. These original works have been reproduced with the permission of the copyright holders.

I Aittokallio J, Virkki A, Aittokallio T, Saaresranta T, Polo-Kantola P and Polo O: Non-invasive respiratory monitoring during wakefulness and sleep in pre- and postmenopausal women. *Respir Physiol Neurobiol* 2006;150:66-74.

II Aittokallio J, Polo O, Hiissa J, Virkki A, Toikka J, Raitakari O, Saaresranta T, Aittokallio T. Overnight variability in transcutaneous carbon dioxide predicts vascular impairment in women. *Exp Physiol* 2008;93: 880-91.

III Aittokallio J, Hiissa J, Saaresranta T, Polo-Kantola P, Aittokallio T, Polo O. Nocturnal transcutaneous carbon dioxide tension in postmenopausal estrogen-users and non-users. *Menopause Int* 2009;15:107-12.

IV Aittokallio J, Saaresranta T, Virkki A, Karppinen N, Heinonen O, Aittokallio T, Polo O. Transcutaneous carbon dioxide profile during sleep reveals metabolic risk factors in postmenopausal women. *Eur Respir J* 2009;34:1132-39.

1. INTRODUCTION

The cessation of ovarian production of female sex hormones has been put forward as a culprit for the increased risk of cardiovascular diseases (Arias 2006) and sleep-disordered breathing (SDB) (Bixler et al. 2001) after menopause. Estrogen and progesterone are known to have an impact on sleep and breathing (Bixler et al. 2001, Saaresranta et al. 1999, Young et al. 2003a). Moreover, the risk of metabolic syndrome (defined as insulin resistance, abdominal obesity, dyslipidemia and elevated blood pressure) increases during the menopausal transition (Davidson et al. 2002, Salpeter et al. 2006). The mechanisms behind these interactions are not fully understood. However, an autonomic nervous system imbalance (increased sympathetic and decreased parasympathetic activity) is likely to be involved (Narkiewicz et al. 1998). The early detection of the risk factors for developing cardiovascular and metabolic abnormalities would be of importance in order to prevent complications of these diseases.

An increased carotid artery intima-media thickness (IMT) is an established marker of subclinical atherosclerosis (Salonen & Salonen, 1990). Arterial endothelial dysfunction is another important early marker of atherosclerosis (Celermajer et al. 1992). Endothelial dysfunction is commonly measured using flow-mediated dilatation (FMD) together with nitroglycerin-mediated dilatation (NMD). Both increased IMT (Drager et al. 2005, Schulz et al. 2005) and impaired FMD (Ip et al. 2004; Nieto et al. 2004; Oflaz et al. 2006) have been linked with SDB. Women with SDB seem to be more vulnerable to endothelial dysfunction than men (Faulx et al. 2004). In previous studies, the degree of vascular impairment has been explained by nocturnal hypoxemia (Nieto et al. 2004, Suzuki et al. 2004, Baguet et al. 2005, Minoguchi et al. 2005). The impact of nocturnal levels of carbon dioxide (CO_2) on vascular impairment has not been studied.

Despite the important influence of menopause on breathing, the physiological respiratory changes during wakefulness and sleep are poorly described. The control of breathing during wakefulness and sleep is critically regulated according to the CO_2 levels. However, the efficacy of nocturnal respiration is often assessed via the arterial oxyhemoglobin saturation (SaO_2) only. The CO_2 levels can be assessed non-invasively with end-tidal carbon dioxide ($EtCO_2$) and transcutaneous carbon dioxide ($TcCO_2$) measurements. The $EtCO_2$ estimates alveolar carbon dioxide levels. Resting high levels of $EtCO_2$ have been linked with a higher systolic blood pressure and a thicker carotid wall in women (Anderson et al. 1999, Anderson et al. 2001). $TcCO_2$ measurements are affected by cutaneous vasoconstriction (Healey et al. 1987), mostly due to changes in blood flow, which affects the capillary CO_2 gradient (Severinghaus et al. 1978, Beran et al. 1981).

Although $TcCO_2$ was used in the assessment of nocturnal hypercapnia in patients with respiratory insufficiency more than two decades ago (Midgren et al. 1984), it has not

gained wide acceptance in sleep research. $TcCO_2$ has been considered to be insensitive to rapid respiratory changes, and not feasible for CO_2 measurements in adults. The information provided by the nocturnal $TcCO_2$ profile has not been studied in detail. Of great interest is the pioneering finding that nocturnal $TcCO_2$ seems to associate with overnight changes in cardiac function and circulation time (Tkacova et al. 2001).

The aim of this study was to investigate the early changes in nocturnal respiratory measurements linked with cardiovascular and metabolic consequences in generally healthy pre- and postmenopausal women. The main focus was on the information derived from the nocturnal $TcCO_2$ profile.

2. REVIEW OF THE LITERATURE

2.1 Sleep

Sleep is the natural state of rest in humans. The need for sleep varies markedly by age, individual need, emotional factors and physical fatigue. However, good and regular sleep is essential for health and well-being.

2.1.1 Sleep regulation

Sleep timing depends upon a balance between homeostatic sleep propensity, the need for sleep as a function of time since the last adequate sleep episode occurred, and circadian rhythms which determine the ideal timing of a correctly structured and restorative sleep episode (Borbély & Achermann, 1999). The homeostatic drive for sleep is usually greatest in the first half of the sleep period and the circadian drive in the latter half. During wakefulness, the concentration of adenosine in the basal forebrain increases, thus promoting sleep, and its concentration falls during sleep (Porkka-Heiskanen et al. 1997). The circadian period is approximately 24-25 hours, but it is only manifested when the person is completely isolated from the outside world (in a windowless room or cave) (Despopoulos & Silbernagl 2003). The main external regulator for 24-hour synchronization is bright light. However, the circadian rhythm is also strongly influenced by environmental, social, genetic and pharmacological aspects, as well as age (Czeisler et al. 2000). The endogenous circadian rhythm is regulated through the suprachiasmatic nuclei of the anterior hypothalamus. The circadian element causes the release of the hormone melatonin and a gradual decrease in core body temperature (Czeisler et al. 2000).

Around the time of sleep onset, sympathetic activation decreases and the parasympathetic tone becomes predominant. Numerous neurotransmitters and neuromodulators are involved in the complex process of falling asleep. The disfacilitation of the thalamocortical systems results in increased slow wave activity in the electroencephalogram (EEG) (Jones 2005).

2.1.2 Sleep architecture

Sleep is commonly divided into cycles of rapid eye movement (REM) sleep and the three stages of non-rapid eye movement (NREM) sleep using a polygraphic sleep recording (polysomnopraphy, PSG), which entails the continuous monitoring of an EEG, electro-oculogram (EOG), and electromyogram (EMG). Stages of REM and NREM (Stages S1-S4) are traditionally determined by criteria of Rechtschaffen and Kales **(**Rechtschaffen & Kales**,** 1968**)** (Figure 1). While falling asleep, S1-S4 normally appear in sequential order. S1 sleep is "light" sleep, actually an intermediate stage between wakefulness and the 'true' sleep stages. S2 typically appears after a short episode of S1, and is characterized by K complexes and sleep spindles (Carskadon & Dement 2000). The deepest sleep stages are

S3 and S4, which are together often referred to as slow wave sleep (SWS). The criterion for scoring SWS is the appearance of at least 20% of high-voltage EEG activity (> 75 μV) in a 30-second epoch (Figure 2). In REM sleep, as during wakefulness, the EEG activity looks desynchronized. At the same time, EMG activity decreases markedly and rapid eye movements are present (Figure 2). These stages of NREM and REM sleep follow each other cyclically during the night, although SWS usually manifests itself more in the beginning of the sleep period and REM sleep dominates in the morning hours (Figure 1).

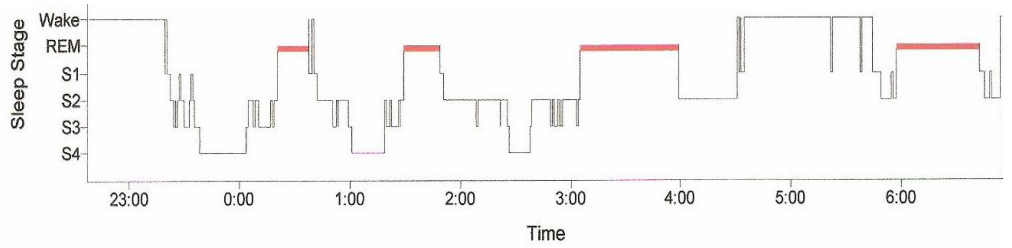

Figure 1. A hypnogram of a 46-year-old woman. Sleep stages S1-4 and rapid eye movement (REM) sleep follow each other cyclically during the night.

The above method has, for a long time, been the gold standard for the definition of sleep stages. It is well-known and widely used in sleep research. Having been, for almost 40 years, the mainstay of sleep medicine, the Rechtschaffen and Kales criteria have now been replaced with the American Academy of Sleep Medicine Visual Scoring Task Force terminology and rules (Iber et al. 2007).

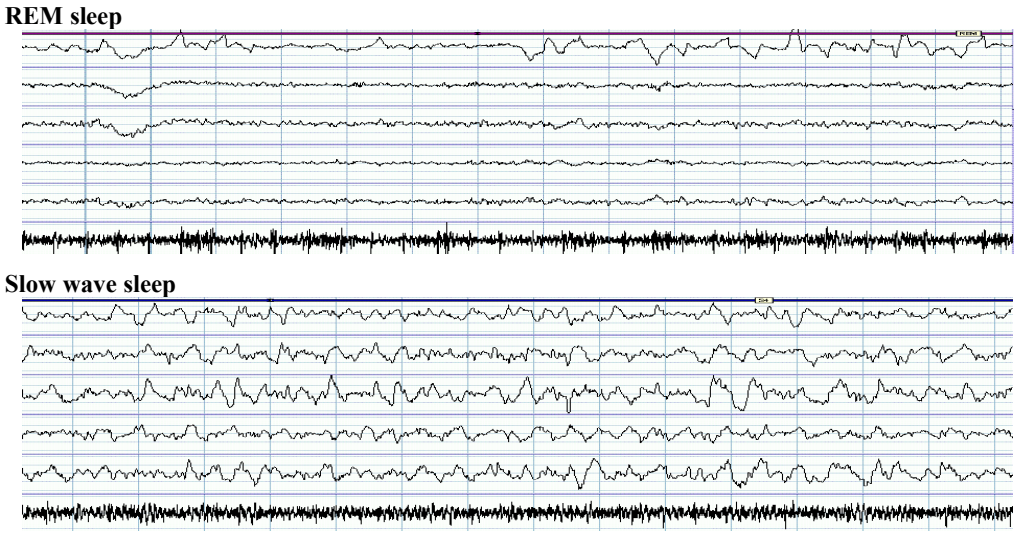

Figure 2. An example of the scoring view of rapid eye movement (REM, superior graph) and Stage 4 sleep (Slow wave sleep, SWS, inferior graph). The upper-most channels of the graphs are electro-oculograms, the next four channels are electroencephalograms and at the bottom are electromyograms. During REM sleep characteristic eye movements are present and during SWS high amplitude delta waves dominate.

2.1.3 Breathing during sleep

Respiration varies considerably between wakefulness, NREM sleep and REM sleep. At sleep onset, upper airflow resistance increases (Hudgel et al. 1984), which results in a reduction in the tidal volume (Colrain et al. 1987). The decreased ventilation increases end-tidal carbon dioxide ($EtCO_2$) and arterial carbon dioxide ($PaCO_2$) tensions (Skatrud et al. 1988). During REM sleep upper airway resistance increases further (Hudgel et al. 1984), mainly due to muscle atonia. Moreover, REM sleep is associated with irregular breathing and an increased frequency of apnea episodes (Krieger et al. 1983), and $EtCO_2$ levels are highest during REM sleep (Douglas et al. 1982).

2.1.4 Nocturnal hypercapnia, hypoxia and hypocapnia

Carbon dioxide is a powerful respiratory stimulant. Normally the partial pressure of arterial CO_2 ranges from 4.5 to 6.0 kPa. An excessively high partial pressure of CO_2 in the blood is referred to as hypercapnia, and is usually caused by hypoventilation (obstructive, central or neuromuscular). Hypercapnia increases respiratory efforts if associated with low pH (the case in obstructive hypoventilation, Douglas et al. 1982). However, during sleep hypercapnic ventilatory responses are weaker (relative central hypoventilation), especially during REM sleep (Douglas et al. 1982). In patients adapted to high CO_2 levels, the sleep-related increase in CO_2 responsiveness may result in excessively high levels of nocturnal CO_2. Hypoxia (too low a partial pressure of oxygen in the blood) also stimulates breathing, but only when the degree of hypoxia is severe enough. In patients habituated to constantly high CO_2 levels (for example in chronic obstructive pulmonary disease), respiration during wakefulness is largely driven by hypoxemia.

Hypocapnia (too low a partial pressure of CO_2 in the blood) counteracts ventilation (Despopoulos & Silbernagl 2003). If hypocapnia is remarkable, it can lead to a complete cessation in breathing (central apnea). This may occur for example in a patient with obstructive sleep apnea (OSA), when low oxygen levels induce rapid episodes of hyperventilation. The oscillation between the hypercapnic, hypoxic and hypocapnic stimuli may lead to periodic breathing during sleep (Polo 1992).

2.1.5 Sleep-disordered breathing (SDB)

The definition of sleep-disordered breathing (SDB) includes a variety of sleep-related breathing abnormalities from snoring to OSA syndrome. According the present criteria, apnea is defined as a 90 % or more drop from the baseline in the amplitude of the nasal flow signal for 10 seconds or more (Iber et al 2007). Hypopnea is defined as the amplitude of the nasal flow signal dropping at least 30 % from the baseline. Further, the hypopnea event has to last 10 seconds or more and it has to be connected with a minimum of 4 % arterial oxyhemoglobin desaturation from the pre-event baseline (Iber et al. 2007). The apnea-hypopnea index (AHI) is the sum of apnea and hypopnea episodes per hour of

sleep. The respiratory disturbance index (RDI) is a combination of the apnea-hypopnea index and the respiratory effort-related arousal (RERA) index. A RERA event is defined as a sequence of breaths characterized by increased respiratory effort or decreased nasal pressure waveform leading to an arousal from sleep (Iber et al. 2007). The severity of SDB is diagnosed based both on findings from sleep recordings and subjective symptoms such as daytime sleepiness (American Academy of Sleep Medicine Task Force 1999). However, although AHI is a widely used measure, it seems to underestimate the severity of SDB (Polo 1992, Cracowski et al. 2001, Anttalainen 2007). Thus, in some studies, analyses using static charge-sensitive beds have successfully been used in the detection of SDB (Polo 1992, Anttalainen 2007).

A static charge-sensitive bed measures intrathoracic pressure variations, thus making it possible to detect an increased respiratory resistance (IRR). An IRR corresponds to obstructive hypoventilation, which manifests itself as an increased spiking in the static charge-sensitive bed curve. Partial upper airway obstruction can be determined in terms of IRR as well as inspiratory flow limitation (Aittokallio et al. 2001). Nasal flow is usually measured with nasal prongs. When the upper airway narrows after sleep onset, the flow limitation can be observed as an alteration in the shape of the inspiratory flow curve (Aittokallio et al. 2001).

The prevalence of SDB (AHI > 5 events/hour plus symptoms) is estimated to be 4 % in men and 2 % in women (Young et al. 1993). However, the amount of undiagnosed SDB is estimated to be high (Young et al. 1993). According to Lindberg and Gislason (2000) the prevalence of undiagnosed SDB in Western countries varied between 0.3 and 5 %. Obesity increases the risk of SDB (Young et al. 1993, Young et al. 2002, Peppard et al. 2000a, Tishler et al. 2003, Anttalainen et al. 2007). With the increasing obesity problem in our society, the prevalence of SDB is likely to increase further. The location of adipose tissue is also essential in calculating the risk of SDB, with the central obesity and adipose tissue around the neck being the most harmful cases (Millman et al. 1995). In addition, male gender and aging further increase the prevalence of SDB (Young et al. 1993).

2.1.6 Aging and sleep

Aging induces many changes in sleep architecture (Bliwise 2000). The amount of SWS and the amplitude of EEG slow waves decrease. Generally, nocturnal sleep duration shortens and becomes more fragmented with more awakenings. Also, the portion of light S1 sleep is enhanced. Sleep disturbances become more frequent in the elderly both in terms of insomnia (Leger et al. 2000) and SDB (Bixler et al. 2001). While SDB is more frequent in men (Young et al. 1993), insomnia more often affects women (Leger et al. 2000). However, after menopause, the prevalence of SDB is similar in both genders (Bixler et al. 2001).

The respiratory system normally changes with increasing age. The chest wall becomes more inelastic, leading to increased effort in breathing (Sharma & Goodwin 2006). Moreover, respiratory muscle strength weakens and lung function declines (Sharma & Goodwin 2006). Elderly subjects also have lower ventilatory responses to CO_2, but their CO_2 sensitivity does not decrease further during sleep (Naifeh et al. 1989). Changes in airway structures with aging are also likely to affect the incidence of SDB.

2.1.7 Effect of menopause on sleep and breathing

At menopause, the endogenous production of female sex hormones (estrogens and progesterones) decreases, whereas SDB becomes more frequent (Bixler et al. 2001, Young et al. 2003a). In addition to aging, the diminished amounts of estrogens and, in particular, progesterones contribute to changes in sleep and breathing (Saaresranta et al. 1999, Bixler et al. 2001, Young et al. 2003a). Progesterone is a powerful respiratory stimulant (Zwillich et al. 1978) and estrogen upregulates progesterone receptors (Brodeur et al. 1986, Camacho-Arroyo et al. 1998). Progesterone stimulates breathing during physiological conditions such as pregnancy and the luteal phase of the menstrual cycle or during progestin therapy. Increased endogenous production or external supplementation of progesterone decreases $EtCO_2$ (Schoene et al. 1981, White et al. 1983, Saaresranta & Polo 2002). The beneficial respiratory effects of high progesterone levels during the luteal phase are likely to be prolonged and at least some residual effect outlasts the three weeks following the cessation of progestin therapy (Saaresranta et al. 1999, Saaresranta et al. 2001). Higher estrogen levels in premenopausal women contribute to the amount of progesterone receptors and mediate the effect further.

The upper airway properties (Kirkness et al. 2008), as well as the compensatory ventilatory response to upper airway obstruction (Schneider et al. 2009), differ between men and women. Aging and menopause may change both of these leading to an inspiratory flow limitation in postmenopausal women, which may be seen in alterations in SaO_2 and arterial CO_2.

Womens' satisfaction with sleep decreases around the time of menopause (Kuh et al. 1997**,** Young et al. 2003b, Kravitz et al. 2003). However, postmenopausal women's subjective experience of poor sleep quality is not seen in PSG recordings**,** for actually postmenopausal women sleep longer and have more SWS than premenopausal women (Sharkey et al. 2003, Young et al. 2003b). Kalleinen et al. have recently shown that aging is likely to affect sleep quality more than menopause (Kalleinen et al. 2008).

2.1.8 Estrogen replacement therapy (ET) and sleep

Estrogen replacement therapy (ET) is used to alleviate menopausal symptoms such as hot flushes, sweating, sleep complaints, and headaches. Estrogen and progestin are often

used as a combined hormone replacement therapy, since unopposed estrogen therapy may cause hyperplacia of the endometrium. ET is given only to hysterectomized women.

The effect of ET on breathing is considered to be weak (Polo-Kantola et al. 2003). Although progesterone has a strong influence on ventilatory control, the role of estrogen seems to be limited to the enhancement of the progestin effect through the up-regulation of progesterone receptors (Brodeur et al. 1986). ET does not reduce partial upper airway obstruction or improve arterial oxyhemoglobin saturation (SaO_2) during sleep (Polo-Kantola et al. 2003). However, long-term ET may slightly improve nocturnal oxygenation (Saaresranta et al. 2006). ET also has implications for the acid-base balance; in particular ET restores the menopausal higher plasma bicarbonate levels (Adami et al. 1992, Orr-Walker et al. 1999).

The effect of ET on sleep quality is conflicting. While ET clearly improves the subjective sleep quality (Thomson & Oswald 1977**,** Polo-Kantola et al. 1999**,** Saletu-Zyhlarz et al. 2003, Polo-Kantola & Erkkola 2004), objective findings are scarce (Polo-Kantola et al. 1999, Saletu-Zyhlarz et al. 2003). Moreover, according to a recent prospective study, women on hormone replacement therapy had even less SWS and more sleep fragmentation than women without hormone therapy (Young et al. 2003b).

2.2 Non-invasive respiratory monitoring during sleep

Although directly measured arterial blood gases are more accurate than non-invasive estimates, such invasive methods are not feasible in sleep research. Non-invasive methods allow the monitoring of blood gases with minimal disturbance to the subject.

2.2.1 Arterial oxyhemoglobin saturation (SaO_2)

A pulse oximeter measures indirectly the arterial oxyhemoglobin saturation in a patient's blood. Usually the sensor is placed on the patient's finger or on the nip of the auricle. Normally, SaO_2 ranges from 95 to 100 %. While SaO_2 is useful in monitoring oxygenation, it does not give enough information about the efficiency of ventilation. Therefore $EtCO_2$ is generally used to evaluate proper gas exchange.

SaO_2 monitoring is a widely used method in sleep research (Clark et al. 1992, Lee-Chiong et al. 2005). It is typically used to assess the severity of SDB in terms of nocturnal average and minimum SaO_2, AHI and desaturation events (for example oxyhemoglobin desaturation of 4% units or more per hour, ODI_4).

2.2.2 End-tidal carbon dioxide ($EtCO_2$)

$EtCO_2$ gives an estimation of the alveolar partial pressure of CO_2. However, $EtCO_2$ also reflects indirectly arterial CO_2 levels (Morley et al 1993). A normal alveolar CO_2 value is

approximately 5.3 kPa (Despopoulos & Silbernagl, 2003). Just as ventilation normally declines during sleep, also $EtCO_2$ levels differ between wakefulness and the sleep states (Douglas et al. 1982). According to Douglas et al., $EtCO_2$ levels normally increase from wakefulness to S2 sleep by 0.26 kPa, to SWS by 0.32 kPa and to REM sleep by 0.4 kPa (Douglas et al. 1982). Hence, in patients with a partial upper airway obstruction, the nocturnal $EtCO_2$ increase is supposed to be even higher (due to obstructive hypoventilation).

Resting high levels of $EtCO_2$ have been associated with higher systolic blood pressure and carotid wall thickening in women (Anderson et al. 1999, Anderson et al. 2001) as well as with the anxious personality trait (Dhokalia et al. 1998).

2.2.3 Transcutaneous carbon dioxide ($TcCO_2$)

$TcCO_2$ measures the amount of carbon dioxide that diffuses across the skin (Baumbach, 1997). The partial pressure of peripheral venous CO_2 is approximately 6.13 kPa (Despopoulos & Silbernagl, 2003). Since carbon dioxide is the end product of the energy metabolism of cells, the warmed-up transcutaneous sensor measures not only the CO_2 of cutaneous blood vessels, but also an additional input from skin metabolism. Therefore, $TcCO_2$ appears slightly to overestimate the arterial CO_2 ($PaCO_2$) values. Several studies have shown that $TcCO_2$ gives a good estimate for $PaCO_2$ (McLellan et al. 1981, Goldman et al. 1982, Mahutte et al. 1984, Janssens et al. 1998, Janssens et al. 2005, Parker & Gibson 2007), yet some studies considered the bias unacceptably large (Sanders et al. 1994, Fanelli et al 2008) or the response to acute respiratory changes too slow (Schachter et al. 1981). Despite the difference between arterial and transcutaneous CO_2, $TcCO_2$ estimates $PaCO_2$ better than $EtCO_2$ (Casati et al. 2006). Both $EtCO_2$ and $TcCO_2$ monitors are likely to give information about different physiological aspects. $EtCO_2$ reflects changes in pulmonary ventilation whereas $TcCO_2$ reflects alterations in peripheral perfusion and/or metabolism. Both of these methods have independent indications but may sometimes also be complementary to each other in patient care (Chhajed et al. 2004).

$TcCO_2$ used to be criticized for producing an upward drift in long-lasting recordings such as those in sleep studies, resulting in artificially high values in the morning hours. However, this is not supported by all studies using the same $TcCO_2$ device at the same temperature for eight hours (Janssens et al. 2001), or another device for four hours (Janssens et al. 1998) (Figure 3). Moreover, neither age nor body mass index (BMI) seems to correlate with the margin between $TcCO_2$ and $PaCO_2$ (Janssens et al. 2005). However, there are some concerns which arise from the monitoring of $TcCO_2$. $TcCO_2$ measurements are affected by cutaneous vasoconstriction (Healey et al. 1987), mainly due to changes in blood flow which affect the capillary CO_2 gradient (Severinghaus et al. 1978, Beran et al. 1981).

$TcCO_2$ is not a widely used method in sleep research, mainly because it is considered to be insensitive to rapid respiratory changes. However, already in 1984, $TcCO_2$ was used in the assessment of nocturnal hypercapnia in patients with respiratory insufficiency (Midgren et al. 1984). Later, changes in $TcCO_2$ levels were registered during episodes of central apnea and OSA (Tkacova et al. 2001). Nocturnal $TcCO_2$ has also been linked with overnight changes in cardiac function and circulation time (Tkacova et al. 2001). In addition, $TcCO_2$ has previously been used for monitoring nocturnal CO_2 differences in patients with and without non-invasive ventilation (Ward et al. 2005). A recent study expanded the usage of nocturnal $TcCO_2$ to the prediction of metabolic variables (Virkki et al. 2008).

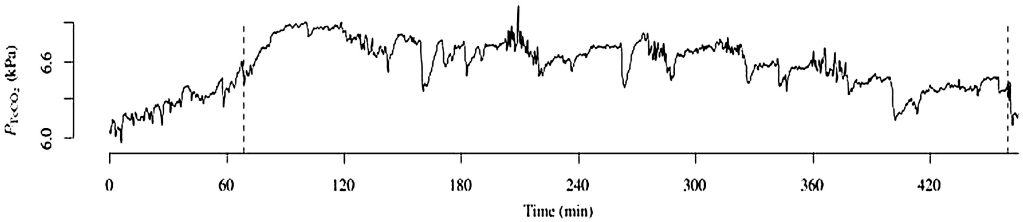

Figure 3. An example of an overnight $TcCO_2$ curve. The first vertical line indicates sleep onset and the second indicates the last awakening in the morning. No additional drift during the night is seen, for the evening and morning levels of $TcCO_2$ are similar.

2.3 Atherosclerosis

Atherosclerosis is defined as the loss of elasticity and hardening of the medium or large arteries, caused by a chronic inflammatory response in the artery walls (Ross et al. 1999). Symptoms of the disease are usually observed, when atherosclerosis affects coronary, carotid or cerebral arteries or the arteries of the legs. Unfortunately, atherosclerotic disease may be asymptomatic for a long time, which makes the early diagnosis challenging and the detection of risk factors important.

2.3.1 Common risk factors for atherosclerosis

Generally, known risk factors for atherosclerosis are diabetes mellitus, hypercholesterolemia, hypertension, obesity, genetic predisposition and smoking. The development process of the disease takes decades, beginning probably already in the early teenage years (Insull 2009). Impairment of endothelium-dependent dilatation occurs in the preclinical phase of vascular disease and is associated with the same risk factors known to predispose to atherosclerosis (Celermajer et al. 1994).

Diabetes mellitus is caused by a disordered metabolism, resulting in high blood glucose levels. Type I diabetes is characterized by insulin deficiency, while type II diabetes is characterized by insulin resistance, yet reduced insulin secretion is common also in type

II. Both type I and type II diabetes increase cardiovascular morbidity (Kuusisto et al. 1994a, Laing et al. 1999), and in women the effect is even more pronounced (Kuusisto et al. 1994b). The long-term balance of blood glucose is assessed using glycosylated haemoglobin A1C (HbA1C). Subjects with HbA1C levels over 7% have an increased risk of coronary heart disease (Kuusisto et al. 1994b). Diabetes is also associated with an increased intima-media thickness (IMT) of carotid arteries (Park et al. 2008) as well as impaired endothelial function of the peripheral arteries (Clarkson et al. 1996, Lekakis et al. 1997).

Cholesterol is an essential component of cell membranes and a precursor of steroidal hormones. In spite of its necessity, high levels of cholesterol are strongly linked with coronary heart disease (Stamler et al. 1986, Chen et al. 1991). Cholesterol is transported in the circulation by lipoproteins, predominantly in low-density lipoprotein (LDL) particles. LDL is considered to be an atherogenic lipoprotein, for large LDL particles trap to the arterial wall (Kovanen & Pentikäinen 1999) leading to LDL particle oxidation (Steinbrecher 1988) and atherosclerotic plaque formation. High cholesterol is related to endothelial dysfunction (Celermajer et al. 1992) and high LDL levels with increased carotid IMT (Bhuiyan et al. 2006). High-density lipoprotein (HDL) cholesterol has an opposite affect to LDL, decreasing the risk of atherosclerosis, probably by facilitating reverse cholesterol transport (Despopoulos & Silbernagl, 2003). Triglycerides are important for energy metabolism, yet they are also atherogenic factors (Durrington 1998). High levels of triglycerides after a fatty meal may impair endothelial function (Vogel et al. 1997).

The term blood pressure (BP) refers to the arterial blood pressure in the systemic circulation. The maximum BP occurs in the aorta during systolic ejection (systolic blood pressure, SBP) and the minimum aortic pressure is referred to as diastolic blood pressure (DBP) (Despopoulos & Silbernagl, 2003). An indirect blood pressure measurement is usually performed using the Riva-Rocci's technique with the Korotkoff method (Riva-Rocci 1896, Korotkov 1956). This method determines the SBP and DBP. Elevated blood pressure is an endemic problem (Kearney et al. 2005) and a major risk factor for cardiovascular events (Stamler et al. 1989). Hypertension is defined to be when the SBP is greater than 140 mmHg and the DBP is over 90 mmHg (Kastarinen et al. 2006). Like other risk factors of atherosclerosis, hypertension is also related to endothelial dysfunction (Panza et al. 1990) and increased IMT (Ghiadoni et al. 1998).

2.3.2 Regulation of the circulation

Blood flow to the organs is mainly regulated by changing the diameter of blood vessels. The muscle tone of the vascular smooth muscles changes in response to local stimuli, and to hormonal and neuronal signals. An oxygen deficiency and an increase in the local concentration of metabolic products (including CO_2) result in local vasodilatation. Also

a number of vasoactive substances such as prostaglandins, are involved. Vasodilatative agents include nitric oxide whereas endothelin-1 and epinephrine (through alpha-receptors) are vasoconstrictors (Figure 5), (Despopoulos & Silbernagl, 2003)

The neuronal regulation of circulation and internal organs are mediated through the autonomic nervous system. The autonomic nervous system is usually divided into the parasympathetic and sympathetic nervous system and their functions are mainly not in the subject's voluntary control. The sympathetic nervous system is more active during wakefulness and stress, whereas the parasympathetic nervous system dominates during rest and digestion. The neuronal regulation of blood flow is usually controlled by the sympathetic nervous system and affects mostly the small arteries and arterioles (Despopoulos & Silbernagl, 2003).

2.3.3 Endothelial function

The layer of endothelial cells covers continuously the lumen of blood vessels (Despopoulos & Silbernagl, 2003)**.** Normal endothelial functions include the control of thrombosis and thrombolysis, platelet and leucocyte interactions with the vessel wall and the regulation of vascular tone (Raitakari & Celermajer 2000). Arterial endothelial dysfunction is an early event in atherosclerosis (Celermajer et al. 1992). Endothelial dysfunction is related to many known risk factors for vascular diseases, including smoking (Celermajer et al. 1992), visceral obesity (Chudek & Wiecek 2006), hypertension (Panza et al. 1990), hypercholesterolemia (Celermajer et al. 1992), diabetes (Clarkson et al. 1996, Mäkimattila et al. 1996, Lekakis et al. 1997) and aging (Andrawis et al. 2000).

Brachial artery endothelial function can be measured non-invasively with ultrasonography (Celermajer et al. 1992). Brachial artery blood flow is increased temporarily via the compression and release of a pneumatic tourniquet, which induces higher wall shear stress, and endothelium-dependent dilatation. In arteries lined with healthy endothelium, an increased flow causes dilatation of the vessels (flow-mediated dilatation, FMD). In nitroglycerin-mediated dilatation (NMD), the diameter of the brachial artery is measured after sublingual nitroglycerine administration. In contrast to FMD, NMD is caused by direct smooth muscle action and is independent of the endothelium. Smooth muscle cell dysfunction or changes in arterial wall structure can cause impaired NMD.

2.3.4 Arterial intima-media thickness (IMT)

Atherosclerosis affects the vascular wall by increasing the wall thickness. Therefore, an increase in IMT is considered as a marker of subclinical atherosclerosis. Carotid IMT is easily measured with ultrasonography (Figure 4) and is a widely used method in the assessment of early changes in atherosclerosis (Salonen & Salonen, 1990). IMT correlates with conventional vascular risk factors (Park et al. 2008, Bhuiyan et al. 2006, Ghiadoni

et al. 1998) as well as predicts the likelihood of cardiovascular and cerebrovascular events (Bots et al. 1997).

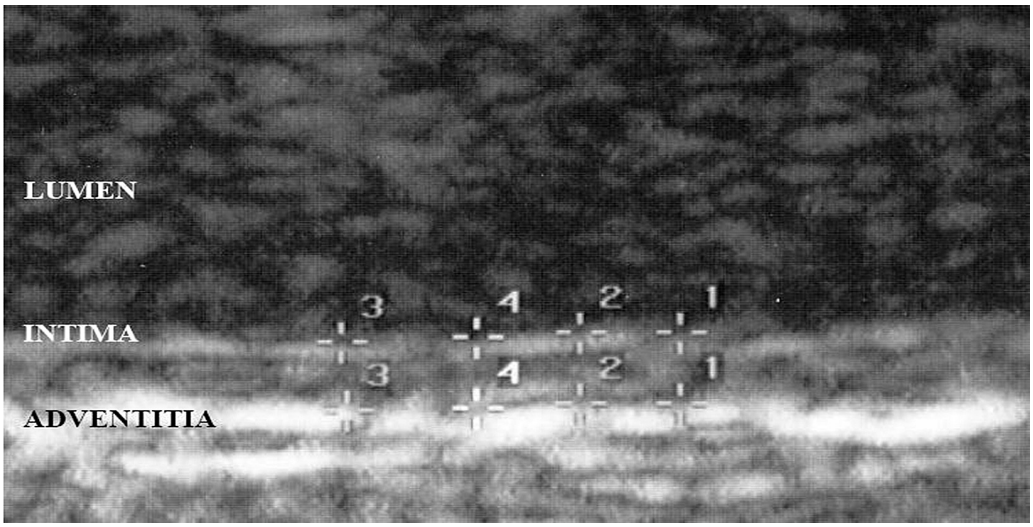

Figure 4. Intima-media thickness (IMT) of the carotid artery. Measurements 1-4 visualize the distance between the lumen-intima interface and the media-adventitia interface.

2.4 SDB and cardiovascular morbidity

Sleep-disordered breathing is highly prevalent in patients with cardiovascular diseases (CVD) (Somers et al. 2008). OSA is a common disorder associated with an increased risk of CVD (McNicholas & Bonsigore 2007). However, OSA strongly associates with known cardiovascular risk factors, including obesity, insulin resistance, dyslipidemia and hypertension (Lattimore et al. 2003). Because both SDB and CVD are prevalent diseases sharing at least partially the same risk factors, it is difficult to evaluate the independent role of SDB in the development of CVD.

2.4.1 SDB and cardiovascular diseases

Episodes of obstructive apnea induce hypoxemia and CO_2 retention during sleep. OSA patients have faster resting heart rates during wakefulness compared to healthy controls, suggesting increased sympathetic activation (Narkiewicz et al. 1998a, Narkiewicz et al. 1998b). Moreover, OSA patients have a diminished heart rate variability (Narkiewicz et al. 1998a), which is associated with the development of hypertension (Singh et al. 1998).

Over half of OSA patients have hypertension (Silverberg et al. 1998). Both OSA and hypertension are common conditions, and may also occur independently. However, OSA has been demonstrated to be an independent risk factor for hypertension, after

controlling for age, sex, BMI, and antihypertensive medications (Peppard et al. 2000b). Even patients with mild SDB had an increased risk of developing new hypertension in a four-year follow-up. According to a study of Bixler et al., SDB, including snoring, was independently associated with hypertension in both genders (2000). In snoring women, postmenopausal hormone therapy was associated with a lower prevalence of hypertension (Bixler et al. 2000).

OSA is also common in patients with heart failure, with a prevalence of 38 % in men and 31 % in women (Sin et al. 1999). The treatment of OSA using continuous positive airway pressure therapy improves systolic function in heart failure patients (Mansfield et al. 2004), which supports the independent role of OSA in the progression of the disease. Stroke (Bassetti et al. 2006), ischemic heart disease (Mooe et al. 1996a, Mooe et al. 1996b) and type 2 diabetes (Ip et al. 2002) are all associated with OSA. Frequent nocturnal arousals in OSA patients cause sleep fragmentation and sleep loss, which has been associated with impaired glucose metabolism (Spiegel et al. 2005).

2.4.2 SDB, endothelial dysfunction and IMT

Table 1 reviews the current data about SDB and vascular impairment.

Recent evidence supports an independent association between OSA and the impairment of vascular function (Budhiraja et al. 2007, Foster et al. 2007, Lévy et al. 2008). OSA-related vascular endothelial dysfunction is probably a consequence of multiple mechanisms, such as hypoxemia, reactive oxygen species production with systemic inflammation, and sympathetic activation (Budhiraja et al. 2007) (Figure 5).

Several studies have shown endothelial dysfunction in patients with SDB (Table 1). Interestingly, the relationship between SDB and FMD is even more pronounced in women (Faulx et al. 2004). Even snoring has been related to an increased risk of CVD in women (Hu et al. 2000), which may suggest that women are highly vulnerable to co-morbidities of SDB. Despite this, most of the studies are focused on men (Table 1).

Impaired NMD has not been demonstrated consistently in OSA patients (Ip et al. 2004), suggesting that vascular smooth muscle function may not be affected in OSA. However, impaired NMD has been related to coronary risk factors (Adams et al. 1998).

The degree of vascular impairment in OSA patients has been explained by hypoxemia during sleep, using mean or minimum SaO_2 values or the percentage of time that SaO_2 stays below 90% (Nieto et al. 2004; Suzuki et al. 2004; Baguet et al. 2005; Minoguchi et al. 2005). The severity and duration of desaturation events, rather than the frequency of obstructive events, has been suggested as an explanation for most of the FMD variation (Nieto et al. 2004).

An increase in the carotid artery intima-media thickness in OSA patients has been shown in several studies as well (Table 1). AHI and IMT correlate in study populations consisting mostly of male patients with OSA (Drager et al. 2005, Szaboova et al. 2007). Similarly with FMD measures, the severity and duration of hypoxemia during OSA events are related to IMT thickening (Suzuki et al. 2004, Minoguchi et al. 2005).

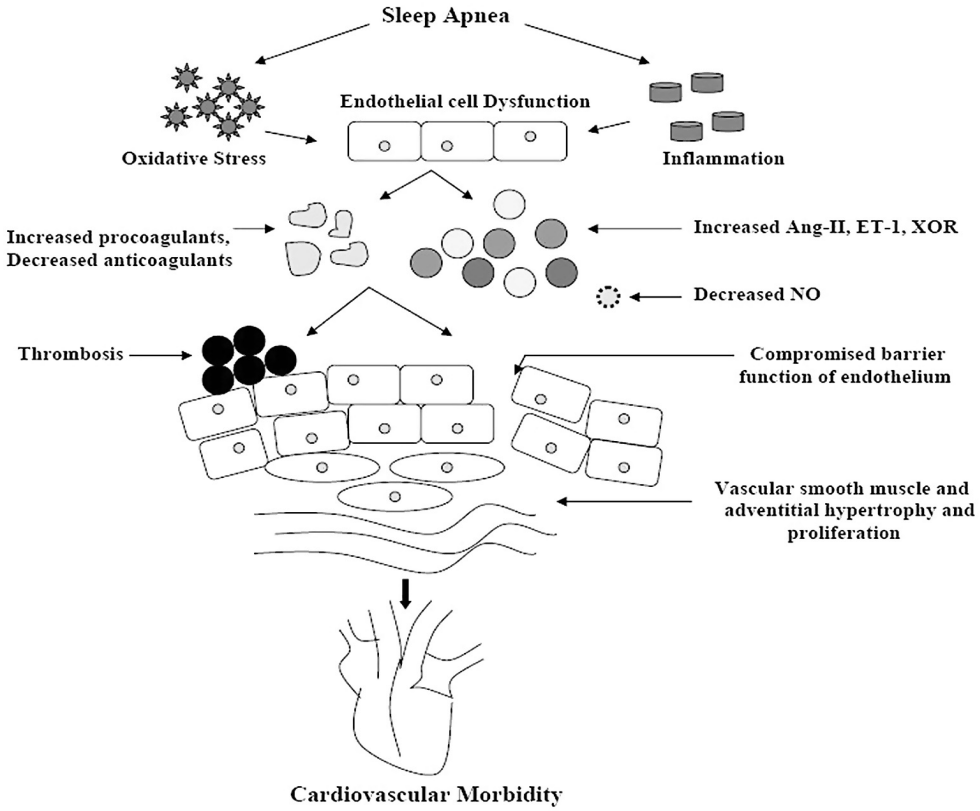

Figure 5. Possible mechanisms linking sleep apnea and cardiovascular diseases. The sources of the endothelial injury are still not clear, but potential etiologies include reactive oxygen species generation and systemic inflammation. Endothelial injury results in an alteration of the endothelial hormones that are responsible for maintaining vascular tone and preventing abnormal cell proliferation. Angiotensin II (Ang-II) causes blood vessel constriction and drives blood pressure up. Endothelin-1 (ET-1) is a protein that constricts blood vessels and raises blood pressure. Xanthine Oxidoreductase (XOR) generates reactive oxygen species. Nitric Oxide (NO) causes vasodilatation through smooth muscle relaxation. Adapted with permission from Budhiraja et al. 2007.

2.5 Metabolic syndrome

2.5.1 Definitions

Metabolic syndrome is defined as a combination of disorders, most of which are risk factors for cardiovascular diseases and diabetes (Wolk & Somers 2007). Its definitions

vary slightly (Eckel et al. 2005), yet all of the definitions include glucose intolerance or insulin resistance, central obesity, dyslipidemia and hypertension. According to the Adult Treatment Panel III, metabolic syndrome is defined as three or more conditions out of the following: central obesity (waist circumference > 102 cm in males, > 88 cm in females), hypertriglyceridemia (triglycerides > 1.7 mmol/L), low HDL cholesterol (< 1.0 mmol/L in males, < 1.3 mmol/L in females), hypertension (BP >135/85 mmHg or medication) and fasting plasma glucose > 6.1 mmol/L (ATP III; Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults, 2001). In addition, metabolic syndrome is also known as syndrome X (Eckel et al. 2005). It is unclear whether metabolic syndrome has a single cause or whether it results from multiple risk factors, which may be environmental, behavioral or genetic (Wolk & Somers 2007).

2.5.2 Metabolic syndrome and sleep

Table 2 reviews the current data about sleep and metabolic co-morbidities.

OSA and metabolic syndrome are related, even if causality is still unproven (Wolk & Somers 2007). Consequently, it has been suggested that OSA is a manifestation of metabolic syndrome (Vgonzas 2005). However, the relation between OSA and insulin resistance seems clear (Punjabi et al. 2003). One hypothesis is that OSA and metabolic syndrome feed each other in a vicious circle: OSA predisposes a person to metabolic syndrome, which impairs ventilatory control by promoting obesity, an inflammatory state and glucose intolerance, finally leading to a worsening of OSA, which further promotes metabolic syndrome (Wolk & Somers 2007).

Sleep quality and duration are known to affect components of metabolic syndrome (Table 2). Both total sleep deprivation and short-term and chronic partial sleep deprivation may increase the risk of metabolic syndrome directly or via obesity (Tasali et al. 2008, Williams et al. 2007, Spiegel et al. 1999). Several studies have shown that self-reported sleep quality is associated with components of metabolic syndrome (Jennings et al. 2007, Hall et al. 2008, Gangwish et al. 2006). In recent studies, also objectively measured short sleep is associated with hypertension and type 2 DM (Vgontzas et al. 2009a, 2009b). The magnitude of the decrease in insulin sensitivity has been shown to correlate with the magnitude of the reduction in SWS in healthy young subjects (Tasali et al. 2008). Moreover, short sleep is also associated with a dysregulation of the neuroendocrine control of appetite, with a reduction of the satiety hormone, leptin, and an increase in the hunger hormone, ghrelin (Mullington et. al 2003, Taheri et al 2004, Knutson et al. 2008). Sleep loss can lead to an increased appetite and increased food intake, which leads to obesity. Sleepiness and fatigue may also result in reduced energy expenditure, in particular through decreased physical exercise, but also through decreases in non-exercise activity thermogenesis (Knutson et al. 2008).

Table 1. Selected studies of SDB, IMT and endothelial function

Author	Study design	Subjects	Age (mean, y)	BMI (kg/m2)	Findings	Comments
Kato et al. 2000	Cross-sectional study of OSA patients and obese controls	n = 8 OSA, M n = 9 obese controls, M	44 (OSA) and 48 (controls)	35 (OSA) and 30 (controls)	Patients with OSA had an impairment of endothelium-dependent vasodilatation (acetylcholine-induced).	Ultrasound studies of brachial artery were also performed, but there were no differences in FMD.
Kraiczi et al. 2001	Cross-sectional study of OSA patients	n = 20 OSA, M	58	29	The severity of apnea-related hypoxemia correlated positively with FMD, but not with NMD	Association between nocturnal norepinephrine and FMD reached borderline significance (p = 0.061)
Kaynak et al. 2003	Cross-sectional study of habitual snorers (I), mild-moderate OSA (II) and severe OSA (III)	n = 37 (I), M n = 41 (II), M n = 36 (III) M	48 (I) 50 (II) 50 (III)	21 (I) 22 (II) 23 (III)	The OSA groups had significantly higher IMT values compared with the snorers.	Age and BMI associated with IMT and age and RDI associated with plaque occurrence.
Nieto et al. 2004	Cross-sectional population based	n = 1037, F/M = 581/456	68-79 (n=720) >79 (n=317)	NA	AHI and hypoxemia index were associated with brachial baseline diameter and FMD. Linear association between hypoxemia index and baseline diameter was strong.	Association between sleep apnea measures and FMD weakened when adjusted with CVD risk factors, particularly BMI. NMD not measured.
Ip et al. 2004	Cross-sectional case-control study, longitudinal case-control study	Part I: n = 28 OSA, M n=12 controls, M Part II: n = 14 OSA-nCPAP and n = 13 OSA-controls	43 (OSA) and 41 (controls), 44 (OSA-nCPAP) and 41 OSA-controls	29 (OSA) and 28 (controls), 30 (OSA-nCPAP) and 29 (OSA-controls)	OSA patients had lower FMD vs. controls, major determinant to FMD were AHI and age. No differences in NMD. After 4 weeks nCPAP significant increase in FMD, but not in NMD.	FMD values revert to the baseline levels or lower after withdrawing the treatment.
Faulx et al. 2004	Cross-sectional study	n = 82, M n = 111, F	41	31	AHI was inverserly associated with FMD and peak blood flow. The relationship was stronger in women.	Association stronger in women
Suzuki et al. 2004	Cross-sectional study, patients with symptoms of OSA	n = 138, M n = 29, F	47	27	AHI, the duration of an oxygen saturation below 90%, and the mean nadir oxygen saturation were associated with the IMT	OSA-related hypoxemia was associated with the IMT independently of the AHI
Baquet at al. 2005	Cross-sectional study	n = 74, M n = 9, F	48	27	The severity of oxygen saturation was one of the best predictors for IMT and plaque occurrence.	30% of patients had carotid wall hypertrophy (> 0.8 mm).
Schulz et al. 2005	Cross-sectional study	n = 35 (OSA) n = 35 (non-OSA)	56 (OSA) 56 (non-OSA)	32 (OSA) 31 (non-OSA)	In the OSA-group IMT was increased compared with the non-OSA.	IMT was related to the degree of nocturnal hypoxia.

Author	Study design	Subjects	Age (mean, y)	BMI (kg/m2)	Findings	Comments
Drager et al. 2005	Cross-sectional	n = 12 controls n = 15 (mild-moderate OSA) n = 15 severe OSA	42 (controls) 43 (moder. OSA) 44 (severe OSA)	29 (controls) 28 (moder. OSA) 29 (severe OSA)	Significant differences between control subjects and OSA subjects in IMT. AHI correlated with IMT.	Control-group and moder. OSA included one woman, and severe OSA 2 women.
Oflaz et al. 2006	Cross-sectional study of OSA patients and healthy controls	n = 23 OSA, F/M = 3/20 and 15 controls, F/M = 3/12	48 (OSA) and 46 (controls)	29 (OSA) and 28 (controls)	FMD values in OSA patients were lower compared those of controls	All the subjects were normotensive. In all subjects FMD was lower in the morning than in the evening.
Tanriverdi et al. 2006	Cross-sectional	n = 40 OSA, F/M 8/32 and 24 controls, F/M 5/19	51 (OSA) 52 (controls)	30 (OSA) 29 (controls)	Subjects with OSA had higher values of IMT and lower values of FMD than the controls.	
Saletu et al. 2006	Cross-sectional	n = 44 controls n = 27 AHI 5-15 n = 25 AHI 15-30 n = 51 AHI > 30 (F/M 102/45)	50 controls 55 AHI 5-15 55 AHI 15-30 54 AHI > 30	27 controls 28 AHI 5-15 29 AHI 15-30 33 AHI > 30	Significant differences between the controls and all three OSA groups in IMT. AHI is a predictor for IMT.	High-sensitivity C-reactive protein was also associated with oxygen saturation events.
Szabóová et al. 2007	Cross-sectional	n = 33 (OSA) n = 16 (non-OSA)	52 (OSA) 48 (non-OSA)	30 (OSA) 29 (non-OSA)	IMT was increased in OSA patients versus controls. IMT correlated with the AHI, minimal oxygen saturation and time spent with SaO_2 < 90% in patients with OSA.	
Drager et al. 2007	Longitudinal case-control study	n = 12 OSA n = 12 OSA with CPAP	47 (OSA) 44 (OSA with CPAP)	30 (OSA) 30 (OSA with CPAP)	A significan decrease in IMT after 4 moths of CPAP.	Changes in IMT were correlated with changes in catecholamines.
de la Peña et al. 2008	Cross-sectional	n = 13 OSA, M n = 13 controls, M	45 (OSA) 45 (controls)	28 (OSA) 26 (controls)	Patients with OSA had lower levels of circulating endothelial progenitor cells and higher levels of vascular endothelial growth factor.	FMD did not differ between OSA and controls.
Wattanakit et al. 2008	Cross-sectional	n = 396 (I, carotid plaque) n = 589 (II, no carotid plaque)	64 (I) 61 (II)	28 (I) 28 (II)	Positive association between SDB and IMT was attributed to confounding by cardiovascular risk factors.	Hypoxemia index was not associated with carotid plaque or IMT

Abbreviations: SDB=sleep-disordered breathing,, OSA= obstruvtive sleep apnea, RDI = respiratory disturbance index, AHI = apnea-hypopnea –index, CPAP = continuous positive airway pressure therapy, IMT= carotid intima media thickness, FMD = flow-mediated dilatation, NMD = nitroglycerine-mediated dilatation, OSA=obstructive sleep apnea, BMI=body mass index, F=female, M=male.

Table 2. Selected studies of sleep and metabolic co-morbidities

Author	Study design	Disease	Subjects	Age (mean, y) BMI (kg/m2)	Findings	Comments
Hla et al. 1994	Cross-sectional	SDB and hypertension	n = 41 (I, healthy) M: 17 n = 53 (II, snorers) M: 21 n = 53 (III, apneics) M: 35	(I) Age 42, BMI 26 (II) Age 43, BMI 28 (III) Age 44, BMI 32	Mean blood pressures were significantly higher among participants with sleep apnea.	Sleep apnea was significantly associated with hypertension in a dose-response fashion
Bixler et al. 2000	Cross-sectional	SDB and hypertension	n = 67 (AHI > 15), M: 49 n = 164 (AHI 0-15), M: 116 n = 238 (snorers), M: 119 n = 1272 (no SDB), M: 457	Age range 20-100 BMI NA	SDB (including snoring) was independently associated with hypertension in both men and women.	The link between SDB and hypertension was strongest in young and lean subjects
Morrell et al. 2000	Cross-sectional	Sleep fragmentation, SDB and hypertension	n = 1021, M: 590	Age 46, BMI 30	The sleep fragmentation was significantly associated with higher awake blood pressure (AHI<1).	When AHI >1, no independent association between sleep fragmentation and blood pressure
Ip et al. 2002	Cross-sectional	SDB and insulin resistance	n = 85 (I, AHI <5), M: 47 n = 185 (II, AHI ≥5), M: 150	(I) Age 42, BMI 24 (II) Age 45, BMI 28	Subjects with OSA were more insulin resistant. Obesity was the major determinant for insulin resistance, but SDB parameters were also involved.	Insulin resistance was also a significant factor for hypertension.
Ekstedt et al. 2004	Cross-sectional	Sleep architecture, hypertension, dyslipidemia	n = 24, M: 10	Age 30 BMI NA	Number of arousals was the best predictor for morning cortisol, blood pressure, total cholesterol, HDL and LDL -cholesterols.	Work stress predicted arousals
Gangwish et al. 2005	Longitudinal (I) and Cross-sectional (II)	Sleep duration and obesity	n = 8073 (Ia) n = 6981 (Ib) n = 9588 (II) M/F ?	Age range 25-86 BMI self-reported or measured	Self-reported sleep duration (< 7h) was associated with obesity in subjects between 32 and 49 years	
Hui et al. 2006	Longitudinal case-controlled	OSA and blood pressure	n = 28 (I,therapeutic CPAP), M: 22 n = 28 (II, subtherapeutic CPAP), M: 21	(I) Age 50, BMI 28 (II) Age 51, BMI 27	3 months with therapeutic CPAP reduced 24h mean and diastolic blood pressure	ESS values improved in both groups
Knutson et al. 2006	Cross-sectional	Sleep duration, sleep quality and DMII	n = 122, M: 35	Age 58, BMI 35	Sleep duration and quality (self-reported) were significant predictors of GHbA1C in DMII patients	African American population
Börgel et al. 2006	Cross-sectional and Longitudinal	OSA and dyslipidemia	n = 366 (I, OSA), F: 62 n = 86 (II, OSA after CPAP), F: 13	(I) Age 56, BMI 31 (II) Age 55, BMI 32	AHI was negatively associated HDL cholesterol (I). After 6 months CPAP therapy (II) HDL cholesterol levels increased	

Author	Study design	Disease	Subjects	Age (mean, y) BMI (kg/m2)	Findings	Comments
Gangwish et al. 2006	Longitudinal	Sleep duration and DMII	n = 802 (I, ≤ 5h sleep), F: 65% n = 1799 (II, 6h), F: 62% n = 2674 (III, 7h), F: 63% n = 2936 (IV, 8h), F: 64% n = 781 (V, ≥9 h sleep), F: 62%	Age range 25-74 Studied between years 1982-1992. Baseline BMI: (I) 27, (II) 27, (III) 26, (IV) 26, (V) 27	Subjects with short self-reported sleep duration (≤ 5h sleep) were likely to have DMII over follow-up period.	Also long sleep duration (≥9 h sleep) was associated with incidence of DMII
Williams et al. 2007	Cross-sectional	Sleep duration, snoring, DMII and dyslipidemia	n = 51 (I, ≤ 5h sleep) n = 600 (II, 6-7h) n = 229 (III, 8h) n = 55 (IV, ≥9 h sleep) All females	(I) Age 58, BMI 32 (II) Age 59, BMI 30 (III) Age 60, BMI 30 (IV) Age 59, BMI 28	Longer self-reported sleep duration was associated with increased C-reactive protein levels. HDL was decreased with short and long sleep duration. Snoring directly associated with triglycerides and inversely with HDL cholesterol.	Nurse's Health Study
Kono et al. 2007	Cross-sectional	OSA, hypertension, dyslipidemia and hyperglycemia	n = 42 (I, OSA) n = 52 (II, controls) All males	(I) Age 52, BMI 23 (II) Age 47, BMI 24	In OSA group, fasting blood glucose was higher and hypertension was more frequent. AHI was a predictor for hypertension, hyperglycemia and dyslipidemia.	BMI and lowest SaO_2 did not predicted metabolic variables.
Barceló et al. 2008	Cross-sectional and longitudinal	OSA, daytime sleepiness, DMII	n = 22 (Ia, OSA with EDS) n = 22 (IIa, OSA, no EDS) n = 23 (III, Controls) n = 20 (Ib, after CPAP) n = 15 (IIb, after CPAP) All males	(I) Age 49, BMI 32 (II) Age 50, BMI 31 (III) Age 48, BMI 25	Patients with EDS had higher plasma levels of glucose and insulin, than OSA patients without EDS or controls. CPAP treatment reduced cholesterol and insulin. EDS in OSA was associated with insulin resistance independently of obesity.	SaO_2 mean and nadir were markedly lower in EDS group
Hall et al. 2008	Cross-sectional community-based	Sleep duration and metabolic syndrome	n = 1214, M:568	Age range 44 BMI 27	The probability for having the metabolic syndrome increased in short sleepers (< 7 h).	After adjustments for antihypertensive medication, prevalence of metabolic syndrome did not increase in long sleepers (> 8h)
Tasali et al. 2008	Empirical	SWS duration and DMII	n = 9, F: 4	Age range 20-31 BMI 19-24	The suppression of SWS resulted in marked decreases in insulin release, leading to reduced glucose tolerance.	1 baseline and 3 SWS suppression nights. Blood samples in 20 min intervals.

Abbreviations: SDB=sleep-disordered breathing,, OSA= obstruvtive sleep apnea, AHI = apnea-hypopnea –index, CPAP = continuous positive airway pressure therapy, DMII = diabetes type II, BMI=body mass index, HDL = high density lipoprotein, LDL = low density lipoprotein, F=female, M=male, EDS = excessive daytime sleepiness, SWS = slow wave sleep.

3. AIMS AND HYPOTHESES OF THE STUDY

The aims of the present study were to investigate the nocturnal non-invasive respiratory parameters and early changes in cardiovascular diseases in women around the time of menopause. The monitoring of $TcCO_2$ was of particular interest because CO_2-mediated vascular autoregulation is mediated through nitric oxide. The main hypothesis of the study was that the nocturnal $TcCO_2$ recording can reveal the abnormal control and function of the peripheral arterial bed which heralds vascular disease. The following questions were to be answered:

I How do nocturnal SaO_2, $EtCO_2$ and $TcCO_2$ measurements differ in pre- and postmenopausal women?
Both estrogen and progesterone are involved in the control of breathing. We hypothesized that postmenopausal women have higher $EtCO_2$ and $TcCO_2$ levels because of lower estrogen and progesterone levels.

II Can nocturnal SaO_2, $EtCO_2$ and $TcCO_2$ measurements predict IMT and endothelial function in healthy premenopausal women?
We hypothesized that nocturnal $TcCO_2$ can predict vascular impairment through its ability to reflect changes in peripheral vasoconstriction and vasodilatation.

III Does $TcCO_2$ differ in postmenopausal ET-users compared with non-users?
We hypothesized that the higher $TcCO_2$ increase during sleep in postmenopausal women is restored to premenopausal levels by ET.

IV Are nocturnal $TcCO_2$ features associated with metabolic risk factors in postmenopausal women?
We hypothesized that features of nocturnal $TcCO_2$ predict metabolic impairment. A secondary aim was to test recently found $TcCO_2$ features in a non-patient population.

4. SUBJECTS AND METHODS

The study protocol was approved by the Commission on Ethics of Turku University Central Hospital. All subjects gave their written informed consent.

4.1 Subjects

4.1.1 Recruitment

Premenopausal women of about 46 years of age and postmenopausal women of about 56 years of age were recruited through a newspaper announcement for a sleep and cardiovascular study (Figure 6). Subjects with a history of malignancies, diabetes, coronary heart disease**,** respiratory insufficiency, known SDB or alcohol abuse were excluded. 133 women were enrolled.

Figure 6. Newspaper announcement used to recruit the subjects.

4.1.2 Characteristics of the premenopausal women (Studies I and II)

Altogether, 107 46-year-old premenopausal women participated in the study (Table 3). The premenopausal women were tested during the follicular phase of the menstrual cycle when progesterone levels are low (between days 3 to 10). During the study, two of the premenopausal women were using ET and 26 women (23 %) had an intrauterine device releasing locally small amounts of progestin. The premenopausal subjects included eight habitual smokers and eight women who smoked occasionally, and 37 women (35 %) reported habitual snoring (at least once a week). Seven women used antihypertensive drugs; three with beta blockers, two with the angiotensin 1 (AT1) receptor inhibitor combined with a thiazide diuretic, one AT1 receptor inhibitor and one angiotensin-converting-enzyme inhibitor. One woman used beta blockers as a preventive medication for migraine. Five women had medication for hypothyroidism and seven women had regular anxiolytic medication (doxepin, lorazepam, amitriptyline, moclobemide, temazepam, venlafaxine or citalopram). One woman used regular inhaled corticosteroids for asthma. Twenty-four women (22 %) had a BMI greater than 30 kg/m^2. Twenty-two women (21 %) had a systolic blood pressure (SBP) greater than 140 mmHg and 32 women (30 %) had a diastolic blood pressure (DBP) greater than 90 mmHg.

4.1.3 Characteristics of the postmenopausal women (Studies I, III and IV)

Twenty-six 56-year-old postmenopausal women participated in the study (Table 3). During the study, nine of the postmenopausal women were using ET. The postmenopausal subjects included three women who smoked occasionally, and 13 (50 %) habitual snorers (at least once a week). Five women used antihypertensive drugs; three with the AT1 receptor inhibitor and two with the AT1 receptor inhibitor combined with a thiazide diuretic. Four women used lipid-lowering medication and three women used low-dose aspirin. Another three women used gastric mucoprotective medication. One woman had betahistine for vertigo. Two women had medication for hypothyroidism and one woman had regular anxiolytic medication (alprazolam). One woman used regular inhaled corticosteroids for asthma. Four women (15 %) had a BMI greater than 30 kg/m^2. Nine women (35 %) had a systolic blood pressure (SBP) greater than 140 mmHg and 12 women (46 %) had a diastolic blood pressure (DBP) greater than 90 mmHg.

4.2 Methods

4.2.1 Demographic measurements

Neck, waist and hip circumferences as well as weight and height were measured before the sleep study. Evening blood pressure was measured from the right arm with a digital blood pressure monitor (Omron M4®, HEM-722C, Omron Health Care, Japan) in seated subjects. The average of three measurements was submitted into the analysis.

4.2.2 Questionnaires

The Basic Nordic Sleep Questionnaire

In Study III, the The Basic Nordic Sleep Questionnaire (BNSQ) was used to determine self-reported sleep quality between ET-users and non-users (Partinen & Gislason, 1995).

The Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS, Johns 1991) was used to evaluate daytime sleepiness (scale 0-24) in Study III. Higher scores represent a greater degree of sleepiness. Subjective sleep quality did not differ between the ET-users and non-users.

Medical history

A personal medical history was collected from all of the subjects. The structured questionnaires included questions about chronic diseases, medications, use of hormone therapies, medical operations, smoking habits, snoring, use of alcohol and socioeconomic status. All questionnaires were completed in the presence of an investigator or a study nurse.

Table 3. Characteristics data of the study groups (total number of women 133).

Study	Subgroup	N	Age, years	FSH, IU/l	BMI, kg/m^2	Waist-to-hip ratio, %	FEV_1,%	SBP, mmHg	DBP, mmHg	Total cholesterol, mmol/l	HbA1C, %
I	Pre	13	45.9 ± 0.8	10.3 ± 7.5	25.2 ± 3.0	83.3 ± 6.0	99.2 ± 16.8	128.9 ± 15.8	86.0 ± 9.2	5.5 ± 0.8	5.4 ± 0.4
	Post	9	55.6 ± 1.3	85.1 ± 24.7	24.5 ± 2.8	82.6 ± 4.4	93.9 ± 17.3	134.6 ± 22.0	89.7 ± 12.3	6.1 ± 1.0	5.5 ± 0.3
	Post-ET	4	55.5 ± 1.3	68.0 ± 39.2	26.5 ± 3.6	85.7 ± 3.8	110.0 ± 22.7	128.0 ± 18.5	83.3 ± 14.6	5.6 ± 0.5	5.4 ± 0.1
II	Pre	103	46.0 ± 0.9	15.1 ± 19.7	26.5 ± 5.4	85.0 ± 8.3	92.1 ± 12.0	127.7 ± 17.5	85.4 ± 10.2	5.3 ± 0.8	5.5 ± 0.3
III	Post	9	55.6 ± 1.1	94.0 ± 32.6	26.8 ± 2.9	81.2 ± 6.6	99.1 ± 26.4	130.8 ± 14.6	85.2 ± 8.7	5.6 ± 1.1	5.7 ± 0.3
	Post-ET	9	56.0 ± 1.0	56.7 ± 29.4	27.2 ± 3.6	87.3 ± 5.1	102.3 ± 22.4	139.9 ± 21.5	90.3 ± 17.3	5.7 ± 0.5	5.6 ± 0.3
IV	Post	17	55.4 ± 1.2	81.8 ± 30.5	25.2 ± 2.4	81.9 ± 4.5	93.6 ± 13.4	131.9 ± 15.9	84.1 ± 10.1	5.8 ± 1.0	5.6 ± 0.3
	Post-ET	5	56.0 ± 1.2	69.0 ± 33.3	25.6 ± 3.7	84.1 ± 4.9	106.6 ± 23.8	127.2 ± 17.7	79.8 ± 11.8	5.5 ± 0.4	5.5 ± 0.3
Total	Pre	107	46.0 ± 0.9	14.9 ± 19.4	26.5 ± 5.3	84.9 ± 8.3	92.9 ± 12.7	127.6 ± 17.5	85.2 ± 10.2	5.3 ± 0.8	5.5 ± 0.3
	Post	17	55.7 ± 1.1	87.8 ± 29.7	25.7 ± 3.2	80.9 ± 4.9	94.9 ± 22.0	135.1 ± 17.6	88.6 ± 10.0	5.8 ± 1.0	5.6 ± 0.3
	Post-ET	9	56.0 ± 1.0	56.7 ± 29.4	27.2 ± 3.6	87.5 ± 5.4	102.3 ± 22.4	139.9 ± 21.5	90.3 ± 17.3	5.7 ± 0.5	5.6 ± 0.3

Data are presented as mean ± SD. Definition of abbreviations: Pre = premenopausal women; Post = postmenopausal women; Post-ET = postmenopausal estrogen users; FSH = follicle stimulating hormone; BMI = body mass index; FEV_1 = forced expiratory volume in one second presented as % of the predicted values; SBP = systolic blood pressure; DBP = diastolic blood pressure; HbA1C = glycosylated hemoglobin A1C.

4.2.3 Laboratory assays

For all of the subjects, venous blood samples for the assessments of plasma follicle stimulating hormone (FSH; AutoDelfia®, Wallac, Turku, Finland), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (Modular Analytics P-analyzer®, Roche/Hitachi, Tokyo, Japan) as well as glycosylated hemoglobin A1C (GHbA1C; Variant II®, Bio-RAD Laboratories, Diagnostics Group, Hercules, CA) were collected after an overnight fast on the morning prior to the sleep study. LDL cholesterol concentrations were calculated using Friedewald's equation (Friedewald et al. 1972).

4.2.4 Forced expiratory volume in one second

The forced expiratory volume in one second (FEV_1) was measured in all subjects prior to the sleep study (handheld spirometer, One Flow® STI, France). The maximum of three consecutive measurements of FEV_1 was entered into the analysis. FEV_1 was presented both as litres and as a percentage of the predicted values.

4.2.5 Sleep studies

The overnight recordings included conventional PSG, SaO_2, $EtCO_2$, ECG, nasal flow pressure and $TcCO_2$ measurements. Sleep studies were done in the sleep laboratory of the Sleep Research Unit at the Department of Physiology of the University of Turku.

Conventional polysomnography

PSG was used for the definition of sleep architecture including sleep stages, sleep latency, sleep efficiency, sleep fragmentation and arousals. PSG recordings included the continuous monitoring of a four channel EEG, an EOG, an EMG and an ECG (Embla®, Medcare Flaga hf. Medical Devices, Reykjavik, Iceland).

Definition of sleep architecture

The sleep architecture of the study subjects was determined in Studies III and IV. Sleep stages were visually analyzed in 30-second epochs by the same scorer (J.Aittokallio) according to the Rechtschaffen and Kales criteria (stage 1, stage 2, combined slow-wave sleep [SWS, stages 3 and 4] and rapid eye movement [REM] sleep) (Rechtschaffen & Kales 1968). Sleep onset (the beginning of the sleep period) was determined as the appearance of the first ten consecutive epochs of sleep (Study III) or the first two consecutive epochs of sleep (Study IV). The end of the sleep period was determined as the final arousal leading to wakefulness. Sleep latency was defined as the period from the beginning of the recording to sleep onset. Sleep efficiency was defined as the percentage of sleep time out of the sleep period time. Arousals were assessed according to the guidelines of the American Sleep Disorder Association (Atlas task force of the ASDA

1992). An arousal was defined as an awakening lasting longer than three seconds. The arousal index was expressed as the average frequency of arousals per hour of sleep.

Measurement of arterial oxyhemoglobin saturation and nasal flow pressure

Nasal flow pressure was measured with nasal prongs attached to the Embla®/Somnologica system. SaO_2 was measured by two finger probe pulse oximeters (Nonin® oximeter built into the Embla®/Somnologica system, Medcare Flaga hf, Reykjavik, Iceland and Ohmeda Biox 3700 Pulse Oximeter®, Biomed Technologies Inc. Louisville, Colorado, US, recorded using the Uniplot® software, Unesta, Turku, Finland).

Mean and nadir SaO_2 values were calculated for all of the subjects. A decrease of 4 % or more from the pre-event baseline in SaO_2 was defined as desaturation. The frequency of at least four percentage desaturations per hour was expressed as the oxyhemoglobin desaturation index (ODI_4). The episodes of ODI_4 were calculated using the Embla® system. In Study II, in addition to ODI_4 being calculated using the Somnologica system, a custom-written algorithm was developed to detect desaturation events larger than 2, 3 and 4 % per hour of recording (ODI_2, ODI_3, and ODI_4, respectively), and the average durations of such events over the night (ODI_d Duration, where d=2, 3, 4). In Study II, the following SaO_2 features were also detected: SaO_2 median, 0.1 % quantile, 90 % quantile, 95 % quantile, proportion of time for which the signal is below 90 %, proportion of time for which the signal is below 95 %, frequency of resaturation events of 3 % or more and average duration of resaturation events over the recording.

Although the RERA index and RDI have recently been introduced (Iber et al. 2007), there is still controversy over their clinical significance. Almost all of the sleep studies examining cardiovascular outcomes still use episodes of apnea and hypopnea, as well as SaO_2. A recent study demonstrated that events without a desaturation were not associated with an increase in mortality and morbidity (Punjabi et al. 2009). In the present work, we used AHI, ODI and SaO_2 as in most of the other studies.

The apnea-hypopnea index was visually determined using the Embla® software (Figure 7). Apnea was scored when the amplitude of the nasal flow signal dropped at least 90% from the baseline and the event lasted 10 seconds or more. Hypopnea was scored when the amplitude of the nasal flow signal dropped at least 30 % from the baseline. Further, the hypopnea event had to last 10 seconds or more and had to be connected with a minimum of 4% desaturation from the pre-event baseline (Iber et al. 2007).

Electrocardiography

An ECG was recorded from all of the subjects with a sampling frequency of 1 Hz throughout the night by the Embla® system. The median heart rate was recorded in Study I. In Study III, the cardiac sympathovagal balance was assessed using the power spectrum analysis of the Embla® software by calculating the low frequency (LF) and

high frequency (HF) ratio. The LF band ranged from 0.04 Hz to 0.15 Hz and the HF band ranged from 0.15 Hz to 0.40 Hz. The LF/HF ratio was analyzed in 5 min epochs of wakefulness and SWS using the Embla® software.

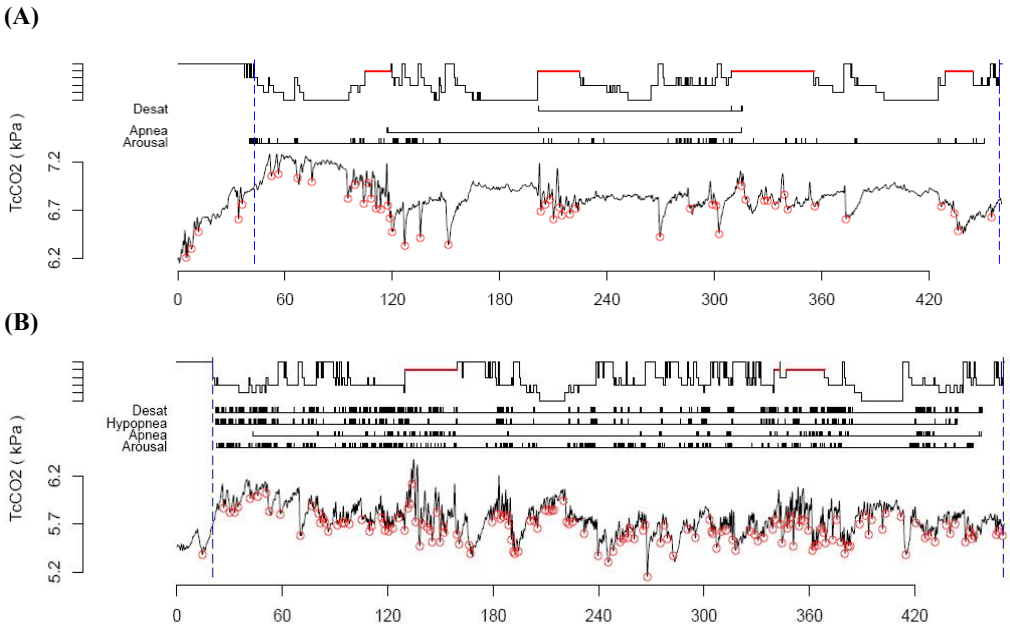

Figure 7. Overnight transcutaneous carbon dioxide ($TcCO_2$) graphs together with hypnograms of two postmenopausal women. Desaturation events, episodes of apnea and hypopnea, and arousals are marked under the hypnogram. Subject A was generally healthy, but subject B was excluded from the study because of severe obstructive sleep apnea. The frequency of pit patterns are markedly greater for subject B (marked with open circles). Desat = arterial oxyhemoglobin desaturation of 4% units or more. The two dotted vertical lines indicate sleep onset and the last awakening in the morning.

Measurement of $EtCO_2$

$EtCO_2$ was measured using nasal prongs attached to a side-stream capnograph (Datex Normocap® CO_2 and O_2 Monitor, Instrumentarium, Finland) in Studies I and II. Before each recording, the $EtCO_2$ signal was calibrated by flushing the sensor with a calibration gas containing a 5 % concentration of CO_2. From the $EtCO_2$ signal, the overnight mean was extracted. A custom-written algorithm was developed to detect all acceptable alveolar plateaus and then construct the $EtCO_2$ envelope curve by joining the plateaus. This pre-processing algorithm, which was adjusted manually, efficiently discards any artefacts from the $EtCO_2$ envelope curve, including short expirations and mouth breathing.

Measurement of $TcCO_2$

$TcCO_2$ was measured with the TCM3 device (Radiometer®; Copenhagen, Denmark) (Baumbach 1997). After cleaning the skin with alcohol, the skin sensor was placed on

the left side of the upper chest parasternally and heated to 43˚C, at which temperature the sensor remained attached continuously during the night for a maximum of eight hours (Janssens 2001). Before each recording, the $TcCO_2$ signal was calibrated by flushing the sensor with a calibration gas (5 % concentration of CO_2).

Signal processing was performed using the R software (R Development Core Team). In the $TcCO_2$ preprocessing phase clear artifacts at the beginning or at the end of the recordings were removed manually. The artifacts in the middle of the recordings were replaced with line segments.

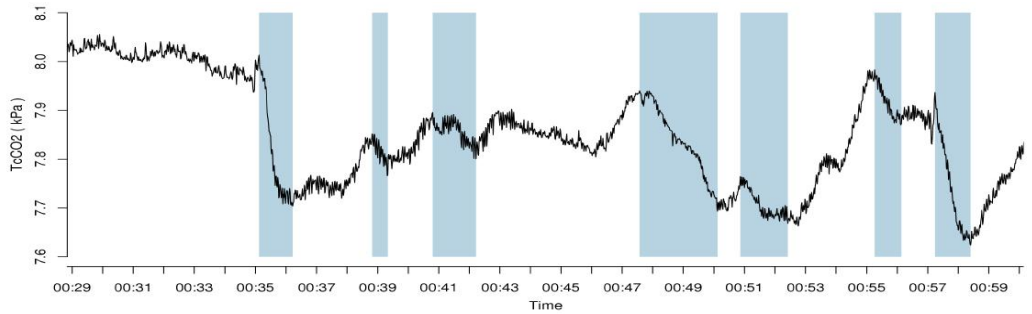

Figure 8. An example of the $TcCO_2$ pit pattern analyzer in a 30 minute interval. Abrupt descents are highlighted in blue. $TcCO_2$ = Transcutaneous carbon dioxide.

From the $TcCO_2$ signal overnight median values were calculated, as well as medians over various sleep stages and evening wakefulness. Median values were used since these give better estimates than averages for the representative $TcCO_2$ levels, for median values are not easily affected by artifacts. In addition to these conventional measures, in Studies II and IV also the overnight variability of $TcCO_2$ was characterized. Special attention was paid to the detection of pit patterns, the abrupt CO_2 descents (Figures 8 and 9). The pit index (the frequency of pit patterns per hour of recording) was calculated, and from these pit patterns the average descents (amplitude, duration and slope) and ascents (amplitude, duration and slope) were calculated. In Study II, also the $TcCO_2$ mean, 90 % quantile, 95 % quantile and initial slope (regression line slope fitted to the initial part) were detected. Different minimum durations and minimum descents were included in the analysis. In Study IV the pit patterns were defined more specifically according to the findings in Study II (Figures 8 and 9). The minimum descent had to last for 90 seconds and to be greater than 0.08 kPa, and the highest $TcCO_2$ plateau (maximal plateau) was defined visually from each curve.

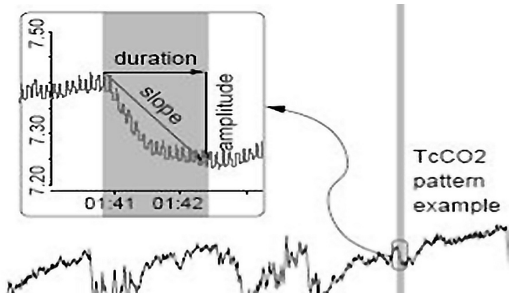

Figure 9. The enlarged insert in the $TcCO_2$ signal corresponds to a single pit pattern.

4.2.6 Ultrasound imaging

Ultrasound studies of the arteries were made in the morning after the overnight recordings. The ultrasound measurements were performed by Acuson Sequoia 512 (Acuson Inc. Mountain View, CA) ultrasonography with a 13 MHz linear array transducer. Ultrasound scans were performed by operators unaware of the results of the overnight recordings. The subjects were in the supine position. Ultrasound scans were recorded and stored in a digital format or in a videotape for subsequent offline analysis.

Endothelial function

Endothelial function was assessed using FMD, and endothelium-independent vasodilatation was assessed using NMD according to the International Brachial Artery Task Force (Corretti et al. 2002). The diameter of the brachial artery was measured in three stages: at rest, after reactive hyperemia, and after sublingual nitroglycerine (isosorbide dinitrate spray 2.5 mg, Dinit®, Leiras, Turku, Finland) (Figure 10). The brachial artery was scanned approximately 10 cm above the elbow. An increased flow was induced by the release of a pneumatic tourniquet placed around the forearm with a pressure of 250 mmHg for 5 minutes. The brachial artery was scanned for a second time approximately 60 seconds after cuff release. After 10 minutes of vessel recovery, sublingual nitroglycerin was administered, and the brachial artery was scanned for the last time 4 to 5 minutes later. The measurements were made at end-diastole, 4 to 8 times at each stage and the average values were used for the results. Reactive hyperemia and nitroglycerin-induced vasodilatation were expressed in terms of the change in the diameter relative to the resting diameter.

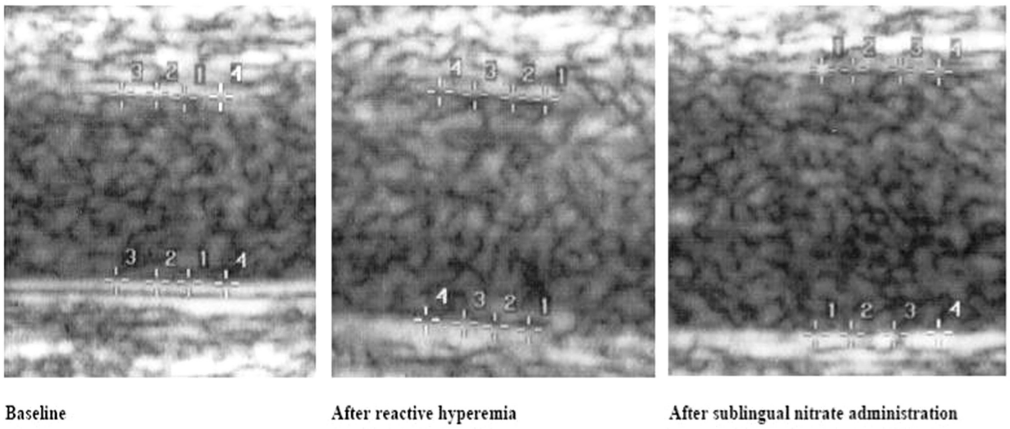

Figure 10. Brachial artery diameter scanning with ultrasound: at rest (baseline), after reactive hyperemia, and after sublingual nitroglycerine. Flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) were calculated as the change in the diameter (%) relative to the baseline.

Fifteen videotapes were analyzed twice by two observers (J. Aittokallio and J. Toikka). The inter-observer variation of the mean FMD was 3.12 ± 0.04 % (mean ± SD) ranging from 0.25 to 12.64 % (coefficient of variation [CV], 42.8 ± 49.6 %). The Pearson's correlation between the FMD measurements was 0.715 ($p = 0.003$). The inter-observer variation of the mean NMD was 3.31 ±0.04 % ranging from 0.61 to 16.8 % (CV, 15.8 ± 13.2 %). The Pearson's correlation between the NMD measurements was 0.938 ($p < 0.001$). Because FMD and NMD are percentage-ratio measures, small differences between observers can appear quite large (Corretti et al. 2002).

Intima-media thickness

The IMT of the left common carotid wall was assessed 1-2 cm proximal to the carotid bulb (Roman et al. 2006) (Figure 4). The common carotid artery wall was scanned longitudinally and the IMT was measured at the site of the far wall during end-diastole. The IMT was measured 4-8 times and the average values were used in the analysis.

Twelve videotapes were analyzed twice by two observers (J. Aittokallio and J. Toikka). The inter-observer variation of the mean IMT was 0.04 ± 0.04 mm ranging from 0 to 0.13 mm (CV, 4.8 ± 2.6 %). The Pearson's correlation between the IMT measurements was 0.923 ($p< 0.001$). The results are in line with previous studies performed in several laboratories (Kanters et al 1997).

4.2.7 Statistical analyses

Statistical analyses were carried out with the SPSS® software (SPSS, Chicago, IL). In all of the tests, a p value < 0.05 was considered to be significant. The results are reported as means with standard deviations (SD). Comparisons between two groups were performed

with Student's t-test and the Wilcoxon signed-rank test. Pairwise associations were tested with the Pearson correlation coefficients. Standard multiple linear regression analyses with stepwise feature selection were used to study independent predictors. In addition, in Study I, the effects of sleep and/or menopause on the median levels were assessed with repeated measurements analysis of variance (ANOVA). In Study II, a predictive modelling technique was used to assess the relative importance of the features in terms of their contribution to the prediction accuracy by which subjects could be distinguished into two classes of ultrasound measurements ("high" and "low" classes by using the median values as the cut-off threshold). The first greedy selection included all SaO_2 features, all $TcCO_2$ features, $EtCO_2$ (Table 4) and the confounders (FSH, BMI, waist-to-hip ratio, total cholesterol, LDL cholesterol and blood pressure). The best subset found was reported. The predictive classification performance was assessed using the receiver operating characteristic (ROC) curves. The overall performance of a classifier was summarized by the area under the ROC curve (AUC).

Table 4. Full list of all features extracted from the overnight measurements in Study II.

Feature name	Unit	Recording	Short description of the feature
ODI_4	1/h	SaO_2 signal*	Frequency of desaturation events of 4% or more
SaO_2 min	%	SaO_2 signal*	Lowest oxyhemoglobin saturation recording
Mean	%	SaO_2 signal	Average of the signal over the recording
Median	%	SaO_2 signal	50% of the values are smaller than this
0.1% quantile	%	SaO_2 signal	0.1% of the values are smaller than this
90% quantile	%	SaO_2 signal	90% of the values are smaller than this
95% quantile	%	SaO_2 signal	95% of the values are smaller than this
Time below 90%	1	SaO_2 signal	Proportion of time the signal is below 90%
Time below 95%	1	SaO_2 signal	Proportion of time the signal is below 95%
ODI_d	1/h	SaO_2 signal	Frequency of desaturation events of d% or more
ODI_d duration	s	SaO_2 signal	Average duration of these events over the recording
RES_3	1/h	SaO_2 signal	Frequency of resaturation events of 3% or more
RES_3 duration	s	SaO_2 signal	Average duration of these events over the recording
Mean envelope	kPa	$EtCO_2$ signal	Overnight average of the envelope signal
Mean	kPa	$TcCO_2$ signal	Average of the signal over the recording
Median	kPa	$TcCO_2$ signal	50% of the values are smaller than this
90% quantile	kPa	$TcCO_2$ signal	90% of the values are smaller than this
95% quantile	kPa	$TcCO_2$ signal	95% of the values are smaller than this
Initial slope	kPa/h	$TcCO_2$ signal	Regression line slope fitted to the initial part
Pit index	1/h	$TcCO_2$ signal	Frequency of pit patterns per hour of recording
Amplitude down	kPa	$TcCO_2$ signal	Average descents in these patterns
Duration down	s	$TcCO_2$ signal	Average duration of the descents
Slope down	kPa/h	$TcCO_2$ signal	Average slope of the descents
Amplitude up	kPa	$TcCO_2$ signal	Average ascents in these patterns
Duration up	s	$TcCO_2$ signal	Average duration of the ascents
Slope up	kPa/h	$TcCO_2$ signal	Average slope of the ascents

*The two features were calculated by the Somnologica® software, whereas the remaining features were extracted with our custom-written computer algorithms (Aittokallio et al. 2008, Supplementary Data).

5. RESULTS

5.1 Effects of age, menopause and estrogen on nocturnal non-invasive blood gas measurements

5.1.1 SaO_2 (Studies I and III)

During wakefulness prior to sleep, the median SaO_2 values were similar in pre- and postmenopausal women. Premenopausal women maintained their wakefulness SaO_2 levels also during sleep, whereas in postmenopausal women SaO_2 levels decreased on average 1.17 % -units from evening wakefulness levels. However, the group/state interaction effect on SaO_2 values reached only borderline significance (p=0.065). ODI_4 did not differ in pre- and postmenopasal women (range 0-1.18 and 0-1.97 events/hour, respectively). Mean and nadir values of ODI_4, AHI and SaO_2 were similar for postmenopausal ET-users and non-users.

5.1.2 $EtCO_2$ (Study I)

$EtCO_2$ levels were similar during wakefulness and sleep in pre- and postmenopausal women. Mean (SD) $EtCO_2$ levels during wakefulness were 4.32 (0.75) kPa and 3.91 (0.53) kPa and during sleep 4.39 (0.97) kPa and 4.00 (0.65) kPa, in pre- and postmenopausal women, respectively. $EtCO_2$ levels were generally lower than $TcCO_2$ levels. The mean difference between $TcCO_2$ and $EtCO_2$ measurements during wakefulness was 1.24 (0.99) kPa and 2.04 (0.81) kPa and during sleep 1.57 (0.67) kPa and 2.59 (0.87) kPa in premenopausal and postmenopausal women, respectively.

5.1.3 $TcCO_2$ (Studies I and III)

$TcCO_2$ monitoring proved to be a more sensitive method for detecting menopause or aging-related alterations in nocturnal CO_2 levels than $EtCO_2$ monitoring. In Study I, $TcCO_2$ levels were similar during wakefulness in pre- and postmenopausal women with means of 5.56 (0.48) kPa and 5.95 (0.72) kPa, respectively. $TcCO_2$ increased from wakefulness to sleep in both groups. Mean $TcCO_2$ levels during sleep were 5.96 (0.68) kPa and 6.57 (0.81) kPa in pre- and postmenopausal women, respectively. In addition, $TcCO_2$ increased more in postmenopausal women than in premenopausal women. This increase was even more pronounced in postmenopausal ET-users, and in particular when comparing the change from wakefulness to SWS (Study III). The wakefulness $TcCO_2$ levels of postmenopausal ET-users and non-users were 6.44 (0.72) kPa and 6.42 (0.51) kPa, and during sleep 7.24 (0.80) kPa and 6.76 (0.55) kPa, respectively.

5.1.4 Sleep architecture, LF/HF ratio and Questionnaires (Study III)

Subjective sleep quality (determined using the BNSQ) or daytime sleepiness (determined using the ESS) did not differ between the postmenopausal ET-users and non-users. Neither ESS nor BNSQ showed a correlation with sleep architecture, SaO_2 data or $TcCO_2$. ET-users had a lower sleep efficiency than non-users, but other sleep architecture parameters did not differ. The LF/HF ratio decreased from wakefulness to SWS in all postmenopausal women. In group comparisons, the LF/HF ratio decrease from wakefulness to SWS reached a borderline significance in ET-users ($p = 0.066$), whereas in non-users the difference was not clear ($p = 0.110$). Further, LF/HF ratio values did not differ between the groups.

5.2 Nocturnal measurements as predictors of vascular function in premenopausal women (Study II)

An advanced mathematical modelling technique was used for the systematic investigation of all possible feature and parameter combinations in the prediction of ultrasound measurements. This greedy search phase included all SaO_2 features, $TcCO_2$ features, mean $EtCO_2$, and the confounders (Table 4). The best subset of the features was reported. The overall performance of a classifier was summarized by the area under the ROC curve (AUC).

5.2.1 SaO_2 features

SaO_2 features did not play a remarkable role. However, despite their modest contribution, SaO_2 features were selected into the subset of best predictors with all three ultrasound variables. ODI_3 duration (contribution 6.79 %) and 95% quantile (contribution 0.23 %) were selected into the subset of best features for predicting NMD. Despite this, when SaO_2 was removed from the NMD prediction model, $TcCO_2$ features provided a similar prediction accuracy to the full set of optimal features (AUC=0.81 vs. 0.83, $p>0.1$) (Table 5). SaO_2 features alone gave a significantly lower prediction accuracy than the full feature set (AUC=0.65, adjusted $p<0.005$) (Table X). The 90% quantile was selected into the subset of best features for predicting FMD (contribution 11.40 %) and the 95% quantile for IMT (contribution 4.28 %).

5.2.2 $EtCO_2$

The $EtCO_2$ mean envelope was selected into the subset of best features when predicting NMD and IMT. However, its contribution to the results was rather low (2.47 % with NMD and 11.05 % with IMT).

5.2.3 $TcCO_2$ features

The $TcCO_2$ measurements offered, by far, the greatest contribution to the prediction of each ultrasound measurement class. However, the absolute levels of $TcCO_2$ had no significance, whereas the features of transient $TcCO_2$ pit patterns showed their importance. $TcCO_2$ features alone could provide a good prediction accuracy for all of the ultrasound measurements (FMD AUC=0.75, IMT AUC=0.70)(Table 5). The prediction accuracy for IMT was the lowest, even with the full set of all optimal features, when compared to the prediction of the FMD and NMD. Some of the demographic confounders (BMI, SBP, waist-hip ratio and smoking) complemented the prediction task. FSH, total cholesterol, LDL-cholesterol and DBP did not contribute to the prediction accuracies.

The single most important event was the pit-form pattern of the $TcCO_2$ measurement (Figures 8 and 9). It should be noted, however, that no single $TcCO_2$ feature alone could provide an accurate prediction, but a set of features was needed to obtain a reasonable prediction accuracy (Figure 11**)**.

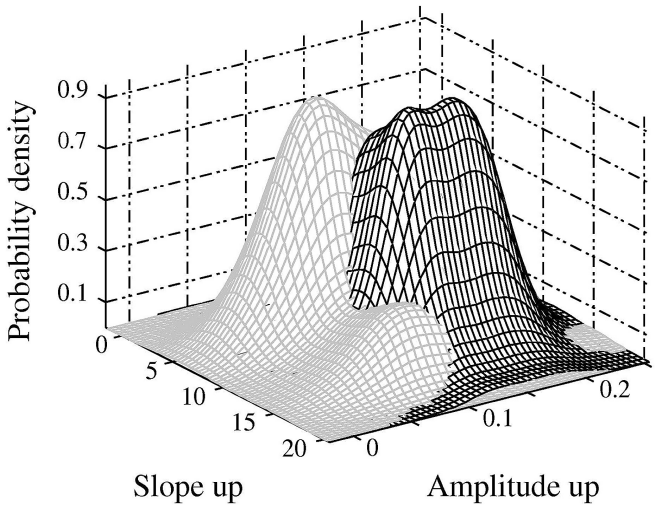

Figure 11. An example of the conditional probability model with 2 features, which was used to predict the NMD class of the subjects as a probabilistic function of two $TcCO_2$ feature values (Slope up and Amplitude up). The probability densities for the two classes were superimposed onto the same graph, in which the grey colour indicates the "low" class and black colour the "high" class, and the maximum of these two densities was used to predict the class of each subject. In many cases, however, two features were not enough and an integration of multiple features was needed to provide an accurate prediction of results (Aittokallio et al. 2008, Supplementary Data). NMD = nitroglycerin-mediated dilatation, $TcCO_2$ = transcutaneous carbon dioxide.

Table 5. Optimal predictor features for the NMD, FMD and IMT classes when using only the demographic and $TcCO_2$ features (upper part) or the demographic and SaO_2 features (lower part) in the non-linear predictive models

(A) NMD			(B) FMD			(C) IMT		
Recording	**Feature name**	**Contribution, %**	**Recording**	**Feature name**	**Contribution, %**	**Recording**	**Feature name**	**Contribution, %**
$TcCO_2$	Slope up	24.3	$TcCO_2$	Duration up	24.2	$TcCO_2$	Amplitude down	40.2
$TcCO_2$	Initial slope	20.4		BMI	23.9	$TcCO_2$	Duration up	20.2
$TcCO_2$	Slope down	13.1	$TcCO_2$	Duration down	16.3	$TcCO_2$	Pit index	15.0
$TcCO_2$	Amplitude up	11.5	$TcCO_2$	Slope down	14.2	$TcCO_2$	Slope up	9.8
	BMI	10.5	$TcCO_2$	Pit index	13.0	$TcCO_2$	90% quantile	5.2
	Smoking	7.1		SBP	8.3	$TcCO_2$	Initial slope	4.9
	SBP	5.5				$TcCO_2$	95% quantile	4.7
$TcCO_2$	Amplitude down	3.1						
	Waist-to-hip ratio	2.9						
$TcCO_2$	Duration down	1.6						
All $TcCO_2$	**AUC=0.81** ($p<0.001$)			**AUC=0.75** ($p<0.001$)			**AUC=0.70** ($p<0.001$)	
All features	**AUC=0.83** ($p>0.1$)			**AUC=0.77** ($p>0.1$)			**AUC=0.72** ($p>0.1$)	

Recording	**Feature name**	**Contribution, %**	**Recording**	**Feature name**	**Contribution, %**	**Recording**	**Feature name**	**Contribution, %**
	Waist-to-hip ratio	37.2		BMI	39.1	SaO_2	95% quantile	28.5
	SBP	35.8	SaO_2	90% quantile	34.0		SBP	16.2
	Smoking	19.0	SaO_2	ODI_2 Duration	15.6	SaO_2	ODI_4 duration	15.3
	FSH	5.3		SBP	9.6		Waist-to-hip ratio	15.0
	DBP	2.6		DBP	1.7		DBP	11.8
						SaO_2	ODI_3 duration	10.6
						SaO_2	0.1% quantile	2.7
All SaO_2	**AUC=0.65** ($p<0.05$)			**AUC=0.64** ($p<0.05$)			**AUC=0.59** ($p>0.1$)	
All features	**AUC=0.83** ($p<0.005$)			**AUC=0.77** ($p<0.05$)				

The first column indicates the recording from which the feature was extracted; empty if the feature is demographic or from the questionnaire. The second column is the feature name used in the text (see Table 4). The third column gives the relative contribution of the feature to the prediction accuracy, that is, the percentage of the change in the AUC value the feature accounts for if it was excluded from the optimal subset. The last row describes the predictive power of the full optimal feature set in terms of the cross-validated AUC and its Bonferroni-adjusted p-value. The row 'All features' gives the AUC value obtained with the full set of optimal features (Table 4), and Bonferroni adjusted statistical significance of the difference between the two cross-validated AUC values. This comparison was not performed for the SaO_2 classifier of IMT since the accuracy of this particular classifier was not significantly different from a random classifier and it was therefore considered meaningless. NMD = nitroglycerin-mediated dilatation, FMD = flow-mediated dilatation, IMT = intima-media thickness, $TcCO_2$ = transcutaneous carbon dioxide, BMI = body mass index, SBP = systolic blood pressure, FSH = follicle stimulating hormone, DBP = diastolic blood pressure, ODI_x = oxygen desaturation index more than 2, 3 or 4% per hour.

5.3 Overnight measurements as predictors of metabolic status in postmenopausal women (Study IV)

The predicted metabolic variables were GHbA1C, cholesterols, triglycerides and blood pressure. The features selected with the stepwise multiple linear regression analyses supported the importance of $TcCO_2$ features, whereas neither AHI, ODI_3, ODI_4, nor any of the SaO_2 or demographic features were selected with any of the metabolic variables. Only the predictors with a p-value under 0.05 were accepted into the final linear regression models. Sleep architecture measures had some importance in the regression model. In addition to the nocturnal $TcCO_2$ features, longer sleep period was found as an important predictor for lower GHbA1C (Beta-value = -0.511, p = 0.009), and increased sleep fragmentation for lower HDL cholesterol (Beta-value = -0.483, p = 0.013).

5.3.1 $TcCO_2$ and metabolic parameters

$TcCO_2$ features were selected with all of the predicted variables. Unlike in Study II, absolute values of $TcCO_2$ had an important role, together with the pit pattern. The percentage of time that $TcCO_2$ stayed over 7 kPa associated with GHbA1C (β = -0.484, p = 0.013) and triglycerides (β = -0.472, p = 0.027). Similarly, the maximal $TcCO_2$ plateau was selected with the HDL/Total cholesterol ratio (β = 0.428, p = 0.047) and evening wakefulness levels of $TcCO_2$ with systolic blood pressure (β = -0.550, p = 0.008). The slope of the $TcCO_2$ pit pattern was the only predictor for total cholesterol (β = -0.500, p = 0.018) and LDL cholesterol (β = -0.439, p = 0.041). The $TcCO_2$ pit index predicted HDL cholesterol (β = -0.487, p = 0.012) and the amplitude of the pit pattern predicted diastolic blood pressure (β = -0.425, p = 0.049).

The nocturnal frequency of pit patterns was computed individually for both REM and NREM sleep, with the pit index being considerably higher in REM sleep (paired *t*-test, $p < 0.001$).

6. DISCUSSION

The present study focuses on the association between early changes in nocturnal breathing disorders and metabolic and cardiovascular diseases. The main emphasis was given to CO_2, the final metabolic end product driving respiration and controlling peripheral vasoconstriction and vasodilatation (autoregulation). A novel approach was used to derive physiologically important information from the nocturnal $TcCO_2$ signal profile. A cross-sectional study design was used for groups of pre- and postmenopausal women. The measurements included sleep recordings, demographic measures, blood samples and ultrasound imaging of blood vessels.

The impact of sleep on breathing was more pronounced in postmenopausal than in premenopausal women. In particular, the change in overnight $TcCO_2$ levels compared to wakefulness levels was markedly higher in postmenopausal women. The effect of sleep on $TcCO_2$ was even greater in postmenopausal ET-users. In addition, the study demonstrated a link between overnight $TcCO_2$ events and early signs of vascular impairment in clinically healthy premenopausal women. This link is much more pronounced with the $TcCO_2$ events than the SaO_2 events that have previously been associated with cardiovascular diseases in OSA patients (Suzuki et al. 2004, Minoguchi et al. 2005). The transient change in the $TcCO_2$ signal, the pit pattern, turned out to be the event of greatest importance in terms of reflecting subtle vascular and metabolic changes. The results suggest that $TcCO_2$ features alone have the potential to identify those individuals at a higher risk of developing atherosclerosis. Moreover, the present study links nocturnal $TcCO_2$ features with metabolic impairment. It was found that the $TcCO_2$ features were the most important predictors of GHbA1C, blood pressure and plasma lipoprotein levels in healthy postmenopausal women. A longer sleep period was linked with a lower GHbA1C, and fragmented sleep with a lower HDL cholesterol as suggested also by previous studies (Eksted et al. 2004, Gangwisch et al. 2007). Interestingly, the conventional measures of SDB (SaO_2, ODI_4, AHI), BMI, and waist circumference were not important predictors of metabolic variables.

According to the present study, nocturnal $TcCO_2$ recording seems to be a sensitive method for revealing differences linked with age or hormonal status in middle-aged women, as well as for evaluating the risk of cardiovascular and metabolic disorders. Subjects were all generally healthy, but despite this, $TcCO_2$ features strongly associated with impairment in metabolic and cardiovascular measures. $TcCO_2$ features might not only measure absolute tissue levels of CO_2, but also reflect changes in peripheral vasodynamics. The bursts of sympathetic nervous system activation cause peripheral vasoconstriction, which may be seen in the $TcCO_2$ signal variation. More studies are needed investigate the mechanisms behind the $TcCO_2$ events.

6.1 Methodology

6.1.1 Subjects

Premenopausal women of around 46 years of age and postmenopausal women of around 56 years of age were recruited through a newspaper announcement for a sleep and cardiovascular study (Figure 6). In total, 133 women were enrolled. The group of premenopausal women (n=107) was markedly larger than the group of postmenopausal women (n=26). All of the subjects were generally healthy. Smokers, habitual snorers and hypertensive women were allowed to participate in the study. However, women with known diabetes or medication for hypercholesterolemia were excluded. The exclusion criteria were rather loose, for the intention was to collect a sample of middle-aged women as representative of the general population as possible. An overly tight selection criteria might have limited the sample to exceptionally healthy women. However, the recruitment process may have skewed the subject selection. The subjects were recruited through newspaper announcements advertising a sleep and cardiovascular study. It is possible that women with subclinical hidden sleep problems or cardiovascular family risk factors were more interested in participating in the study. Moreover, health conscious or health oriented women may be more likely to respond to this kind of newspaper announcement.

The subjects were relatively heterogeneous in terms of hormonal status. Thirteen women in the "premenopausal group" were defined to be perimenopausal due to their FSH levels over 30 IU/L and two women were on ET. Moreover, 23 premenopausal women were using an intrauterine device which releases progesterone locally. In menstruating premenopausal women, the effect of fluctuating estrogen and progesterone levels was reduced by performing measurements in the follicular phase of the menstrual cycle (between the 3rd and the 10th day of the menstrual cycle).

In the postmenopausal group, nine women were on ET. Although the subjects were all of around the same age, the time from menopause varied. The serum FSH measurements confirmed cessation of ovarian hormonal activity in all of the postmenopausal women.

The postmenopausal women were ten years older than the premenopausal women. Aging has been associated with decreased minute ventilation and increased arterial CO_2 during SWS in dogs (Phillipson et al. 1993). In a human study, similar age-related changes are reported as well (Nishimura et al. 1991). After the withdrawal of endogenous progesterone at menopause, a decreased responsiveness to hypoxic and hypercapnic stimuli may allow chronic hypoventilation and higher CO_2 during sleep. With this study design, however, it is impossible to separate the influence of age and hormonal deficiency at menopause on nocturnal respiratory phenomena. By investigating pre- and postmenopausal women of similar ages, one could separate the effects of age and menopause on the results.

After menopause the risk of SDB increases (Bixler et al. 2001, Young et al. 2003). SDB is also known to be a risk factor for metabolic and cardiovascular disorders (Vgontzas et al. 2005, Hamilton et al. 2004). Moreover, aging as well as a greater BMI, increase the risk of nocturnal breathing abnormalities in women (Anttalainen et al. 2007). Our study population was a group of clinically healthy middle-aged women with age and menopausal status as risk factors, which gave us the intriguing possibility to search incipient changes of cardiovascular and metabolic diseases.

6.1.2 Questionnaires

Sleep complaints are frequent in postmenopausal women (Polo-Kantola 2008).The widely-used questionnaire BNSQ was used in Study III for the detection of self-reported sleep quality (Partinen & Gislason, 1995). Snoring at least once a week was selected as a cut-off point of habitual snoring, resulting in quite a high frequency of self-reported snoring in both the pre- and postmenopausal groups of women. The well-known and validated Epworth Sleepiness Scale (ESS, Johns 1991) was used to evaluate daytime sleepiness in Study III. The pre- and postmenopausal women did not differ either in terms of the ESS scale or BNSQ responses. However, since the study sample was rather small (nine pre- and nine postmenopausal women), the results should be interpreted with caution. Moreover, the women were clinically healthy and therefore not supposed to differ in terms of excessive daytime sleepiness.

6.1.3 Sleep recordings and analyses

The sleep studies were single-night recordings without an adaptation night. Formerly adaptation to the laboratory environment was considered essential for sleep recordings (Agnew et al. 1966), but a more recent study found no first-night effect (Mourtazaev et al. 1995). Moreover, the situation and environment in the laboratory were similar for all of the subjects. On the day before the sleep study, the use of alcohol and caffeine was prohibited.

The sleep studies were scored manually according to the Rechtschaffen & Kales criteria (Rechtschaffen & Kales 1968) in 30-second epochs in Studies III and IV for altogether 26 postmenopausal women by the same scorer (J. Aittokallio). The Rechtschaffen and Kales criteria have been a gold standard in sleep research since the 1960's. The scorings of the present study were performed before the year 2007, when the new modified scoring rules were finally introduced (Iber et al. 2007). In spite of the new scoring rules, the basics of the scoring system are the same. The standard system has been criticized for inter alia its poor usability in elderly and patient populations, and for its inflexible timing with 30 s epochs, which ignores more rapid sleep stage changes (Himanen & Hasan 2000). In addition, the EEG measurement itself might have limitations, for it shows only

cortical electrical events and cannot measure the activity of deeper brain structures (Polo 2003).

The new scoring rules have been recently compared with the old Rechtschaffen and Kales criteria (Moser et al. 2009). The sleep latency, total sleep time, and sleep efficiency were not affected by the new classification standard. However, the amount of light (S1) and deep (SWS) sleep increased, whereas stage 2 sleep decreased according to the AASM rules (Iber et al. 2007). The amount of REM sleep decreased with increasing age (Moser et al. 2009). It is unclear, how this new scoring standard would have affected our results. In group comparisons (Study III), however, the new rules probably would have had no importance. In Study IV, only the sleep fragmentation and the length of the sleep period were selected into the linear regression models, which are not markedly influenced by the AASM rules.

In the present study, sleep onset was determined as the appearance of the first ten consecutive epochs of sleep (Study III) or the first two consecutive epochs of sleep (Study IV). The end of the sleep period was determined as the final arousal leading to wakefulness. The criterion of sleep onset differs between Studies III and IV, for in Study III we wanted to ensure that sleep onset was not immediately followed by an awakening. In Study III, the respiratory recordings before and after sleep onset were compared. Hence, the wake period in this study was also significantly shorter than the sleep period.

6.1.4 Ultrasound imaging

Ultrasound images of the arteries were taken in the morning after the overnight sleep recordings for 103 premenopausal women. The recordings were done by three observers (J. Aittokallio for 41 % of the measurements). In ultrasound imaging, inter-observer variations can be quite large. Ideally, all of the analysis should be completed by the same observer. For the validation of the measurements, fifteen videotapes of FMD and NMD and twelve videotapes of IMT were analyzed twice by two observers (J. Aittokallio and J. Toikka). The assessments of the different observers did not differ (see the Methods section).

6.1.5 Study design

The study design was cross-sectional, with two age groups of women (46- and 56-year-olds). Therefore it was not possible to separate the influence of age and menopause, or address the question of causality. CO_2 can play a causative role or be a surrogate for some underlying pathophysiological process.

In Studies I, III and IV the sample size was rather modest (n= 26, 18 and 22, respectively). The laborious methodology in sleep studies often results in a small amount of subjects.

However, in Study II the sample size was over one hundred subjects, which is a remarkable size in clinical sleep research and enabled us to search early changes in sleep and vascular parameters. The careful matching of groups by age and demographic measurements is a strength of the study.

6.1.6 Data processing

$TcCO_2$ signal processing was performed using the R software (R Development Core Team). In the preprocessing phase, clear artifacts were removed manually and replaced with line segments. This procedure is not likely to affect $TcCO_2$ median values.

In Study II, a mathematical modelling technique was used in the feature selection and prediction of vascular parameters. Physiological correlates behind the newly-found $TcCO_2$ features and their underlying relationships with the vascular impairment are not known. Therefore, an alternative approach was selected, in which mathematical modelling was first used to suggest potential overnight features of $TcCO_2$. In contrast to many previous studies that have identified the key features from overnight measurements by exploring their linear association to the vascular parameters within the study population only, our aim was to find such features that can predict the correct class of a new subject as accurately as possible by exploiting non-linear relationships between the features. Mathematical models can serve as a step towards learning the mechanistic insights into the factors which are most important for a particular marker.

However, mathematical models also have their limitations. One technical limitation of the present testing procedure concerns the subject classification. In the absence of relevant clinical cut-off thresholds for the ultrasound measurements, the subjects were simply divided into two classes using medians as cut-off points. Another technical concern present in each prediction study is the possibility of over-fitting the classifier to a limited amount of data. In total, there were 34 distinct features extracted from the PGS signals; 13 features from the SaO_2 signal, 12 from the $TcCO_2$ signal, one from the $EtCO_2$ signal, and eight from the demographic data. Both model-based feature selection and data-driven feature reduction procedures were used together with cross-validation to limit the feature set sizes of the optimal classifiers and the risk of their over-fitting.

6.2 Nocturnal non-invasive blood gas measurements

6.2.1 SaO_2

In Study I, nocturnal SaO_2 levels of postmenopausal women were slightly lower than those of premenopausal women, and the postmenopausal women reported more habitual snoring. These results suggest that postmenopausal women might have more mild SDB than premenopausal women, even though the ODI_4 values did not differ.

The women participating in the study were generally healthy, which may explain the minor role of the SaO_2 measurements in our results. Conventional indexes of the severity of SDB (AHI, ODI_4 and SaO_2) did not play an important role in our analyses.

It is known that the blood pressure of OSA patients is higher than in healthy controls (Hla et al. 1994) and nocturnal hypoxia elevates blood pressure (Arabi et al. 1999). In addition, several previous works have demonstrated a strong association between hypoxia and vascular impairment (Ip et al. 2004, Nieto et al. 2004, Oflaz et al. 2006). However, the results of this study suggest that it is perhaps not hypoxemia per se, but other nocturnal events like alterations in sympathetic activation, which may result in cardiovascular disease. On the other hand, the healthiness of the subjects may explain the low importance of conventional severity indexes of SDB.

6.2.2 $EtCO_2$

In Studies I and II, the overnight mean $EtCO_2$ was calculated. All acceptable alveolar plateaus of $EtCO_2$ were used in the construction of the $EtCO_2$ envelope curve by joining the plateaus. However, it is possible that during some episodes (for example during some short periods of REM sleep) the respiratory rate may have increased and the tidal volume decreased so that the $EtCO_2$ readings were artificially low because of insufficient alveolar plateau. However, such episodes represent a marginal proportion of the sleep period and are unlikely to influence significantly the robust median values over the long recording periods.

Sleep onset associates with a marked and rapid decrease in minute ventilation independently of gender or the phase of the menstrual cycle of women (Colrain et al. 1987, 1990). It is well established that the decrease of minute ventilation after sleep onset results in an elevation of $EtCO_2$ and $PaCO_2$ (Skatrud et al. 1988). Based on these findings we expected higher $EtCO_2$ levels during sleep. Although in Study I there was typically an initial $EtCO_2$ increase after sleep onset, an overall $EtCO_2$ increase from wakefulness to sleep was not observed when analyzing simply the median value over the sleep period.

The mean alveolar partial pressure of CO_2 is about 5.3 kPa (Despopoulos & Silbernagl, 2003). In Study I, pre- and postmenopausal women had wakefulness values of 4.32 kPa and 3.91 kPa, respectively. During sleep $EtCO_2$ values were 4.38 kPa and 4.00 kPa, respectively. The results suggest that our method might slightly underestimate alveolar CO_2 values. Moreover, an interesting finding was that postmenopausal women had slightly lower values compared to premenopausal women. However, our $EtCO_2$ results are in accordance with another study performed in healthy subjects (Douglas et al. 1982). $EtCO_2$ levels in that study were approximately 4.53 kPa during wakefulness, 4.85 kPa during SWS and 4.93 kPa during REM sleep.

6.2.3 $TcCO_2$

CO_2, which is the final metabolic end product, turned out to play a major role in our results. The $TcCO_2$ sensor measures the CO_2 that diffuses through the skin, and is affected by the central respiratory drive, peripheral vascular perfusion and local tissue metabolism (Baumbach 1997, Lubbers 1981). Overnight $TcCO_2$ measurements were performed for all of the subjects. The absolute $TcCO_2$ values were markedly higher than $EtCO_2$ values (as expected), but the quantity of increase between wakefulness and sleep was comparable to the change observed in $EtCO_2$ values by Douglas et al. (1982).

In almost all of the subjects, $TcCO_2$ increased after sleep onset and remained at the higher average level during the entire sleep period. This could be due to an upward drift, which is thought to disturb long-term $TcCO_2$ recordings, resulting in artificially high values towards the morning hours. However, such a drift was not seen in our study population, for usually after awakening $TcCO_2$ levels dropped to the same level as the evening values (Figure 3). Due to the eight hour limitation in $TcCO_2$ recording time, we do not have $TcCO_2$ morning values for all of the subjects. However, previous studies have shown that the $TcCO_2$ device provides reliable measures for between 4 and 8 hours at the same temperature as was used in the present study (Janssens et al. 1998, 2001*)*.

In study I, the sleep-related increase in $TcCO_2$ was higher in postmenopausal women than in premenopausal women, and also the SaO_2 values were slightly lower. According to the gas equation rules, any increase in partial pressure of CO_2 is associated with a fall in partial pressure of O_2. In addition, postmenopausal women reported slightly more snoring (7 vs. 4), which may indicate the higher prevalence of upper airway collapse. However, snoring was not objectively measured in the present study. In the future, it would be of interest to compare $TcCO_2$ levels with inspiratory flow shapes in snoring subjects.

$TcCO_2$ has recently been demonstrated to enable the accurate estimation of arterial CO_2 (Casati et al. 2006, Parker & Gibson, 2007). However, $TcCO_2$ measurements have not become very popular in sleep studies, because they are often considered to have too slow a response time to rapid respiratory events. Despite these suspicions, the pit pattern of $TcCO_2$ seems to reflect very rapid nocturnal events. These events might be associated with arousals and, further, arousals are associated with transient activations of the sympathetic nervous system. Since it is known that $TcCO_2$ measurements are adversely affected by cutaneous vasoconstriction (Healey et al. 1987), it is possible that the rapid pit patterns reveal the local CO_2 changes resulting from neurally mediated episodes of cutaneous vasodynamics. The most interesting theory concerning $TcCO_2$ measurements in this study, is the effect of sympathetic activity. Sympathetic activity is normally dominant both during wakefulness and REM sleep, while in NREM sleep a parasympathetic tone is prevailing. Sympathovagal imbalance is related to cardiovascular

morbidity and components of metabolic syndrome, but a clear causative role has not been demonstrated (Frontoni et al. 2005, Narkiewicz et al. 1998a, Narkiewicz et al. 1998b). Pit patterns may be manifestations of sudden bursts of sympathetic activity that produce peripheral vasoconstriction. These bursts may appear more frequently during sympathetic dominance. In the present study, $TcCO_2$ features were classified according to the sleep stages and we found out that $TcCO_2$ pit patterns are often present during REM sleep. They also occur during wakefulness, which strongly supports the theory. The relationship between $TcCO_2$ and the sympathetic nervous system has not been studied further, and needs comparisons for example with heart rate variation analyses.

The transient $TcCO_2$ features performed well in predicting endothelial function and structural vascular changes in premenopausal women. The most important $TcCO_2$ features linked with vasodilatation capacity (slope up and initial slope for NMD and duration up and amplitude up for FMD) are all upwards events, whereas the clearly most powerful predictors of IMT were events operating in the opposite direction, amplitude down and the pit index. This might imply that an increase in $TcCO_2$ indicates episodes of vasodilatation and a sudden decrease indicates vasoconstriction. NMD represents smooth muscle-dependent dilatation, whereas FMD depends on the endothelium released nitric oxide in response to shear stress. An impairment of NMD has earlier been demonstrated in the presence of coronary risk factors (Adams et al. 1998), suggesting that even in the early stages of atherosclerosis, the changes in the vessel wall are already present. The strong link between IMT and a sudden decreases of $TcCO_2$ may suggest that the more powerful the nocturnal vasoconstrictive events are, the thicker the intima-media grows. On the other hand, a thicker IMT produces more powerful vasoconstriction.

Nocturnal $TcCO_2$ features played a remarkable role in predicting metabolic variables (GHbA1C, HDL and LDL cholesterol, triglycerides and blood pressure) in healthy postmenopausal women. The features used in Study IV were originally described in the mathematical modelling study of Virkki et al (2008). The longer the subjects could keep their $TcCO_2$ over 7 kPa, the lower GHbA1C and triglycerides they had. Furthermore, the visually detected maximal plateau of the $TcCO_2$ curve associated positively with the HDL/total cholesterol ratio. These results are encouraging, yet they still need validation, since we have no explicit data about "the normal" $TcCO_2$ ranges during sleep. We may presume that $TcCO_2$ levels change during a persons life span and may also differ between genders. However, the findings are in line with the earlier $TcCO_2$ profile results, and in particular with the fact that the proportion of high $TcCO_2$ levels measured during sleep was one of the most important features for classifying insulin resistance (Virkki et al. 2008). Subjects with a high pit index had a lower HDL cholesterol concentration, further confirming the previous findings (Virkki et al. 2008).

Another interesting finding in the $TcCO_2$ pit patterns was the size of the drops, in particular the amplitude and the sharpness. The fast and deep descents associated with low LDL and total cholesterol. In addition, a high amplitude of the pit pattern was the only predictor for lower diastolic blood pressure. This could be explained through the effects of vasodilatation and vasoconstriction on $TcCO_2$. By monitoring the transient $TcCO_2$ events against the prevailing parasympathetic tone (vasodilatation), the bursts of sympathetic nervous system activity (vasoconstriction) might be seen more clearly. The dominance of the sympathetic activity may diminish the signal amplitude.

The only predictor of systolic blood pressure was the evening wakefulness level of $TcCO_2$. This could be due to vasodilatative effects of CO_2 (Atkinson et al. 1990), or another explanation could be the above described theory that $TcCO_2$ reflects local vasodynamics. If so, in parasympathetic dominance when peripheric blood vessels are dilated, blood pressure is low and $TcCO_2$ seems to be high. Despite this, somewhat opposite results have also been demonstrated: Andersson et al. have previously shown that high $EtCO_2$ predicts high systolic blood pressure in women (Anderson et al. 1999). However, $EtCO_2$ measures CO_2 concentration in the alveoli and is strongly affected by changes in ventilation, whereas $TcCO_2$ is affected by tissue metabolism and local vasodynamics. Hence, $EtCO_2$ and $TcCO_2$ are likely to measure different phenomena.

6.3 Effect of hormonal status

The study population contained women with different hormonal statuses. In the premenopausal group, some women were already perimenopausal (n=13) and several women were using a intrauterine device releasing small amounts of progesterone (n=23). However, the progesterone of intrauterine devices is considered clinically insignificant, for the released amounts of progesterone are minimal and are released locally inside the uterus. In long-term use, levonorgestrel plasma concentrations are between 150 and 200 pg/ml (Mirena®, Bayer Schering Pharma, Turku, Finland). A small minority of premenopausal women were on ET (n=2). The number of premenopausal ET-users was so small that statistical analyses could not be performed. In the postmenopausal group, nine women were on ET. The rather heterogeneous hormonal status of the participants is one of the confounders of the study. However, estrogen usage was not selected as an exclusion criteria since estrogen has only a weak effect on breathing (Polo-Kantola et al. 2003). Systemic progestagens were not allowed.

Progesterone has a strong impact on breathing and the levels of endogenous progesterone decrease at menopause. An increased endogenous production or external supplementation of progesterone decrease $EtCO_2$ (Saaresranta & Polo 2002, Schoene et al. 1981, White et al. 1983). In the present study, however, we failed to demonstrate the difference in $EtCO_2$ levels between pre- and postmenopausal women. Only $TcCO_2$ showed a group

difference. The premenopausal women were investigated during their follicular phase when progesterone levels are low. However, it is unlikely that breathing even during the follicular phase, with its low progesterone level, would be similar to that during the postmenopause. The respiratory effects of high progesterone levels during the luteal phase are likely to be prolonged and at least some residual effect should outlast the two week low progesterone period (Saaresranta et al. 1999, Saaresranta et al. 2001). Moreover, higher estrogen levels in premenopausal women predict more progesterone receptors which then mediate the effect (Brodeur et al. 1986).

The present study demonstrates that the difference in $TcCO_2$ levels between wakefulness and sleep is higher in postmenopausal women than in premenopausal women, and the effect is even more pronounced in postmenopausal ET-users. Some studies suggest a decreased responsiveness to hypercapnia in elderly subjects (Chapman et al. 1987, Peterson et al. 1981). However, according to Naifeh et al, healthy elderly subjects have a lower ventilatory response to CO_2 than younger subjects during wakefulness, but their CO_2 sensitivity does not decrease further during sleep (Naifeh et al. 1989). Lower ventilatory responses to CO_2 would explain higher CO_2 levels overall in postmenopausal women, but not the increase in the awake-sleep difference. Estrogen has minor positive effects on breathing (Polo-Kantola 2008). However, it is possible that aging rather than the hormonal status explains the observed differences in $TcCO_2$ in the pre- and postmenopausal groups, for $TcCO_2$ increased even more in postmenopausal ET-users. This can be explained by the fact, that unlike arterial CO_2 or $EtCO_2$, the $TcCO_2$ actually measures the peripheral tissue pH, which is the product of the CO_2 of the feeding artery and the peripheral tissue perfusion and metabolism. Estrogen is a known vasodilatator (Parker et al. 2008). Thus, the greater sleep-induced changes in $TcCO_2$ in ET-users could indicate enhanced peripheral vasodilatation. On the other hand, ET-usage may also have an effect on upper airway function by improving the ventilatory response to arousals (Jordan et al. 2004), which could lead to higher arterial CO_2 levels and lower arousability. This could also induce a lower $TcCO_2$ pit index in ET-users. Unfortunately, the pit index was not measured in Study II. However, this will be considered in future research.

In Study III, the sleep architecture measures were compared between ET-users and non-users. ET-users were found to have impaired sleep efficiency. However, all of the other sleep architecture parameters were similar between the groups, and this finding is in line with previous reports showing no marked improvement in sleep architecture in ET-users (Polo-Kantola 2008).

Sex hormones, and in particular estrogen, might explain some of the differences in impairment of endothelial function in women. It is known that in premenopausal women FMD is highest in the follicular phase (Kawano et al. 1996). Also ET might improve endothelial function (Lee et al. 2001). In our study, regularly menstruating women were

investigated during their follicular phase with low progesterone and high estradiol levels to avoid effects of hormonal alteration between the subjects. Moreover, none of the ultrasound measurements showed significant differences between the subgroups of pre- and perimenopausal women (categorized according to FSH >30 IU/L) or individuals with or without an intrauterine device releasing small amounts of progestagens. In addition, although FSH was included in the predictive models as a potential confounding factor, it remained an unimportant predictor for the ultrasound measurements (NMD, FMD and IMT).

6.4 SDB and vascular function

Several ultrasound-polysomnographic studies have associated the FMD or IMT with the traditional measures of SDB and oxyhemoglobin saturation. The key observation made in most studies is that rather than the frequency of obstructive events, the severity and the duration of the oxygen desaturation events can explain most of the variation both in FMD (Nieto et al. 2004) and IMT (Suzuki et al. 2004, Baguet et al. 2005, Minoguchi et al. 2005, Schulz et al. 2005). In study populations consisting mostly of male patients with OSA, a significant correlation has been found between the AHI and FMD (Ip et al. 2004) and between the AHI and IMT (Drager et al. 2005; Szaboova et al. 2007).

In contrast to most previous studies, Study II focused on a population of relatively healthy women, which may at least partly explain why the SaO_2 features gave a relatively poor contribution to the prediction power. However, even if our study subjects had no history of SDB, there were subtle but systematic differences in the ultrasound recordings that could be attributed to the overnight $TcCO_2$ events. These new findings, together with the studies showing stronger effects in women than in men (Anderson et al. 1999; Anderson et al. 2001; Faulx et al. 2004), suggest that women with even the mildest forms of SDB are susceptible to SDB-induced cardiovascular consequences. This higher risk might be possible to detect with nocturnal $TcCO_2$ measurements.

6.5 Sleep and metabolic measures

The risk of metabolic syndrome increases after menopause (Davidson et al. 2002, Reckelhoff et al. 2004, Salpeter et al. 2006). Therefore we expected to find some variation in metabolic parameters in our study group of postmenopausal women. Despite the initial expectations, the great contribution of $TcCO_2$ features in the prediction of metabolic parameters in Study IV was rather surprising. For example central obesity is a known risk factor for insulin resistance (Greenfield & Campbell 2004), but in the present study, BMI and waist to-hip ratio did not turn out to be important contributors in the prediction of metabolic variables. Similarly, sleep fragmentation, arousals and short self-reported sleep duration have been shown to be associated with high blood pressure

(Morrell et al. 2000, Ekstedt et al. 2004, Gangwisch et al. 2006), but these variables did not predict blood pressure in Study IV. In contrast, $TcCO_2$ features were linked with each predicted metabolic value. These conflicting findings may be due to our healthy and rather lean study population.

SDB is associated with insulin resistance and impaired lipid profile (Ip et al. 2002, Borgel et al. 2006, Williams et al. 2007). Van Cauter et al. have previously shown that a short SWS duration is associated with an increased risk of diabetes (Tasali et al. 2008). Also a self-reported short sleep duration has been linked with obesity and diabetes (Gangwisch et al. 2005, Gangwisch et al. 2007). These studies are in line with our results showing that a long sleep period was the most important predictor for lower GHbA1C. In addition, sleep fragmentation was a predictor for decreased HDL cholesterol. A recent study showed that a high arousal index predicted lower HDL cholesterol (Ekstedt et al. 2004). These results together suggest the importance of an adequate length and quality of sleep in the prevention of metabolic disorders. The $TcCO_2$ signal seems to be sensitive to subtle nocturnal changes, possibly affected by changes in local vasodynamics. Monitoring $TcCO_2$ events against the prevailing parasympathetic tone during sleep may reveal the abnormal activation of the sympathetic nervous system (increased vasoconstriction). This might be one of the key early changes in metabolic syndrome.

6.6 Future considerations

The standard commercially available $TcCO_2$ device, originally developed for non-invasive arterial CO_2 monitoring, was used in the present studies. By analyzing the "signal artefacts" associated with vasodynamic events, the nocturnal $TcCO_2$ device was unconventionally used to evaluate endothelial function and the risk factors of CVD and metabolic syndrome. This method could be of great clinical value for the early detection of vascular disease. It could also be used to assess the efficacy of new medication in controlling early abnormalities in vascular function. The nocturnal $TcCO_2$ monitoring would offer a promising tool for assessing sleep quality, particularly in terms of the sympathovagal balance during sleep, which is for the moment difficult to measure. Finally, the $TcCO_2$ signal is likely to contain additional information on the control of peripheral perfusion, the significance of which remains to be discovered.

In its present form, overnight $TcCO_2$ monitoring remains laborious to use. The $TcCO_2$ devices are produced in low quantities and have therefore a high unit price. Setting up the recording requires experience and the subject needs to be hospitalized. The sensor and signal processing are optimized for recording arterial pCO_2 and filtering artifacts. Based on the current results, the great potential of this method should foster further development of the technical solutions targeted at a robust portable home device, which could be manufactured in large quantities to reduce the unit price. The sensor and

the signal processing should be optimized for the recording of the vascular tone and the sensitive detection of transient events. Further development of the method would certainly also stimulate vascular and sleep research and provide a better understanding of atherosclerosis and sleep disturbances.

7. CONCLUSIONS

The main conclusions were:

I. Menopause is associated with respiratory changes, which occur particularly during sleep. During wakefulness, the median SaO_2 values did not differ in pre- and postmenopausal women. During sleep, premenopausal women maintained their wakefulness SaO_2 levels, whereas in postmenopausal women SaO_2 levels decreased. $EtCO_2$ levels were similar during wakefulness and sleep in pre- and postmenopausal women. The sleep-related $TcCO_2$ increase was markedly stronger in postmenopausal women than in premenopausal women. Based on different awake-sleep profiles, $EtCO_2$ and $TcCO_2$ are likely to measure different phenomena.

II. Nocturnal SaO_2 and $EtCO_2$ levels seem to play a minor role in a model predicting vascular impairment in clinically healthy middle-aged premenopausal women. The nocturnal $TcCO_2$ signal contains significant information according to which it is possible to classify clinically healthy premenopausal women with different degrees of vascular impairment. These results may be useful in understanding the potential mechanisms underlying the disease pathogenesis. The $TcCO_2$ may not only reflect the changes in CO_2 homeostasis, but also the changes in local vasodynamics.

III. Contrary to our initial hypothesis, ET-users have a higher sleep-induced increase in $TcCO_2$ than postmenopausal women who are not using ET. This is more likely to be due to ET-related vasodilatation than absolute higher arterial CO_2 levels, for in earlier studies ET has shown slight beneficial effects on breathing.

IV. With nocturnal $TcCO_2$ features it is possible to predict the levels of metabolic variables including GHbA1C, HDL and LDL cholesterol, triglycerides and blood pressure in clinically healthy postmenopausal women. The longer the $TcCO_2$ was maintained above the cut-off point of 7 kPa, the lower the GHbA1C and serum triglycerides values were. Moreover, the visually detected maximal plateau of the $TcCO_2$ curve linked directly with the HDL/total cholesterol ratio. The conventional measures such as waist circumference and nocturnal hypoxia were not important predictors. Monitoring $TcCO_2$ events against the prevailing parasympathetic tone (which appears in vasodilatation) during sleep may reveal abnormal endothelium responses to the activation of the sympathetic nervous system. This might be an early change in an abnormal metabolic process.

APPENDICES

Appendix 1. The Epworth Sleepiness Scale (ESS) is a questionnaire intended to measure daytime sleepiness (Johns 1991). 0 - 9 - average score in normal population.

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation:

0 = no chance of dozing
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

SITUATION	CHANCE OF DOZING
Sitting and reading	_____
Watching TV	_____
Sitting inactive in a public place (e.g a theater or a meeting)	_____
As a passenger in a car for an hour without a break	_____
Lying down to rest in the afternoon when circumstances permit	_____
Sitting and talking to someone	_____
Sitting quietly after a lunch without alcohol	_____
In a car, while stopped for a few minutes in traffic	_____

Appendix 2. 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

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