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Sleep-disordered breathing in women

Associations with cardiovascular disease and the significance of sleep apnea during REM sleep

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Abstract

Ljunggren, M. 2019. Sleep-disordered breathing in women. Associations with cardiovascular disease and the significance of sleep apnea during REM sleep. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1567. 75 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0640-7.

Background: Sleep-disordered breathing (SDB) is associated with an increased risk of cardiovascular disease, but it is unclear which elements of SDB that are most harmful to the cardiovascular system and whether the associations observed in men also apply to women.

Aim: To investigate associations between different aspects of SDB and cardiovascular disease in women

Methods and results: All four papers were based on participants in "Sleep and Health in Women" (SHE), a population-based cohort study of women.

Paper I is a cross-sectional study of 349 women with polysomnographic assessments of obstructive sleep apnea (OSA) and measurements of plasma BNP, clinically used as a marker of heart failure, in the morning. There was a dose-response relationship between the severity of OSA and levels of BNP.

In Paper II, with a study population of 5,990 women, questionnaire data on symptoms of obstructive sleep apnea were combined with register data from the Swedish National Patient Register regarding a diagnosis of heart failure (mean follow-up 11.4 years). Women with the combination of snoring and daytime sleepiness had a two-fold increase in the risk of incident heart failure after adjustment for confounding.

Paper III was based on 201 women without known cardiovascular disease, with a polysomnography at baseline, assessing OSA during REM sleep, and a carotid artery ultrasound with measurements of intima thickness at follow-up. Severe OSA during REM sleep was associated with a thicker carotid intima.

Paper IV comprised 253 women with polysomnographic data on severe OSA and severe OSA during REM sleep, as well as proteomic analyses of cardiac and inflammatory proteins. After adjustment for confounding and multiple testing, severe OSA during REM sleep was associated with decreased levels of Sirt2, LAP-TGF-β_1 and Axin1, while there were no significant associations for OSA based on a whole night and protein levels.

Conclusions: Women with symptoms of OSA run an increased risk of developing heart failure and OSA is associated with increased levels of BNP. Severe OSA during REM sleep is associated with an early sign of atherosclerosis and reduced levels of proteins with anti-inflammatory effects linked to atherosclerosis and metabolic regulation.

Keywords: Obstructive sleep apnea, REM sleep, cardiovascular disease, heart failure

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Till Tomas

Till Siri, Eira och Alvar

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Ljunggren M, Lindahl B, Theorell-Haglöw J, Lindberg E. (2012) Association between Obstructive Sleep Apnea and Elevated Levels of Type B Natriuretic Peptide in a Community-Based Sample of Women. *Sleep*, 35:1521-1527.

II Ljunggren M, Byberg L, Theorell-Haglöw J, Lindahl B, Michaëlsson K, Lindberg E. (2016) Increased risk of heart failure in women with symptoms of sleep-disordered breathing. *Sleep Medicine*,17:32-37.

III Ljunggren M, Lindberg E, Franklin K, Öhagen P, Larsson M, Theorell-Haglöw J, Naessén T. (2018) Obstructive sleep apnea during rapid eye movement sleep is associated with early signs of atherosclerosis in women. *Sleep*, 41(7).

IV Ljunggren M, Theorell-Haglöw J, Freyhult E, Sahlin C, Franklin K, Malinovschi A, Janson C, Lindberg E. Sleep apnea during REM sleep matters! A proteomic approach to studying the impact of different measurements of sleep-disordered breathing (submitted).

Contents

Abbreviations

AASM	American academy of sleep medicine
AHI	Apnea-hypopnea index
BMI	Body mass index
BNP	Type B natriuretic peptide
BP	Blood pressure
CAGE	Cut down, annoyed by criticism, guilty about drinking and eye-opener drinks
CI	Confidence interval
CIMT	Carotid intima media thickness
CPAP	Continuous positive airway pressure
CRP	C-reactive protein
DAG	Directed acyclic graph
DM	Diabetes mellitus
ECG	Electrocardiogram
EDS	Excessive daytime sleepiness
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
ESS	Epworth Sleepiness Scale
FDR	False discovery rate
FSH	Follicle-stimulating hormone
HAD	Hospital anxiety and depression
HbA1c	Haemoglobin A1c
HR	Hazard ratio
HRT	Hormone replacement therapy
HT	Hypertension
Hif-1	Hypoxia-inducible factor-1
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Revision
IGC	Interstitial glucose concentration
IQR	Interquartile range
LAP-TGF-β_1	Latency-associated peptide transforming growth factor beta-1
LDL	Low density lipoprotein
LOD	Limit of detection
MAD	Mandibular advancement device

MAI	Microarousal index
MI	Myocardial infarction
NF-κB	Nuclear factor-κB
NPX	Normalized protein expression
NREM	Non-rapid eye movement
NT-pro-BNP	N-terminal pro-B natriuretic peptide
ODI	Oxygen desaturation index
OGTT	Oral glucose tolerance test
OR	Odds ratio
OSA	Obstructive sleep apnea
OSAS	Obstructive sleep apnea syndrome
$PaCO_2$	Arterial carbon dioxide tension
PaO_2	Arterial oxygen tension
PCR	Polymerase chain reaction
PEA	Proximity extension assay
PSG	Polysomnography
RAS	Reticular activating system
RCT	Randomised controlled trial
RDI	Respiratory disturbance index
REM	Rapid eye movement
RERA	Respiratory effort related arousal
RTN	Retrotrapezoid nucleus
SD	Standard deviation
SDB	Sleep-disordered breathing
SHE	Sleep and Health in Women
Sirt2	SIR2-like protein 2
Ti90	Sleep time with saturation <90%
TST	Total sleep time
VLPO	Ventrolateral pre-optic

Introduction

> Dionysius, the son of Clearchus, who was the first tyrant of Heracleia, who was himself afterwards tyrant of his country, grew enormously fat without perceiving it, owing to his luxury and to his daily gluttony; so that on account of his obesity he was constantly oppressed by a difficulty of breathing and a feeling of suffocation. On which account his physicians ordered thin needles to be run into his sides and chest whenever he fell into a deeper sleep than usual.[1]

Even though descriptions of what might be sleep apnea date back to 340 BC,[1] obstructive sleep apnea (OSA) is a relatively young diagnosis, not defined until the mid-1960s.[2] Soon after, it was reported that treatment with tracheostomy not only improved daytime sleepiness but also reduced systemic and pulmonary blood pressure.[3] In the following decade, epidemiological studies started to report an association between the main symptom of OSA, snoring, and cardiovascular disease.[4,5] Since then, researchers have attempted to determine the adverse effects of sleep-disordered breathing on the cardiovascular system.

Initially, OSA was regarded as a condition that mainly affected men and it was not until in the 1990s that women were also included in epidemiological studies of OSA.[6]

Sleep and breathing

Sleep architecture and regulation

Both sleep and wake are actively generated states regulated by a complex interaction of multiple neuroregulatory systems.

Sleep regulation is thought to be an interaction between two regulatory processes, the circadian process (Process C) and the homeostatic process (Process S).[7] The circadian regulation of sleep depends on the suprachiasmatic nucleus, the central circadian clock, and operates on a cycle of about 24 hours affected by light input from the retina and other zeitgebers. The homeostatic drive to sleep, on the other hand, increases with an increasing duration of wakefulness and it is also affected by the quality of the preceding sleep. The drive to sleep might be mediated by an accumulation of adenosine and other sleep-promoting substances.[8]

Sleep can be divided into rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep, with NREM further divided into stage 1, stage 2 and stage 3 sleep with increasing sleep depth.[9] REM sleep, first described by Aserinsky and Kleitman in 1953 and associated with vivid dreaming,[10] is characterised by a wake-like electroencephalography (EEG) pattern, high sympathetic activity and the suppression of motor activity.[11] Normal sleep consists of cycles of NREM and REM sleep that repeat throughout the night. REM sleep, occupying about 20 per cent of total sleep time, occurs at approximately 90-minute intervals during sleep, with longer episodes at the end of the night.[12] The functional role of REM sleep is not fully understood, but it is thought to be important for learning and memory consolidation.[11]

The ascending reticular activating system (RAS) is thought to play a central role in the regulation of wakefulness.[12] In adults, sleep onset is normally initiated through NREM sleep. NREM sleep onset is characterised by decreased ascending RAS activation, in combination with increased neural activity in the ventrolateral pre-optic (VLPO) area, anterior hypothalamus and basal forebrain. REM sleep is triggered by the activation of cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei, while the suppression of motor activity in REM sleep is generated by the glutamate-mediated activation of descending medullary reticular formation.[12]

Breathing during sleep

During eupnoea, normal quiet breathing, inspiration is generated by the activation of the diaphragm and external intercostal muscles, while expiration is passive.[13] The preBötzinger complex, located in the ventrolateral medulla, is considered to be the respiratory rhythm generator in eupnoea. A second cluster of respiratory neurons called the retrotrapezoid nucleus (RTN) is believed to regulate the generation of active expiration when necessary, e.g. during exercise.

The neural groups involved in sleep-wake regulation mentioned above also have descending projections to neurons of the respiratory network and sleep onset is associated with a reduction in neural activity to respiratory muscles and the upper airway muscles.[14] The tidal volume and minute ventilation decrease at sleep onset and are lowest during REM sleep.[15] There is also a reduction in chemosensitivity and slightly higher levels of $PaCO_2$ and lower levels of PaO_2 during sleep.[15]

Sleep-disordered breathing

Sleep-disordered breathing (SDB) is an umbrella term that describes respiratory disturbances that occur during sleep characterised by a narrow upper airway or the loss of a normal respiration pattern during sleep. The most common form of SDB is OSA, characterised by recurrent episodes of airway

narrowing with airflow reduction (hypopnea) or upper airway collapse with airflow cessation (apnea), despite continued respiratory effort.

In humans unlike in other mammals the hyoid bone, which is an important anchorage site for pharyngeal dilator muscles, is not rigidly attached to any skeletal structures.[14] This predisposes the human pharynx to upper airway obstruction and, in the presence of other risk factors affecting upper airway lumen size, the changes during sleep with a reduction in upper airway dilator muscle tone, in combination with a decrease in tidal volume, could cause the pharynx to collapse causing an obstructive apnea.[16]

When an obstructive apnea occurs, the hypoxia, hypercapnea and increased negative pharyngeal pressure stimulate an arousal, the dilator muscle tone increases and the airway is re-opened.[17]

Obstructive sleep apnea

Definition

Obstructive sleep apnea (OSA) is defined as the presence of at least five obstructive respiratory events (apneas, hypopneas or respiratory effort-related arousals) per hour of sleep, in combination with signs, symptoms or associated medical disorders (i.e. cardiovascular disease).[18] In the absence of associated symptoms and disorders, OSA can be diagnosed in the presence of 15 or more obstructive respiratory events per hour of sleep. The definition of OSA has changed over time and much research is based on a definition distinguishing OSA, defined solely on the presence of at least five obstructive respiratory events per hour of sleep, from obstructive sleep apnea syndrome (OSAS), defined as the combination of symptoms and at least five obstructive respiratory events per hour of sleep.[19]

Pathogenesis and risk factors

Factors that narrow the pharyngeal airway lumen may contribute to the development and progression of OSA. These factors include retrognathia, macroglossia, an inferiorly positioned hyoid bone and tonsillar hypertrophy.[14] Obesity is a strong risk factor for OSA and this could be explained both by fat deposition in the upper airway and by a reduction in lung volume also predisposing to upper airway collapse.[20] In the Wisconsin sleep cohort, a 10% increase in body weight was associated with a six-fold increase in the incidence of moderate to severe OSA.[21]

Factors that affect the muscular tone in the pharynx, e.g. alcohol and age, also increase the risk of upper airway obstruction.[22,23]

It has been suggested that smoking is associated with an increased risk of sleep apnea, with upper airway inflammation and overnight withdrawal from

nicotine as hypothesised explanations. The evidence in favour of smoking as a risk factor for OSA is, however, limited.[24]

In patients with heart failure or renal failure, oedema with fluid redistribution from the legs to the pharynx during sleep might contribute to increased pharyngeal resistance and upper airway collapsibility.[14,20]

Snoring is regarded as the main symptom of OSA, but it might also contribute to the development of OSA.[25] The vibration from snoring causes inflammation in the upper airway, resulting in upper airway neuropathy, contributing to the development and progression of OSA.

The link to cardiovascular disease

There are a number of possible ways OSA may contribute to the development of cardiovascular disease. The obstruction of the upper airway during sleep with subsequent oxygen desaturation and hypercapnia leads to the activation of the sympathetic nervous system and arousal.[26] The repetitive cycles of increased sympathetic activity during sleep cause sympathetic over-activity that remains while awake and may contribute to the development of hypertension.[26,27] This sympathetic over-activity is illustrated by increased urine and serum catecholamine levels in OSA and with Continuous Positive Airway Pressure (CPAP) treatment withdrawal[28,29] and, in randomised controlled trials (RCT), CPAP therapy has been shown to reduce catecholamine levels.[30] Sympathetic over-activity in combination with the effects of increased negative intrathoracic pressure has been suggested as a trigger for cardiac arrhythmias.[31]

OSA is regarded as a disease of low-grade inflammation and is associated with increased levels of several inflammatory markers.[32] This is mainly believed to be an effect of intermittent hypoxia activating the transcription factors hypoxia-inducible factor-1 (Hif-1) and nuclear factor-κB (NF-κB), which in turn increase the expression of several inflammatory proteins.[33] It is possible that the inflammation and oxidative stress over time contribute to the development of endothelial dysfunction and atherosclerosis.[34-36] On the other hand, most randomised controlled trials investigating the effect of CPAP therapy on inflammatory markers have failed to show any reduction with active CPAP therapy.[30,37] Confounding by age and obesity have been suggested as factors that might in part explain some of the observed associations between OSA and inflammatory markers.

Obstructive sleep apnea also has momentary effects on the heart. Trying to breathe against a closed airway causes an increase in myocardial work and oxygen demand and delays the necessary increase in coronary blood flow.[38] In combination with the hypoxia, this may lead to impaired cardiac contractility.[39] The negative intrathoracic pressure swings caused by the apneas also cause an increase in venous return and filling of the right ventricle, leading to right ventricle distension and a leftward shift of the interventricular septum, resulting in impaired filling of the left ventricle.[40] This re-

duction in preload, in combination with an increase in afterload, also a consequence of the negative intrathoracic pressure, causes a reduction in stroke volume and cardiac output.[41] An association between OSA and heart failure might be explained by the long-term impact of these effects.

In proteomic studies of clinic-based male study populations, changed levels of proteins involved in the complement system, lipid metabolism, oxidative stress, vascular pathways and acute phase response have also been reported.[42-44]

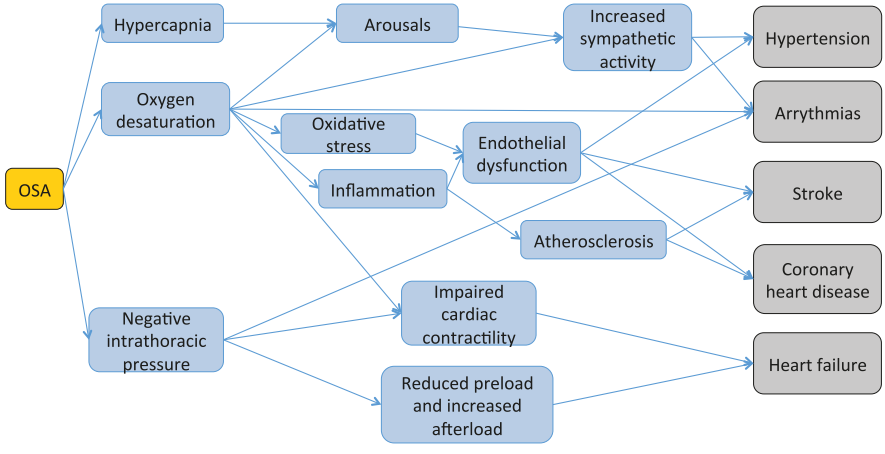

Figure 1. Possible mechanisms by which obstructive sleep apnea might contribute to the development of cardiovascular disease.

Prevalence

When defined as an apnea-hypopnea index (AHI) of ≥5, in combination with excessive daytime sleepiness, the prevalence of obstructive sleep apnea is about 6% in men and 4% in women, based on population based studies published between 1993 and 2013.[23] The sole occurrence of an increase in AHI is much higher. In a recent Swiss study, an AHI of ≥ 15 was seen in 50% of men and 23% of women.[45] This is also in accordance with data from the Sleep and Health in Women (SHE) study, showing the prevalence of an AHI of ≥ 15 to be 20% in women.[46]

In the Wisconsin Sleep Cohort Study in 1997 an estimated 82% of men and 92% of women with moderate or severe OSA were not diagnosed clinically.[47] Even though awareness of OSA has increased since then, a large percentage of those fulfilling the criteria for OSA are probably still undiagnosed.

Recent studies report a higher prevalence of OSA than earlier. This might be explained by the increasing prevalence of obesity and by methodological differences, but a change in scoring criteria also contributes.[23,48]

Symptoms

Symptoms of OSA often develop gradually over time and increase in association with weight gain and ageing. OSA can be associated with a wide range of symptoms including snoring, witnessed apneas, choking, nycturia, sweating, gastroesophageal reflux and disturbed unrefreshing sleep, together with daytime symptoms such as excessive daytime sleepiness, fatigue, morning dry mouth, morning headache, irritability and difficulty concentrating.[49]

Snoring

The sound of snoring is generated by vibrations in the narrow, unstable pharynx. The prevalence of habitual snoring in the general population ranges from 9-50% in men and 4-17% in women.[50] As a single symptom, snoring is a poor predictor of obstructive sleep apnea.[24] Combining snoring with other symptoms increases its ability to identify individuals with sleep apnea. The combination of no snoring and no obesity makes a diagnosis of moderate to severe OSA unlikely.[24] Snoring has also been used in combination with excessive daytime sleepiness as a proxy for OSA.[51,52]

Daytime sleepiness

Excessive daytime sleepiness (EDS) is regarded as one of the main consequences of OSA, but the relationship between OSA and EDS is not fully understood. In the Wisconsin Sleep Cohort Study, 16% of men and 23% of women with an AHI of $\geq$5 reported EDS compared with 3% of men and 10% of women without snoring or sleep apnea.[6] In randomised controlled trials, the treatment of OSA with CPAP improves daytime sleepiness, suggesting a causal relationship between sleep apnea and EDS.[53] On the other hand, the correlation between AHI and measurements of EDS is weak, suggesting that the individual susceptibility to EDS differs.[54]

The presence or absence of EDS has also been reported to be associated with other consequences of SDB. In a cross-sectional analysis of the Sleep Heart Health study, severe OSA with EDS was associated with hypertension, while severe OSA without EDS was not[55] and Gooneratne *et al.* reported that SDB with EDS was associated with increased mortality, while SDB without EDS was not.[56] Young *et al.,* on the other hand, reported that EDS status had no impact on mortality risk in severe OSA.[57]

The cause of EDS in OSA is believed to be multifactorial. Apneas and hypopneas with subsequent arousals cause sleep fragmentation and a reduced deep-sleep percentage. The effect of intermittent hypoxia, interleukins or comorbid conditions such as depression might also contribute.[58,59]

Several scales for the measurement of subjective sleepiness exist. One of the most widely used is the Epworth Sleepiness Scale (ESS) that evaluates the tendency to doze off or fall asleep in eight different situations commonly encountered in daily life.[60] Each question is scored with 0-3 points, with a

total score of 0-24 points. A sum of ten points or more is regarded as excessive daytime sleepiness. There are also tests to measure objective sleepiness, such as the Multiple Sleep Latency Test and the Maintenance of Wakefulness Test. The American Academy of Sleep Medicine (AASM) recommends the ESS for assessments of sleepiness severity in patients suspected of OSA while objective sleepiness tests are not routinely indicated.[49]

Diagnostic procedures

The standard method for diagnosing OSA is polysomnography (PSG), in conjunction with a comprehensive sleep evaluation.[61] Simplified sleep apnea recording is often used instead of PSG in clinical practice. Both methods measure cardiorespiratory functions during sleep, including measurements of airflow, respiratory effort, oxygen saturation, heart rate and body position. In contrast to the simplified sleep apnea recording, PSG also includes electroencephalography (EEG), electromyography (EMG) and electrooculography (EOG). This enables measurements of sleep architecture, with the scoring of sleep stages and arousals, and correct assessment of sleep time.

The frequency of obstructive events is reported as the AHI, the mean number of apneas and hypopneas per hour of sleep. Alternatively, the term respiratory disturbance index (RDI), also including respiratory effort-related arousals (RERAs), is sometimes used. Simplified sleep apnea recordings, without sleep staging channels, might result in an underestimation of the true AHI.[61]

The definitions of apneas and hypopneas have varied over time. The AASM manual for the Scoring of Sleep and Associated Events updated in 2017 recommends that an apnea should be scored when there is a reduction in airflow of $\geq$ 90% compared with baseline for $\geq$10 seconds and a hypopnea should be scored when there is a reduction of $\geq$ 30% compared with baseline for $\geq$ 10 seconds in association with either $\geq$ 3% arterial oxygen desaturation or an arousal.[9]

Treatment

The treatment of OSA is indicated to reduce symptoms such as daytime sleepiness and to prevent accidents and cardiovascular disease. Possible treatment options include lifestyle changes, positional therapy, mandibular advancement devices (MAD), CPAP therapy and surgery. The choice of treatment depends on OSA severity, risk factors, the presence or absence of daytime sleepiness and other symptoms and on patient's anatomy, weight and own preferences.

CPAP, providing pneumatic splinting of the upper airway, is the most effective and well-studied treatment option.[62] It is indicated for the treatment of moderate to severe OSA and can be considered for mild OSA, especially

if the patient is obese or has drug-resistant hypertension or other comorbidities.[49] It is effective in reducing AHI and improving subjective daytime sleepiness.[53,62] In randomised controlled trials, it has also been shown to have a modest yet significant effect on blood pressure.[63] The incidence of adverse cardiovascular outcomes is lower in CPAP-treated OSA compared with untreated in non-randomised studies,[64,65] but randomised controlled trials have failed to show any cardioprotective effect of CPAP treatment.[66-68] Adherence to CPAP therapy has been reported to range from 40% to 84% in different study populations.[69]

In patients with mild to moderate OSA, or when CPAP therapy is not tolerated, an MAD, which holds the mandible in an advanced position, could be an alternative to CPAP.[49,70] An MAD reduces AHI and EDS compared with placebo devices, but it is less effective than CPAP therapy.[71] Short-term compliance with MAD treatment ranges from 76% to 95%, but with time the compliance decreases and about half the patients have been reported to still be using their MADs after a few years.[71] Compared with CPAP therapy, its effects on cardiovascular morbidity are less well studied.[62]

Positional therapy can be a treatment option in selected patients who have most of their breathing abnormalities in the supine position, while weight loss and the avoidance of alcohol and sedatives before bedtime are regarded as supplementary treatments. Surgical treatments, aiming to correct anatomical abnormalities in the upper airway, are rarely first-line treatment in adult OSA, but they could be an option when non-invasive treatments have failed and in patients with mild OSA with severe obstructing anatomy that is surgically correctable, e.g. tonsillar hypertrophy.[49]

Association with cardiovascular disease

Obstructive sleep apnea is associated with an increased risk of cardiovascular disease,[64,72] all-cause mortality[57,73,74] and cardiovascular mortality.[57,64,73]

One of the most well-established relationships is with hypertension. In 1997 in an OSA model in dogs, Brooks and colleagues saw a progressive increase in nighttime and daytime blood pressure when mimicking obstructive apneas with tracheostomy with an opening and closing valve.[75] More recently, similar results have also been reported in experiments with healthy humans; exposure to intermittent hypoxia during the night resulted in an increase in daytime blood pressure and sympathetic activity.[27] The association between OSA and an increased risk of hypertension has also been shown in epidemiological cross-sectional[76] and prospective[77] studies. The association appears to be stronger at younger age.[78,79] Blood pressure is normally lower during the night than in the day, but OSA patients with hypertension often have a non-dipping pattern with blood pressure remaining at higher levels even during the night.[80]

In observational studies, CPAP-treated OSA is associated with a lower incidence of hypertension than in untreated OSA[81] and the results of randomised controlled trials show an overall reduction in systolic blood pressure of 2-3 mmHg and diastolic blood pressure of about 1 mmHg with CPAP treatment.[62] The effects depend on hypertension status and are more pronounced in patients with uncontrolled hypertension.[62,82]

In patients with coronary heart disease, OSA has been associated with nocturnal angina[83] and myocardial infarction is more likely to occur between midnight and 6 am in patients with OSA than in patients without OSA.[84] Untreated severe OSA has also been associated with an increased incidence of cardiovascular events defined as the combined endpoint of stroke, myocardial infarction, coronary artery bypass surgery or percutaneous transluminal coronary angiography,[64] but, in studies looking prospectively at coronary heart disease as a separate endpoint, the association is less clear.[85] Gottlieb *et al.* reported an association between severe sleep apnea and incident coronary heart disease only in men younger than 70 years.[86]

While an association with coronary heart disease remains unclear, more and more evidence is emerging in favour of OSA as a risk factor for stroke. In a meta-analysis of prospective cohort studies, Dong *et al.* reported that OSA significantly increased the risk of developing stroke (RR = 2.02, 1.40-2.90)[85] and, in a randomised controlled trial, CPAP treatment in patients with first-time ischaemic stroke and moderate to severe OSA significantly improved cardiovascular survival.[87] Secondary analyses in the SAVE and RICCADSA trials revealed a reduced stroke incidence in patients compliant with CPAP, although this was not seen in the intention-to-treat analyses.[66,68]

A number of small clinical studies have reported an association between OSA and signs of atherosclerosis defined as an increase in carotid intima media thickness,[88] but in epidemiological studies, the association is less clear.[89,90] In the Sleep Heart Health study, there was no association between RDI and carotid intima media thickness or carotid plaques after adjustment for cardiovascular disease risk factors.[89] The Wisconsin Sleep Cohort, on the other hand, reported an association between AHI at baseline and carotid intima media thickness and carotid plaques measured 13 years later also after adjusting for confounders.[90]

OSA has also been recognised as a risk factor for atrial fibrillation.[91] Gami *et al.* reported an increased incidence of atrial fibrillation in OSA patients younger than 65 years[92] and, in a meta-analysis, OSA patients had a 25% greater risk of atrial fibrillation recurrence after catheter ablation than those without OSA.[93] The CPAP treatment of OSA was reported to reduce the risk of atrial fibrillation recurrence after catheter ablation in a non-randomised clinical study.[94]

While obstructive sleep apnea is a prevalent finding in heart failure patients and is associated with a poorer prognosis,[95] it is less clear whether OSA may play a role in the pathogenesis and progression of heart failure. In

human cross-sectional studies, OSA is associated with increased odds of experiencing heart failure[96] and, in a canine model, Parker *et al.* reported a reduced left ventricular ejection fraction after one to three months of untreated OSA.[97] Echocardiographic studies of OSA have yielded somewhat conflicting results,[98] but an association between moderate to severe OSA and diastolic dysfunction has been reported in several studies.[99,100] In a cohort study, Gottlieb *et al.* reported an association between OSA and future incident heart failure in men but not in women.[86]

Kita *et al.*[101] reported increasing levels of Type B natriuretic peptide (BNP), used clinically as a diagnostic and prognostic marker of heart failure, during sleep in patients with OSAS and reduced BNP levels with CPAP treatment, but subsequent studies have produced conflicting data, as a significant association between sleep apnea and natriuretic peptides has been reported by some[102-105] but not by others.[106-111]

Obstructive sleep apnea in women

OSA is more common in men than in women. In the general population, the male-to-female ratio is about 2:1, but, in clinical populations, the male predominance is even higher.[23] It has been suggested that this might be explained by a different symptom profile in women,[112] but, even when symptoms are similar at baseline, women are less likely to be diagnosed and treated for sleep-disordered breathing.[113]

The reasons for the difference in OSA prevalence between men and women are not fully understood. Anatomic differences, differences in risk factor profiles and the effects of sex hormones might contribute.[114] Women have a smaller upper airway volume, which could be expected to be associated with a greater risk of apneas, but they also have a stiffer upper airway with a shorter length. This might explain why the female airway appears to be less disposed to collapse. Sex differences in body-fat distribution, with a higher tendency towards upper-body and neck fat accumulation in men, might also contribute.[114] OSA increases with age and, in women, the prevalence of OSA is higher in postmenopausal women than in premenopausal.[45] Post-menopausal women display more risk factors for SDB, such as central obesity, while there is little evidence of a direct link between menopause *per se* and the prevalence of sleep apnea.[115]

Polysomnographically, at the same AHI, women have a greater proportion of hypopneas and fewer apneas than men.[116] The obstructive events also tend to be shorter and associated with less severe desaturations in women. Women have a greater proportion of their apneas and hypopneas in REM sleep.[117,118]

The evidence that OSA increases the risk of cardiovascular disease and mortality is weaker in women than in men. Since women were not included in epidemiological studies until the 1990s, are underrepresented in clinic-

based studies and randomised controlled trials (RCT), have a lower prevalence and severity of OSA and, since cardiovascular disease often present later in life in women, this might be explained by a lack of statistical power. It has also been hypothesised that this might represent a true difference, that women are less vulnerable to the effects of OSA.

Obstructive sleep apnea during REM sleep

REM sleep, occurring in about 20% of total sleep time,[119] with a higher concentration the hours before morning awakening,[120] is thought to be important for learning and memory consolidation.[11] REM sleep is associated with decreased upper-airway dilator muscle activity, increased upper-airway collapsability[121] and a reduced hypoxic ventilator drive.[122] As a result, apneas in REM sleep are longer and are associated with deeper desaturations.[123] REM sleep is also a state of high sympathetic activity. Whether these sleep-state-specific features result in different consequences of OSA during REM sleep compared with OSA during NREM sleep is unclear, but it has been suggested that OSA during REM sleep might have more severe cardiovascular consequences than OSA during NREM sleep. Table 1 summarises studies investigating sleep-disordered breathing divided by sleep stage in association with cardiometabolic outcomes.

In studies of adult populations analysing REM OSA and NREM OSA separately, REM OSA was associated with an increased risk of prevalent and incident hypertension[124,125] and incident non-dipping blood pressure,[126] while NREM OSA was not. In children, on the other hand, NREM AHI but not REM AHI was associated with higher blood pressure in two cross-sectional studies.[127,128] The reason for this discrepancy is unclear; it might be explained by the small number of children with moderate to severe OSA in the two studies (AHI > 5: n=8 and n=30 respectively). Alternatively, it might reflect a true difference between adult and childhood OSA.

The effects of SDB on glucose metabolism have also been reported to differ by sleep stage. REM sleep is associated with a decline in glucose levels, but Bialasiewicz *et al.* reported that this decline was abolished by episodes of SDB in REM sleep, while NREM SDB had no effect on glucose levels.[129] Chami *et al.* reported that REM AHI was associated with insulin resistance, while NREM AHI was associated with fasting and postprandial glucose levels.[130] REM OSA has also been associated with an increased risk of prevalent[131] and incident[132] diabetes and higher haemoglobin A1c (HbA1c) in patients with diabetes.[120]

Table 1. *Studies investigating sleep stage-dependent sleep-disordered breathing and cardiometabolic outcomes*

Study	Study population & design	Outcome	Main findings
Bixler 2008[127]	Children (5-12 years), n=700. Community-based, cross-sectional	Evening BP	NREM AHI was associated with elevated systolic and mean BP, while REM AHI was not.
Mahmood 2009[131]	n=1,008 (53% women). Clinic-based, cross-sectional	Type II diabetes	REM OSA was associated with increased odds of diabetes (OR 2.1 95%CI1.3-3.3). For OSA, OR 1.3 95% CI 0.9-2.0
Bialasiewicz 2011[129]	n=11 (18% women). Clinic-based, within-subject.	Interstitial glucose concentration (IGC)	There was a decline in IGC in REM sleep, which SDB in REM sleep abolished. NREM SDB had no effect on IGC.
Au 2013[128]	Children (6-13 years), n=292. Community-based, cross-sectional	24-hour ambulatory BP	NREM OAHI but not REM OAHI was associated with increased daytime and nighttime systolic BP.
Grimaldi 2014[120]	n=115 (57% women). Clinic-based, cross-sectional	HbA1c	REM AHI, REM ODI and REM MAI were associated with increased HbA1c, while NREM SDB was not.
Mokhlesi 2014[124]	n=1,451 (46% women). Community-based, cross-sectional & longitudinal	Hypertension, prevalent and incident	REM AHI was associated with an increased risk of prevalent and incident hypertension, while NREM AHI was not.
Chen 2014[133]	n=79 (20% women). Clinic-based, cross-sectional	Cardiac function and signs of remodelling	An REM AHI of $\geq$ 32.3 was associated with diastolic dysfunction, while AHI and NREM AHI were not.
Kendzerska 2014[132]	n=8,678 (48% women). Clinic-based, longitudinal	Incident diabetes	AHI, REM AHI, Ti90, daytime sleepiness and TST were associated with incident diabetes.

Mokhlesi 2015[126]	n=269 (38% women). Community-based, longitudinal	Non-dipping nocturnal BP	An REM AHI of ≥ 15 was associated with new-onset non-dipping BP, while NREM AHI was not.
Chami 2015[130]	n=3,310 (54% women). Community-based, cross-sectional	Fasting and post-prandial glucose, insulin resistance	REM AHI was associated with insulin resistance, NREM AHI with fasting and post-prandial glucose levels.
Appleton 2016[125]	n= 739 (0% women). Community-based, cross-sectional	Prevalent and recent-onset hypertension	An REM AHI of ≥ 30 was associated with prevalent and recent-onset hypertension, while NREM OSA was not.
Sasaki 2018[134]	n=42 (14% women). Clinic-based, cross-sectional	Systolic BP at the end of each apnea or hypopnea	OSA during REM sleep caused higher BP at the end of an OSA episode compared with OSA during NREM sleep.
Lin 2018[135]	n=275 (38%women). Clinic-based, cross-sectional	Arterial stiffness	REM AHI, but not AHI or NREM AHI, was associated with the peripheral arterial stiffness index.
Acosta-Castro 2018[136]	n=2,074 (52% women). Community-based, cross-sectional	Hypertension, metabolic syndrome, diabetes, depression	An REM AHI of ≥ 20 was associated with the metabolic syndrome and BP. In exclusive REM SDB subgroups, an REM AHI of ≥ 20 was associated with diabetes.
Aurora 2018[137]	n=3,265(63% women). Community-based, longitudinal	Composite cardiovascular endpoint [1]	An REM AHI of ≥ 30 was associated with the endpoint in those with but not without prevalent CVD
Kurosawa 2018[138]	n=115 (13% women). Clinic-based, cross-sectional	HbA1c, glucose levels	NREM AHI was associated with HbA1c and mean glucose levels, while REM AHI was not.

BP=blood pressure, AHI=apnea hypopnea index, OR=odds ratio, CI=confidence interval, CVD=cardiovascular disease, IGC=interstitial glucose concentration, SDB=sleep disordered breathing, OAHI= obstructive apnea hypopnea index, ODI=oxygen desatruration index, MAI=microarousal index, Ti90=sleep time with saturation of <90%, TST=total sleep time. 1) Fatal or non-fatal myocardial infarction, coronary artery revascularisation, heart failure episode or stroke.

In the HypnoLaus Sleep Cohort, an REM AHI of ≥ 20 was associated with increased odds of having the metabolic syndrome, but a significant association between an REM AHI of ≥ 20 and diabetes was only seen when the study-population was restricted to participants with an NREM AHI or an AHI of < 10.[136] Unlike most studies, a Japanese study of predominantly male patients with OSA reported that NREM AHI, but not REM AHI, was associated with higher HbA1c and glucose levels.[138]

Only a few studies have investigated OSA during REM sleep in association with other cardiovascular outcomes. Chen *et al.* reported that severe OSA during REM sleep (REM AHI ≥ 32.3) was associated with left ventricular diastolic dysfunction after adjustment for confounders, while AHI and NREM AHI were not.[133] An association between REM AHI and arterial stiffness, a sign of biological ageing and atherosclerosis, has also been reported.[135] In a study restricted to participants with an NREM AHI of < 5 in the Sleep Heart Health Study, the adjusted hazard ratio for a composite cardiovascular endpoint of fatal or non-fatal myocardial infarction, coronary artery revascularisation, heart failure episode or stroke for REM AHI ≥ 30 was 1.35 (95% confidence interval (CI) 0.98-1.85). In stratified analyses, there was an association between an REM AHI of ≥ 30 and the endpoint in those with prevalent cardiovascular disease but not in those without.[137]

There is no uniform definition of REM SDB. Some studies use the term REM-related OSA, defined as an REM AHI/NREM AHI ratio of > 2. In other studies, REM AHI is used as a continuous variable or with different cut-offs, sometimes in combination with a maximum value of NREM AHI or AHI. An association between OSA during REM sleep and cardiometabolic disease appears to be most evident for severe OSA during REM sleep; REM AHI ≥ 15,[124,126] REM AHI ≥ 20,[125,136] REM AHI ≥ 30.[133,137]

Cardiovascular disease in women

Cardiovascular disease accounts for one third of all deaths and is the leading cause of death globally.[139] More women than men die of cardiovascular disease, but women develop cardiovascular disease later in life than men.[140] This might be explained by both sex and gender differences.[141]

The exact effect of sex hormones on the cardiovascular system is not fully understood and is controversial, but oestrogens appear to have protective effects in premenopausal women.[142] Early menopause is associated with an increased risk of coronary heart disease but not stroke.[143]

The risk factor profile and the relative importance of different risk factors differ between men and women. Hypertension is less common in premenopausal women compared with men of the same age and premenopausal women have better lipid profiles than men.[141] The prevalence of diabetes is higher in men than women, but, on the other hand, the risk of developing

cardiovascular disease is greater in women with diabetes than in men with diabetes.[139,144] Smoking is also associated with a greater risk of developing cardiovascular disease in women than in men.[144] Globally, more men than women smoke,[139] but in Sweden smoking is now more common in women than in men.

Heart failure

Heart failure, affecting 1-2% of the adult population in the developed countries and > 10% of the population > 80 years of age is a clinical syndrome that can have many different causes.[145] The European Society of Cardiology defines heart failure as "a clinical syndrome characterised by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that might be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress".[146]

Globally, the incidence of heart failure is one to nine cases per 1,000 person-years, depending on definitions and the population studied.[145] In Sweden, the incidence has been estimated at 3.7 per 1,000 person-years in women and 3.9 per 1,000 person-years in men.[147] About half of the patients are dead within five years of diagnosis.[147]

The diagnosis of heart failure is based on symptoms and signs in combination with cardiac functional and structural alterations.[146] An electrocardiogram (ECG) and measurement of natriuretic peptides can be used as a first diagnostic procedure and to decide which patients require echocardiography.[146] Heart failure is divided into heart failure with a reduced ejection fraction, heart failure with a normal ejection freaction and the newly defined heart failure with a mid-range ejection fraction.[146] Heart failure with a normal ejection fraction, which is more common in women, has been less studied than heart failure with a reduced ejection fraction and no treatments have been shown to reduce morbidity or mortality in patients with heart failure with a normal ejection freaction.[146,148]

Causes of heart failure include hypertension, ischaemic heart disease and valvular heart diseases.[145] The risk of developing heart failure due to hypertension is higher in women and hypertension is a more common aetiology of heart failure in women than in men.[144] Women with ischaemic heart disease also run a higher risk of developing heart failure than men, but the proportion of heart failure caused by ischaemic heart disease is still lower in women than in men.[141,145]

Sleep-disordered breathing occurs in more than one-third of women with heart failure, with a higher prevalence with increasing age.[149] Both central and obstructive sleep apnea are associated with a poorer prognosis in heart failure.[95] An association between OSA and an increased risk of heart failure

has been reported in men, but it is unclear whether this is also true in women.[86]

Even though the recommendations for diagnosis and treatment do not differ between men and women, a diagnosis of heart failure is less frequently based on echocardiography in women than in men and women are often undertreated, even for treatments like cardiac resynchronisation therapy, which has been shown to be more effective in women.[141,150]

Atherosclerotic cardiovascular disease

Premenopausal women have less atherosclerotic disease than men, but after menopause the gender gap narrows.[141]

The diagnosis of acute myocardial infarction might be more challenging in women. Central chest pain is the most common symptom of acute myocardial infarction in both men and women, but atypical symptoms are more common in women than in men.[144] Women also wait longer before seeking medical care and unrecognised myocardial infarction is more common in women.[144,151]

Prognosis and pathophysiology may also differ between men and women. Young women diagnosed with an ST-elevation myocardial infarction have a higher in-hospital mortality rate than men of the same age.[141] Female gender is also associated with increased short-term and long-term mortality after percutaneous coronary intervention.[152] On the other hand, in women with acute myocardial infarction examined with a coronary angiogram, the absence of stenosis is more common than in men.[153] Microvascular disease, coronary artery spasm and coronary artery dissection are alternative mechanisms of angina and myocardial infarction that are more frequent in women.[141] Attacks of coronary artery spasm often occur during the night and are more common during REM sleep than during NREM sleep.[154]

Markers of cardiovascular disease

Natriuretic peptides

BNP and N-terminal pro-B natriuretic peptide (NT-pro-BNP) serve as diagnostic and prognostic markers of heart failure and inversely relate to left ventricle ejection fraction.[155] In community-based studies, elevated levels of natriuretic peptides have also been reported to represent an increased risk of cardiovascular events and death.[156,157]

BNP has a diuretic and vasodilatory effect and is secreted by the cardiac ventricles in response to volume expansion and pressure load.[158] Hypoxia and ischaemia have also been reported to induce BNP secretion.[159] Plasma levels of B-type natriuretic peptides increase with age and are higher in

women than in men. The levels are also affected by body mass index (BMI) and glomerular filtration rate.[155]

Carotid artery ultrasound

Atherosclerosis may progress silently for decades before resulting in a cardiovascular event. An ultrasound of the carotid artery is able to detect atherosclerotic plaques and measure the thickness of the layers of the vessel wall. The number of plaques and plaque volume are independent predictors of future cardiovascular disease and mortality but develop late in the atherosclerotic process. Increased intima media thickness (CIMT) can be detected earlier and is also associated with the risk of cardiovascular events.[160]

Intimal thickening, caused by inflammation and recruitment of inflammatory cells, occurs early in the atherosclerotic process. Recent studies suggest that the measurement of the individual layers of the common carotid wall with high-resolution ultrasound may be an even more sensitive way to detect early signs of atherosclerosis and that the individual intimal thickness, as well as the ratio between the intimal and medial thickness, correlate with cardiovascular disease.[161]

Proteomics

The development of proteomic techniques, enabling the detection of multiple proteins in small samples, offers new opportunities to detect markers of cardiovascular stress. From classical technologies such as mass spectrometry, flow cytometry and immunoassays, refined techniques, allowing high multiplexing with high sensitivity and specificity, have been developed. Immuno-polymerase chain reaction (immuno-PCR) technology, first developed almost three decades ago, combines the high specificity of immunoassays and the high sensitivity of PCR, but it is limited by the risk of non-specific binding. To overcome this problem, proximity ligation assays and proximity extension assays have been developed.[162]

Proximity extension assays (PEA) use matched antibody pairs attached to single-stranded DNA sequences.[163,164] Only when both antibodies bind to the same protein do the matched DNA sequences hybridise and are extended by a DNA polymerase. The created double-stranded DNA is then amplified by PCR and quantified by quantitative PCR. This enables both high specificity and a high degree of multiplexing without the risk of cross-reactivity.[163]

Aims

The overall aim of this thesis was to investigate associations between different aspects of sleep-disordered breathing and cardiovascular disease in women.
The specific aims of the four papers were to:

I analyse the possible association between obstructive sleep apnea during the night and levels of BNP the following morning in women

II investigate whether symptoms of obstructive sleep apnea, i.e. snoring and EDS, are able to predict incident heart failure in women

III investigate whether OSA during REM sleep is associated with early signs of atherosclerosis, defined as increased intima thickness, in women

IV investigate whether severe OSA and severe OSA during REM sleep are associated with changed levels of inflammatory and cardiac disease-related proteins in a population-based cohort of women.

Methods

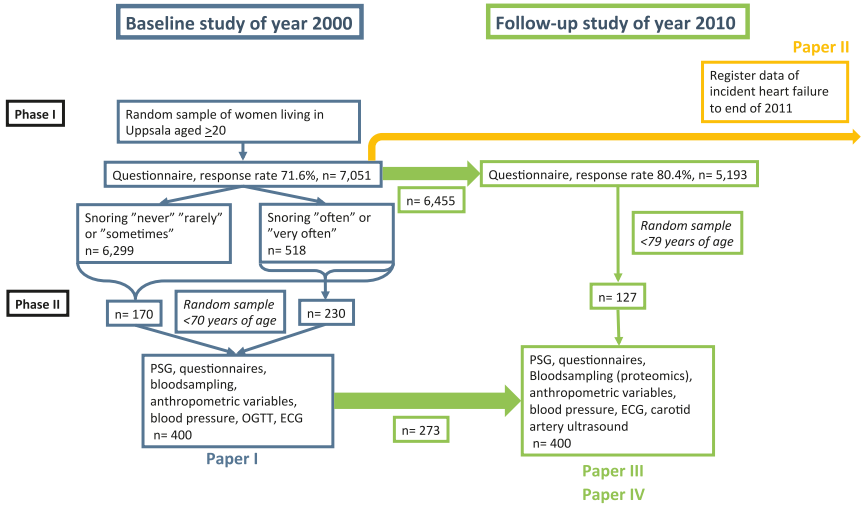

Figure 2. Study design. PSG=polysomnography, OGTT=oral glucose tolerance test, ECG= electrocardiogram

Population

The population-based cohort study "Sleep and Health in Women" (SHE) was initiated in April 2000, when a postal questionnaire was sent to randomly selected females aged ≥ 20 years from the population registry of the City of Uppsala, Sweden. The questionnaire included questions on snoring, sleep disturbances, daytime symptoms, lifestyle factors, anthropometric variables and medical history. The response rate was 71.6%, with a total of 7,051 women responding to the questionnaire.

Based on their answer to the question "How often do you snore loudly and disturbingly?", the participants were categorised as non-snorers (answering "never", "seldom" or "sometimes", n = 6,299) or snorers (answering "often" or "very often", n = 518). Of the total study population aged 70 years and younger (n = 6,112), a random sample of 170 women from the entire study population and a random sample of 230 snorers participated in the second phase of the study. The second phase of the study, initiated in 2003, included a whole-night polysomnography, questionnaires, anthropometric

measurements, blood sampling, blood pressure measurements, an ECG and an oral glucose tolerance test (OGTT). Women who were expected not to manage to perform the ambulatory recordings because of severe somatic or psychiatric disease were excluded.

In 2010, the first phase of the follow-up study was initiated when a new questionnaire was sent out to all the women answering the baseline questionnaire. In the second phase of the follow-up study, initiated in 2011, the participants in the second phase of the baseline study were asked to perform a new polysomnography, as well as a carotid artery ultrasound, blood sampling, anthropometric measurements, blood pressure measurements and an ECG and to answer new questionnaires. Out of the 400 original participants, 273 accepted the invitation. An additional 127 women, randomly selected from the participants answering the questionnaire in the follow-up study, also participated in the second phase of the follow-up study.

Paper I is based on the second phase of the baseline study, with a total study population of 349 subjects who had plasma BNP levels measured the morning after the polysomnography.

Paper II is based on questionnaire data from the baseline study in combination with register data. All the women who were pregnant (n = 104), suffered from heart failure (n = 27) or were receiving CPAP treatment (n=8) at baseline were excluded, as well as women who had omitted to respond to any of the questions on exposure variables or to any of the main confounding factors of smoking, alcohol use or waist circumference, leaving a total study population of 5,990 women.

Paper III is based on the women with a polysomnography at baseline who also participated in the second phase of the follow-up study and underwent a carotid artery ultrasound. Women with atherosclerotic disease at baseline, CPAP treatment or less than 30 minutes of REM sleep were excluded, leaving a final study population of 201 women.

Paper IV is based on a subsample of the women in the second phase of the follow-up study in which proteomic analyses were performed. The subsample included all the women with severe OSA (AHI $\geq$ 30 or oxygen desaturation index (ODI) $\geq$ 30), all the women with asthma and a random sample of women with no (AHI < 5), mild (AHI 5 -< 15) and moderate (AHI 15-< 30) OSA. One woman was excluded because of low-quality samples in both the Inflammation panel and the Cardiovascular II panel, leaving a total study population of 253 women.

Measurements

Table 2. *Measurements of sleep-disordered breathing, cardiovascular disease, markers of cardiovascular disease and covariates in Papers I-IV*

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Measurements of SDB	AHI, ODI4, lowest & mean saturation Additional: AHI with different degree of desaturation	Snoring and EDS	REM AHI categories Additional: AHI categories, ODI3 categories, mean sat, mean sat during REM sleep, % of TST with sat < 90%, apnea length	AHI<15 vs ≥30, ODI3<15 vs ≥30, REM AHI <15 vs ≥30
Cardiovascular disease/marker	BNP	Incident heart failure	Carotid artery intima thickness	Cardiac and inflammatory proteins
Adjustments for confounding	1) Unadjusted 2) Age and BMI 3) 2 + BP, anti-hypertensive drugs, creatinine	1) Age 2) 1+ waist circumference, smoking, alcohol dependence 3) 1+2 + HT, DM, previous MI, menopause, HRT, inactivity, depressive symptomes	1) Age 2) 1 + BMI, alcohol, smoking 3) 1 + 2 + BP, LDL, CRP, DM	1) Unadjusted 2) Age, BMI, plate

SDB=sleep-disordered breathing, AHI= apnea-hypopnea index, ODI4=oxygen desaturation index with desaturations of ≥ 4%, EDS=excessive daytime sleepiness, ODI3= oxygen desaturation index with desaturations of ≥ 3%, TST=total sleep time, BMI=body mass index, BP=blood pressure, HT=hypertension, DM=diabetes mellitus, MI=myocardial infarction, HRT=hormone replacement therapy, LDL=low density lipoprotein, CRP=C-reactive protein

Polysomnography

The 400 participants in the second phase of the baseline study and in the follow-up study underwent full-night polysomnography in their own homes or at the patients' hotel at the hospital. During the evening before the polysomnography, the participants arrived at the Sleep Laboratory at Uppsala University Hospital, where the polysomnography equipment was set up. The polysomnography was conducted using the ambulatory EMBLA system (Flaga Inc., Iceland) with 16 channels: two electroencephalography leads (C3-A2, C4-A1), two electrooculography leads, three electromyography leads (sub mental, left and right anterior tibialis muscles), two airflow leads (oronasal thermistor and nasal flow pressure sensor), two respiratory effort

leads from piezoelectric belts (thoracic and abdominal), two electrocardiography leads, one piezo vibration sensor for snoring, one oximeter lead and one body position lead. Data were downloaded to the Somnologica reviewing analysis software (Version 2.0; Flaga Inc.) and sleep was scored manually in 30-sec epochs according to the standard criteria of Rechtschaffen and Kales.[165] An obstructive apnea was defined as the complete cessation of nasal and oral airflow lasting 10 sec or more with continuing abdominal and thoracic movements. An obstructive hypopnea was defined as a ≥ 50% reduction in both oronasal thermistor and nasal pressure for at least 10 sec, compared with baseline, accompanied by abdominal and thoracic movements, in combination with a desaturation of ≥ 3% or an arousal. The apnea-hypopnea index (AHI) was calculated as the mean number of apneas and hypopneas per hour of sleep. The oxygen desaturation index (ODI) was defined as the mean number of desaturations of ≥ 4% per hour of sleep in Paper I, while it was defined as the mean number of desaturations of ≥ 3% per hour of sleep in Paper III and IV. Central apneas were scored at the cessation of both oronasal thermistor and nasal pressure for 10 sec without respiratory movements. The REM AHI was calculated as the numbers of apneas and hypopneas during REM sleep divided by the hours spent in REM sleep. In addition, data on mean oxygen saturation, mean oxygen saturation in REM sleep, lowest oxygen saturation, percentage of total sleep time with saturation below 90% and AHI with different levels of accompanying hypoxia were obtained from the polysomnography recordings.

Questionnaires

The baseline questionnaire in 2000 consisted of 109 questions. To assess snoring and EDS, participants were asked to state how often they used to "snore loudly and disturbingly?" and "fall asleep involuntarily for a short period during the day, for example when there is a pause at work?". In Paper II, snoring and EDS were defined as answering "sometimes", "often" or "very often" (in contrast to "never" or "seldom") to the respective question. The women were then categorised into four groups; non-snorers without EDS, snorers without EDS, non-snorers with EDS and snorers with EDS.

The baseline questionnaire was also used to identify co-variables and exclusion criteria in Paper II. Subjects with heart failure or a previous myocardial infarction at baseline were identified by questions about medical conditions requiring either a regular medical check-up or for which they had been admitted to a hospital in the past ten years. Women with hypertension or diabetes were identified by either answering "yes" to the question "Do you have high blood pressure?" or "Do you have diabetes?" or stating "hypertension" or "diabetes" to the questions about the medical conditions. The women were asked whether they had had any treatment for snoring with the mul-

tiple choices of operation, CPAP or other treatment. The questionnaire also included a question on current pregnancy.

BMI was calculated from self-reported height and weight. The women were given a tape measure and instructions on how to measure their waist circumference along with the questionnaire. The women also reported their level of physical activity during leisure time on a four-point scale.[166] In the statistical analysis, the level of physical inactivity was defined as score 1: spending most of the time watching television, reading and being sedentary for most of their leisure time. Alcohol dependence was investigated using the CAGE questionnaire[167] and participants answering "yes" to $\geq$ two questions were defined as alcohol dependent. Based on six questions about smoking habits, the participants were classified as never, former or current smokers.[168] Depression was assessed using the Hospital Anxiety and Depression (HAD) scale.[169] Based on 15 questions relating to menopausal and hormonal status,[168] the women were divided into premenopausal, postmenopausal or postmenopausal on hormone replacement therapy (HRT).

In the second phase of the baseline study, the 400 participants completed a second questionnaire, which included specific questions about hypertension, myocardial infarction, chronic heart failure, angina pectoris, stroke, diabetes and current medication. Daytime sleepiness was assessed using the Epworth Sleepiness Scale[60] and a value of 10 or more was regarded as excessive daytime sleepiness. Alcohol consumption was assessed with a question in which the participants were asked to state how many units of different kinds of alcoholic beverage they drank per week. Smoking was assessed with questions identical to those in the baseline questionnaire. The baseline data, co-variables and exclusion criteria in Paper I and Paper III are based on the answers from this second questionnaire.

In the second phase of the follow-up study, the women also answered a questionnaire including questions similar to those in the second phase of the baseline study. The baseline data in Paper IV are based on the answers from this questionnaire.

Laboratory analyses and anthropometric measurements

The morning after the polysomnography, the subjects returned to the hospital while fasting. Blood samples were drawn and blood pressure was taken in the right arm after 15 min of rest in a supine position. The participants' height and weight were measured and their BMI was calculated. An electrocardiogram was taken to detect atrial fibrillation. The women had also collected their urine during the night and, in the morning after the polysomnography, the urine volume was measured.

The blood samples for glucose, C-reactive protein (CRP), creatinine, low-density lipoprotein (LDL), follicle-stimulating hormone (FSH) and BNP, all taken between 7 am and 9 am the morning after the polysomnography, were

analysed at the Department of Clinical Chemistry and Pharmacology at Uppsala University.

The plasma levels of BNP were analysed using an immunoradiometric assay (Shionoria BNP, Shionogi, Japan). The lower detection limit was 4 ng/L and a value of ≥ 20 ng/L was regarded as elevated, in accordance with the reference value of the method. Plasma BNP values are available for 349 of the 400 women. This is due to a change in the analysis method for BNP at the laboratory during the study period and problems obtaining blood from some participants.

Based on the FSH levels, the women's menopausal status was assessed (Paper III).[170]

Women without known diabetes also underwent an oral glucose tolerance test and were then categorised as having diabetes, impaired glucose tolerance, impaired fasting glucose or no impairment of glucose metabolism.[171,172]

Blood samples for the proteomic analysis in Paper IV were drawn the morning after the polysomnography in the follow-up study and stored at -70°C until analysis. The proteomic analyses were performed at Olink Analysis Service (Olink Proteomics, Uppsala, Sweden) using PEA technology. Two panels, each measuring 92 proteins, were analysed: the Olink Proseek® Multiplex Inflammation kit and the Olink Proseek® Cardiovascular II ^{95x96} kit. Data are presented as normalized protein expression (NPX) values, on a log2 scale. Proteins with more than 90% of the values below the limit of detection (LOD) were excluded from the analyses, leaving 78 proteins in the Inflammation panel and 92 proteins in the Cardiovascular II panel. Proteins with 20-90% of the values below the LOD (eight proteins in the Inflammation panel and three proteins in the Cardiovascular II panel) were discretised as detectable (above the LOD) or undetectable (below the LOD). For all proteins with less than 20% values below the LOD, the values below the LOD were imputed by replacing the missing value with the value of the LOD. The samples were analysed on three different plates.

Register data

Register data, used in Paper II, were obtained from the Swedish National Patient Register and the Swedish Cause of Death Register regarding all-cause mortality and a diagnosis of heart failure (International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) codes I50, I42.0 and I11.0) between 1 April 2000 and 31 December 2011. The Swedish National Patient Register contains all inpatient care in Sweden and, since 2001, also outpatient visits but not primary care visits. The registers list the diagnoses for which the patients have been treated, the first being the principal cause of hospitalisation or outpatient visit. Since studies have shown a higher validity for main diagnoses in the register than

for secondary diagnoses (for heart failure, positive predictive value 95% compared with 81.7%),[173] only main diagnoses were included in the analysis.

Carotid artery ultrasound

In the second phase of the follow-up study, the women were examined with high-resolution ultrasound (Collagenoson Minhorst Company, Meudt, Germany) of the left common carotid artery, with separate measurements of the intima and the media layers.[161] The examination was performed after 15 minutes of rest with the participant sitting in an upright position looking straight ahead, using a broad-banded probe with 22MHz centre frequency. The transducer was applied at the point of maximum pulsation of the common carotid artery in front of the sternocleidomastoid muscle and the near carotid artery wall was identified. Point estimates of the artery wall were obtained and means of 10 separate measurements were calculated and used in the analysis. Measurements of the intima were made using the brightest echoes from leading edge to far edge and the thickness of the media layer was measured as the distance between the two brightest echoes.

Statistical analyses

Statistical analyses were performed using Stata 10.0, Stata 12.0, Stata 13.0 (Stata Corporation, College Station, TX) and R version 3.4.4.

To compare baseline data between groups, the unpaired t-test was used for normally distributed variables, the Mann-Whitney U test for not normally distributed variables and the chi-square test for categorical variables. Data are presented as the mean $\pm$ standard deviation (SD) for normally distributed data, as the median (interquartile range (IQR)) for not normally distributed data or as n (per cent (%)).

Associations between measurements of sleep-disordered breathing and markers of cardiovascular disease were analysed using linear regression for continuous dependent variables (Papers I, III and IV) and logistic regression for dichotomous dependent variables (Papers I and IV). The results from regression analyses are presented as regression coefficients or odds ratios (OR) with 95% confidence intervals (CI). In Paper IV, the p-values were adjusted for multiple testing using the Benjamin and Hochberg method for controlling the false discovery rate (FDR), setting the false discovery rate at 10%. The q-value is the FDR-adjusted p-value.

In Paper II, Kaplan-Meyer curves were used to estimate heart failure incidence and the association between the combination of snoring and EDS with incident heart failure was analysed using Cox proportional hazard regression analysis. The results are presented as hazard ratios (HR) with 95% CI. The time at risk for each participant was calculated from the date of the baseline

questionnaire to the date of the first-time heart failure diagnosis, date of death or the end of the study period, whichever came first.

Possible confounders included in the regression models in Papers I-IV were selected on the basis of existing literature, on the results of directed acyclic graph (DAG) analyses,[174] created at dagitty.net, and on the results in previous papers. Table 2 summarises the covariates included in the regression models in Paper I-IV. A DAG used in Paper II is shown in Figure 3.

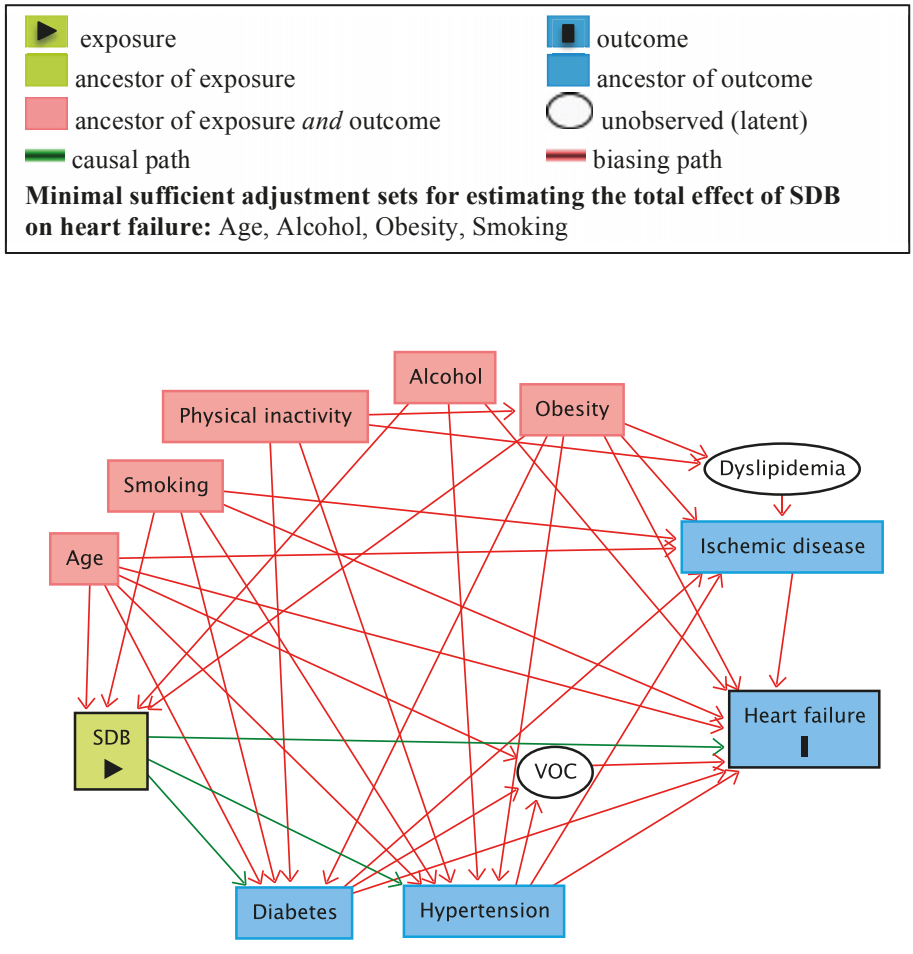

Figure 3. Directed acyclic graph (DAG) of proposed associations between sleep-disordered breathing (SDB) and heart failure. According to this model, to assess the total effect of sleep-disordered breathing on heart failure, adjustments for age, alcohol, obesity and smoking are needed. (created on dagitty.net).

Ethics

The written informed consent of all participants was obtained and the Ethics Committee at the Medical Faculty at Uppsala University approved the studies.

Results

Table 3. *Baseline characteristics of the participants in Papers I-IV*

	Paper I	Paper II	Paper III	Paper IV
Population, n	349	5,990	201	253
Age	49.8±11.3	44.4 ± 16.8	49.8 ± 10.4	60.1 ± 10.7
BMI	26.5 ± 4.9	24.0 ± 4.1	26.1 ± 4.1	27.2 ± 4.8
AHI	7.6 (3.1 – 17.8)		7.6 (3.3 - 16.2)	12.6 (4.1-24.8)
ESS	8.6 ± 4.2		8.8 ± 4.0	7.9 ± 4.3
Current smoking, n (%)	73 (21.2)	1018 (17.0)	42 (21.1)	23 (9.1)
Hypertension, n (%)	55 (16.2)	655 (10.9)	25 (12.8)	69 (27.3)

Data presented as the means ± SD for normally distributed data, as the median (IQR) for not normally distributed data or as n (%). AHI= apnea-hypopnea index, BMI=body mass index, ESS=Epworth Sleepiness Scale

Paper I

No sleep apnea, defined as an AHI of < 5, was seen in 34.7% of the women (n=121), while 32.4% (n=113) had an AHI of 5-14.9, 22.1% (n=77) had an AHI of 15-29.9 and severe sleep apnea with an AHI of ≥ 30 was seen in 10.9% (n=38). There was a significant increase in mean BNP as the severity of sleep apnea increased, from a mean value of 8.5 ng/L among women with a normal AHI to 18.0 ng/L in women with severe sleep apnea. A box plot showing the distribution of BNP level in the AHI groups is presented in Figure 4.

There was an association between variables of sleep apnea and plasma BNP, using a univariate linear regression model. For AHI and ODI, but not for lowest or mean oxygen saturation, this association remained after adjustment for age, BMI, systolic blood pressure, antihypertensive drugs and plasma creatinine (for AHI; coeff 0.117 (95% CI 0.03-0.21) and for ODI; coeff 0.110 (95% CI 0.02-0.20) in the fully adjusted model).

Elevated plasma BNP levels (≥ 20 ng/L) were found in 29.8% of the study population. Participants with elevated plasma BNP levels were significantly older, had higher blood pressure and were more frequently on antihypertensive medication, while there was no significant difference in BMI, central obesity or levels of creatinine. OSA, defined as an AHI of ≥ 5, was more common in women with increased BNP, but there was no difference in daytime sleepiness.

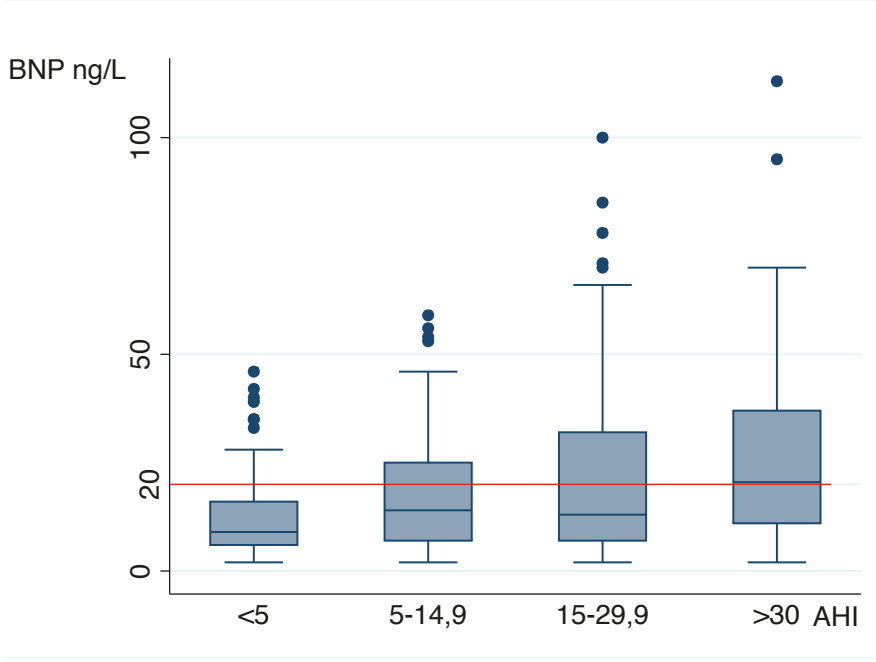

Figure 4. Distribution of BNP by AHI group. The box is the 25th-75th percentile (including 50% of the participants), the line in the box is the median, the whiskers the 5th to 95th percentiles, and the dots the outliers.

There was a dose-response relationship between increasing severity of sleep apnea, defined with either the AHI or ODI, and elevated BNP. This relationship remained after adjustment for confounders. The results from logistic regression models for AHI are shown in Table 4. Additional analyses of AHI with different levels of hypoxia showed no clear associations with BNP after adjustment for confounders.

Table 4. *Results from logistic regression analysis with dependent variable BNP ≥ 20*

	Unadjusted			**Adjusted for age and BMI**			**Adjusted for age, BMI, systolic blood pressure, antihypertensive drugs and plasma creatinine**		
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value
AHI <5	1.0			1.0			1.0		
AHI 5< 15	2.7	(1.4 – 5.3)	0.002	2.2	1.1– 4.4	0.023	2.2	(1.1 – 4.3)	0.027
AHI 15<30	4.4	(2.2 – 8.6)	<0.001	3.0	1.4 – 6.3	0.004	3.0	(1.4 – 6.5)	0.004
AHI ≥30	6.8	(3.0 – 15.4)	<0.001	4.9	1.9 – 12.6	0.001	4.6	(1.8 – 12.1)	0.004

AHI= apnea hypopnea index, BMI=body mass index

Paper II

The distribution of snoring and EDS in the study population is shown in Figure 5. Snoring was reported by 25.5% (n=1,528) and EDS by 12.3% (n=735). A combination of the two symptoms was reported by 4.1% (n=246), while 66.3% (n=3,973) of the women had no symptoms of OSA. Compared with the non-snorers without EDS, the snorers with EDS were older and more often postmenopausal and on HRT. Physical inactivity was more common among the women with the combination of snoring and EDS. They also had a higher mean BMI and waist circumference and were more often smokers. They had a higher prevalence of diabetes, hypertension and depressive symptoms, while there was no difference in the prevalence of alcohol dependence.

Of the total study population, 1.2% were diagnosed with heart failure during a total follow-up of 11.8 years (mean 11.4 years). Among the women with both snoring and EDS, 5.3% (n=13) developed heart failure compared with 0.9% (n=36) in the reference group with neither snoring nor EDS (Figure 6).

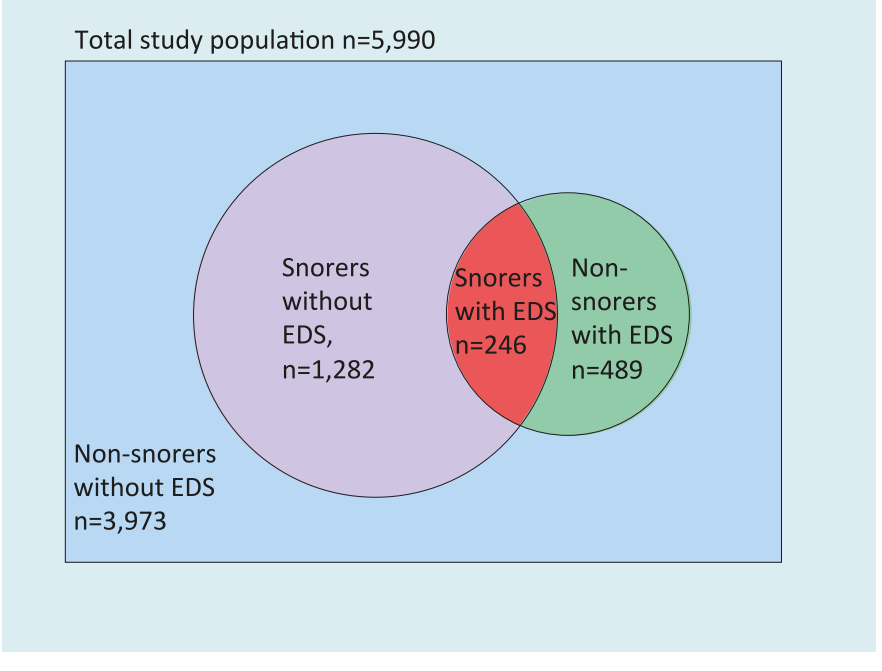

Figure 5. Distribution of sleep apnea symptoms in the study population.

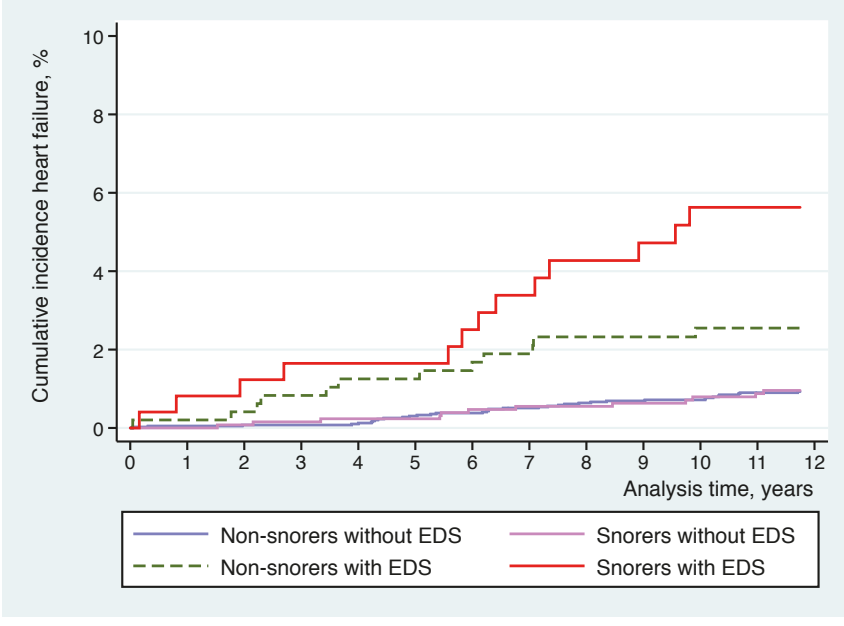

Figure 6. Incidence of heart failure in relation to follow-up time. EDS=excessive daytime sleepiness

In Cox proportional hazard regression analyses, the combination of snoring and EDS was associated with a higher rate of heart failure. After adjustment for confounding by age, waist circumference, smoking and alcohol, women with the combination of snoring and excessive daytime sleepiness had a two-fold increase in the risk of incident heart failure (HR 2.2, 95% CI 1.1-4.2). The association remained after further adjustments for hypertension, diabetes, previous myocardial infarction, physical inactivity, depressive symptoms, menopausal status and hormone replacement therapy (Table 5).

Table 5. *Snoring and excessive daytime sleepiness (EDS) as risk factors for incident heart failure. Cox proportional hazards regression model adjusted for age, waist circumference, smoking, alcohol dependence, hypertension, diabetes mellitus, previous myocardial infarction, menopausal status, HRT, physical inactivity and depressive symptoms.*

	Incident heart failure		
Exposure	**HR**	**95% CI**	**p-value**
Non-snorers without EDS	1.00		
Snorers without EDS	0.84	0.41 - 1.71	0.63
Non-snorers with EDS	1.14	0.53 - 2.46	0.73
Snorers with EDS	2.17	1.07 - 4.40	0.032

Paper III

Severe OSA during REM sleep (REM AHI≥30) was associated with a thicker intima, while there was no association between severe OSA, defined as an AHI of ≥ 30 or an ODI ≥ 30, and intima thickness (Table 6). The group with severe OSA based on a whole night was, however, small (n=13) compared with the group with severe OSA during REM sleep (n=58).

The association between severe OSA during REM sleep and intima thickness remained after adjustment for age, BMI, alcohol and smoking and also after further adjustment for possible intermediators (Table 7).

The association between severe OSA during REM sleep and intima thickness also remained when trying to isolate the effect of OSA during REM sleep by restricting the analysis to women with an overall AHI of < 15 and an NREM AHI of < 5.

Table 6. *Associations between measurements of obstructive sleep apnea and carotid artery-intima thickness (mm). Mean values and results of age-adjusted linear regression models.*

		Mean intima thickness (SD), mm	**Linear regression model, age adjusted**		
			β-coeff	(95% CI)	p-value
AHI					
0-4.9	n=69	0.084 (0.012)			
5-14.9	n=70	0.089 (0.016)	0.004	(-0.002-0.009)	0.175
15-29.9	n=49	0.090 (0.016)	0.002	(-0.003-0.008)	0.401
≥30	n=13	0.092 (0.018)	0.004	(-0.006-0.013)	0.438
ODI					
0-4.9	n=100	0.085 (0.013)			
5-14.9	n=65	0.091 (0.016)	0.006	(0.001-0.010)	0.023
15-29.9	n=24	0.090 (0.015)	0.002	(-0.005-0.009)	0.506
≥30	n=11	0.091 (0.019)	0.003	(-0.006-0.013)	0.477
REM AHI					
0-4.9	n=47	0.082 (0.010)			
5-14.9	n=42	0.088(0.014)	0.006	(-0.001-0.012)	0.096
15-29.9	n=54	0.089 (0.015)	0.005	(-0.001-0.011)	0.117
≥30	n=58	0.092 (0.017)	0.007	(0.0004-0.014)	0.038

AHI= apnea-hypopnea index, ODI=oxygen desaturation index with desaturations of ≥ 3.

Table 7. *Associations between sleep apnea during REM sleep and intima thickness (mm) after adjustment for confounders.*

	Adjusted for age, BMI, alcohol and smoking			**Adjusted for age, BMI, alcohol, smoking, systolic blood pressure, LDL, CRP and diabetes**		
REM AHI	β-coeff	(95% CI)	p-value	β-coeff	(95% CI)	p-value
5-14.9	0.006	(-0.001-0.013)	0.081	0.004	(-0.003-0.011)	0.239
15-29.9	0.006	(-0.001-0.012)	0.097	0.005	(-0.002-0.012)	0.158
≥30	0.009	(0.002-0.016)	0.013	0.008	(0.001-0.016)	0.022

AHI=apnea-hypopnea index, BMI=body mass index, LDL=low-density lipoprotein, CRP=C-reactive protein

Analyses of hypoxia measurements revealed no independent associations with intima thickness, but mean apnea length was associated with a thicker intima.

It has been suggested that OSA might be more harmful to the cardiovascular system at younger age. Stratifying by age, an association between OSA during REM sleep and intima thickness was only seen in those aged < 70 years (for REM AHI ≥ 30 in the fully adjusted model ≤ 70 years vs > 70 years; coeff 0.085 p=0.033 vs coeff 0.0004 p=0.983).

Paper IV

In unadjusted models, 57 proteins were associated with AHI, 56 proteins with ODI and 64 proteins with REM AHI.

After adjustment for age, BMI and plate, there was no significant association between AHI or ODI and any of the proteins. The top association for both AHI and ODI was with Cystatin D (CST5) (for AHI coeff 0.31 (0.11-0.50) p=0.0019 q=0.32, for ODI coeff 0.23 (0.04-0.43) p=0.018 q=0.72).

Severe OSA during REM sleep was still associated with decreased levels of SIR2-like protein 2 (Sirt2), latency-associated peptide transforming growth factor beta-1 (LAP-TGF-β_1) and Axin1, after adjustment for age, BMI and plate (Table 8).

Figures 7-9 summarise associations between different measurements of severe sleep-disordered breathing and proteins after adjustment for age, BMI and plate.

Stratifying by age, an association between protein levels and OSA during REM sleep was only seen in those aged < 70 years (for REM AHI ≥ 30, adjusting for age and BMI ≤ 70 years vs > 70 years: Sirt2 coeff -0.67 p<0.001 vs coeff -0.15 p=0.74, LAP-TGF-β_1 coeff -0.29 p<0.001 vs coeff -0.01 p=0.97, Axin1 coeff -0.46 p<0.001 vs coeff 0.06 p=0.84).

Table 8. *Top associations of an REM AHI of ≥ 30 with plasma proteins. Results from linear regression models adjusted for age, BMI and plate. The ratio (high REM AHI/low REM AHI)=2^coeff. The q-value is the FDR-adjusted p-value*.

	Regression coefficient	**Ratio**	**p-value**	**q-value**
Sirt2	-0.60	0.66	0.00016	0.016
LAP-TGF-β_1	-0.23	0.85	0.00019	0.016
Axin1	-0.37	0.77	0.0017	0.095
CD84	-0.20	0.87	0.0025	0.11
CD244	-0.17	0.89	0.0039	0.11
STAMBP	-0.33	0.79	0.0040	0.11
ITGB1BP2	-0.46	0.73	0.0047	0.11
CASP-8	-0.29	0.82	0.0059	0.12
ST1A1	-0.57	0.67	0.0084	0.14
STK4	-0.38	0.77	0.0085	0.14

Sirt2=SIR2-like protein 2, LAP-TGF-β_1=latency-associated peptide transforming growth factor beta-1, CD84=SLAM family member 5, CD244=natural killer cell receptor 2B4, STAMBP=STAM binding protein, ITGB1BP2=melusin, CASP-8=caspase-8, ST1A1= sulphotransferase 1A1, STK4=serine/threonine-protein kinase 4

AHI>30

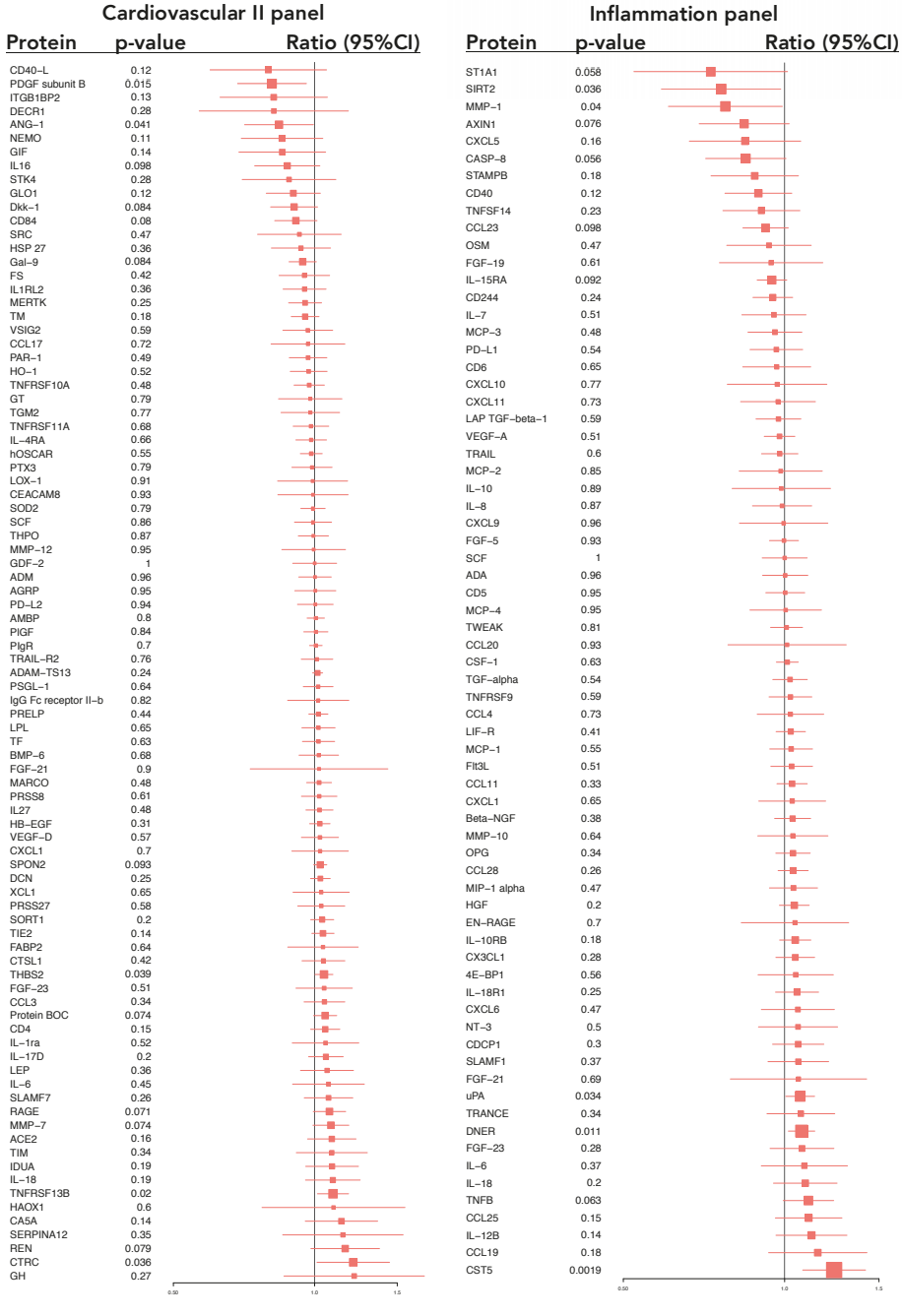

Figure 7. Associations between an AHI ≥ 30 and plasma proteins after adjustment for age, BMI and plate. The bars represent the ratio with a 95% confidence interval and the p-value is presented.

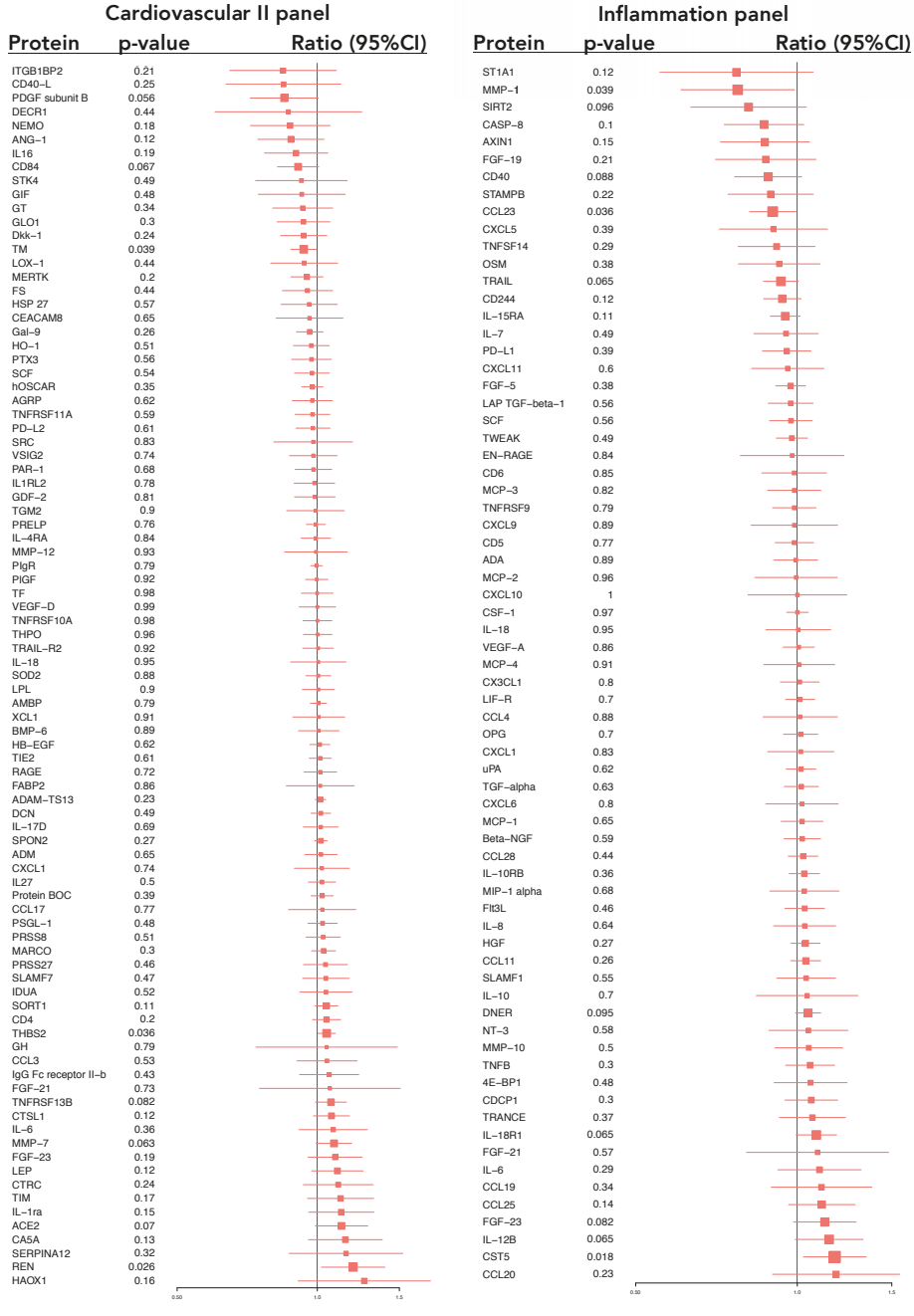

Figure 8. Associations between an ODI $\geq$ 30 and plasma proteins after adjustment for age, BMI and plate. The bars represent the ratio with a 95% confidence interval and the p-value is presented.

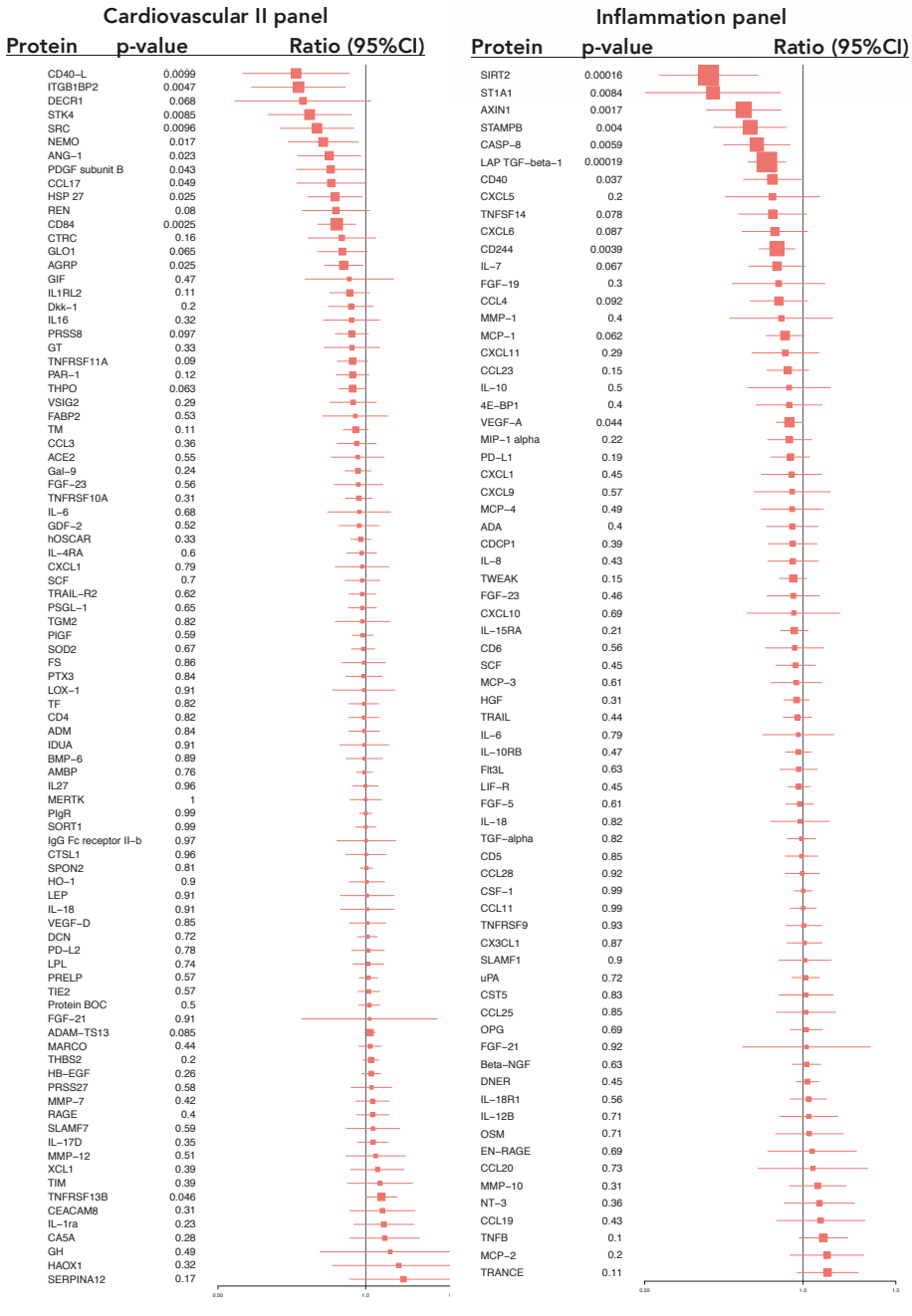

Figure 9. Associations between an REM AHI $\geq$ 30 and plasma proteins after adjustment for age, BMI and plate. The bars represent the ratio with a 95% confidence interval and the p-value is presented.

Discussion

The main findings are that, in a community-based sample of women, there is a dose-response relationship between the severity of obstructive sleep apnea during the night and levels of plasma BNP in the morning, that women with symptoms of obstructive sleep apnea run an increased risk of developing heart failure and that severe obstructive sleep apnea during REM sleep is associated with early signs of atherosclerosis and decreased levels of proteins with anti-inflammatory effects linked to the atherosclerotic process and metabolic regulation.

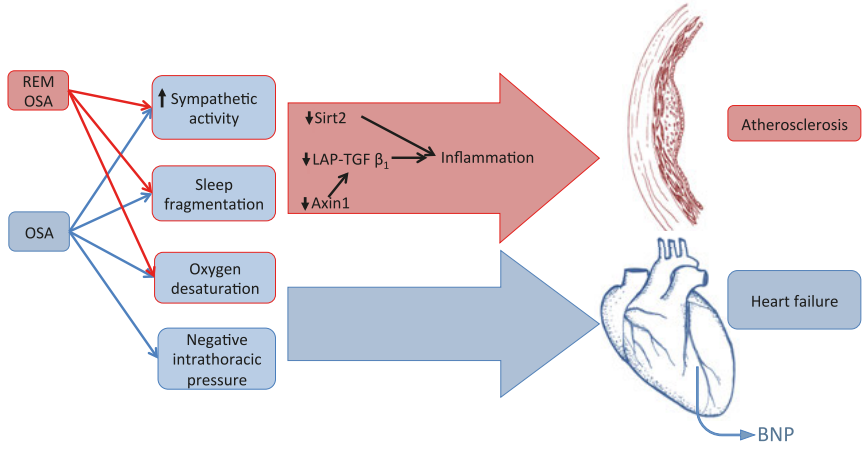

Figure 10. Graphic summary and explanatory model of the findings in Papers I-IV. Obstuctive sleep apnea is associated with negative intrathoracic pressure swings and causes increased myocardial work and oxygen demand, a reduction in preload and increased afterload. In combination with the hypoxia, this might lead to impaired cardiac contractility and contribute to the development of heart failure and elevated levels of BNP. OSA during REM sleep is associated with longer apneas, deeper desaturations, REM sleep fragmentation and a high sympathetic activity. These features of REM OSA might explain why OSA during REM sleep, but not OSA based on a whole night, was associated with decreased levels of anti-inflammatory proteins and increased intima thickness.

OSA and heart failure

The finding in Paper I that there is a relationship between the severity of obstructive sleep apnea during the night and levels of plasma BNP in the morning is in accordance with some previous studies,[101,103-105] while others reported no association.[106-108] Most previous studies are clinic-based studies of predominantly male study populations[101,104,106,107] or children,[103,105] while few other community-based studies on OSA and BNP existed when Paper I was published.[108] The subjects with OSA are generally leaner in the studies reporting an association between OSA and BNP levels,[101,103-105] than in those reporting no association.[107,108] Since BNP is affected by BMI, with lower levels in obese subjects,[155] the cardiac stress caused by OSA might be more difficult to detect with BNP in obese populations.

After Paper I was published, the relationship between OSA and NT-pro-BNP has been investigated in a large cohort study where no association was seen.[175] This is in accordance with some previous studies of OSA and NT-pro-BNP.[109,110] The reason for the discrepancy between the results for BNP and NT-pro-BNP is unclear, but it might be related to differences in half-life and stability between the active peptide, BNP, and its inactive split metabolite, NT-pro-BNP.[176]

In spite of the somewhat conflicting results regarding the association between OSA and natriuretic peptide levels, CPAP treatment has recently been shown to reduce the levels of natriuretic peptides in patients with OSA in both non-randomised studies[177,178] and also in a randomised trial.[179] This was not, however, true in a trial of minimal symptomatic mild to moderate OSA, where no effect on BNP levels or cardiac functional parameters was seen with CPAP treatment.[180]

The finding in Paper II that women from the general population with symptoms of obstructive sleep apnea run an increased risk of developing heart failure is in accordance with the results in Paper I and previous experimental[97] and cross-sectional studies,[96,99,100] but it differs from the results of a study in the Sleep Heart Health cohort reporting an association between OSA and incident heart failure in men but not in women.[86] Interestingly, since Paper II was published, the Sleep Heart Health cohort published another study, with a study population consisting of participants that also participated in the Atherosclerosis Risk in the Communities study. In this study population, OSA was associated with incident heart failure in women but not in men in unadjusted models and with left ventricular hypertrophy in women but not in men even after adjustment for confounders.[181]

Although definite conclusions about causality cannot be drawn, the results from Papers I and II indicate that the association between obstructive sleep apnea and heart failure, first reported in men, also exists in women. The levels of BNP seen in Paper I are low compared with the thresholds used to diagnose heart failure clinically, but, even in asymptomatic individu-

als without known cardiovascular disease, there is an association between levels of BNP and future risk of cardiovascular events, heart failure and death.[182]

Intermittent hypoxia has been suggested as the main cause of long-term cardiovascular consequences in OSA,[183] but the effect of obstructive sleep apnea on the development and progression of heart failure is probably caused by a combination of factors, where both the effect of repetitive episodes of negative intrathoracic pressure, increased sympathetic activity and intermittent hypoxia contribute.[184] BNP is secreted from the cardiac ventricles in response to cardiac wall stretch.[158] The dose-response association between AHI and levels of BNP is most probably explained by negative intrathoracic pressure swings during the apneic cycles causing pressure load and volume expansion. In combination with hypoxia, this might in time lead to impaired cardiac contractility[39] and the development of heart failure. Intermediate mechanisms might also contribute. Non-dipping blood pressure during the night has been associated with an increased incidence of heart failure, even after adjustment for daytime blood pressure measurement.[185] The association between OSA and BNP levels and symptoms of OSA and heart failure remained after adjustment for blood pressure or known hypertension, but it is still possible that OSA promotes heart failure through non-dipping nocturnal blood pressure that was not discovered with morning blood pressure measurement.

The impact of daytime sleepiness on cardiovascular risk in OSA is the subject of debate. In Paper II, only the combination of snoring and daytime sleepiness was associated with an increased incidence of heart failure. This could be because the combination of snoring and daytime sleepiness is a better proxy for OSA than snoring alone, but, alternatively, it could indicate an impact of daytime sleepiness on cardiovascular risk. In accordance with the latter, an independent association between ESS and impaired cardiac function has been reported in OSA[186] and a recent study reported an increased risk of cardiovascular disease and heart failure in the excessively sleepy OSA subtype.[187] On the other hand, when heart failure is established, individuals with the combination of heart failure and OSA have been reported to be less sleepy than those with OSA without heart faiure.[188] This is thought to be a consequence of the high sympathetic tone in heart failure.[189]

Heart failure is a condition with a poor prognosis. Five-year survival after the diagnosis of heart failure is 50-60%.[139] Identifying risk factors and treating them might prevent the development of heart failure, although threating risk factors is not always associated with risk reduction. While the treatment of hypertension with anti-hypertensive medication reduces the risk of heart failure, smoking cessation and most kinds of diabetic treatment have not been shown to reduce the risk.[146] Whether treatment for OSA with CPAP could help prevent heart failure remains to be investigated. In a randomised controlled trial of patients with severe OSA, an improvement in diastolic

function was seen with CPAP therapy.[190] However, in the SAVE trial, investigating the effect of CPAP on a composite endpoint of cardiovascular death, myocardial infarction, stroke or hospitalisation for heart failure, unstable angina or transient ischaemic attacks in patients with cardiovascular disease, no risk reduction was seen with CPAP.[66]

Although heart failure patients with OSA have been reported to have a poorer prognosis than those without sleep-disordered breathing[95,191] it is also unclear whether the treatment of coexisting OSA affects the prognosis in established heart failure. In observational studies of patients with the combination of heart failure and OSA, CPAP compliance is associated with increased hospitalisation-free survival[192] and, in randomised trials of heart failure patients, treating OSA with CPAP was associated with improved left ventricular ejection fraction[193,194] and reduced BNP levels.[195] CPAP also reduces blood pressure and sympathetic activity, but no improvement in terms of survival has been shown. Screening for OSA in heart failure patients has been suggested, but, since evidence of survival benefits is lacking, there is currently no recommendation for screening in European guidelines.[146]

OSA during REM sleep and cardiovascular disease

In Papers III and IV, we found that severe OSA during REM sleep was associated with an early sign of atherosclerosis and changes in the levels of proteins involved in metabolic regulation and atherosclerosis. These results suggest that, even though REM sleep only accounts for about 20% of total sleep time, OSA during REM sleep might have negative effects on the cardiovascular system.

These results are in accordance with several recent studies.[120,124-126,129-135] Severe OSA during REM sleep has been associated with hypertension,[124-126] diastolic dysfunction,[133] the metabolic syndrome[136] and cardiovascular insults.[137] Some studies reported that OSA during REM sleep had a greater impact on the outcome than OSA during non-REM sleep,[120,124-126,129,131,133-135] while others only investigated the effect of OSA during REM sleep.[136,137] Since NREM accounts for about 80% of total sleep time, a greater impact of OSA during NREM on cardiovascular outcomes could be expected. However, very few studies have found an association between OSA during NREM sleep but not during REM sleep and cardiometabolic outcomes.[127,128,138] The greater impact of OSA during REM sleep might be explained by longer apneas with deeper desaturations in REM sleep or might be related to the negative effects of REM sleep fragmentation.

Few other studies have investigated the association between OSA during REM sleep and atherosclerotic disease. Lin *et al.* reported that REM AHI was associated with arterial stiffness[135] and, in patients with cardiovascular

disease, severe OSA during REM sleep was associated with an increased risk of cardiovascular insults.[137]

The association between OSA during REM sleep and signs of atherosclerosis might be explained by the activation of inflammatory pathways that over time contribute to the development of atherosclerosis. Decreased levels of Sirt2, LAP-TGF-β_1 and Axin1 might be a part of this altered inflammatory state, although it is only possible to hypothesise about the exact mechanisms. The NF-κB pathway is activated in OSA, causing an increase in the expression of several inflammatory proteins.[33] Sirt2 has been shown to exhibit anti-inflammatory effects by inhibiting NF-kB activity.[196] LAP-TGF-β_1 is also an anti-inflammatory protein and decreased serum levels of TGF-β_1 have been correlated to clinical atherosclerosis,[197] while Axin1 has been reported to facilitate TGF-β signalling.[198] Intermittent hypoxia, the activation of the sympathetic nervous system and REM sleep fragmentation are all possible ways that OSA during REM sleep could cause decreased levels of Sirt2, Axin1 and LAP-TGF-β_1.

The importance of obesity as a major contributor to the increased risk of cardiovascular disease seen in OSA is illustrated by the results in Paper IV, where the vast majority of the associations between sleep-disordered breathing and protein levels were no longer significant after adjustment for BMI. Obesity is a state of low-grade inflammation and is associated with changes in the levels of adipokines like leptin. Leptin has also been reported to be increased in OSA,[199] but in our study the association between AHI and leptin (coeff 0.53 $p<0.001$ for AHI>30) was explained by obesity (coeff 0.08 $p=0.359$ for AHI>30 after adjustment for BMI). Interestingly, for the proteins significantly associated with OSA during REM sleep after adjustment for confounding, OSA during REM sleep and BMI had the opposite effect on protein levels, suggesting a mechanism separate from obesity.

It has been suggested that OSA might be more harmful to the cardiovascular system at younger age. Punjabi *et al.* reported that, in age-stratified analyses, OSA was only associated with increased mortality in those younger than 70 years[73] and Gottlieb reported an association with myocardial infarctions only in men aged < 70 years.[86] In accordance with this, if stratifying by age, an association between protein levels and OSA during REM sleep was only seen in those aged < 70 years. The same pattern was also observed for intima thickness. These results should, however, be interpreted with caution since the group of women older than 70 years was small (Paper III n=26, Paper IV n=31) and we might not have enough power to detect an association in this subgroup.

Methodological considerations

The SHE study is a well-characterised cohort based on a sample from the general population. In Papers I, III and IV, we used PSG, the gold-standard diagnostic method for OSA, to assess sleep-disordered breathing. The measurements of the dependent variables were also assessed with well-established laboratory techniques (Paper I), register based diagnoses with high validity (Paper II), high-resolution ultrasound measurement (Paper III) and a high quality proteomic technique (Paper IV). However, the studies also have several limitations.

While Papers I, III and IV are based on PSG-verified OSA, the exposure variable in Paper II is based on self-reported symptoms. The combination of snoring and EDS is a somewhat crude proxy for OSA. Validation of its ability to identify women with an AHI of > 15 showed a sensitivity of 85% and a specificity of 37%.

Because of the cross-sectional design of Papers I, III and IV, conclusions on causality cannot be drawn. Based on the dose-response relationship between OSA and BNP levels, we speculate that OSA causes an increase in BNP, but, in heart failure patients, fluid shift from the legs to the neck during the night might cause pharyngeal narrowing and contribute to the appearance of obstructive events. On the other hand, the levels of BNP in our healthy population-based cohort were low compared with those seen in established heart failure with oedema and excluding women with known heart failure did not change the results. In Paper III we had no carotid ultrasound at baseline and we cannot be certain that the intimal thickening did not precede the occurrence of OSA during REM sleep, even though we attempted to address this issue by excluding women with known cardiovascular disease. There is, however, no plausible biological explanation as to why atherosclerosis would contribute to OSA during REM sleep.

The associations between SDB and biomarkers that was seen in Papers I, III and IV do not necessarily translate into an increased risk of heart failure or cardiovascular disease. They do, however, illustrate the cardiovascular stress and point toward possible mechanisms by which SDB could contribute to cardiovascular disease. The prospective design in Paper II, with a register-based diagnosis of heart failure as the endpoint, strengthens the evidence of OSA as a risk factor for heart failure. To address the problem of possible reverse causation caused by undiagnosed heart failure at baseline, sensitivity analyses excluding heart failure diagnoses within the first year of follow-up were performed.

In Paper IV, multiple proteins were studied. We adjusted for multiple testing, but the findings should ideally be confirmed in an independent cohort and with absolute quantification. In the absence of a confirmatory cohort, the results should be regarded as hypothesis generating. Nonetheless, even though the decreased levels of Sirt2, LAP-TGF-β_1 and Axin1 need to be

further confirmed, the pattern of a greater impact of OSA during REM sleep on protein levels than for OSA based on a whole night highlights the significance of OSA during REM sleep.

We chose confounding variables based on literature research and DAG analyses, but there is a risk of residual confounding. Obesity is the most important risk factor for OSA and it is also an important risk factor for cardiovascular disease. The best way to adjust for obesity to avoid residual confounding is the subject of debate. We used BMI in Papers I, III and IV, while, in Paper II, we used waist circumference, as self-reported waist circumference, measured with a tape measure that was sent to the women along with the questionnaire, was expected to give a more satisfactory measurement than BMI based on self-reported weight. However, European guidelines on cardiovascular disease prevention conclude that both BMI and waist circumference are similarly strong and continuously associated with cardiovascular disease and type II diabetes[140] and using self-reported BMI instead of waist circumference in Paper II did not change the results.

Smoking is an important risk factor for cardiovascular disease and is thought to be a risk factor for OSA, although this remains to be proven. We adjusted for smoking in Papers II and III. Smoking had, however, no real impact on the results and was not included as a confounder in Paper IV.

The difference between a confounder and an intermediator is not always clear. The inclusion of hypertension, diabetes and other cofactors that might not be confounders but intermediators in the multivariable models increases the risk of type II errors. In Papers II and III, we created separate models also considering the effect of intermediators, but these models might underestimate the true effect of the exposure variable on the outcome.

The cohort is based on a random sample of women from the general population, thereby minimising the risk of selection bias. There is, however, always a risk of non-participation introducing bias.

General discussion

The pathophysiology of OSA is seemingly simple – repeated episodes of complete or partial obstruction of the upper airway during sleep causes apneas and hypopneas. From this perspective, the AHI, quantifying the number of apneas and hypopneas per hour of sleep, is a reasonable measurement of OSA. However, to fully reveal the consequences of OSA, mediated by the disruption of functions essential for human life, like sleep and oxygenation, factors other than the number of apneas and hypopneas per hour of sleep might need to be taken into consideration. While the evidence for SDB as an independent risk factor for cardiovascular and metabolic disease has increased rapidly in the last decade, the usefulness of the AHI as the sole measurement of OSA has been increasingly questioned. The inability of the

AHI to fully reflect the impact that sleep apnea might have is illustrated by the weak association between the AHI and daytime sleepiness,[54] by the finding that an OSA severity classification that also accounted for the duration of the apneas and the area of desaturations was a better predictor of cardiovascular mortality than the AHI[200] and by the findings in this thesis and other studies that the impact of sleep apnea might differ by sleep stage.

Identifying factors other than the overall AHI that have a prognostic impact would also have consequences for treatment. Even though CPAP treatment adherence is often defined as CPAP use for at least four hours a night on 70 per cent of nights,[201] RCTs have failed to show any cardioprotective effect of CPAP for three to four hours a night.[66-68] Since REM sleep increases through the night, with the highest concentration during the hours before awakening, treatment for three to four hours a night leaves much of OSA during REM sleep untreated. It is possible that an improved cardioprotective effect could be obtained with longer CPAP usage that covers REM sleep more effectively. This is supported by secondary analyses in RCTs.[67,68]

Appart from the question regarding what is harmful about SDB, it is also important to answer to whom it is harmful. The impact of gender and age on cardiovascular risk in OSA is still not fully understood. Fortunately, the gap in knowledge regarding the effects of SDB in women has been increasingly recognised in the past few years and more studies with female participants or stratified by gender have been published. As a result, we now know that the association with hypertension first reported in men also exists in women, that CPAP treatment lowers blood pressure in women as well[202] and that CPAP treatment is associated with improvements in EDS and mood in women.[203] While the results of early studies including few women led to the interpretation that SDB might not be as harmful in women, recent studies report the opposite, that OSA is associated with cardiovascular events in women[65] and that the effects of SDB on the cardiovascular system might be even more pronounced in women than in men.[181,204,205] In spite of this, in RCTs investigating the effects of CPAP treatment, women are underrepresented. The fact that women have a greater proportion of their apneas and hypopneas during REM sleep, with a greater concentration during the second half of the night, might also require alternative study designs to truly assess the effect of OSA treatment on cardiovascular endpoints in women.

Future perspectives

While the results from Papers I & II strengthen the evidence that the associations between OSA and heart failure that have previously been reported in men also exist in women, the findings in Papers III and IV are novel and the question of whether there is an association between OSA during REM sleep and atherosclerosis or decreased levels of Sirt2, LAP-TGF-β_1 and Axin1 in

men needs to be investigated. It is possible that OSA during REM sleep is a more important risk factor in women, as women have a larger percentage of their apneas and hypopneas during REM sleep. The Men in Uppsala; a Study of sleep, Apnea and Cardiometabolic Health (MUSTACHE) study, with men matched by age and BMI to the participants in SHE, will provide an opportunity to address these questions and to study gender differences in sleep apnea.

There is real need to redefine OSA. With the current definition, the prevalence of OSA is very high in the population in general and even more so in patients with cardiovascular disease, while the AHI gives little information about which patients run the greatest risk of OSA-related consequences and will benefit the most from treatment. Future studies also have to consider the impact of measurements other than the AHI on symptoms and health outcomes. In addition to sleep stage-dependent OSA, algorithms also considering the lengths of the apneas and the depths of the desaturations might provide more accurate measurements of the impact of sleep apnea.

While we are far from identifying biomarkers that can help us diagnose OSA, biomarker studies are needed to reveal the pathophysiological mechanisms linking OSA to cardiometabolic disease. Biomarkers might also be a future tool to identify individuals running an increased risk of cardiovascular consequences. Established cardiovascular disease markers like natriuretic peptides and troponins would be the first-hand choice, with some studies already examining their potential to predict cardiovascular risk in OSA.[181] Newer biomarkers might add additional information. Few studies so far have investigated associations between SDB and sirtuins. Since sirtuins are involved in metabolic[196] and circadian regulation,[206] they offer an intriguing possible link between SDB and cardiometabolic dysregulation that deserves to be studied in greater detail. There are seven human sirtuins and so far only two have been investigated in relation to SDB.[207]

The emerging body of evidence on the importance of OSA during REM sleep also calls for differently designed treatment studies to investigate the effect of effective treatment during REM sleep on cardiovascular events or markers of cardiovascular disease. A randomised CPAP withdrawal trial, investigating the effect on cardiovascular biomarkers of CPAP withdrawal for a few weeks in patients with severe OSA during REM sleep and good CPAP compliance, could be the first step.

The significance of daytime sleepiness for OSA prognosis and consequences also deserves further investigation. The Swedevox project, with data on more than ten thousand CPAP-treated patients with the ESS at baseline and follow-up and register data from the Swedish National Patient Register and the Swedish Cause of Death Register, provides a good opportunity to study this.

Conclusions

In a community-based sample of women

I There is a dose-response relationship between the severity of obstructive sleep apnea during the night and levels of plasma BNP in the morning that cannot be explained by known confounding factors

II Those with the combination of snoring and daytime sleepiness, symptoms of obstructive sleep apnea, run an increased risk of developing heart failure

III Severe obstructive sleep apnea during REM sleep is associated with early signs of atherosclerosis with a thicker carotid intima

IV Severe obstructive sleep apnea during REM sleep is associated with decreased plasma levels of Sirt2, LAP-TGF-β_1 and Axin1, proteins with anti-inflammatory effects involved in numerous cellular functions, in metabolic regulation and in the atherosclerotic process. For OSA based on a whole night the associations with cardiac and inflammatory proteins are weaker and are to a large extent explained by age and BMI.

Sammanfattning på svenska

Obstruktiv sömnapné betraktades länge som ett tillstånd som framför allt drabbar män, men senare studier har visat att det i allra högsta grad även drabbar kvinnor. Sömnapné leder till dagsömnighet och sänkt livskvalitet och allt fler studier visar att sömnapné också är en riskfaktor för hjärt-kärlsjukdom. Eftersom sömnapné länge betraktades som en manlig sjukdom är en stor del av befintliga studier gjorda på män, medan vi vet mindre om vilka konsekvenser sömnapné kan ha hos kvinnor.

Även om vi nu vet att andningsstörningar under sömnen är associerade med en ökad risk för hjärt-kärlsjukdom vet vi ännu förhållandevis lite om vad det är med dessa andningsstörningar som är skadligt och vilket eller vilka mått på andningsstörningarna som bäst förutspår risken att drabbas av hjärt-kärlsjukdom. Andningsuppehållen är associerade med återkommande sänkningar i blodets syresättning, fragmenterad sömn och ökad aktivitet i det sympatiska nervsystemet. Att försöka andas mot en sluten luftväg medför också stora tryckförändringar i bröstkorgen. Alla dessa faktorer skulle potentiellt kunna bidra till den ökade risken för hjärt-kärlsjukdom.

Sömnen kan delas in i REM-sömn, även kallad drömsömn, och i non-REM-sömn. Det finns studier som talar för att andningsstörningar under REM-sömn skulle kunna vara mer skadligt än andningsstörningar under nonREM-sömn.

Syftet med denna avhandling var att undersöka samband mellan olika mått på sömnrelaterade andningsstörningar och tecken på hjärt-kärlsjukdom hos kvinnor.

Alla delarbeten är baserade på ”Sömn och hälsa hos kvinnor” (SHE-studien), där 7051 kvinnor bosatta i Uppsala har svarat på frågor om sömn och hälsa första gången år 2000. 400 av dessa kvinnor undersöktes sedan med nattlig andnings- och sömnregistrering (polysomnografi) för att detektera sömnrelaterade andningsstörningar. Frågeformulär och undersökningar upprepades sedan tio år senare.

Studie I undersökte sambandet mellan sömnapné under natten och nivåer av hjärtsviktsmarkören B-typ natriuretisk peptid (BNP) i blodet efterföljande morgon hos 349 kvinnor.

Studie II inkluderade totalt 5990 kvinnor och jämförde risken att insjukna i hjärtsvikt hos kvinnor med de två huvudsymptomen på sömnapné, snarkning och dagsömnighet, med risken hos kvinnor som inte hade dessa symptom under en uppföljningstid på drygt 11 år.

I studie III undersöktes sambandet mellan sömnapné under REM-sömn och tecken på ateroskleros. 201 kvinnor, som inte hade någon känd hjärt-kärlsjukdom när de genomgick polysomnografin, undersöktes ca 10 år senare med ultraljud av halspulsådern. Tjockleken på blodkärlets innersta lager, intiman, användes som en markör för ateroskleros.

Studie IV undersökte sambandet mellan svår sömnapné, svår sömnapné under REM-sömn och nivåerna av 170 olika proteiner hos 253 kvinnor.

Studie I och II visade att det finns ett samband mellan sömnapné under natten och nivåer av hjärtsviktsmarkören BNP efterföljande morgon och att kvinnor med kombinationen dagsömnighet och snarkning, typiska symptom vid sömnapné, har en fördubblad risk att drabbas av hjärtsvikt jämfört med dem som inte snarkar eller är dagsömniga. I studie III och IV fann vi att svår sömnapné under REM-sömn, med mer än 30 andningsstörningar per timmes REM-sömn, är associerat med tecken på ateroskleros och med minskade nivåer på tre av de undersökta proteinerna: Sirt2, LAP-TGF-β_1 och Axin1. Ovan beskrivna samband kunde inte förklaras med faktorer såsom ålder och vikt. Svår sömnapné baserad på en hel natts sömn var däremot inte associerat med ändrade proteinnivåer efter att hänsyn tagits till effekten av ålder, vikt och multipla test.

Resultaten i studie I och II skulle kunna förklaras med att de tryckförändringar i bröstkorgen som sömnapnén orsakar, och kanske även dipparna i syresättning, påverkar hjärtat och resulterar i frisättning av BNP och på sikt en ökad risk att insjukna i hjärtsvikt. Dessa resultat talar för att vi bör ha en ökad uppmärksamhet beträffande symptom på sömnapné hos kvinnor med risk för hjärtsvikt och vara liberala med utredning.

Ateroskleros är en inflammatorisk process och sambandet mellan frekventa andningsstörningar under REM-sömn och tecken på ateroskleros skulle kunna förklaras med aktivering av inflammatoriska signalvägar. Två av proteinerna som uppvisade sänkta nivåer vid svår sömnapné under REM-sömn, Sirt2 och LAP-TGF-β_1, har antiinflammatoriska effekter. De lägre nivåerna av dessa proteiner kan vara en del av förklaringen till ökad inflammation och ateroskleros. Tidigare studier har visat att inflammation vid sömnapné delvis är medierad via nuclear factor-κB (NF-κB), som i sin tur aktiverar en rad med inflammatoriska ämnen, och en av Sirt2:s effekter är att förhindra NF-κB-aktivitet.

Vilka mekanismer som orsakar de sänkta nivåerna av Sirt2, LAP-TGF-β_1 och Axin1 kan vi bara spekulera om, men man vet att sömnapné under REM-sömn är associerat med längre andningsuppehåll med djupare dippar i syresättningen och med en hög aktivitet i det sympatiska nervsystemet. Därutöver är det möjligt att den fragmentering av REM-sömn som sömnapné under REM-sömn orsakar har betydelse.

Om sömnapné under REM-sömn är särskilt skadligt för hjärt-kärlsystemet får det konsekvenser för hur vi ska behandla sömnapné. REM-sömn utgör ca 20 % av total sovtid och återkommer cykliskt under natten med högre kon-

centration timmarna före uppvaknande. Vid behandling med övertrycksandning via mask (CPAP) blir det därför viktigt att behandlingen används hela natten. I studier som har tittat på effekten av CPAP-behandling på risken att drabbas av hjärt-kärlsjukdom har man inte kunna påvisa någon tydlig effekt. Detta kan kanske förklaras med att längre behandlingstider än de uppnådda behövs för att även täcka REM-sömnen. Resonemanget stärks av iakttagelsen i ovan nämnda studier att de grupper av studiedeltagare som använde sin CPAP mest, faktiskt fick viss skyddande effekt. Detta kan vara särskilt viktigt hos kvinnor, som har en större andel av sina andningsuppehåll under REM-sömn.

Sammanfattningsvis visar studierna i denna avhandling att det finns ett samband mellan obstruktiv sömnapné under natten och nivåer av hjärtsviktsmarkören BNP efterföljande morgon hos kvinnor, att kvinnor med symptom på obstruktiv sömnapné har en ökad risk att drabbas av hjärtsvikt och att svår sömnapné under REM-sömn är associerat med tecken på ateroskleros och med sänkta nivåer av proteiner med antiinflammatorisk effekt.

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References

1. Krahn, L.E., Silber, M.H, Morgenthaler, T.I. *Atlas of Sleep Medicine*, (Taylor & Francis Group, 2010).
2. Kryger, M.H. Sleep apnea. From the needles of Dionysius to continuous positive airway pressure. *Archives of internal medicine* **143**, 2301-2303 (1983).
3. Camacho, M.*, et al.* Tracheostomy as treatment for adult obstructive sleep apnea: a systematic review and meta-analysis. *The Laryngoscope* **124**, 803-811 (2014).
4. Norton, P.G. & Dunn, E.V. Snoring as a risk factor for disease: an epidemiological survey. *British medical journal (Clinical research ed.)* **291**, 630-632 (1985).
5. Koskenvuo, M.*, et al.* Snoring as a risk factor for hypertension and angina pectoris. *Lancet* **1**, 893-896 (1985).
6. Young, T.*, et al.* The occurrence of sleep-disordered breathing among middle-aged adults. *The New England journal of medicine* **328**, 1230-1235 (1993).
7. Saper, C.B., Scammell, T.E. & Lu, J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* **437**, 1257-1263 (2005).
8. Porkka-Heiskanen, T. Sleep homeostasis. *Current opinion in neurobiology* **23**, 799-805 (2013).
9. Berry RB, B.R., Gamaldo CE, et al. *The AASM manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.4*, (Darien, IL, 2017).
10. Aserinsky, E. & Kleitman, N. Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science* **118**, 273-274 (1953).
11. Peever, J. & Fuller, P.M. The Biology of REM Sleep. *Curr Biol* **27**, R1237-R1248 (2017).
12. Morrell M.J, P.P., Levy P, DeBacker W. Neuroanatomy and neurobiology of sleep. *ERS Handbook: respiratory Sleep Medicine*, 1-5 (2012).
13. Feldman, J.L., Del Negro, C.A. & Gray, P.A. Understanding the rhythm of breathing: so near, yet so far. *Annual review of physiology* **75**, 423-452 (2013).
14. Dempsey, J.A., Veasey, S.C., Morgan, B.J. & O'Donnell, C.P. Pathophysiology of sleep apnea. *Physiological reviews* **90**, 47-112 (2010).
15. Morrell M.J, P.P., Levy P, DeBacker W. Breathing during sleep and wakefulness. in *ERS Handbook: Respiratory Sleep Medicine* (ed. Simonds A.K, d.B.W.) 6-12 (2012).
16. Horner, R.L., Hughes, S.W. & Malhotra, A. State-dependent and reflex drives to the upper airway: basic physiology with clinical implications. *Journal of applied physiology* **116**, 325-336 (2014).

17. Ramirez, J.M.*, et al.* Central and peripheral factors contributing to obstructive sleep apneas. *Respiratory physiology & neurobiology* **189**, 344-353 (2013).
18. *International Classification of Sleep Disorders. 3rd ed.*, (American Academy of Sleep Medicine, 2014).
19. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. *Sleep* **22**, 667-689 (1999).
20. Gaudette E, K.R.J. Pathophysiology of OSA. *European Respiratory Monograph*, 31-50 (2010).
21. Peppard, P.E., Young, T., Palta, M., Dempsey, J. & Skatrud, J. Longitudinal study of moderate weight change and sleep-disordered breathing. *Jama* **284**, 3015-3021 (2000).
22. Kolla, B.P.*, et al.* The impact of alcohol on breathing parameters during sleep: A systematic review and meta-analysis. *Sleep medicine reviews* **42**, 59-67 (2018).
23. Franklin, K.A. & Lindberg, E. Obstructive sleep apnea is a common disorder in the population-a review on the epidemiology of sleep apnea. *Journal of thoracic disease* **7**, 1311-1322 (2015).
24. Myers, K.A., Mrkobrada, M. & Simel, D.L. Does this patient have obstructive sleep apnea?: The Rational Clinical Examination systematic review. *Jama* **310**, 731-741 (2013).
25. Friberg, D.*, et al.* Histological indications of a progressive snorers disease in an upper airway muscle. *American journal of respiratory and critical care medicine* **157**, 586-593 (1998).
26. Somers, V.K., Dyken, M.E., Clary, M.P. & Abboud, F.M. Sympathetic neural mechanisms in obstructive sleep apnea. *The Journal of clinical investigation* **96**, 1897-1904 (1995).
27. Tamisier, R.*, et al.* 14 nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans. *The European respiratory journal* **37**, 119-128 (2011).
28. Kohler, M.*, et al.* Effects of continuous positive airway pressure therapy withdrawal in patients with obstructive sleep apnea: a randomized controlled trial. *American journal of respiratory and critical care medicine* **184**, 1192-1199 (2011).
29. Carlson, J.T.*, et al.* Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest* **103**, 1763-1768 (1993).
30. Jullian-Desayes, I.*, et al.* Impact of obstructive sleep apnea treatment by continuous positive airway pressure on cardiometabolic biomarkers: a systematic review from sham CPAP randomized controlled trials. *Sleep medicine reviews* **21**, 23-38 (2015).
31. Rossi, V.A., Stradling, J.R. & Kohler, M. Effects of obstructive sleep apnoea on heart rhythm. *The European respiratory journal* **41**, 1439-1451 (2013).
32. Svensson, M., Venge, P., Janson, C. & Lindberg, E. Relationship between sleep-disordered breathing and markers of systemic inflammation in women from the general population. *Journal of sleep research* **21**, 147-154 (2012).
33. Arnardottir, E.S., Mackiewicz, M., Gislason, T., Teff, K.L. & Pack, A.I. Molecular signatures of obstructive sleep apnea in adults: a review and perspective. *Sleep* **32**, 447-470 (2009).

34. Kent, B.D., Ryan, S. & McNicholas, W.T. Obstructive sleep apnea and inflammation: relationship to cardiovascular co-morbidity. *Respiratory physiology & neurobiology* **178**, 475-481 (2011).
35. Dumitrascu, R., Heitmann, J., Seeger, W., Weissmann, N. & Schulz, R. Obstructive sleep apnea, oxidative stress and cardiovascular disease: lessons from animal studies. *Oxidative medicine and cellular longevity* **2013**, 234631 (2013).
36. Wang, J.*, et al.* Impact of Obstructive Sleep Apnea Syndrome on Endothelial Function, Arterial Stiffening, and Serum Inflammatory Markers: An Updated Meta-analysis and Metaregression of 18 Studies. *J Am Heart Assoc* **4**(2015).
37. Thunstrom, E.*, et al.* CPAP Does Not Reduce Inflammatory Biomarkers in Patients With Coronary Artery Disease and Nonsleepy Obstructive Sleep Apnea: A Randomized Controlled Trial. *Sleep* **40**(2017).
38. Hamilton, G.S., Meredith, I.T., Walker, A.M. & Solin, P. Obstructive sleep apnea leads to transient uncoupling of coronary blood flow and myocardial work in humans. *Sleep* **32**, 263-270 (2009).
39. Okuda, N.*, et al.* Depressed myocardial contractile reserve in patients with obstructive sleep apnea assessed by tissue Doppler imaging with dobutamine stress echocardiography. *Chest* **131**, 1082-1089 (2007).
40. Shiomi, T., Guilleminault, C., Stoohs, R. & Schnittger, I. Leftward shift of the interventricular septum and pulsus paradoxus in obstructive sleep apnea syndrome. *Chest* **100**, 894-902 (1991).
41. Orban, M.*, et al.* Dynamic changes of left ventricular performance and left atrial volume induced by the mueller maneuver in healthy young adults and implications for obstructive sleep apnea, atrial fibrillation, and heart failure. *The American journal of cardiology* **102**, 1557-1561 (2008).
42. Kim, J.*, et al.* Increase in serum haptoglobin and apolipoprotein M in patients with obstructive sleep apnoea. *Journal of sleep research* **18**, 313-320 (2009).
43. Jurado-Gamez, B.*, et al.* Serum proteomic changes in adults with obstructive sleep apnoea. *Journal of sleep research* **21**, 139-146 (2012).
44. Feliciano, A.*, et al.* Evening and morning peroxiredoxin-2 redox/oligomeric state changes in obstructive sleep apnea red blood cells: Correlation with polysomnographic and metabolic parameters. *Biochim Biophys Acta Mol Basis Dis* **1863**, 621-629 (2017).
45. Heinzer, R.*, et al.* Impact of sex and menopausal status on the prevalence, clinical presentation, and comorbidities of sleep-disordered breathing. *Sleep Med* **51**, 29-36 (2018).
46. Franklin, K.A., Sahlin, C., Stenlund, H. & Lindberg, E. Sleep apnoea is a common occurrence in females. *The European respiratory journal* **41**, 610-615 (2013).
47. Young, T., Evans, L., Finn, L. & Palta, M. Estimation of the clinically diagnosed proportion of sleep apnea syndrome in middle-aged men and women. *Sleep* **20**, 705-706 (1997).
48. Ho, V., Crainiceanu, C.M., Punjabi, N.M., Redline, S. & Gottlieb, D.J. Calibration Model for Apnea-Hypopnea Indices: Impact of Alternative Criteria for Hypopneas. *Sleep* **38**, 1887-1892 (2015).
49. Epstein, L.J.*, et al.* Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **5**, 263-276 (2009).
50. Lindberg, E. Epidemiology of OSA. in *European Respiratory Monograph*, Vol. 50 51-68 (2010).

51. Lindberg, E., Carter, N., Gislason, T. & Janson, C. Role of snoring and daytime sleepiness in occupational accidents. *American journal of respiratory and critical care medicine* **164**, 2031-2035 (2001).
52. Lindberg, E., Berne, C., Franklin, K.A., Svensson, M. & Janson, C. Snoring and daytime sleepiness as risk factors for hypertension and diabetes in women--a population-based study. *Respiratory medicine* **101**, 1283-1290 (2007).
53. Gay, P., Weaver, T., Loube, D. & Iber, C. Evaluation of positive airway pressure treatment for sleep related breathing disorders in adults. *Sleep* **29**, 381-401 (2006).
54. Arnardottir, E.S., Bjornsdottir, E., Olafsdottir, K.A., Benediktsdottir, B. & Gislason, T. Obstructive sleep apnoea in the general population: highly prevalent but minimal symptoms. *The European respiratory journal* **47**, 194-202 (2016).
55. Kapur, V.K., Resnick, H.E., Gottlieb, D.J. & Sleep Heart Health Study, G. Sleep disordered breathing and hypertension: does self-reported sleepiness modify the association? *Sleep* **31**, 1127-1132 (2008).
56. Gooneratne, N.S.*, et al.* Sleep disordered breathing with excessive daytime sleepiness is a risk factor for mortality in older adults. *Sleep* **34**, 435-442 (2011).
57. Young, T.*, et al.* Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin sleep cohort. *Sleep* **31**, 1071-1078 (2008).
58. Jacobsen, J.H., Shi, L. & Mokhlesi, B. Factors associated with excessive daytime sleepiness in patients with severe obstructive sleep apnea. *Sleep & breathing = Schlaf & Atmung* **17**, 629-635 (2013).
59. Chen, R.*, et al.* Daytime sleepiness and its determining factors in Chinese obstructive sleep apnea patients. *Sleep & breathing = Schlaf & Atmung* **15**, 129-135 (2011).
60. Johns, M.W. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* **14**, 540-545 (1991).
61. Kapur, V.K.*, et al.* Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **13**, 479-504 (2017).
62. Jonas, D.E.*, et al.* Screening for Obstructive Sleep Apnea in Adults: Evidence Report and Systematic Review for the US Preventive Services Task Force. *Jama* **317**, 415-433 (2017).
63. Fava, C.*, et al.* Effect of CPAP on blood pressure in patients with OSA/hypopnea a systematic review and meta-analysis. *Chest* **145**, 762-771 (2014).
64. Marin, J.M., Carrizo, S.J., Vicente, E. & Agusti, A.G. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* **365**, 1046-1053 (2005).
65. Campos-Rodriguez, F.*, et al.* Role of sleep apnea and continuous positive airway pressure therapy in the incidence of stroke or coronary heart disease in women. *American journal of respiratory and critical care medicine* **189**, 1544-1550 (2014).
66. McEvoy, R.D.*, et al.* CPAP for Prevention of Cardiovascular Events in Obstructive Sleep Apnea. *The New England journal of medicine* **375**, 919-931 (2016).

67. Barbe, F.*, et al.* Effect of continuous positive airway pressure on the incidence of hypertension and cardiovascular events in nonsleepy patients with obstructive sleep apnea: a randomized controlled trial. *Jama* **307**, 2161-2168 (2012).
68. Peker, Y.*, et al.* Effect of Positive Airway Pressure on Cardiovascular Outcomes in Coronary Artery Disease Patients with Nonsleepy Obstructive Sleep Apnea. The RICCADSA Randomized Controlled Trial. *American journal of respiratory and critical care medicine* **194**, 613-620 (2016).
69. Russell, T. Enhancing adherence to positive airway pressure therapy for sleep disordered breathing. *Seminars in respiratory and critical care medicine* **35**, 604-612 (2014).
70. Ramar, K.*, et al.* Clinical Practice Guideline for the Treatment of Obstructive Sleep Apnea and Snoring with Oral Appliance Therapy: An Update for 2015. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **11**, 773-827 (2015).
71. Marklund, M., Verbraecken, J. & Randerath, W. Non-CPAP therapies in obstructive sleep apnoea: mandibular advancement device therapy. *The European respiratory journal* **39**, 1241-1247 (2012).
72. Redline, S.*, et al.* Obstructive sleep apnea-hypopnea and incident stroke: the sleep heart health study. *American journal of respiratory and critical care medicine* **182**, 269-277 (2010).
73. Punjabi, N.M.*, et al.* Sleep-disordered breathing and mortality: a prospective cohort study. *PLoS Med* **6**, e1000132 (2009).
74. Marshall, N.S., Wong, K.K., Cullen, S.R., Knuiman, M.W. & Grunstein, R.R. Sleep apnea and 20-year follow-up for all-cause mortality, stroke, and cancer incidence and mortality in the Busselton Health Study cohort. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **10**, 355-362 (2014).
75. Brooks, D., Horner, R.L., Kozar, L.F., Render-Teixeira, C.L. & Phillipson, E.A. Obstructive sleep apnea as a cause of systemic hypertension. Evidence from a canine model. *The Journal of clinical investigation* **99**, 106-109 (1997).
76. Nieto, F.J.*, et al.* Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. *Jama* **283**, 1829-1836 (2000).
77. Peppard, P.E., Young, T., Palta, M. & Skatrud, J. Prospective study of the association between sleep-disordered breathing and hypertension. *The New England journal of medicine* **342**, 1378-1384 (2000).
78. Haas, D.C.*, et al.* Age-dependent associations between sleep-disordered breathing and hypertension: importance of discriminating between systolic/diastolic hypertension and isolated systolic hypertension in the Sleep Heart Health Study. *Circulation* **111**, 614-621 (2005).
79. Sjostrom, C.*, et al.* Prevalence of sleep apnoea and snoring in hypertensive men: a population based study. *Thorax* **57**, 602-607 (2002).
80. Davies, C.W.*, et al.* Case-control study of 24 hour ambulatory blood pressure in patients with obstructive sleep apnoea and normal matched control subjects. *Thorax* **55**, 736-740 (2000).
81. Marin, J.M.*, et al.* Association between treated and untreated obstructive sleep apnea and risk of hypertension. *Jama* **307**, 2169-2176 (2012).

82. Iftikhar, I.H., *et al.* Effects of continuous positive airway pressure on blood pressure in patients with resistant hypertension and obstructive sleep apnea: a meta-analysis. *Journal of hypertension* **32**, 2341-2350; discussion 2350 (2014).
83. Franklin, K.A., Nilsson, J.B., Sahlin, C. & Naslund, U. Sleep apnoea and nocturnal angina. *Lancet* **345**, 1085-1087 (1995).
84. Kuniyoshi, F.H., *et al.* Day-night variation of acute myocardial infarction in obstructive sleep apnea. *Journal of the American College of Cardiology* **52**, 343-346 (2008).
85. Dong, J.Y., Zhang, Y.H. & Qin, L.Q. Obstructive sleep apnea and cardiovascular risk: meta-analysis of prospective cohort studies. *Atherosclerosis* **229**, 489-495 (2013).
86. Gottlieb, D.J., *et al.* Prospective study of obstructive sleep apnea and incident coronary heart disease and heart failure: the sleep heart health study. *Circulation* **122**, 352-360 (2010).
87. Parra, O., *et al.* Efficacy of continuous positive airway pressure treatment on 5-year survival in patients with ischaemic stroke and obstructive sleep apnea: a randomized controlled trial. *Journal of sleep research* **24**, 47-53 (2015).
88. Nadeem, R., *et al.* Patients with obstructive sleep apnea display increased carotid intima media: a meta-analysis. *International journal of vascular medicine* **2013**, 839582 (2013).
89. Wattanakit, K., Boland, L., Punjabi, N.M. & Shahar, E. Relation of sleep-disordered breathing to carotid plaque and intima-media thickness. *Atherosclerosis* **197**, 125-131 (2008).
90. Gunnarsson, S.I., *et al.* Obstructive sleep apnea is associated with future subclinical carotid artery disease: thirteen-year follow-up from the Wisconsin sleep cohort. *Arteriosclerosis, thrombosis, and vascular biology* **34**, 2338-2342 (2014).
91. January, C.T., *et al.* 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. *Journal of the American College of Cardiology* **64**, e1-76 (2014).
92. Gami, A.S., *et al.* Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. *Journal of the American College of Cardiology* **49**, 565-571 (2007).
93. Ng, C.Y., *et al.* Meta-analysis of obstructive sleep apnea as predictor of atrial fibrillation recurrence after catheter ablation. *The American journal of cardiology* **108**, 47-51 (2011).
94. Fein, A.S., *et al.* Treatment of obstructive sleep apnea reduces the risk of atrial fibrillation recurrence after catheter ablation. *Journal of the American College of Cardiology* **62**, 300-305 (2013).
95. Damy, T., *et al.* Prognostic impact of sleep-disordered breathing and its treatment with nocturnal ventilation for chronic heart failure. *European journal of heart failure* **14**, 1009-1019 (2012).
96. Shahar, E., *et al.* Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *American journal of respiratory and critical care medicine* **163**, 19-25 (2001).
97. Parker, J.D., *et al.* Acute and chronic effects of airway obstruction on canine left ventricular performance. *American journal of respiratory and critical care medicine* **160**, 1888-1896 (1999).

98. Sascau, R.*, et al.* Review of Echocardiographic Findings in Patients with Obstructive Sleep Apnea. *Can Respir J* **2018**, 1206217 (2018).
99. Dursunoglu, D.*, et al.* Impact of obstructive sleep apnoea on left ventricular mass and global function. *The European respiratory journal* **26**, 283-288 (2005).
100. Otto, M.E.*, et al.* Comparison of cardiac structural and functional changes in obese otherwise healthy adults with versus without obstructive sleep apnea. *The American journal of cardiology* **99**, 1298-1302 (2007).
101. Kita, H.*, et al.* The nocturnal secretion of cardiac natriuretic peptides during obstructive sleep apnoea and its response to therapy with nasal continuous positive airway pressure. *Journal of sleep research* **7**, 199-207 (1998).
102. Ybarra, J.*, et al.* Association between sleep-disordered breathing, aminoterminal pro-brain natriuretic peptide (NT-proBNP) levels and insulin resistance in morbidly obese young women. *European journal of internal medicine* **20**, 174-181 (2009).
103. Kaditis, A.G.*, et al.* Overnight change in brain natriuretic peptide levels in children with sleep-disordered breathing. *Chest* **130**, 1377-1384 (2006).
104. Koga, S., Ikeda, S., Urata, J. & Kohno, S. Effect of nasal continuous positive airway pressure in men on global left ventricular myocardial performance in patients with obstructive sleep apnea syndrome. *The American journal of cardiology* **101**, 1796-1800 (2008).
105. Sans Capdevila, O., Crabtree, V.M., Kheirandish-Gozal, L. & Gozal, D. Increased morning brain natriuretic peptide levels in children with nocturnal enuresis and sleep-disordered breathing: a community-based study. *Pediatrics* **121**, e1208-1214 (2008).
106. Moller, D.S., Lind, P., Strunge, B. & Pedersen, E.B. Abnormal vasoactive hormones and 24-hour blood pressure in obstructive sleep apnea. *American journal of hypertension* **16**, 274-280 (2003).
107. Svatikova, A.*, et al.* Plasma brain natriuretic peptide in obstructive sleep apnea. *The American journal of cardiology* **94**, 529-532 (2004).
108. Patwardhan, A.A.*, et al.* Obstructive sleep apnea and plasma natriuretic peptide levels in a community-based sample. *Sleep* **29**, 1301-1306 (2006).
109. Vartany, E.*, et al.* N-terminal pro-brain natriuretic peptide for detection of cardiovascular stress in patients with obstructive sleep apnea syndrome. *Journal of sleep research* **15**, 424-429 (2006).
110. Hubner, R.H.*, et al.* NT-proBNP is not elevated in patients with obstructive sleep apnoea. *Respiratory medicine* **102**, 134-142 (2008).
111. Tasci, S.*, et al.* NT-pro-BNP in obstructive sleep apnea syndrome is decreased by nasal continuous positive airway pressure. *Clinical research in cardiology : official journal of the German Cardiac Society* **95**, 23-30 (2006).
112. Ralls, F.M. & Grigg-Damberger, M. Roles of gender, age, race/ethnicity, and residential socioeconomics in obstructive sleep apnea syndromes. *Current opinion in pulmonary medicine* **18**, 568-573 (2012).
113. Lindberg, E.*, et al.* Women with symptoms of sleep-disordered breathing are less likely to be diagnosed and treated for sleep apnea than men. *Sleep Med* **35**, 17-22 (2017).
114. Kapsimalis, F. & Kryger, M.H. Gender and obstructive sleep apnea syndrome, part 2: mechanisms. *Sleep* **25**, 499-506 (2002).
115. Krystal, A.D., Edinger, J., Wohlgemuth, W. & Marsh, G.R. Sleep in peri-menopausal and post-menopausal women. *Sleep medicine reviews* **2**, 243-253 (1998).

116. Kapsimalis, F. & Kryger, M.H. Gender and obstructive sleep apnea syndrome, part 1: Clinical features. *Sleep* **25**, 412-419 (2002).
117. O'Connor, C., Thornley, K.S. & Hanly, P.J. Gender differences in the polysomnographic features of obstructive sleep apnea. *American journal of respiratory and critical care medicine* **161**, 1465-1472 (2000).
118. Basoglu, O.K. & Tasbakan, M.S. Gender differences in clinical and polysomnographic features of obstructive sleep apnea: a clinical study of 2827 patients. *Sleep & breathing = Schlaf & Atmung* **22**, 241-249 (2018).
119. Sahlin, C., Franklin, K.A., Stenlund, H. & Lindberg, E. Sleep in women: Normal values for sleep stages and position and the effect of age, obesity, sleep apnea, smoking, alcohol and hypertension. *Sleep Med* **10**, 1025-1030 (2009).
120. Grimaldi, D., Beccuti, G., Touma, C., Van Cauter, E. & Mokhlesi, B. Association of obstructive sleep apnea in rapid eye movement sleep with reduced glycemic control in type 2 diabetes: therapeutic implications. *Diabetes Care* **37**, 355-363 (2014).
121. Carberry, J.C., Jordan, A.S., White, D.P., Wellman, A. & Eckert, D.J. Upper Airway Collapsibility (Pcrit) and Pharyngeal Dilator Muscle Activity are Sleep Stage Dependent. *Sleep* **39**, 511-521 (2016).
122. White, D.P., Douglas, N.J., Pickett, C.K., Weil, J.V. & Zwillich, C.W. Hypoxic ventilatory response during sleep in normal premenopausal women. *Am Rev Respir Dis* **126**, 530-533 (1982).
123. Findley, L.J., Wilhoit, S.C. & Suratt, P.M. Apnea duration and hypoxemia during REM sleep in patients with obstructive sleep apnea. *Chest* **87**, 432-436 (1985).
124. Mokhlesi, B.*, et al.* Obstructive sleep apnea during REM sleep and hypertension. results of the Wisconsin Sleep Cohort. *American journal of respiratory and critical care medicine* **190**, 1158-1167 (2014).
125. Appleton, S.L.*, et al.* Hypertension Is Associated With Undiagnosed OSA During Rapid Eye Movement Sleep. *Chest* **150**, 495-505 (2016).
126. Mokhlesi, B.*, et al.* Obstructive sleep apnoea during REM sleep and incident non-dipping of nocturnal blood pressure: a longitudinal analysis of the Wisconsin Sleep Cohort. *Thorax* **70**, 1062-1069 (2015).
127. Bixler, E.O.*, et al.* Blood pressure associated with sleep-disordered breathing in a population sample of children. *Hypertension* **52**, 841-846 (2008).
128. Au, C.T., Ho, C.K., Wing, Y.K. & Li, A.M. The effect of childhood obstructive sleep apnea on ambulatory blood pressure is modulated by the distribution of respiratory events during rapid eye movement and nonrapid eye movement sleep. *Sleep Med* **14**, 1317-1322 (2013).
129. Bialasiewicz, P., Czupryniak, L., Pawlowski, M. & Nowak, D. Sleep disordered breathing in REM sleep reverses the downward trend in glucose concentration. *Sleep Med* **12**, 76-82 (2011).
130. Chami, H.A., Gottlieb, D.J., Redline, S. & Punjabi, N.M. Association between Glucose Metabolism and Sleep-disordered Breathing during REM Sleep. *American journal of respiratory and critical care medicine* **192**, 1118-1126 (2015).
131. Mahmood, K.*, et al.* Prevalence of type 2 diabetes in patients with obstructive sleep apnea in a multi-ethnic sample. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **5**, 215-221 (2009).

132. Kendzerska, T., Gershon, A.S., Hawker, G., Tomlinson, G. & Leung, R.S. Obstructive sleep apnea and incident diabetes. A historical cohort study. *American journal of respiratory and critical care medicine* **190**, 218-225 (2014).
133. Chen, Y.L.*, et al.* Influence and predicting variables of obstructive sleep apnea on cardiac function and remodeling in patients without congestive heart failure. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **10**, 57-64 (2014).
134. Sasaki, N.*, et al.* Associations Between Characteristics of Obstructive Sleep Apnea and Nocturnal Blood Pressure Surge. *Hypertension* **72**, 1133-1140 (2018).
135. Lin, C.Y., Ho, C.S., Tsai, W.C. & Chen, J.Y. Different effects of apnea during rapid eye movement period on peripheral arterial stiffness in obstructive sleep apnea. *Atherosclerosis* **269**, 166-171 (2018).
136. Acosta-Castro, P.*, et al.* REM-associated sleep apnoea: prevalence and clinical significance in the HypnoLaus cohort. *The European respiratory journal* **52**(2018).
137. Aurora, R.N., Crainiceanu, C., Gottlieb, D.J., Kim, J.S. & Punjabi, N.M. Obstructive Sleep Apnea during REM Sleep and Cardiovascular Disease. *American journal of respiratory and critical care medicine* **197**, 653-660 (2018).
138. Kurosawa, H.*, et al.* Association between severity of obstructive sleep apnea and glycated hemoglobin level in Japanese individuals with and without diabetes. *Endocr J* **65**, 121-127 (2018).
139. Joseph, P.*, et al.* Reducing the Global Burden of Cardiovascular Disease, Part 1: The Epidemiology and Risk Factors. *Circulation research* **121**, 677-694 (2017).
140. Piepoli, M.F.*, et al.* 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* **37**, 2315-2381 (2016).
141. Group, E.U.C.C.S.*, et al.* Gender in cardiovascular diseases: impact on clinical manifestations, management, and outcomes. *Eur Heart J* **37**, 24-34 (2016).
142. Morselli, E.*, et al.* The effects of oestrogens and their receptors on cardiometabolic health. *Nat Rev Endocrinol* **13**, 352-364 (2017).
143. Muka, T.*, et al.* Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. *JAMA Cardiol* **1**, 767-776 (2016).
144. Humphries, K.H.*, et al.* Sex differences in cardiovascular disease - Impact on care and outcomes. *Front Neuroendocrinol* **46**, 46-70 (2017).
145. Ziaeian, B. & Fonarow, G.C. Epidemiology and aetiology of heart failure. *Nat Rev Cardiol* **13**, 368-378 (2016).
146. Ponikowski, P.*, et al.* 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* **37**, 2129-2200 (2016).

147. Zarrinkoub, R.*, et al.* The epidemiology of heart failure, based on data for 2.1 million inhabitants in Sweden. *European journal of heart failure* **15**, 995-1002 (2013).
148. Meyer, S.*, et al.* Sex differences in new-onset heart failure. *Clinical research in cardiology : official journal of the German Cardiac Society* **104**, 342-350 (2015).
149. Arzt, M.*, et al.* Prevalence and Predictors of Sleep-Disordered Breathing in Patients With Stable Chronic Heart Failure: The SchlaHF Registry. *JACC Heart Fail* **4**, 116-125 (2016).
150. Arshad, A.*, et al.* Cardiac resynchronization therapy is more effective in women than in men: the MADIT-CRT (Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy) trial. *Journal of the American College of Cardiology* **57**, 813-820 (2011).
151. van der Ende, M.Y.*, et al.* Prevalence of electrocardiographic unrecognized myocardial infarction and its association with mortality. *International journal of cardiology* **243**, 34-39 (2017).
152. Kunadian, V.*, et al.* Gender Differences in Outcomes and Predictors of All-Cause Mortality After Percutaneous Coronary Intervention (Data from United Kingdom and Sweden). *The American journal of cardiology* **119**, 210-216 (2017).
153. Johnston, N.*, et al.* Effect of Gender on Patients With ST-Elevation and Non-ST-Elevation Myocardial Infarction Without Obstructive Coronary Artery Disease. *The American journal of cardiology* **115**, 1661-1666 (2015).
154. Lanza, G.A., Careri, G. & Crea, F. Mechanisms of coronary artery spasm. *Circulation* **124**, 1774-1782 (2011).
155. Troughton, R.W. & Richards, A.M. B-type natriuretic peptides and echocardiographic measures of cardiac structure and function. *JACC. Cardiovascular imaging* **2**, 216-225 (2009).
156. Wang, T.J.*, et al.* Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *The New England journal of medicine* **350**, 655-663 (2004).
157. Zethelius, B.*, et al.* Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *The New England journal of medicine* **358**, 2107-2116 (2008).
158. Boerrigter, G., Costello-Boerrigter, L.C. & Burnett, J.C., Jr. Natriuretic peptides in the diagnosis and management of chronic heart failure. *Heart failure clinics* **5**, 501-514 (2009).
159. Woodard, G.E. & Rosado, J.A. Natriuretic peptides in vascular physiology and pathology. *International review of cell and molecular biology* **268**, 59-93 (2008).
160. Steinl, D.C. & Kaufmann, B.A. Ultrasound imaging for risk assessment in atherosclerosis. *International journal of molecular sciences* **16**, 9749-9769 (2015).
161. Rodriguez-Macias, K.A., Lind, L. & Naessen, T. Thicker carotid intima layer and thinner media layer in subjects with cardiovascular diseases. An investigation using noninvasive high-frequency ultrasound. *Atherosclerosis* **189**, 393-400 (2006).
162. Greenwood, C.*, et al.* Proximity assays for sensitive quantification of proteins. *Biomol Detect Quantif* **4**, 10-16 (2015).
163. Assarsson, E.*, et al.* Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PloS one* **9**, e95192 (2014).

164. Lundberg, M., Eriksson, A., Tran, B., Assarsson, E. & Fredriksson, S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res* **39**, e102 (2011).
165. Rechtschaffen A, K.A. A manual of standardized terminology, techniques, and scooring system for sleep stages in human subjects. *US National Public Health Service, US Government Printing Office: Washington DC* (1968).
166. Lissner, L., Bengtsson, C., Bjorkelund, C. & Wedel, H. Physical activity levels and changes in relation to longevity. A prospective study of Swedish women. *American journal of epidemiology* **143**, 54-62 (1996).
167. Mayfield, D., McLeod, G. & Hall, P. The CAGE questionnaire: validation of a new alcoholism screening instrument. *The American journal of psychiatry* **131**, 1121-1123 (1974).
168. Svensson, M., Lindberg, E., Naessen, T. & Janson, C. Risk factors associated with snoring in women with special emphasis on body mass index: a population-based study. *Chest* **129**, 933-941 (2006).
169. Snaith, R.P. & Zigmond, A.S. The hospital anxiety and depression scale. *British medical journal (Clinical research ed.)* **292**, 344 (1986).
170. Wesstrom, J., Ulfberg, J., Sundstrom-Poromaa, I. & Lindberg, E. Periodic limb movements are associated with vasomotor symptoms. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **10**, 15-20 (2014).
171. Theorell-Haglow, J., Berne, C., Janson, C. & Lindberg, E. Obstructive sleep apnoea is associated with decreased insulin sensitivity in females. *The European respiratory journal* **31**, 1054-1060 (2008).
172. *Diagnosis and Classification of Diabetes Mellitus.*, (World Health Organization, Geneva, 1999).
173. Ingelsson, E., Arnlov, J., Sundstrom, J. & Lind, L. The validity of a diagnosis of heart failure in a hospital discharge register. *European journal of heart failure* **7**, 787-791 (2005).
174. Textor, J., Hardt, J. & Knuppel, S. DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology* **22**, 745 (2011).
175. Querejeta Roca, G.*, et al.* Sleep apnea is associated with subclinical myocardial injury in the community. The ARIC-SHHS study. *American journal of respiratory and critical care medicine* **188**, 1460-1465 (2013).
176. Steiner, J. & Guglin, M. BNP or NTproBNP? A clinician's perspective. *International journal of cardiology* **129**, 5-14 (2008).
177. Monneret, D.*, et al.* Glucose tolerance and cardiovascular risk biomarkers in non-diabetic non-obese obstructive sleep apnea patients: Effects of long-term continuous positive airway pressure. *Respiratory medicine* **112**, 119-125 (2016).
178. Muller, P.*, et al.* Reverse Remodelling of the Atria After Treatment of Obstructive Sleep Apnoea with Continuous Positive Airway Pressure: Evidence from Electro-mechanical and Endocrine Markers. *Heart Lung Circ* **25**, 53-60 (2016).
179. Chang, Y.S.*, et al.* The effects of continuous positive airway pressure therapy on Troponin-T and N-terminal pro B-type natriuretic peptide in patients with obstructive sleep apnoea: a randomised controlled trial. *Sleep Med* **39**, 8-13 (2017).

180. Craig, S., *et al.* Effect of CPAP on Cardiac Function in Minimally Symptomatic Patients with OSA: Results from a Subset of the MOSAIC Randomized Trial. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **11**, 967-973 (2015).
181. Roca, G.Q., *et al.* Sex-Specific Association of Sleep Apnea Severity With Subclinical Myocardial Injury, Ventricular Hypertrophy, and Heart Failure Risk in a Community-Dwelling Cohort: The Atherosclerosis Risk in Communities-Sleep Heart Health Study. *Circulation* **132**, 1329-1337 (2015).
182. Di Angelantonio, E., *et al.* B-type natriuretic peptides and cardiovascular risk: systematic review and meta-analysis of 40 prospective studies. *Circulation* **120**, 2177-2187 (2009).
183. Dewan, N.A., Nieto, F.J. & Somers, V.K. Intermittent hypoxemia and OSA: implications for comorbidities. *Chest* **147**, 266-274 (2015).
184. Kasai, T. & Bradley, T.D. Obstructive sleep apnea and heart failure: pathophysiologic and therapeutic implications. *Journal of the American College of Cardiology* **57**, 119-127 (2011).
185. Ingelsson, E., Bjorklund-Bodegard, K., Lind, L., Arnlov, J. & Sundstrom, J. Diurnal blood pressure pattern and risk of congestive heart failure. *Jama* **295**, 2859-2866 (2006).
186. Choi, J.B., *et al.* Sleepiness in obstructive sleep apnea: a harbinger of impaired cardiac function? *Sleep* **29**, 1531-1536 (2006).
187. Mazzotti, D.R., *et al.* Symptom Subtypes of Obstructive Sleep Apnea Predict Incidence of Cardiovascular Outcomes. *American journal of respiratory and critical care medicine* (2019).
188. Arzt, M., *et al.* Sleepiness and sleep in patients with both systolic heart failure and obstructive sleep apnea. *Archives of internal medicine* **166**, 1716-1722 (2006).
189. Parati, G., *et al.* Heart failure and sleep disorders. *Nat Rev Cardiol* **13**, 389-403 (2016).
190. Shim, C.Y., *et al.* Effects of continuous positive airway pressure therapy on left ventricular diastolic function: a randomised, sham-controlled clinical trial. *The European respiratory journal* **51**(2018).
191. Khayat, R., *et al.* Sleep disordered breathing and post-discharge mortality in patients with acute heart failure. *Eur Heart J* **36**, 1463-1469 (2015).
192. Kasai, T., *et al.* Prognosis of patients with heart failure and obstructive sleep apnea treated with continuous positive airway pressure. *Chest* **133**, 690-696 (2008).
193. Kaneko, Y., *et al.* Cardiovascular effects of continuous positive airway pressure in patients with heart failure and obstructive sleep apnea. *The New England journal of medicine* **348**, 1233-1241 (2003).
194. Sun, H., Shi, J., Li, M. & Chen, X. Impact of continuous positive airway pressure treatment on left ventricular ejection fraction in patients with obstructive sleep apnea: a meta-analysis of randomized controlled trials. *PloS one* **8**, e62298 (2013).
195. Zhang, X.B., Yuan, Y.T., Du, Y.P., Jiang, X.T. & Zeng, H.Q. Efficacy of positive airway pressure on brain natriuretic peptide in patients with heart failure and sleep-disorder breathing: a meta-analysis of randomized controlled trials. *Lung* **193**, 255-260 (2015).
196. Gomes, P., Fleming Outeiro, T. & Cavadas, C. Emerging Role of Sirtuin 2 in the Regulation of Mammalian Metabolism. *Trends Pharmacol Sci* **36**, 756-768 (2015).

197. Toma, I. & McCaffrey, T.A. Transforming growth factor-beta and atherosclerosis: interwoven atherogenic and atheroprotective aspects. *Cell Tissue Res* **347**, 155-175 (2012).
198. Furuhashi, M.*, et al.* Axin facilitates Smad3 activation in the transforming growth factor beta signaling pathway. *Mol Cell Biol* **21**, 5132-5141 (2001).
199. Ip, M.S., Lam, K.S., Ho, C., Tsang, K.W. & Lam, W. Serum leptin and vascular risk factors in obstructive sleep apnea. *Chest* **118**, 580-586 (2000).
200. Muraja-Murro, A.*, et al.* Adjustment of apnea-hypopnea index with severity of obstruction events enhances detection of sleep apnea patients with the highest risk of severe health consequences. *Sleep & breathing = Schlaf & Atmung* **18**, 641-647 (2014).
201. Sawyer, A.M.*, et al.* A systematic review of CPAP adherence across age groups: clinical and empiric insights for developing CPAP adherence interventions. *Sleep medicine reviews* **15**, 343-356 (2011).
202. Campos-Rodriguez, F.*, et al.* Effect of continuous positive airway pressure on blood pressure and metabolic profile in women with sleep apnoea. *The European respiratory journal* **50**(2017).
203. Campos-Rodriguez, F.*, et al.* Continuous Positive Airway Pressure Improves Quality of Life in Women with Obstructive Sleep Apnea. A Randomized Controlled Trial. *American journal of respiratory and critical care medicine* **194**, 1286-1294 (2016).
204. Strausz, S.*, et al.* Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland. *BMJ Open* **8**, e022752 (2018).
205. Hla, K.M.*, et al.* Coronary heart disease incidence in sleep disordered breathing: the Wisconsin Sleep Cohort Study. *Sleep* **38**, 677-684 (2015).
206. Chen, X. & Rosbash, M. MicroRNA-92a is a circadian modulator of neuronal excitability in Drosophila. *Nat Commun* **8**, 14707 (2017).
207. Chen, W.J.*, et al.* Effect of Nasal CPAP on SIRT1 and Endothelial Function in Obstructive Sleep Apnea Syndrome. *Lung* **193**, 1037-1045 (2015).

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