Obstructive Sleep Apnea and Cardiovascular Disease - Mechanisms and Impact of Treatment

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Cover illustration: A long and winding road by Per Östgärd

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“Now, blessings light on him that first invented this same sleep! It covers a man all over, thoughts and all, like a cloak; it is meat for the hungry, drink for the thirsty, heat for the cold, and cold for the hot. It is the current coin that purchases all the pleasures of the world cheap, and the balance that sets the king and the shepherd, the fool and the wise man, even.”

- Miguel de Cervantes, Don Quixote, 1605

To Sofia, the love of my life

and

to Iris, Axel and Hannes, the meaning of it

Obstructive Sleep Apnea and Cardiovascular Disease - Mechanisms and Impact of Treatment

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ABSTRACT

Background: Scientific understanding of obstructive sleep apnea (OSA) has increased exponentially during recent decades, suggesting a link between OSA and cardiovascular disease. Few randomized controlled trials exist within the field.

Aim: To study the effect of continuous positive airway pressure (CPAP) on mechanisms contributing to cardiovascular disease deterioration.

Methods and Results: Paper I is a cross-sectional analysis of revascularized patients with coronary artery disease (CAD). Patients with concomitant OSA had higher levels of inflammatory markers, independent of obesity. In paper II, the effect of losartan on blood pressure (BP) was investigated in patients with new-onset hypertension and OSA compared to patients with hypertension only. In addition, the effect on blood pressure of CPAP treatment in addition to losartan was investigated. Losartan reduced BP significantly in OSA but the reductions were less than in patients without OSA. Add-on CPAP treatment reduced night-time blood pressure in OSA patients in the intention-to-treat population, and all 24-h measurements in those compliant with CPAP. Paper III demonstrates that inflammatory markers decreases after one year in all CAD patients, and this was independent of CPAP in OSA. In paper IV, hypertensive patients with OSA responded with smaller reductions in aldosterone than patients without OSA after losartan. Add-on CPAP treatment tended to lower aldosterone, but the reductions were more robust in the sympathetic activity. No effect was seen on the inflammatory markers.

Conclusions: Inflammatory markers are high in newly revascularized CAD patients with OSA, but the levels decrease over time independent of CPAP treatment, suggesting that the initial increase in inflammatory activity in CAD with concomitant OSA is most probably driven by other factors. Blood pressure in new-onset hypertension seems to be reduced by CPAP as add-on treatment to losartan; this may be attributed mainly to sympathetic activity and, to a lesser extent, to RAAS activity, whereas inflammation seems to be of minor importance.

Keywords: Obstructive sleep apnea, coronary artery disease, hypertension, inflammation, RAAS activity, sympathetic activity

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LIST OF PAPERS

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

I Thunström, E, Glantz, H, Fu M, Yucel-Lindberg, T, Petzold M, Lindberg K, Peker, Y. Increased Inflammatory Activity in Nonobese Patients with Coronary Artery Disease and Obstructive Sleep Apnea.
Sleep 2015 Mar 1;38 (3) 463-71.

II Thunström, E, Manhem, K, Rosengren, A, Peker, Y. Blood Pressure Response to Losartan and CPAP in Hypertension and Obstructive Sleep Apnea.
Am J Respir Crit Care Med 2015 Sep 28. [Epub ahead of print]

III Thunström, E, Glantz, H, Yucel-Lindberg, T, Lindberg, K, Saygin, M, Peker Y. Effect of CPAP on Inflammatory Biomarkers in Non-Sleepy Patients with Coronary Artery Disease and Obstructive Sleep Apnea: A Randomized Controlled Trial.
In manuscript

IV Thunström, E, Manhem, K, Yucel-Lindberg, T, Rosengren, A, Peker, Y. Neuroendocrine and Inflamatory Responses to CPAP in Hypertension with Obstructive Sleep Apnea: A Randomized Controlled Trial.
In manuscript

CONTENTS

ABBREVIATIONS

AASM	American Academy of Sleep Medicine
ABPM	Ambulatory blood pressure monitoring
AHI	Apnea–hypopnea index
AI	Apnea index
ARB	Angiotensin receptor blocker
BMI	Body mass index
CHF	Congestive heart failure
CABG	coronary artery bypass graft
CAD	Coronary artery disease
CPAP	Continuous positive airway pressure
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EEG	Electroencephalography
ESC	European Society of Cardiology
ESH	European Society of Hypertension
Hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
IL-8	Interleukin-8
MAP	Mean arterial pressure
MESAM	A digital recording device developed to monitor heart rate and breathing sounds (snoring)
MSLT	Multiple Sleep Latency Test
MWT	Maintenance of Wakefulness Test
ODI	Oxygen desaturation index
OSA	Obstructive sleep apnea
OSAS	Obstructive sleep apnea syndrome
OR	Odds ratio
PCI	Percutaneous coronary intervention
PG	Polygraphy
PSG	Polysomnography
RAAS	Renin-angiotensin-aldosterone system

RICCADSA	Randomized Intervention with CPAP in Coronary Artery Disease and Sleep Apnea
RCT	Randomized controlled trial
RDI	Respiratory disturbance index
RERA	Respiratory effort-related arousal
SBP	Systolic blood pressure
SD	Standard deviation
TNFα	Tumor necrosis factor alpha
UARS	Upper airway resistance syndrome
WHO	World Health Organization

DEFINITIONS IN SHORT

Apnea	Two criteria must be fulfilled: (1) amplitude reduction: there is a drop in the peak thermal sensor excursion by ≥90% of baseline (2) the duration of the event is at least 10 seconds
Hypopnea (based on the American Academy of Sleep Medicine guidelines from 1999)	Criterion (1) or (2) must be fulfilled in combination with criterion (3): (1) a clear decrease (≥50%) from baseline in the amplitude of a valid measure of breathing during sleep (2) a clear amplitude reduction on a validated measure of breathing during sleep that does not reach criterion but is associated with either an oxygen desaturation of ≥4% and/or an arousal (3) the event lasts 10 seconds or longer
AHI	The apnea–hypopnea index is based on the number of apneas and/or hypopneas per hour of registered sleep.
ODI	The oxygen desaturation index is based on the number of desaturations (≥4%) per hour of sleep time.
OSA	Obstructive sleep apnea is a laboratory diagnosis with three levels: mild (AHI 5-14.9/h), moderate (AHI 15-29.9/h), and severe (AHI ≥30/h).
OSAS	Obstructive sleep apnea syndrome is a clinical diagnosis of OSA with symtoms, mainly excessive daytime sleepiness.
RDI	An index created by adding RERA to the AHI.

RERA	A sequence of breaths characterized by increasing respiratory effort leading to an arousal from sleep, but which does not meet criteria for an apnea or hypopnea. RERA events must fulfill both of the following criteria: (1) a pattern of progressively lower esophageal pressure, terminated by a sudden change in pressure to a higher level and an arousal (2) a duration of 10 seconds or longer
Hypertension	Having systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg
Optimally treated 24-h blood pressure	Mean 24-h ABPM of a systolic blood pressure ≥130 mmHg and diastolic blood pressure ≥80 mmHg[1]
Dipping blood pressure pattern	A difference of ≥10% between day and night blood pressure.

INTRODUCTION

"The amount of sleep required by the average person is five minutes more."

- Wilson Mizener

Sleep is defined as follows: "Sleep is a recurring, reversible neurobehavioral state of relative perceptual disengagement from and unresponsiveness to the environment. Sleep is typically accompanied (in humans) by postural recumbence, behavioral quiescence, and closed eyes"[2].

To sleep is of vital importance to maintaining existence[3], as well as to keeping a lucid mind[4,5]. The human species sleeps on average less than seven hour per night and studies in adults have not been able to show any substantial or consistent change in this over the last 50 years[6,7]. There is overwhelming evidence that short sleep duration or poor sleep quality is associated not only with a risk of increased pain, impaired performance, increased errors, greater risk of accidents, and increased risk of depression, but also with cardiovascular morbidity and mortality, such as obesity, diabetes, hypertension, heart disease, stroke, and increased risk of death, as well as impairment of the immune system[8-10]. As everyone who has ever missed a good night's sleep intuitively perceives, sleep is essential for health.

Accordingly, any conditions that alter sleep duration as well as sleep quality could be supposed to increase vascular morbidity, and treating them might be beneficial from a cardiovascular perspective.

Obstructive sleep apnea (OSA) is a common sleep impairment which, apart from sleep fragmentation, produces oscillations in intra-thoracic pressure, increases in the transmural pressure on the heart and the aorta , and leads to intermittent desaturations[11]. All these are factors that give rise to an endocrine, paracrine, and autocrine hormonal upregulation, as well as a change in genetic and inflammatory activity[12] and a change in the coagulations system response[13]. This can result in cardiovascular remodeling, endothelial dysfunction and metabolic impairment [14-16], thus increasing the risk of developing hypertension[17], diabetes[18], coronary artery disease (CAD)[19,20], atrial fibrillation[21], stroke[22,23], renal disease[24], pulmonary hypertension[25], and congestive heart failure[26]. An association between OSA and this condition has been shown in cohort studies in sleep clinics as well as in cohorts from the general population and sub-cohorts from each of the different diagnoses. This has been done both cross-sectionally and longitudinally[9,25,27,28].

The growing body of evidence showing an association between OSA and cardiovascular disease (CVD) has led to physicians starting to search for signs and/or symptoms of OSA in patients with CVD. Moreover, OSA is now addressed in international guidelines on CVD[29].

However, more needs to be done to establish a causal relation between OSA and CVD. Randomized controlled trials (RCTs) need to be conducted to investigate whether treatment of OSA reduces the incidence of hard endpoints, such as death and new cardiovascular events. It is also important to investigate softer endpoints such as inflammatory activity, endocrine activity, and blood pressure responses. This will contribute to the identification of the mechanism that mediates the relationship between OSA and CVD. Given that many of the existing RCTs have been conducted in sleep clinic cohorts, less is known regarding the effect of OSA treatment in other clinical populations, where the diagnosis is common, but where physicians until recently have not been aware of it.

This thesis explores the effect of OSA treatment in two different clinical populations with different degrees of CVD: patients with newly diagnosed hypertension (new-onset CVD), and revascularized patients with CAD (established CVD). The thesis addresses what effect OSA treatment has on the mechanisms which have previously been shown to be linked with CVD.

Obstructive sleep apnea

Historical perspective

OSA is defined as a condition characterized by repetitive episodes of complete or partial collapse of the upper airway (mainly the oropharyngeal tract) during sleep, with a consequent cessation or reduction of the airflow[30]. The progressive asphyxia induced by apneas or hypopneas causes an increased stimulation to breathe against the collapsed airway, typically until the person is awakened. OSA is a diagnosis which has gained interest in the medical community over the last four decades. However, according to Lavie[31] the first descriptions in the medical literature are from the late 19th century; these are case reports of obese patients with daytime sleepiness and a particular breathing pattern when asleep, presented by WH Broadbent and entitled *On Cheyne-Stokes' respiration in cerebral haemorrhage*[32] and the case report *Case of narcolepsy* by R Caton in 1889[33]. Sleep hypersomnolence was nicely described by Charles Dickens in *The Pickwick Papers* from 1836, in which the fat boy Joe is depicted as follows:

> *"The object that presented itself to the eyes of the astonished clerk, was a boy – a wonderfully fat boy – habited as a serving lad, standing upright on the mat, with his eyes closed as if in sleep… 'Sleep!' said the old gentleman, 'he's always asleep. Goes on errands fast asleep, and snores as he waits at table'."*

In even earlier literature a condition similar to what we now know as OSA was described by Aelinaus (1666)[34]. However, Kryger claims in a historical overview published in 1983 that the first to touch on the subject were the ancient Greeks, who described Dionysius of Heraclia as an epicurean who *"increased to an extraordinary degree of corpulence and fatness"* so that he had *"much ado to take breath"*[34].

This indicates that OSA, stimulated by the weight gain we associate with our modern lifestyle of overindulgent eating in combination with decreased exercise, has in fact existed since ancient times.

In the early 20th century, apneas were thought to be directly linked to obesity, and in 1956 the term *Pickwickian syndrome* was coined in an article by Burwell[35]. However, the first use of this term has been contested, because other researchers had described patients with obesity, hypersomnolence and breathing disturbances previously[31]. Hitherto, the hypersomnolence observed in the Pickwickian syndrome was considered to be due to carbon dioxide poisoning and not due to obstruction of the upper airways. In parallel with these clinical observations, other landmarks in the development towards modern sleep medicine were the performance of the first EEG by Berger et al. in 1929[36] (publication in German), and during the years just after the discovery that brain activity during sleep showed a different electrical wave pattern compared to that in subjects who were awake[37-39]. However, the first real step in a new era of sleep medicine was taken by Gerardy in Germany (1959 published 1960)[40] and one year later by Druchman and Gummit[41] in the USA. Both groups conducted an EEG on a patient with Pickwickian syndrome in daytime, which showed repeated fluctuation from sleep to awakening during the EEG recording. Gerardy modified the EEG so that it also measured breathing and pulse rate at the same time as brain activity. By doing so, he found that the patient, at the time of falling asleep, suffered from an apnea and bradycardia followed by tachycardia when the breathing started again. Similar results were seen in the American patient. However, both groups still attributed the daytime sleepiness to carbon dioxide poisoning rather than poor sleep quality.

It was not until after Gerardy et al. and Druchman et al. had published their work in 1960 when Kuhl et al. performed a full-night EEG recording on a Pickwickian patient that they realized that it was sleep fragmentation rather than carbon dioxide poisoning that caused the daytime sleepiness. The results were only published at a conference in 1964, but were replicated and published soon after by others[42]. This paper also showed that it was obstruction of the upper airways that triggered the apneas. Since then, research in the field has started to pick up pace. In 1972 Coccagna et al. showed that the apneas in the Pickwickian patients were associated with severe blood pressure swings in both pulmonary and systemic blood pressure[43], which further stressed the importance of treating the condition. Weight reduction had been the only treatment option up until then, but Kuhl had published a case report (in German) three years earlier in which a tracheostomy was used to cure the condition; this was now replicated by Coccagna[44,45], but the procedure could only be used on those with severe complications to their Pickwickian syndrome.

In 1976 Guilleminault et al. published a paper showing that non-obese as well as obese individuals could suffer from apneas during sleep caused by obstruction of the upper airway. In the same paper, they used the term obstructive sleep apnea syndrome (OSAS) for the first time and defined it based on their sleep registration findings. The criterion was at least 30 apneas of minimum duration of 10 seconds each, detected during sleep, in combination with hypersomnolence. They found that a high proportion of their patients with OSAS were men (34 of 35) and that fifty percent had hypertension[46].

When the pathophysiology behind the collapse of the upper airways was understood[47], followed by the discovery of new treatment options for OSAS, such as continuous positive airway pressure treatment (CPAP)[48] and surgery[49], the research around OSA

intensified, and during the following decades there has been an exponential growth of publications in the field (Figure 1). The most important paper on the subject was without a doubt the publication by Young et al. in 1993 showing how common OSA was in the general population[50]. This led to a paradigm shift in how the health care profession addressed OSAS, since it might have a direct impact on public health. These breakthroughs have shaped modern sleep medicine.

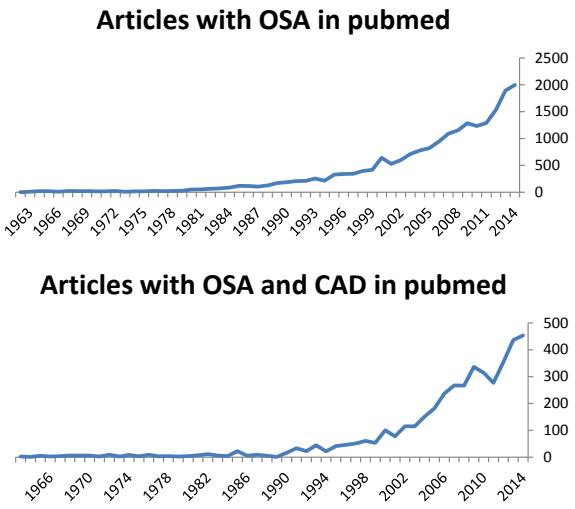

Figure 1. Number of published articles on the search" Obstructive Sleep Apnea" and on the search "Obstructive Sleep Apnea and Coronary Artery Disease" showing articles tagged in Pubmed for each year. The exponential increase during the last 20 years, reflects the increased interest in the field.

Definition of OSA and OSAS

Obstructive sleep apnea syndrome (OSAS) was first introduced by Guilleminault et al. in 1976. They defined it as the combination of daytime sleepiness and a minimum of 30 polysomnographically verified obstructive apneas per nights each lasing at least 10 seconds[30]. However, to adjust for sleep duration, the apnea index (AI), apneas per hour of sleep, was soon adopted instead of apneas per night. The AI cutoff for OSA was set to five apneas per hour. The concept of hypopneas, defined as reduced ventilation but not complete cession of airflow, was also introduced a few years after OSAS was first defined[51], which soon led to the use of the apnea–hypopnea index (AHI) instead of AI; however the cutoff for abnormal breathing was kept at five apneas and or hypopneas per hour.

During the 15 years that followed, many groups conducted research using sleep recordings and several different scoring definitions came to be used, making it difficult to make comparisons of the absolute values of the results from different studies. Some studies used the oxygen desaturation index (ODI), which was defined as the number

of desaturations per hour of sleep, instead of AHI. Others used respiratory disturbance indices (RDI) which were different from study to study, though many used the same definition: the sum of apneas, hypopneas and the respiratory effort-related arousals (RERAs) per hour of sleep; RERA was defined as a pattern of progressively more negative esophageal pressure, terminated by a sudden change in pressure to a less negative level and an arousal, and the event lasts 10 seconds or longer. RERA was usually applied when a patient had desaturations but no hypopnea or apnea that could explain them, indicating that the patient had upper airway resistance syndrome (UARS). Moreover, there was a problem defining overlapping conditions such as OSAS, Pickwickian syndrome (apneas, under-ventilation, and daytime sleepiness), central sleep apnea (CSA), and UARS[52]. Therefore the American Academy of Sleep Medicine established a task force to describe and define the key features and specific events of four separate syndromes associated with abnormal breathing events during sleep previously described in the literature: the OSA–hypopnea syndrome, CSA, UARS, and the sleep hypoventilation syndrome (including the Pickwickian syndrome). The task force made recommendations for methods of measuring the key features of these four syndromes, and developed a standard system for rating their severity. In 1999 they published their first report[53] which provided the definitions used for the work of this thesis, since it was these definitions and scoring methods that were in clinical use at the time of the design of the studies (2005–2007).

In accordance with the report of 1999 by the American Association of Sleep Medicine, the diagnostic criteria for a diagnosis of OSAS are listed below. Either (a) or (b) in combination with (c) must be fulfilled:

(a) excessive daytime sleepiness that is not better explained by other factors;

(b) two or more of the following that are not better explained by other factors:

- choking or gasping during sleep
- recurrent awakenings from sleep
- unrefreshing sleep
- daytime fatigue
- impaired concentration
- obstructive central and mixed sleep apnea;

(c) five or more obstructed breathing events per hour during sleep, demonstrated by overnight monitoring. These events may include any combination of obstructive apneas, hypopneas or RERAs[53,54].

An apnea is defined as an almost complete (at least 90%) cessation of airflow, and hypopnea is defined as a reduction in nasal pressure amplitude of at least 50% and/or a reduction in thoracoabdominal movement of 50% or more for a minimum of 10 seconds[53].

Severity of OSA

Just as OSAS is composed of two components, daytime sleepiness and number of night-time apneas and hypopneas, the definitions of severity can also be divided in

these two categories. Whereas the AASM uses the reports from the Wisconsin cohort, which show a higher risk of hypertension in individuals with an AHI >30[55], to define severe OSA as AHI >30, the cut-off point between mild and moderate OSA is set at 15, which is an arbitrary definition by the task force.

Severity of sleep-related obstructive breathing events

- Mild: 5 to 15 events per hour
- Moderate: 15 to 30 events per hour
- Severe: greater than 30 events per hour

The definitions of daytime sleepiness outlined in the report from 1999 are completely arbitrary and they are difficult to apply because they are formulated vaguely. Instead, it has been customary to objectively assess sleepiness either with sleep-or-awake tests such as MSLT (multiple sleep latency test) or with MWT (maintenance of wakefulness test) or by using sleep questionnaires such as Epworth Sleepiness Scale (ESS) or Berlin Questionnaire[53]. A simple definition used in many sleep clinics is to define daytime sleepiness as an ESS score above 10 (on a scale from 0-24, see appendix).

Sleep scoring

The first definition of apnea and hypopneas was provided by Guilleminault et al. in 1976 (see the section Historical perspective above)[30]. During the first two decades of sleep scoring, there was no true consensus about the definitions of the variables that were scored when analyzing a sleep recording. The AASM task force recommendations published 1999 provided standard definitions, criteria and severity ratings for abnormal breathing events during sleep, but the purpose of these definitions was "to facilitate comparability of studies for research purposes and their associated clinical syndromes". Thus, the sleep clinicians were not bound to apply the recommendations in their clinical work. They could continue to apply their clinical judgment for each patient they saw. The recommendations did however become guidelines for how to score sleep recordings in many studies[53].

The definition for hypopnea was not very precise in the 1999 definition, and in 2007 AASM published their guidelines for sleep scoring. In that document the authors suggested two different definitions of hypopnea, one recommended and one alternative definition (Table 1). When comparing the 2007 guidelines with the 1999 definition of hypopnea there were vast differences in AHI. Many patients with clinical symptoms of OSA would not get the diagnosis if the 2007 recommended guidelines were applied. Ruehland et al. compared the 1999 guidelines and the *recommended* and *alternative* guidelines of 2007 and found that approximately 40% of those who had OSA according to the 1999 guidelines would not get a diagnosis of OSA if the recommended definition of hypopnea in the 2007 guidelines was used; even if the alternative definition of hypopnea in the 2007 guidelines was used, 25% of the patients who had OSA according to the 1999 criteria would not get the diagnosis[56]. The AASM sleep apnea task force reviewed the guideline again in 2012, partly because there was such a vivid discussion regarding whether the definitions of 2007 were too harsh[56]. After a literature review, the task force concluded that, even if the cardiovascular risk

was mainly associated with desaturation, frequent arousal from sleep could lead to reduced quality of life, possibly associated with cardiovascular events. The task force concluded that it was more important to relax the inclusion threshold, and the definition of hypopnea in use after 2012 was a 30% drop in the nasal pressure excursion for 10 seconds or more, associated with at least 3% oxygen desaturation or an arousal (Table 1).

This problem with defining hypopneas bears great significance, since it makes it vital in all studies conducted on OSA to clearly state which guidelines were used when scoring a sleep recoding in a study, since this might influet AASM guidelines.

Table 1. Definitions of hypopnea according to different AASM guidelines

Hypopnea 1999 guidelines	1 or 2 in combination with 3 1. A clear decrease (more than 50%) from baseline in the amplitude of a valid measure of breathing during sleep. Baseline is defined as the mean amplitude of stable breathing and oxygenation in the two minutes preceding onset of the event (in individuals who have a stable breathing pattern during sleep), or the mean amplitude of the three largest breaths in the two minutes preceding onset of the event (in individuals without a stable breathing pattern). 2. A clear amplitude reduction of a validated measure of breathing during sleep that does not reach the above criterion but is associated with either an oxygen desaturation of at least 4% or an arousal. 3. The event lasts 10 seconds or longer.
Hypopnea (recommended) 2007 guidelines	A 30% or greater drop in flow for 10 seconds or longer, associated with at least 4% oxygen desaturation
Hypopnea (alternative) 2007 guidelines	At least 50% decreased flow for 10 seconds or longer, associated with at least 3% oxygen desaturation or an arousal.
Hypopnea 2012 guidelines	A 30% drop in the nasal pressure excursion for 10 seconds or longer, associated with at least 3% oxygen desaturation or an arousal.

Pathogenesis of OSA

Obstructive sleep apnea

OSA is generally defined as having sleep apnea independent of coexisting daytime sleepiness, in contrast to the syndrome OSAS, in which daytime sleepiness is present[53]. OSA is considered to be caused by pharyngeal collapse of the upper airways. This is thought to be due to craniofacial differences or higher body fat causing a decrease in the air flow lumen and increasing the likelihood of a pharyngeal collapse[57]. However other factors might also influence the risk of developing OSA (Figure 2).

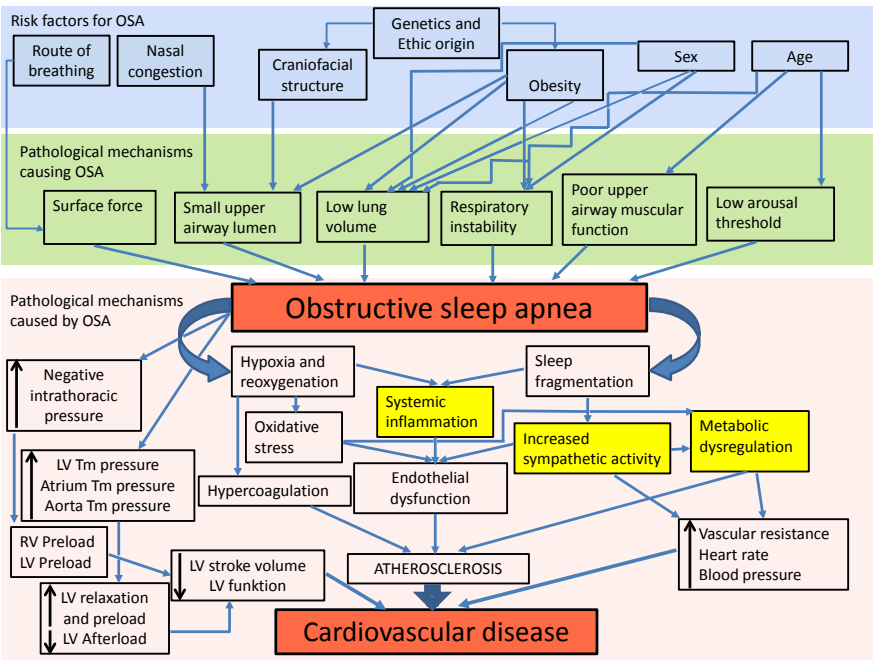

Figure 2. Risk factors, pathophysiological mechanism inducing OSA, and the pathophysiological mechanism induced by OSA that causes CVD.

Maintaining stability in the respiratory control system is essential to preventing the development of OSA. The upper airway dilatory muscle activity varies in accordance with the respiratory control system. Thus, low respiratory central drive is associated with low upper air dilatory muscle activity, high airway resistance and predisposition for collapse of the airways. Poor precision in respiratory control could therefore be the cause of OSA in some patients[58].

The susceptibility to arousals from sleep is another important factor in the pathophysiology of sleep apnea. Since most people hyperventilate after an arousal, the CO_2 in blood might decrease below the chemoreceptor threshold for central apneas in some

individuals. The dilatory muscles of the upper airways also receive respiratory input as a response to chemoreceptors. Therefore, hypercapnia (excessive carbon dioxide in the bloodstream) could lead to collapse of the upper airways, mediated through decreased upper airway dilatory muscle activity secondary to hypercapnia[59], leading to obstructive sleep apneas.

It has been proposed that small lung volumes are associated with a high risk of OSA, since lung volumes are correlated with the cross-sectional area of the upper airways. An increase in lung volume pulls the mediastinum forward and thereby increases the area of the upper airways[60]. Moreover, a recently presented theory is that greater lung volume stabilizes the respiratory control system by increasing the concentrations of O_2 and CO_2, thereby buffering the system and diminishing the destabilizing effect on breathing[61]. Because the functional residual capacity decreases during sleep, the pharyngeal airways are more prone to collapse than when awake[62]. Likewise, a neuromuscular dysfunction in the muscles controlling the tonus in the upper airways can also induce apneas. The most important muscle for this is the genioglossus, and adequate contraction in this muscle seems to be crucial to avoid apneas during sleep[63]. Finally, direct surface force applied by tissue or fluid surrounding the airway could increase the risk of a collapse. In obese individuals, adipose tissue could operate such force on the airways, and in patients with edemas, redistributed fluid due to horizontal positioning could induce the apneas[64,65].

Epidemiology of OSA

Prevalence

The prevalence of OSAS is approximately 3-7% for men and 2-5% for women in the general adult population[66]. This is supported by several cross-sectional studies in USA, China, Australia and Korea[50,67-73]. The difference in prevalence seen in between different studies could be attributed to differences in the definitions of apneas and hypopneas used, and other confounding factors in the different studies (Table 2).

The highest prevalence was reported in the Indian study, but a selection bias could be suspected in this group because they report that screening for eligible study participants was conducted among patients that come for a routine general medical exam, whereas in all the other studies shown in Table 2, the subjects were contacted and asked for participation independent of a visit to a physician. Thus, one could suspect that in the Udawadia cohort the participants might not reflect the general population but rather a somewhat sicker group of individuals[74].

At the other end of the spectrum, the Australian cohort, which shows the lowest prevalence, had a different registration device (MESAM) compared to the other studies which could possibly underestimate the prevalence since they did not measure actual sleep duration with EEG, and thus could not know whether the patients were awake during parts of the night. Usually a registration with polygraphy instead of polysomnography underestimates the numbers of apneas and hypopneas[75].

Interestingly, the remaining studies, which were conducted in different parts of the world but with similar inclusion criteria, showed comparable prevalence values for

Table 2. Prevalence of OSAS according to different studies

Country	Author	Nr	Screening device	OSAS Prevalence Men	Women
USA	Young	602	Polysomnography	4.0%	2.0%
	Bixler	1,741	Polysomnography	3.9%	1.2%
			Polysomnography		
Australia	Bearpark	485	Polygraphy (MESAM)	3.1%	-
India	Udwadia	250	Polysomnography	7.5%	4.5%
China	Ip	258	Polysomnography	4.1%	-
	Ip		Polysomnography	-	2.1%
Korea	Kim	457	Polysomnography	4.5%	2.3%

Abbreviations: MESAM = a sleep recording device.

both men and women. There is very little data on prevalence of OSAS among Africans, but there is (albeit contradictory) data on African Americans suggesting a higher prevalence of OSAS than among Caucasians[76]; this stresses the importance of conducting prevalence studies in Africa.

The prevalence of OSA independent of daytime sleepiness is much higher. However, due to variability in measurements, definitions, sample construction, and statistical techniques used for analyses, it is difficult to establish the prevalence of OSA from the studies that have measured it. Prevalence for moderate or severe OSA (AHI ≥15) has been reported as 9-14% for men and 4-7% for women, increasing with age for both genders[50,67,68,73,77]. In the sleep heart health study, which is the largest cohort study on patients from the general population (n=6,294), the prevalence of moderate or severe OSA was even higher: 24% of all men and 9% of all women in the study had AHI ≥15[9].

Incidence

The risk of developing OSA is associated with body mass index (BMI)[50,78,79] and age[73,79,80]. In a large cross-sectional analysis of the sleep heart health study, the association between BMI and OSA seemed to decrease with increasing age[81]. Longitudinal studies have also found an independent association between OSA and BMI, as well as between age and OSA and between sex and OSA[82], where the effect of age and BMI was attenuated with increasing age. The large increase in sleep apnea in older individuals appears to be driven by two different phenomena: an increase in mild and moderate OSA but a rather consistent prevalence of severe OSA in combination with an increase in CSA, where the majority of cases have a high AHI[73].

The incidence of OSAS also increases with age but seems to reach a plateau at around 65 years of age, mimicking the incidence curve for severe OSA without daytime sleepiness[73].

Treatment of OSA

The first line treatment of OSA is continuous positive airway pressure (CPAP), applied via an external air pump. By mechanically stretching out the upper airways,

CPAP, as long as applied, essentially abolishes obstructive events in a majority of patients[48]. Other treatment modalities include active weight loss for obese individuals, intraoral devices (enlarging the pharyngeal airway by moving tongue or mandible anteriorly[61]) and surgical approaches (uvulopalatopharyngoplasty[49], and in severe cases, tracheostomy[45]).

Obstructive sleep apnea and comorbidities

OSA has been associated with several CVDs[11,83], such as atrial fibrillation[21,84,85], stroke[27], heart failure[28,86,87], pulmonary hypertension[25], aortic disease[88,89], the metabolic syndrome[90], diabetes[18,91], and death[9]. The studies addressed within the scope of this thesis analyze the relationship between OSA and hypertension as well as between OSA and CAD and therefore the thesis will focus on the relationship of those two diagnoses to OSA, and the pathophysiological mechanism caused by OSA that could induce them.

OSA and hypertension

A direct link between OSA and hypertension is well documented both through cross-sectional[55,92,93] and longitudinal studies[94] in the general population. However, the association is more pronounced in younger individuals. Moreover, in the young and middle-aged patients with OSA, increased BMI seems to play a minor role in the increased risk of having hypertension[93,95,96], though there are longitudinal studies that could not confirm this. Thus, the data for the general population is inconclusive[97,98].

In a cross-sectional analysis of a sleep clinic cohort, the odds ratio (OR) of having hypertension increased by 1% for each unit increase in AHI, independent of other risk factors[99]. Grote et al. showed in another cross-sectional study on sleep clinic patients that the risk of uncontrolled hypertension increases by 2% for each unit increase in the respiratory disturbance index (RDI) which was used in this study in a similar way as other studies have used AHI. A good quality longitudinal cohort study by Marin et al. followed 1,889 patients from a sleep clinic cohort for 12.2 years to detect whether treatment of OSA with CPAP reduced the incidence of hypertension. The results of this study showed that OSA patients had higher relative risk of hypertension, but treatment of OSA with CPAP removed this risk[100]. Finally Barbé et al. studied 725 patients from a sleep clinic who had OSA but not daytime sleepiness, and randomized them to CPAP or no CPAP for 4.5 years to study whether CPAP decreased the risk of developing hypertension or cardiovascular events. This study could not confirm the result Marin showed, although the authors argued that their study may have been underpowered.

Several RCTs have been conducted addressing the effect of CPAP on hypertension. In a meta-analysis of 30 RCTs from 2013, Fave et al. concluded that a modest but significant effect of CPAP on blood pressure could be expected (2.6 ± 0.5 for systolic blood pressure and 2.0 ± 0.5 for diastolic blood pressure)[101]. However, there were very few trials that had hypertension among the inclusion criteria, which might bias the result towards non-significance. Only one study, conducted by Duran Cantolla et al., was based on a cohort of patients with newly diagnosed hypertension, where the par-

ticipants were recruited from primary care facilities and not from a sleep clinic cohort or from a resistant hypertension cohort[102]. In this study they found a slight reduction of blood pressure: 3 mmHg in main ambulatory blood pressure in response to CPAP treatment.

There are however, reports, on subgroups with high OSA prevalence who might benefit more from CPAP treatment e.g. resistant hypertension[103,104], those with high blood pressure at baseline[101], those with OSA and daytime sleepiness[105] , and those without blood pressure treatment[106].

A *dipping* blood pressure pattern is defined as a 10% decrease in mean night-time blood pressure compared to daytime blood pressure[1]. Patients with OSA have to a greater degree a non-dipping blood pressure pattern during night-time[107]. Not having this decrease has been shown to predict a poor outcome. In patients from a sleep clinic cohort, treatment with CPAP can eliminate the non-dipping pattern. Other factors that predict a poor outcome in patients with hypertension are increased night-time blood pressure and increased blood pressure rise in the morning[1].

OSA and coronary artery disease

Most of the early studies of a possible correlation between OSA and CAD report some type of association, but the results are inconsistent, hampered by small sample size or have problems adjusting for confounding factors such as age, comorbidity, and BMI[108-110]. However, later studies have shown an association between OSA and increased CAD in both sleep lab cohort studies and in case-control studies[111-115].

Longitudinal cohort studies of the general population have shown an association between OSA and CAD events, especially in men with severe OSA who are below 70 years of age[9].

In cohort studies of patients with CAD, the risk of new cardiovascular events was increased[116-118]. However, there is a lack of RCTs addressing this issue.

Inflammation

Low-grade inflammation has been defined as “a complex set of interactions among soluble factors, and cells that can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic or autoimmune injury”[119]. It is one of at least three essential factors involved in the development of an arteriosclerotic plaque. In addition to inflammation, oxidized low density lipoprotein (LDL) and hemodynamic strain are also vital components[120-123]. Circulating inflammatory markers have lately been proposed as a factor to evaluate the risk of CVD in several disease cohorts. An association between low-grade inflammatory markers in blood and CAD, as well as with hypertension, has been suggested[124-128]. Four markers were studied in the scope of this thesis, as outlined below.

Hs-CRP

The most studied marker of low-grade inflammation is high sensitive C-reactive pro-

tein (hs-CRP). The first acute-phase marker to be discovered, it is mainly produced in the liver, primarily under the transcriptional control of interleukin-6 (IL-6). It has a fast production onset with concentrations rising above 5mg/L in only six hours and peaks after approximately 48 hours. The half-life is 19 h and it remains unchanging through health or disease. Thus, the only factor influencing its concentrations is the production rate. It is therefore a good predictor of the intensity in the pathophysiological process stimulating this production[129].The concentrations of hs-CRP in the general population are low and increases somewhat with age, from 1mg/L in the youngest age to 2 mg/L in the oldest, with women tending to have higher values than men[130]. The values seen in a healthy blood-donor cohort were somewhat lower[131] than what had been reported in the general population[130], reflecting a higher level of subclinical disease. This is also thought to account for the increase in hs-CRP with age[130]. Moreover, Pepys et al. report that an individual's hs-CRP concentrations are very stable over time with little variability other than occasional spikes induced by minor or subclinical infections or inflammations[129].

Hs-CRP increase, already at very low levels, has been associated with increased risk of having both hypertension[128] and CAD, both in the general population and in subcohorts with ischemic heart disease[132,133]. Furthermore, using hs-CRP to identify patients at high risk and then treating them with statins has been shown to reduce the incidence of major cardiovascular events[134,135].

Patients with OSA have increased concentrations of hs-CRP[136]. Treatment of OSA with CPAP seems to reduce these concentrations[137], even though there are studies that failed to show this effect of CPAP treatment[137].

Little is known about hs-CRP concentrations in patients from a CAD cohort screened for OSA, or whether OSA influences the increased hs-CRP levels seen in CAD patients.

Interleukin-6

Interleukin-6 (IL-6) is a cytokine secreted by several cells (macrophages, smooth muscle cells, T-cells, adipose tissue, and endothelial cells). It induces differentiation of myeloid cells, proliferation of smooth muscle cells and secretion of acute-phase proteins. In animal models a dual function has been observed: on the one hand, treatment of mice with IL-6 showed increased fatty streak size, and on the other hand, IL-6 knockout mice suffered from more atherosclerotic plaque at one year of age[138].

A recent meta-analysis by Danesh et al. showed a 1.61 OR for developing myocardial infarct or death for each 2 SD increase in IL-6 compared to baseline[125]. Moreover, IL-6 is higher in patients with unstable angina compared to those with stable angina[139]. Furthermore, in patients treated for acute coronary syndrome in the FRISC II trial, increased levels of IL-6 predicted death independently of whether CPR was elevated[140]. A meta-analysis by Nadeem et al. showed that in many case-control and cross-sectional studies, IL-6 levels are elevated in patients with OSA[136]. However, not all studies could replicate this correlation, and the most important confounding factor for this is probably obesity, because it also correlates with OSA and with IL-6[141,142].

Interestingly, IL-6 inversely correlates with cognitive performance in subjects from the general population[143]. Something that is seen in OSA patients as well[144], opening for a possible mechanism through which OSA influences the brain.

Tumor necrosis factor alpha (TNFα)

Tumor necrosis factor alpha (TNFα) is a cytokine that among other things induces the NFκB genetic inflammatory pathway, which leads to increased inflammatory activity[138]. Even though less studied than hsCRP and IL-6, TNFα has been shown to be associated with risk of CVD[145]. In OSA patients, CPAP treatment reduces TNFα concentrations[137].

Interleukin-8

Of the four cytokines studied in this thesis, interleukin-8 (IL-8) is the least well documented. However there are reports indicating an association with CAD in otherwise healthy individuals[146]. In sleep lab cohorts, the concentration of IL-8 seems to be increased and declines after CPAP treatment; however, the number of studies conducted on this marker is sparse[136].

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone System (RAAS) is vital for salt regulation as well as hemodynamic control in the body. A dysregulation of the system causes stimulation and perpetuation of arteriosclerotic plaque and hypertension[147,148]. The system is not as fully studied as the sympathetic system is in the context of OSA, but OSA is especially prevalent in resistant hypertension. A study by Gonzaga et al. found high levels of plasma aldosterone levels in the patients with OSA and resistant hypertension, and the aldosterone levels were correlated with AHI among those who had elevated aldosterone levels but not in the group with normal aldosterone concentrations[149].

In another study by Gaddam et al. on patients with resistant hypertension and OSA, treatment with aldosterone antagonists improved not only hypertension but also OSA with decreases in AHI[150]. This has also been observed in patients with OSA and heart failure[64]. In this study, the observed improvement of OSA after treatment with spironolactone in heart failure patients was explained by decreased fluid volumes rather than decreased aldosterone levels per se. If the aldosterone or renin levels are not increased, the treatment of OSA with CPAP does not reduce the concentrations of any of the hormones[151]. However, in a study on patients with resistant hypertension, the aldosterone levels were elevated, and in that cohort the concentrations were associated with OSA[152], indicating that it is in those with resistant hypertension that the link between RAAS and OSA exists. However, in a recently published study by Nicholl et al.[153] on normotensive patients with OSA showed that CPAP treatment reduces RAAS activity in normotensives as well. Albeit the data is sparse and inconclusive it indicates a possible link between OSA and hypertension in some individuals.

Sympathetic activity

Obstructive apneas often end with an arousal accompanied by an increase in the sympathetic activity[154]. Repetitive hypoxia and large oscillations in intrathoracic pressure

due to collapse of the upper airways in OSA patients may cause an overactive sympathetic system[155]. Strikingly, OSA patients continue to have repetitive bursts of sympathetic activity and increased sympathetic activity even during the day[155], demonstrated by microneurography, and increased catecholamine levels both in plasma and urine. Indeed, increased and variable heart rate and blood pressure have been observed in OSA patients compared to normal subjects during wakefulness[156]. As the altered cardiovascular variability due to the dysfunction of autonomic cardiovascular regulation predicts morbidity and mortality in patients with hypertension, diabetes, heart failure, and CAD; this may be the case even for OSA patients who experience CVD. Obesity can, in this context, be considered the main confounding factor. However, it has been shown that obesity in the absence of OSA is not accompanied by increased sympathetic activity[157].

Sympathetic overactivity can be diminished, and impaired autonomic dysfunction reversed, by effective CPAP therapy[158]. Moreover, CPAP withdrawal even for one week has been found to be associated with a marked increase in sympathetic activity[154]. On the other hand, CPAP leads to significant reductions in plasma norepinephrine levels both by increases in norepinephrine clearance and decreases in diurnal and nocturnal excretion compared with placebo or oxygen therapy[155]. CPAP has been shown to be effective in lowering of daytime muscle sympathetic nerve activity and reducing sensitivity of the arterial baroreflex both during wakefulness and sleep[159,160]. The increase in baroreflex control of heart rate during sleep may be of clinical relevance since it is accompanied by reduced cardiovascular variability which is an independent cardiovascular risk factor.

Gaps in knowledge

In spite of the growing evidence regarding the independent relationship between OSA and CVD, the cardiovascular mechanisms and impact of CPAP treatment are poorly understood. Moreover, a causal relation between OSA and CVD is not definitely established. To date, few well-designed clinical trials have addressed this subject. Therefore the research programme of this thesis focuses on prospective, randomized, controlled trials studying interventions with CPAP. The research was conducted with two specific cohorts of patients: one with newly diagnosed, untreated hypertension, and the other, consisting of newly revascularized patients with established CAD.

LosartanPAP – research questions

There is, as previously described, a well-established association between hypertension and OSA. However, whether the increase in blood pressure during the whole 24-hour period, and not just in association with the apneas, is a direct cause of OSA is currently debated. Several mechanisms have been proposed to support a causal association. However, this is poorly studied, especially in cohorts of untreated hypertensive patients who have never attended a sleep clinic. Both inflammation, and sympathetic activity have been found to be pathologically influenced by OSA. How much the RAAS activity, and the medication that affects RAAS activity, is influenced by OSA and treatment of OSA is poorly understood.

RICCADSA – research questions

There are no previous RCTs which address whether OSA in CAD patients increases the risk of new cardiovascular events or death. Moreover, most studies of the mechanisms that could be involved in the link between OSA and CVD have been carried out on patients from a sleep lab cohort. Information about how the pathophysiological mechanisms induced by OSA are manifested in patients with already established severe CAD is sparsely described.

AIM

"Sleep is the interest we have to pay on the capital which is called in at death; and the higher the rate of interest and the more regularly it is paid, the further the date of redemption is postponed."

- Arthur Schopenhauer

The overall aim of this study was to explore the association between OSA and CVD, with special focus on hormonal and inflammatory mechanisms that could mediate this linkage. A further aim was to investigate the impact of CPAP treatment on these mechanisms.

Specific aims

Paper I To study whether circulating inflammatory markers are elevated in subjects with OSA and stable newly revascularized CAD, compared with similar subjects without OSA.

Paper II A: To study whether patients with new-onset hypertension and concomitant OSA respond differently to blood pressure treatment with the angiotensin receptor blocker (ARB) losartan, compared to the hypertensive patients without OSA.
B: To study the impact of add-on CPAP treatment on blood pressure in patients with new-onset hypertension and concomitant OSA.

Paper III A: To study whether CPAP treatment of OSA in patients with revascularized CAD decreases levels of circulating inflammatory markers.
B: To study whether sleepy and non-sleepy patients with OSA and revascularized CAD differ with regard to circulating inflammatory markers at baseline and after CPAP treatment.

Paper IV A: To study whether levels of circulating neurohormonal and inflammatory markers differ between patients with new-onset hypertension depending on whether they have concomitant OSA.
B: To study whether CPAP treatment of OSA reduces circulating neurohormonal and inflammatory markers in patients with new-onset hypertension.

Study overview (what it adds to the field)

Paper I

The first paper is a cross-sectional analysis in which we compared levels of circulating inflammatory markers (IL-6, IL-8, TNFα, and hs-CRP) in patients from the RIC-CADSA trial with revascularized CAD with and without OSA. It gives new insight into the association between OSA and CAD inflammation, because to date, very few studies have been conducted on how these three entities are associated in a cohort where all patients had CAD.

Paper II

The second paper is an RCT which investigating how well ARB works on patients with hypertension and OSA, and whether add-on treatment with CPAP loweres blood pressure further. It is the first RCT in the field that investigates the effect of CPAP as an add-on treatment in patients with new-onset hypertension, and one of very few RCTs that investigates the effect of CPAP on blood pressure in a cohort of patients with newly discovered hypertension.

Paper III

In the third paper we have focused on how CPAP affects circulating inflammatory markers in revascularized patients with CAD and OSA. It is to date, one of the largest RCTs addressing the effect of CPAP on circulating inflammatory markers, and the first conducted in a CAD cohort.

Paper IV

In the fourth paper, we studied the same cohort as in paper II but now addressed how neurohormonal and inflammatory activity was influenced by CPAP treatment. This RCT adds new information to the field of OSA and hypertension regarding these cardiovascular mechanisms involved in a cohort of new-onset CVD.

PATIENTS AND METHODS

"Sleep: a poor substitute for caffeine!"

- Wally Shawn

Evaluation of literature

Systemic searches of literature were performed before and during the period of the studies. The following search terms were used: "hypertension and OSA", "Coronary artery disease and OSA", "Cardiovascular disease and OSA, inflammation and OSA", "Renin-Angiotensin-Aldosterone System and OSA", and "Sympathetic activity and OSA". Searches were restricted to English literature and for some of the topics limited to core clinical journals. Literature not directly linked to the research questions of this thesis were searched in a more general manner. The main database used was PubMed. However, occasionally Scopus, Cochrane, and Google Scholar were used.

Study design

The results of the four papers in this thesis originate from two different RCTs: the RICCADSA trial (paper I and paper III) and the LosartanPAP trial (paper II and paper IV). Thus, unless otherwise specified, references to the RICCADSA trial relate to paper I and paper III, and references to the LosartanPAP trial relate to paper II and paper IV.

RICCADSA (paper I)

This was a cross-sectional, case-control study on a subsample of the baseline population of the RICCADSA trial. It investigated differences in circulating inflammation in a revascularized cohort of patients with CAD, comparing patients with concomitant OSA to those without. The endpoints of this study were pre-specified in the protocol of the main RICCADSA trial[161].

LosartanPAP (paper II)

Phase 1: This was a 2:1 multicenter case-control (OSA vs non-OSA) trial, investigating the difference in blood pressure response after 50mg losartan treatment for six weeks in patients with newly diagnosed hypertension, comparing patients with concomitant OSA to those without. Phase 2: This was a 1:1 randomized, open, multicenter case-control (CPAP treatment versus no CPAP) trial with an additional follow-up arm, investigating the difference in blood pressure response to CPAP treatment for six weeks on top of losartan in patients with newly diagnosed hypertension and with concomitant OSA.

RICCADSA (paper III)

This was a 1:1 randomized, open, multicenter case-control trial with additional passive non randomized control arms. The study duration was one year follow-up from inclusion. It is a substudy pre-specified in the protocol of the main RICCADSA trial[161].

LosartanPAP (paper IV)

Phase 1: This was a 2:1 multicenter case-control (OSA vs non-OSA) trial, investigating the response in RAAS activity, sympathetic activity, and circulating inflammatory markers in response to 50mg losartan treatment for six weeks in patients with newly diagnosed hypertension comparing patients with concomitant OSA to those without.

Phase 2: This was a 1:1 randomized, open, multicenter case-control (CPAP treatment versus no CPAP) trial with an additional follow-up arm, investigating the RAAS activity, sympathetic activity and circulating inflammatory markers in response to CPAP treatment for six weeks on top of losartan in patients with newly diagnosed hypertension and with concomitant OSA.

The rationale and design of the RICCADSA trial has been published as well as the baseline characteristics of the whole RICCADSA populations[161,162]. The design of the LosartanPAP study has not been published in a peer-reviewed article.

Both studies are registered with ClinicalTrials.gov (NCT 00519597 and NCT 00701428, respectively), as well as with the national researchweb.org (FoU i Sverige – Research and development in Sweden; nr VGSKAS-4731; 04.29.2005, and nr VGSKAS-10375; 04.27.2007, respectively).

Participants and settings

RICCADSA

The target population of the main RICCADSA study comprised adult patients with angiography-verified CAD who had undergone percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) in Skaraborg County, West Sweden, in the previous six months, and had an AHI <5 or AHI ≥15 during a sleep study. Patients with existing OSA, an AHI of 5.0-14.9/h, and predominantly central apneas with Cheyne-Stokes respiration were excluded (Figure 3).

Patients were included in the study between December 2005 and November 2010, and follow-up was completed in May 2013. Patients were recruited from two hospitals with training and research facilities serving a population of approximately 250,000 living in the Skaraborg county of Västra Götaland, Sweden. PCI was performed either as an elective or acute/subacute procedure at the hospital in Skövde or at the Sahlgrenska University Hospital in Gothenburg, which is the regional hospital. CABG was performed in Gothenburg, and all patients were moved to the study hospitals in Skövde or Lidköping when clinically stable after revascularization. Eligible patients who gave informed consent to participate in the study were referred to the Sleep Medicine Unit in Skövde for a sleep studies. Fasting blood samples were collected in the morning at time of study inclusion. The procedure was repeated after three months and after one year (only the one-year repetition has been used in the article IV).

Patients for whom the blood pressure could not be analyzed, (e.g. hemolysis in the blood sample) were excluded from the sub-analyses presented in this thesis (paper I and paper III).

Patients with obesity (BMI ≥30 kg/m^2) and systolic heart failure (LVEF <50%) were excluded from the analysis conducted in Paper I because these are both major confounders which could induce low-grade inflammation. Since the main analysis in paper III is based on a comparison of randomized groups, the randomization was expected to adjust for these factors, and therefore patients with systolic heart failure and patients with obesity were included in the cohort of this paper.

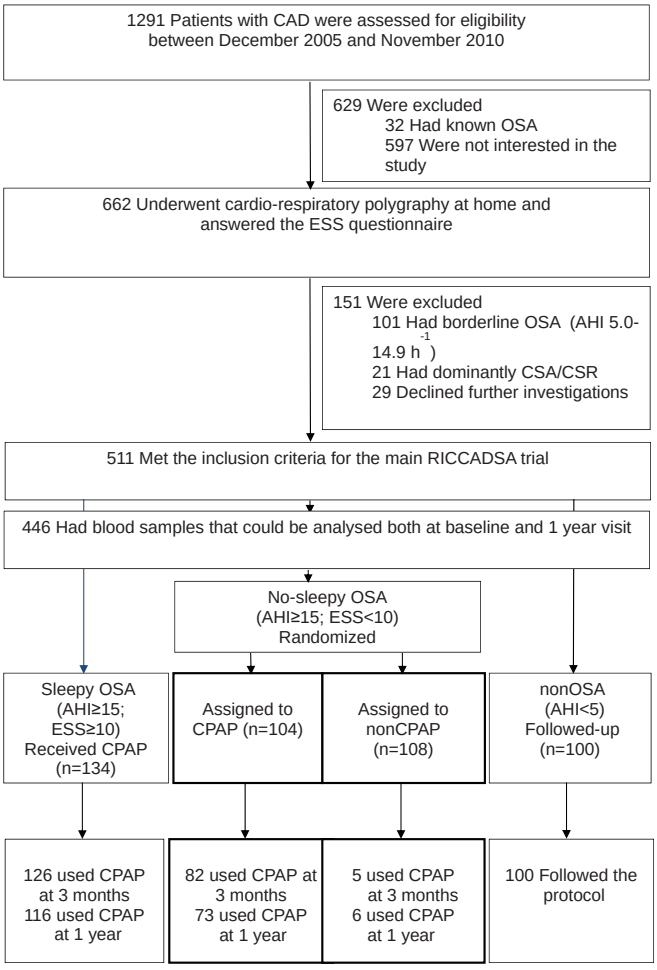

Figure 3. Flow chart of the patients included in the RCT of the RICCADSA trial (paper III).

LosartanPAP

Subjects were recruited from the participants in the Prospective Urban Rural Epidemiology (PURE) study (an epidemiologic multinational study of cardiovascular risk factors in the general population)[163] and through newspaper advertisements, as well as through referrals to the study from general or private practitioners. Two centers from the west region of Sweden were involved in the recruitment: Skaraborg hospital, with a catchment area of 250 000 individuals from a mixed rural and urban population of mainly Caucasian inhabitants, and Sahlgrenska University hospital, with a catchment area of 250 000 individuals from an urban population of mainly Caucasian inhabitants.

The subjects with newly diagnosed and never-treated hypertension (office-based SBP ≥140 mm Hg, DBP ≥90 mm Hg, or both) were invited to participate. After the initial screening, patients were excluded if they had normal blood pressure at study entry, uncontrolled malignant hypertension, borderline OSA, e.g. an AHI between 5 and 14.9, morbid obesity (BMI ≥35), or other serious disease (e.g. cancer, cardiac disease, manifest diabetes, or pulmonary embolism). Patients were also excluded if they were unable to give informed consent or if they had received any medical treatment for hypertension for more than six months. Subjects who were hypertensive and had been on a single antihypertensive agent for less than six months were allowed to participate if they stopped the treatment two weeks prior to entering the study. The hypertension diagnosis was established by the patient's general practitioner or independently by another study physician. The diagnosis of hypertension was based on the definition in the 2007 ESH/ESC hypertension guidelines[164] (Figure 4).

In paper IV, if a patient had insufficient blood samples that subject were excluded from the analysis where that variable we evaluated.

Changes to methods after trial commencement

LosartanPAP

The original research plan was to conduct two studies with 90 participants (60 with OSA and 30 without OSA) in each study. Since we did not want to adjust for gender, we planned first a study on 90 men followed by the same study on women. We started with the male subjects since OSA is more common among men and we therefore thought that the inclusion rate would be higher. Moreover, published studies at the time indicated that hypertension might be more strongly associated with OSA among men than among women. We planned to then compare the two study results from a gender perspective (which could have been post-doctoral research).

However the inclusion rate was low in the first (male) LosartanPAP study, and we realized in 2010, that it would not be possible to include 90+90 individuals as we initially hoped. We therefore amended the protocol including women in the same study. By doing so, the inclusion rate was increased to address the primary research question but the sample size was not sufficient to address the possible gender differences in that context.

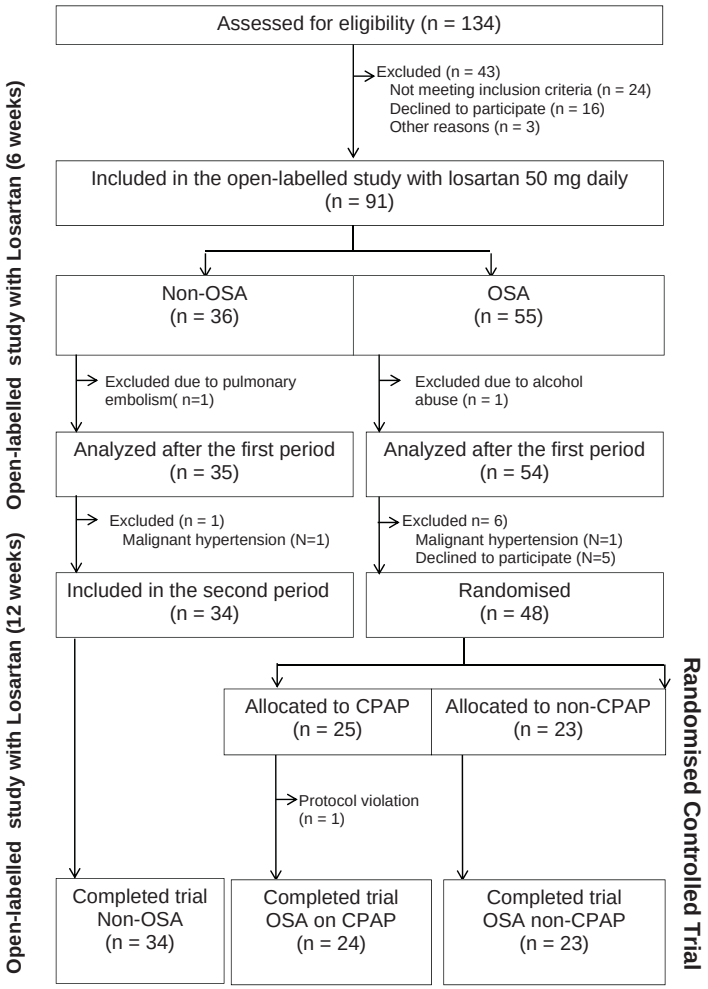

Figure 4. Flow chart of the patients included in the RCT of the RICCADSA trial (paper II and paper IV).

RICCADSA

In the main RICCADSA trial, an interim analysis blinded to randomization group in non-sleepy OSA patients that was performed in February 2010 revealed an incident rate of 21%, and a CPAP adherence rate of 60% at one year, resulting in a protocol amendment. With an enlarged sample size of 242 patients (121 in each of the randomization arms) and an extended follow-up period for the main study of between two years and seven years, a significant risk reduction for the primary endpoint from 25%

to 12% was hypothesized (instead of the 10% initially assumed). Since the current thesis addresses secondary endpoints of the RICCADSA trial, this change directly influences the sample size of the two papers in this thesis (paper I and paper III).

Interventions

RICCADSA baseline (paper I)

No intervention.

RICCADSA follow-up (paper III)

Randomized to intervention with CPAP for one year.

LosartanPAP (papers II and IV)

Phase 1: Intervention with 50 mg losartan for six weeks.

Phase 2: Randomized to intervention with CPAP in addition to 50 mg losartan for six weeks.

Outcomes

RICCADSA baseline (paper I)

Cross-sectional difference in concentrations of circulating inflammatory markers (hs-CRP, IL-6, TNFα, and IL-8) at baseline.

LosartanPAP (paper II)

Difference in 24-h mean systolic blood pressure (SBP), mean diastolic blood pressure (DBP) and mean atrial blood pressure (MAP); mean night-time (22:00–07:00) SBP, DBP, and MAP; mean daytime (07:00–22:00) SBP, DBP, MAP; difference in the proportion of patients who had dipping blood pressure pattern; and the proportion of patients with optimally treated 24-h blood pressure.

RICCADSA follow-up (paper III)

Difference in circulating inflammatory markers (hs-CRP, IL-6, TNFα and IL-8) after one year of CPAP treatment.

LosartanPAP (paper IV)

Difference in RAAS activity: renin and aldosterone concentrations in blood (baseline, after six weeks, and after 12 weeks) as well as difference in change after six weeks and after 12 weeks.

Difference in sympathetic activity: adrenaline and noradrenaline concentrations in blood (baseline, after six weeks and after 12 weeks) as well as difference after six weeks and after 12 weeks.

Difference in circulating inflammatory activity: hs-CRP, IL-6, TNFα and IL-8, concentrations in blood (baseline, after six weeks, and after 12 weeks) as well as difference after six weeks and after 12 weeks.

Adjustments for potential confounders

A confounder is a factor which is associated both with the effect variable and the outcome variable, thus it is difficult to conclude which of two variables could cause an effect on the outcome variable. Furthermore, the confounding variable should not be an effect of the effect variable.

To avoid confounders we used several methods:

- We stratified the randomization (for gender in LosartanPAP and for gender and type of intervention in RICCADSA).
- We excluded patients with major confounders in the cross-sectional study.
- We adjusted for variables associated with the outcome variable in the multivariate analysis.

Potential effect modifiers

The link between OSA and CVD has in some studies been reported to differ with age, gender, and BMI. Thus, these three variables could possibly be effect modifiers. However, when trying to do subgroup analyses stratified for these variables, the subgroups became very small. In the RICCADSA population with obesity, only 8 out of 140 did not have OSA. In the LosartanPAP trial, the cohort was even smaller, which made it even more difficult to do subgroup analyses. We therefore refrained from doing such analyses.

Diagnostic criteria

In all four papers, OSA was defined as having an AHI ≥15/h. However, in Paper I an additional parallel definition was used for OSA based on ODI ≥5/h to investigate whether desaturation per se or hypopneas with or without desaturations is the most important link in the association between OSA and inflammation.

Hypertension diagnosis as an inclusion criterion in the LosartanPAP trial was based on a systolic blood pressure above 140 mmHg and/or a diastolic blood pressure above 90 mmHg on three or more different occasions, or a documented hypertension diagnosis set by a study-independent physician. Moreover, no patients were included in the study if they did not have hypertension (a mean blood pressure above 130/80 mmHg) at the baseline 24-hour ABPM[1].

In the RICCADSA trial, the hypertension diagnosis was less rigorously controlled since it was not an outcome variable but only included in the study as comorbidity. A patient was defined as hypertensive if it was documented in the medical records that they suffered from hypertension.

Sample size

RICCADSA (paper I and paper III)

The sample size was calculated for the power of the main RICCADSA trial. No power calculation was conducted to verify the numbers needed to detect a certain difference in inflammatory markers. The sample size was thus defined by the number in the main RICCADSA trial (n=511) and excluding those without analyzable blood samples.

LosartanPAP (paper II)

In a substudy of the LIFE trial on otherwise healthy individuals, losartan, 100 mg by mouth daily, reduced SBP by 30.2 mmHg and DBP by 16.7 mmHg[165]. In another study by Minami and colleagues[166], losartan, 50 mg by mouth daily, reduced the mean SBP and DBP by 11 mmHg and 10 mmHg, respectively, in patients with hypertension. Based on these findings, our initial sample size was calculated on an assumption that losartan would reduce mean 24-h SBP and DBP, each by approximately 10 mmHg. Based on this assumption, the study was designed to detect whether CPAP treatment as add-on therapy to losartan would further reduce SBP and DBP by at least 5 mmHg (±5 mmHg), compared with patients treated for the second six weeks with only losartan.

Our initial calculation was based on a power of 90% (with a two-sided significance level of 0.05), which required at least 22 patients in each arm to detect the above-mentioned levels of blood pressure changes. After accounting for the probability of dropout, we initially planned to include 30 patients in each arm. However, due to the slow recruitment rate, we had to make a new calculation based on 80% power with the same significance level, which required at least 16 patients in each arm. Adding eight dropout and/or noncompliant subjects resulted in at least 24 patients in each group. With this sample size we would be able to detect a difference in reduction of a mean of 5 mmHg with a SD of 5 mmHg in the first phase of the study as well.

LosartanPAP (paper VI)

No power calculation was conducted for this study. The sample size was defined by the number in the main LosartanPAP trial (paper II).

Randomization

RICCADSA (paper I)

In this cross-sectional study, no randomization was conducted.

LosartanPAP (paper II and IV)

During the second part of the trial, the cohort was stratified according to sex. After six weeks of treatment with losartan, patients with OSA continued treatment with losartan and were randomly assigned 1:1 to treatment either with or without CPAP. The CPAP allocation sequence was manually generated by the senior physician responsible for the trial (YP), who was not involved in the recruitment or randomization procedure. Participants were randomly assigned in order of appearance (random number table: CPAP versus a control group with no CPAP).

RICCADSA (paper III)

The 1:1 random assignment of patients with CAD and non-sleepy OSA was scheduled by the sealed envelope system with a block size of eight patients (four CPAP, four controls) stratified by gender and revascularization type (PCI/CABG). Thus, four groups of sealed envelopes (eight in each group: PCI men, PCI women, CABG men, and CABG women) were prepared in advance by the senior physician responsible for the trial (YP) and the study nurse. The patients were enrolled in the randomization procedure in the morning after overnight PSG, scheduled by the study nurse with no knowledge about the details of the patient characteristics and comorbidity data. Non-sleepy OSA patients randomized to treatment, and CAD patients with sleepy OSA phenotype (ESS score ≥10) who were offered CPAP treatment were informed about the technical procedure in the morning after overnight PSG and provided with an automatic (self-titrating) CPAP device (S8®, or S9®; ResMed, San Diego CA, USA) plus a nasal or full-face mask and humidifier by trained staff at the study center. All patients assigned to CPAP treatment were instructed to use the device at home every night for at least four hours, contacted by telephone after one week, and given a check-up in the clinic after one month, three months, six months, one year, and then yearly until the end of the main study. Non-sleepy OSA patients who were randomized to the control group and who were obese were given advice about weight reduction, and all OSA patients randomized to the control group were informed about the tennis ball technique to avoid the supine position during sleep.

Data sources

Ambulatory blood pressure measurements

The 24-h ABPM was conducted using Spacelabs 90217 (Spacelabs Medical, Snoqualmie, WA). The first ABPM was conducted on a separate day after the sleep recording. The 24-h ABPM levels were recorded every 15 minutes during the day (07:00-22:00) and every 20 minutes during the night (22:00-07:00). The average 24-h blood pressure was calculated from the average blood pressure each hour divided by 24. The following parameters were studied: the mean systolic blood pressure (SBP), the mean diastolic blood pressure (DBP), and the mean arterial pressure (MAP) over 24 hours as well as during the day (07:00-22:00) and during the night (22:00-07:00). In addition, morning blood pressure (06:00-08:00), dipping versus non-dipping patterns (with subjects labeled as dippers if their blood pressure declined by more than 10% during the night [22:00-07:00] compared with during the day [07:00-22:00]), and hour-to-hour blood pressure variability were recorded. Dipping pattern is a well-established concept used in many previous studies[167,168]. Patients' hypertension was considered to be optimally treated if the patient had an average SBP of less than 130 and an average DBP of less than 80 on 24-h ABPM[1]. Patients who worked shifts (n=9) were working the day shift during the time of inclusion in the study as well as at randomization and at the final 24-h ABPM.

Polygraphy

The sleep study was performed in subjects' homes using the Embletta Portable Digital System (Embla, Broomfield, CO, USA) at baseline. The portable, limited-channel, sleep recording device consisted of a nasal pressure detector using a nasal cannula/

pressure transducer system, thoracoabdominal movement detection through two respiratory inductance plethysmography belts, and a finger pulse oximeter detecting heart rate and oxyhemoglobin saturation (SpO2), as well as body position and movement detection.

Blood samples

In both the LosartanPAP and RICCADSA trials, all blood samples were collected in the morning after the patients had been fasting. Clinical routine blood test such as blood glucose, hemoglobin (Hb), creatinine, sodium, and potassium (LosartanPAP only), lipids, white and red blood count, thrombocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), N-terminal prohormone of brain natriuretic peptide (nt proBNP) Alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) (liver status was not included in RICCADSA). In addition, blood and plasma were collected, centrifuged and frozen to -70° Celsius and stored for later analysis. For analysis of inflammatory markers, blood samples were sent to Stockholm for analysis at the Karolinska Institutet, Huddinge.

In Stockholm, serum levels of hs-CRP were measured by immunoturbidimetry using the infrared immunoassay rate method and the near-infrared particle immunoassay at the Karolinska University Laboratory (Solna, Sweden) in a routine clinical analysis. The detection limit for hs-CRP was 0.20 mg/L with a measuring range of 0.20–380 mg/L. The levels of the proinflammatory biomarkers IL-6, IL-8, and TNFα were analyzed in undiluted plasma samples using commercially available Milliplex MAP (based on Luminex technology) human serum adipokine assay kits in accordance with the manufacturer's instructions (Merck Millipore, Billerica, MA). Minimum detectable concentrations (the assay sensitivities) for IL-6, IL-8, and TNFα were 0.6, 0.2, and 0.14 pg/mL, respectively.

The concentrations of all samples (undiluted) were observed within the standard curve, ranging from 0 to 10,000 pg/mL for the biomarkers IL-6, IL-8, and TNFα. The intra-assay and interassay variability (generated from the mean of the percentage coefficient of variability from multiple reportable results across two different concentrations of analytes in one experiment, or from two results each for two different concentrations of analytes across several different experiments) were 1.4-7.9% and <21%, respectively. Other markers which were beyond the scope of this thesis were analyzed in parallel.

In the LosartanPAP study the hormones renin, aldosterone, adrenalin, and noradrenalin were analyzed as well. This was done at Sahlgrenska University Hospital in Gothenburg. Serum levels of renin, aldosterone, adrenaline, and noradrenaline were measured.

For renin a chemiluminescence immunoassay of sandwich type method was applied to do the analysis using the LIAISON® analysis instrument SN 2229002564, DiaSorin. The detection limit for renin was 2.0mIU/L with a measuring range of 2.0–46 mIU/L where the blood sample will be diluted to values within the range.

For Aldosterone, a Coat-A-Count procedure was used, which is a fast-phase radioimmunoassay, based on an aldosterone specific antibody attached to the wall of a polypropylene tube. Concentration calculations were made using a gamma counter linked to MultiCalc calculation program. The detection limit for aldosterone was <30 pmol/L with a measuring range of 30–3000 pmol/L.

The catecholamines, adrenaline, and noradrenaline were extracted and concentrated from the plasma through absorption, adding aluminum oxide. Unwanted integrations such as phosphor lipids were washed off using a diluted base solution. The catecholamines was desorbed from the aluminum oxide powder by adding a diluted acid water solution. The elute was analyzed using a high pressure chromatographic system with reversed phase column (C18) and electronic detection linked to the Chromeleon data system. The detection limit for adrenaline was 0.002 nmol/L with a measuring range of 0.02-500 nmol/L. The detection limitation for noradrenaline was 0.18 nmol/L with a measuring range of 0.18-500 nmol. The intra-assay variables were 6-11% for renin, 10% for aldosterone, 8.2% for adrenaline, and 11.6% for noradrenaline[169-171].

Epworth sleepiness scale

Epworth sleepiness scale (ESS) was used to define subjective sleepiness. ESS consists of eight questions concerning the propensity to fall asleep during the day. Respondents have to rate their risk from 0 (not at all) to 3 (high risk) of falling asleep under different circumstances described by each question. The maximum combined score is thus 24. The instrument has been validated as a measure of the risk of falling asleep during the day[172,173].The cut-off defining daytime sleepiness (ESS ≥10) was the cut-off used in clinical practice at Skaraborg sleep clinic at the time for the design of both the RICCADSA and LosartanPAP study (see appendix for the scale).

Echocardiography

Echocardiography was conducted at baseline, after three months, and after one year in all RICCADSA participants. All investigations were scored by the same physician with echocardiography experience (HG). The LVEF which was used to define systolic heart failure in paper I was derived from the baseline examinations. All other date from these examinations is not the scope of this article.

Statistical methods

IBM Statistical Package for Social Sciences verison 20.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses.

Quality control

All data was extensively checked for data entry errors when they were manually imputed in to the SPSS. In RICCADSA, 10% of all the data was re-evaluated to find errors by an external monitor which rendered a few errors, but the numbers were very low. For LosartanPAP, an external monitor form Gothia Forum manually double-checked all endpoint values (over 7000 independent values) and found a very low number of errors; he also did a random spot-check in the other variables in the SPSS file with equally low rate of errors. All monitor findings in RICCADSA as well as

in LosartanPAP were corrected before analysis of the data started. To find data entry errors not found by the monitors, all analyzed variables were checked for minimum and maximum values, and all values that could suggest an error were checked against the raw data if possible; if this was not possible, they were omitted from the analysis. Analyzed variables were also checked for normal distribution and, if not normally distributed, data transformation was considered; where appropriate, it was transformed to the natural logarithm of the original variable.

Baseline characteristics

The baseline characteristics of the studies in all four papers were presented as mean ± SD (standard deviation), for continuous variable or as percent of the total number of the categorical variables.

Differences between groups

Independent sample T-tests were used for comparison of continuous variables between two groups (paper I, II, III, and IV), and Mann-Whitney U-test was used for variables with non-normal distribution (paper I). For comparison of dichotomous variables, Chi-squared test or Fisher's exact test (two-tailed) was used, depending on group sizes in the analysis. For analysis of change in one variable in the same individual on two different occasions, paired T-test was used. Comparison of continuous variables between more than two groups was carried out with one-way analysis of variance (ANOVA) with post hoc Bonferroni correction if equal variance was assumed and with Gammes-Howell if unequal variance was assumed.

Multivariate models were constructed by first doing univariate analysis with all variables considered as clinically relevant prior to conducting the statistical analysis. Multivariate models were than created from those variables that were significant in the univariate analysis (paper I).

Ethical considerations

The main protocols of the RICCADSA trial and the LosartanPAP trial were originally approved by the ethical committee of Västra Götaland the 15th of August 2005 and 26th of March 2008 respectively. Several minor amendments were submitted over the course of the study. The LosartanPAP study was also approved by the European Medicines Agency and the Medical Products Agency (Sweden) since a drug (losartan) was used. Both studies comply with the Declaration of Helsinki[174].

At the first contact with the study staff, all participants in both the RICCADSA and the LosartanPAP trial received both oral and written information about the study they were to participate in. They all gave written informed consent before inclusion in the study. Pathological findings discovered in any patient eligible for RICCADSA or LosartanPAP were addressed as soon as they were discovered; if necessary, a referral was made to a subspecialist. After the end of the trial, all patients were referred to their general practitioner for further assistance regarding blood pressure control and secondary prevention after revascularization, and to their sleep lab if they were interested in treatment for their OSA.

SUMMARY OF RESULTS

"Sleep is not on good terms with broken hearts. It will have nothing to do with them."
- Christopher Pike

LosartanPAP

Figure 4 (page 37) shows the flow chart of the LosartanPAP study, both the first non-randomized phase, in which patients with newly discovered hypertension and OSA were compared to patients with newly discovered hypertension without concomitant OSA, and the second phase where OSA patients with and without CPAP treatment were compared.

Paper II

Blood pressure response to losartan and CPAP in hypertension and obstructive sleep apnea

In paper II, 24-h ABPM included an assessment every 15 minutes during daytime hours and every 20 minutes during the night. Of the 91 patients eligible for the trial, two patients were excluded before the first analysis was conducted; thus, 89 patients (54 with OSA and 35 without OSA) took part in the analysis. Baseline characteristis of the study population is shown in Table 3.

Table 3. Baseline characteristics of the LosartanPAP study

Characteristic	All patients (n = 89)	Hypertension (n = 35)	Hypertension + OSA (n = 54)	p Value*
Age, y	58.6 ± 5.3	58.2 ± 5.6	58.9 ± 4.8	0.558
Male sex, %	67.4	65.7	68.5	0.783
Body mass index, kg/m^2	27.1 ± 3.5	26.2 ± 3.3	27.8 ± 3.4	0.034
Waist circumference, cm	97.4 ± 12.6	95.0 ± 14.0	98.9 ± 11.6	0.240
Abdominal obesity, %	75.4	63.6	82.9	0.100
Current smoker, %	9.3	6.1	11.5	0.610
Consumes ≥14 U alcohol/week, %	10.3	11.4	9.6	0.785
Shift worker, %	10.1	8.6	11.1	0.930
Epworth Sleepiness Scale, score	7.9 ± 4.7	5.9 ± 3.1	9.1 ± 4.7	<0.001
Apnea Hypopnea Index, events/h	18.4 ± 16.5	3.0 ± 1.5	28.3 ± 12.4	NA
Oxygen Desaturation Index, events/h	8.5 ± 9.6	1.8 ± 1.3	12.9 ± 10.2	NA
Nocturnal SpO_2, %				
Mean	94.0 ± 1.2	94.3 ± 1.1	93.8 ± 1.2	NA
Time <90 % saturation	2.3 ± 5.5	0.4 ± 1.1	3.7 ± 6.8	NA
Nadir	85.4 ± 5.0	88.7 ± 3.0	83.4 ± 4.7	NA
Office-based blood pressure reading, mm Hg				
Systolic	162.0 ± 15.4	157.8 ± 11.8	164.9 ± 16.2	0.035
Diastolic	95.6 ± 10.4	94.1 ± 9.5	96.5 ± 10.9	0.317
Laboratory values, mmol/L				
Fasting blood glucose	5.4 ± 0.8	5.2 ± 0.5	5.4 ± 1.0	0.420
HDL cholesterol	1.6 ± 0.5	1.6 ± 0.5	1.5 ± 0.5	0.599
LDL-cholesterol	3.6 ± 0.9	3.6 ± 0.8	3.7 ± 1.0	0.616
Triglycerides	1.2 ± 0.6	1.1 ± 0.5	1.2 ± 0.7	0.616

Abbreviations: OSA refers to obstructive sleep apnea; SpO_2, peripheral oxygen saturation; HDL, high-density lipoprotein; LDL; low-density lipoprotein. *Continuous variables are expressed as mean ± SD. Means between groups were compared with independent sample student t tests, and categorical variables were compared using 2-tailed Pearson χ^2 tests.

As shown in Table 4, at baseline, patients with OSA, compared with those without OSA, had higher 24-h ABPM values, and the differences were most pronounced during the night. Moreover, the proportion of patients with a non-dipping BP pattern was higher in the OSA group than in the no-OSA group.

Table 4. Blood pressure results in the first part of the study in response to losartan treatment

Variable, mm Hg	No-OSA (n = 35)	OSA (n = 54)	p Value*	BP change difference† (p value)
Mean 24-h BP				
Systolic				
Baseline	142.2 ± 10.3	146.9 ± 11.9	0.058	
After 6 weeks of treatment	129.6 ± 10.5	137.1 ± 14.3	***0.009***	2.8 (0.230)
Diastolic				
Baseline	87.6 ± 7.8	90.2 ± 7.8	0.140	
After 6 weeks of treatment	80.4 ± 7.2	84.5 ± 7.1	***0.014***	1.5 (0.270)
Mean arterial				
Baseline	105.8 ± 6.9	108.0 ± 8.0	0.199	
After 6 weeks of treatment	96.8 ± 6.8	101.9 ± 8.7	***0.004***	2.8 (0.052)
Mean daytime BP				
Systolic				
Baseline	151.3 ± 11.0	154.8 ± 12.7	0.172	
After 6 weeks of treatment	137.1 ± 10.8	145.2 ± 15.6	***0.005***	4.6 (0.064)
Diastolic				
Baseline	94.0 ± 7.1	96.1 ± 7.9	0.145	
After 6 weeks of treatment	85.9 ± 7.0	90.1 ± 7.9	***0.016***	2.3 (0.097)
Mean arterial				
Baseline	112.3 ± 7.0	115.0 ± 7.9	0.111	
After 6 weeks of treatment	102.6 ± 7.0	107.9 ± 9.2	***0.001***	2.6 (0.107)
Mean nighttime BP				
Systolic				
Baseline	127.7 ± 12.1	134.8 ± 12.9	***0.012***	
After 6 weeks of treatment	116.8 ± 12.8	123.7 ± 14.2	***0.022***	0.0 (0.996)
Diastolic				
Baseline	77.9 ± 9.7	81.3 ± 8.7	0.098	
After 6 weeks of treatment	71.2 ± 8.8	74.8 ± 8.5	0.064	0.2 (0.891)
Mean arterial				
Baseline	95.1 ± 8.9	99.2 ± 8.9	***0.037***	
After 6 weeks of treatment	87.3 ± 9.1	91.7 ± 9.6	***0.033***	0.4 (0.750)
Mean morning BP				
Systolic				
Baseline	143.4 ± 15.3	145.9 ± 15.8	0.341	
After 6 weeks of treatment	127.3 ± 16.9	137.6 ± 17.1	***0.007***	***7.4 (0.015)***
Diastolic				
Baseline	89.6 ± 10.7	91.8 ± 10.4	0.341	
After 6 weeks of treatment	79.7 ± 10.5	86.1 ± 9.8	***0.006***	3.9 (0.052)
Mean arterial				
Baseline	107.6 ± 11.2	109.6 ± 11.3	0.437	
After 6 weeks of treatment	96.1 ± 11.5	103.0 ± 11.0	***0.006***	***4.8 (0.030)***
Nondippers†, %				
Baseline	14.7	32.7	0.062	
After 6 weeks of treatment	17.4	20.4	0.753	(0.778) §
Optimally treated, % of all				
Baseline	0	0	N/A	
After 6 weeks of treatment	28.6	13.0	0.067	(0.067) §

Abbreviations: BP refers to blood pressure; MAB, mean arterial pressure. Continuous variables are expressed as mean ± SD. Means between groups were compared with independent sample student t tests, and categorical variables were compared using 2-tailed Pearson χ^2 tests. *P value for differences in mean between subjects with and without OSA. †P value for the difference in change in blood pressure between baseline and final visit in subjects in the obstructive sleep apnea (OSA) vs no-OSA groups. §P value for the difference in change in the ratio: baseline visit/6 week visit, between the OSA and no-OSA groups

Losartan significantly reduced SBP, DBP, and MAP in both groups, but the reduction was less in the OSA group (without OSA: 12.6, 7.2, and 9.0 mmHg, respectively; with OSA: 9.8, 5.7, and 6.1 mmHg, respectively). This difference in response to losartan was especially pronounced during the early morning hours (Figure 5).

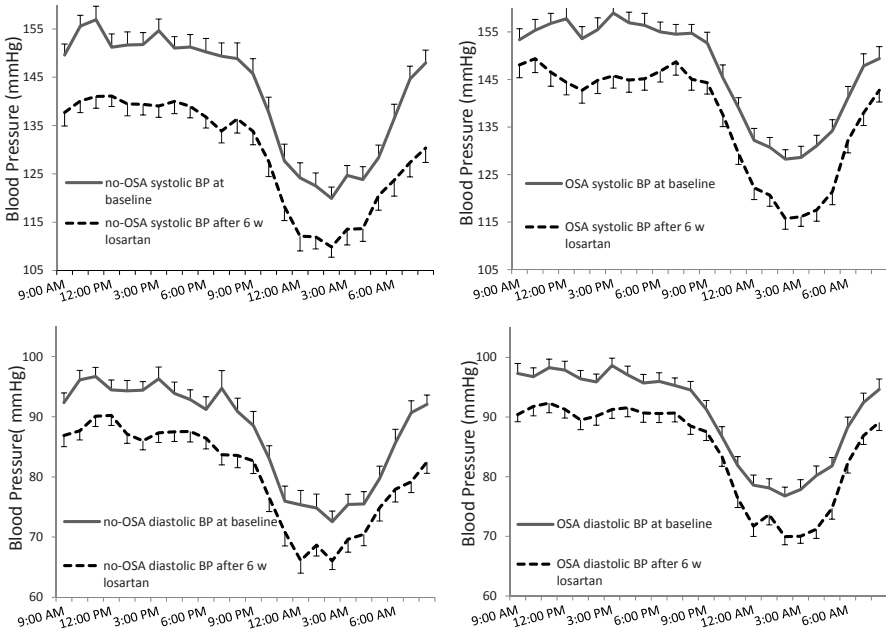

Figure 5. Ambulatory blood pressure (BP) profile of hypertensive patients with or without obstructive sleep apnea (OSA) at baseline and after 6 weeks of losartan treatment.

Add-on CPAP treatment in the patients with OSA led to no significant changes in 24-h blood pressure values except for in their night-time SBP (4.7 mmHg) (Figure 6).

However, all 24-hour blood pressure values were reduced significantly in the 13 patients with OSA who used CPAP at least four hours per night.

If the cut-off level for CPAP compliance instead was set to a CPAP use of at least three hours per night, the reduction in blood pressure was only significant in 24-h SBP and at a lower level of significance, indicating a dose–response effect of CPAP treatment on blood pressure.

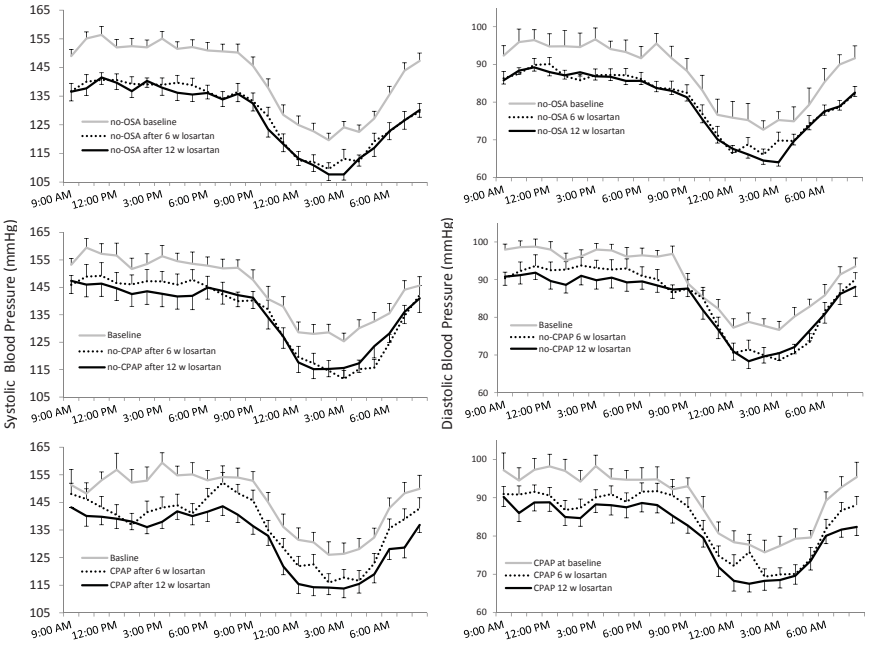

Figure 6. Ambulatory systolic and diastolic blood pressure profile of the subgroups (no-OSA, OSA-CPAP OSA-no-CPAP) at baseline, after 6 weeks of losartan treatment, and after 12 weeks of losartan treatment.

Paper IV

Neuroendocrine and inflammatory responses to CPAP in hypertension with obstructive sleep apnea: A randomized controlled trial

Blood samples were collected from all participants at baseline, after six weeks, and after 12 weeks for analysis of renin, aldosterone, noradrenaline, adrenaline, and inflammatory markers. Fifty-four patients with OSA and 35 without OSA who had analyzable blood samples were included in the analysis. Losartan significantly increased the renin and reduced the aldosterone levels in the non-OSA group with newly diagnosed hypertension, whereas there was no significant decrease in aldosterone among OSA patients with newly diagnosed hypertension. A trend towards a significant reduction was seen in adrenaline as well.

Add-on CPAP treatment tended to lower both aldosterone ($p=0.080$) and noradrenaline ($p=0.053$). No significant changes in inflammatory markers were observed following treatment with losartan and CPAP (Figures 7 and 8).

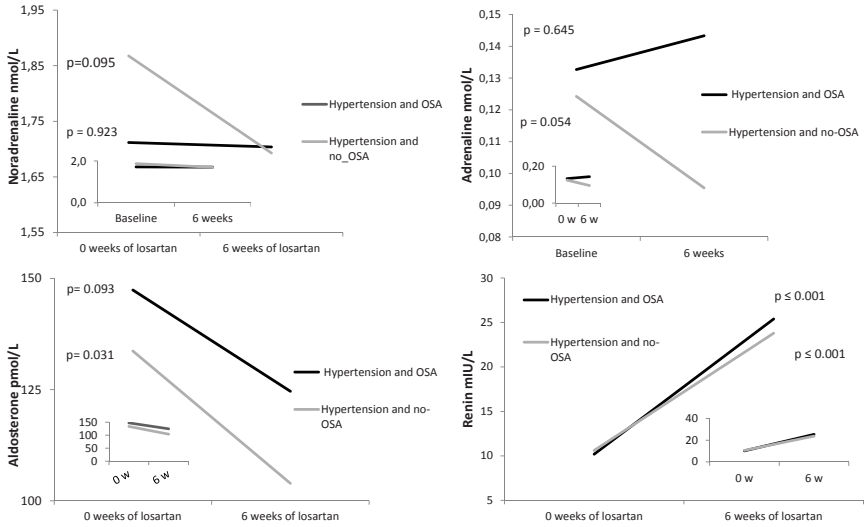

Figure 7. Effect of 6 weeks of losartan treatment on noradrenaline, adrenaline, aldosterone and renin in OSA and no-OSA patients.

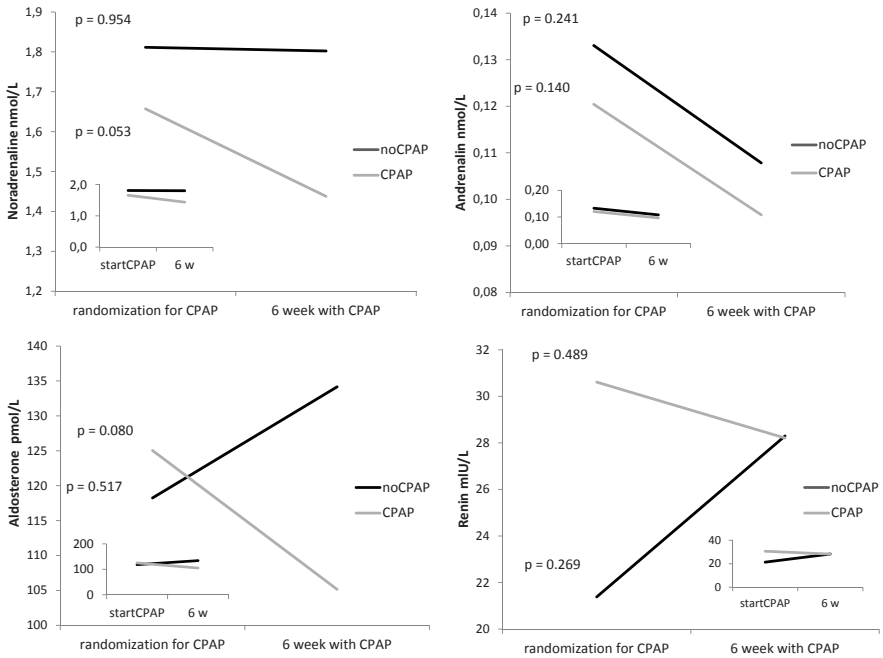

Figure 8. Effect on noradrenaline, adrenaline, aldosterone and renin add-on treatment of CPAP on top off losartan treatment for 6 weeks.

RICCADSA

Paper I

As shown in Table 5, the nonobese CAD group with OSA was older, consisted of more men, had higher BMI and waist circumference, and the proportion of subjects with abdominal obesity, hypertension and CABG at baseline was higher whereas concomitant lung disease was less common compared with nonobese CAD patients without OSA. The proportion of lean patients was also lower in the nonobese OSA group. When comparing obese OSA patients with the nonobese OSA group, the obese subjects were slightly younger, and had higher AHI, ODI and ESS. The proportion of women and patients with diabetes mellitus as well as lung disease were also higher in the obese group.

Table 5. Demographic and clinical characteristics of the revascularized CAD patients

	Nonobese		Obese	
	Non-OSA (AHI<5) (N=95)	**OSA (AHI≥15) (N=234)**	**OSA (AHI≥15) (N=110)**	**P-value (ANOVA or x^2)**
Male sex (%)	74.7	87.2**	78.2†	0.012
Age (years)	61.4 ± 9.5	65.3 ± 7.1***	62.9 ± 8.6†	<0.001
BMI (kg/m^2)	25.2 ± 2.5	26.8 ± 2.1***	33.5 ± 3.3†††	<0.001
Lean/ BMI<25 (%)	44.2	18.8***	0.0†††	<0.001
Waist-Hip-Ratio	1.10 ± 0.98	0.95 ± 0.06	1.01 ± 0.08†††	0.151
Abdominal obesity (%)	78.9	91.6**	100††	<0.001
Waist Circumference	91.5±8.5	98.1±8.0***	113.2±9.8†††	<0.001
AHI (n/h)	3.1 ± 1.3	28.9 ±13.7***	31.5 ± 16.2	<0.001
ODI (n/h)	1.6 ± 1.3	16.9 ±12.3***	19.9 ± 16.9	<0.001
ESS score	5.8 ± 3.0	7.8 ± 3.9***	8.8 ± 4.2†	<0.001
Current smokers (%)	22.1	15.0	15.5	0.268
Prior revascularization (%)	15.8	19.0	21.3	0.605
CABG intervention (%)	16.8	28.2*	20.0	0.052
Hypertension (%)	45.3	59.4*	69.1	0.002
Diabetes mellitus (%)	12.6	15.4	40.9†††	<0.001
Atrial fibrillation (%)	1.1	2.2	3.7	0.458
Lung disease (%)	12.6	5.2*	13.6††	0.013
Stroke (%)	3.2	7.3	2.8	0.125
Depression (%)	3.3	6.2	2.9	0.316
Lipid lowering treatment	94.4	94.3	96.4	0.707

*p <0.05; **p <0.01; ***p <0.001 (for comparisons within the nonobese group). †p<0.05; ††p<0.01; †††p<0.001 (comparing nonobese OSA and obese OSA patients). Continuous variables are expressed as mean ± Standard deviation, and dichotomic variables as numbers. One way analysis of variance (ANOVA) for continuous variables with post hoc analyzes with Bonferroni if equal variance and with Games Howell if no equal variance was found. Pearson chi-square test was used for variables in percentage. Definition of the abbreviations: CAD=Coronary Artery Disease; OSA=Obstructive Sleep Apnea; BMI=Body Mass Index; AHI=Apnea Hypopnea Index; ODI=Oxygen Desaturation Index; ESS=Epworth Sleepiness Scale; CABG=Coronary Artery Bypass Grafting.

Circulating levels of hs-CRP, IL-6, IL-8, and TNFα were assessed in relation to the OSA diagnosis-based ODI ≥5 events/h. Non-obese patients with OSA had significantly higher levels of hs-CRP and IL-6 than non-obese patients without OSA. The values did not differ significantly between obese and non-obese patients with OSA. In bivariate regression analysis, AHI ≥15 events/h was associated with all four biomarkers but not so in the multivariate model after adjustment for confounders (Table 6).

Table 6. Association between OSA, based on AHI and ODI cut-off values, and the inflammatory biomarkers adjusted for the confounding variables in the non-obese CAD cohort

	Multivariate odds ratios with 95% CI		
	AHI ≥ 15 events/h (95 % CI)	**ODI ≥ 5 events/h (95 % CI)**	**ODI ≥ 15 events/h (95 % CI)**
LnHs-CRP	1.20 (0.91;1.57)	1.49 (1.13;1.99)**	1.38 (1.07;1.78)*
LnIL-6	1.27 (1.00;1.55)	1.30(1.05;1.60)*	1.12 (0.92;1.36)
LnIL-8	1.29 (0.88;1.90)	1.32 (0.91;1.90)	1.09 (0.78 ;1.52)
LnTNFα	1.22 (0.77;1.93)	1.19 (0.77;1.84)	0.90 (0.60;1.36)

*p <0.05; **p<0.01. Variables used in the model: age, gender, body mass index, waist circumference, abdominal obesity, hypertension, and lung disease. (OSA=obstructive sleep apnea; AHI=apnea-hypopnea index; ODI=oxygen desaturation index; CI=confidence interval; Ln=natural logarithm; Hs-CRP=high sensitivity C-reactive protein; IL-6=interleukin-6; IL-8=interleukin-8; TNFα=tumor necrosis factor alpha).

Paper III

Circulating levels of hs-CRP, IL-6, IL-8, and tumor necrosis factor-α were assessed at baseline and after one year in 446 patients. CPAP treatment had no significant impact on levels of the inflammatory biomarkers in non-sleepy OSA patients in intention-to-treat or per-protocol analysis. Significantly higher mean levels of IL-6 were found at baseline in OSA patients with daytime sleepiness (18.5 pmol/L) compared to levels in non-sleepy OSA patients (5.6 pmol/L in the CPAP group vs 10.0 pmol/L in the no-CPAP group), and in the non-OSA group (6.8 pmol/L; p=0.011). Corresponding values after one year were 4.3, 2.8, 4.4, and 4.3 pmol/L, respectively (p=0.010 for change from baseline between groups). No linear association was observed between changes in the inflammatory biomarkers and CPAP hours in patients with OSA (Table 7).

When only comparing sleepy and non-sleepy patients with OSA without any adjustments, there was a difference in the decrease of IL-6 at the 1 year follow-up, however the difference were not statistically significant. Furthermore, the patients with no-OSA had a lower decrease in IL6 compared to those with OSA and daytime sleepiness both in absolute values and in percent of baseline values for percentile change in IL-6 (Figure 9).

Table 7. Demographic and clinical characteristics of the revascularized patients in paper IV

Variable*	**Positive controls** Sleepy OSA on CPAP (n = 134)	**RCT group** Nonsleepy OSA on CPAP (n =104)	Nonsleepy OSA no-CPAP (n =108)	**Negative controls** No-OSA (n =100)	**p-value** Nonsleepy OSA on CPAP *vs* no-CPAP	**p-value** Sleepy *vs* Nonsleepy OSA on CPAP	**p -value** All groups (n =446)
Age (y)	62.6±7	65.4±8	66.6±8	61.5±10	1.0	0.081	*<0.001*
AHI, events/h	32.7±16	28.0±12	29.2±14	3.0±1	1.0	*0.033*	*<0.001*
ODI events/h	21.6±16	16.2±10	16.4±11	1.5±1	1.0	*0.006*	*<0.001*
ESS score	12.2±2.4	5.6±2.3	5.5±2.2	5.73.0	1.0	*<0.001*	*<0.001*
BMI, kg/m^2	29.6±4.5	28.3±3.7	28.5±3.5	25.6±3.0	1.0	*0.019*	*<0.001*
Obesity (%)	39.6	26.9	27.8	7.0	0.889	*0.041*	*<0.001*
Female sex (%)	9.7	17.3	13.9	25.0	0.492	0.084	0.015
Current smoker (%)	15.4	17.5	14.3	24.2	0.529	0.679	0.256
Pulmonary disease (%)	8.2	9.3	3.9	13.0	0.117	0.175	0.139
Hypertension (%)	56.5	57.9	70.9	48.0	0.051	*0.024*	*0.10*
Prior myocardial infarction (%)	61.9	67.0	69.2	65.0	0.736	0.422	0.683
CABG before inclusion (%)	23.9	25.0	26.9	17.0	0.758	0.842	0.365
Diabetes mellitus (%)	23.9	28.2	18.5	13.8	0.097	0.456	*0.045*
p-NT-proBNP, ng/L	353	462	622	321	0.561	0.475	*0.004*
Medication use							
β-blocker (%)	91.0	90.9	84.9	80.2	0.189	0.986	0.056
ASA	86.6	93.9	85.8	96.9	0.056	0.067	0.013
Clopidogrel	60.4	63.0	50.0	67.4	0.691	0.060	0.073
Diuretic (%)	21.8	21.2	22.9	9.5	0.777	1.0	0.057
CCB (%)	21.1	21.2	15.1	10.4	0.255	0.977	0.116
ACE inhibitor (%)	34.6	43.4	48.1	38.5	0.502	0.170	0.175
ARB (%)	17.2	14.1	16.0	4.2	0.705	0.517	0.023
Lipid-lowering agent (%)	94.8	97.0	92.5	89.5	0.152	0.414	0.166

*Continuous variables are expressed as mean ± SD, and comparison of means between groups was done by one-way analysis of variance with post hoc Bonferroni if equal variance was assumed and with Gammes-Howell if unequal variance was assumed. Categorical variables are expressed as percent of the total and comparsion between the groups were done by chi-squared test or Fisher's exact test (two tailed). *Abbreviations:* RCT refers to randomized controlled trial; OSA, obstructive sleep apnea; CPAP, continuous positive airway pressure; AHI, apnea hypopnea index; ODI, oxygen desaturation index; ESS, Epworth Sleepiness Scale; BMI, body-mass-index; MI, myocardial infarction; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; CCB, calcium channel blocker; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker. †Anticoagulant use refers to aspirin and/or clopidogrel and/or warfarin use.

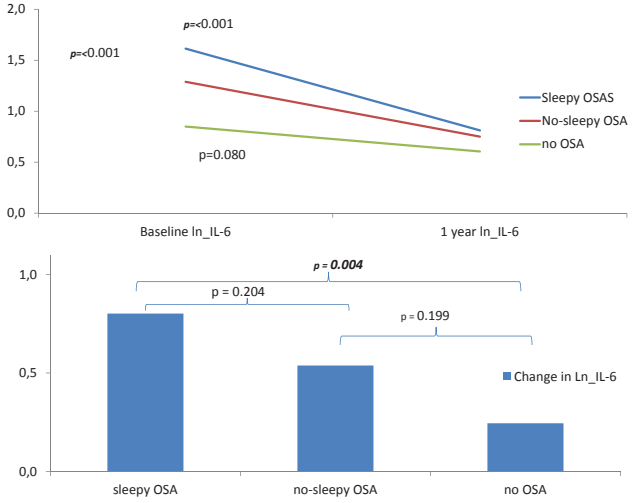

Figure 9. Difference in natural logarithm of IL-6 between baseline and one year visit in all three groups measured with paired T-test. Difference in change in natural logarithm of IL-6 between the sleepy OSA, no sleepy OSA, and noOSA independent of CPAP treatment or not, measured with ANOVA and adjusted for multiple comparisons with Games-Howell due to skewed distribution. *Abbreviations*: OSA; obstructive sleep apnea, ln_IL-6; natural logarithm of interleukin 6.

DISCUSSION

"I'm not a very good sleeper. But you know what? I'm willing to put in a few extra hours every day to get better. That's just the kind of hard worker I am."

- Jarod Kintz

Discussion of methods

When designing a study there are several factors that need to be considered:

- What type of study is best suited to answerer the research question?
- Do we have the experience and capability to complete the study?
- Does one specific study design add something novel to the field?

In the research field of OSA and CVD and the interactions between the two conditions, many mechanistical studies both on animals and humans (recruited from a sleep clinic cohort) have been conducted to establish whether OSA could induce CVD. Epidemiological observational studies, both cross-sectional and longitudinal, have established the prevalence of OSA in the general population[50,67,73,81,175] as well as in subgroups with different comorbidities, e.g. hypertension and CAD among others[117,176]. Furthermore some longitudinal cohort studies have investigated the effect of CPAP on the risk of developing CVD mainly in longitudinal follow-up of sleep clinical cohorts. There are a few studies that have screened for OSA in hypertensive and CAD cohorts and then observed what happened with those who were treated for their OSA with CPAP in a observational design[19]. However, in 2005 (at the time of the start of the RICCADSA trial) there were no RCTs which had studied the effect of CPAP on hard endpoints such as death, new cardiovascular events or new revascularization in patients with severe CAD. Although CPAP seemed to reduce circulating inflammatory markers (as a marker for increased risk of CAD)[137] in subjects from a sleep lab cohort, nothing had been done to study whether this was true for a cohort where all patients already had established CAD. For hypertension, CPAP treatment had been shown to have a modest effect in several case-control studies[101], even though some studies showed greater effect . There were however many limitations to these studies, the greatest being that hypertension was not present at baseline in most of them. This will be discussed in more detail later.

We thought RCTs would be the best way to answer our research questions, mainly because it is generally considered to have the highest research validity when studying efficacy of treatment. Moreover since it had not been done previously, our studies would fill a gap in scientific understanding in the research field.

Sample size considerations

The major concern when designing an RCT is that it easily could be underpowered, which we were aware of, especially for the RICCADSA trial. Since the paper III investigated secondary outcomes, and the sample size was decided from the power calculation for the primary endpoints, this might be a limitation. However, this is a relatively large trial, and in sleep clinic cohorts, significant differences in change

between a group on CPAP treatment and a group without CPAP treatment has been observed in studies with smaller sample sizes[177]. Thus, we consider that a type II error is less likely to be present in paper III (where negative results were observed). For LosartanPAP, the first power calculation applied a power of 90%. However, due to feasibility problems (hard to recruit the pre-specified number of patients with OSA), a reduced power of 80% was accepted. This lower power led to an increased risk of missing a true difference between the groups. However, the fact that we found differences between the groups in paper II further strengthens our study results even though we had less power than originally planned. For paper IV, no power calculation was performed, and we cannot exclude the possibility of a type II error, since the confidence intervals are rather wide, especially for aldosterone.

Potential sources of random error

There are some sources for potential random error in the variable used to define which group an individual belonged to; the OSA variable, both in the RICCADSA and the LosartanPAP study. This was based on AHI manually scored after one night of polygraph recording. Since there is some night-to-night variability in sleep recordings, a patient could have AHI >5 one night and AHI <5 another night. Such an error would have most grave consciences for those patients with borderline OSA (with an AHI around 5). To avoid patients without OSA being assigned to the OSA group and vice versa, all patients with mild OSA (those with AHI 5-14.9) were excluded from both LosartanPAP and the RICCADSA study. By doing so, the probability of having patients with moderate OSA in the non-OSA group was also reduced.

In paper II, the outcome variable, hypertension, was based on 24-h ABPM, which could be influenced by shift work, high alcohol consumption, salt intake, as well as other medications. To avoid any effect of varying work hours, patients on shift work ensured that they worked day shifts in association with the study check-ups. Patients were asked about their alcohol consumption, eight of those who completed the LosartanPAP trial had a risk-level consumption of alcohol (more than 14 units of alcohol per week), but there was no significant difference in how they were distributed between the groups (four in the non-OSA group, one in the group without CPAP treatment, and three in the CPAP group). It was not possible to require restrained salt intake since this would make it hard to recruit patients to the study. However, the randomization might in part level off these errors by distributing these subjects equally in both groups.

In papers I, III, and IV, temporary infection or inflammations could led to a random error, but in the RICCADSA study the sample size was so large that temporary changes in a few patients would probably not influence the results, especially since all inflammatory variables were converted to the natural logarithm of the original variable. In LosartanPAP, occasional high values could influence the results, but specifically excluding extreme values that were 10 times higher than all others did not result in a different outcome. Therefore, we do not think that this should be considered a major source of random error.

In paper IV, differences in aldosterone and renin might be caused by changes in salt intake and body position when the blood samples were collected. We strived to collect

the blood samples from the patients by having them in a horizontal position to minimize errors secondary to position changes. However, we could not limit salt intake .

Potential sources of systemic error

We used the scoring criteria from AASM published in 1999[53], because that was what was clinically in use in the sleep unit when the RICCADSA study started. Since we wanted to be able to compare the sleep recordings from LosartanPAP study and the sleep recordings from RICCADSA study the same scoring criteria was used in the LosartanPAP study, even though new guideline had just been published when this study was designed[178]. The 1999 definitions of hypopneas were less strict, and thus more episodes with disturbed breathing could be scored as hypopneas, resulting in a higher AHI score compared to the later guidelines. As previously described, all patients with mild OSA (AHI 5-14.9) were excluded and for this reason a possible systemic overestimation of OSA patients in the study would be much less likely. Furthermore, in RICCADSA, polysomnography was conducted in all patients who had AHI ≥15 on their polygraph. Only five out of 399 had AHI <5, which was attributed to night-time variability since most of these five had moderate or severe OSA and thus could have lower AHI if they did not sleep in supine position on the tpolysomnography-night.

For the 24-h ABPM, the registration of all data values was double-checked by a monitor blinded to which group the participants were in. Given that the blood samples were objective measurements that were analyzed by study-independent labs with long clinical experience, we therefore think there is a low probability for type I error or systematic errors in any of the studies.

Self-selection bias

In the RICCADSA trial, we found that patients who did not participate in the study were slightly older, had more comorbidity, and more were women compared to those who did not participate but went through a mechanical revascularization in Skaraborg county during 2005 until 2010. This has already been published and does not make this RCT different from other RCTs which show similar results[162]. In the LosartanPAP study there could have been some self-selection since we advertised in newspapers for patients with hypertension and thus we might get patients with greater health awareness than the average hypertensive individual.

Investigator selection bias

Because of the strict inclusion criteria there is same degree of investigatory selection bias in the LosartanPAP study since we only recruited patients between 50-69 years of age, without medication or comorbidities. The rational for this was clear: since we had a small sample size, we did not include too much potential confounders and, so long as the results are not extrapolated to other groups, we do not think this would be a problem.

The RICCADSA trial had very loose inclusion criteria, making it relatively representative of the typical revascularized population, but it does introduce other potential confounding factors because the cohort was so heterogeneous.

The risk of statistical bias

Statistical bias is often caused when random statistical analyses are conducted and a hypothesis is produced based on the statistical results. We tried to avoid this bias by formulating clear research questions, and one or two hypotheses for each paper in advance of the analyses. We therefore think there is very little chance of type I error and statistical bias in our papers.

External validity

Applicability

Extrapolating results derived from soft endpoints (inflammatory markers and neuroendocrine hormones) to hard endpoints such as death or new cardiovascular events should be treated with great caution. However, the results can be used to draw conclusions regarding the possible mechanisms in which OSA escalates the development of hypertension and CAD. Since we used very strict inclusion criteria for the patients with early CVD (newly discovered, untreated hypertension), we think that the study was well designed to examine the effect of both losartan and CPAP on hypertension and the mechanisms that could mediate the effect. Since the group studied was uniform the risk of biased results due to comorbidity are low. The RICCADSA population might have the problem of comorbidity, but on the other hand it very well represents a revascularized population, which makes the study clinically very relevant.

Generalizability

We compared the patients in the RICCADSA study with all patients revascularized in Skaraborg during the same period[161] without finding any significant differences except what was previously shown to be typical for comparison of individuals who participate in studies and those who chose not to. We therefore think that our results are generalizable to a revascularized cohort. However, since most participants were of Caucasians origin, the results may not be applicable to other ethnicities.

The recruitment period of the LosartanPAP study was much longer than expected when the study was designed. It could be argued that the cohort therefore might not be truly representative, since it should not take that long to find individuals with hypertension. This could indicate that those we found constitute a population that is particularly willing to participate in studies.

However, we consider the long recruitment period a logistical problem rather than a problem with representativeness. Most patients with newly discovered, untreated hypertension are found by the general practitioners and we had not established a good enough contact with these physicians before the start of the study, which resulted in a long inclusion period. Nonetheless, we do think that the results can be applied to most patients with newly discovered, untreated hypertension that meet the rather strict inclusion criteria of this study.

Finally, the LosartanPAP population were also mainly of Caucasian origin and therefore other ethnicities might react differently to both losartan and CPAP treatment.

Discussion of results

Effect of losartan on blood pressure in OSA and new-onset hypertension

In one small study comparing the effect of different types of antihypertensive medication on patients with OSA, Kraiczi et al. showed that beta-blockers (atenolol) had the best effect, and that ARB blocker (losartan) had the least effect, on blood pressure. The study should be regarded with caution, since the subgroups were very small, but the finding is interesting in terms of generating new hypotheses[179]. This difference in effect of two types of blood pressure medication in patients with OSA might either be due to the increased sympathetic activity seen in patients with OSA[154, 155] or due to an alteration in the RAAS activity[149,180], making the patient partly resistant to medication that affects RAAS activity.

Several studies have shown that sympathetic activity is upregulated in patients with OSA[154-156]. However, studies on RAAS activity and OSA, as well as on RAAS activity, OSA, and hypertension, are scarce and the results presented are disparate. Some found no difference in RAAS activity in patients with OSA and some found an association between RAAS activity and OSA severity, while other studies could only find this in patients with OSA and hypertension[149,151,152,153,180].

We found that low-dose losartan (50 mg) reduces blood pressure significantly in both OSA and non-OSA patients, but those with OSA had higher blood pressure already at baseline and this difference between the groups was enhanced after six weeks of losartan treatment. Thus, losartan might be slightly less effective in individuals with OSA. This is further supported by the aldosterone response to losartan treatment seen in the first phase of paper IV (see below).

Interestingly, patients with OSA did not have the same positive effect of losartan on morning blood pressure. This morning surge in blood pressure has previously been shown to increase risk of CVD, so it is important to treat it effectively[181]. Recently, Cho et al. showed that morning rise in blood pressure was higher in patients with OSA and untreated hypertension compared those with untreated hypertension only, which supports our results of the association between morning blood pressure and OSA[182]. How much of these morning surges in blood pressure is driven by dysregulation of the RAAS activity, and how much can be attributed to increased sympathetic activity, is an open question. However, losartan did not have much effect on morning blood pressure in the OSA group, which indicates that OSA could play an important role in inducing these surges. The fact that CPAP treatment seemed to reduce morning blood pressure and noradrenaline concentrations further strengthens a link between morning surges and OSA, possibly though increased sympathetic activity. Our data on morning surge is rather speculative, especially since we only looked at the mean blood pressure two hours after awakening and did not subtract the mean blood pressure two hours before awakening, as would normally be the case when taking these measurements[181]. It has been proposed that the non-dipping pattern in some patients with hypertension is driven by excessive aldosterone concentrations, and Satoh et al. indirectly showed that a dysfunction in the RAAS system could be involved in driving this[183]. Our results

further support this, even though the significance was borderline: we found almost twice as many OSA patients with hypertension (one in three) with a non-dipping pattern compared to patients with hypertension only (one in six). This difference was attenuated after six weeks of losartan treatment, indicating that it could be excessive aldosterone production that causes the non-dipping pattern. Nonetheless, the fact that we did not see any decrease in the aldosterone levels in the patients with OSA during the first six weeks on losartan treatment contradicts this.

Effect of add-on CPAP treatment on blood pressure in novel hypertension

Previous studies investigating the effect of CPAP on hypertension have shown disparate results, ranging from no effect at all to relatively good reduction[177,184]. In a recent meta-analysis of 30 RCTs conducted up to 2013 addressing the effect of CPAP on blood pressure in a total of 1,906 subject, Fava et al. showed that CPAP seemed on average to give a modest reduction in blood pressure, reducing SBP by 2.6 ±0.5 and DBP by 2.0 ± 0.4 ($p<0.001$)[101]. However, there are major limitations to many of the studies. Only 16 studies had blood pressure as an endpoint. Of these, only 11 used 24-h ABPM which is an important tool for comparing differences in night and day blood pressure[1]. Moreover, there were only four studies in which hypertension at baseline was an inclusion criterion[102,184-186]. Two of these studies could not find any significant effect of CAP on hypertension. Robinson et al. included patients who were referred to the sleep clinic for OSA assessment and who did not have daytime sleepiness. They could not find any effect of CPAP on blood pressure after four weeks of CPAP treatment; however, in this study the placebo group also received sub-therapeutic treatment with CPAP and their blood pressure decreased by 3 mmHg. Sub-therapeutic CPAP treatment has been criticized for not being a suitable placebo treatment because it partly reduces AHI, which could give a blood pressure reduction in the control group as well. In the other study that could not show a blood pressure decrease after CPAP treatment, by Campos-Rodriguez et al., patients with hypertension from a sleep clinic were treated with CPAP for four weeks. There are several possible explanations for the lack of effect on blood pressure, in particular that all patients were taking at least one hypertensive drug and were already almost optimally treated at the start of the study; thus, they did not have high blood pressure in the sense that it was uncontrolled.

Among the two studies that did show significant results, one was conducted on patients with resistant hypertension, and in that Lozano et al. observed a reduction in DBP of 4.9 ± 6.4 in intention-to-treat (ITT) analysis of those randomized to CPAP, and those who followed their CPAP treatment had an even greater reduction (9.7 for SBP and 7.0 for DBP) compared to the control group[186]. Finally, the largest study on patients with hypertension and OSA where 24-h ABPM was used was conducted by Durán-Cantolla et al. They studied 340 patients who had recently been diagnosed with hypertension by a general practitioner and they found CPAP to reduce SBP by 2.1 and DBP by 1.3. It was statistically significant but was considered to have uncertain clinical relevance. Interestingly, even though these were newly diagnosed hypertensive individuals, they had almost normal blood pressure at baseline[102]. This indicates that different subgroups of hypertensive individuals (well-controlled vs uncontrolled) could responded very different to CPAP treatment, possibly due to different mechanisms involved at difference stages in the development of high blood pressure.

The greater effect seen in patients with resistant hypertension is supported by a more recently published meta-analysis of studies (observational and RCT) investigating resistant hypertension. Iftikar et al. found an SBP reduction of 7.2 mmHg 95% confidence interval (CI): 9.0 to 5.4; p<0.001) and 5.0 mmHg (95% CI: 6.0 to 4.0; p<0.001) for DBP. Thus, the benefit of CPAP on blood pressure reduction seems to be much greater for patients with resistant blood pressure than for subjects with well-controlled blood pressure.

Only one study has so far been conducted in which CPAP treatment was compared to a hypertensive medication. Pepin et al. showed that valsartan (160 mg) was superior to CPAP treatment in individuals with hypertension and OSA where everyone was native to any treatment for their blood pressure or their OSA. Interestingly in the same trial, they studied the effect of the combination of CPAP and valsartan in an open substudy in some patients after the RCT and found an additive effect of combining the two treatments[187], with net reduction in SBP of approximately 4 mmHg in 24-h SBP, 1.5 mmHg in 24-h DBP, and 2.5 in 24-h MAP. However, they report that these trends toward reduction were only significant in office blood pressure, and not for the 24-h ABPMs. A possible explanation for this could be that office blood pressures are usually taken in the morning, and the results of the LosartanPAP study would confirm the figures published in the paper by Pepin et al. that the effect of CPAP on blood pressure seem to be greatest in the morning hours[187].

The LosartanPAP study is the first RCT in which all patients were recruited from a primary care cohort and all had uncontrolled high blood pressure. Our results indicate a slightly weaker blood pressure effect of CPAP on newly diagnosed untreated hypertension compared with the CPAP effect on resistant hypertension[104]. Interestingly, we found a greater effect of CPAP than was seen in well-treated hypertensive patients[102], further supporting the hypothesis that the response to CPAP can be dependent on multiple factors, such as other medication and the fact that patients who are in different stages of the hypertension disease progress when CPAP treatment is applied could respond very differently.

Moreover, the fact that our result is similar to the results seen in the open study by Pepin et al. on the combination of valsartan and CPAP further supports an additive effect of the combination of a RAAS-blocking agent and CPAP. This possible additive effect indirectly supports the idea of some type of influence of OSA on the RAAS system.

Effect of losartan and CPAP on mechanisms that could induce hypertension

Several mechanisms have been proposed to play a vital role in the development of hypertension mediated or enhanced by concomitant OSA. We considered upregulated sympathetic activity, increased low-grade inflammation, and dysfunctional RAAS activity to be the three major systems that were most likely to be involved in a possible relationship between OSA and hypertension.

Sympathetic activity has been shown to be upregulated in OSA both when studying neural burst and circulating catecholamines, as well as urine catecholamines[154,155,188,189]. It is increased in patients with OSA independently of possible major confounder such

as obesity[157]. Treatment of OSA with CPAP has been shown to decrease sympathetic activity[158]. In animal models, losartan has also been reported to reduce stress-induced catecholamines in plasma[190].

When treating patients with hypertension and OSA with losartan, there was only a reduction of borderline significance in both noradrenaline and adrenaline in the non-OSA group. The adrenaline concentrations in the OSA group were unchanged and an increase in noradrenaline was observed. This indicates that if losartan has a reducing effect on stress hormones, concomitant untreated OSA might obliterate this effect.

A reduction in aldosterone concentrations after losartan treatment has been observed in hypertensive individuals[191]. However, it seems to be attenuated by factors that up-regulate the RAAS system, such as high salt intake[192]. Very interestingly, we found a significant reduction of aldosterone concentrations only in those hypertensive individuals who did not have concomitant OSA. This might suggest that OSA induces a subclinical resistance in the RAAS. This is further supported by the correlation observed between increase in aldosterone levels and severity of OSA in patients with resistant hypertension[152].

Treatment with CPAP has been shown to reduce sympathetic activity in patients with OSA[158]. Moreover, in a recent analysis, Nicholl et al. showed a reduction of plasma aldosterone after CPAP treatment in normotensive OSA subjects[193]. Thus, in normotensive OSA patients, both sympathetic and RAAS activity is reduced after CPAP treatment. Our findings that there was a tendency towards reduction both in aldosterone concentrations and in noradrenaline concentrations after CPAP treatment were therefore in line with these previous findings in non-hypertensive subjects. We attribute the fact that it did not reach significance to small sample size in combination with the lack of salt restrictions, which probably explains the rather wide confidence intervals observed in the aldosterone concentrations at baseline, after six weeks, and after 12 weeks in the LosartanPAP study.

A recently published article demonstrated a less pronounced effect of losartan in combination with a thiazide diuretic in patients who had higher levels of inflammation at baseline. This implies that it could be an association between low-grade inflammation and the RAAS[194]. This is further supported by the fact that angiotensin receptor blockers have been shown to reduce hs-CRP levels in patients with hypertension. However, this could not be shown in patients from the LosartanPAP cohort independently of whether OSA was present or not. Furthermore, even though hs-CRP, IL-6, TNFα, and IL-8 all have been shown to decrease in some studies after CPAP treatment in patients with OSA from a sleep clinic cohort[137] . We could not find such an effect on the LosartanPAP cohort. This is possibly due to a selection bias, given that we had selected rather healthy hypertensive individuals without other comorbidities or medications. This is speculative and against this hypothesis is the fact that no reduction was seen in the RICCADSA cohort in response to CPAP either. Moreover, a recent meta-analysis of the effect of CPAP on inflammatory markers actually questions whether there is an effect of CPAP on inflammatory markers, even in subjects from a sleep clinic cohort[195].

Differences in inflammatory activity in patients with CAD depending on OSA and CPAP treatment

Low-grade inflammation is an essential part of the development of CAD[121,196]. An elevation of circulating inflammatory markers as a sign of low-grade inflammation is associated with increased risk of developing cardiovascular events. This association is seen both in the general population[133,197] and in patients with already established CAD[145,198]. Nevertheless, it is not yet recommended to screen for low-grade inflammation in order to reclassify patients at low CAD risk to higher CAD risk[135,199], partly because the number of patients who would have to be screened to stop one event would be very high[133]. In secondary risk management, however, hs-CRP has been proposed in addition to lipid screening so that statin treatment could be intensified based on the degree of low-grade inflammation[135,200].

Patients with OSA from sleep clinical cohorts have been reported to have elevated inflammatory markers[201] which can be reduced by CPAP treatment[202]. However, obesity is a major confounder when studying inflammation and OSA, due to the overlap of the two conditions[203,204] and it is thus important to adequately adjust for that. Furthermore, in a recent meta-analysis, Jullian-Desayes et al. concluded that, to date, no RCTs have been able to show reduction of inflammatory markers (IL-6, IL-8, TNFα or hs-CRP). To the knowledge of the author there are no RCTs on patients with OSA and CAD investigating the effect of CPAP on inflammatory markers.

From a Chinese cohort with both OSA and CAD, Zhao et al. showed that in 151 consecutive patients with optimally treated CAD there was an increase in hs-CRP with increasing severity of OSA. Moreover, those patients who were treated for their OSA with CPAP showed a significant decline in their hs-CRP. A weakness of this study was that they did not have any control group to describe the natural progress of hs-CRP in patients who have had instable CAD, and thus the study could not show whether the decline was due to CPAP treatment or not[205]. Our results from the baseline data of the RICCADSA trial support the results of Zhao et al., indicating that there seems to be an association between prevalence of OSA and increased inflammatory markers. We also saw a reduction in inflammatory markers in the patients with revascularized CAD and OSA treated with CPAP. However, this decline was seen in all patients who participated in the study, independent of whether they were treated with CPAP or not. Thus, we concluded that the decline is probably the natural development of the concentrations of inflammatory markers after a revascularization, rather than a consequence of CPAP treatment.

An interesting result of the study was that IL-6 was significantly higher at baseline in patients with OSA and daytime sleepiness compared those who had OSA but no daytime sleepiness. This is an exciting finding that needs to be further explored in studies addressing this specific research question.

CONCLUSION

"There is life, and there is death, and in between there is me. Please don't wake me up."
- Jarod Kintz

Main conclusion

In patients with recently discovered untreated hypertension, losartan lowers blood pressure both in patients with and without OSA. However, the effect seems to be less pronounced in those with OSA. When CPAP was added to losartan, blood pressure was reduced further, especially in those who used their CPAP device regularly for more than four hours (paper II). The less prominent effect of losartan on blood pressure in patients with OSA could hypothetically be explained by a subclinical upregulation of RAAS activity resulting in no decline in aldosterone after losartan treatment (paper IV). We propose that the reducing effect of CPAP on blood pressure could in part be explained by removing this upregulation of the RAAS activity, and in part by decreasing the sympathetic activity (paper IV).

Circulating markers of inflammation do not seem to be influenced by CPAP treatment in patients with newly discovered untreated hypertension (paper IV). Moreover, in patients who were newly revascularized, the concentrations of inflammatory markers were higher in those with OSA compared to those without OSA (paper I). The inflammatory markers were reduced after CPAP treatment, but this could not be attributed to the treatment because a similar decline was observed in the control groups (paper III). Thus, although inflammation has been shown to be reduced by CPAP in open cohort studies of patients from sleep clinic cohorts as well as from CAD cohorts, this RCT on the RICCADSA population could not find an effect, and thus, any effect in patients with different degrees of CVD seems to be modest at most.

Scientific relevance of this thesis

In this thesis we add several important pieces of evidence to the ongoing research into OSA and how it is related to CVD. The LosartanPAP trial is the first study to investigate the add-on effect of CPAP in addition to losartan on blood pressure in newly discovered, previously untreated hypertensive individuals. Though the difference was not statistically significant, the initial response to losartan was less in the OSA group, and CPAP treatment had an additive effect. This is further supported by the lack of decline in aldosterone levels in the OSA patients after losartan treatment, and the trend towards a reduction in aldosterone after add-on CPAP therapy. Finally, it may indicate that individuals with new-onset CVD have more benefits of CPAP treatment, before irreversible organ damages develop, compared to the benefits in patients with established CVD. Further studies on the topic are, thus, warranted.

We could not confirm our hypothesis that low-grade inflammation is reduced by CPAP treatment in patients with OSA and CVD. However, this does not mean that CPAP treatment has no effect on inflammation. There is a risk that the LosartanPAP study sample would have been too small to detect a possible difference in change between those who used their CPAP and those who had conventional treatment. The

RICCADSA trial would probably not miss an effect due to samples size, however, the greatest difference between OSA and non-OSA patients was seen where ODI ≥5 was used as cut-off for OSA diagnosis. In future RCTs, ODI rather than AHI, may be preferable to define OSA when studying the association with inflammation.

Clinical relevance of this thesis

The high prevalence of OSA in both hypertensive patients and CAD indicates that clinicians should be aware of the condition when evaluating these patients and actively ask about symptoms of OSA. The effect of CPAP on blood pressure underscores this further. A possible interaction between lower efficacies of losartan on blood pressure in combination with a poor reduction in aldosterone implies an interesting explanation to recent data suggesting good effect of aldosterone antagonists in resistant hypertension[206].

Public health relevance of this thesis

In epidemiologic studies, OSA has been associated with an increased health burden for society. In the near future we aim to look at the health burden of OSA in a CAD cohort (RICCADSA), but that is beyond the scope of this thesis. To date, we see no justification for screening for OSA to reduce inflammatory activity and thus reduce risk of new cardiovascular events. Nonetheless, there might be other public health reasons to screen for OSA among ischemic patients. To be able to answer whether it would be warranted, the main, primary results of the RICCADSA trial need to be published. However, it can be argued that at least the major research trials that study CVD patients should do some simple screening for OSA to be able to adjust for this factor, given that it is so common in CVD and seems to interact on many different levels. Researchers need to know how prevalent it is in their cohorts so that they can adjust for OSA in the same way that they adjust for other potential risk factors that could bias the results.

Hypertension is one of the leading causes of death worldwide[207]. Reducing DBP by 2 mmHg would result in a reduction of the yearly incidence of stroke or transient ischemic attack (TIA) by 13%, and the incidence of CAD events by 6% in an American population[208]. The observation of increased effect on blood pressure in the morning could indicate that treating OSA with CPAP in patients with newly discovered hypertension is especially effective in treating morning rise - an effect that we know is associated with increased risk of CVD[181].

Limitations

There are some limitations to the studies presented in this thesis. Due to the composition of the population in the area where the studies were conducted, the large majority of participants both in LosartanPAP and in RICCADSA were of Caucasian origin, making it difficult to apply our results to populations with other ethnicities. Furthermore, no people over 70 year of age or older were included in the in the LosartanPAP study. This was due to the reports that in older individuals the association between OSA and hypertension might be questioned[93]. Our results from the LosartanPAP study

could thus only be applied to hypertensive individuals under 70 years of age. Since the size of the RICCADSA population was reached by doing a power calculation for the endpoints of death or new cardiovascular events, there could be some uncertainty about whether the study was adequately powered for answering the research question: whether CPAP reduces inflammatory markers in patients with CAD. However, we were bound by the size of the main study and, as this is still one of the largest cohort studies measuring circulating inflammatory markers, we think that if the effect had been great we would have detected it.

The fact that we used polygraphy instead of polysomnography to diagnose OSA in both studies could be seen as a limitation. However, polysomnography is difficult to manage in a home sleep recording and it is also time-consuming to analyze the recordings. In the diagnosis of moderate and severe OSA, polygraphy has been shown to a good alternative[75]. Moreover, another argument for using polygraphy instead of polysomnography recordings is the feasibility. If future screening for OSA in patients with CVD are to be conducted outside a sleep clinic, less advanced devices would be used for sleep recordings, because general practitioners and cardiologists would probably not have access to, or be interested in, handling the more sophisticated polysomnography recorders.

A minor limitation is the fact that we used blood samples instead of sympathetic neural activity to measure sympathetic activity. Even though sympathetic neural activity would be preferable, it was not feasible to do that in the LosartanPAP study.

FUTURE PERSPECTIVES

"Of all the things a man may do, sleep probably contributes most to keeping him sane. It puts brackets about each day. If you do something foolish or painful today, you get irritated if somebody mentions it, today. If it happened yesterday, though, you can nod or chuckle, as the case may be. You've crossed through nothingness or dream to another island in Time."

- Roger Zelazny, Isle of the Dead

In the field of OSA and CVD there is much left to do. Up until now much work has been done to study patients from sleep clinic cohorts, but we do think that OSA should be considered a possible confounding factor in most studies that investigate CVD, in the same manner that hypertension, diabetes, hyperlipidemia, and smoking are.

Moreover, more RCTs need to be conducted to study the effect of CPAP on CVD, as well as on specific mechanisms inducing CVD. They should be carried out in several different populations, both the general population, especially with different age groups and both sexes, and in subgroups with specific diseases. It would be of great interest to study whether patients with resistant hypertension show similar results to the patients with newly discovered untreated hypertension in the research reported here, concerning hypertension, RAAS and sympathetic activity, as well as inflammation in response to CPAP treatment.

Much research is yet to be done in the RICCADSA trial, beyond the results already published or presented in this thesis. We aim to investigate the effect of CPAP on cardiovascular morbidity and mortality. (The primary outcomes of the RICCADSA trial have been submitted for publication at the time of the preparation of the current thesis). We will also study the following issues in the RICCADSA trial: the effect of CPAP treatment on echocardiographic variables, exercise testing, quality of life as well as on the health economy. The natural process of OSA over time in CAD patients not randomized to CPAP as well as and polysomnographic features associated with poorer outcome in any of the variables measured in RICCADSA trial are among the further analyses to work on for our research group.

Finally, it would be of interest to match patients with different degrees of OSA from different cohorts (general population, CAD population, and sleep clinic population) to see how IL-6 correlates with excessive daytime sleepiness in these cohorts.

In memory of Jan Östgärd (1952–2014):
the best dad there ever was.

SAMMANFATTNING PÅ SVENSKA

”Folk som säger att de sover som ett barn är i regel barnlösa”

Leo J Burke

Obstruktiv sömnapné (OSA) är ett tillstånd med upprepade andningsuppehåll under sömn sekundärt till att övre luftvägarna obstrueras när man slappnar av i muskulaturen runt dem. Epidemiologiska studier har visat att detta tillstånd är associerat med ökad risk för kardivaskulär sjukdom. Flera fysiologiska mekanismer har visat sig vara påverkade hos individer med OSA. Vad som har bedömts som viktigt och som studeras extra i denna avhandling är det låggradiga inflammatoriska påslag som setts hos patienter med sömnapné samt om detta bidrar till kranskärlssjukdom och högt blodtryck. Även ökade nivåer av stresshormon (adrenalin och noradrenalin) som ett tecken på en kronisk stressbelastning har noterats hos individer med OSA. Huruvida även den mineralkortikoida hormon-axeln (RAAS) är påverkad vid OSA är mindre känt. Dock skull en eventuell påverkan kunna ha stor effekt på utveckling av högt blodtryck, men även på effekten av blodtrycksbehandling. Med anledning av detta studeras om behandling av OSA har effekt på dessa system.

Denna avhandling berör två studier RICCADSA och LosartanPAP. I RICCADSA studien, vilken är en randomiserad kontrollerad studie vars huvudsyfte är att svara på om CPAP-behandling av OSA påverkar kardiovaskulära händelser. Vi fann en ökad nivå av inflammatorisk aktivitet bland kranskärlsjuka individer med OSA som nyligen revaskulariserats jämfört med patienter från samma grupp som inte hade OSA (artikel I). Ett år efter revaskularisering var det inte längre någon skillnad mellan grupperna avseende inflammatoriska parametrar. Bland individerna med OSA fick hälften behandling för sin OSA med kontinuerlig positiv övertryck andning på mask så kallad CPAP varje natt när de sov. Detta hade dock ingen betydelse för hur mycket de sjönk i inflammatoriska markörer (artikel III).

I LosartanPAP studien är en randomiserad kontrollerad studie, vars syfte var (1) att undersöka om blodtrycket hos individer med nyupptäck högt blodtryck sjönk mindre efter behandling med blodtryckspläkemedlet losartan om man samtidig hade OSA jämför med om man bara hade högt blodtryck, samt (2) att undersöka om tillägg med CPAP till losartan kunde bidra till ytterligare blodtrycksänkning och slutligen (3) att studera om svaret på losartan och CPAP skiljer sig mellan grupperna (ej OSA, OSA med CPAP och OSA utan CPAP) avseende inflammatorisk aktivitet, stresshormonnivåer (adrenalin och noradrenalin) eller förändringar på den mineralkortikoida axeln (renin och aldosteron).

Individer med OSA visade sig ha en tendens till mindre blodtryckssänkning vid behandling med Losartan. Om man behandlade dessa personer med CPAP sjönk blodtrycket dock ytterligare (artikel II). Den inflammatoriska aktiviteten påverkades inte av losartan eller av CPAP däremot sjönk aldosteron som en effekt av losartanbehandling detta skedde dock bara hos dem utan OSA. Behandlade man OSA gruppen med

CPAP sågs en tendens till att aldosteron började sjunka även i denna grupp. Stresshormonet norandrenalin hade också en tendens att sjunka efter CPAP behandling (artikel IV).

Inflammatorisk aktivitet är ökad bland individer som nyligen revaskulariserat och som samtidig har OSA jämfört med de som inte har OSA. Behandling av OSA påverkar inte den naturliga minskningen av inflammatorisk aktivitet över tid. Minskad kardiovaskulär risk i denna grupp är sannolikt inte medierad via sänkning av inflammatorisk aktivitet i någon större utsträckning. Den blodtryckessänkande effekten av losartan bland patienter med nyupptäck högt blodtryck är något sämre hos individer med OSA jämför med dem som inte har det. Behandling med CPAP hos individer som redan står på losartan får en ytterligare blodtrycketsänkande effekt av denna behandling. Detta sker sannolikt mer genom förändringar i sympatikusaktivitet och genom förändringar i det mineralkortikoida svaret än genom en minskad inflammation.

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- Jarod Kintz

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REFERENCES

"There is no hope for a civilization which starts each day to the sound of an alarm clock."
- Unknown Author

1. Group JJW. Guidelines for the clinical use of 24 hour ambulatory blood pressure monitoring (ABPM) (JCS 2010): - digest version. Circulation journal : official journal of the Japanese Circulation Society 2012;76:508-19.
2. Carskadon MA DWNhs. Normal human sleep: an overview. In Kryger MH, Roth T, Dement WC, eds. Principles and practice of sleep medicine Elsevier Saunders,, 2005.
3. Everson CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: III. Total sleep deprivation. Sleep 1989;12:13-21.
4. Petrovsky N, Ettinger U, Hill A, et al. Sleep deprivation disrupts prepulse inhibition and induces psychosis-like symptoms in healthy humans. The Journal of neuroscience : the official journal of the Society for Neuroscience 2014;34:9134-40.
5. Freeman D, Pugh K, Vorontsova N, Southgate L. Insomnia and paranoia. Schizophrenia research 2009;108:280-4.
6. Bin YS, Marshall NS, Glozier N. Secular trends in adult sleep duration: a systematic review. Sleep medicine reviews 2012;16:223-30.
7. Ford ES, Cunningham TJ, Croft JB. Trends in Self-Reported Sleep Duration among US Adults from 1985 to 2012. Sleep 2015;38:829-32.
8. Konecny T, Kara T, Somers VK. Obstructive Sleep Apnea and Hypertension: An Update. Hypertension 2013.
9. Punjabi NM, Caffo BS, Goodwin JL, et al. Sleep-disordered breathing and mortality: a prospective cohort study. PLoS medicine 2009;6:e1000132.
10. Turgut Celen Y, Peker Y. Cardiovascular consequences of sleep apnea: I -Epidemiology. Anadolu Kardiyoloji Dergisi/The Anatolian Journal of Cardiology 2010:75-80.
11. Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. Lancet 2009;373:82-93.
12. McNicholas WT. Obstructive sleep apnea and inflammation. Progress in cardiovascular diseases 2009;51:392-9.
13. Turgut Celen Y, Peker Y. Cardiovascular consequences of sleep apnea: II-Cardiovascular mechanisms. Anadolu kardiyoloji dergisi : AKD = the Anatolian journal of cardiology 2010;10:168-75.
14. Hoyos CM, Melehan KL, Liu PY, Grunstein RR, Phillips CL. Does obstructive sleep apnea cause endothelial dysfunction? A critical review of the literature. Sleep medicine reviews 2015;20:15-26.
15. Colish J, Walker JR, Elmayergi N, et al. Obstructive sleep apnea: effects of continuous positive airway pressure on cardiac remodeling as assessed by cardiac biomarkers, echocardiography, and cardiac MRI. Chest 2012;141:674-81.

16. Hedner J, Grote L, Bonsignore M, et al. The European Sleep Apnoea Database (ESADA): report from 22 European sleep laboratories. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 2011;38:635-42.

17. Phillips CL, O'Driscoll DM. Hypertension and obstructive sleep apnea. Nature and science of sleep 2013;5:43-52.

18. Botros N, Concato J, Mohsenin V, Selim B, Doctor K, Yaggi HK. Obstructive sleep apnea as a risk factor for type 2 diabetes. The American journal of medicine 2009;122:1122-7.

19. Cassar A, Morgenthaler TI, Lennon RJ, Rihal CS, Lerman A. Treatment of obstructive sleep apnea is associated with decreased cardiac death after percutaneous coronary intervention. Journal of the American College of Cardiology 2007;50:1310-4.

20. Peker Y, Kraiczi H, Hedner J, Loth S, Johansson A, Bende M. An independent association between obstructive sleep apnoea and coronary artery disease. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 1999;14:179-84.

21. Braga B, Poyares D, Cintra F, et al. Sleep-disordered breathing and chronic atrial fibrillation. Sleep medicine 2009;10:212-6.

22. Valham F, Mooe T, Rabben T, Stenlund H, Wiklund U, Franklin KA. Increased risk of stroke in patients with coronary artery disease and sleep apnea: a 10-year follow-up. Circulation 2008;118:955-60.

23. Yaranov DM, Smyrlis A, Usatii N, et al. Effect of obstructive sleep apnea on frequency of stroke in patients with atrial fibrillation. The American journal of cardiology 2015;115:461-5.

24. Hanly PJ, Ahmed SB. Sleep Apnea and the Kidney. CHEST Journal 2014;146:1114.

25. Arias MA, Garcia-Rio F, Alonso-Fernandez A, Martinez I, Villamor J. Pulmonary hypertension in obstructive sleep apnoea: effects of continuous positive airway pressure: a randomized, controlled cross-over study. European heart journal 2006;27:1106-13.

26. Kasai T, Bradley TD. Obstructive sleep apnea and heart failure: pathophysiologic and therapeutic implications. Journal of the American College of Cardiology 2011;57:119-27.

27. Redline S, Yenokyan G, Gottlieb DJ, et al. Obstructive sleep apnea-hypopnea and incident stroke: the sleep heart health study. American journal of respiratory and critical care medicine 2010;182:269-77.

28. Gottlieb DJ, Yenokyan G, Newman AB, et al. Prospective study of obstructive sleep apnea and incident coronary heart disease and heart failure: the sleep heart health study. Circulation 2010;122:352-60.

29. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European heart journal 2013;34:2159-219.

30. Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndromes. Annual review of medicine 1976;27:465-84.

31. Lavie P. Who was the first to use the term Pickwickian in connection with sleepy patients? History of sleep apnoea syndrome. Sleep medicine reviews 2008;12:5-17.

32. Broadbent WH. On Cheyne-Stokes´ respiration in cerebral haemorrhage. The Lancet 1877;109:307-9.

33. R C. Case of narcolepsy. Clin Soc Trans 1889:133–7.

34. Kryger MH. Sleep apnea. From the needles of Dionysius to continuous positive airway pressure. Archives of internal medicine 1983;143:2301-3.

35. Bickelmann AG, Burwell CS, Robin ED, Whaley RD. Extreme obesity associated with alveolar hypoventilation; a Pickwickian syndrome. The American journal of medicine 1956;21:811-8.

36. H B. Ueber das Elektroenkephalogramm des Menschen. Psychiatrie Neurologie und Medizinische Psychologie Zeitschrift Fuer die Gesa 1930;40:160–79.

37. Loomis AL, Harvey EN, Hobart G. Potential rhythms of the cerebral cortex during sleep. Science (New York, N.Y.) 1935;81:597-8.

38. Davis H, Davis PA, Loomis AL, Harvey EN, Hobart G. Changes in human brain potentials during the onset of sleep. Science 1937;86:448-50.

39. Loomis AL, Harvey EN, Hobart G. Further observations on the potential rhythms of the cerebral cortex during sleep. Science (New York, N.Y.) 1935;82:198-200.

40. Gerardy W, Herberg D, Kuhn HM. [Comparative studies on pulmonary function and the electroancephalogram in 2 patients with Pickwick's syndrome]. Zeitschrift fur klinische Medizin 1960;156:362-80.

41. Drachman DB, Gumnit RJ. Periodic alteration of consciousness in the "pickwickian" syndrome. Archives of neurology 1962;6:471-7.

42. Gastaut H, Tassinari CA, Duron B. Polygraphic study of the episodic diurnal and nocturnal (hypnic and respiratory) manifestations of the Pickwick syndrome. Brain research 1966;1:167-86.

43. Coccagna G, Mantovani M, Brignani F, Parchi C, Lugaresi E. Continuous recording of the pulmonary and systemic arterial pressure during sleep in syndromes of hypersomnia with periodic breathing. Bulletin de physio-pathologie respiratoire 1972;8:1159-72.

44. Kuhlo W, Doll E, Franck MC. [Successful management of Pickwickian syndrome using long-term tracheostomy]. Deutsche medizinische Wochenschrift (1946) 1969;94:1286-90.

45. Coccagna G, Mantovani M, Brignani F, Parchi C, Lugaresi E. Tracheostomy in hypersomnia with periodic breathing. Bulletin de physio-pathologie respiratoire 1972;8:1217-27.

46. Guilleminault C, Eldridge FL, Dement WC. Insomnia with sleep apnea: a new syndrome. Science (New York, N.Y.) 1973;181:856-8.

47. Remmers JE, deGroot WJ, Sauerland EK, Anch AM. Pathogenesis of upper airway occlusion during sleep. Journal of applied physiology: respiratory, environmental and exercise physiology 1978;44:931-8.

48. Sullivan CE, Issa FG, Berthon-Jones M, Eves L. Reversal of obstructive sleep apnoea by continuous positive airway pressure applied through the nares. Lancet 1981;1:862-5.

49. Fujita S, Conway W, Zorick F, Roth T. Surgical correction of anatomic azbnormalities in obstructive sleep apnea syndrome: uvulopalatopharyngoplasty. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery 1981;89:923-34.

50. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. The New England journal of medicine 1993;328:1230-5.

51. Block AJ, Boysen PG, Wynne JW, Hunt LA. Sleep apnea, hypopnea and oxygen desaturation in normal subjects. A strong male predominance. The New England journal of medicine 1979;300:513-7.

52. Guilleminault C, Stoohs R, Clerk A, Cetel M, Maistros P. A cause of excessive daytime sleepiness. The upper airway resistance syndrome. Chest 1993;104:781-7.

53. Report AAoSMTF. 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 1999;22:667-89.

54. Gould GA, Whyte KF, Rhind GB, et al. The sleep hypopnea syndrome. The American review of respiratory disease 1988;137:895-8.

55. Young T, Peppard P, Palta M, et al. Population-based study of sleep-disordered breathing as a risk factor for hypertension. Archives of internal medicine 1997;157:1746-52.

56. Ruehland WR, Rochford PD, O'Donoghue FJ, Pierce RJ, Singh P, Thornton AT. The new AASM criteria for scoring hypopneas: impact on the apnea hypopnea index. Sleep 2009;32:150-7.

57. Schwab RJ, Pasirstein M, Pierson R, et al. Identification of upper airway anatomic risk factors for obstructive sleep apnea with volumetric magnetic resonance imaging. American journal of respiratory and critical care medicine 2003;168:522-30.

58. Wellman A, Jordan AS, Malhotra A, et al. Ventilatory control and airway anatomy in obstructive sleep apnea. American journal of respiratory and critical care medicine 2004;170:1225-32.

59. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. Physiological reviews 2010;90:47-112.

60. Van de Graaff WB. Thoracic influence on upper airway patency. Journal of applied physiology (Bethesda, Md. : 1985) 1988;65:2124-31.

61. Jordan AS, McSharry DG, Malhotra A. Adult obstructive sleep apnoea. Lancet 2014;383:736-47.

62. Ballard RD, Irvin CG, Martin RJ, Pak J, Pandey R, White DP. Influence of sleep on lung volume in asthmatic patients and normal subjects. Journal of applied physiology (Bethesda, Md. : 1985) 1990;68:2034-41.

63. Jordan AS, White DP, Lo YL, et al. Airway dilator muscle activity and lung volume during stable breathing in obstructive sleep apnea. Sleep 2009;32:361-8.

64. Kasai T, Bradley TD, Friedman O, Logan AG. Effect of intensified diuretic therapy on overnight rostral fluid shift and obstructive sleep apnoea in patients with uncontrolled hypertension. Journal of hypertension 2014;32:673-80.

65. Yumino D, Redolfi S, Ruttanaumpawan P, et al. Nocturnal rostral fluid shift: a unifying concept for the pathogenesis of obstructive and central sleep apnea in men with heart failure. Circulation 2010;121:1598-605.

66. Punjabi NM. The epidemiology of adult obstructive sleep apnea. Proceedings of the American Thoracic Society 2008;5:136-43.

67. Duran J, Esnaola S, Rubio R, Iztueta A. Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. American journal of respiratory and critical care medicine 2001;163:685-9.

68. Bixler EO, Vgontzas AN, Lin HM, et al. Prevalence of sleep-disordered breathing in women: effects of gender. American journal of respiratory and critical care medicine 2001;163:608-13.

69. Ip MS, Lam B, Lauder IJ, et al. A community study of sleep-disordered breathing in middle-aged Chinese men in Hong Kong. Chest 2001;119:62-9.

70. Ip MS, Lam B, Tang LC, Lauder IJ, Ip TY, Lam WK. A community study of sleep-disordered breathing in middle-aged Chinese women in Hong Kong: prevalence and gender differences. Chest 2004;125:127-34.

71. Bearpark H, Elliott L, Grunstein R, et al. Snoring and sleep apnea. A population study in Australian men. American journal of respiratory and critical care medicine 1995;151:1459-65.

72. Kim J, In K, You S, et al. Prevalence of sleep-disordered breathing in middle-aged Korean men and women. American journal of respiratory and critical care medicine 2004;170:1108-13.

73. Bixler EO, Vgontzas AN, Ten Have T, Tyson K, Kales A. Effects of age on sleep apnea in men: I. Prevalence and severity. American journal of respiratory and critical care medicine 1998;157:144-8.

74. Udwadia ZF, Doshi AV, Lonkar SG, Singh CI. Prevalence of sleep-disordered breathing and sleep apnea in middle-aged urban Indian men. American journal of respiratory and critical care medicine 2004;169:168-73.

75. Dingli K, Coleman EL, Vennelle M, et al. Evaluation of a portable device for diagnosing the sleep apnoea/hypopnoea syndrome. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 2003;21:253-9.

76. Durrence HH, Lichstein KL. The sleep of African Americans: a comparative review. Behavioral sleep medicine 2006;4:29-44.

77. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. American journal of respiratory and critical care medicine 2002;165:1217-39.

78. Peppard PE, Young T, Palta M, Dempsey J, Skatrud J. Longitudinal study of moderate weight change and sleep-disordered breathing. JAMA : the journal of the American Medical Association 2000;284:3015-21.

79. Redline S, Schluchter MD, Larkin EK, Tishler PV. Predictors of longitudinal change in sleep-disordered breathing in a nonclinic population. Sleep 2003;26:703-9.

80. Ancoli-Israel S, Kripke DF, Klauber MR, Mason WJ, Fell R, Kaplan O. Sleep-disordered breathing in community-dwelling elderly. Sleep 1991;14:486-95.

81. Young T, Shahar E, Nieto FJ, et al. Predictors of sleep-disordered breathing in community-dwelling adults: the Sleep Heart Health Study. Archives of internal medicine 2002;162:893-900.

82. Tishler PV, Larkin EK, Schluchter MD, Redline S. Incidence of sleep-disordered breathing in an urban adult population: the relative importance of risk factors in the development of sleep-disordered breathing. JAMA : the journal of the American Medical Association 2003;289:2230-7.

83. McNicholas WT, Bonsigore MR. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 2007;29:156-78.

84. Mehra R, Stone KL, Varosy PD, et al. Nocturnal Arrhythmias across a spectrum of obstructive and central sleep-disordered breathing in older men: outcomes of sleep disorders in older men (MrOS sleep) study. Archives of internal medicine 2009;169:1147-55.

85. Gami AS, Hodge DO, Herges RM, et al. Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. Journal of the American College of Cardiology 2007;49:565-71.

86. Schulz R, Blau A, Borgel J, et al. Sleep apnoea in heart failure. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 2007;29:1201-5.

87. Wang H, Parker JD, Newton GE, et al. Influence of obstructive sleep apnea on mortality in patients with heart failure. Journal of the American College of Cardiology 2007;49:1625-31.

88. Saruhara H, Takata Y, Usui Y, et al. Obstructive sleep apnea as a potential risk factor for aortic disease. Heart and vessels 2012;27:166-73.

89. Naito R, Sakakura K, Kasai T, et al. Aortic dissection is associated with intermittent hypoxia and re-oxygenation. Heart and vessels 2012;27:265-70.

90. Coughlin SR, Mawdsley L, Mugarza JA, Calverley PM, Wilding JP. Obstructive sleep apnoea is independently associated with an increased prevalence of metabolic syndrome. European heart journal 2004;25:735-41.

91. Qian X, Yin T, Li T, et al. High levels of inflammation and insulin resistance in obstructive sleep apnea patients with hypertension. Inflammation 2012;35:1507-11.

92. Nieto FJ, Young TB, Lind BK, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. JAMA : the journal of the American Medical Association 2000;283:1829-36.

93. Bixler EO, Vgontzas AN, Lin HM, et al. Association of hypertension and sleep-disordered breathing. Archives of internal medicine 2000;160:2289-95.

94. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. The New England journal of medicine 2000;342:1378-84.

95. Haas DC, Foster GL, Nieto FJ, 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 2005;111:614-21.

96. Grote L, Hedner J, Peter JH. Sleep-related breathing disorder is an independent risk factor for uncontrolled hypertension. Journal of hypertension 2000;18:679-85.

97. O'Connor GT, Caffo B, Newman AB, et al. Prospective study of sleep-disordered breathing and hypertension: the Sleep Heart Health Study. American journal of respiratory and critical care medicine 2009;179:1159-64.

98. Cano-Pumarega I, Duran-Cantolla J, Aizpuru F, et al. Obstructive sleep apnea and systemic hypertension: longitudinal study in the general population: the Vitoria Sleep Cohort. American journal of respiratory and critical care medicine 2011;184:1299-304.

99. Lavie P, Herer P, Hoffstein V. Obstructive sleep apnoea syndrome as a risk factor for hypertension: population study. BMJ (Clinical research ed.) 2000;320:479-82.

100. Marin JM, Agusti A, Villar I, et al. Association between treated and untreated obstructive sleep apnea and risk of hypertension. JAMA : the journal of the American Medical Association 2012;307:2169-76.

101. Fava C, Dorigoni S, Dalle Vedove F, et al. Effect of continuous positive airway pressure (CPAP) on blood pressure in patients with obstructive sleep apnea/hypopnea. A systematic review and metaanalysis. Chest 2013.

102. Duran-Cantolla J, Aizpuru F, Montserrat JM, et al. Continuous positive airway pressure as treatment for systemic hypertension in people with obstructive sleep apnoea: randomised controlled trial. BMJ (Clinical research ed.) 2010;341:c5991.

103. Muxfeldt ES, Margallo VS, Guimaraes GM, Salles GF. Prevalence and associated factors of obstructive sleep apnea in patients with resistant hypertension. American journal of hypertension 2014;27:1069-78.

104. Iftikhar IH, Valentine CW, Bittencourt LR, 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 2014;32:2341-50; discussion 50.

105. Torres G, Sanchez-de-la-Torre M, Barbe F. Relationship Between OSA and Hypertension. Chest 2015;148:824-32.

106. Borgel J, Sanner BM, Keskin F, et al. Obstructive sleep apnea and blood pressure. Interaction between the blood pressure-lowering effects of positive airway pressure therapy and antihypertensive drugs. American journal of hypertension 2004;17:1081-7.

107. Pankow W, Nabe B, Lies A, Kohl FV, Lohmann FW. Influence of obstructive sleep apnoea on circadian blood pressure profile. Journal of sleep research 1995;4:102-6.

108. Ancoli-Israel S, Kripke DF, Klauber MR, et al. Morbidity, mortality and sleep-disordered breathing in community dwelling elderly. Sleep 1996;19:277-82.

109. Partinen M, Jamieson A, Guilleminault C. Long-term outcome for obstructive sleep apnea syndrome patients. Mortality. Chest 1988;94:1200-4.

110. Bliwise DL, Bliwise NG, Partinen M, Pursley AM, Dement WC. Sleep apnea and mortality in an aged cohort. American journal of public health 1988;78:544-7.

111. Peker Y, Hedner J, Norum J, Kraiczi H, Carlson J. Increased incidence of cardiovascular disease in middle-aged men with obstructive sleep apnea: a 7-year follow-up. American journal of respiratory and critical care medicine 2002;166:159-65.

112. Ou Q, Chen YC, Zhuo SQ, et al. Continuous Positive Airway Pressure Treatment Reduces Mortality in Elderly Patients with Moderate to Severe Obstructive Severe Sleep Apnea: A Cohort Study. PloS one 2015;10:e0127775.

113. Peker Y, Carlson J, Hedner J. Increased incidence of coronary artery disease in sleep apnoea: a long-term follow-up. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 2006;28:596-602.

114. Wu X, Lv S, Yu X, Yao L, Mokhlesi B, Wei Y. Treatment of OSA reduces the risk of repeat revascularization after percutaneous coronary intervention. Chest 2015;147:708-18.

115. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. Lancet 2005;365:1046-53.

116. Mooe T, Franklin KA, Holmstrom K, Rabben T, Wiklund U. Sleep-disordered breathing and coronary artery disease: long-term prognosis. American journal of respiratory and critical care medicine 2001;164:1910-3.

117. Peker Y, Hedner J, Kraiczi H, Loth S. Respiratory disturbance index: an independent predictor of mortality in coronary artery disease. American journal of respiratory and critical care medicine 2000;162:81-6.

118. Milleron O, Pilliere R, Foucher A, et al. Benefits of obstructive sleep apnoea treatment in coronary artery disease: a long-term follow-up study. European heart journal 2004;25:728-34.

119. Nathan C. Points of control in inflammation. Nature 2002;420:846-52.

120. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. The New England journal of medicine 1989;320:915-24.

121. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. The New England journal of medicine 2005;352:1685-95.

122. Skalen K, Gustafsson M, Rydberg EK, et al. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 2002;417:750-4.

123. Dai G, Kaazempur-Mofrad MR, Natarajan S, et al. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. Proceedings of the National Academy of Sciences of the United States of America 2004;101:14871-6.

124. Tsai SS, Lin YS, Lin CP, Hwang JS, Wu LS, Chu PH. Metabolic Syndrome-Associated Risk Factors and High-Sensitivity C-Reactive Protein Independently Predict Ar-

terial stiffness in 9903 Subjects With and Without Chronic Kidney Disease. Medicine 2015;94:e1419.

125. Danesh J, Kaptoge S, Mann AG, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS medicine 2008;5:e78.

126. Kilic T, Ural D, Ural E, et al. Relation between proinflammatory to anti-inflammatory cytokine ratios and long-term prognosis in patients with non-ST elevation acute coronary syndrome. Heart (British Cardiac Society) 2006;92:1041-6.

127. Sung KC, Suh JY, Kim BS, et al. High sensitivity C-reactive protein as an independent risk factor for essential hypertension. American journal of hypertension 2003;16:429-33.

128. Hage FG. C-reactive protein and hypertension. Journal of human hypertension 2014;28:410-5.

129. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. The Journal of clinical investigation 2003;111:1805-12.

130. Hutchinson WL, Koenig W, Frohlich M, Sund M, Lowe GD, Pepys MB. Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. Clinical chemistry 2000;46:934-8.

131. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. Clinica chimica acta; international journal of clinical chemistry 1981;117:13-23.

132. He LP, Tang XY, Ling WH, Chen WQ, Chen YM. Early C-reactive protein in the prediction of long-term outcomes after acute coronary syndromes: a meta-analysis of longitudinal studies. Heart (British Cardiac Society) 2010;96:339-46.

133. Kaptoge S, Di Angelantonio E, Pennells L, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. The New England journal of medicine 2012;367:1310-20.

134. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. The New England journal of medicine 2008;359:2195-207.

135. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. The New England journal of medicine 2005;352:20-8.

136. Nadeem R, Molnar J, Madbouly EM, et al. Serum inflammatory markers in obstructive sleep apnea: a meta-analysis. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine 2013;9:1003-12.

137. Baessler A, Nadeem R, Harvey M, et al. Treatment for sleep apnea by continuous positive airway pressure improves levels of inflammatory markers - a meta-analysis. Journal of inflammation (London, England) 2013;10:13.

138. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiological reviews 2006;86:515-81.

139. Yamashita H, Shimada K, Seki E, Mokuno H, Daida H. Concentrations of interleukins, interferon, and C-reactive protein in stable and unstable angina pectoris. The American journal of cardiology 2003;91:133-6.

140. Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. JAMA : the journal of the American Medical Association 2001;286:2107-13.

141. Arnardottir ES, Maislin G, Schwab RJ, et al. The interaction of obstructive sleep apnea and obesity on the inflammatory markers C-reactive protein and interleukin-6: the Icelandic Sleep Apnea Cohort. Sleep 2012;35:921-32.

142. Vgontzas AN. Does obesity play a major role in the pathogenesis of sleep apnoea and its associated manifestations via inflammation, visceral adiposity, and insulin resistance? Archives of physiology and biochemistry 2008;114:211-23.

143. Marsland AL, Petersen KL, Sathanoori R, et al. Interleukin-6 covaries inversely with cognitive performance among middle-aged community volunteers. Psychosomatic medicine 2006;68:895-903.

144. Olaithe M, Bucks RS. Executive dysfunction in OSA before and after treatment: a meta-analysis. Sleep 2013;36:1297-305.

145. Cesari M, Penninx BW, Newman AB, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation 2003;108:2317-22.

146. Boekholdt SM, Peters RJ, Hack CE, et al. IL-8 plasma concentrations and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Arteriosclerosis, thrombosis, and vascular biology 2004;24:1503-8.

147. Ferrario CM, Strawn WB. Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. The American journal of cardiology 2006;98:121-8.

148. Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH. Hypertension: renin-angiotensin-aldosterone system alterations. Circulation research 2015;116:960-75.

149. Gonzaga CC, Gaddam KK, Ahmed MI, et al. Severity of obstructive sleep apnea is related to aldosterone status in subjects with resistant hypertension. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine 2010;6:363-8.

150. Gaddam K, Pimenta E, Thomas SJ, et al. Spironolactone reduces severity of obstructive sleep apnoea in patients with resistant hypertension: a preliminary report. Journal of human hypertension 2010;24:532-7.

151. Svatikova A, Olson LJ, Wolk R, et al. Obstructive sleep apnea and aldosterone. Sleep 2009;32:1589-92.

152. Pratt-Ubunama MN, Nishizaka MK, Boedefeld RL, Cofield SS, Harding SM, Calhoun DA. Plasma aldosterone is related to severity of obstructive sleep apnea in subjects with resistant hypertension. Chest 2007;131:453-9.

153. Nicholl DD, Hanly PJ, Poulin MJ, et al. Evaluation of continuous positive airway pressure therapy on renin-angiotensin system activity in obstructive sleep apnea. American journal of respiratory and critical care medicine 2014;190:572-80.

154. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. The Journal of clinical investigation 1995;96:1897-904.

155. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. Chest 1993;103:1763-8.

156. Narkiewicz K, Montano N, Cogliati C, van de Borne PJ, Dyken ME, Somers VK. Altered cardiovascular variability in obstructive sleep apnea. Circulation 1998;98: 1071-7.

157. Narkiewicz K, van de Borne PJ, Cooley RL, Dyken ME, Somers VK. Sympathetic activity in obese subjects with and without obstructive sleep apnea. Circulation 1998;98:772-6.

158. Maser RE, Lenhard MJ, Rizzo AA, Vasile AA. Continuous positive airway pressure therapy improves cardiovascular autonomic function for persons with sleep-disordered breathing. Chest 2008;133:86-91.

159. Bonsignore MR, Parati G, Insalaco G, et al. Continuous positive airway pressure treatment improves baroreflex control of heart rate during sleep in severe obstructive sleep apnea syndrome. American journal of respiratory and critical care medicine 2002;166:279-86.

160. Noda A, Nakata S, Koike Y, et al. Continuous positive airway pressure improves daytime baroreflex sensitivity and nitric oxide production in patients with moderate to severe obstructive sleep apnea syndrome. Hypertension research : official journal of the Japanese Society of Hypertension 2007;30:669-76.

161. Peker Y, Glantz H, Thunstrom E, Kallryd A, Herlitz J, Ejdeback J. Rationale and design of the Randomized Intervention with CPAP in Coronary Artery Disease and Sleep Apnoea--RICCADSA trial. Scandinavian cardiovascular journal : SCJ 2009;43:24-31.

162. Glantz H, Thunstrom E, Herlitz J, et al. Occurrence and predictors of obstructive sleep apnea in a revascularized coronary artery disease cohort. Annals of the American Thoracic Society 2013;10:350-6.

163. Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S. The Prospective Urban Rural Epidemiology (PURE) study: examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. American heart journal 2009;158:1-7 e1.

164. Mancia G, De Backer G, Dominiczak A, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension 2007;25:1105-87.

165. Devereux RB, Dahlof B, Kjeldsen SE, et al. Effects of losartan or atenolol in hypertensive patients without clinically evident vascular disease: a substudy of the LIFE randomized trial. Annals of internal medicine 2003;139:169-77.

166. Minami J, Abe C, Akashiba A, et al. Long-term efficacy of combination therapy with losartan and low-dose hydrochlorothiazide in patients with uncontrolled hypertension. International heart journal 2007;48:177-86.

167. Shivpuri S, Allison MA, Macera CA, Lindsay S, Gallo LC. Associations between nocturnal blood pressure dipping and the metabolic syndrome in high- vs. low-acculturated Mexican American women. American journal of hypertension 2013;26:1030-6.

168. Hermida RC, Chayan L, Ayala DE, et al. Association of metabolic syndrome and blood pressure nondipping profile in untreated hypertension. American journal of hypertension 2009;22:307-13.

169. Eriksson BM, Persson BA. Determination of catecholamines in rat heart tissue and plasma samples by liquid chromatography with electrochemical detection. Journal of chromatography 1982;228:143-54.

170. Anton AH, Sayre DF. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. The Journal of pharmacology and experimental therapeutics 1962;138:360-75.

171. Eisenhofer G, Goldstein DS, Stull R, et al. Simultaneous liquid-chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma, and their responses to inhibition of monoamine oxidase. Clinical chemistry 1986;32:2030-3.

172. Johns MW. Reliability and factor analysis of the Epworth Sleepiness Scale. Sleep 1992;15:376-81.

173. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.

174. Assembly tWG. World Medical Association. Declaration of Helsinki: ethical principle 79 for medical research involving human subjects. . In: 64 th General Assembly, Fortaleza Brazil, October 2013;http://www.wma.net/en/30publications/10policies/b3/(accessed 23 of june 2015), 2013.

175. 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 1997;20:705-6.

176. Worsnop CJ, Naughton MT, Barter CE, Morgan TO, Anderson AI, Pierce RJ. The prevalence of obstructive sleep apnea in hypertensives. American journal of respiratory and critical care medicine 1998;157:111-5.

177. Becker HF, Jerrentrup A, Ploch T, et al. Effect of nasal continuous positive airway pressure treatment on blood pressure in patients with obstructive sleep apnea. Circulation 2003;107:68-73.

178. Iber C, Ancoli-Israel S, Chesson A, S Q. for the American Academy of Sleep Medicine; The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. 1st ed. ed. Westchester, IL: American Academy of Sleep Medicine, 2007.

179. Kraiczi H, Hedner J, Peker Y, Grote L. Comparison of atenolol, amlodipine, enalapril, hydrochlorothiazide, and losartan for antihypertensive treatment in patients with obstructive sleep apnea. American journal of respiratory and critical care medicine 2000;161:1423-8.

179. Barcelo A, Pierola J, Esquinas C, et al. Relationship between aldosterone and the metabolic syndrome in patients with obstructive sleep apnea hypopnea syndrome: effect of continuous positive airway pressure treatment. PloS one 2014;9:e84362.

180. Lloberes P, Sampol G, Espinel E, et al. A randomized controlled study of CPAP effect on plasma aldosterone concentration in patients with resistant hypertension and obstructive sleep apnea. Journal of hypertension 2014;32:1650-7; discussion 7.

181. Sheppard JP, Hodgkinson J, Riley R, Martin U, Bayliss S, McManus RJ. Prognostic significance of the morning blood pressure surge in clinical practice: a systematic review. American journal of hypertension 2015;28:30-41.

182. Cho JS, Ihm SH, Kim CJ, et al. Obstructive Sleep Apnea Using Watch-PAT 200 Is Independently Associated With an Increase in Morning Blood Pressure Surge in Never-Treated Hypertensive Patients. J Clin Hypertens (Greenwich) 2015;17:675-81.

183. Satoh M, Hosaka M, Asayama K, et al. Aldosterone-to-renin ratio and nocturnal blood pressure decline assessed by self-measurement of blood pressure at home: the Ohasama Study. Clin Exp Hypertens 2014;36:108-14.

184. Campos-Rodriguez F, Grilo-Reina A, Perez-Ronchel J, et al. Effect of continuous positive airway pressure on ambulatory BP in patients with sleep apnea and hypertension: a placebo-controlled trial. Chest 2006;129:1459-67.

185. Robinson GV, Smith DM, Langford BA, Davies RJ, Stradling JR. Continuous positive airway pressure does not reduce blood pressure in nonsleepy hypertensive OSA patients. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 2006;27:1229-35.

186. Lozano L, Tovar JL, Sampol G, et al. Continuous positive airway pressure treatment in sleep apnea patients with resistant hypertension: a randomized, controlled trial. Journal of hypertension 2010;28:2161-8.

187. Pepin JL, Tamisier R, Barone-Rochette G, Launois SH, Levy P, Baguet JP. Comparison of continuous positive airway pressure and valsartan in hypertensive patients with sleep apnea. American journal of respiratory and critical care medicine 2010;182:954-60.

188. Hedner J, Darpo B, Ejnell H, Carlson J, Caidahl K. Reduction in sympathetic activity after long-term CPAP treatment in sleep apnoea: cardiovascular implications. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 1995;8:222-9.

189. Elmasry A, Lindberg E, Hedner J, Janson C, Boman G. Obstructive sleep apnoea and urine catecholamines in hypertensive males: a population-based study. European Respiratory Journal 2002;19:511-7.

190. Uresin Y, Erbas B, Ozek M, Ozkok E, Gurol AO. Losartan may prevent the elevation of plasma glucose, corticosterone and catecholamine levels induced by chronic stress. Journal of the renin-angiotensin-aldosterone system : JRAAS 2004;5:93-6.

191. Goldberg MR, Bradstreet TE, McWilliams EJ, et al. Biochemical Effects of Losartan, a Nonpeptide Angiotensin II Receptor Antagonist, on the Renin-Angiotensin-Aldosterone System in Hypertensive Patients. Hypertension 1995;25:37-46.

192. Gandhi SK, Ryder DH, Brown NJ. Losartan blocks aldosterone and renal vascular responses to angiotensin II in humans. Hypertension 1996;28:961-6.

193. Fukutomi M, Hoshide S, Eguchi K, Watanabe T, Kario K. Low-grade inflammation and ambulatory blood pressure response to antihypertensive treatment: the ALPHABET study. American journal of hypertension 2013;26:784-92.

194. Jullian-Desayes I, Joyeux-Faure M, Tamisier R, 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 2015;21:23-38.

195. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nature immunology 2011;12:204-12.

196. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. The New England journal of medicine 2004;350:1387-97.

197. Sabatine MS, Morrow DA, Jablonski KA, et al. Prognostic significance of the Centers for Disease Control/American Heart Association high-sensitivity C-reactive protein cut points for cardiovascular and other outcomes in patients with stable coronary artery disease. Circulation 2007;115:1528-36.

198. Helfand M, Buckley DI, Freeman M, et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Annals of internal medicine 2009;151:496-507.

199. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1999;100:230-5.

200. Arnardottir ES, Mackiewicz M, Gislason T, Teff KL, Pack AI. Molecular signatures of obstructive sleep apnea in adults: a review and perspective. Sleep 2009;32:447-70.

201. Steiropoulos P, Tsara V, Nena E, et al. Effect of continuous positive airway pressure treatment on serum cardiovascular risk factors in patients with obstructive sleep apnea-hypopnea syndrome. Chest 2007;132:843-51.

202. Vgontzas AN, Bixler EO, Chrousos GP. Metabolic disturbances in obesity versus sleep apnoea: the importance of visceral obesity and insulin resistance. Journal of internal medicine 2003;254:32-44.

203. Carter R, 3rd, Watenpaugh DE. Obesity and obstructive sleep apnea: or is it OSA and obesity? Pathophysiology : the official journal of the International Society for Pathophysiology / ISP 2008;15:71-7.

204. Zhao Q, Liu ZH, Zhao ZH, et al. Effects of obstructive sleep apnea and its treatment on cardiovascular risk in CAD patients. Respiratory medicine 2011;105:1557-64.

205. Vaclavik J, Sedlak R, Jarkovsky J, Kocianova E, Taborsky M. Effect of spironolactone in resistant arterial hypertension: a randomized, double-blind, placebo-controlled trial (ASPIRANT-EXT). Medicine 2014;93:e162.

207. Roth GA, Nguyen G, Forouzanfar MH, Mokdad AH, Naghavi M, Murray CJ. Estimates of Global and Regional Premature Cardiovascular Mortality in 2025. Circulation 2015;132:1270-82.

208. Cook NR, Cohen J, Hebert PR, Taylor JO, Hennekens CH. Implications of small reductions in diastolic blood pressure for primary prevention. Archives of internal medicine 1995;155:701-9.

"*There is a time for many words, and there is also a time for sleep*."

- Homer, The Odyssey

APPENDIX

ESS

Namn:

Dagens datum:

Hur troligt är det att du skulle slumra till eller somna i följande situationer, till skillnad från att bara känna dig trött? Det avser ditt vanliga levnadssätt på senaste tiden. Även om du inte gjort allt detta nyligen, så försök att komma på hur det skulle ha påverkat dig. Använd följande skala för att välja den lämpligaste siffran för varje situation.

0 = skulle *aldrig* slumra
1 = *liten* risk att slumra
2 = *måttlig* risk att slumra
3 = *stor* risk att slumra

Situation	***Risk att slumra***
Sitter och läser	_____
Tittar på TV	_____
Sitter overksam på allmän plats (t ex teater eller ett möte)	_____
Som passagerare i en bil i en timme utan paus	_____
Ligger ner och vilar på eftermiddagen om omständigheterna tillåter	_____
Sitter och pratar med någon	_____
Sitter stilla efter att ha ätit lunch (utan alkohol)	_____
I en bil som stannat några minuter i trafiken	_____

Tack för din medverkan

Ref.: Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. Sleep 1991;14:540-545.
Svensk översättning gjord av Jan-Erik Broman, Uppsala 2000 och antagen av Svensk förening för sömnforskning, 2001.