Pharmacological therapy in obstructive sleep apnea

Methodology and interventional aspects of carbonic anhydrase modulation

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ABSTRACT

BACKGROUND: Obstructive sleep apnea (OSA) is a highly prevalent disease with potential severe short- and long-term consequences such as daytime sleepiness and cardiovascular disease. Recurrent total or partial collapse of the upper airway during sleep leads to sleep fragmentation and intermittent hypoxia/hypercapnia. The underlying mechanisms include a collapsible upper airway, unstable ventilation, low arousal threshold and insufficient muscle compensation in the pharynx region. The main treatments, positive airway pressure (PAP) and mandibular advancement devices, mainly address anatomical causes of OSA. In many patients, these treatments are not tolerated or not efficient enough. Improved understanding of pathophysiological mechanisms in OSA have paved the way for new treatment targets. In this thesis, carbonic anhydrase (CA) activity as a biomarker for OSA is investigated together with studies on CA inhibition as a therapy in OSA, in addition to a systematic evaluation of placebo effects in drug treatments of OSA.

METHODS AND RESULTS: In paper I, we studied a cohort of 33 PAP-treated subjects with OSA and 9 OSA patients treated with PAP, the CA inhibitor acetazolamide (ACT) and a combination of PAP+ACT. We could show that CA activity in whole blood was higher in hypertensive compared to normotensive participants and PAP treatment per se did not reduce CA activity. Further, CA activity was numerically reduced by ACT.

Paper II contained a detailed analysis of 58 patients participating in a randomised controlled trial of the CA inhibitor sulthiame (STM) in moderate to severe OSA. By using a PSG-based analysis method, we investigated the effects of STM on

underlying pathomechanisms of OSA. STM reduced ventilatory instability and increased the upper airway stability.

In paper III, the effect of STM on potential biochemical markers of OSA was investigated. CA activity in whole blood was reduced by STM and this effect remained two weeks after the last drug intake. Hypoxia-inducible factor 1α, a key factor in the cellular response to hypoxia, was reduced during treatment and at two weeks after last drug intake, suggesting an overall improvement of overnight O_2 saturation following STM.

In paper IV we analysed the placebo effect of drug treatments in OSA by means of a systematic review with meta-analyses. Objective measures of OSA were not systematically affected by placebo whereas placebo effects in subjective symptoms like excessive daytime sleepiness need to be accounted for.

CONCLUSIONS: The CA system plays an important part in OSA pathophysiology and appears to be a promising treatment target. Further studies are needed to evaluate CA activity in whole blood as a diagnostic biomarker in OSA. The CA inhibitor STM had dual effects in OSA including a reduction of ventilatory instability and an increase in upper airway stability. Future studies in pharmacological treatment of OSA need to be significantly sized and placebo controlled for subjective outcomes.

Keywords: obstructive sleep apnea, carbonic anhydrase, endotype, drug therapy, hypoxia, hypoxia-inducible factor 1α, placebo

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SAMMANFATTNING PÅ SVENSKA

Obstruktiv sömnapné, OSA, är en folksjukdom som leder till dagtrötthet och på längre sikt ökad risk för hjärtkärl-sjuklighet. Vid OSA blir andningen instabil under sömnen, svalget faller ihop, och man får svårt att få luft. Vi vet idag att flera olika mekanismer kan orsaka OSA: en anatomiskt trång övre luftväg, otillräcklig muskelaktivitet i svalget, en instabil andningsreglering samt en känslig väckreflex. Fram tills nu har behandlingen vid OSA främst varit inriktad mot öppna svalget med mekaniska hjälpmedel, som övertrycksandning (PAP) eller framdragande bettskena. Tyvärr är det många som inte trivs med sin behandling (PAP) eller har otillräcklig effekt (bettskena). Med ökad förståelse kring sjukdomens underliggande mekanismer finns det nu även potentiella farmakologiska behandlingsmål. Enzymsystemet karbanhydras (CA) har en betydelsefull roll vid omsättningen av koldioxid i kroppen. Tidigare studier har visat att en blockering av CA-aktiviteten minskar OSA. I denna avhandling undersöks om mätning av CA-aktivitet i blod kan användas för att förutsäga svårighetsgrad av OSA och behandlingssvar, på vilket sätt CA-blockering minskar OSA samt hur placebo bör användas i läkemedelsstudier av OSA. I **projekt I** undersöktes två grupper: Grupp A behandlades med PAP i sex månader och grupp B fick PAP samt CA-blockering i två veckor. Individer med högt blodtryck hade högre nivåer av CA-aktivitet. PAP påverkade ej CA-aktiviteten, men läkemedelsblockering tenderade att sänka nivåerna. I **projekt II och III**, blockerades CA med sultiam (STM). Resultaten visade att STM minskar OSA genom en mer stabil andningsreglering och en stabilare luftväg. CA-aktiviteten och syrebrist-markören HIF-1α minskade vid behandling, i linje med en förbättring av syresättningen. I **projekt IV** sammanställdes tidigare läkemedelsstudier av OSA med fokus på effekten av placebo. Effekterna på objektivt mätt OSA (svårighetsgrad och syrebrist) var försumbara men det kan finnas en placebo-effekt för subjektiva symptom som dagtrötthet.

Sammanfattningsvis kan en blockering av CA med STM stabilisera andningsregleringen och luftvägen, vilket förklarar hur OSA reduceras och syresättningen förbättras. Det är dock fortfarande osäkert om CA-aktivitetsmätning i blod har en plats i utredning och behandling av OSA. Framtida studier av läkemedel mot OSA bör placebo-kontrolleras för en bättre utvärdering av patienternas symptom.

LIST OF PAPERS

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

I. Erik Hoff, Ding Zou, Sophia Schiza, Yeliz Demir, Ludger Grote, Izolde Bouloukaki, Sükrü Beydemir, Davoud Eskandari, Kaj Stenlöf, Jan Hedner

Carbonic anhydrase, obstructive sleep apnea and hypertension: Effects of intervention

Journal of Sleep Research 2019; 29: e12956

II. Erik Hoff, Christian Strassberger, Ding Zou, Ludger Grote, Kaj Stenlöf, Jan Hedner

Modification of endotypic traits in obstructive sleep apnea by the carbonic anhydrase inhibitor

CHEST 2023, *in press*

III. Erik Hoff, Saliha Musovic, Ali M. Komai, Ding Zou, Christian Strassberger, Kaj Stenlöf, Ludger Grote, Jan Hedner

The effect of sulthiame on potential biomarkers in moderate to severe obstructive sleep apnea

Submitted 2023

IV. Erik Hoff, Ding Zou, Ludger Grote, Kaj Stenlöf, Jan Hedner

The placebo effect in pharmacological treatment of obstructive sleep apnea, a systematic review and meta-analysis

Sleep Medicine 2023; 106: 1-7

CONTENTS

ABBREVIATIONS

5-HT	Serotonin
AASM	American Academy of Sleep Medicine
ACT	Acetazolamide
AF	Atrial fibrillation
AHI	Apnea-hypopnea index
ANCOVA	Analysis of covariance
ArTh	Arousal threshold
Ato-Oxy	Atomoxetine oxybutynin
BMI	Body mass index
CA	Carbonic anhydrase
CHF	Congestive heart failure
CI	Confidence interval
CNS	Central nervous system
CPAP	Continuous positive airway pressure
ECG	Electrocardiogram
EDS	Excessive daytime sleepiness
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyography
EOG	Electrooculogram
ERS	European respiratory society

ESADA	European Sleep Apnea Database
ESC-ESH	European society of Cardiology – European society of Hypertension
ESS	Epworth sleepiness scale
GABA	Gamma-aminobutyric acid
GLP-1	Glucagon-like-peptide 1
HIF-1α	Hypoxia-inducible factor 1α
HGNS	Hypoglossal nerve stimulation
HR	Hazard ratio
IQR	Interquartile range
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LG_1	Loop gain, response to one cycle/min disturbance
LG_n	Loop gain, response to a disturbance at natural frequency
MAD	Mandibular advancement device
MSLT	Multiple sleep latency test
NREM	Non-rapid eye movement
ODI	Oxygen desaturation index
OSA	Obstructive sleep apnea
P-down	Downstream pressure
P-up	Upstream pressure
PAP	Positive airway pressure
pCO_2	Partial pressure of CO_2
Pcrit	Critical closing pressure

PG	Polygraphy
pO_2	Partial pressure of oxygen
PSG	Polysomnography
PUPBeta	Phenotyping Using Polysomnography Beta
RCT	Randomised controlled trial
REM	Rapid eye movement
RERA	Respiratory effort-related arousal
RR	Relative risk
SCAPIS	Swedish CArdioPulmonary bioImage Study
SD	Standard deviation
STM	Sulthiame
TASK-1 / 3	(TWIK)-related acid-sensitive K^+ channel 1 / 3
UPPP	Uvulopalatopharyngoplasty
V_{active}	Median ventilation at arousal threshold, % of eupneic ventilation
V_{comp}	Compensatory muscle activation, ($V_{active} - V_{passive}$)
V_{min}	Median ventilation at lowest decile of drive, % of eupneic ventilation
$V_{passive}$	Median ventilation at eupneic ventilatory drive, % of eupneic ventilation
VRA	Ventilatory response to arousal

1 INTRODUCTION

1.1 OBSTRUCTIVE SLEEP APNEA

Obstructive sleep apnea (OSA) is a common disorder with disturbed breathing during sleep leading to periodic hypoxia and increased CO_2 load. The breathing disturbances cause disruptions of regular sleep and result in daytime sleepiness, reduced quality of life and an increased risk of cardiovascular disease [1-5]. The main symptoms include snoring, frequent awakenings, morning headaches, nocturia and daytime sleepiness [6].

As previously summarised by Lavie [7], one of the earliest descriptions of a sleepy, obese patient, presumably with OSA, was presented in 1889 by Dr Richard Caton from Liverpool, England, at the clinical society of London [8]. In "A case of Narcolepsy" the patient is described as:

> *"The patient, S., age 37, a poulterer, was admitted into my wards in Liverpool Royal Infirmary on January 12, 1888, complaining of intense drowsiness and chronic psoriasis. (p. 133)."*

During the preceding two years the patient had gained seven stones (~45 kgs) in weight, which coincided with an increased tendency to fall asleep. Going to the theatre was no longer an option, "*he slept soundly through the most exciting drama*"; he quickly fell asleep when sitting down, *"Reading and writing became quite impossible",* and he would even fall asleep while serving his customers in his poultry shop. With today's insight the clinical description of Dr Caton is an obvious case of OSA:

> *"When in sound asleep a very peculiar state of the glottis is observed, a spasmodic closure entirely suspending respiration. The thorax and abdomen are seen to heave from fruitless contractions of inspiratory and expiratory muscles; their efforts increase in violence for about a minute or a minute and a half, the skin meantime becoming more and more cyanosed, until at last, when the condition to the onlooker is most alarming, the glottic obstruction yields, a series of long inspirations and expirations follows, and the cyanosis disappears. This acute dyspnoeic attack does not awaken the patient. If in the midst of the dyspnoeic attack he be forcibly aroused, the glottic spasm at once relaxes."*

The resemblance with the character Joe, the sleepy fat boy, from Charles Dickens' *The Posthumous papers of the Pickwick club* [9] was evident for the clinical society. However, the patient's symptoms were unexplained.

In the early 1960s, the first physiological recording of a patient with Pickwickian syndrome during sleep was conducted. The patient presented with morning headaches, reduced work capacity and periodic breathing with continuous movements in the abdomen and thorax. The researchers concluded that the disorder was due to "carbon dioxide poisoning" [10].

The hypothesis that the sleepiness in Pickwickian syndrome was due to disturbed sleep and frequent awakenings was first presented at a conference in 1964 by Kuhl and Jung [7]. Later, this was confirmed by Gastaut and Lugaresi with colleagues. With the addition of mouth and nostril airflow measurements it was concluded that the breathing disturbances were due to blockage of the upper airway, with continued respiratory movements [11, 12].

By chance, the theory of upper airway blockage was finally confirmed when a patient with Pickwickian syndrome was tracheostomised due to severe hypercapnia. This cured both the respiratory failure and the severe daytime sleepiness [13]. Several patients with Pickwick syndrome were later tracheostomised with similar results [14].

Another milestone was the first demonstration of insomnia associated with upper airway obstruction in patients with normal weight [15]. In "The Sleep Apnea Syndromes" the disorder was later defined by polysomnographic measurements as at least 30 apneas, with a minimum duration of 10 seconds, each detected during 7 hours of sleep [16]. This publication started the modern era of investigations in OSA and its consequences.

1.1.1 DISEASE DEFINITIONS AND DIAGNOSE

1.1.1.1 DEFINITIONS

The intermittent partial (hypopnea) or complete (apnea) collapses of the upper airway during sleep are what define OSA. The recommended guidelines for apnea/hypopnea scoring have mainly been developed by the American Academy of Sleep Medicine (AASM). The latest guidelines, from 2023 [17], define the breathing disturbances as:

- Apnea: a drop in peak nasal pressure ≥90% with a duration of ≥10 seconds
- Hypopnea: defined in two ways, both definitions include a drop in peak nasal pressure ≥30% with a duration of ≥10 seconds in addition to one of the rules below:
    - ≥3% oxygen desaturation from pre-event baseline OR event associated with an arousal
    - ≥4% oxygen desaturation from pre-event baseline (optional)

For apneas, the event is defined as obstructive (in contrast to central) based on whether there is continued or increased respiratory effort during the entire period of absent airflow. In the scoring of hypopneas, snoring, increased inspiratory flattening or thoracoabdominal paradox during the event are signs of obstructive events. In a mixed apnea, respiratory effort is absent in the first part of the event, but returns in the second part, before the airflow has resumed [17].

An additional type of breathing disturbance is the "respiratory effort-related arousal" (RERA). This type of event constitutes an obstruction of the airway with increased respiratory effort (or flattening of the airflow signal) lasting ≥10 seconds, resulting in an arousal without desaturation or any other apnea/hypopnea criteria [17].

The number of apneas and hypopneas during the night is used to calculate the respiratory events/hour of sleep, the apnea-hypopnea index (AHI). The AHI has for long been used as the main severity classification, even though its relevance is debated [18]. Mild OSA in adults is defined as AHI ≥5 events/h, moderate OSA as ≥15 AHI <30 events/h and severe OSA as AHI ≥30 events/h. The cut-offs go back to the first studies on OSA, where 30 respiratory events/night (roughly 5 events/h) was considered abnormal [16]. The use of 30 events/h as the cut-off for severe OSA is based on data from the Wisconsin Sleep Cohort where the risk for hypertension was substantially increased at AHI >30 events/h [19].

The recommendation on whether to use 3% or 4% as the desaturation rule has been changed repeatedly. The same goes for whether the flow reduction should be 30% or 50% in the hypopnea definition. In addition, the instruments used in sleep studies have greatly improved, with subsequent increases in sensitivity and specificity. These developments has had the unfortunate effect that it is difficult to compare OSA severity and studies over longer time periods [20].

1.1.1.2 DIAGNOSE

The diagnose of OSA can be made in two ways according to the International Classification of Sleep Disorders 3rd edition, text revision [21]. Either A+B+D or C+D:

A. The presence of day-time/night-time symptoms or habitual snoring/breathing interruptions as observed by bedpartner
B. Polysomnography (PSG) with an AHI $\geq$5 events/h, predominantly obstructive events
C. AHI $\geq$15 events/h, predominantly obstructive events, irrespective of symptoms
D. The symptoms are not better explained by another sleep or medical disorder or medications

1.1.2 EPIDEMIOLOGY

The prevalence of OSA is strongly influenced by the population measured, the definition of breathing disturbances and if symptoms are required, see above.

In the American Wisconsin sleep cohort, a 4% oxygen desaturation rule was used to define hypopnea. It was estimated that among 30- to 70-year-olds, 17% of women and 34% among men had at least mild sleep apnea in the years 2007-2010. If you include only individuals with moderate sleep apnea, the number was 6% and 13% respectively. Adding of daytime sleepiness (Epworth sleepiness scale, ESS, score >10) and an AHI>15 events/h, the prevalence was lower, 2% in women and 6% in men [22].

In a population-based study, performed in Switzerland during the years 2009-2013, 3 000 participants, 40-85 years old, were screened for OSA using the AASM 2012 criteria with a hypopnea definition of 3% oxygen desaturation *or* an arousal. AHI >15 events/h was prevalent in 23% of women and 50% of men [23]. In Sweden it is estimated that roughly 500 000 individuals have moderate to severe OSA [24].

In 2019, Benjafield and colleagues summarised OSA prevalence studies in an attempt to estimate the global burden of OSA in the age group 30-69 years old. The study results were converted to AASM 2012 criteria and countries without prevalence estimations were compared to similar countries. The overall prevalence was thereafter calculated. Globally, moderate OSA (AHI >15 events/h) had an estimated prevalence of 425 million and AHI >5 events/h 936 million based on data from 16 countries. The research group did not specifically investigate symptoms, since this was rarely reported in studies and population data [25].

1.1.3 RISK FACTORS

Typical risk factors for OSA include a narrow upper airway – caused by e.g., obesity and fat deposition or craniofacial abnormalities. The abnormalities can sometimes be easily seen like micro- or retrognathia or inferior positioning of the hyoid bone. However, the specific anatomy in an individual cannot determine if someone develops OSA, but the anatomy may increase the risk. Across the world the risk factors vary, in Asia it is more common with mandibular or bony causes of a narrow upper airway, and in western countries obesity is the major cause. With body mass index (BMI) 30-39.9 the prevalence of OSA is roughly 45% among men and 14% among women, compared to 7% and 1.4% with a BMI <25 in the age group 30-49 years [6].

Men are more prone to develop OSA compared to women. Hormonal differences may be an important factor causing this difference as, for example, progesterone affects the responsiveness to hypercapnia and hypoxia. In illnesses with high androgen levels in women, such as polycystic ovary syndrome, the risk for OSA is increased. The sex differences diminish after menopause but hormonal replacement therapy may reduce the risk in women [26].

The risk of OSA increases during the life and plateaus after 60 years of age. The increase in incidence is driven by a reduced muscle activity, reduced reflexes in the upper airway, decreased lung elasticity, increased sleep fragmentation and respiratory instability [27].

Potentially modifiable risk factors of OSA, other than body weight, include high alcohol intake and physical inactivity. Whether smoking is a risk factor or not is debated [28-30].

1.1.4 SLEEP PHYSIOLOGY

Reduced responsiveness, metabolism and motor activity defines the state of sleep [31]. The major theory explaining sleep regulation is the two-process model, initially proposed by Borbély et al in 1982 [32]. In short, two different processes interact to regulate sleep: the circadian process (C) and the homeostatic process (S). Process C is the circadian rhythm with changes in e.g., body temperature and melatonin production, modulated by the suprachiasmatic nuclei in response to light and darkness. The S process describes the relation of sleep versus wake, with increasing drive to sleep during wakefulness and a reduction during sleep [33].

Human sleep can be divided into two states: rapid eye movement (REM) and non-REM (NREM), as defined by electroencephalogram (EEG) and electromyography (EMG) measurements in a PSG according to AASM criteria.

NREM can be further divided into N1, N2 and slow wave sleep N3. During sleep, the brain alternates between these sleep stages to form 90 min cycles (often N1, N2, N3, N2, REM), with a total of 4-6 cycles during the night. In the beginning of the sleeping period, REM is relatively short and NREM longer, while the opposite occurs later in the night [34].

The function of sleep is still debated but theories and hypotheses include an important role for memory consolidation, brain development and/or recovery of wakefulness-induced neuronal changes and metabolites [31]. However, we do know that lack of sleep has negative effects: an increase in cardiovascular risk, metabolic changes and obesity, cognitive decline and an induction of a pro-inflammatory state [35].

1.1.5 UPPER AIRWAY AND RESPIRATORY PHYSIOLOGY

The human upper airway, spanning from the nasal septum to epiglottis, is rigid only at the upper and lower end, with bone and cartilage respectively, and would therefore be prone to collapse unless there were dilating powers. The two main forces to keep the airway patency are dilatory muscles in the pharynx and, to a lesser extent, the traction force on the airway from the inflating lungs. These forces prevent collapse by counteracting intraluminal negative pressure during inspiration and the external pressure from extraluminal tissues surrounding the airway [36].

1.1.5.1 FORCES KEEPING THE AIRWAY OPEN

1.1.5.1.1 Airway dilation by muscles

23 muscles are responsible for the dilation of the airway, but the best studied are *m genioglossus* and *m tensor palatini*. The muscles have two basic working modes, either by intermittent activation during inspiration, or constant activation during the full respiratory cycle. The *m genioglossus* activity can be modulated via three pathways:

1. Mechanoreceptor activation by negative pressure during inspiration
2. Respiratory pattern neurons – affecting both respiratory and pharyngeal muscles
3. Arousal neurons (primarily wakefulness modulation) innervating the m genioglossus [36]

1.1.5.1.2 Traction force on the airway

When the lungs expand, the caudal traction on the airway stiffens the airway and reduces the extraluminal pressure from surrounding tissue [36].

1.1.5.1.3 Modulation of the opening forces during sleep

During sleep, the activity modulation on the muscles in the upper airway changes dramatically: the mechanoreceptor response to negative pressure is slower and the arousal neurons are less active. The respiratory pattern neurons are probably less affected by sleep, but the overall result is an airway more vulnerable to collapse. In addition, shallow breathing and a recumbent position reduce the traction force and makes the airway more prone to collapse. Overall, the forces that keep the airway open are reduced [36].

1.1.5.2 FORCES PROMOTING AIRWAY COLLAPSE

1.1.5.2.1 Underlying anatomy

The airway anatomy, the relation between the airway lumen and surrounding soft and skeletal tissues, is of great importance for the airway patency. Individual anatomical differences may increase the extraluminal pressure or reduce the underlying airway lumen size. "Impaired" anatomy, i.e., a more collapsible airway, can be a result of e.g., fat deposition surrounding the lumen in obesity, a retrognathic mandible or enlarged tonsils protracting into the airway [37].

1.1.5.2.2 Airway pressure

During inspiration, the pressure in the airway drops, increasing the tendency of a reduced or collapsed lumen. Whether there is a partial or total collapse is a result of the pressure *upstream* (*P-up*) and *downstream* (*P-down*) the collapsible fragment. Collapse of biological lumens occur if the *P-up* of the conduit falls below a certain pressure, the critical closing pressure, *Pcrit* [38]. A lower *P-down* of the collapsible fragment (the inspiratory negative pressure) will mainly reduce the lumen, but not cause it to collapse. This is shown in the Starling resistor modelling of the airway, a tube with a collapsible middle between two rigid ends, see Figure 1. *P-up - Pcrit* defines the pressure gradient of the airflow, independently of the *P-down*. Healthy subjects have a much lower (negative) passive *Pcrit* (<-10 cm H_20) compared to subjects with OSA (often >0 cm H_2O). A greater reduction of intraluminal pressure is thereby needed to collapse a healthy airway [36, 37].

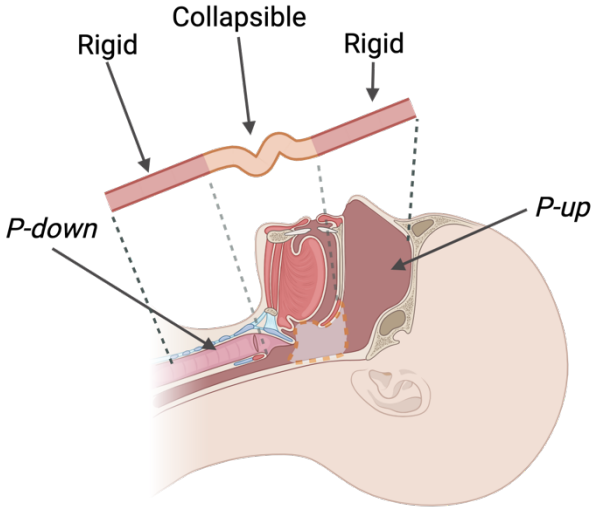

Figure 1. Overview over the collapsible segment in the human upper airway as described in the Starling resistor model. *P-up*: upstream pressure, *P-down*: downstream pressure.

1.1.5.3 RESPIRATORY CONTROL AND CHEMORECEPTION

The respiratory centre, located in the pons of the brain stem and medulla oblongata, tightly controls the ventilation to regulate the partial pressure of O_2 (pO_2) and partial pressure of CO_2 (pCO_2). The regulation of respiratory rhythm is complex, and the exact mechanisms are still investigated. Simplified, current models suggest that the neuronal regulation can be divided into phases: inspiratory, post-inspiratory and late expiratory. The inspiratory phase starts with a rapid increase in nervous signalling that ramps up and diminishes quickly. This is followed by a post-inspiratory phase, with a declining burst of neuronal discharge. The post-inspiratory phase is an important active part in the otherwise mostly "passive" exhalation. Active exhalation may occur in the late expiratory phase. The three phases are coordinated from the ventral medulla in the pre Bötzinger complex (inspiratory, pacemaker for the respiratory rhythm) and the Bötzinger complex (post-inspiratory and expiratory). Excitatory and inhibitory input from e.g., central chemosensitive neurons, peripheral chemoreceptors and lung stretch receptor via nucleus tractus solitarius in addition to higher central nervous system (CNS) control, modulate the three-phase neuronal output, and thereby the tonic respiratory drive and the phasic respiratory rhythm [39-41] .

The chemosensory receptors located peripherally (primarily carotid bodies) and centrally (medulla oblongata, retrotrapezoid nucleus) regulate the respiratory

neurons. The function of the different chemoreceptor groups are highly integrated and the overall ventilatory effects of gas changes are dependent on both peripheral and central chemoreception [42]. In addition to ventilatory effects, the chemoreceptors also have a considerable influence on the sympathetic nervous system via the nucleus tractus solitarius and ventral lateral medulla leading to e.g., modulation of blood pressure [43].

The multimodal carotid bodies main input is pO_2 and pCO_2/H^+ in arterial blood but also temperature, glucose, insulin and osmolarity play a role. The carotid body function seems to be important for the eupneic drive to breathe. The sensory signals are transmitted to respiratory neurons in nucleus tractus solitarius. The effects of changes in pO_2 and CO_2 are closely linked. For example, animal models show that hypercapnia without hypoxia induces a relatively slow activation. In contrast, a combination of hypoxia and hypercapnia will give rise to a powerful activation of neuronal signalling. On the other hand, hypocapnia during sleep elicits a fast reduction in signalling and ventilation [42].

The central chemoreceptors in the nucleus retrotrapezoid in the medulla oblongata mainly sense changes in pCO_2/H^+. In contrast to general brain vasculature, the blood vessels in this area do not react to changes in CO_2, preserving the responsiveness to overall pCO_2. The central chemoreceptors response to hypoxia is less pronounced than the corresponding response in the carotid bodies [37].

1.1.5.3.1 Respiratory control during sleep

Several changes take place in the respiratory regulation during sleep. The wakefulness stimuli and behavioural stimuli on respiratory drive are diminished and the respiratory drive is thereby highly dependent on metabolic control. In addition, the ventilatory response to both O_2 and CO_2 is reduced. Altogether, this leads to a lower minute ventilation and an increase in pCO_2 and apneic threshold, increasing the risk of a hypocapnic reduction of respiratory drive. The loss of wakefulness stimuli also reduces the tonic activity in the upper airway, increasing collapsibility. Sleep stage is another important parameter, with differences in hypoxic/hypercapnic response and muscle tone [41]. How these physiological changes contribute to OSA is discussed further below.

1.1.6 THE PATHOPHYSIOLOGICAL MECHANISMS OF OSA

In OSA, the upper airway is repetitively partially or fully collapsed, leading to reduction or obstruction of the airflow, even though the respiratory effort is continued. The fundamental problem in OSA is that the dilating forces of the

airway are weaker than the collapsing forces, causing an airway occlusion. In contrast, *central* sleep apnea is characterised by a diminished ventilatory drive and respiratory muscle activation. The upper airway can be open or closed during central apneas and hypopneas. In reality, the distinction between obstructive and central sleep apnea is less clearcut, there are considerable changes in central ventilatory drive also in OSA. The underlying pathophysiology behind the pharyngeal obstructions is based on both structural and functional deficiencies, often in combination [44].

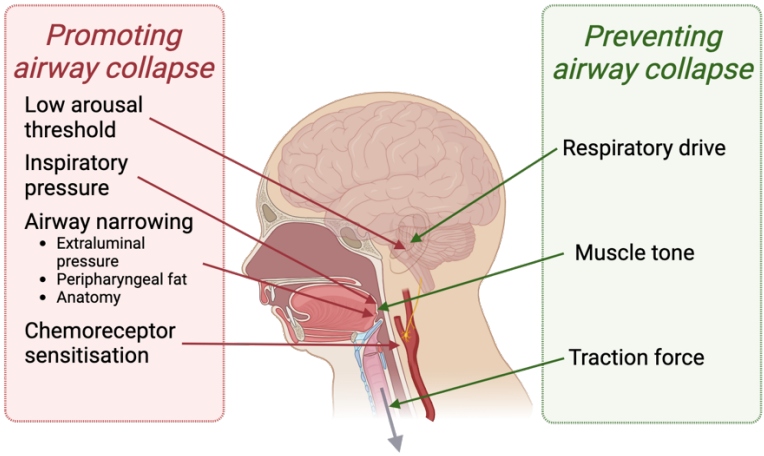

Figure 2. Forces affecting the upper airway collapsibility, underlying OSA development.

1.1.6.1 UPPER AIRWAY NARROWING IN OSA

As previously discussed, the size of the upper airway canal is determined by skeletal/cartilage structures and soft tissues. In OSA, there are two main causes for the increased collapsibility [37, 44]:

- Skeletal structures that are relatively small e.g. shorter maxilla, retrognathic mandible, a posteriorly placed hyoid [45]
- Higher total soft tissue volume e.g., increased fat deposition in the oral cavity, adenoids and enlarged tonsils [37, 46]

Other causes of upper airway narrowing include nocturnal fluid redistribution and accumulation in the upper airways due to congestive heart failure (CHF) or renal failure [47] and potentially nasal obstruction [48]. In obese subjects, increased abdominal fat may reduce the traction on the airway due to lower lung volumes, in addition to the airway fat deposition [37].

How much the anatomical factors contribute to the development of OSA differ. Despite a similar anatomical structure, the severity of OSA may vary considerably.

1.1.6.2 INSUFFICIENT MUSCLE COMPENSATION

Despite a compromised pharyngeal anatomy, patients with OSA do not have an upper airway obstruction while awake. The problem arises only during sleep. Physiologically, the activity in the upper airway dilatory muscles is reduced during sleep (lowest during REM sleep), resulting in increased airway resistance also in healthy subjects [44, 49]. The reduction of dilatating forces during sleep, in combination with a more collapsible airway, can induce a full occlusion. The muscle activity in OSA subjects may still be higher than in healthy individuals, but the compensation is insufficient to keep the airway open [44, 50].

1.1.6.3 RESPIRATORY CONTROL DEPENDENT MECHANISMS

Apneas and hypopneas in OSA show a rhythmicity, a respiratory disturbance increase the propensity of a new disturbance. There are several mechanisms underlying this phenomenon: induced changes in ventilatory regulation during sleep, a sensitised chemoreception and the ventilatory response to arousals.

During NREM sleep, the dependence on chemical/metabolic stimuli (pCO_2) is high. Even small reductions in pCO_2 will elicit reduced respiratory drive (ventilatory undershoot) and an apnea, the "CO_2 reserve" is low. Sensitised chemoreception, discussed below, increases the risk of hypocapnia after ventilatory overshoot. Activation of lung stretch receptors during hyperventilation and baroreceptor stimulation from increased blood pressure adds to a fast reduction of ventilatory drive leading to consequent apneas and hypercapnia provoking a new ventilatory overshoot [37, 44].

1.1.6.3.1 Sensitised chemoreception

The chemoreceptors activity is dependent both on short-term changes in pO_2 or pCO_2 but also on long-term changes in the partial pressures. Merely a few hours of hypoxia will increase the overall receptor sensitivity leading to hyperventilation, increased sympathetic drive and hypertension. These effects are sustained for several days after returning to normoxia. Intermittent hypoxia has an even higher potential to sensitise chemoreception due to the oxidative stress on the receptors. Hypertension, with increased vascular tone, may also sensitise receptors, potentially via low blood flow or sheer stress. A similar effect can be seen in CHF where the low blood flow reduce antioxidative processes in the carotid bodies [42].

An increased sensitivity in the chemoreception makes the respiratory system more unstable and prone to respond too much and too fast to a change in blood gas pressures. This instability can be explained by the engineering term "loop gain", describing the sensitivity of a variable system. The mechanism is described by the controller gain, the plant gain and the delay gain. The loop gain ratio is the size of a response to a disturbance (ventilatory response/ventilatory disturbance). If the response is higher than the disturbance, the system becomes unstable. A lower response will stabilise the system with decreasing response amplitudes, see Figure 3. The loop gain may be resembled with a room with a radiator in one end, and the thermostat in the other. The radiator (plant gain) heats the room air, which diffuses across the room with some delay (delay gain). After the delay, the thermostat transmits a feedback signal to the radiator in a response to the change in temperature (controller gain). The temperature stability of the room is dependent on a thermostat that is not hypersensitive and a radiator that is not too powerful (heats too much too quickly). A hypersensitive thermostat, a radiator that is too powerful or a long delay between the thermostat signal and radiator response, may increase the propensity for the system to oscillate [51, 52].

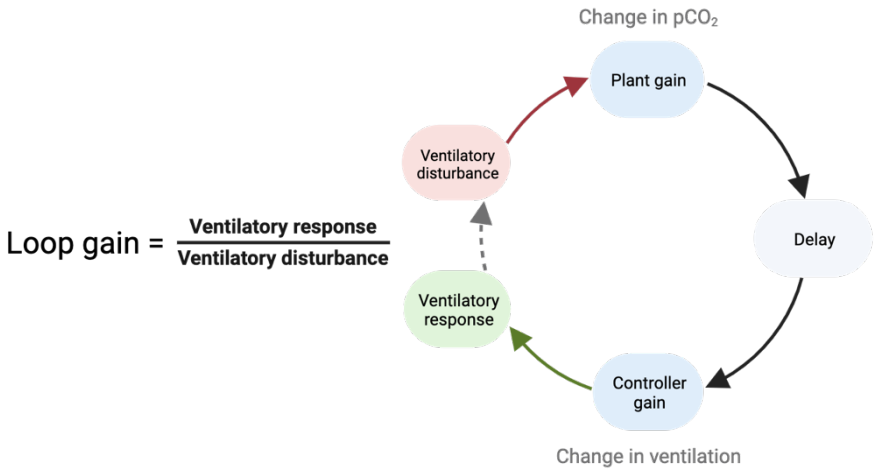

Figure 3. Simplified overview of the ventilatory regulation with focus on loop gain. Modified from Orr et al 2014 [53].

In the ventilatory system, the *controller gain* reflects the ventilatory response to blood gas changes (medullary receptors and carotid bodies). The *plant gain* reflects the blood gas change in response to a change in ventilation (effectiveness of lung function). *Delay gain* describes the time of the feedback signal to reach the controller (describing circulation and potentially also chemosensory response time). In OSA, the time to open the airway during an apnea also affects delay gain,

as there is no ventilation before the airway opens. An increased loop gain ratio in the ventilatory system will lead to an excessively high ventilation for a given disturbance. Instead of reducing the hypercapnia to normal values, there is an overshoot and the ventilation results in hypocapnia. The hypocapnia reduces the ventilatory drive, in turn inducing hypotonia in upper airway muscles. If the individual is predisposed to airway collapse, this leads to an obstruction and an apnea ensues. The airway collapse reduces the ventilation, causing a hypercapnic period which initiates a ventilatory response, opening the airway. If the ventilatory response is, again, too high, the overshoot results in hypocapnia and a new cycle starts [52].

In individuals with OSA, the loop gain is increased to a varying degree. It is not fully known if the loop gain change causes OSA or if OSA is causing the increased loop gain. The studies show conflicting and inconclusive results both regarding whether the loop gain generally is increased in OSA compared to healthy subjects, and if the controller or plant gain is changed. Several factors may affect the loop gain in OSA:

- Intermittent hypoxia may induce increased chemoreceptor sensitivity, as described above, increasing the controller gain by increased sensitivity to hypoxia and hypercapnia
- Obesity reduces the functional residual lung volume, resulting in higher fluctuations of gas pressures, increasing plant gain

It is difficult to physiologically determine loop gain in OSA and healthy subjects but estimation methods include positive airway pressure (PAP) pressure drops and approximation via PSG [51, 54, 55].

With deepening of NREM sleep, controller gain is reduced with the lowest levels seen in REM sleep. In REM, the respiratory muscles are tonically inhibited with intermittent periods of increased activity. During the activity phase, the respiration is more prone to overshoot due to increased muscle activity with subsequent irregularity of breathing, even though controller gain is lowered [51].

1.1.6.3.2 Arousal threshold

A high ventilatory response with increased risk of ventilatory overshoot can be induced by an arousal from sleep, as the arousal adds "extra force" to the upper airway dilator activity. The level of blood gas pressure disturbance (chemical drive) needed to elicit an arousal, the arousal threshold, differs between subjects. By having a low threshold, i.e., a relatively small increase in pCO_2 will induce arousals, the risk of overshoot and subsequent hypocapnia is higher. A low arousal threshold also means a shorter time for the upper airway muscles to react before an arousal starts. This limits the possibility for upper airway muscle tone

to increase and stabilise breathing, without arousals. Together, these mechanisms may contribute to development of OSA [44].

1.1.6.4 PATHOPHYSIOLOGY IN CLINICAL RESEARCH

The main pathophysiological mechanisms can be summarised as compromised anatomy, insufficient muscle compensation, high loop gain and low arousal threshold. The contributions of these four main mechanisms, or endotypic traits, were determined in a study cohort (58 OSA subjects and 17 controls) by Eckert et al in 2013: 37% had low arousal threshold, 36% insufficient muscle compensation, 37% high loop gain and most subjects (80%) had a highly collapsible airway, see Figure 4. 69% of the included subjects had multiple deranged mechanisms. This pivotal study influenced the field towards more mechanistic studies in OSA, addressing the underlying endotypes in OSA treatment [56, 57].

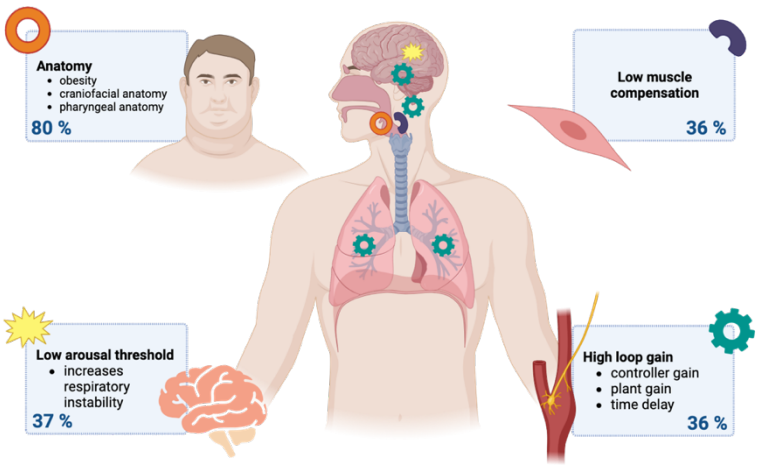

Figure 4. The main endotypic traits proposed in OSA with estimated prevalence according to Eckert et al 2013 [56].

1.1.7 THE CLINICAL INVESTIGATION

The investigation of suspected OSA is based on three steps according to Swedish guidelines [58]:

1. Symptom assessment and comorbidities
2. Physical examination
3. Sleep and respiratory recordings

1.1.7.1 SYMPTOMS

The most common symptom of OSA is excessive daytime sleepiness (EDS) or unrefreshing sleep which is reported by up to 90% of the patients. However, not all patients are sleepy, lack of energy or fatigue can be equally common. Excessive sleepiness is reported in 15-50% of the OSA population. The frequent arousals during sleep cause the tiredness, even though the individual may not fully wake up [6].

Witnessed *pauses of breathing, choking or gasping* during sleep is reported in only 10-15% of the patients, but the symptom is twice as common with OSA compared to general population. *Snoring* is also common, 50-60% of the OSA population snores. But there is also a considerable group of snorers that do not have OSA. Nocturnal *gastroesophageal reflux* is seen in 50-75% of OSA patients. Recurring *morning headaches*, resolving within a few hours, is reported by approximately 15% of OSA patients which is twice as common as compared to the general public [6]. *Nocturia,* two or more times per night is relatively common (30%) and may be a sensitive symptom, especially in the younger population. The mechanism underlying the nocturia is an increased venous pressure due to a low intrathoracic pressure, resulting in more increased atrial natriuretic peptide and subsequently more diuresis [59].

In addition to the patient history, several questionnaires are available e.g., ESS (daytime sleepiness), Berlin questionnaire (OSA screening) and STOP-Bang (OSA screening) [60-62].

1.1.7.2 COMORBIDITIES

Other illnesses closely linked to OSA include hypertension [63], atrial fibrillation (AF) [64], type 2 diabetes [65] and stroke [2]. See details under *Consequences and treatment effects*.

1.1.7.3 PHYSICAL EXAMINATION

According to Swedish guidelines for investigation of OSA [58] the physical examination can include:

- BMI
- Blood pressure
- Oral status, assessment before potential mandibular advancement device (MAD)
- Pharynx anatomy (tonsil enlargement etc)
- Nasal anatomy (polyposis)

1.1.7.4 SLEEP AND RESPIRATORY RECORDINGS

If the screening for symptoms and/or the physical examination suggest a risk for OSA, a night-time breathing examination is indicated. Several modalities are available, with different sensitivity and specificity.

1.1.7.4.1 Polysomnography – type 1 and type 2

Monitors $\geq$7 channels: EEG, eye movements (electrooculogram, EOG), chin muscle tone (EMG) for assessment of wake and sleep stages. In addition, abdominal and thoracic movements (effort), oronasal airflow, microphone for sound and pulse oximetry are recorded for scoring of respiratory events. A continuous electrocardiogram (ECG) and activity in anterior tibial muscle and body position is frequently measured [58].

- Type 1 PSG – investigation in a sleep laboratory
    - the gold standard for diagnosing OSA
    - video, oesophageal pressure (for measurement of respiratory drive) or CO_2 measurement is sometimes added
- Type 2 PSG – no surveillance, outside of the sleep laboratory
    - Similar standard montage as type 1

1.1.7.4.2 Polygraphy – type 3

No surveillance during the recording, often in the patients home using a portable device. The major difference compared to type 1/2 is the lack of EEG. At least four (but sometimes up to seven) variables are measured: oximetry, pulse, breathing movements, airflow, body position, EMG, ECG, actigraphy and/or sounds. The type 3 polygraphy (PG) recording is, according to Swedish guidelines, acceptable for diagnosis if combined with examination of an OSA competent medical specialist. In comparison to PSG, the PGs lack of EEG makes it less sensitive and specific because arousals are not detected and usually no sleep time quantification can be performed. The inability to detect arousals may lower the respiratory event index since hypopnea events without desaturations and RERAs are not scored [58].

1.1.7.4.3 Limited polygraphy – type 4

Measurement of one or two variables: oximetry, airflow and/or pulse. This level of investigation cannot be used to diagnose OSA according to international guidelines, as the sensitivity is too low. It may be used for follow-up of hypoxemia during OSA treatment [6, 58].

1.1.7.5 THE AHI AS A SEVERITY MEASURE AND FUTURE DIRECTIONS

The focus on AHI for sleep apnea classification has been criticised as the hypoxic and sleep fragmenting properties of OSA are poorly reflected in the metric [18, 66, 67]. In addition, the Wisconsin sleep study, as well as other studies (e.g. Swedish CArdioPulmonary bioImage Study [SCAPIS]) have reported high numbers of patients with OSA in the general population, but only a minority of those had daytime symptoms [22]. There are several underlying issues with the AHI: the definition has changed considerably over the years without corresponding changes in the OSA classification, different denominators ("sleep time" or "time in bed") are used for the index and the sensitivity differs in different types of sleep studies [18, 68]. However, the AHI is not useless. Rather than a single metric for OSA, AHI should be interpreted in relation to other measurements. One example is the Baveno classification proposed by Randerath et al, see Figure 5. In short, this classification is based on AHI >15 events/h in combination with daytime symptoms (ESS ≥11, hypersomnia, insomnia) and comorbidities or end-organ impact (AF, hypertension, CHF, stroke, diabetes mellitus). The different combinations categorise the patient into groups: A (mild symptoms, minor end-organ impact), B (severe symptoms, minor end-organ impact), C (mild symptoms, major end-organ impact) or D (severe symptoms, major end-organ impact). In an evaluation within the European Sleep Apnea Database (ESADA) cohort, OSA treatment in group A did not improve daytime symptoms or blood pressure but improvements in both or one of the two categories were seen in group B, C and D [69]. In the Swedish national guidelines for treatment of OSA, a modified classification has been introduced [70].

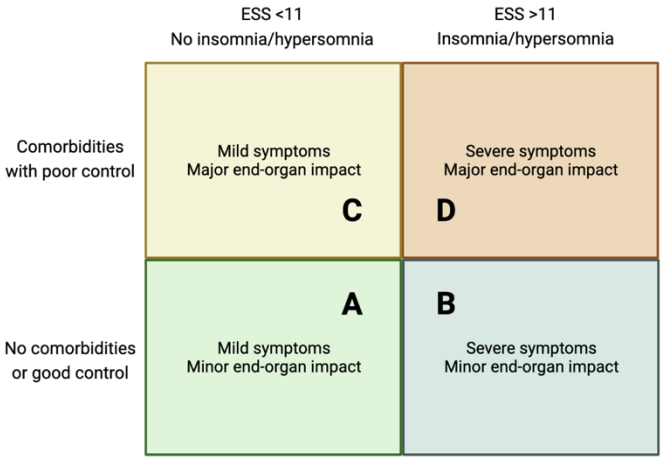

Figure 5. Revised Baveno classification. Picture modified from Randerath et al 2021 [69].

New measures are needed to describe the disease severity in OSA, in addition to AHI. To start with, more information can be extracted from the PSG, the investigation contains much more information than what we currently use. Different types of signal analysis and machine learning are likely to play a bigger part in the future diagnoses and severity classification of OSA [71]. In addition, wearables or new "non-contact" systems using cameras, radar, audio and sheet-type systems will probably be more commonly used to recognise OSA or risk of OSA. In a recent systematic review, the sensitivity and specificity of these types of systems were not inferior compared to portable PSG/PG, but most had only been evaluated in single studies. The sensitivity was often relatively high (>80%) but the specificity range was wide. It should also be noted that a majority of the studies evaluated the portable devices in sleep laboratories, which is not the intended setting. It remains to be seen where these systems have a place in OSA diagnostics [72].

1.1.8 TREATMENTS

The main, currently available, treatment options for OSA include continuous positive airway pressure (CPAP) therapy, MAD and in some cases upper airway surgery. Several new treatment modalities are under investigation but so far there are no additional established alternatives to CPAP or MAD. In a recent guideline of non-CPAP therapies, only carbonic anhydrase (CA) inhibitors (for use in randomised controlled trials [RCTs]) and surgical weight reducing strategies were granted conditional recommendations, with low evidence level. Other potential treatments such as myofunctional therapy, maxillo-mandibular osteotomy, hypoglossal nerve stimulation (HGNS) and positional therapy were either recommended against or did not have enough evidence to support a recommendation/non-recommendation [73]. This new guideline emphasises the considerable interest for the development of new treatment principles that are well tolerated by the patients.

1.1.8.1 CONTINUOUS POSITIVE AIRWAY PRESSURE

Sullivan and colleagues published the first description of the technique that came to revolutionise treatment of OSA, the CPAP, in 1981 [74]. CPAP prevents the collapse of the airway by an increase in the intrapharyngeal air pressure delivered via a nasal or nasal+oral mask. This "air splint" mechanism prevents obstructive apneas and/or hypopneas, and in most cases, completely eliminates OSA. The high efficacy makes it the primary treatment for moderate to severe OSA [6].

The recommended use of CPAP is throughout the sleeping period but many patients struggle to use the CPAP all night. Common practice is that a usage ≥4 h/night at least five nights per week is acceptable, a number that originates from CPAP adherence studies from the mid 1990s where mean usage was 4.7 h/night. Although these studies did not suggest that this level was adequate, it influenced studies to come [75]. The "adequate" usage level needs to be related to the outcome [76]. A systematic review recently summarised the effects on daytime function and, as expected, the positive effects are stronger along with increasing usage with study investigators often recommending ≥6 h/night [77]. For cardiovascular outcomes, observational studies have showed that a usage ≥4 h/night have positive effects [78-82]. ≥4 h/night should not be seen as a definitive level, keeping in mind the dose-response relationship with increased time on treatment.

The rate of patients who use their CPAP as prescribed vary in different studies. The non-adherence rate (<4 h/night cut-off) was 29% to 83% in 12 different studies, summarised by Weaver and Grunstein [83]. More recent studies show a 90 days compliance of 75% [84], long-term (three and four years respectively) compliance of roughly 50-80% [85-87] and no obvious increase in usage after the year 2000 [88]. Although compliance is lower than recommended, it is not far off from "regular" medications where compliance is reported to average around 50% [89]. Predictors of low compliance are: residual AHI during treatment, small nasal volume, black race (probably at least somewhat due to health disparities), claustrophobic tendencies, negative perceptions to treatment and low usage during the first two weeks of treatment [90].

1.1.8.2 NON-PAP TREATMENTS

1.1.8.2.1 Mandibular advancement devices

MAD increase the volume of the upper airway by protruding the mandible, resulting in a reduced collapsibility of the pharynx. The efficacy of MAD treatment is lower than CPAP. In a meta-analysis with 597 patients, MAD reduced the AHI less than CPAP (mean difference 7.8 events/h). The lower efficacy is important mainly in severe OSA, whereas in mild and moderate OSA the AHI may be normalised using MAD. In favour of MAD, the usage level is generally higher than CPAP and the treatment is often preferred by the patients [73]. The side effects of MAD include temporomandibular joint pain or tooth discomfort and occlusal changes due to the movement of the maxilla [6]. The high usage of MAD increases the mean disease alleviation even though the efficacy during use is lower. This may explain why the effects on sleepiness and quality of life is similar to CPAP [91]. Over the entire OSA severity spectrum, blood pressure effects are similar between CPAP and MAD. However, when

looking at moderate to severe OSA, the CPAP is more efficient in reducing night-time systolic blood pressure. Considering this, the MAD is not recommended as first-hand treatment in severe OSA or in individuals with comorbidities, but can be seen as equally effective in mild to moderate OSA [73].

1.1.8.2.2 Surgery

The first treatment systematically applied for OSA was surgical, the tracheostomy. In general, a surgical procedure can be seen as an alternative in patients with specific anatomic anomalies and in those not tolerating CPAP treatment [92]. There are several more or less extensive surgical treatment options. Uvulopalatopharyngoplasty (UPPP) is the most common soft tissue surgical treatment of OSA, reducing the tissue volume in the pharynx via resection of the uvula, tonsils and soft palate. The efficacy is often lower than CPAP, and if the patient gains weight, the effect may be transient. In addition, there are side effects such as postoperative pain [6].

A less invasive surgical treatment is provided by HGNS, a relatively new addition to the treatment arsenal. By increasing the muscle tone in pharyngeal dilators, the collapsibility of the pharynx is reduced. So far, there are few RCTs on HGNS in OSA but the evidence is increasing. The treatment can be invasive (surgically placing a "pacemaker" electrode on n. hypoglossus) or non-invasive with transcutaneous stimulation applied during the night. In a recent European respiratory society (ERS) guideline, HGNS treatment was recommended only as a salvage treatment in specific cases when CPAP or MAD are insufficient or do not provide an option [6, 73].

1.1.8.2.3 Weight reduction / exercise

Obesity is the major risk factor contributing to OSA and observational studies have shown that 1% weight reduction reduces the AHI by 2.4%-2.8% [93]. Importantly, weight reduction may have additional positive effects on comorbidities and quality of life. However, diets and other weight-reducing interventions do not always work. The ERS recommends bariatric surgery over weight-reducing diets in patients with BMI $\geq$35 kg/m^2 [73].

Interestingly, individuals that exercise have been shown to have lower risk of moderate to severe OSA compared to individuals that do not exercise, with similar BMI. Even with a relatively short total weekly exercise time of 1-2 hours, the adjusted odds ratio for moderate to severe OSA was 0.62 [30]. Similar results have been shown in small RCTs where OSA was reduced without weight change [6]. Pharmacological weight loss regimes in relation to OSA are discussed below, see *Development of pharmaceutical therapies in OSA*.

1.1.8.2.4 Myofunctional therapy

Myofunctional therapy may reduce upper airway collapsibility by improvement of nasal breathing, lip seal and tongue position with specific exercises / electrical muscle stimulation. Although the exercises have similar goals, the techniques used differ [94]. The evidence is limited but treatments have been shown to be safe and have positive effects in both AHI (-8 events/h) and ESS (-2.7) in a meta-analysis of RCTs. Long-term data is lacking. The ERS does not recommend myofunctional treatment in favour of CPAP but states that it may be an alternative for patients reluctant to mechanical therapy [73].

1.1.8.2.5 Positional therapy

Some patients have a more severe OSA when lying supine, due to the posterior positioning of the tongue. If the AHI is twice as high in supine position compared to non-supine, the OSA is positional. Many of these patients do not have a significant disease when sleeping on their side, and positional therapy may be an option. There are several different techniques; e.g., tennis balls sewn into the back of a t-shirt and more intricate devices that vibrate when the patient is supine. Positional therapy with vibratory stimulation can be used in favour of CPAP in cases with positional OSA and no or low number of non-supine breathing disturbances. Other forms of positional therapy are not recommended due to low long-term adherence. Compared to MAD, the effects are similar, but evidence is limited [73].

1.1.8.2.6 Pharmacological treatments

The use of pharmaceutical compounds to treat OSA is discussed below, see *Development of pharmaceutical therapies in OSA*.

1.1.9 CONSEQUENCES AND TREATMENT EFFECTS

OSA has both short-term and long-term consequences. The short-term consequences are mainly disturbed sleep with resulting cognitive effects. In the long-run, the risk of metabolic and cardiovascular dysfunction is increased in addition to higher all-cause mortality.

1.1.9.1 COGNITIVE EFFECTS

1.1.9.1.1 Daytime sleepiness and cognitive function

In contrast to what one may think, the severity level of OSA is not highly correlated to EDS [95]. It is also important to remember that EDS can be caused by other diseases than OSA and that 4-20% of the general population report EDS,

at least a few days a week [96]. Studies of EDS prevalence in OSA have shown mixed results. In the American Wisconsin sleep cohort (adults with AHI ≥5 events/h), 19% were subjectively sleepy on a 5-point scale and a European study showed that 50% of individuals with AHI ≥15 events/h feel sleepy (defined as ESS >10) [97, 98]. Sleepiness can be measured objectively (multiple sleep latency test, MSLT) and/or subjectively (e.g. ESS). Unfortunately, the correlation between the objective and subjective sleepiness in OSA is weak [99]. Different measurements affect the study definitions of sleepiness, resulting in highly variable study outcomes and difficulties to combine study results [95].

Using more objective measurements, OSA has been shown to affect multiple cognitive domains. The most disturbed domains include psychomotor speed and executive function but other domains such as memory, motor control and attention are also affected [100]. On a more general scale, OSA is associated with a decrease in productivity, an increase in traffic accidents, injuries and risk of work accidents [101, 102]. The mechanisms for these cognitive deficits are not fully understood but the sleep fragmentation and intermittent hypoxia may potentially induce brain cell injury and reduce plasticity of the synapses [100].

In a study from 2009 Sànchez et al reviewed the literature of OSA treatments on daytime sleepiness. The included trials had different methodologies, control groups and mostly participants with EDS. The majority of the trials did show improvement in subjective EDS following CPAP treatment, also in comparison to placebo, but in the objective outcomes the results were inconsistent. In the cognitive functioning measurements, studies suggest an improvement mainly in executive function [103]. Balk et al investigated the effects on cognitive (executive functioning) and included RCTs and non-randomised controlled studies. The consistency among studies was low and the authors state that it is unclear if the cognitive tests used are ideal for evaluation in OSA. The overall results are inconclusive with insufficient evidence [104, 105].

1.1.9.1.2 Cognitive impairment and Alzheimer's disease

OSA has been associated with mild cognitive impairment as well as Alzheimer's disease dementia, mostly in retrospective observational studies. The proposed mechanisms include inducing or aggravating neurodegeneration, synaptic dysfunction, inflammation and tissue injury following oxidative stress, in addition to sleep fragmentation and EDS [106]. Trials of CPAP treatment for these outcomes are in general small and mainly observational, with inconsistent results [106].

1.1.9.1.3 Mental health

There is an increased risk of psychiatric disorders in OSA, especially when the treatment is suboptimal [107]. A systematic review by Gupta et al in 2015 indicated that OSA prevalence was increased in major depressive disorder and posttraumatic stress disorder, but not in psychotic, bipolar or anxiety disorders [108]. The considerable symptom overlap between mood disorders and OSA complicates the diagnosis [107].

CPAP treatment has in some meta-analyses shown positive effects in major depressive disorder and anxiety disorders, but often the outcomes are not compared to placebo or not statistically significant from placebo [109]. In a recent meta-analysis of long-term effects (>6 months) by CPAP treatment, a small but clinically insignificant effect was seen, with low strength of evidence [104].

1.1.9.2 CARDIOVASCULAR DISEASES

The increased risk of several cardiovascular diseases such as hypertension, ischaemic heart disease, cardiac rhythm disorders and stroke are mechanistically closely interlinked in patients with OSA. In addition to the direct association to cardiovascular diseases, OSA also increases the risk of metabolic dysfunction which may further increase cardiovascular morbidity. It is important to remember that even though AHI is the most widely used and accepted metric for OSA severity, other measurements such as hypoxia, total sleep time, frequency of awakenings during the night and daytime sleepiness have been shown to better predict cardiovascular outcomes [3].

Recently three large RCTs of cardiovascular secondary prevention with CPAP treatment of OSA failed to reduce composite outcomes of cardiovascular events [110-112]. In short, McEvoy et al (SAVE study, n=2 717) included patients 45-75 years, with oxygen desaturation index (ODI, with 4% desaturation rule) $\geq$12 events/h and coronary or cerebrovascular disease, without severe daytime sleepiness (ESS>15) or severe hypoxemia. Peker et al (RICCADSA study, n=244) included revascularised patients with coronary artery disease and AHI $\geq$15 events/h without daytime sleepiness (defined as ESS$\geq$10). Sanchez-de-la-Torre et al (ISAACC study, n=1 264) included patients aged $\geq$18 years admitted for acute coronary syndrome with ESS $\leq$10 and AHI $\geq$15 events/h. Several aspects have been raised that may potentially explain the lack of effects in primary outcomes [113]: participants had established cardiovascular disease, a large share of the patients did not have symptomatic OSA (individuals with daytime sleepiness were excluded), the CPAP adherence was low (in SAVE study 41% and in ISAACC 36% used CPAP $\geq$4 h/night, in RICCADSA 62% used CPAP after one year) and the sample sizes were relatively small. Several views on how

to overcome these problems in future studies have been published, such as inclusion of sleepy patients, more severe OSA (AHI >30 events/h), younger patients, using a single cardiovascular end-point and efforts for higher CPAP adherence [114] or propensity score matching to balance covariates [113].

1.1.9.2.1 Hypertension

OSA and hypertension are often coexisting, with a relationship that is bidirectional. The prevalence of hypertension in OSA subjects ranges from 35-80% depending on study and setting. From the opposite viewpoint, the prevalence of OSA in hypertensive subjects ranges from 40% to almost 90% in resistant hypertension. The causality of OSA to hypertension is debated, some studies show a causal relationship independent of confounders, while others do not [63].

There are several proposed mechanisms behind the relationship: hypoxemia and hypercapnia induce an *increase in the sympathetic activity* via sensitisation of chemoreceptors and changes in the baroreflex that continue during the daytime [42, 63]. The intermittent hypoxia increases reactive oxygen species, promoting *inflammation* and *endothelial dysfunction* [115]. *Hormonal changes* with aldosterone promote fluid retention, increasing OSA [47, 63]. *Intrathoracic pressure swings* during respiratory disturbances increase cardiac afterload, putting pressure on the ventricular and atrial walls, promoting cardiac re-modelling [63].

The effect of CPAP treatment in OSA on blood pressure is generally modest, systolic pressure drops 2.6 mmHg and diastolic 2 mmHg compared to no CPAP [116]. Withdrawal studies have shown increases in blood pressure when discontinuing CPAP (systolic +9 mmHg and diastolic +5 mmHg) [117]. MAD have shown blood pressure reducing effects similar to CPAP. However, in more severe OSA, CPAP is more efficient [73, 118].

1.1.9.2.2 Stroke

The association between ischaemic stroke and OSA have been shown in both observational and cross-sectional studies [2, 119, 120]. Further supporting this association is the increased risk of wake-up stroke in individuals with OSA, the 60% prevalence of OSA in stroke patients and the increased risk of recurrent stroke and/or worse outcomes post-stroke in individuals with OSA. The same underlying mechanisms as in OSA-related hypertension are believed to play a role in the increased risk for ischaemic stroke, in addition to changes in cerebral blood flow and increased platelet aggregation [121].

Observational data on CPAP treatment effects in reducing the risk of stroke show a that untreated severe OSA patients have a 3.4 times higher risk of incident stroke

compared to treated severe OSA or untreated mild to moderate OSA [122]. Other observational studies have shown no effect of CPAP treatment. RCTs have failed to show reduction of primary endpoints but when comparing CPAP treatment with usage >4 h/night to untreated, there are positive treatment effects in some studies [121]. Overall, the controlled trials performed so far cannot provide evidence of treatment effects but this does not imply that treatment is ineffective [104].

1.1.9.2.3 Ischaemic heart disease

Observational studies show a relation between OSA and coronary artery disease and/or cardiovascular mortality. The relationship is more pronounced in severe OSA (meta-analysis, hazard ratio [HR] 2.73) and some studies suggest that mild or moderate OSA do not increase the risk of cardiovascular mortality [123]. Patients with myocardial infarction and OSA have been shown to have a lower 18-month event-free survival compared to patients without OSA [124]. The underlying mechanisms are proposed are the same as in hypertension in addition the oxidative stress from hypoxemia/reoxygenation that may induce coronary atherosclerosis [64, 125]. It is important to remember that the association is confounded by other risk factors for cardiovascular disease and adjustment of comorbidities is necessary when analysing the data.

Even though the relation is evident in observational studies, RCTs with CPAP treatment have failed to show reductions of myocardial infarctions, angina, hospitalisation for unstable angina or revascularisation [104, 110-112]. There are ongoing discussions on why these RCTs have failed to show effects when an association is seen in the observational data.

1.1.9.2.4 Atrial fibrillation

AF is common in OSA (ranging from 17-82% in different studies), with a higher prevalence compared to normal population, also after adjusting for confounders. The risk of failure of anti-arrhythmic medications is higher and recurrence of AF after cardioversion is more common in patients with AF and OSA. The underlying mechanisms include changes in intrathoracic pressure during respiratory events (with functional and structural re-modelling of the atria), hypoxia and hypercapnia, autonomic dysregulation and increased sympathetic tone. Small, observational studies have shown that CPAP treatment can reduce burden of AF such as recurrence and transition to permanent AF [64, 125]. However, a recent systematic review and meta-analysis did not find evidence of effects on AF risk in controlled studies of CPAP versus no CPAP [104].

1.1.9.2.5 Congestive heart failure

Sleep disordered breathing is associated with CHF in several ways. The prevalence of both OSA and central sleep apnea in the CHF population is 40-60%, with OSA constituting roughly 30-50% of these cases [64]. This section will focus on the relationship with OSA.

Prospective observational data suggests that the incidence of CHF is increased 1.58-fold in male subjects with severe OSA compared to no OSA after adjustment for risk factors [1]. The relationship is bidirectional: the fluid retention in CHF may narrow the upper airways due to rostral fluid shift [47] and, theoretically, prolonged lung-chemoreceptor circulation time may increase loop gain. OSA may aggravate CHF by increased afterload and right ventricular distention due to intrathoracic pressure swings, myocyte dysfunction due to sympathetic tone increase and endothelial dysfunction [64, 126].

There is limited evidence regarding the effect of CPAP treatment of OSA in CHF, but continuation of CPAP therapy for OSA was associated with a lower HR (0.77) of incident CHF compared to CPAP termination [127]. A recent systematic review concluded that "there is insufficient evidence to determine the effect of CPAP on risk of CHF or hospitalization for CHF" [104].

1.1.9.3 METABOLIC COMORBIDITY

Obesity is a major risk factor for both metabolic disorders and OSA. However, studies show that also lean OSA patients have a high risk for metabolic comorbidities [128, 129].

1.1.9.3.1 Diabetes mellitus and impaired glucose tolerance

Several observational studies have shown an association with OSA and insulin resistance and/or type 2 diabetes, independent of obesity. A meta-analysis by Reutrakul et al estimated the adjusted relative risk (RR) for type 2 diabetes in OSA to be 1.35. This can be compared to physical inactivity with a RR 1.20. The prevalence of type 2 diabetes in OSA is approximately 15-30%, and higher in severe OSA [65]. Intermittent hypoxia and sleep fragmentation lead to activation of reactive oxygen species, activated hypothalamic-pituitary-adrenal axis, increased sympathetic tone and changes in adipokine levels [130]. The interaction between hormones regulating metabolism and OSA is complex. For example, the adipokine leptin, a satiety regulating hormone, discovered by the Friedman group in 1994 [131], is increased by obesity but also intermittent hypoxia. In addition to its main effects, leptin may also act pro-inflammatory, stimulate respiration and potently activate the sympathetic tone. These widely different effects are induced

by the intermittent hypoxia of OSA and can further amplify OSA consequences and increase dysmetabolism [130, 132].

Studies investigating the effect of CPAP on glycaemic markers in non-diabetic or pre-diabetic patients with OSA show inconsistent results. In diabetic patients, CPAP treatment has in some studies modestly reduced HbA1c [65]. As for RCTs, these have only investigated the risk of incident type 2 diabetes and a meta-analysis did not show effects on the incidence [104].

1.1.9.3.2 Hyperlipidaemia

In line with increased prevalence of diabetes in OSA patients, other signs of dysmetabolism such as a dyslipidaemia are more common as well. With increasing OSA severity, the dysregulation of lipid metabolism and hyperlipidaemia are more pronounced [133-135].

Animal studies show that the intermittent hypoxia in OSA increase lipolysis in adipose tissue and increase the biosynthesis in the liver during fasting state. After a meal, intermittent hypoxia delays the lipoprotein clearance. These mechanisms are proposed to constitute the major factors behind the dyslipidaemia. In addition, oxidative stress changes the metabolic function of the lipids [135].

Barros et al summarised observational studies of OSA treatment and dyslipidaemia in 2019, with mixed conclusions. Some studies show improved lipid variables and others do not [135].

1.1.9.4 ALL-CAUSE MORTALITY

Several systematic reviews with meta-analysis have investigated the evidence for an increased risk of all-cause mortality in OSA, the two latest published in 2017. Both Fu et al (observational studies, retrospective and prospective) and Xie et al (observational studies, only prospective) show a significantly increased risk of all-cause mortality in the group with severe OSA (HR 2.13 and RR 1.54, respectively). However, there was no statistically significant difference for the mild and moderate OSA group [123, 136].

In Fu et al, CPAP treatment was shown to reduce all-cause mortality in OSA, even though the relative risk was still higher than the control group [123]. However, RCTs of CPAP treatment in OSA have failed to show beneficial effects on all-cause mortality [104, 110-112]. On the contrary, a recent study by Pépin et al investigated all-cause mortality following termination of CPAP treatment in the French national health reimbursement system. Using propensity score matching, patients who terminated CPAP were compared to patients who continued their treatment. Overall, continuation of CPAP treatment showed a significantly lower HR (0.61) for all-cause death compared to therapy termination [127]. A recent

systematic review and meta-analysis prepared by the Brown Evidence-based Practice center showed similar results: CPAP treatment did not reduce all-cause mortality in RCTs, but an effect was seen when including not randomised controlled studies (effect size 0.61) [104].

1.1.10 POTENTIAL BIOMARKERS IN OSA

There is considerable interest to find techniques that may facilitate diagnosis and treatment in OSA as well as prognosis prediction. Some of these characteristics may be provided by biomarkers. A biomarker is a "…defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention". *Diagnostic* biomarkers detect or confirm the presence of a disease or condition, *predictive* biomarkers predict a higher likelihood of favourable/unfavourable effect of treatment and *prognostic* biomarkers may indicate the likelihood of clinical events [137].

The key focus in OSA have been to identify diagnostic biomarkers that may reduce the need of a PSG measurement. These have often been identified in blood chemistry within the inflammatory, cardiometabolic or hypoxic field and several inflammatory parameters like IL-6, IL-10, Vimentin and Endocan show potential as diagnostic biomarkers. However, meta-analyses of the current status in this research area concluded that few diagnostic biomarker candidates have been evaluated repeatedly, many reports lack important data such as sensitivity and specificity, the control group is often insufficient and most are studies are small single-centre trials [138, 139]. Studies on different biochemical measures with focus on underlying mechanisms, aimed at prediction of treatment effect or disease prognosis, are more common. Several studies support e.g. a higher concentration of inflammatory parameters such as C-reactive protein, lower/higher levels of the metabolic markers adiponectin/leptin, higher levels of the hypoxic marker hypoxia-inducible factor 1α (HIF-1α) and higher CA activity in severe OSA [140-143].

Other types of biomarkers include PSG-related variables such as endotypic traits [55] or PSG hypoxia severity [144, 145]. Parameters like these may play a role in future treatment decisions [146, 147]. There is a great potential to increase treatment efficacy, but prospective studies investigating these markers are needed.

As the possibility to handle, store and analyse big data sets is rapidly increasing, the field of biomarkers is developing. The human biology is complex and it is rarely one single mechanism that explains a disease. OSA, like many other diseases, is a cause of many interlinked mechanisms. Biomarkers need to be seen as a system, with several markers contributing to diagnose, treatment response prediction or prognosis. This makes studies of biomarkers complex, but is

probably necessary for further development in the field [137]. Using biomarkers from blood/and or PSG can perhaps make the OSA patient care more efficient (potentially reducing unnecessary investigations) and may enable a more personalised treatment recommendation to the patient.

1.2 CARBONIC ANHYDRASES

In the early 1930s, researchers realised that the rate of uncatalysed HCO_3^- dehydration was too slow to explain how the production of CO_2 could be excreted in the lungs during the short capillary transit time. The explanation turned out to be a class of enzymes called carbonic anhydrases (CA) [148-150]. There are 15 isoforms in humans, all of the α class. CA catalyses the reaction

$$CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+$$

This reaction is fundamental in many physiologic processes and with CA, the reaction rate can be up to 10^6 times faster. The catalytic activity is dependent on zinc hydroxide that binds to CO_2, creating zinc-bound HCO_3^-. The HCO_3^- is later displaced by a water molecule after which H^+ is transferred to the surrounding solution and the zinc hydroxide is regenerated, the last step being the rate limiting [151]. The CAs also catalyse several other reactions, such as hydrolysis of esters, valuable for studies in vitro [152]. Interestingly, three of the CAs (VIII, X and XI) are catalytically inactive [153]. The human CAs are divided into cytosolic (I, II, III, VII, VIII, X, XI, and XIII), membrane-associated (IV, IX, XII, XIV), mitochondrial (V_A and V_B) and secretory (VI). The isoforms have different functions and specific locations throughout tissues and organs, see Table 1. The CAs play important roles in the elimination of CO_2 from cells, transport in the blood and subsequent elimination in lungs and kidneys. In addition, CAs are involved in a wide range of processes such as gluconeogenesis, ureagenesis, tumorigenesis, physiologic proliferation and have a key role in pH- and fluid balance [154].

	ISOFORM	TISSUE	CATALYTIC ACTIVITY	FUNCTION
CYTOSOL	I	Erythrocytes, gastrointestinal tract, eye, sweat glands, adrenal glands	Low	CO_2 turnover, acid-base balance
	II	Erythrocytes, eye, gastrointestinal tract, bone osteoclasts, kidney, lung, testis, brain, salivary glands, endometrium, adrenal glands	High	CO_2 turnover, acid-base balance
	III	Skeletal muscle, adipocytes, uterus, prostate, lungs, kidneys, colon, erythrocytes	Low	CO_2 turnover, acid-base balance, resistance to oxidative stress
	VII	CNS, liver, skeletal muscles, stomach, intestines	High	CO_2 turnover, acid-base balance
	VIII	CNS, liver, lungs, heart, intestines, thymus, kidney	Acatalytic	modulating calcium-signalling
	X	CNS	Acatalytic	myelin sheath development
	XI	CNS, gastrointestinal tract, ovary, pancreas, kidneys, adrenal gland, thyroid, salivary glands	Acatalytic	not known
	XIII	Kidney, brain, lung, intestines, reproductive tract, uterus, endometrium	Low	CO_2 turnover, acid-base balance
MEMBRANE	IV	Kidney, lung, pancreas, brain capillaries, colon, heart muscle, eye	Medium	CO_2 turnover, acid-base balance
	IX	Gastrointestinal mucosa, CNS. Tumours	High	CO_2 turnover, hypoxic cell survival
	XII	Kidney, intestine, reproductive epithelia, eye, CNS, endometrium, sweat glands. Tumours	Low	CO_2 turnover, hypoxic cell survival
	XIV	Kidney, brain, liver, eye, heart, skeletal muscles, lungs	Low	CO_2 turnover, acid-base balance, response to chronic hypoxia
MITOCHONDRIA	V	V_a: Liver V_b: Heart and skeletal muscle, pancreas, kidney, spinal cord, brain, gastrointestinal tract	Low High	Function in gluconeogenesis, lipogenesis, ureagenesis
SECRETED	VI	Salivary and mammary gland	Low	neutralises acid from food and oral bacteria

Table 1. Carbonic anhydrase (CA) isoforms with tissue distribution and localisation, catalytic activity and summarised function. CNS: central nervous system. Adapted from Mishra et al 2020 and Zamanova et al 2019 [152, 155].

The activity of CA in whole blood has been proposed to have a relation to ventilation and ventilatory changes. For example, an increased activity was shown after six weeks of supervised exercise and patients with hyperventilation syndrome has been reported to have higher activity. In addition, higher activity was recorded in severe OSA patients compared to controls [143, 156, 157].

1.2.1 ACID-BASE BALANCE IN RELATION TO CARBONIC ANHYDRASE

The pH is tightly controlled and regulated by two main pathways, the respiratory and metabolic/renal. These processes can be described using a physiological approach where the pCO_2 and HCO_3^- are in constant equilibrium, dependent on the concentration of pH. This reaction is catalysed by CA as described above. In the respiratory pathway, a higher pCO_2/H^+ (lower pH) induces hyperventilation via the chemoreceptors. This will reduce the pCO_2, and subsequently the overall H^+ concentration. The renal pathway is slower than the respiratory but may increase or reduce the secretion of H^+ in the tubules and can modify the reabsorption of HCO_3^-. These mechanisms are dependent on CA function for substrate modification, enabling diffusion and active transport. Modification of the HCO_3^- level is important in the metabolic response to acid-base changes [158, 159].

1.2.2 CARBONIC ANHYDRASE INHIBITION

The role in many physiologic reactions and widespread expression in tissues have opened possibilities for treatment in a diverse range of diseases. Even though there is a potential for more specific treatment targets, most clinically used CA inhibitors have a non-specific binding. There are considerable differences in *in vitro* binding characteristics for the CA inhibitors which in part may explain the differences in effects. However, the *in vivo* effects are further explained by the location of the CA enzyme (membrane, intracellular etc.), the "CA isomer mix" in the tissue and pharmacological factors (metabolites, protein binding, excretion). In addition, non-CA effects may also be important to explain the full effects of a compound [160].

CA inhibition is used in diseases such as glaucoma where the increased intraocular pressure can be lowered by reducing the secretion of aqueous humour, a process in which CAs have an important role. By using topically administered CA inhibitors, systemic side effects are limited. Other uses for CA inhibitors include high altitude sickness, where acetazolamide (ACT) has been shown to reduce symptoms and blood pressure increase and minimise periodic breathing during

sleep at high altitude [161-163]. CA inhibition can be used in the treatment of obesity as CA V_A, V_B and cytosolic CA II, play a role in the cell metabolism. The two CA inhibitors topiramate and zonisamide have been shown to induce weight loss, potentially by affecting lipogenesis and suppressing the appetite. The CA inhibitory effects of topiramate (in addition to Gamma-aminobutyric acid [GABA] enhancement) are also used in the treatment of epilepsy. CAs are widespread in the brain and crucial for controlling pH, thereby affecting cell excitability [152].

Much of the current interest in CA inhibitors is focused on developing more specific and selective compounds. For example, the insufficient angiogenesis in tumours results in a hypoxic environment, and subsequent acidosis. To prevent intracellular acidosis, tumour cells may increase the expression of CA IX and XII. An increased level of CA IX and XII has been associated with more malignant tumours. In this case, inhibition of CA IX and XII could potentially be anti-tumoral by reduction of the tumour cells resistance to acidosis [152, 160].

Most treatment indications include a single tissue or organ system, where an inhibition of CA may be of use. But with systemic administration, a widespread expression of CA and a non-specific binding pattern of inhibitors, the risk of side effects is increased. The majority of studies on systemic CA inhibition used ACT and many of the findings on adverse effects are therefore heavily biased towards this specific drug. It should also be noted that the side effect for one patient may be a desired effect for another, e.g. unwanted weight loss versus treatment of obesity. In general, the most common side effects include paraesthesias, dyspepsia and fatigue. For most patients, the effects are tolerable, but may occur in up to 50% of treated subjects. More serious side effects include respiratory failure, acidosis and encephalopathy in patients with underlying illnesses such as lung, renal or hepatic disease [160].

1.3 DEVELOPMENT OF PHARMACEUTICAL THERAPIES IN OSA

The first studies of drug treatment in OSA were performed already in the 1980s, but these trials were often small, short-term and mostly explorative. Today, a deeper knowledge of underlying pathophysiological mechanisms has opened doors for more specific treatment targets. Rather than focusing on downstream mechanisms, such as splinting the airway open with PAP, researchers have now turned their interest to upstream mechanisms, preventing the collapse of the airway.

Since there is no prior drug for OSA, there is a lack of regulatory guidelines for drug development in this disease. In addition, there are several OSA specific challenges: Firstly, which OSA outcome should be used? For a long time, the AHI was the only primary efficacy measure in studies of OSA. Now, the focus has broadened and the trend is to, in addition to AHI, use more hypoxia-focused outcomes (e.g., ODI, AHI with 4% desaturation rule and hypoxic burden [144, 145]) and subjective outcomes (e.g., EDS). More rarely, comorbidity outcomes (e.g., blood pressure) have been used. However, different outcomes complicate comparisons between studies. Secondly, the variability of OSA measurements increases the risk of both type 1 and type 2 errors. The variability within and between studies is increased by individual scorers, different sensor techniques and type of overnight measurements (PSG or PG?). This can potentially make multi-centre studies problematic. Thirdly, the night-to-night variability of OSA is considerable at the individual level. Previous small-scale exploratory studies have been hampered by this high variability of results, with promising developments failing in subsequent trials [164, 165]. Fourthly, the pathophysiology of OSA (endotypic traits) is diverse and differs between subjects, which may affect the drug efficacy in a single patient [57]. The characterisation of these mechanisms is still relatively laboursome and incompletely understood. At this stage, you cannot in a clinical setting reliably group patients according to underlying mechanisms / endotypic traits. A small sample size in pilot drug trials may lead to a skewed distribution of different endotypic traits, and thus, a lower generalisability [164]. So far, attempts to pre-select participants based on individual endotypic traits have not been successful, possibly due to changes in several underlying traits in a single patient.

1.3.1 PLACEBO AS A COMPARATOR IN DRUG TRIALS

In OSA, many drug trials have been relatively small, with few sleep measurements and often without sufficient control groups. The considerable intra-individual night-to-night variability in OSA poses specific needs for trial design and power calculations since this may have a big impact on the results [166]. Using placebo is a way to adjust for potential confounders from the *administration* of a treatment, and to differentiate these effects from drug effects. In a disease where subjective measures such as daytime sleepiness are important, the placebo is vital.

1.3.1.1 DEFINITIONS AND MECHANISMS

There are several definitions of *placebo*. In controlled trials, *placebo* is the control intervention with a similar appearance of the study treatment, but without the

active component. The placebo effect is believed to be a result of the interaction between healthcare provider and the study participant and not the inactive compound (e.g., sugar). Another definition of *placebo* is based on the patient's expectations concerning their health. These expectations, negative or positive, affect the way he or she respond to the intervention given. In this case, *placebo* is the beneficial effect whereas *nocebo* is a harmful effect for the individual. The effects may be pronounced, not only for treatments with active/inactive drugs in clinical trials but also when the patient is given information such as public health campaigns or information about treatments [167, 168]. In the following section *placebo* is defined as a control intervention.

The individual's expectation or anticipation of effects are important. Conscious expectations can be modulated by prior experiences, information, instructions or observations (e.g., observing symptom relief in another individual by the same intervention). The effects are thought to be a result from the release of endogenic neurotransmitters such as endocannabinoids, endogenous opioids, oxytocin, dopamine and vasopressin which may affect e.g., the sensation of pain, nausea or motor function. Inhibition of these pathways have been shown to block the effects of placebo and imaging studies have indicated that for example, pain nocebo effects can be seen in spinal cord imaging as an increased pain signalling to the brain [168]. Even unconscious expectations may play a role as was shown in patients taking immunosuppressive medications. Previous stimulus given at the same time as the immunosuppressant also reduced T-cell proliferation without administering the active medication [169]. Similarly, one study showed that using open label (!) placebo between active drug doses reduced glucocorticoid use in psoriasis without increasing the risk of relapse [170].

1.3.1.2 INVESTIGATING THE PLACEBO EFFECT

To understand the effect of an intervention, the regression to the mean has to be accounted for. Regression to the mean describes the phenomenon that an extreme value tend to be closer to the mean if the measurement is repeated [171]. This is due to the random measurement variability, or the natural course of disease and symptoms. You can adjust for regression to the mean by using a control group, often together with a placebo. In line with this, to investigate the effects of placebo (where the placebo itself is the "active" intervention) the control intervention would be to do nothing, a no-treatment group. Nevertheless, many studies investigating the effects of placebo interventions have not used no-treatment comparisons, which may introduce bias due to the regression to the mean. This may have affected the results in many of trials investigating placebo effects [167].

Hróbjartsson and Gøtzsche summarised the effects of placebo interventions for all clinical conditions in a systematic review in 2010. This included observer-reported outcomes such as hypertension, dementia and obesity in addition to patient-reported outcomes such as pain and nausea, all with a no-treatment control. In general, placebo interventions did not have clinically relevant effects. However, in patient-reported outcomes such as pain or nausea, there may be beneficial effects of placebo although biased reporting may have played a role [167].

Within the field of sleep medicine many placebo investigations are not controlled with a no-treatment arm, and results are thereby not clearly distinguished from regression to the mean. But even though these effects are not separated, the studies show that placebos may have considerable effect on the interpretation of results. A meta-analysis of insomnia trials showed that >60% of the effect (objective and subjective) from active treatments could also be seen in the placebo group (no-treatment comparisons not available) [172]. Similar effects have been shown in restless legs in mainly subjective but also objective outcomes [173].

1.3.2 INVESTIGATED DRUGS IN OSA

Most of the current strategies in drug development of OSA therapy can be categorised based on the four main endotypic traits: anatomy, upper airway responsiveness, ventilatory instability and arousal threshold. Some studies have the primary goal of OSA reduction whereas others focus on other outcomes with OSA severity as a secondary goal. Currently investigated drug therapies and trials of particular physiological interest, are briefly described below.

1.3.2.1 CARBONIC ANHYDRASE INHIBITORS

An increased loop gain/ventilatory instability is one of the proposed mechanisms for OSA. By stabilising the ventilation with CA inhibiting drugs, OSA can be reduced. For ACT the main underlying mechanism is thought to be a slight metabolic acidification after administration, thereby reducing loop gain (mainly plant gain) [174-176]. Additional mechanisms of CA inhibition may include a diuretic effect [177]. The effect of CA inhibitors seems to be class-wide as not only ACT reduces AHI (-40% to -50%), but also sulthiame (STM, AHI -32% to -41%) and zonisamide (AHI -30%) [174, 175, 178-180]. In addition to reducing AHI, CA inhibitors may have additional effects, including blood pressure reduction (ACT) and body weight reduction (zonisamide) [175, 178, 181]. Any additional effects on cardiometabolic comorbidities would strongly favour the use in OSA because of the cardiovascular and metabolic comorbidities.

1.3.2.1.1 Sulthiame

STM is a CA inhibitor used in Rolandic epilepsy, a specific childhood epilepsy subtype. Studies of STM suggest that the respiratory effects are similar to those of ACT (inhibition of loop gain), but in addition there appears be added effects via the central respiratory regulation, supposedly by central nervous chemosensory mechanisms [182]. Recently a phase II double-blind, placebo-controlled trial of safety and tolerability of STM in OSA in 68 subjects was completed. STM was found to be safe and showed an AHI reduction of -32% to -41%, depending on dosage [179].

A more general discussion on compounds with CA inhibitory properties can be found above, see *Carbonic anhydrases*.

1.3.2.2 NORADRENERGIC MECHANISMS

The noradrenergic system tone is tightly related to wakefulness and sleep stages. With an arousal from sleep, the genioglossus muscle drive is increased via excitatory noradrenergic effects. Stimulation of the noradrenergic system has been shown to have excitatory properties on the hypoglossal muscle neurons which may reduce upper airway collapsibility. With this in mind, several drugs with agonistic noradrenergic properties have been tested [164, 165]. One of the first drugs to be studied in OSA was protriptyline. Protriptyline increased O_2 saturation but also significantly reduced REM sleep. Subsequent small size studies with this drug have shown mixed results [183-185].

A more recent development is atomoxetine, a selective norepinephrine reuptake inhibitor, which in the first studies did not reduce OSA when administered alone [186, 187]. However, substantial OSA reducing effects were seen when combined with an antimuscarinic drug (oxybutynin), as proposed in preceding experimental studies. The combination, Ato-Oxy, resulted in a reduction of AHI by 63% in a cohort with moderate to severe OSA [187]. Similar noradrenergic + antimuscarinic combinations have thereafter been tested, e.g., reboxetine + oxybutynin (AHI -59%), reboxetine + hyoscine butylbromide (AHI -33%) and atomoxetine + fesoterodin (no effect overall effect on AHI) [188-190]. A lower efficacy was seen when the combination atomoxetine + aroxybutynin was recently tested in a large trial (n=181) in moderate to severe OSA with an overall median AHI (4% desaturation rule) reduction of 7 events/h (placebo controlled) or -43% to -47% in both dosage groups. In this study, atomoxetine alone also reduced AHI [191]. The results emphasise the differences in outcomes depending on OSA severity and how the results are computed. Despite a relatively high reduction of OSA, the potential cardiovascular effects of noradrenergic stimulation could be a possible concern in relation to OSA comorbidities [164].

1.3.2.3 K^+ CHANNEL BLOCKERS

The effects of neurotransmitters as serotonin (5-HT), acetylcholine and norepinephrine are modulated by opening of neuronal K^+ channels that mediate cellular hyperpolarisation, thereby reducing excitatory signalling. Conversely, by blocking these channels, the cells are more easily excitable, increasing motor neuron stimulation. The increased excitability may also increase the upper airway pressure reflex, increasing muscle compensation. The widespread location of these channels increases the risk of adverse effects, and systemic doses probably need to be kept low [164, 165]. Using systemic administration, two small scale human trials showed modest effects on breathing disturbance length and a small increase in m genioglossus activity in healthy volunteers during REM sleep [192, 193]. However, local application may improve bioavailability in the target tissue. In animal studies, local administration of potassium channel blocker was shown to increase m genioglossus activity [164, 194]. The first human parallel group trial in OSA patients with TASK-1 and TASK-3 ([TWIK]-related acid-sensitive K^+ channel) blocker BAY2253651 did however not show any effects on the AHI [195]. Recently, a modified version of the compound (BAY2586116) was shown to reduce pharyngeal collapsibility in severe OSA patients [196]. For now, the potential effect on respiratory measurements is unknown but intense research is ongoing in the area.

1.3.2.4 DIURETICS AND MINERALOCORTICOID ANTAGONISTS

Neck fluid accumulation reduces the upper airway diameter, increasing the collapsibility and OSA. By reducing oedema with diuretics or mineralocorticoid antagonists, the upper airway collapsibility may be reduced [47]. Several trials have showed reduction of AHI with combination treatments aiming at fluid retention: spironolactone -45% [197], spironolactone + metolazone -16% [198], furosemide + spironolactone -16% in comparison to -24% in sodium-restricted diet [199]. In the right patient group, these types of drugs may be beneficial [165].

1.3.2.5 SEROTONERGIC MECHANISMS

The serotonergic system with several receptor families is complex with both inhibitory and excitatory effects. In addition, 5-HT modulates several other neurotransmitters such as glutamate, dopamine, GABA, noradrenalin and acetylcholine. In the CNS, 5-HT seem to have excitatory effects on respiration whereas inhibitory effects are seen with peripheral stimulation. The central 5-HT effect is mediated by a stimulation of m genioglossus activity via the hypoglossal nerve. This effect is reduced during sleep, and lowest during REM sleep [164, 165]. When administration of the 5-HT3 antagonist ondansetron significantly

reduced OSA in a dog model, human experiments were quickly started [200]. However, no effect was seen in the human trial [201]. Potentially, the effects from preclinical models were overexaggerated due to vagotomy of study animals. Thereby the vagal inhibitory effects on neurons were reduced and central excitatory effects overestimated [202].

By increasing 5-HT concentration, the 5-HT reuptake inhibitors such as fluoxetine and paroxetine have showed AHI reductions of 20-40% during NREM sleep in small human trials. Mirtazapine (5-HT1 agonist and 5-HT2/5-HT3 antagonist) showed promising effects in an early small study, but the results could not be replicated in subsequent longer trials [203, 204]. In addition, mirtazapine often induces weight gain, a side effect that may worsen OSA [164, 165].

1.3.2.6 ACETYLCHOLINE MECHANISMS

Acetylcholine is an important neurotransmitter for ventilation during sleep. Earlier studies showed that patients with multisystem atrophy and OSA had a lower activity in this system, indicating that low levels of acetylcholine may be predisposing for OSA. Trials with acetylcholinesterase inhibitor donepezil initially showed promising potential but later studies could not confirm the findings [205-208]. Physostigmine, also an acetylcholinesterase inhibitor, reduced AHI during a single night administration with more pronounced effects in REM sleep [164, 209].

1.3.2.7 CANNABINOIDS

The cannabinoid type 1 and 2 receptor agonist dronabinol was tested in OSA after animal studies had shown a stabilised autonomic response and reduction of sleep apnea. The compound significantly reduced OSA in a pilot study and in the subsequent placebo-controlled trial AHI was reduced by -40% compared to placebo, in addition to an ESS reduction. However, this effect was largely driven by an increase of AHI in the placebo group [210-212]. Overall, the usefulness and mechanisms of cannabinoids in OSA are uncertain and warrant further studies. Combination therapies with e.g. ACT or atomoxetine and cannabinoids (dronabinol) are currently under investigation [164].

1.3.2.8 NASAL DECONGESTANTS

Nasal congestion increases the negative inspiratory pressure, promoting collapse of the airway. By reducing nasal resistance, a lower suction pressure is needed. Several trials have tested this hypothesis with varying results. For example, a low reduction of AHI was seen in the 4-arm trial by Acar et al, with mometasone, desloratadine and a combination versus placebo. In the "mometasone only" arm,

AHI was reduced by 17%, an effect not seen in the combination arm [213]. Fluticasone reduced AHI by 21% in one study, but no effect was seen a study with a combination of fluticasone + montelukast [214, 215]. Overall, studies of nasal decongestants show modest to low reduction of AHI but may have a place in certain patient groups [165].

1.3.2.9 GABA RECEPTOR AGONISTS

A low arousal threshold may in some individuals limit the possibility to reach stable sleep and respiration, inducing periodic breathing. Compounds that increase the arousal threshold can prolong the time for compensatory mechanisms in the pharynx, thereby stabilising the upper airway without an arousal and subsequent periodic breathing. For long, GABA receptor agonists were considered contraindicated in OSA as they were expected to reduce respiratory drive and promote relaxation of the upper airway muscles, worsening OSA. Subsequent data suggest that this does not occur, the drugs can be used without deleterious effects during shorter periods in normal dosages. In a study by Eckert et al, eszopiclone increased the arousal threshold in OSA, resulting in a reduction of AHI from 31 to 24 events/h [216]. However, subsequent trials have failed to show clinically relevant effects with similar drugs. Higher efficacy may potentially be reached in patients with low arousal threshold and in combination treatments [164, 165].

1.3.2.10 XANTHINES

Caffeine, a xanthine, is used as a respiratory stimulant in infants. The respiratory effects of xanthines are multifactorial, but include adenosine antagonism in the CNS and a stimulatory effect on the diaphragm. Theophylline, another xanthine, reduced the AHI by 20% in adult OSA, but unfortunately in tandem with a reduction of sleep quality. Similar relatively weak effects have been shown in other xanthines and overall, the effects seem to be low for this group of drugs [164, 217, 218].

1.3.2.11 PHARMACOLOGICAL WEIGHT REDUCTION

Pharmacological weight loss regimes have also been investigated in relation to OSA [219]. Most recently, liraglutide, a glucagon-like-peptide 1 (GLP-1) analogue, was compared to placebo during 32 weeks in subjects with obesity and moderate to severe OSA (n=180+179, mean AHI 49 events/h and mean BMI 39.1 kg/m^2). In addition to the drug, all participants were also put on a 500-kcal deficit diet and exercise program. The liraglutide group showed a greater weight loss compared to placebo (-5.7% vs -1.6%) and reduced AHI (-12.2 events/h vs -6.1 events/h) [220]. The combination phentermine + topiramate has been investigated in

adjunction to lifestyle counselling, in a smaller trial (moderate to severe OSA, BMI 30-40 kg/m^2, placebo n=23 and drug n=22). In the active drug group, AHI was reduced -31.5 events/h compared to placebo -16.6 events/h in addition to weight loss (10% vs 4%, respectively) [221].

There are currently several ongoing development programs for pharmaceutical treatments of OSA. As mentioned, many drugs have been theoretically interesting but failed to show clinically relevant effects. Compounds focused on OSA comorbidities such as antihypertensives and acid reflux medication have also been tested, so far without clinically significant results [222, 223].

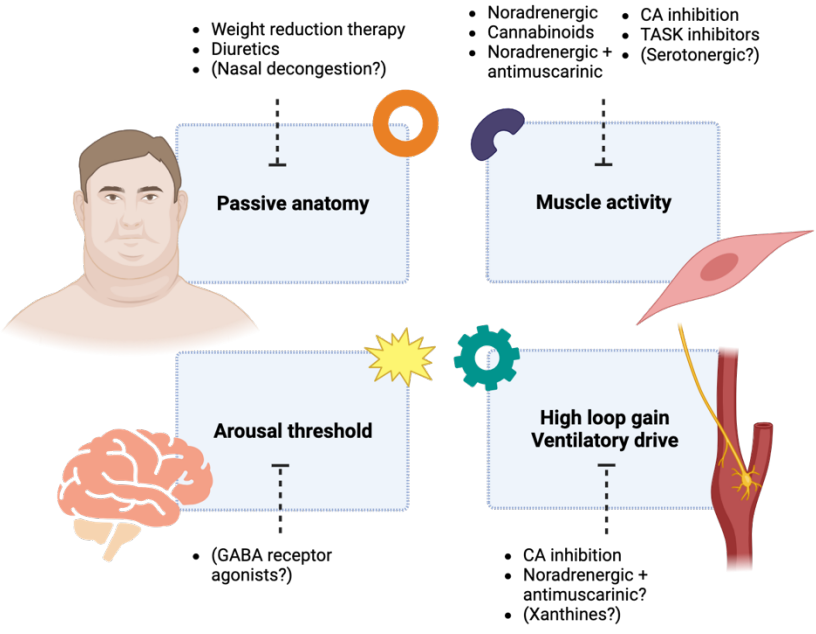

Figure 6. Investigated pharmacological treatments in OSA in relation to endotypic traits as potential treatment targets.

2 AIM

The overall aim of this thesis was to further investigate the potential role of a new pharmacological treatment principle of OSA in the following areas:

- CA activity as an underlying mechanism in OSA and/or predictor of treatment response with CA inhibition
- CA inhibition in OSA
    - how the treatment response is related to patient phenotypes/endotypes
- The potential placebo effect in drug treatment of OSA

With data from two separate trials of CA inhibition in OSA and an investigation on how a potential placebo effect may influence drug trials of OSA, we hoped to come a few steps closer to a drug treatment in OSA.

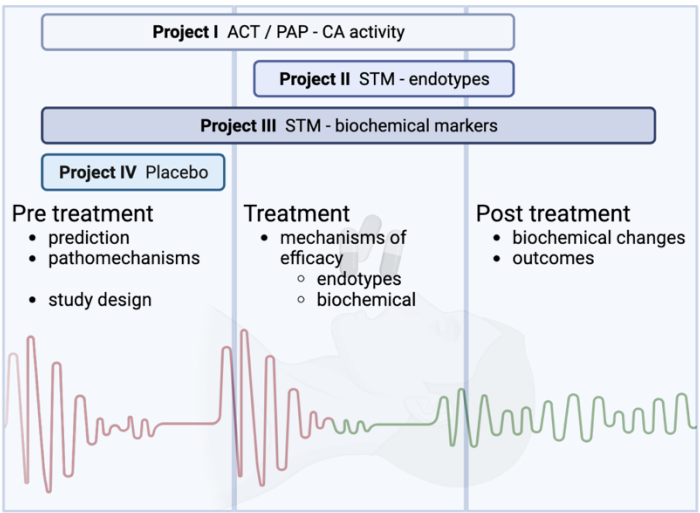

Figure 7. Thematic overview of the included papers in the PhD thesis.

More specifically, the aims of each paper were the following:

- **Paper I**
  Previous data suggested that CA activity in whole blood was higher in patients with severe OSA. If the higher level was secondary to OSA, or if the increased activity was a potential cause of OSA was unknown. By reducing OSA with PAP, we could study if CA activity levels were secondary to OSA. ACT had previously been shown to significantly

reduce blood pressure in OSA subjects. In this study we also investigated CA activity in normotensive and hypertensive subjects.

- Does PAP or CA inhibition by ACT, alone or in combination, reduce whole blood CA activity?
- How does CA activity relate to comorbid hypertension in OSA subjects?

- **Paper II**

Previous data suggested that the effect of CA inhibition in OSA was mainly by a reduction of loop gain. However, STM has in previous studies also shown potential as a central respiratory stimulant.

- How does STM modify endotypic traits in OSA and can this explain the OSA reducing effect?
- How does the endotypic traits change at different plasma concentrations of STM?

- **Paper III**

In paper I, the effects on CA activity by ACT was investigated. In this study we continued these investigations with the CA inhibitor STM. In the main analysis, STM improved mean SaO_2 but the minimum oxygen saturation and time below 90% SaO_2 were largely unchanged [179]. By analysing HIF-1α we aimed to investigate biochemical changes related to hypoxia. Both CA activity and HIF-1α have been proposed as potential biomarkers in OSA.

- What are the effects on CA activity and hypoxic marker HIF-1α during STM treatment of OSA?

- **Paper IV**

Many potential pharmacologic treatments of OSA have not been evaluated in RCTs of high quality. OSA has a considerable night-to-night variability, increasing the importance of an adequate control group in studies. In the placebo-controlled studies previously performed, not rarely, there is no baseline without treatment. Rather, the placebo administration is used as the baseline, and not as a separate control arm. The effect size of placebo medication on sleep apnea indices and patient related outcome measures have never been systematically evaluated. In order to improve future investigations of pharmacologic treatments in OSA it is of great importance to understand the influence of a potential placebo effect.

- Is there a significant placebo effect on objective sleep apnea indices and subjective patient-related outcome measures in OSA?

3 METHODS

3.1 STUDY DESIGN AND COHORTS

	PAPER I COHORT A	PAPER I COHORT B	PAPER II	PAPER III	PAPER IV
DESIGN	Longitudinal observational cohort	Cross-over trial with 3 arms	Placebo controlled, double-blind, randomised study		Systematic review with meta-analyses
COHORT / DATASET	PAP longitudinal follow-up	Cross-over PAP + ACT	RCT of STM treatment in OSA		OSA drug trials with placebo
INCLUSION	Adults with OSA starting PAP treatment *n = 33*	Adults with moderate to severe OSA and hypertension *n = 9*	Adults with moderate to severe OSA, age 18-75 years, BMI >20 to <35 kg/m^2, ESS >6, and non-acceptance of PAP treatment *n = 58*	*n = 43 / 53*	P - adults with OSA I - placebo C - baseline values in placebo arm O – AHI, ODI, SaO_2, ESS
EXCLUSION	Central sleep apnea, unstable gastrointestinal or pulmonary disease, CHF, significant renal disease, mental illness, substance abuse, ongoing oncological treatment, severe cognitive impairment	Allergies to sulphonamides, epilepsy, hepatic or renal dysfunction, depression, substance abuse, unstable cardiovascular, pulmonary gastrointestinal illness, treatment resistant hypertension	Central sleep apnea, other sleep disorders, severe night-time hypoxia or hypertension not under control		Studies investigating oxygen-therapy, treatments in the peri-operative period, central sleep apnea or high altitude related illnesses
OUTCOME	CA activity	CA activity	Endotypic traits	CA activity HIF-1α	AHI, ODI, mean SaO_2, ESS
FOLLOW-UP LENGTH	6 months	2 weeks	4 weeks	4 weeks + 2 weeks	1 to 120 nights

Table 2. Overview of included cohorts and study designs. Shortened versions of inclusion and exclusion criteria. PAP: positive airway pressure, ACT: acetazolamide, STM: sulthiame, RCT: randomised controlled trial, OSA: obstructive sleep apnea, BMI: body mass index, ESS: Epworth sleepiness scale, AHI: apnea-hypopnea index, ODI: oxygen desaturation index, SaO_2: oxygen saturation, CHF: congestive heart failure, CA: carbonic anhydrase, HIF-1α: hypoxia-inducible factor 1α.

3.1.1 PAPER I

Longitudinal observational cohort + Cross-over trial with 3 arms

The study consisted of two separate cohorts with long term (Cohort A) and short-term cross-over trial (Cohort B), see Table 3.

The long-term cohort with 33 participants was based on an adult OSA cohort from the University of Crete. Study participants were included when starting PAP treatment and followed for a median of six months. Blood pressure and CA activity was measured before starting PAP treatment and at follow-up.

The short-term cohort included 9 participants with moderate to severe OSA and established hypertension in an open cross-over design with three arms (*PAP treatment*, *ACT only* and *ACT + PAP*). The cohort was a subgroup of a previously reported trial [175]. Before starting the study, antihypertensive medications were stopped with a two-week washout, to enable investigations in subjects without antihypertensive treatment. Blood pressure and CA activity was measured at baseline and follow-up of each treatment.

	COHORT A	COHORT B
n	33	9
Age (years)	51±10	65±6
Males %	82%	100%
AHI (events/h)	47±31	39±20
Mean SaO_2 %	91±3%	92±2%
Hypertensive %	36%	100%
Blood pressure systolic (mmHg)	133±16	156±11
Blood pressure diastolic (mmHg)	83±10	84±9
Hyperlipidaemia %	48%	44%
Diabetes %	9%	0
BMI (kg/m^2)	37±7	29±4

Table 3. Cohorts in paper I at baseline. Anthropometric, physical and sleep apnea related variables, mean±standard deviation. AHI: apnea-hypopnea index, SaO_2: oxygen saturation, BMI: body mass index. Adapted from paper I.

3.1.2 PAPER II AND III

Placebo controlled, double-blinded, randomised, dose escalation study

These studies are based on data from a placebo controlled, double-blinded, randomised, dose escalation study (phase II). The study included dose escalation

in steps of 100-200 mg and 200-400 mg once daily, during 2 + 2 weeks in 68 subjects [179]. Subjects were randomised and treated during 4 weeks with placebo or STM. PSG was applied twice at baseline and at the 4-week follow-up. Blood samples were drawn at baseline, at the 4-week follow-up and 2 weeks after drug treatment was completed. Paper II and III included subgroups of the main analysis with participants finishing the trial per protocol.

3.1.2.1 PAPER II

An analysis focused on the effects of STM on underlying pathophysiological mechanisms (endotypes), trying to explain the OSA reduction. A total of 58 participants had full sets of data to enable analysis (Table 4). Algorithms for calculation of endotypic traits from PSG recordings were used on baseline and follow-up sleep studies. Endotypic traits were compared between treatment groups and to STM plasma concentration.

	PLACEBO	STM 200 MG	STM 400 MG
n	22	12	24
Age (years)	61±10	60±11	60±9
Males %	77%	58%	71%
AHI (events/h)	54±21	61±24	54±22
Mean SaO_2 %	93±2%	93±2%	94±2%
Hypertensive %	27%	50%	38%
Blood pressure systolic (mmHg)	134±11	134±13	131±14
Blood pressure diastolic (mmHg)	81±6	85±8	80±11
Hyperlipidaemia %	14%	0%	8%
Diabetes %	9%	0%	4%
BMI (kg/m^2)	29±3	28±3	26±3

Table 4. Cohorts in paper II at baseline. Anthropometric, physical and sleep apnea related variables, mean±standard deviation. STM: sulthiame, AHI: apnea-hypopnea index, SaO_2: oxygen saturation, BMI: body mass index. Note that the cohort include subjects from the same trial as paper III. Adapted from paper II.

3.1.2.2 PAPER III

This project investigated the effects of STM on CA activity and the hypoxia biomarker HIF-1α, both previously proposed as potential biomarkers for OSA diagnose and treatment response. Levels of CA activity and HIF-1α concentrations were compared between treatment groups at follow-up and 2 weeks after drug treatment completion. A total of 43 + 53 (CA activity + HIF-1α, respectively) subjects had full sets of data to enable analysis, see Table 5.

	CA ACTIVITY ANALYSIS			HIF-1α ANALYSIS		
	Placebo	*STM 200 mg*	*STM 400 mg*	*Placebo*	*STM 200 mg*	*STM 400 mg*
n	15	8	20	22	7	24
Age (years)	60±11	63±10	60±10	61±10	60±11	60±9
Males %	73%	63%	70%	77%	57%	71%
AHI (events/h)	51±22	56±22	55±23	54±2	64±30	54±22
Mean SaO_2 %	93±2%	92±2%	94±2%	93±2%	92±2%	94±2%
Hypertensive %	20%	75%	40%	27%	71%	38%
Blood pressure systolic (mmHg)	134±12	136±12	131±15	134±11	134±12	131±14
Blood pressure diastolic (mmHg)	80±5	85±8	80±11	81±6	87±8	80±11
Hyperlipidaemia %	7%	0%	0%	14%	0%	8%
Diabetes %	7%	0%	5%	9%	0%	4%
BMI (kg/m^2)	29±3	27±3	27±3	29±3	29±3	26±3

Table 5. Cohorts in paper III at baseline. Anthropometric, physical and sleep apnea related variables, mean±standard deviation. CA: carbonic anhydrase, HIF-1 α: hypoxia-inducible factor 1α, STM: sulthiame, AHI: apnea-hypopnea index, SaO_2: oxygen saturation, BMI: body mass index. Note that both CA activity cohort and HIF-1α cohort include patients from the same trial as paper II. Adapted from paper III.

3.1.3 PAPER IV

Systematic review with meta-analyses

In this systematic review we evaluated OSA outcomes in the placebo arm compared to baseline values in trials of pharmacologic treatments of OSA. The systematic literature search was conducted in cooperation with health sciences librarians in four databases from inception to 2021-01-19. Two reviewers individually performed study selection, risk of bias assessment and data extraction according to pre-defined criteria.

Study inclusion criteria were:

- **Patients** – adults with OSA (AHI ≥5 events/h)
- **Intervention** – placebo
- **Control** – baseline values in placebo arm
- **Outcomes**
    - Objective outcomes:
        - Primary: AHI
        - Secondary: ODI, mean SaO_2
    - Subjective outcome: ESS

It was pre-specified that a narrative review would be written and if data was deemed homogenous, several pre-specified meta-analyses were to be conducted. Exploratory meta-analyses were also performed. The results were compared to previous studies investigating night-to-night variability in OSA. The protocol was reviewed and published at PROSPERO (CRD42021229410).

3.2 OVERVIEW OF METHODS

3.2.1 STUDY INTERVENTIONS

3.2.1.1 ACETAZOLAMIDE

In paper I, ACT (Diamox®) was used as an inhibitor of CA activity. ACT is the most studied CA inhibitor in OSA and is used clinically mainly for acute altitude sickness, but also glaucoma, CHF, oedema and epilepsy. Dosing is indication dependent, but generally 250-1000 mg daily in the adult population. In this paper, ACT was titrated from 250 to 750 mg daily, and continued at highest tolerable dose for two weeks. Administration was per oral and 2-3 times daily depending on dose. The night-time dose was to be taken two hours before bedtime. Compliance was determined by tablet count.

3.2.1.2 SULTHIAME

The CA inhibitor sulthiame (STM, Ospolot®) was administered in the trial of paper II and III. STM is currently used as an anticonvulsant, mainly in childhood Rolandic epilepsy. In addition to CA inhibition, it has been reported that the compound also inhibit sodium channels which reduces the risk of seizures [224]. The dosage for the dose-finding trial was based on recommended dosages for epilepsy of 5-10 mg/kg bodyweight per day corresponding to 350-700 mg/day for an adult of 70 kg. Doses of 200 and 400 mg once daily at bedtime were finally

studied, based on reviews by a data safety monitoring board [179]. The study subjects started with 50% of the target dose for two weeks after which the full dose was administered for an additional two weeks.

3.2.1.3 POSITIVE AIRWAY PRESSURE TREATMENT

In paper I, PAP treatment was used as a means to reduce OSA to investigate if a possible CA activity increase was secondary to OSA. In the long-term cohort, PAP was started according to local routines at the Sleep Disorders Center, Medical School, University of Crete, Greece. Treatment use was followed and mean use was 4.7h/night during the follow-up period. In the short-term cohort, PAP treatment was used as a single treatment and as a combination with ACT. The compliance was fair, 4.8 to 5.0 h/night. The compliance in both cohorts was in general higher than the recommended shortest usage of 4 h/night.

3.2.2 SLEEP STUDIES

3.2.2.1 PAPER I

Type 1 PSG (Alice 5, Diagnostics System, Respironics, USA) was applied at baseline for the long-term observational cohort using standardised techniques. In the short-term cross-over study, type 3 ambulatory PG (Embletta X10, Embla, USA) was applied at baseline and follow-up of each treatment arm. The results were scored by experienced sleep technicians blinded to treatment allocation, where applicable. The scoring was done in accordance to AASM criteria [225]. Apneas were defined as >90% reduction in airflow during $\geq$10 seconds. Hyponeas from PSGs in the long-term cohort were scored with "recommended" AASM criteria: $\geq$30% nasal pressure drop during $\geq$10 seconds + $\geq$4% SaO_2 reduction. In the cross-over cohort, hypopneas were defined as $\geq$50% reduction in airflow during $\geq$10 seconds + $\geq$3% SaO_2 reduction. In the PG recording, the AHI was calculated with time from "lights off to lights on" as the denominator.

3.2.2.2 PAPER II AND III

The study subjects were measured with ambulatory PSG (type 2, Embla A10 system, Flaga, Iceland) during two consecutive nights at baseline and 4-week follow-up. The recordings were scored by an experienced sleep technician, blinded to treatment allocation, according to standard regulations [226]. Apneas were defined as >90% reduction in airflow during $\geq$10 seconds and hypopneas as $\geq$50% nasal pressure drop during $\geq$10 seconds + $\geq$3% SaO_2 reduction OR an EEG arousal. The means of the OSA indices from the two PSGs were used.

3.2.3 ANTHROPOMETRICS INCLUDING BLOOD PRESSURE

Information on comorbidities and medications were collected for all study participants (self-reported medical history or based on study clinician diagnosis). Office blood pressure measurements were performed according to the current European society of Cardiology – European society of Hypertension (ESC-ESH) guidelines [227] after 5 min (paper II and III) or 15 min (paper I) rest in supine position. The mean of three automatic recordings were used in the studies.

3.2.4 EPWORTH SLEEPINESS SCALE

The ESS for daytime sleepiness was used as a patient reported outcome measure in paper II and IV. The questionnaire consists of eight descriptions of situations in which the responder is asked to judge the "chance of dozing" [60]. The plausibility is scored on a 0-3 scale, with 0 being no risk of dozing and 3 points a high risk. A score >10 points is usually deemed abnormal. The questionnaire is frequently used in studies of OSA even though the scale is not sufficiently validated for repeated measures, and the variability is high with low repeatability [228, 229]. A clinically relevant change in ESS is often considered a change $\geq$2 points [230, 231].

3.2.5 BIOCHEMICAL ANALYSIS

3.2.5.1 SULTHIAME PLASMA CONCENTRATION

Plasma concentrations of STM from the end of treatment visit were analysed in paper II. The analysis was performed at the Analytisches Zentrum Biopharm GmbH (Berlin, Germany) with a Liquid chromatography with tandem mass spectrometry (LC-MS/MS). The method was validated for linearity and selectivity in accordance with Good Clinical Practice Guidelines. The method is a combination of liquid chromatography for separation of components in a sample and identification of the separated components with mass spectrometry.

3.2.5.2 CARBONIC ANHYDRASE ACTIVITY ANALYSIS

Two different analysis techniques have been used to determine whole blood CA activity. In the first analysis, the CO_2 hydration activity was measured and in the second analysis the esterase activity was measured. Both enzymatic reactions are catalysed at the same active site of the enzyme, but with different substrates.

1. In paper I, a CO_2 hydration method originally described by Wilbur and Anderson, modified by Rickli et al was used [232]. The

assessment was performed by collaborators at the Atatürk University in Turkey. In this method, the CO_2 hydratase enzymatic activity is determined by measurement of pH change in a veronal buffer with addition of CO_2. The experiment was conducted three times and the means of the measurements were used for further analysis.

2. For paper III, a commercially available kit (BioVision, catalog #K472-100) was used. This kit measures the esterase activity of the CA enzyme on an ester substrate. A chromophore is released which can be spectrophotometrically measured. Results are presented as the highest enzymatic activity over time, V_{max}. Assessments were run in duplicates and the means were used for further analysis.

3.2.5.3 HYPOXIA-INDUCIBLE FACTOR-1α ANALYSIS

The analysis of HIF-1α protein concentrations in paper III were determined with a sandwich ELISA (enzyme-linked immunosorbent assay, biotechne Catalog #DYC1935-2). In short, microtiter wells were coated with capture antibodies. After this, non-specific antibody bindings were blocked with reagent diluent (5% bovine serum albumin [BSA*] in wash buffer). Antigen (serum sample with HIF-1α) was added, binding to the capture antibodies. Detection antibody was added to the wells, binding to HIF-1α. Enzyme solution, Streptavidin-HRP A (horse-radish peroxidase), was added to the plate, binding to the detection antibody. After this step, a substrate (3,3',5,5'-Tetramethylbenzidine) was added. This substrate changes colour in a reaction with Streptavidin-HRP. After 20 min, the reaction was stopped and the result was read in a spectrophotometer. The results were compared to a two-fold standard curve. All analyses were run in duplicates and means were used for further analysis.

3.2.6 ANALYSIS OF ENDOTYPIC TRAITS

Endotypic traits were determined using an automated analysis of PSG recordings. The analysis tool, PUPBeta ("Phenotyping using Polysomnography", version 02/2022), has been shown to correlate to standard measurements of endotypic traits [55, 233-235]. This type of technique has the advantage that it may be used without laboursome and complicated research sleep study setups that may disturb the patient during sleep. In short, the main model input is respiratory flow and apnea/hypopnea/arousal scoring. The model approximates the ventilatory drive at a given (7 min) timepoint and calculates nine endotypic traits:

1. **Loop gain**
   - **LG_1** - response to one cycle/min disturbance
   - **LG_n** - response to a disturbance at natural frequency
2. **Time delay** - time from beginning of respiratory event to start of increased respiratory drive, seconds
3. **Arousal threshold** - % of eupneic drive at the onset of arousal events (ArTh)
4. **V_{min}** - median ventilation at lowest decile of drive, % of eupneic ventilation
5. **$V_{passive}$** - median ventilation at eupneic ventilatory drive, % of eupneic ventilation
6. **V_{active}** - median ventilation at arousal threshold, % of eupneic ventilation
7. **V_{comp}** - compensatory muscle activation (transformed V_{active} – transformed $V_{passive}$)
8. **Ventilatory response to arousal** - increase in ventilation explained by arousal, % eupneic ventilation (VRA)

The results for $V_{passive}$ and V_{active} are transformed due to ceiling effects [235]. It has previously been shown that you can reduce night-to-night variation and variability of the results by using NREM + all positions as input. The original method was however validated in NREM + supine position and therefore we did several separate calculations including NREM + all positions, NREM + supine position only and all sleep (NREM+REM) + all positions [235].

3.2.7 STATISTICS

A summary of statistical methods used in the thesis are presented in Table 6. The statistical analysis was performed in SPSS statistics (IBM Corp, Armonk, NY, USA) in paper I, II and III if not otherwise stated. In the meta-analyses for paper IV, a specific program for meta-analysis was used, Review Manager 5.4 (The Cochrane Collaboration, 2020).

Paper I, II and III were experimental studies with limited number of participants which limited the possibility to adjust for confounders in the statistical tests. The two cohorts in paper I were analysed separately.

Paper IV was a systematic review with meta-analysis. Studies were summarised and if data was homogenous enough, meta-analysis was performed. Some studies presented summary measures that could not be added to the meta-analysis and were therefore summarised separately.

	PAPER I	PAPER II	PAPER III	PAPER IV
Data presentation				
- *Normal distribution*	mean (SD)	mean±SD	mean±SD	-
- *Not normal distribution*	median [IQR]	median [IQR] median (95% CI)	median [IQR] median (95% CI)	-
- *Categorical data*	-	numbers (% of total)	numbers (% of total)	-
Normal distribution/outliers	Kolmogorov-Smirnov	histograms and Shapiro-Wilk's	histograms and Shapiro-Wilk's	-
Statistical sign.	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
Difference 2 groups				
- *Normal distribution*	paired t-test	-	t-test	-
- *Not normal distribution*	-	-	Mann Whitney U	-
Difference >2 groups				
- *Normal distribution*	-	ANCOVA, baseline-adjusted. Fisher's LSD test	-	-
- *Not normal distribution*	-	Kruskal-Wallis H-test. Dunn's (1964) procedure	Kruskal-Wallis H-test. Dunn's (1964) procedure	-
Correlative analysis				
- *Normal distribution and no sign. outliers*	-	Pearson correlation	Pearson correlation	-
- *Not normal distribution and/or sign. outliers*	Spearman rank correlation	Spearman rank correlation	Spearman rank correlation	-
Graphical presentation	bar diagrams	bar diagrams, box plots, scatter plots	bar diagrams	forrest plots
Other		95% CI for medians bootstrapped	95% CI for medians bootstrapped	median recalculations according to Wan 2014 [236]
Meta-analysis	-	-	-	results: mean difference (95% CI)
Random effects model	-	-	-	DerSimonian Laird
Heterogeneity				I^2
Risk of bias	-	-	-	Cochrane RoB 2.0, Funnel plots, Egger's test
Statistics analysis software	SPSS Statistics 25	SPSS Statistics 29	SPSS Statistics 29	Covidence systematic review software, ReviewManager 5.4, SPSS Statistics 29

Table 6. Overview of statistical methods used. SD: standard deviation, IQR; interquartile range, CI: confidence interval, ANCOVA: analysis of covariance, LSD: least significant difference, RoB: risk of bias.

3.3 ETHICS

Informed written and oral consent was provided by all subjects included in paper I, II and III. Paper IV was a systematic review of previously published studies where group-level data was handled and no biological material analysed. No ethical permit was thereby necessary.

Ethical permits are provided below:

- **Paper I**
  - Approved by the Ethics Committee at the University of Gothenburg (977-13) (cohort B) and Ethics Committee at University of Crete, Greece (7370/23-5-2014) (cohort A). The main study for cohort B was registered at ClinicalTrials.gov (NCT02220803) as well as European Clinical Trials Database (EudraCT: 2013-004866-33).

- **Paper II and III**
  - Approved by the Ethics Committee at the University of Gothenburg (Dnr: 045-18, 2018-02-07) and the Swedish Ethical Review Authority (2020-06237). The study was registered at European Clinical Trials Database (EudraCT: 2017-004767-13).

4 RESULTS

4.1 PAPER I

- PAP treatment does not change CA activity
- CA activity may be related to hypertension development in OSA

CA activity did not change during PAP treatment (median 6 months [IQR 6, 6], compliance of 4.7±1.5 h/day). Similarly, in the short-term cohort (B), cross-over study *PAP* treatment (compliance 5.1±2.4 h/day) did not change CA activity. In contrast, *ACT* and *ACT+PAP* reduced CA activity, although without statistical significance (842±139 vs. 726±119 units, p=0.081, and 818±152 vs. 720±135, p=0.056. In the long-term longitudinal cohort with PAP treatment (A), baseline CA activity did not correlate to OSA severity in the full cohort but a correlation (Spearman 0.539, p=0.047) was seen in the subgroup without hypertensive medication.

Hypertensive OSA subjects had a higher CA activity in whole blood samples compared to normotensive, despite a similar degree of OSA (1033±204 versus 861±201 units, p =0.028). Significant blood pressure reductions were evident in the longitudinal PAP treatment group (Cohort A), (-9±11/-5±7 mmHg, both p <0.001), stronger effects than what is commonly expected. In the cross-over cohort (B), *PAP alone* did not change blood pressures (-3±9/2±9 mmHg, all non-significant). In contrast, treatment with *ACT* and *ACT+PAP* significantly reduced blood pressure (*ACT alone* -10±10/-5±7 mmHg, and *ACT+PAP* -5±5/-13±13 mmHg, all p<0.05).

4.2 PAPER II

- STM stabilises the ventilation and upper airway

In the endotypic trait analysis, the effect on OSA by STM was attributed to several mechanisms, both ventilatory stabilisation and increased upper airway stability, see Table 7 below for overview. The effects were similar in sensitivity analyses in NREM/REM + all positions and NREM + supine position.

DOSE	VENTILATORY			UPPER AIRWAY				AROUSAL	
	LG_1	LG_n	Time delay	V_{min}	$V_{passive}$	V_{active}	V_{comp}	VRA	ArTh
200 MG	↓		↑	↑	↑				
400 MG	↓	↓	↑	↑	↑			↓	

Table 7. Endotypic traits (NREM + all position), statistically significant versus placebo marked in blue with direction of change. LG_1: loop gain, response to one cycle/min disturbance, LG_n: loop gain, response to a disturbance at natural frequency, Time delay: time from beginning of respiratory event to start of increased respiratory drive (seconds), V_{min}: median ventilation at lowest decile of drive (% of eupneic ventilation), $V_{passive}$: median ventilation at eupneic ventilatory drive (% of eupneic ventilation), V_{active}: median ventilation at arousal threshold (% of eupneic ventilation), V_{comp}: compensatory muscle activation (transformed V_{active} – transformed $V_{passive}$), VRA: ventilatory response to arousal (% eupneic ventilation), ArTh: arousal threshold (% of eupneic drive at the onset of arousal events.

The endotypic traits changed in a dose-response relationship to STM concentration, with a majority of the effect seen already in the lower dose interval. The dose-concentration slope was specific for each endotypic trait with ceiling effects reached at different concentration levels.

Correlation analysis between significant endotypic traits and change in OSA indices showed correlations for LG_1, V_{min} and $V_{passive}$ but not for time delay, VRA or LG_n. The change in LG_1 correlated to % change in AHI and ODI in the 200 mg group but not in the 400 mg group. For V_{min}, correlations were seen versus the absolute change in AHI and ODI in both treatment groups. $V_{passive}$ correlated to change in AHI only in the 400 mg group, with numerical trends in the 200 mg group. Results were comparable in NREM + supine position.

- The AHI effect is evident already in lower STM concentrations

Most of the AHI reduction was already seen in the lower dose interval of STM, with a ceiling effect after quartile 2, see Figure 8.

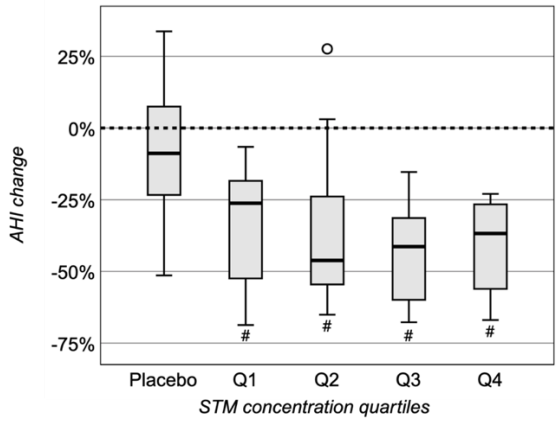

Figure 8. Apnea-hypopnea index (AHI) changes were associated with sulthiame (STM) concentrations. The main effects of STM are evident already in dosage quartile 1 (Q1) and Q2 (1.3-4.2 microg/mL), with a flatter concentration-response curve in the upper dosage quartiles (STM versus placebo, $p<0.05$ in all quartiles, between quartiles all non-significant).

4.3 PAPER III

- STM reduces CA activity in whole blood with effects still evident 2 weeks after administration

A full set of measurements from baseline, treatment and 2-week follow-up was available for 43 participants. CA activity was significantly reduced in the STM 400 mg group, whereas the 200 mg group showed numerical differences. The reduction in CA activity was less prominent, but still evident, at the follow-up 2-week after treatment, see Table 8. The reductions correlated to changes in mean SaO_2, but not AHI, in a full cohort analysis including all participants on STM and placebo (Spearman r -0.341, $p<0.05$).

		END OF TREATMENT	FOLLOW-UP VISIT
CA ACTIVITY	Placebo	+15% (-15% to 23%)	+9% (-4% to 35%)
	200 mg	-16% (-34% to 2%)	-3% (-22% to 30%)
	400 mg	**-26% (-32% to -6%)**	**-13% (-23% to -4%)**

Table 8. Changes in carbonic anhydrase (CA) activity during treatment and at follow-up after treatment. Median change (95% confidence interval). Bold text indicates statistically significant versus placebo.

- STM reduces HIF-1α concentration, with prolonged effects

Data (including baseline, treatment and 2-week follow-up) was available for 53 participants. As with CA activity, the HIF-1α concentration was reduced in the 400 mg group, both during treatment and at the 2-week follow-up after last administration. For the 200 mg group, a numerical trend was seen during treatment, with a statistically significant change at 2-week follow-up, see Table 9. In contrast to CA activity, the reductions were stable at the follow-up visit. The correlation analysis was similar to CA activity, with a significant correlation versus change in mean SaO_2 when including the full cohort with placebo and STM treated (Spearman r -0.273, $p<0.05$).

		END OF TREATMENT	FOLLOW-UP VISIT
HIF-1α	Placebo	0 (-1% to 0%)	0 (0% to 0%)
	200 mg	-2% (-6% to 0%)	**-2% (-6% to -0.4%)**
	400 mg	**-4% (-8% to -2%)**	**-4% (-7% to -2%)**

Table 9. Changes in hypoxia-inducible factor 1α (HIF-1α) during treatment and at follow-up after treatment. Median change (95% confidence interval). Bold text indicates statistically significant change versus placebo.

- The STM induced changes in CA activity and HIF-1α were correlated

The reductions of CA activity and HIF-1α correlated during the STM treatment, Pearson $r = 0.443$, $p = 0.023$.

- Neither CA activity nor HIF-1α were correlated to baseline OSA indices

In this relatively narrow spectrum of moderate to severe OSA severity, no correlations were seen between baseline levels of CA activity or HIF-1α and AHI, ODI, mean SaO_2, time under 90% SaO_2, venous pCO_2, blood pressure or hypertension diagnose.

4.4 PAPER IV

- Drug placebo tended to reduce the subjective, but not the objective outcomes

In this systematic review with meta-analysis there was a trend for a placebo effect on the subjective outcome ESS whereas no placebo effect was seen in the objective measures AHI, ODI or mean SaO_2. The mean differences in objective outcomes were similar to what is expected due to night-to-night variability [166].

When adding studies published after the initial search to the meta-analysis, there were still no significant differences seen in the objective outcomes of OSA severity (AHI, see Figure 9) whereas the mean difference in ESS was statistically significant, but with a similar effect size (Table 10, Figure 10 and 11) [179, 237, 238].

OUTCOME	STUDIES INCLUDED	STUDIES IN META-ANALYSIS	MEAN DIFFERENCE (PUBLISHED DATA)	MEAN DIFFERENCE (UPDATED DATA)
AHI	29	17	-0.84 (-2.98 to 1.30)	-1.13 (-3.18 to 0.92)
ODI	4	4	-0.76 (-6.78 to 5.76)	-1.57 (-6.15 to 3.0)
MEAN SAO_2	12	10	0.14% (-0.36% to 0.65%)	0.36% (-0.04% to 0.77%)
ESS	15	9	-0.94 (-2.02 to 0.14)	**-1.28 (-2.12 to -0.44)**

Table 10. Overview of inclusion and results from systematic review and meta-analysis. Updated data with added studied published after original search in the right column. Bold text indicates statistically significant results. AHI: apnea-hypopnea index, ODI: oxygen desaturation index, SaO_2: oxygen saturation, ESS: Epworth sleepiness scale.

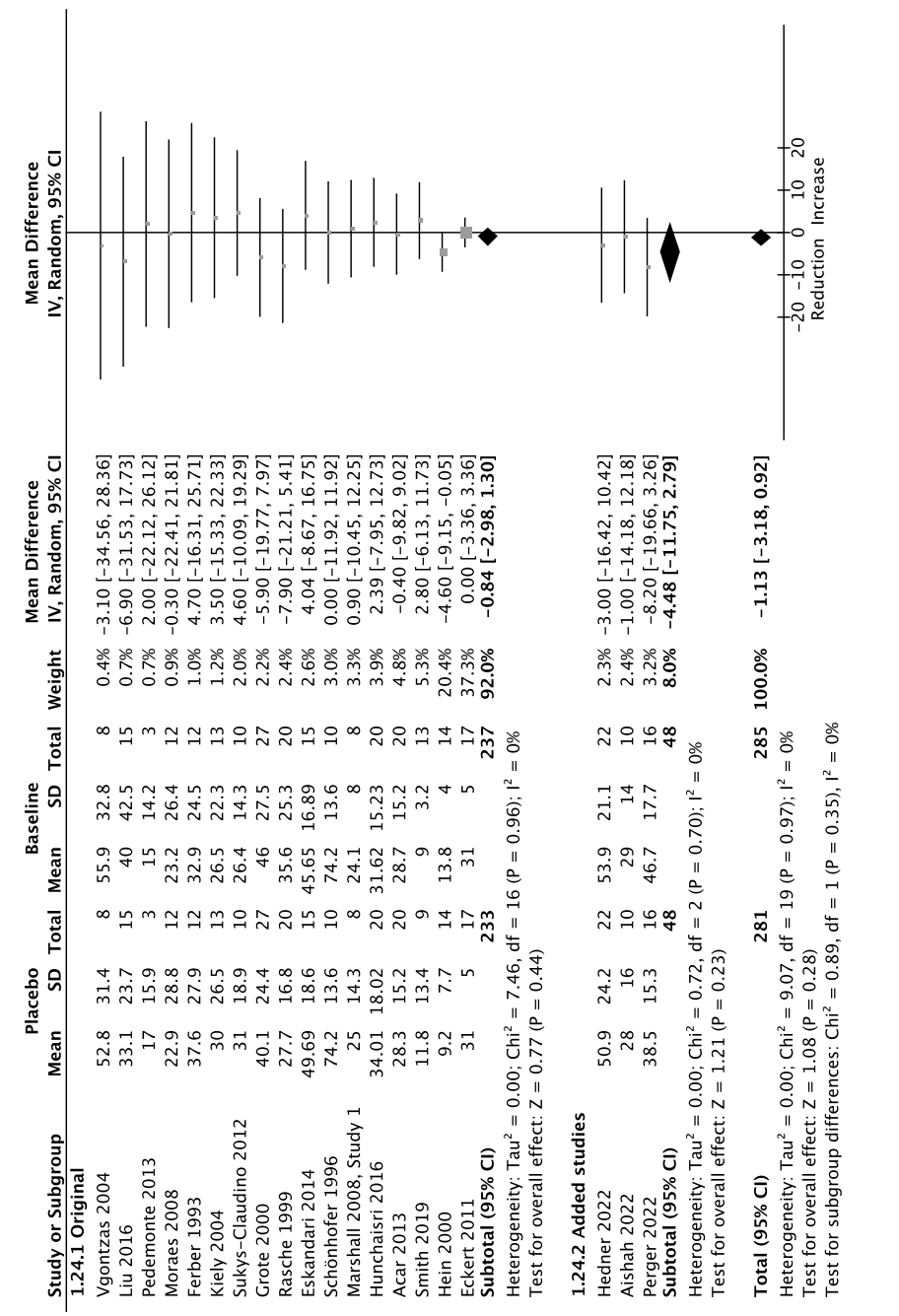

Figure 9. Forest plot of apnea-hypopnea index (AHI) meta-analysis, including more recent studies. SD: standard deviation, IV: Inverse variance, Random: random-effects model, 95% CI: 95% confidence interval.

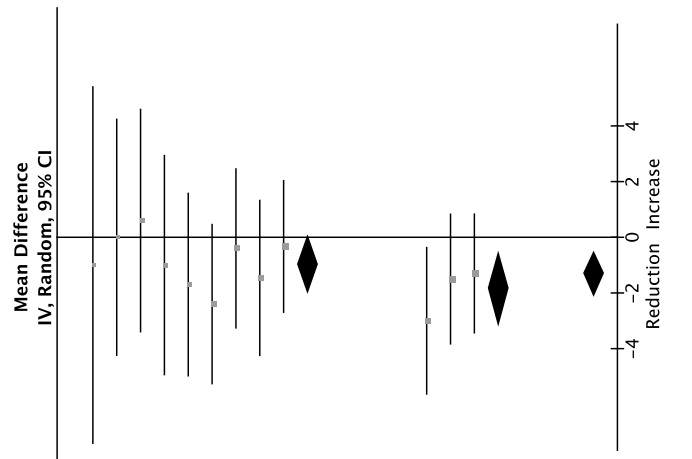

Figure 10. Forest plot of Epworth sleepiness scale (ESS) meta-analysis, including more recent studies. SD: standard deviation, IV: Inverse variance, Random: random-effects model, 95% CI: 95% confidence interval.

5 DISCUSSION

We may be close to a paradigm shift in the treatment of OSA. The improved understanding of anatomical and functional pathomechanisms has added new potential treatment targets after many years with PAP treatment as the main viable alternative. Many patients do not only have anatomical causes underlying the disease, altered muscle responsiveness in addition to low arousal threshold and unstable ventilatory regulation may also contribute to the sleep and breathing disorder. Such non-anatomical traits may be modified by pharmacological therapies. The OSA treatment strategy is moving from a one-size-fits-all approach with PAP to a more diverse and personalised medicine. The results of this thesis further support this evolution.

5.1 THE EFFECTS OF SULTHIAME IN OSA

Two approaches were used to characterise the effect of STM in OSA: firstly, the changes in endotypic traits during treatment and secondly, the effects on CA activity and HIF-1α.

5.1.1.1 THE VENTILATION AND UPPER AIRWAY IS STABILISED

The STM effect on underlying mechanisms was analysed using a PSG method for approximation of endotypic traits in paper II. The results showed a stabilisation of the ventilation (reduced loop gain, LG_1 and LG_n) and a stabilised passive upper airway (increased V_{min} and $V_{passive}$) in addition to a prolonged time delay and reduced VRA. These changes all contribute to a reduction of the breathing disturbances.

5.1.1.1.1 Mechanisms

Previous investigations of CA inhibition by ACT have mainly showed reduced loop gain and VRA [174, 239]. The effects have been attributed to a left-shift of the isometabolic curve, in which a metabolic acidosis (from reduced HCO_3^- reabsorption in the kidneys) increases the basal ventilation, with a higher CO_2-reserve and the ventilatory reserve as a consequence, see Figure 11. Due to this, a higher ventilation is needed to reach the apneic threshold (and a new apnea), as recently shown in a meta-analysis of Schmickl et al [176]. The authors proposed that the reduction of the loop gain is mainly due to lower plant gain (efficiency of CO_2 excretion in the lung), and not controller gain (chemosensitivity). However, the study did not take the chemoreflex delay (time delay) into account. Inhibitory effects on chemosensitivity have been shown in animal studies using carotid body

preparations [240-242]. Such inhibition could potentially affect both the controller gain and the chemoreflex delay. In humans, the ACT effects on ventilatory regulation are insufficiently understood, and studies on whether plant gain, controller gain or both changes are conflicting. Overall, the loop gain is reduced [174, 176] and the overall ventilation is increased due to the metabolic acidosis, despite a potential inhibitory effect on chemoreceptors [243]. It should also be emphasised that most of the studies in this field include healthy volunteers and experiments have mostly been performed during wakefulness, not in OSA patients during sleep.

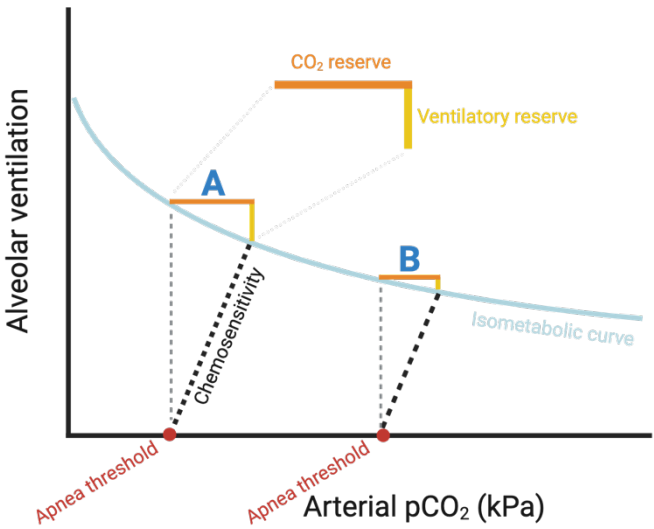

Figure 11. Schematic illustration of the isometabolic curve. In situation A (lower arterial pCO_2), a higher increase in alveolar ventilation is needed for a change in arterial pCO_2 (higher ventilatory reserve), compared to situation B where the curve is flatter. If the chemosensitivity is not changed, the risk of reaching the apnea threshold is lower in situation A compared to B (CO_2 reserve is higher). Carbonic anhydrase inhibitors have been proposed to cause a left-shift (B to A) in the isometabolic curve, increasing the CO_2 reserve and ventilatory reserve, reducing plant gain. Adapted from Schmickl et al 2021[176].

STM reduced loop gain, but with the PSG based method for estimation of endotypic traits, we cannot discriminate between controller gain and plant gain. In addition, an increase in time delay was demonstrated after STM. Overall, the effects may be attributed to chemoreceptor inhibition and a shift of the isometabolic curve, similar to the effect described for ACT. In addition, the increase in passive upper airway stability could potentially result from a higher basal respiratory drive, in which the basal muscle tone is increased [44]. In contrast

to ACT, the reduction of HCO_3^- was lower after STM compared to previously published ACT data (STM -3 mmol/l vs ACT -6 to -7 mmol/l [175, 179, 244]). This suggests that STM increases basal respiratory drive through additional mechanisms, other than acidification, which may also affect the upper airway. Interestingly, a direct central respiratory effect has previously been proposed [182]. Diuretic effects of CA inhibitors could also act to reduce collapsibility of the upper airway, but current data suggest that the diuretic effects of STM are small [245] and there was no significant change in bodyweight during treatment [179]. It is important to remember that compounds mainly seen as CA inhibitors may have pharmacological effects that are not CA-related. These properties may also play a role for the efficacy in OSA. An overview of mechanisms potentially involved in beneficial effects of CA inhibition in OSA therapy is provided in Figure 12.

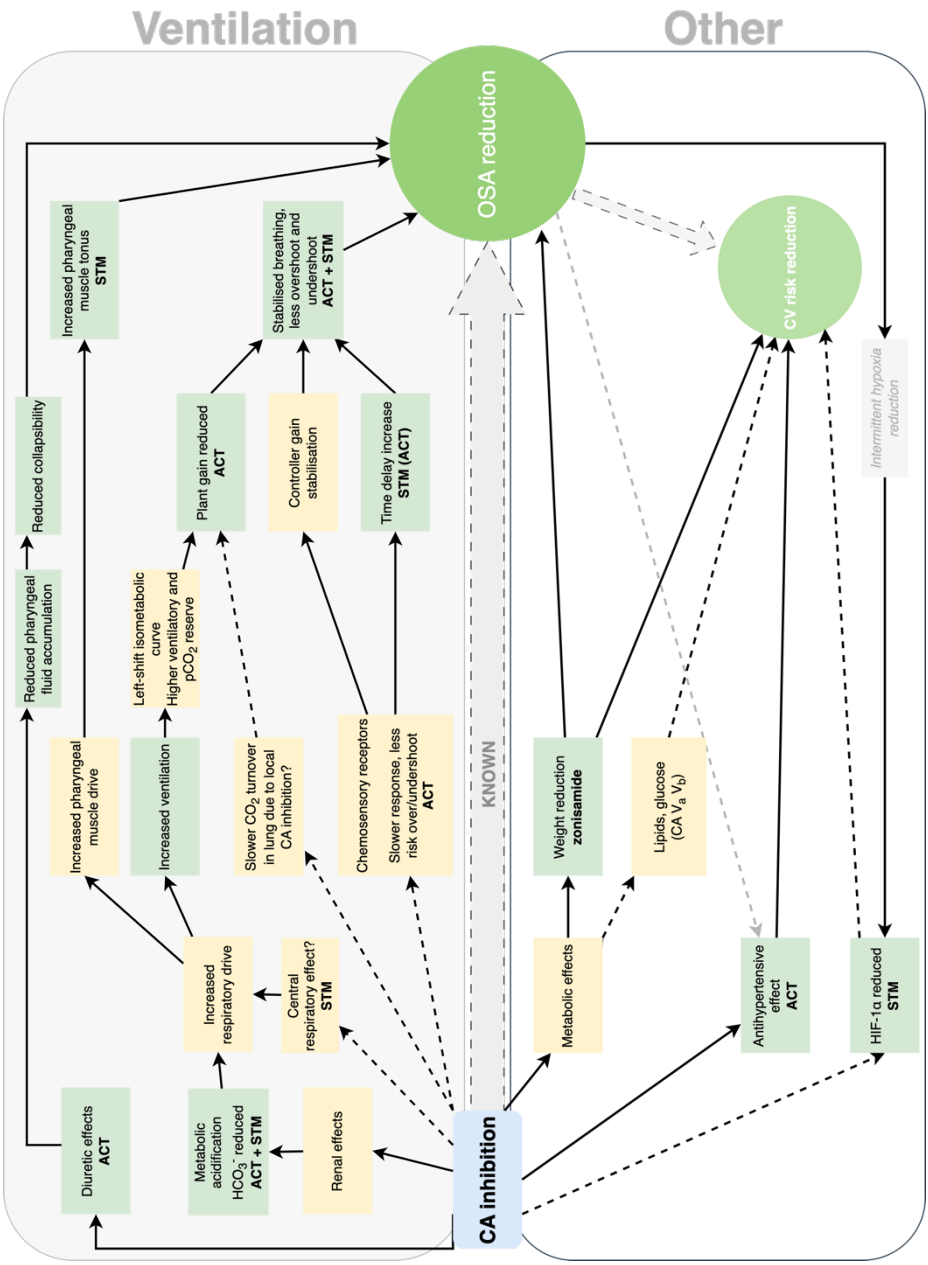

Figure 12. Overview of mechanisms potentially involved in the beneficial effects of carbonic anhydrase (CA) inhibition in obstructive sleep apnea (OSA). Green boxes indicate established effect/mechanism, yellow boxes theoretical effects/mechanisms. Dashed arrows: potential pathways. Grey arrows: known effects. ACT: acetazolamide, STM: sulthiame, HIF-1α: hypoxia-inducible factor 1α, CV: cardiovascular.

5.1.1.1.2 Methods

Previous investigations of the ACT effects on endotypic traits have mostly used other techniques than those employed in this thesis. This could potentially explain some of the differences between STM and ACT on OSA. The main advantage of the PSG method with PUPBeta is that endotypic traits measurements are now available in traditional PSG recordings. However, it should also be noted that the PSG-based method has been criticised [246]. In general terms, the criticism includes disagreements on several areas but, a main point is whether the respiratory drive can be calculated from ventilation, since the ventilation at a certain level of respiratory drive is highly dependent on the upper airway resistance, which is not accounted for in the analysis. In addition, the arousal threshold is not constant during the night, the approximation of passive airway collapsibility is lacking a measurement without dilator drive (this does not happen without PAP) and the loop gain measurement showed a modest correlation to standard measurements when validated [246]. On the other hand, the overall validation against gold standard shows reasonable agreement [55, 233, 234]. In addition, the method shows physiologically reasonable results in investigations of e.g., HGNS responders (higher arousal threshold – higher efficacy), a higher loop gain in non-responders to surgery, and a lower loop gain in MAD responders [247-249]. The measures show relatively high repeatability within the same subject over several nights [235], and the measurement results vary during sleep stages in a manner that is theoretically adequate [249]. Overall, there are advantages and disadvantages of this technique and a continued discussion on strengths and limitations is important. When interpreting the results, one should be aware of that the reported values are approximations, or surrogates, for the actual underlying mechanisms. To strengthen our results, the endotypic traits are calculated for different combinations of sleep stage and position. This showed repeatability in different settings and reduced spontaneous variability. We believe that the PSG method with PUPBeta, interpreted in relation to data from other methods, adds to the understanding of the treatment effect in STM.

5.1.1.1.3 Concentration matters

In this thesis we describe for the first time the changes in endotypic traits in relation to plasma drug concentrations. Indeed, the STM plasma concentration needed to change the endotypic trait varied between the different traits. For example, the effect on LG_1 reached a ceiling in concentration quartiles 1 and 2 whereas *time delay* continued to increase in all quartiles. Overall, it seems that lower STM concentrations may be sufficient to reach "full effect" in most endotypic traits. This is in line with the data for the AHI changes, which also reached a ceiling effect in the mid-range of concentration. Since most of the AHI reduction

is seen in the lower dose interval, a reduction of the STM dosage may be possible (Figure 8). This could potentially reduce side effects. The bivariate correlation data showed varying results dependent on endotypic trait and dosing. As the underlying mechanisms are complex, it is likely that a bivariate model is an oversimplification of the physiology where most subjects may have several underlying mechanisms. In addition, the different concentration-effect curves add to the complexity and non-linearity of associations. Differences in STM dose versus endotypic effect may explain why the correlations are only seen in specific dose groups for LG_1 and $V_{passive}$.

The OSA reducing effect varied considerably between subjects and dosage groups. The STM concentration matters, but patient-related differences in underlying pathomechanisms may also be important. We are still looking for the variables to predict a favorable treatment response, trying to treat the right patient with the right therapy. Larger studies, allowing multivariate analysis, are needed to further understand these complex mechanisms.

5.1.1.2 HIF-1α IS REDUCED BY STM

A dose response reduction of HIF-1α concentration was seen during treatment. This emphasises the positive effects on hypoxia by STM.

5.1.1.2.1 At baseline

Previous studies have shown that the HIF-1α levels are higher in OSA compared to healthy controls, with increasing levels in more severe OSA [140, 141, 250]. In our study, there was no correlation with OSA severity, but the cohort included mostly severe OSA patients. This prevented an analysis of the HIF-1α levels in the full OSA spectrum, with both healthy subjects and subjects with different OSA severity levels.

5.1.1.2.2 During treatment

The HIF-1α is increased by intermittent hypoxia [251]. The reduction of OSA and subsequent reduction of intermittent hypoxia may provide a pathway for the reduced HIF-1α concentration observed during STM treatment. Indeed, the reduction also correlated with the changes in SaO_2 in the full cohort (STM + placebo subjects). In line with this, a previous study suggested that 2 months of PAP treatment also reduce HIF-1α [250]. The correlation to changes in CA activity during treatment suggests a closer relation between CA and HIF-1α, but this needs to be further investigated. HIF-1α has been proposed to play a role in several cardiometabolic diseases, like insulin resistance, hypertension and atherosclerosis, that all are of importance in OSA as comorbidities [252-254]. However, in this study we could not show an association to comorbidities,

potentially due to the proportionally a narrow spectrum of OSA severity and low comorbidity frequency. If STM, and CA inhibitors in general, reduce HIF-1α in a different way compared to other OSA treatments and if this has implications in cardiometabolic outcomes remains to be investigated. Potential mechanisms and pathways are illustrated in Figure 13.

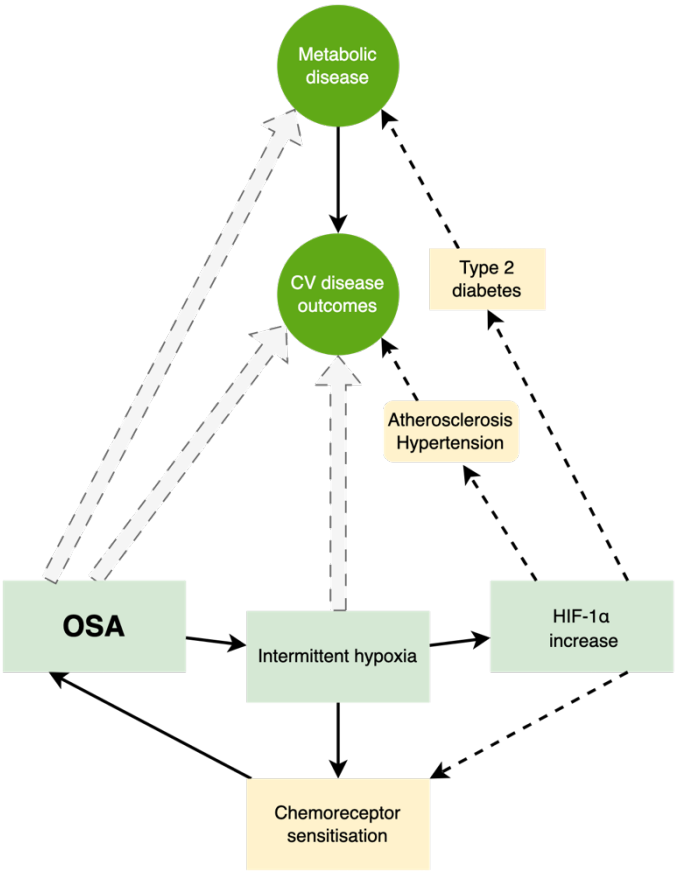

Figure 13. Overview of mechanisms potentially involved in the relation of HIF-1α (hypoxia-inducible factor 1α) to obstructive sleep apnea (OSA). Green boxes indicate established effect/mechanism, yellow boxes theoretical effects/mechanisms. Dashed arrows: potential pathways. Grey arrows: known effects. CV: cardiovascular.

5.1.1.2.3 After treatment completion

Interestingly, HIF-1α was reduced at least two weeks after the active treatment was discontinued. If this is a result of a prolonged pharmacodynamic half-life of STM, or if other factors like a delayed response in HIF-1α regulation are involved, remains to be investigated. If improved mean SaO_2 drives the reduction the HIF-

1α concentration, this could suggest that the OSA reduction is also prolonged. Reports of STM serum/plasma half-life ranges from 9 to 90 h, depending on dosage, with a terminal half-life of up to 13 days [255, 256]. The results need to be replicated in other materials, preferably with a sleep study after discontinuing treatment. Elevated levels of HIF-1α, or pronounced reductions during treatment in future studies, would also suggest that current methods for estimating biological impact of hypoxia on health in patients with OSA would need a reappraisal.

5.2 CA ACTIVITY AS A BIOMARKER IN OSA

The study by Wang et al, showing higher CA activity in whole blood in severe OSA subjects [143], in addition to several studies on CA inhibition as a potential treatment for OSA [174, 178, 180, 185] provided a rational for continued investigation of CA activity in OSA. With these studies in mind, the CA enzyme appeared to modulate fundamental mechanisms in OSA and we hypothesised that CA activity in blood could serve as a biomarker for OSA development or as a predictor for treatment effects of CA inhibition. In addition, the antihypertensive effect of CA inhibitors in OSA was seen as a potential link to cardiometabolic comorbidities. For now, we have still not fully understood if and how the CA activity can be used as a biomarker in OSA.

5.2.1.1.1 At baseline

In paper I, there was a correlation between CA activity and AHI in the longitudinal cohort for the normotensive subgroup in Cohort A, but this finding was not replicated in paper III. In the study by Wang et al the difference was seen between the *no OSA* group and *severe OSA* groups [143], whereas the cohorts in this thesis did not include subjects with mild or without OSA. The fact that we have no cohort replicating the setting from Wang et al. may explain the largely negative findings. At this point, the question on whether CA activity in whole blood could be used as a diagnostic marker for OSA remains largely unclear and cohorts with a wider inclusion of both *no OSA* and *severe OSA* cases should be investigated. Preferably, such a study would also include a longitudinal follow-up of patients initiated on PAP treatment in order to study whether there is a change in CA activity when OSA is reduced, similar to the study design in paper I.

5.2.1.1.2 During treatment

Whether CA activity in whole blood could be used as a biomarker to follow treatment effects/mechanisms of CA inhibition was investigated in paper I and III. The CA activity did not change during PAP treatment but was reduced during

CA inhibitory treatment in both paper I (ACT -14% to -12%, numerical change) and paper III (STM -26% to -16%, statistically significant in 400 mg group). The reduction of CA activity in whole blood correlated with the changes in mean SaO_2 in a full cohort analysis (STM + placebo) in paper III, enforcing the connection between CA inhibition and reduction of OSA. However, if it is the inhibitory effect on blood CA, or CA in other tissues (e.g, kidneys, lungs), that explains the improved SaO_2 remains uncertain. For AHI, there was no correlation, although CA inhibition evidently has a potential to reduce OSA. We also know that the AHI and mean overnight SaO_2 capture different characteristics of OSA. There may be several reasons behind the overall findings: the main effects of CA inhibition may involve other compartments or tissues such as lungs, chemoreceptors and/or brain and it may be that the activity in whole blood (mainly the activity level in erythrocytes rich in high activity CA II [257]) does not fully capture the pathophysiological changes in OSA. It is reasonable to believe that the CA levels in blood are not secondary to OSA, as PAP during 6 months with relatively high treatment adherence did not change the levels.

5.2.1.1.3 After treatment completion

In line with the HIF-1α concentration, the CA activity was reduced up to two weeks after the completion of the STM treatment. However, the CA activity did, in contrast to HIF-1α, show a tendency to return to pre-treatment values. As previously discussed, the reported half-life of STM varies considerably and the functional half-life is unknown. These results need to be replicated but are interesting in relation to dose and administration interval of the drug.

5.2.1.1.4 In hypertension

The CA activity was higher in hypertensive subjects compared to normotensive subjects in the longitudinal cohort of paper I. However, this result was not replicated in the cohort of paper III. The cohorts differ in several regards: the subjects in cohort A are younger (51±10 vs 61±10 years), have a higher BMI (37±7 vs 28±3 kg/m^2), more hyperlipidaemia (48% vs 2%) and somewhat lower AHI (47±31 vs 54±22 events/h). These differences could potentially play a role in the contrasting results. A previous study also showed a low-grade linear relation to diastolic blood pressure [143]. At this time, the CA activity in whole blood cannot be used as a diagnostic biomarker for comorbid hypertension in OSA. The antihypertensive effect of CA inhibition in systemic hypertension outside OSA is generally weak, but it has been shown to be more pronounced at high altitude [257]. Interestingly, ACT had considerable antihypertensive effects in the untreated hypertensive cohort in paper I, whereas in the study STM, the blood pressure levels were unchanged, despite a similar change in CA activity [175, 179]. However, the latter cohort included individuals with well-treated hypertension

and the potential to further lower blood pressure may have been reduced. Another explanation could be differences in ACT/STM CA isoform binding and other non-CA effects. For example, ACT has been shown to cause vasodilation, possibly via a mechanism related to calcium-activated potassium-channels [258] and has several other binding targets [257]. In OSA, an additive antihypertensive effect would be of great use. The pronounced antihypertensive effect of ACT suggests that the secondary hypertension in OSA is different from essential hypertension. Future studies of CA inhibitors could include 24-hour blood pressure assessments to investigate this potential and/or inclusion of untreated hypertensive OSA patients as in paper I.

5.2.1.1.5 Methods

The two analysis methods of CA activity in paper I and III measure the activity in the tissue, in contrast to protein concentration levels. This has both strengths and limitations. The strength includes a measure of the total activity of CA isoforms in the sample. An analysis of protein levels would most likely focus on one or a few different isoforms. If certain CA isoforms are more or less important in the OSA pathophysiology is largely unknown. Only one study on CA IX levels in OSA has been published so far showing increased levels in OSA compared to healthy controls [259]. The methods to assess CA activity are labour intensive and has a high intraindividual variability which are significant limitations.

5.3 A PHARMACOLOGICAL TREATMENT OF OSA

5.3.1.1 PAP DOES NOT FIT ALL

PAP provides great symptomatic relief for many patients, but randomised controlled trials have not shown reductions in hard cardiovascular endpoints [104, 110-112]. Despite technical advances in PAP machines, the compliance to treatment remains relatively low [88]. Having a "good PAP compliance" is often defined as >4 h/night, leaving many patients untreated during 50% of their sleep. New treatments are now being developed, aiming to address separate endotypic mechanisms [165]. Often, tolerability and compliance are higher, but the efficacy during treatment is lower than PAP that "splint open" most airways.

5.3.1.2 COMPARISON BETWEEN TREATMENT MODALITIES

To compare treatments with different treatment efficacy and compliance requires a broader picture than *efficacy during treatment*. Mean disease alleviation describes the overall efficacy, combining efficacy during treatment and adherence to

treatment [260, 261]. By using this method, you can also take sleep time into account, 4 h/night can be 67% for someone sleeping six hours, but only 44% if nine hours is the preferred sleep time. We also need to define "efficacious therapy" and who should be characterised as a "treatment responder". Is a 50% AHI reduction reasonable, or perhaps an AHI <10 events/h? Is it important that the effects can be seen throughout the night or only during treatment? Should we keep looking at AHI as a single metric? Is symptom relief a more important outcome? Using "mean disease alleviation" in addition to other clinical data such as patient reported outcomes and cardiovascular outcomes can increase comparability between treatments and the possibility to choose therapies that help patients in the long run, see Figure 14.

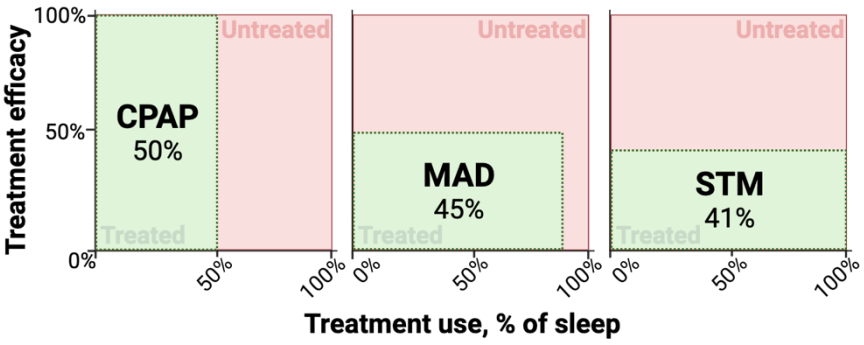

Figure 14. Summarised mean disease alleviation at different degrees of adherence (treatment use) and treatment efficacy (mean AHI reduction). The mean disease alleviation in green (Treated) with % alleviation indicated. "Untreated" proportion in pink. CPAP: continuous positive airway pressure, efficacy 100% - use 50%. MAD: mandibular advancement device, efficacy 50% - use 90%. STM: sulthiame, efficacy 41% - use 100%.

5.3.1.3 PLACEBO MAY AFFECT SUBJECTIVE OUTCOMES

The systematic review in paper IV showed that objective outcomes in OSA are not subject to systematic changes after intake of a placebo drug, whereas a potential placebo effect can be seen in subjective outcomes. These data are in line with previous studies on placebo effects in a number of diseases [167]. In OSA therapy, placebo in the form of sham-PAP has previously been studied [262, 263]. The results from these studies are comparable to our results from drug placebo. The ESS outcome in the study by Reid et al shows a numerical decrease versus baseline in the sham-PAP group, similar to the meta-analysis results, and the objective outcomes are not changed in a clinically relevant way.

The results of the updated meta-analysis should be treated with caution as no systematic search was conducted for inclusion of the new articles. Although we are confident that the added studies include the main new publications in the field,

published after the original systematic search, there may be studies with contrasting results that could have affected the results.

Overall, the results are important to keep in mind when designing and interpreting studies addressing OSA therapy. The effects on patient related outcome measures need to be significantly improved, more than what would be expected by placebo. An ESS increase of 2 units may in an uncontrolled setting be largely driven by placebo effect. On the other hand, no significant effects are seen on objective measures and previous efficacy data on objective outcomes, without control, can be interpreted in relation to night-to-night variability only. In smaller studies this can be of major importance since the intra-individual disease severity varies considerably between nights making small studies vulnerable to false positive and false negative results [166].

5.3.1.4 THE FUTURE OF OSA TREATMENTS

If the concept of "mean OSA alleviation" (the combination of efficacy and adherence) is implemented, the overall therapeutic effects of drugs such as STM compare favourably to PAP and other non-PAP treatments like MAD. But in many cases, we should be aiming higher than 50% disease alleviation. With this in mind, there are several knots that need to be untied in order to optimise the treatment in OSA. Firstly, whom to treat? At this moment, the treatment indications and modalities are subject to change after the lack of effects in cardiovascular outcomes in long-term RCTs, despite what we thought was relatively good treatment compliance and efficacy [104, 110-112]. The ongoing discussions on how to design studies of OSA treatments are important. Study cohorts need to better match the overall patient population and the outcomes should include long-term effects and comorbidities, short-term outcomes are often insufficient. Secondly, how to treat? We need to acknowledge that PAP treatment in many cases may not be the ideal way forward. Since treatments focusing on separate endotypic traits, at this moment, are not as efficient as PAP, combinations of treatment modalities with separate endotypic focus may be a way to increase the total efficacy. For instance, a drug-induced loop gain reduction could be combined with a MAD. A similar concept was recently investigated with different add-on treatments for residual AHI during MAD treatment [264]. When PAP treatment is used, a high compliance is important. Thirdly, predictive biomarkers for treatment outcomes are needed. For example, studies indicate that anatomically oriented treatments such as MAD have a higher effect in individuals with low loop gain, and HGNS may work better if the individual has a higher arousal threshold [146, 147, 248]. Finding these predictive biomarkers can guide clinicians in selecting the correct treatment for a specific patient and increase the adherence and efficacy.

5.4 ETHICAL CONSIDERATIONS AND REFLECTIONS

5.4.1.1 PAPER I

This study consisted of two separate cohorts with long-term treatment of PAP and a short-term study with PAP, ACT and a combination of the two.

The long-term cohort was an observational study where no treatment intervention outside regular routine was used. The only difference compared to regular routine was blood sampling at follow-up which may have caused discomfort for the included subjects.

The short-term cohort was a subgroup of a previous cross-over trial. All participants were hypertensive and before study start, antihypertensive medications were paused. The withdrawal was closely monitored and if an individual had a blood pressure exceeding grade 2 hypertension (>179/110 mmHg) [265], he or she re-started their medication and was excluded from further participation. Pausing a preventive treatment is a risk for the participant but in this setting the potential gains were deemed higher. OSA is closely related to cardiovascular disease, especially hypertension. 35-80% of OSA patients have hypertension and among individuals with resistant hypertension 90% have OSA [63]. ACT can prevent hypertension in high altitude sickness, which also includes sleep disordered breathing. To fully understand the effects of ACT on blood pressure in OSA, it was important to investigate the effects without interaction of other antihypertensives. In summary, we believe that the risks for the study participants were lower than the potential gains of this investigation.

5.4.1.2 PAPER II AND III

These papers were based on a phase IIb study on safety and tolerability of STM treatment in OSA. STM has been used for over 50 years as an anti-epileptic, but the experience in an OSA patient group was limited. The focus was therefore to evaluate safety and tolerability in this specific group. The long period of use for another indication ensured extensive knowledge of both side effects and dosing regimens. However, without previous use in the OSA patient group, there was a potential of OSA-specific side effects. All participants were clinically examined before study inclusion and followed closely during the trial to ensure their safety and to monitor potential side effects. To be included in this trial, participants had terminated CPAP, either by non-acceptance or by non-tolerance. Thereby, no participant discontinued an efficient treatment, which otherwise might have worsened the consequences of their disease.

All techniques and methods used in the study were non-invasive beside blood sampling and carried out by experienced registered nurses. As described earlier, there is a great need for an efficient and well tolerated treatment of OSA. Since there are no pharmacological treatments available, all studies within this area will investigate new and previously untested drugs. By investigating compounds that have been used for other indications, there are less uncertainties of potential adverse effects. We believe that the potential gains from this study were higher than the risks.

5.4.1.3 PAPER IV

This study was a systematic review including data from published studies, no ethical permit was necessary since no individual data was be handled and no biological material analysed.

5.5 SUMMARY OF LIMITATIONS

Overall
- In the interventional studies (paper I, II and III) the small cohort sizes were a potential limitation. The trials were not originally designed, and sized, for the outcomes analysed in this thesis. - The studies mainly included male subjects; this reduces the generalisability as a substantial proportion of the OSA patients are women. This may be especially important in studies of drug therapies in OSA.
Paper I
- The higher CA activity in the long-term cohort A, despite the same analysis method, remains unexplained. Unidentified confounders may play a role.
Paper II
- The PUP Beta PSG analysis tool approximates the endotypic traits. Even though correlation to standard measurements is good, it is based on mathematical modelling with limitations. - Previous studies on the CA inhibitor ACT's effect on endotypic traits were performed with other techniques for estimation of endotypic traits, making direct comparisons between the study results more complex. - The relatively low number of participants in the study prevented multivariate analysis to adjust for potential confounders. Larger studies are needed to confirm our findings and to better understand the underlying pathomechanisms.
Paper III
- The CA analysis methods used in paper I and III are different. - The absolute concentrations of HIF-1α differed considerably compared to previously published data. Different analysis techniques and/or reagents may explain the findings, but the difference remains incompletely understood.
Paper IV
- A number of studies could not be included in the meta-analyses due to incompatible data presentation. - No study with a "no treatment" or "waiting list" comparator was found. It is thereby difficult to fully separate placebo effects from *regression to the mean* effects. - As with all systematic reviews, the included studies are a snapshot of the literature at a specific timepoint. Additional data is constantly published.

Table 11. Summary of limitations in the papers of the thesis.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

The CA system is an important part of the pathophysiology in OSA and inhibition of the CAs appears to be a significant future target for reduction of this disease. CA inhibition with STM has been shown to not only reduce the AHI, but also HIF-1α, a biochemical marker for hypoxia. The mechanistic changes underlying the OSA reduction by STM include an increased stability of ventilation in addition to a stabilised upper airway. The exact pharmacodynamic pathways for these effects remain to be further investigated. Our study populations did not allow us to fully determine if measurement of CA activity in blood can be used as a diagnostic biomarker in OSA. Placebo control remains important in trials of OSA drug therapy as subjective outcomes may be significantly affected.

We are entering an exciting period of OSA research. Common beliefs of treatment effects have recently been questioned, but new discoveries have opened pathways that were previously closed. Future clinical trials of CA inhibition in OSA need to focus on larger cohorts. This would open possibilities for multivariate analysis in this complex disease, to further investigate predictors for higher efficacy. In addition, the variability of the results would be reduced, keeping the high night-to-night variability of OSA in mind. Trials need to be based on cohorts that mimic the OSA patient population, for generalisable results. Inclusion of only low-symptomatic patients does not reflect the wide spectrum of OSA patients. Effort needs to be put into investigations of biomarkers, biochemical and PSG-related, that can enable further classification of whom to treat and how. We need to study both subjective patient-reported outcomes, in addition to a broad selection of objective outcomes to paint a complete picture of disease alleviation, enabling correct comparisons between treatments. Placebo controls are needed, not only to properly assess subjective positive outcomes but also potential side effects. Gradually, we will move on from the "one treatment fits all" of PAP and future treatment indications will be based on the underlying OSA endotypes. Combination treatments will play an important role in many patients as a multifactorial pathogenesis is common and new drug-based treatment principles focus on specific underlying mechanisms.

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