SCUOLA DI DOTTORATO
UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

Department of Medicine and Surgery

PhD program in Public Health

Cycle XXXV

Curriculum in Pathophysiology and Disease Prevention

SLEEP APNEA AND HYPOXIA: NEW THERAPEUTIC PROSPECTIVES

Surname: Perger

Name: Elisa

Registration number: 854303

Tutor: Prof. Gianfranco Parati

Supervisor: Prof. Carolina Lombardi

Coordinator: Prof. Guido Grassi

ACADEMIC YEAR 2021/2022

UNIVERSITA' di MILANO-BICOCCA
Dipartimento di Medicina e Chirurgia
Corso di Dottorato in Sanità Pubblica - Ciclo XXXV
Curriculum in Fisiopatologia Clinica e Prevenzione delle Malattie

Candidata: Dott.ssa Elisa Perger
Tutor: Prof. Gianfranco Parati Supervisore: Prof. Carolina Lombardi

APNEE DEL SONNO ED IPOSSIA: NUOVE PROSPETTIVE TERAPEUTICHE

Introduzione: Un terzo della popolazione europea è affetta da apnee ostruttive del sonno (OSA), patologia che ha conseguenze negative su morbilità cardiovascolare e qualità della vita. L'OSA è caratterizzata da ripetuti episodi di collasso delle alte vie respiratorie che determinano ipossia intermittente, modifiche della pressione intratoracica e risvegli corticali. L'ipossia intermittente ha un ruolo chiave nel determinare le conseguenze cardiovascolari dei disturbi del respiro nel sonno e può sovrapporsi, peggiorandone la prognosi, a condizioni caratterizzate da ipossia tonica quali l'alta quota o le patologie respiratorie croniche o infettive, esacerbando lo stress ossidativo, l'angiogenesi e quindi l'attivazione del sistema nervoso simpatico con conseguenti incrementi della pressione arteriosa, della frequenza cardiaca, dell'infiammazione e della disfunzione endoteliale.

Il trattamento gold standard per l'OSA è la terapia ventilatoria che risulta però non tollerata dalla metà dei pazienti che ne fanno uso. Nuove strategie terapeutiche sono pertanto auspicabili.

Con l'obiettivo di meglio delineare l'eziopatogenesi dell'OSA e quindi di poter disporre di nuovi target terapeutici, sono stati identificati specifici fattori fisiopatologici tra i quali: un'elevata collassabilità delle vie aeree superiori, l'instabilità del sistema di controllo del respiro, una ridotta soglia di arousal ed una ridotta risposta compensatoria dei muscoli dilatatori della faringe. Quest'ultima è dovuta alla perdita di attività noradrenergica e aumento delle influenze muscariniche alle alte vie aeree. Il riconoscimento di questi tratti fisiopatologici ha permesso di ipotizzare e sviluppare nuove strategie terapeutiche per l'OSA.

Obiettivo: Valutare l'efficacia della somministrazione per 1 settimana della combinazione di reboxetina (noradrenergico) ed ossibutinina (antimuscarinico) sul trattamento dell'OSA e dell'effetto dei farmaci sugli endotipi fisiopatologici.

Metodi: E' stato condotto uno studio randomizzato controllato cross-over in doppio cieco per comparare 4mg di reboxetina più 5mg di ossibutinina (reb-oxy) in pazienti con OSA. I pazienti sono stati sottoposti ad

una polisonnografia basale (PSG), una dopo 7 notti di assunzione di reb-oxy ed una dopo 7 notti di assunzione di placebo per confrontare l'indice di apnea-ipopnea (AHI–outcome primario). Outcome secondari comprendevano il carico ipossico, modifiche degli endotipi, la variabilità della frequenza cardiaca, test di vigilanza.

Risultati: Hanno completato lo studio 16 pazienti con età 57 [51-61] anni (mediana [range interquartilico]) ed indice di massa corporea 30 [26-36] kg/m^2. Reb-oxy ha determinato una riduzione di AHI da 49 [35-57] eventi/h al basale a 18 [13-21] eventi/h (59% di riduzione mediana) e 39 [29-48] eventi/h (6% riduzione mediana) confrontato con il placebo (p<0·001). La frequenza cardiaca mediana durante la PSG è stata 65 [60-69] bpm al basale ed è aumentata fino a 69 [64-77] bpm dopo reb-oxy e 66 [59-70] bpm dopo placebo (p=0.02). La somministrazione di reb-oxy non ha comportato modifiche di variabilità della frequenza cardiaca, pressione arteriosa nelle 24 ore. Il test di vigilanza si è ridotto da 250 [239-312] ms al basale a 223 [172-244] ms dopo reb-oxy versus 264 [217-284] ms dopo placebo (p<0·001).

Conclusioni: Il miglioramento delle conoscenze della fisiopatologia dell'OSA ha permesso di identificare la responsività muscolare delle alte vie come target principale di strategia terapeutica per l'OSA, predisponendo il percorso verso la medicina di precisione anche nel contesto dei disturbi del respiro nel sonno. Il nostro studio ha evidenziato il dato pionieristico dell'effetto positivo della somministrazione di reboxetina più ossibutinina sulla gravità dell'OSA e sull'ipossia associata agli eventi ostruttivi nel sonno. I risultati del nostro studio sottolineano la possibilità di una terapia personalizzata con farmaci per trattare l'OSA ed il carico ipossico ad essa relato.

UNIVERSITY of MILANO-BICOCCA
Department of Medicine and Surgery
PhD program in Public Health - Cycle XXXV
Curriculum in Pathophysiology and Disease Prevention

PhD Candidate: Dr. Elisa Perger
Tutor: Prof. Gianfranco Parati Supervisor: Prof. Carolina Lombardi

SLEEP APNEA AND HYPOXIA: NEW THERAPEUTIC PROSPECTIVES

Introduction: Obstructive sleep apnea (OSA) affects one third of the population in Europe and has major negative consequences for cardiovascular disease and quality of life. OSA is characterized by recurrent episodes of apneas and hypopneas associated with repetitive episodes of intermittent hypoxemia, intrathoracic pressure changes, and arousals. Intermittent hypoxemia, particularly with concomitant hypercapnia, activates the sympathetic nervous system and it is the major contributor to negative cardiovascular consequences. Intermittent hypoxia might also worsen concomitant tonic hypoxia due to high altitude or due to acute or chronic respiratory diseases by promoting oxidative stress and angiogenesis, thus increasing sympathetic activation with blood pressure elevation, inflammation and endothelial dysfunction. Although OSA and its hypoxic consequence are effectively alleviated with positive airways pressure, this treatment is yet unsatisfactory, being poorly tolerated by up to half of patients. Thus, new treatment strategies are strongly needed.

With the aim of better understand OSA physiopathology, key contributors of its development have been identified and include upper airway collapsibility, ventilatory instability, low arousal threshold and reduced pharyngeal dilator muscle responsiveness during sleep, due to loss of noradrenergic drive and enhanced muscarinic influences to upper airway muscles. The recognition of these pathophysiological traits permitted to advance the research in the field of OSA new therapeutic perspectives.

Aim: The aim of this study was to evaluate the effect of 1-week of reboxetine (a noradrenergic) plus oxybutynin (an antimuscarinic) on OSA severity (primary outcome) and their effect on endotypic traits and cardiovascular autonomic modulation.

Methods: We performed a randomized, placebo-controlled, double-blind, crossover trial comparing 4 mg reboxetine plus 5 mg oxybutynin (reb–oxy) to placebo in OSA subjects. After a baseline in-lab polysomnogram

(PSG), patients performed PSGs after 7 nights of reb-oxy and 7 nights of placebo to compare apnea-hypopnea index (AHI, primary outcome). Secondary outcomes included hypoxic burden, heart rate variability, blood pressure and heart rate changes and psychomotor vigilance test. Home oximetry evaluated overnight oxygen desaturation throughout treatment.

Results: 16 subjects aged 57[51-61] years (median [interquartile range]) with body mass index 30[26-36] kg/m^2 completed the study. Reb-oxy lowered AHI from 49[35-57] events/h at baseline to 18[13-21] events/h (59% median reduction) compared with 39[29-48] events/h (6% median reduction) on placebo (p<0·001). Response rate for reb-oxy was 81% versus 13% for placebo p<0·001). Median nocturnal heart rate during the PSG was 65 [60-69] bpm at baseline and increased to 69 [64-77] bpm on reb-oxy vs 66 [59-70] bpm on placebo (p=0.02). Reb-oxy administration was not associated with any modification in heart rate variability, 24-hour, day-time and night-time systolic and diastolic blood pressure. The psychomotor vigilance test decreased from 250[239-312] ms on baseline to 223[172-244] ms on reb-oxy versus 264[217-284] ms on placebo (p<0·001). Home oximetry illustrated acute and sustained improvement in oxygen desaturation index on reb-oxy versus placebo.

Conclusions: The recent understanding of OSA pathophysiological mechanisms brought to hypothesize that, among the others, muscle responsiveness would be the main target to develop a precision medicine to treat OSA. We demonstrated that OSA severity and OSA-related hypoxic consequences are greatly decrease by the administration of reboxetine-plus-oxybutynin. These results highlight potential possibilities for personalized medicine with pharmacological therapy to treat OSA and its related hypoxic burden.

1 TABLE OF CONTENTS

2 INTRODUCTION

Hypoxia has deleterious effects on the cardiovascular system, on the central nervous system and on all the organs of the human body. For example, hypobaric hypoxia is responsible for acute and chronic mountain sickness, which are linked to respiratory and cardiovascular consequences. On the other hand, many chronic respiratory diseases, such as chronic obstructive pulmonary disease, interstitial lung disease, asthma, pneumonitis as the one caused by the recent COVID-19, determine normobaric hypoxia based on different pathophysiological mechanisms (1). The night-time presence of sleep disordered breathing can further worsen tonic hypoxia adding intermittent episodes of oxygen reduction (2, 3). Intermittent hypoxia is a typical feature of obstructive sleep apnea (OSA), one of the most common sleep-related breathing disorders. Due to repetitive collapse of the pharyngeal airway during sleep, OSA leads to intermittent oxygen desaturations, sleep fragmentation, excessive daytime sleepiness and cardiovascular impairment, and long-term OSA is associated with increased morbidity and mortality (4, 5).

Although OSA is effectively alleviated with the use of continuous positive airway pressure (CPAP) and, for some individuals, with oral appliances or multimodal approaches(6), these treatments are often poorly tolerated by patients and compliance is relatively low(7). Efforts to develop pharmacological therapies for the treatment of OSA have accelerated over the last two decades, but currently no pharmacological intervention has been approved for clinical use.

With the goal of identifying possible therapeutic targets, research in the last decade has identified key pathophysiological *traits* that contribute to the development of OSA (Figure 1) (8-11). These not only include an anatomically small, collapsible upper airway (high passive critical closing pressure of the upper airway [Pcrit]) (*1*) (12), but also: (*2*) inadequate responsiveness of the upper-airway dilator muscles during sleep (minimal increase in muscle activity to negative pharyngeal pressure)(13) ; (*3*) waking up prematurely in concomitance with airway narrowing (a low respiratory arousal threshold)(14) and (*4*) having an oversensitive ventilatory control system (high loop gain) (15).

One of the key pathophysiological traits in OSA is the loss of upper airway dilator muscle activity at sleep onset and the lack of reactivation (muscle compensation) during sleep in response to upper airway obstruction.

Research in animals improved the understanding of the state-dependent neurotransmitters involved in pharyngeal muscle activation during sleep, providing evidence that both noradrenergic and antimuscarinic processes are involved. Specifically, the impairment of noradrenergic activity is now thought to play a key role in the sleep-related hypotonia of pharyngeal muscles mostly during Non-Rapid Eye Movement (NREM) (16-18) sleep, and muscarinic activity is primarily involved in Rapid Eye Movement (REM) muscle atonia (19, 20).

It has been shown that a fixed dose combination of atomoxetine (a noradrenergic agent) and oxybutynin (an antimuscarinic compound) (21) reduced the frequency of obstructive events by 63% and improved genioglossus responsiveness in 20 OSA patients over a single night of treatment. In a study on healthy individuals, a combination of reboxetine (a noradrenergic agent) and hyoscine butylbromide (an antimuscarinic drug) improved activity of the *tensor palatini* muscle and upper airway resistance during NREM sleep (22). Preliminary data also suggested an improvement in OSA severity of approximately 35% after a single night of reboxetine plus hyoscine butylbromide (23). Given that the putative mechanism of action of this combination is the stimulation of the hypoglossal motor pool in the brainstem, we considered that a combination of reboxetine plus oxybutynin might have greater therapeutic potential than reboxetine plus hyoscine, since the latter can reach higher concentration in the brainstem parenchyma (24). Since these processes have been identified only recently, there has not yet been any attempt to stimulate the pharyngeal muscles with these drugs for longer than a single night.

2.1 Hypoxia

Hypoxia is a hallmark feature of respiratory disease and has multiple effects on all the human body, included the central nervous and the cardiovascular system. Interest in the effects of hypoxia is both clinically relevant to discern the pathophysiological mechanisms of cardiorespiratory diseases and physiologically relevant to understand the adaptive changes that occur in response to high altitude. As climbers have ventured into high altitude, it became apparent that humans adapt to counter the chronic effects of hypoxia in ways highly beneficial for maximizing the efficient use of oxygen for metabolic demand. On ascent to altitude, acclimatization to hypoxia is reflected by progressive increases in ventilation, adaptations in the cardiovascular

system that enhance oxygen delivery to tissues, and alterations at the tissue level that allow for better extraction of oxygen and more efficient utilization of oxygen for metabolic processes (25). Substantial nocturnal hypoxemia has been reported in highlanders (26-30), and sleep disordered breathing is frequently observed in lowlanders ascending to high altitude (31, 32) especially in the populations of the Andes (33). The severity, frequency, and duration of sleep related intermittent desaturations on top of chronic hypoxemia might lead to more pronounced chemoreceptor stimulations and thereby might influence erythropoiesis (34). Thus, among the adaptation process that highlanders adopt to respond to hypobaric hypoxia there is the increase in erythropoiesis. However, exaggerated erythrocytosis as maladaptive response to hypobaric hypoxia characterize chronic mountain sickness that is often associated with increased pulmonary artery pressure and systemic vascular dysfunction (35). To evaluate the pathophysiological effects of hypobaric hypoxia on chronic mountain sickness, we performed an international expedition in 2019 at 5100m altitude in Perù (36). We found that highlanders at 5,100m with moderate-severe chronic mountain sickness presented the lowest daytime and night-time SpO_2, with increased blood pressure variability during the night and with a tendency towards worse oxygen desaturation index compared to highlanders without chronic mountain sickness (2). We also showed that lower SpO_2 nadir was independently associated with more severe chronic mountain sickness. Nocturnal oxygen saturation drops in susceptible highlanders characterized by low arterial oxygen pressure (PaO_2) may be explained by being in the steepest part of the SpO_2/PaO_2 curve, where small changes in PaO_2 due to respiratory instability can induce significant reductions in SpO_2. Besides such tonic reduction, this phenomenon might easily determine chronic intermittent drops of SpO_2, which are known to increase haemoglobin concentrations in highlanders with chronic mountain sickness (28, 37). The evidence of higher nocturnal blood pressure variability supports such an interpretation and suggests a slight contribution of episodes of phasic events rather than the implication of tonic desaturations only (2).

In the same way, intermittent hypoxia related to sleep disordered breathing might superimpose chronic or acute hypoxia due to respiratory disease. In 2020 our department was temporarily transformed in a COVID-19 Unit during the first wave of pandemic in Italy, The novel coronavirus SARS-CoV-2 responsible for COVID-19 determine an highly variable clinical presentation, with some of affected individuals exhibiting

severe and progressive pneumonia that requires intensive care (38). In this contest of acute hypoxia due to a respiratory virus, we had the opportunity to evaluate the presence of sleep disordered breathing among hospitalized patients due to COVID-19. We showed that among hospitalized patients due to COVID-19 75% presented sleep disordered breathing and that, together with higher body mass index, the presence of OSA, was associated with the worst hospitalization outcome (3).

The high rate of underdiagnosed – and undertreated – OSA in various conditions of hypoxia, might act as cofactor in the worsening of the patients' clinical conditions, representing itself a risk factor for increase susceptibility to symptomatic disease, such as for worse COVID-19 sequelae or for worse adaptation to hypobaric hypoxia due to high altitude.

Although much is known about the acute and chronic effects of sustained hypoxia, less is known about the effects of intermittent hypoxia. Repeated exposures to hypoxia have been examined for both their beneficial and adverse effects(39). For example, the functional benefits of repetitive hypoxia have been explored for both its therapeutic value in patients and performance value in athletes. However, the clinical pathology of the intermittent hypoxemia associated with sleep apnea syndrome and the apnea of prematurity suggests that there may be long-term adverse consequences of chronic cyclical hypoxia. The pathophysiology of apnea syndromes has spurred a recent surge of interest in the pathophysiological effects of recurrent episodes of intermittent hypoxemia that produce a variety of comorbid disorders. The effects of repetitive intermittent hypoxia, as occurs nightly in OSA, are generally considered deleterious for the cardiovascular system. Thus, intermittent hypoxia promotes oxidative stress by increased production of reactive oxygen species and angiogenesis, increased sympathetic activation with blood pressure elevation, and systemic and vascular inflammation with endothelial dysfunction that contributes to diverse multiorgan chronic morbidity and mortality affecting cardiovascular disease, metabolic dysfunction, cognitive decline, and progression of cancer. Data from observational studies in large population groups also support the role for hypoxia in the pathogenesis of OSA comorbidity. For instance, 2–4 weeks of nightly intermittent hypoxia increase daytime blood pressure and sympathetic nerve activity in healthy individuals (40). In addition, the overnight sleep apnea–related hypoxic burden metric, which includes both hypoxemia frequency and magnitude components, predicts cardiovascular

mortality (41-43). This recent evidence that the desaturation parameters related to apneas are a more adequate index of cardiovascular outcome than the number of apneas or hypopneas highlights the role of the qualitative quantification of hypoxia consequent to OSA in the general management of the disease and in the future treatment directions.

2.2 Obstructive Sleep Apnea

OSA is one of the most common sleep disorders and it affects one third of the population aged between 30 to 70 years in Europe (44). Recent estimates suggest that nearly 1 billion adults aged 30–69 years worldwide could have OSA, including a huge amount of people who have not received a diagnosis yet (44-46). It has been estimated that up to 50% of men from a community sample had clinically important OSA (based on an apnea hypopnea index [AHI] above 5 events per hour and associated daytime consequences) (47).

OSA was identified to affect numerous health outcomes and pathophysiological processes, particularly cardiovascular diseases. The repetitive collapse of the pharyngeal airway during sleep leads to impaired gas exchange and intermittent oxygen desaturations that result in arousal from sleep. Although these arousals generally do not awake the patient, together with hypoxia, sleep fragmentation is the primary cause of excessive sleepiness in individuals with OSA. Intermittent hypoxemia, particularly with concomitant hypercapnia, activates the sympathetic nervous system and it is the major contributor to both acute and chronic elevation of blood pressure (48). These surges in sympathetic activity were shown to result not only in acute blood pressure elevations, but also in release of inflammatory mediators, lipolysis, and worsened insulin resistance (49). Cardiac remodelling, common in patients with OSA, was attributed to exposures to hypoxemia, catecholamine excess, blood pressure elevation, and intrathoracic pressure swings affecting preload and afterload and left atrial and ventricular transmural pressures (5, 50). Thus, OSA has major adverse consequences for the cardiovascular system**,** leading to potentially severe complications such as stroke, cardiac arrhythmias, and heart failure (4, 51-53). The relationship with hypertension is the best established (50) with a linear, dose-dependent relationship between the severity of OSA at baseline and the relative risk of developing hypertension during follow-up (5). Extensive literature describe the pathophysiologic link between OSA and cardiovascular

disease (54-58), demonstrating, for example, that risk of atrial fibrillation occurrence is two times higher in patients with OSA with respect to non-OSA subjects and that the association between arrhythmias and OSA is consistent regardless coexistent conditions. The mechanisms linking sleep breathing disorders with arrhythmias are triggered by sympathetic overactivity during the post-apnea period, arousals, intermittent hypoxemia/hypercapnia and the mechanic strain related to the respiratory muscle efforts against the collapsed upper airway (56). Moreover, sleep fragmentation may interfere with neurocognitive activity, that can significantly affect daytime functioning, leading to excessive daytime sleepiness and fatigue (59).

2.2.1 Currently available treatment for obstructive sleep apnea

Treatment for OSA changed little over the past 40 years, with the overwhelming majority of patients treated with positive airway pressure, the most common of which is Continuous Positive Airway Pressure (CPAP), provided by a machine that mechanically maintains an open airway by pushing positive pressure into the upper airways contrasting the collapse.

CPAP is the primary choice of treatment for individuals with symptomatic OSA of any severity (60). CPAP delivers pressure to the airway through a mask worn over the nose or the nose and mouth. This pressure acts as a splint to prevent airway collapse during inspiration. CPAP benefits strictly depend on adherence to therapy, with not enough hours of use per night being associated with lack of effectiveness in improving OSA consequences and related symptoms (61).

Oral appliances (mandibular advancement devices) are effective treatment options for individuals with mild OSA(62). Beside their indication for only mild to moderate OSA, these devices require adequate dental and periodontal structure and can cause temporomandibular joint discomfort and occlusal abnormalities due to teeth movements.

Surgical modification of the upper airway is suitable for selected patients. The most common surgical procedures for managing OSA modify soft tissue, including palate, tongue base, and lateral pharyngeal walls. However, selection criteria for this procedure are not clearly established yet. Besides the elevated costs, these

invasive procedures include possible post-operative complications and relapses of the airway collapse is not uncommon.

Being the stimulation of the upper airways one of key pathogenic factors for OSA, hypoglossal nerve stimulation has been proposed as a surgical procedure to increases pharyngeal dilator muscle tone during sleep. This technique is invasive and expensive and is still not available in many European countries. Potential complications include temporary tongue weakness and soreness and discomfort from stimulation.

Other treatment options include specific OSA phenotypes such as the positional treatment for supine-related apneas or weight loss for those affect by obesity (63, 64). Other alternative therapies available for subject with overload of fluid such as renal insufficiency, heart failure or venous insufficiency might be targeting fluid shift with diuretics, compression stocking or physical activity (6, 65).

While CPAP and related therapies are effective in improving sleep characteristics and oxygenation, many, perhaps most, patients find these devices uncomfortable, and most estimates indicate that fewer than 50% of patients prescribed CPAP use it more than 4 hours per night (7, 66-68). Efforts to develop pharmacologic therapies using antidepressants, ventilatory stimulants, and hormonal agents for the treatment of OSA have been ongoing for at least 20 years, with no approved pharmacologic products thus far.

Although increased hours of CPAP therapy are associated with greater improvements in symptoms, quality of life and blood pressure, the low adherence to this treatment can be problematic. As many patients do not use CPAP, this represents a significant health concern, as OSA is associated with numerous co-morbidities and increased mortality. Alternative options, such as drugs that activate the pharyngeal muscles are needed.

2.3 Pathophysiology of obstructive sleep apnea

OSA is characterized by repetitive partial or complete collapse of the upper airway during sleep, resulting in episodic reduction (hypopnea) or cessation (apnea) of airflow despite increased respiratory effort. Contraction of pharyngeal dilator muscles is necessary to maintain airway patency during inspiration. The most

important upper airway dilator muscle is the genioglossus muscle, which activates with each inspiration to prevent posterior collapse of the tongue, assisted by the *levator* and *tensor palatini* muscles (advancing and elevating the soft palate) and the geniohyoid and stylopharyngeus muscles (opposing medial collapse of the lateral pharyngeal walls) (48).

A narrow upper airway importantly contributes to development of OSA, typically worsened by fat deposit in the parapharyngeal fat pads and pharyngeal muscles(69). In the presence of a small pharyngeal airway, upper airway collapse is prevented when an individual is awakened by increased CO_2 levels during apneas and hypopneas. A decrease in both basal and compensatory dilator muscle tone during sleep also facilitates airway collapse (48, 70).

The occurrence of upper airway obstruction during sleep reflects an interplay between the removal of the wakefulness drive (which helps to maintain airway patency) and an individual susceptibility to collapse. Although individual risk factors are known, the precise pathophysiologic pathways leading to upper airway obstruction and their reciprocal influence in patients with obstructive sleep apnea (OSA) need further investigation.

Pharyngeal muscles relaxation during sleep and lack of sufficient reactivation are key primary pathophysiological events leading to OSA (71). The reduced ventilation consequent to an obstructive event increases CO_2 and ventilatory drive, which could lead to the pharyngeal muscles' activation (muscle responsiveness) followed by the reduction of the upper airway resistance. Waking up prematurely (low arousal threshold) to a relatively modest level of airway narrowing can limit the ability to build up sufficient respiratory stimuli to recruit the pharyngeal dilator muscles to open the upper airway, thereby achieving breathing stability (72-74) . Thus, while arousals have in some patients an important role in protecting from asphyxia during sleep,

they may disrupt breathing stability and worsen OSA severity in patients with a low arousal threshold (Figure 1).

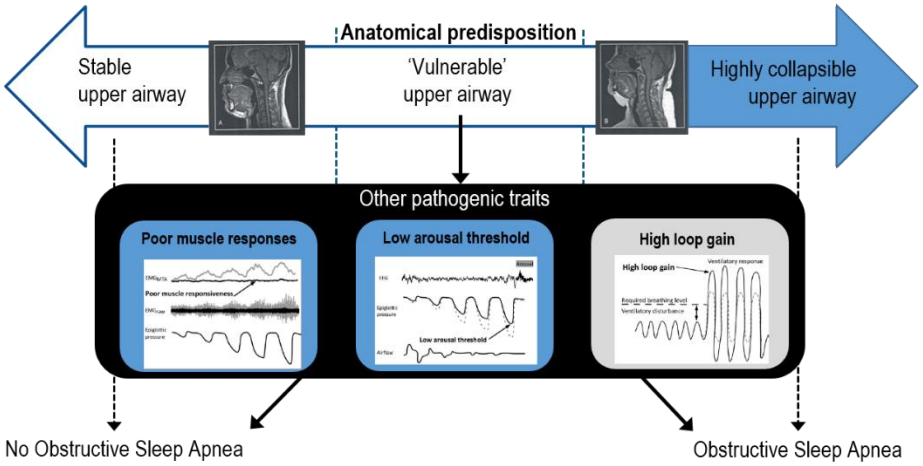

Figure 1*: The four key pathophysiological mechanisms of OSA. Pharmacological avenues for improving OSA via muscle physiology will act on collapsibility, muscle responsiveness and arousal threshold (in blue). Reduced* ***resting muscle tone*** *raises collapsibility; reduced* ***muscle responsiveness*** *impairs recovery from reduced airflow; a lowered* ***arousal threshold*** *prevents the rise in ventilatory drive needed to increase muscle tone and airflow. Reboxetine and oxybutynin acted in our preliminary results via increased muscle tone, and trazodone is expected to improve the arousal threshold*

Pathophysiology studies have shown that interventions aiming to lower upper airway collapsibility (6), to increase the activity to pharyngeal muscles (21, 75, 76), to increase the arousal threshold (77) or lower loop gain (78) can reduce OSA severity. Moreover, recent evidence has demonstrated that treatment efficacy for oral appliances and pharmacological therapies are dependent on the severity of underlying pharyngeal *collapsibility* in individual patients (76, 79, 80). Endotypes measurements were originally obtained challenging the upper airway function through CPAP manipulation during sleep (81), using sophisticated procedures that remained limited to specialized physiology laboratories (9, 10). Further research permitted to develop noninvasive clinically applicable techniques to quantify OSA traits (82-84). Specifically, it recently became possible to estimate the ventilatory control contribution to OSA severity (loop gain and arousal threshold), together with the pharyngeal collapsibility and muscle compensation using automated techniques applied to respiratory signals collected in routine clinical polysomnography (82, 83, 85).

2.3.1 Functional anatomy of the upper airway

The human upper airway is a multipurpose anatomical region serving respiration, deglutition, and vocalization. Because of this multiple functions, the pharyngeal airway lacks a rigid bony or cartilaginous support which would make it more stable but also less adaptable to different tasks; therefore, it is a deformable tube susceptible to collapse if sufficient transmural pressure is applied across a compliant pharyngeal wall (86). The importance of an abnormal pharyngeal susceptibility to collapse in the pathogenesis of obstructive apneas was demonstrated by studying the pharyngeal critical pressure (Pcrit) in patients with OSA and in control subjects (87). Flow cannot occur until the pressure upstream of the collapsible segment exceeds the surrounding pressure, i.e. Pcrit. There is a progressive increase in Pcrit from normal subjects to snorers and apnoic patients, with normal being a negative Pcrit (88) (the airways stay open) and apnoic having a positive Pcrit leading to upper airway collapse (89).

The loss of wakefulness drive to breathe results in decreased upper airway neuromuscular activity and responsiveness, leading to decreased caliber, increased resistance, and to an increased probability of pharyngeal collapse (90). The response to these physiologic changes partly depends on the underlying susceptibility to pharyngeal collapse, which is determined by baseline upper airway caliber, surrounding tissue pressure i.e. adipose tissue, vascular and mucosal factors, craniofacial structure, and the intrinsic properties of the upper airway. The thoracic-upper airway link via caudal (tracheal) traction might also influence the upper airway patency.

Structural factors: Support for the role of bony structures in the propensity for upper airway obstruction comes from observational and experimental studies. Differences in position of the hyoid bone between patients with OSA and controls was largely determined by tongue volume, suggesting that inferior displacement of the hyoid bone in patients with OSA is due to relative tongue volume and increased surrounding pressure(91).

In a case-control study that used three-dimensional magnetic resonance imaging, increased mandibular length was associated with decreased risk for OSA in men but not in women (92). Indeed, OSA is more prevalent in men than in women, and the prevalence increases in both sexes with age, independent of body weight, yet it is

not clear what the precise mechanism behind this difference is. It is unlikely that the variability in airway cross-sectional area or volume, which is actually larger in men then in women (93), plays a role. A more relevant measurement may be airway length, which seems to be predictive of pharyngeal collapsibility based on experimental measurements and computational modeling (94, 95). Because a longer airway is more collapsible, pharyngeal airway length may explain at least in part the male predisposition to pharyngeal collapse. Epidemiological studies also highlighted that differences in craniofacial indices may contribute to racial and ethnic differences in the prevalence of sleep apnea (96). For example, compared to Icelandic OSA patients, Chinese patients smaller combined soft tissue volume but have larger soft palate volume (in males), as well as smaller retropalatal airway areas and smaller mandibular and maxillary structures(97).

Soft tissue characteristics: The upper airway lumen is surrounded by the soft tissue of the neck, including connective, adipose, vascular, and lymphatic tissue. Consequently, factors that increase surrounding tissue pressure tend to promote its narrowing. Several soft tissue factors are associated with higher risk of OSA, including increased tongue size, increased size of lateral pharyngeal walls, and increased total soft tissue volume(98). Enlarged tonsils can raise the susceptibility to upper airway obstruction by encroaching on the pharyngeal lumen (99). Increased soft tissue may be a heritable trait, as evidenced by the familial aggregation of soft tissue structure in normal individuals and those with OSA, independent of body mass index (BMI) and neck circumference (100). Increased adipose tissue in the upper airway or the tongue secondary to obesity may also increase collapsing tissue pressure (101). Confirming diagnostic evidences, interventions that decrease surrounding tissue pressure such as tonsillectomy or weight loss have been seen to reduce OSA severity (102).

Overnight rostral fluid shift: During the day fluid accumulates in the intravascular and interstitial spaces of the legs due to gravity, and upon lying down at night redistributes rostrally. Some of this fluid may accumulate in the neck, increasing tissue pressure and causing the upper airway to narrow, thereby increasing its collapsibility and predisposing to OSA(103). This pathogenic mechanism is particularly important for patients with fluid retaining states, such as heart failure, renal failure or venous insufficiency(58, 65, 104) and reducing lower extremity fluid volume (eg, with compression stockings, diuresis) may attenuate this process(6).

Lung Volume: Changes in lung volume during the respiratory cycle are related with changes in upper airway caliber (105). Independent of dilating muscle activity, there is an inspiratory increase and an expiratory decrease in upper airway luminal size. In fact, pharyngeal cross-sectional area reaches a nadir at end expiration, especially in patients with sleep apnea (106). Decreased lung volumes during sleep are associated with increased upper airway collapsibility, perhaps via a reduction in the longitudinal tension of the pharyngeal airway (107). This could be attributed to tracheal displacement playing a role in upper airway stability: indeed, inspiratory activity displaces the trachea caudally and stretches the connective tissue linking the trachea to the upper airway (108). From a mechanical standpoint, caudal traction promotes upper airway patency by increasing transmural pressure and stiffening the pharyngeal wall (109).

2.3.2 Upper airway muscles

Since humans have no fixed bone to support the pharynx, that is maintained open by the surrounding musculature. The most important and studied pharyngeal dilator muscle is genioglossus, whose activity is characterized by a tonic (measured during expiration) and a phasic component (displaying during inspiration) which is essential to prevent posterior collapse of the tongue when the pressure becomes negative in the compliant pharynx. The levator and tensor palatini advance and elevate the soft palate, while the geniohyoid and stylopharyngeus oppose medial collapse of the lateral pharyngeal walls (48). Relaxation of these muscles during sleep and lack of sufficient reactivation are key primary pathophysiological events leading to OSA (71).

Thus, to maintain a patent upper airway while awake, OSA patients activate more the upper airway dilators during wakefulness compared to healthy subjects. At sleep onset, all individuals physiologically reduce the activity of pharyngeal dilator muscle (110). This, together with anatomic predisposition and/or unstable control of breathing, often leads to OSA during lighter stages of sleep. Epiglottic pressure swings and CO_2 increase with deeper stages of sleep and during obstructive events (111), generally determining a reflexive reactivation of pharyngeal muscle with the consequent restoration of upper airway patency. This reflex is widely variable between individuals, with some showing effective recruitment and the ability to partially reopen the upper

airway and increase ventilation during an obstructive respiratory event (muscle compensation), and others showing minimal or no upper airway muscle compensation during sleep (Figure 2) (48, 112).

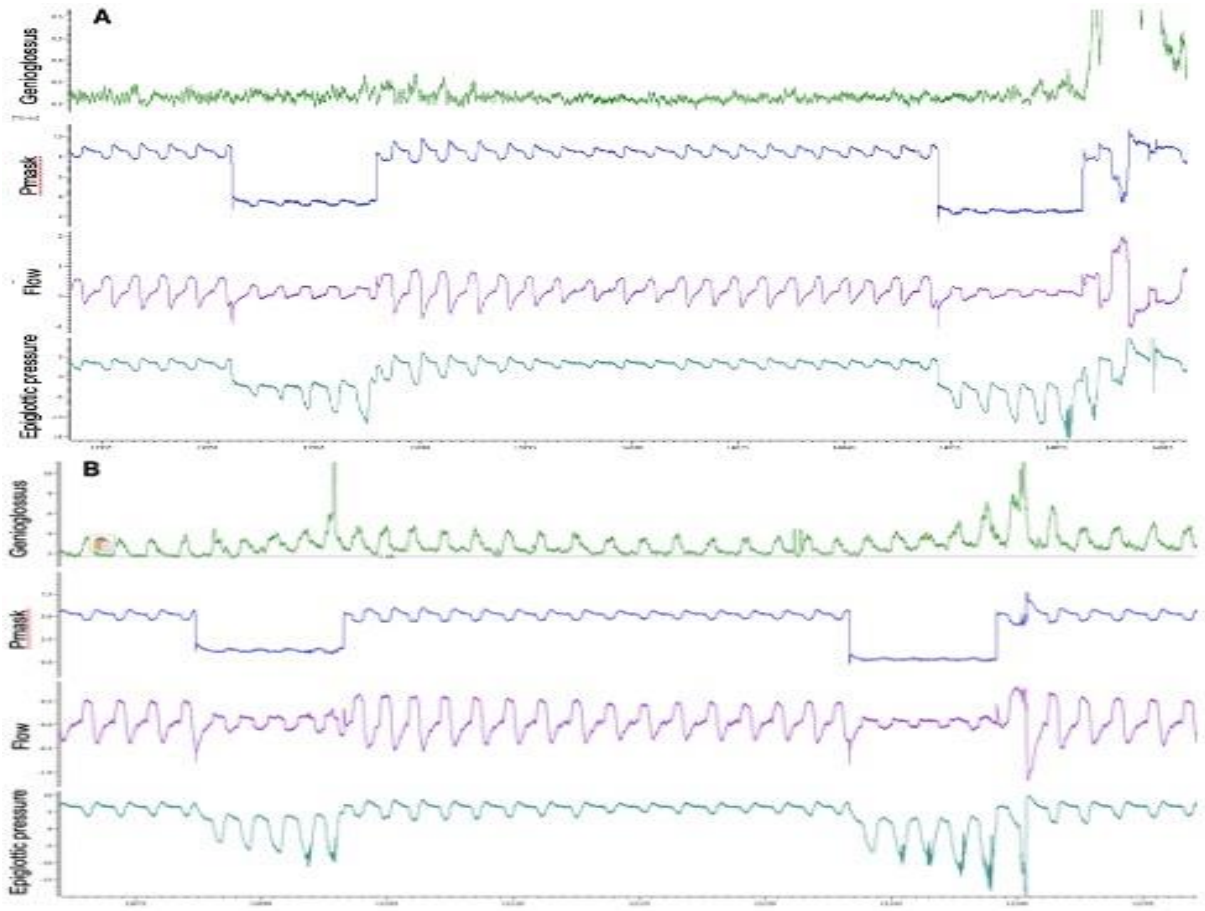

Figure 2*: Examples of OSA patients with low vs high genioglossus responsiveness to negative upper airway pressure reflex during sleep.* ***Panel A*** *(top) shows genioglossus activity of a patients with OSA during manipulation of upper airway physiology using the CPAP drop technique. The patient is sleeping on CPAP at therapeutic level that avoids flow limitation (see Flow channel) when CPAP pressure is abruptly reduced for 5 breaths, inducing flow limitation. In this patient, the response of the genioglossus activity to the progressively more negative epiglottic pressure is minimal or absent.* ***Panel B*** *(bottom) shows the genioglossus response to the same upper airway challenge performed with the CPAP drop in a patient with simple snoring without OSA. In this case, the progressive lowering of epiglottic pressure during the CPAP drop induces a proportional increase in genioglossus activity. Presented with permission from "E. Perger et al; Upper airway muscles: influence on obstructive sleep apnoea pathophysiology and pharmacological and technical treatment options; Curr Opin Pulm Med 2021 27(6):505-513. doi: 10.1097/MCP.0000000000000818*

2.3.3 Ventilatory drive and loop gain

Breathing is a complex but automated process that, in passive, healthy conditions, goes unnoticed. Respiration originates in the brainstem, in different areas that are intimately connected (113, 114). These neural networks contribute to generating and pace making the rhythm of respiration, i.e. ventilatory drive. Ventilatory drive is influenced by mechanoreceptors within the lungs (i.e., stretch receptors, j-receptors, C-fibers (115, 116)) and peripheral and central chemoreceptors (117, 118). The activity of mechanoreceptors is also conditioned by behavior such as vocalization, sneezing, coughing, etc. (119) and emotions (120). Therefore, ventilatory drive can be subdivided in *mechanical-behavioral* drive (or *wake* drive), which overall quantifies the mechanical load on the respiratory muscles and, in healthy conditions, is exquisitely state dependent (121), and *chemical* drive; of note, since O_2 contributes to chemosensitivity only during severe hypoxemia, CO_2 is the main determinant of chemical drive (117).

Chemical drive is fine-tune regulated by a precise negative feedback mechanism (loop gain) that keeps gas tension levels in the blood within homeostatic limits (122, 123). Loop gain is the result product of different components; 1) the *plant*, namely the pulmonary response that processes gas tension variations following a ventilatory disturbance, 2) the *controller*, which drives the ventilatory response to changes in gas tensions, 3) the *mixing* (with negligible physiological impact on breathing instability), which further adjusts gas tensions when the blood passes from the pulmonary capillary to the larger chest vessels (73).

A low loop gain leads to negligible changes in ventilation in response to larger variations in blood gas tensions; vice versa, a high loop gain, a marker of breathing instability, responds to minimal changes in blood gas tensions with an exaggerated increase in ventilation. Subsequently, this response becomes a disturbance itself and will propagate breathing instability at a rate determined by the plant (i.e., if the plant is high, it will lead to CO_2 accumulation in the lungs in response to a minimal disturbance, which favors frequent further ventilatory disturbance) and at an amplitude determined by the controller (124). Of note, a high baseline chemical drive does not necessarily mean a high loop gain, rather it can lead to decreased loop gain in healthy conditions (through reducing the plant). However, if the controller is increased by a larger magnitude than the decrease in the plant, a high drive will reflect a high loop gain.

Loop gain is also a function of the lung-to-chemoreceptor circulation time, or *circulatory delay*, which influences the duration of each sleep apnea cycle (125); however, since circulatory delay increases when cardiac output decreases (126, 127), in otherwise healthy individuals with OSA circulatory delay is generally not prolonged and does not contribute to greater loop gain and sleep apnea severity.

During sleep, in healthy conditions, wake drive falls to minimal levels and chemical drive becomes predominant (128). Chemical drive during sleep is also reduced compared to wakefulness, which leads to slightly higher CO_2 levels (~2-4 mmHg) during sleep (129). A decreased chemical drive during sleep is generally accompanied by a lower or unchanged controller gain vs. wake and a modestly higher plant gain (129, 130). This may overall result in a higher loop gain during sleep vs. wake (130) that, along with other mechanisms, increases susceptibility for a greater ventilatory instability during sleep (119). Such instability may be the precipitating factor for OSA exacerbation in predisposed individuals (131). Indeed, a high loop gain cyclically drives CO_2 under the threshold for apnea through recurring swings in chemical drive (132). This may seem reminiscent of central sleep apnea, however, in OSA, differently than central, flow reductions are always larger in magnitude than the reductions in drive (133), likely due to a mismatch between the neural drive to the pump and the pharyngeal muscles (134).

An elevated loop gain was accounted for by as one of the non-anatomical determinants of OSA, however, in the prevailing view, OSA was mainly though to onset following a state-dependent loss of pharyngeal muscle function, with subsequent flow reduction and reflex increase in ventilatory drive (9). REM OSA specifically seemed to be highly influenced by a loss of preferential neural drive to the upper airway muscles (135), with lower controller and loop gain being instead protective mechanisms (129, 136). Only recently, withdrawal of chemical drive has been identified as a key feature of NREM (133) and REM OSA pathogenesis (137), where drive falls closely accompany flow and pharyngeal muscle activity reductions.

2.3.4 Respiratory arousal threshold

The respiratory arousal threshold is the level of ventilatory drive or inspiratory mechanical effort required to wake up an individual in response to the narrowing of the upper airway during sleep (138). Traditionally, respiratory arousals were considered necessary to reinstate upper airway patency after a ventilatory disturbance,

thus a low arousal threshold was long thought to be protective from deep event-related hypoxemia (57, 139). It is now clear that the pharyngeal muscles can overcome respiratory obstruction *without* the onset of an arousal (140, 141), yet the arousal is often concurrent due to similar thresholds for upper airway reopening and arousal inception (142). In addition, respiratory arousals are responsible for increased sympathetic activation at event termination (143, 144). Therefore, it has been postulated that a low arousal threshold is not only non-protective in most cases, but also increases the risk of OSA exacerbation (8, 10) and OSA-related cardiovascular morbidity (145, 146).

Several are the reasons by which a low arousal threshold is responsible for OSA pathogenesis in a third of cases (147) and even more frequently in certain populations (148). First, it produces sleep fragmentation and prevents deep sleep, notoriously protective against respiratory events (149). Second, with an arousal abruptly interrupting a respiratory event, there are less time and respiratory stimuli (e.g. CO_2 levels that "do not have time" to increase enough) for the pharyngeal muscles to be effectively recruited (140); consequences are shorter event duration and more frequent event cycling (150). Third, arousals imply a ventilatory response with quick intrusions of wake drive and subsequent rapid CO_2 swings (see above), which can contribute to further breathing instability and greater OSA severity (151); not surprisingly, shorter event duration is associated with elevated loop gain (150). Fourth, arousals are also characterized by different levels of intensity (140) and greater arousal intensity is accompanied by larger ventilatory responses (152).

Since the respiratory arousal threshold can be quantified as the ventilatory drive needed to awaken, gold-standard measures include esophageal catheterization , or, although less reliable, surrogates such as recording of epiglottic (153) or esophageal pressure (21). Sensitive alternative methods to calculate the arousal threshold have also been introduced using polysomnography (83) or clinical parameters (154).

2.3.5 Assessment of endotypic traits

Gold-standard measurement of pharyngeal collapsibility and other endotypic traits were, in the past years, based on manipulation of CPAP during sleep to assess the critical pressure at which the airway completely collapses (Pcrit) (81). These procedures remained limited to specialized physiology laboratories. An attempt has recently been made to identify simpler ways to measure the entity of pharyngeal collapse (Pcrit) and the

related muscles compensation\responsiveness with the goal to allow a widespread clinical use of these metrics (10, 84, 155, 156). These models are based on the estimation of ventilatory drive during spontaneous breathing and subsequently assess pharyngeal collapsibility, upper airway muscle compensation, arousal threshold and loop gain (9, 10). Pharyngeal collapsibility has been identified as the level of ventilation that can be achieved at eupneic ventilatory drive (Vpassive, the ventilatory equivalent of critical collapsing pressure), where a more collapsible airway is captured by a lower ventilation. Muscle compensation is the increase in ventilation that occurs in conjunction with a rise in ventilatory drive (from eupneic levels to the level that triggers arousal from sleep). Thus, compensation can be taken as the simple difference between Vactive and Vpassive (10, 157), where Vactive is the level of ventilation at maximum drive that can be achieved during sleep (arousal threshold)(82). More recently other polysomnographic surrogate measures of collapsibility have been presented as candidate to clinically quantify Vpassive and responsiveness (158).

2.4 Alternative non-pharmacological intervention to increase pharingeal muscle responsiveness

The decrease in both basal and compensatory dilator muscle activity during sleep is the key factor that determines airway collapse (48, 70). Thus, among the others, muscle responsiveness seems to be the predominant trait responsible for OSA development. Preventing the sleep-related relaxation of upper airway dilator muscles, and in particular the genioglossus recently became a successful strategy to develop alternative treatments for OSA (159)

Hypoglossal nerve stimulation consists in a surgically implanted device, which permits the recruitment of tongue protruding muscles determining an enlargement of the retroglossal and retropalatal airway spaces (160, 161). It is a suitable option to treat OSA in patients who are unable to tolerate CPAP or other appropriate therapy devices (161, 162). New devices acting on the neurostimulation of other upper airway muscles are currently under exploration (163). While it is known that patients with complete concentric collapse do not typically benefit from hypoglossal nerve stimulation, this characteristic is currently assessed during a drug induced sleep endoscopy performed by specialized personnel (164). Indeed, upper airway scans did not demonstrate enough discriminant predictive ability (165). Thus, less invasive and expensive methods are

needed to identify hypoglossal nerve stimulation implant candidates. Moreover, despite careful patient selection, approximately one third of the patients are only partial responders. While older patients tend to benefit the most of this therapy, among the polysomnographic characteristics, AHI does not seem to be associated with response rate to hypoglossal nerve stimulation (166, 167). A recently published analysis of baseline endotypic traits from the STAR (Stimulation Therapy for Apnea Reduction) trial (168) showed that a complete response to therapy was independently associated with higher arousal threshold, greater upper airway muscles compensation and lower loop gain at baseline.

Myofunctional treatment: Orofacial myofunctional treatment consists of isotonic and isometric exercises targeted to oral and oropharyngeal structures, with the aim of increasing muscle tone, endurance, and coordination of pharyngeal and peri-pharyngeal muscles (169, 170). As a result of direct training of the genioglossus and other pharyngeal muscles, myofunctional treatment determine tongue fat and neck circumference reduction(170). Thus, myofunctional therapy helps to reposition the tongue, improve nasal breathing, and increase muscle tone and responsiveness in pediatric and adult OSA patients (169, 170). The improvement in nasal breathing and the consequent reduction in mouth breathing during the night seems to contribute to the reduction of OSA severity and snoring (171). A major problem for myofunctional treatment is the difficulty to measure subjects' adherence. Indeed, patients are normally instructed to perform exercises regularly, often by themselves at home without supervision (172).To date, none of the studies reported in literature on patients with moderate-to-severe OSA demonstrated complete efficacy of myofunctional treatment alone in resolving the disorder. However, the concomitant implication of myofunctional treatment with conventional treatments has showed improvements of success rates and of compliance to therapy (169, 170).

2.5 Biological Rationale for pharmacological intervention on upper airway responsiveness

OSA pathogenesis consists in the interaction between unfavourable upper airway anatomy and sleep-related changes in dilator muscle function. The sleep-related reduction in upper airway muscles activity associated with an inadequate responsiveness of these muscles to increased ventilatory drive and more negative

pharyngeal pressure swings during sleep are the key events triggering the repetitive collapse of the upper airway in OSA (13).

The genioglossus muscle is controlled by the hypoglossal nerve and is the main pharyngeal dilator muscle with a critical role in defending upper airway patency during sleep (142). New research in animals improved understanding of the state-dependent neurotransmitters involved in pharyngeal muscle activation during sleep, namely that both noradrenergic and antimuscarinic processes are involved. Specifically, the loss of noradrenergic activity is now thought to play a key role in the sleep-related hypotonia of pharyngeal muscles during NREM sleep and muscarinic activity is involved in REM atonia.

Chan and colleagues (16) showed in rats that the noradrenergic antagonist terazosin substantially reduced genioglossus (a major muscle of the upper airway) activity (i.e., genioglossal electromyographic activity) during wakefulness and produced REM-like atonia during NREM sleep, illustrating the importance of noradrenergic mechanisms. Other studies (17, 18) also support the notion that progressive withdrawal of noradrenergic tone, from wakefulness to NREM and REM sleep, is the major mechanism causing sleep-related pharyngeal hypotonia. While noradrenergic withdrawal is thought to be the main cause of pharyngeal hypotonia in NREM sleep, there are additional mechanisms that cause further reduction during REM sleep. Chan and colleagues (16) failed to reverse REM atonia with alpha-1 receptor agonists applied to the hypoglossal nucleus, suggesting that another, possibly inhibitory, mechanism is at work. Grace, Horner and colleagues identified this inhibitory process as muscarinic by demonstrating restoration of genioglossal electromyographic activity during REM sleep with the muscarinic antagonist scopolamine applied directly to the hypoglossal nucleus in rats (19, 20).

Recently, it has been shown that a fixed dose drug combination of atomoxetine (noradrenergic agent) and oxybutynin (an antimuscarinic) is effective in short-term OSA treatment (21). Efficacy of this combination for the signs and symptoms of OSA has been demonstrated in a small number of patients in National Institutes of Health-supported studies in the academic setting. In a study on healthy individuals, a combination of reboxetine and hyoscine butylbromide improved upper airway function during non REM sleep (22) and an improvement of OSA severity in patients taking this combination (23). However, hyoscine butylbromide has a low

permeability for the blood-brain barrier compared to oxybutynin (24). As the mechanism of action of this combination is the stimulation of the hypoglossal motor pool in the brainstem, we hypothesize that a combination of reboxetine and oxybutynin may be more efficacious than reboxetine and hyoscine in treating OSA, since it can reach higher concentration in the brainstem. Oxybutynin is an antispasmodic drug that inhibits the muscarinic action of acetylcholine on smooth muscle and is indicated for the treatment of symptoms of bladder instability associated with voiding in patients with uninhibited neurogenic or reflex neurogenic bladder such as urgency, frequency, urinary leakage, urge incontinence and dysuria (173). Reboxetine is a norepinephrine reuptake inhibitor approved in Italy and Europe for the treatment of major depression disturbs (174)

Reboxetine and oxybutynin are approved and have a long history of safe clinical use in Italy. However, due to the only recent identification of these processes, until now there has not yet been an attempt to stimulate the pharyngeal muscles with these drugs in sleeping humans. Reboxetine and oxybutynin as individual drugs have well-established pharmacokinetics, tolerability and safety profiles, but have not previously been studied in combination.

As known, noradrenaline is the main neurotransmitter of the sympathetic nervous system and muscarinic receptors are responsible for vagal activity. Thus, a pending issue, not yet explored, is the possibility that the administration of noradrenergic reboxetine together with antimuscarinic oxybutynin might negatively affect the autonomic control of circulation (24). On the other hand, the substantial decrease of the AHI with the resulting improvement of nocturnal hypoxemia induced by reb-oxy, might be also associated with beneficial effects on sympathetic over-activity, thus counterbalancing the direct sympathetic stimulation of these noradrenergic and antimuscarinic drugs. Thus, in order to assess the safety of reboxetine plus oxybutynin (reb-oxy) on cardiovascular autonomic modulation in OSA patients, we also performed 24h ambulatory blood pressure monitoring and nocturnal heart rate variability (V) after 1-week of treatment in the frame of our randomized controlled cross-over trial.

3 AIMS

Given the role of OSA-related intermittent hypoxia on human health and given the evidence of upper airway muscle responsiveness as the main pathophysiologic trait responsible for OSA development, we designed a trial to evaluate the efficacy of a combination of reboxetine (a noradrenergic) and oxybutynin (an antimuscarinic) (reb-oxy) on the severity of OSA over 1-week of treatment. We hypothesized that reb-oxy would reduce AHI (primary outcome) and oxygen desaturation indices. We investigated the effects of this combination on OSA pathophysiological traits (82, 85), also exploring whether patients' baseline characteristics could predict the treatment success. Moreover, we evaluated the effect of reb-oxy on heart rate and blood pressure parameters.

4 METHODS

4.1 Patients

Both male and female patients between 18 and 70 years of age with a diagnosis of OSA were eligible for study enrollment. Subjects treated with CPAP were included in the study (Table 1) only if they showed poor compliance (use of CPAP less than 4 hours per night for 70% of nights) and they were asked to completely stop the treatment at least 2 weeks prior to the baseline PSG. Exclusion criteria included any clinically significant neurological, psychiatric or cardiovascular disorder, untreated narrow angle glaucoma, hypertension requiring more than 3 drugs to be controlled, use of respiratory stimulants or depressants, hypnotics, central nervous system stimulants or other medicaments known to interact with study drugs, central sleep apnea, pregnancy, history of benign prostatic hyperplasia or urinary retention, which may be exacerbated by antimuscarinic medications.

Participants were enrolled from July 2020 to October 2020 through our sleep clinic (Istituto Auxologico Italiano, Milan, Italy) after a pre-screening evaluation for inclusion and exclusion criteria based on the clinical history. The trial ended when the previously calculated sample size was reached.

The study was approved by the Ethics Committee and by the Italian drug agency AIFA (Agenzia Italiana del Farmaco). Informed consent in writing was obtained from all study participants. The study was registered at ClinicalTrials.gov (NCT04449133).

4.2 Study Design

This was a randomized, double blind, placebo-controlled, cross-over, phase II, single center efficacy study of the combination of reboxetine and oxybutynin in adults with OSA.

Study participants underwent further eligibility screening with a one-night in-lab baseline in-lab PSG (Embla, Reykjavik, Iceland), which served as the baseline for AHI and other PSG endpoints. Participants were eligible for randomization if AHI on baseline PSG was>15 events/hr. Eligible participants were then randomized equally to first receive 4 mg reboxetine plus 5 mg oxybutynin (reb–oxy) or matching placebo (2 capsules). Subjects started taking study drug at home the day after the baseline PSG immediately prior to bedtime for 7 days. A washout of 7-10 days preceded the switch to the other arm of the study. During the entire

at-home period (6 nights on placebo and 6 nights on reb-oxy), the patients underwent full night pulse-oximetry testing (Nonin Medical Inc., 3150, Minnesota, USA). On the final night of dosing for each arm, participants performed an in-lab PSG to evaluate OSA severity. The predefined primary outcome variable was the change in AHI from baseline. Secondary outcomes were: response rate based on ≥50% reduction in AHI; proportion of participants with AHI<15/hour; change in subjective sleepiness with Epworth Sleepiness Scale (ESS), Psychomotor Vigilance Test (PVT), change from baseline in these PSG parameters: Oxygen Desaturation Index (ODI) at 3% threshold and hypoxic burden. Karolinska Sleepiness Scale (KSS), Patient Global Impression of OSA Severity (PGI-S), arousal index, periodic limb movement (PLM) index) and descriptive summary of nightly change with at-home pulse oximetry (ODI 4%) were also assessed. 24h ambulatory blood pressure monitoring and nocturnal heart rate variability (V) was also performed.

4.3 Randomization and blinding

Study medications were prepared by the ST Pharma PRO SRL (Milan, Italy) and were placed in identical capsules that could not be identified by study personnel or participants. Participants were randomly assigned in a 1:1 equal allocation ratio to receive the active treatment dose or placebo first using a blocked randomization (block size of 2). Each participant was assigned a unique number (randomization number) that encoded the participant's assignment to 1 of the 2 arms of the study. The randomization list was produced and validated by a statistician not involved in patient recruitment and external to the hospital. No stratification was expected for any characteristics. Subjects, care providers, investigators, and outcomes assessors were blinded to the treatment allocation (quadruple blinding). Study treatment was dispensed the morning after PSG screening. Once all data analyses were completed and reviewed, the database was locked and the intervention allocations were unblinded for statistical analysis

4.4 Data analyses and measurements of outcomes

Overnight in-lab polysomnography: PSG recordings and scoring were performed in accordance with the American Academy of Sleep Medicine rules (175). All studies were scored by the same specialized sleep

clinician, blinded to treatment assignment (176). AHI, ODI 3%, arousal index, and periodic leg movement index were calculated from the PSG. The OSA specific hypoxic burden (respiratory event–related oxygen desaturation area under pre-event SpO_2 baseline curve, per hour) was also calculated (41, 42). ODI at 4% threshold level (ODI 4%) was collected during at-home pulse oximetry for each night of treatment. Adverse events were recorded at each visit.

Pathophysiological traits causing sleep apnea (endotypes) were estimated during NREM sleep using established automated methods and executed using custom software (Endo-Phenotyping Using Polysomnography; MATLAB, Mathworks, Natick MA) (82, 83, 85). Each trait is defined by spontaneous fluctuations in ventilation (from nasal pressure, mean-normalized) and ventilatory drive (intended ventilation estimated using a chemoreflex model and least-squares regression). Collapsibility was based on the median ventilation during sleep at normal/eupneic ventilatory drive (Vpassive); lower values of Vpassive indicate greater collapsibility. Compensation, the increase in ventilation with rising ventilatory drive, was determined by calculating Vactive (ventilation when ventilatory drive is at the arousal threshold); greater Vactive, for any given Vpassive, reflects greater dilator muscle compensation. Loop gain (LG1, ventilatory control sensitivity) was determined from the ventilatory drive response to reduced ventilation; higher values represent a greater ventilatory control instability. Arousal threshold was measured as the ventilatory drive preceding each scored arousal; low values reflect greater arousability.

Questionnaires and vigilance test: The ESS questionnaire was taken to evaluate subjective somnolence over the preceding week of treatment (177) and the KSS was taken to measure the situational sleepiness in the late afternoon before the in-lab PSG. The PGI-S was used to rate the participants' impression of disease severity. A validated 3-minutes PVT evaluated the sustained-attention and reaction-time by measuring the speed with which subjects responded to a visual stimulus (178). The reaction time, the number of lapses (defined as reaction time > 500 ms, i.e. inability to respond in a timely fashion when a stimulus was present and the reciprocal reaction time as a measure of speed (1/ reaction time) (lapses included) were studied. The above-mentioned evaluations together with respiratory rate, ECG and three measurements of blood pressure, were performed without coffee intake in the previous 3 hours and at the same time of the day before the PSG.

Heart Rate Variability: The beat-by-beat series of R-R intervals were extracted from 1 ECG channel of each PSG through a derivative-and-threshold algorithm, selecting a segment of at least 10-minute duration without respiratory events in the supine position during NREM stage 2 (N2) sleep for heart rate variability analysis. The powers in the very-low-frequency (VLF, between 0.0025 and 0.04 Hz), low-frequency (LF, between 0.04 and 0.15 Hz), and high-frequency (HF, between 0.15 and 0.40 Hz) bands, as well as the LF/HF powers ratio, were obtained by integrating the periodogram. More details on heart rate variability analysis are provided in the online supplemental file.

Ambulatory Blood Pressure Monitoring was performed using a validated oscillometric device (TM2430; A&D Medical, Japan) to evaluate blood pressure changes over 24 hours. The 24h blood pressure monitoring was assessed in the pre-screening visit at least 2 days before baseline PSG and between the 2nd and the 5th night of the two weeks of treatment to avoid disturbing the PSG night sleep.

4.5 Statistical analysis

Individuals were enrolled until 16 completed baseline and both treatment nights. The study was powered to detect an AHI reduction with reboxetine plus oxybutynin (percent reduction from baseline) by 50+/-50 % more than placebo (alpha 5%, power 80%); SD of the effect was estimated from a previous trial (21).

Data are presented as median [interquartile range]. Continuous variables were compared using a two-tailed Wilcoxon matched-pairs signed-rank test. Categorical data were analyzed using Fisher's exact test.

For the endotypic traits, the effect of the reb-oxy combination and placebo vs baseline were modelled by using linear mixed effects models, with treatments as fixed effects and subjects as a random effect. See supplemental material for further details. Effects on Vpassive (collapsibility) were modelled by using a sigmoidal transformation function (slope of 1 at Vpassive = 50%) to handle the known floor and ceiling effects(82) ; changes in collapsibility using our method are only linearly related to underlying collapsibility in the flow-limited range between Vpassive = 0% (apnea) and Vpassive = 100% (open airway). Effects on muscle compensation were estimated by modelling Vactive (same sigmoidal function) while adjusting for Vpassive. Effects on LG and arousal threshold were modelled by using simple linear models.

The effects of placebo and reb-oxy on repeated measures of ODI 4% at home were analyzed using a mixed effects model testing treatment, time, and the interaction between treatment and time as fixed effects and subjects as random effects. Comparisons between ODI on reb-oxy *vs.* placebo at individual time points (days 1-6) were corrected for multiplicity using the Sidak method.

To evaluate the predictors of response to reb-oxy from baseline characteristics, we performed a univariate linear regression analysis including baseline age, body mass index, PSG characteristics (AHI, ODI, fraction of events that were hypopneas, mean desaturation associated with an event) and each endotype as independent variables. The percent change in AHI was the dependent variable. Associations were exploratory and were not adjusted for multiple comparisons.

Regarding ambulatory blood pressure and heart rate variability continuous variables were compared as change from baseline after 1-week of placebo and 1-week of reb-oxy using a two-tailed Wilcoxon matched-pairs signed-rank test.

A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using Graph Pad Prism 6.0 (McKiev Software, Boston, MA) and MATLAB (MathWork).

5 RESULTS

5.1 Subjects

Eighteen subjects were enrolled in the study and performed a baseline PSG night; all individuals were eligible for randomization based on AHI>15 events/hr (Consort diagram in Figure 3). One subject dropped out prior to starting the first treatment period (second wave of Sars-Cov2 disease in Milan; active drug period). One subject dropped out at the end of the first treatment period (also active drug period) as the patient was unable to continue (personal problems).

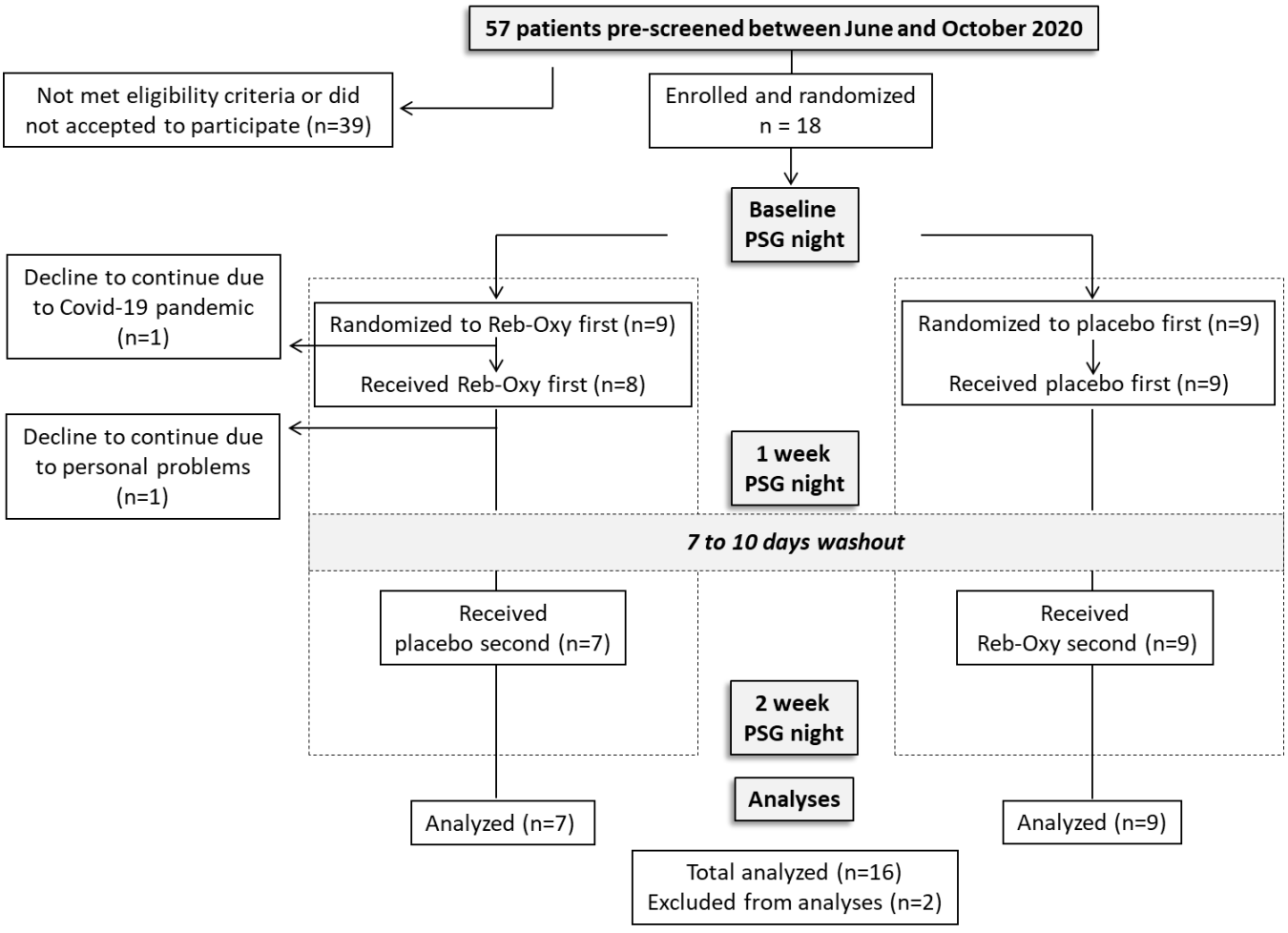

Figure 3*: Consolidated Standards of Reporting Trials diagram of the clinical trial.*

Data from 16 participants were available for analysis of OSA severity at baseline and on both nights after the week of drug or placebo intake. The characteristics of these subjects are shown in Table 1. None had previous history of upper airway surgery.

The results relative to primary and secondary outcomes were upheld when adjusted for sequence and period effects in a linear mixed effect model analysis (see paragraph 4.10). A significant sequence effect was found in the analysis of AHI %reduction. Adjusted results showed a reduction of placebo effect, suggesting a possible mild carry-over effect on placebo when it was administered after reboxetine plus oxybutynin, see the supplement for the detailed model. Secondary outcomes such as hypoxic burden or PVT were not affected by period or sequence.

***Table 1**: General characteristics of the population studied*

CHARACTERISTICS	**VALUES**
Age, years	57 [51-61]
Male, N (%)	14 (87.5)
Height, cm	180 [171-184]
Weight, Kg	94 [77-105]
BMI, Kg/m^2	30 [26-36]
Waist circumference, cm	116 [103-123]
Neck circumference, cm	43 [39-46]
Mallampati score (1 / 2 / 3 / 4)	1 (6.3) / 10 (62.5) / 4 (25) / 1 (6.3)
Tonsils score (1/ 2 / 3 / 4)	15 (93.7) / 1 (6.3) / 0 / 0
Smoke	8 (50)
Previous OSA treatment, n (%)	
C-PAP	5 (31.2)
Comorbidities, N (%)	
Hypertension	7 (44)
Diabetes	1 (6.3)
Dyslipidemia	7 (44)
Hypothyroidism	3 (18.8)

Rheumatoid arthritis	1 (5.6)
Medications, N (%)	
ACE-I/ARB	6 (35)
CCB	1 (6.3)
Diuretics	1 (6.3)
Antilipidemics	4 (25)
Antidiabetics	1 (6.3)
Antithrombotics	2 (12.6)

Definition of abbreviations: BMI = body mass index; OSA = obstructive sleep apnea; CPAP = continuous positive airway pressure; ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CCB = calcium channel blocker Data are expressed as number (%), median [interquartile range] unless otherwise specified

5.2 Effect of reb-oxy on AHI, oxygen saturation and sleep architecture

Reb–oxy reduced the AHI by a median of 26 events/h, or 59% (expressed as the median value of all reductions), compared with baseline and by 20 events/h, or 59% compared to placebo (Table 2; see Figure 4 for individual data). The vast majority of patients (81%) experienced a reduction in AHI > 50% on the treatment night, and 37% of the patients on reb-oxy had an AHI<15. Effects of the intervention on AHI specific to REM and NREM sleep stages, hypoxic burden, ODI, arousal index and sleep architecture are shown in Table 2. Reb-oxy significantly reduced hypoxic burden and ODI ($p<0.001$ and $p=0.021$, respectively). Considering that an hypoxic burden >53%min/h has been previously associated with higher cardiovascular-related mortality (41), reb-oxy reduced the hypoxic burden below this threshold in the 69% of our sample. Individual data on hypoxic burden are reported in Figure 5A and 5B. Reb-oxy significantly reduced the number of arousals compared to baseline and placebo, and sleep architecture was unchanged with the exception of a trend for reduced REM sleep and increased N2 on reb-oxy compared to placebo. No difference in periodic leg movements were observed among the three nights.

***Table 2**: Obstructive Sleep Apnea Severity and Sleep Architecture Baseline, on Placebo and on Drug Combination for All the Participants (n = 16)*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
AHI total, events/h	48.7 [34.8 to 56.6]	38.7 [29.0 to 47.8]	18.0 [12.5 to 21.4]	<0.001
%change from baseline		5.9 [-4.5 to 37.5]	59.2 [53.3 to 68.1]	<0.001
AHI supine, events/h	60.4 [52.7 to 81.9]	56.3 [44.9 to 76.0]	33.7 [25.3 to 48.1]	<0.001
%change from baseline		7.0 [0.4 to 27.2]	51.1 [30.9 to 64.3]	<0.001
Proportions of patients with AHI reduction>50% from baseline		13%	81%	<0.001
Proportion of patients with AHI<15 events/h		6%	37%	0.080
Hypoxic burden, %min/h	90.8 [69.5 to 154]	75.5 [68.1 to 168]	39.7 [25.4 to 55.3]	<0.001
%change from baseline		7.7 [-17.3 to 44.5]	61.5 [38.2 to 72.5]	<0.001
ODI 3%, events/h	42.7 [32.3 to 53.0]	36.8 [23.8 to 43.2]	31.4 [19.1 to 37.7]	0.021
%change from baseline		11.1 [-4.6 to 25.3]	29.0 [13.3 to 42.6]	0.025
ODI 4%, events/h	34.8 [23.9 to 43.9]	30.1 [17.4 to 40.0]	20.1 [13.3 to 28.2]	0.001
%change from baseline		7.7 [-7.7 to 38.2]	38.5 [21.1 to 49.7]	0.016
Arousal index, events/h	30.6 [20.7 to 47.7]	26.6 [14.1 to 34.7]	10.7 [7.6 to 16.8]	0.003
Total Sleep time, min	329 [301 to 368]	323 [274 to 351]	322 [283 to 363]	0.376
Sleep efficiency, %TIB	71.2 [59.9 to 76.2]	71.7 [60.8 to 83.5]	69.7 [64.0 to 73.3]	0.504
N1, %TST	3.7 [2.4 to 7.3]	3.5 [2.8 to 4.5]	5.4 [2.7 to 9.9]	0.102
N2, %TST	63.5 [55.3 to 68.1]	62.9 [58.5 to 68.7]	68.0 [58.4 to 75.8]	0.051
N3, %TST	16.2 [10.9 to 22.1]	17.4 [9.5 to 26.3]	15.9 [6.8 to 23.0]	0.117
REM, %TST	18.1 [13.8 to 21.4]	16.2 [13.2 to 17.9]	10.2 [5.1 to 15.5]	0.057
PLM index, events/h	0.0 [0.0 to 2.8]	0.0 [0.0 to 2.8]	0.5 [0.0 to 2.8]	0.457
Heart Rate, bpm	78 [71 to 90]	82 [72 to 93]	79 [69 to 87]	0.700
Systolic blood pressure, mmHg	133 [124 to 145]	126 [118 to 135]	120 [115 to 138]	0.234
Diastolic blood pressure, mmHg	82 [75 to 89]	84 [75 to 92]	80 [73 to 88]	0.065

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; AHI = apnea–hypopnea index; ODI = oxygen desaturation index; TIB = time in bed; N1-2-3 = non-REM stage 1-2-3; TST = total sleep time; REM = rapid eye movements sleep; PLM = periodic legs movements. Data are presented as median (interquartile range). % changes are expressed as the median of the group percentage change. P values compare placebo versus reb-oxy.

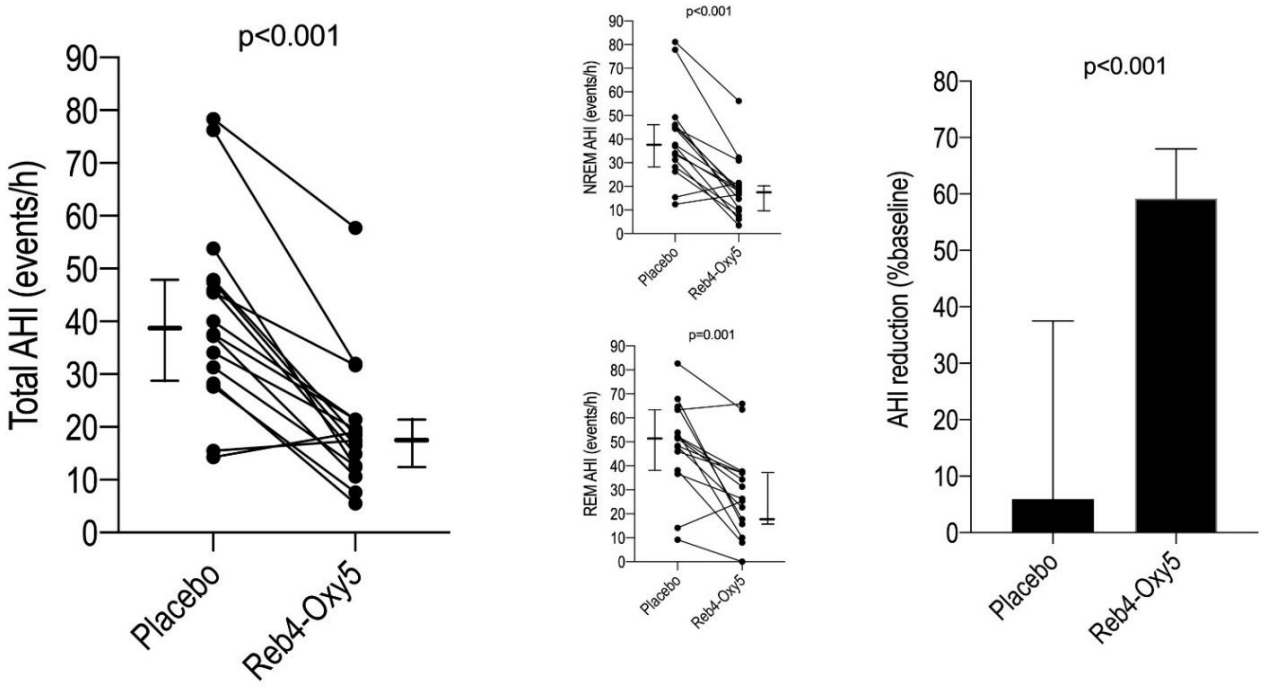

Figure 4: *Individual data showing the effect of reboxetine plus oxybutynin (reb–oxy) on (A) total apnea–hypopnea index (AHI), during NREM (B) or REM (C) sleep stages. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles. (D) Group data showing percentage of apnea–hypopnea index (AHI) changes from baseline on placebo and on reb-oxy.*

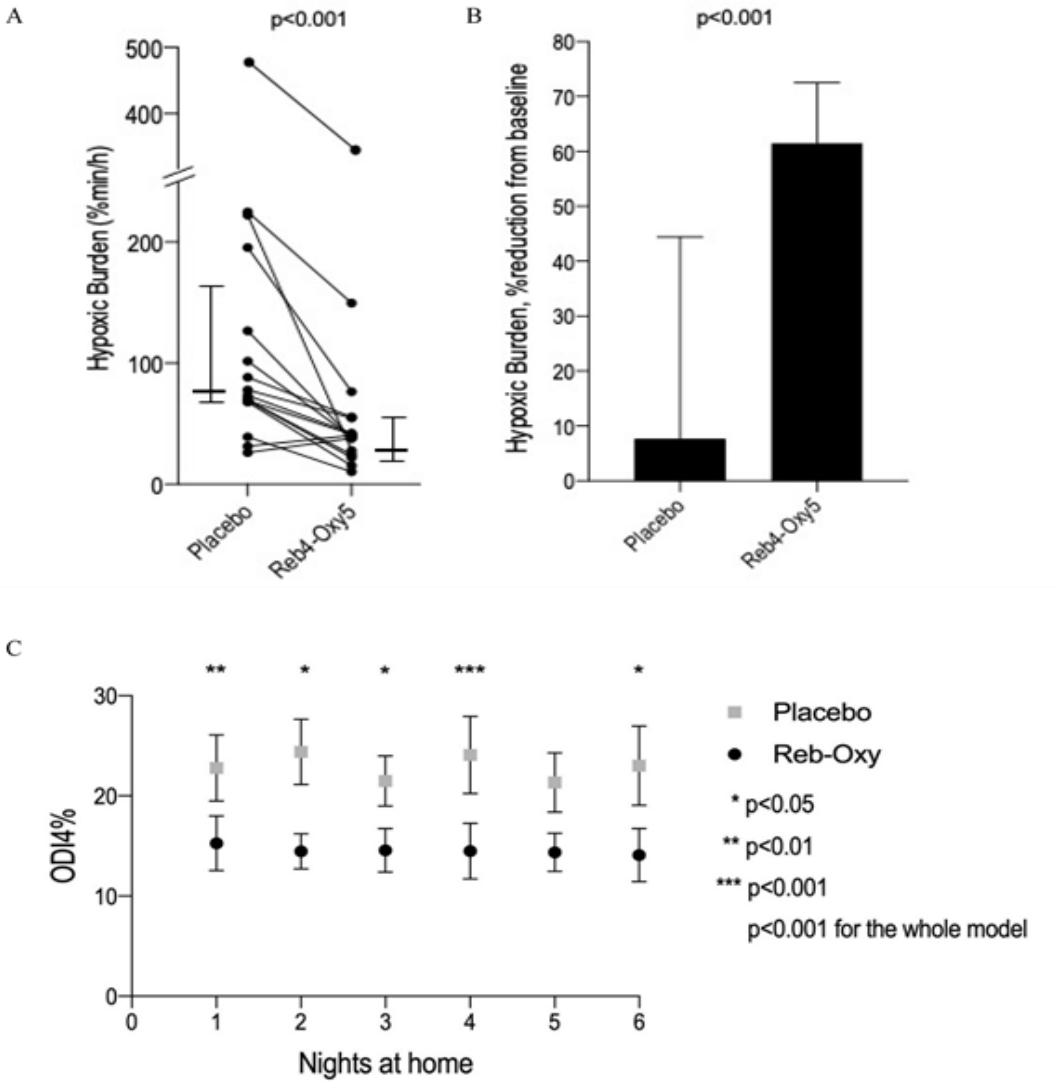

Figure 5*: Effect of reboxetine plus oxybutynin (reb–oxy) on desaturation index: (A) hypoxic burden as individual data. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles. (B) Group data showing percentage of hypoxic burden changes from baseline on placebo and on reb-oxy are shown in panel. (C) Analysis of repeated measures of ODI 4% obtained during at-home pulse oximetry during placebo (grey squares) and during reboxetine plus oxybutynin (reb-oxy) weeks (black dots). Data were compared using a mixed effect model including treatment, time and time x treatment interaction as fixed effects and subjects as a random effect. Only treatment effect was significantly associated with ODI4% (dependent variable). P value for day-by-day multiple comparison between placebo and reb-oxy arms are adjusted using Sidak method.*

5.3 Effect of reb-oxy on ODI at home

ODI 4% obtained during at-home pulse oximetry was collected on average (SD) 5.7 (0.8) nights on reb-oxy and 5.4 (1.0) nights on placebo. Group results are shown in Figure 3C for each night. In the mixed effects model, only treatment (reb-oxy vs placebo) was associated with a significant change in ODI 4% ($p<0.001$), while there were no effects related to time or to the interaction between time and treatment.

5.4 Effect of reb-oxy on subjective questionnaires and vigilance

Reb-oxy did not significantly improve subjective indices related to sleepiness, impression of disease severity or vigilance when considering group data (Table 3).

***Table 3**: Results of questionnaires regarding subjective indices related to sleepiness and impression of disease severity and objective vigilance test (n = 16).*

	Baseline	**Placebo**	**Reb-oxy**	**p-value**
ESS	5.0 [4.3 to 9.3]	5.0 [3.0 to 6.0]	5.0 [3.0 to 7.5]	0.75
%change from baseline		0 [-15 to 30]	25 [-10 to 42]	0.45
KSS	2.0 [1.0 to 2.8]	1.5 [1.0 to 3.0]	2.0 [1.0 to 2.8]	0.53
%change from baseline		0 [-75 to 25]	0 [-100 to 54]	0.75
PGI-S	7.0 [4.0 to 8.0]	4.0 [3.0 to 7.8]	3.5 [2.3 to 6.5]	0.184
%change from baseline		0 [-7 to 33]	21 [-14 to 56]	0.59
PVT, reaction time, msec	250 [239 to 312]	264 [217 to 284]	223 [172 to 244]	<0.001
%change from baseline		5 [-7 to 11]	19 [6 to 30]	0.02
PVT, lapses	2 (1.0%)	0	3 (1.6%)	0.33
PVT, 1/RT	4.0 [3.33 to 4.17]	3.8 [3.5 to 4.5]	4.5 [4.1 to 5.7]	0.02

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; ESS = Epworth Sleepiness Scale; KSS = Karolinska Sleepiness Scale; PGI-S = Patient Global Impression of OSA; RT: reaction time; Severity; PVT = Psychomotor Vigilance Test. Data are presented as median [interquartile range]. % changes are expressed as the median of the group percentage change. P values compare placebo versus reb-oxy.

Regarding subjective sleepiness, 4/5 patients with ESS>6 at baseline experienced improvement in the score from 11 [3 to 12.5] to 6 [1.5 to 6.5], although this did not reach statistical significance (p=0.19). PGI-S improved on reb-oxy compared to baseline, but again this difference did not reach statistical significance (p=0.087). Despite KSS revealing no change in subjective alertness between treatments, PVT as response time and 1\response time performance significantly improved on reb-oxy compared to placebo, as shown in Figure 6.

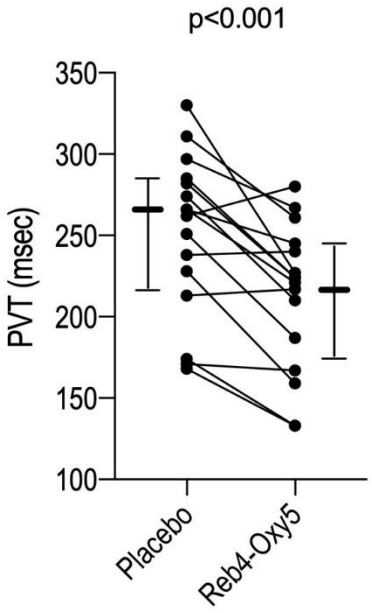

Figure 6: *Effect of reboxetine plus oxybutynin (reb–oxy) on Psychomotor Vigilance Test (PVT) reaction time. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles.*

5.5 Effect of reb-oxy on pathophysiological traits

Group data from the mixed effects model of endotypic traits at baseline, on placebo, and on reb-oxy are shown in Table 4. Compared to placebo, reb-oxy increased muscle compensation by 30% of normal/eupneic ventilatory drive (eupnea), supporting the effect of this combination on upper airway muscle responsiveness. However, reb-oxy reduced the arousal threshold by 27% of eupnea, i.e. patients woke more easily on active treatment. Vactive was increased on reb-oxy by 20% of eupnea compared to baseline but not compared to placebo. No changes were found in loop gain (i.e. ventilatory control sensitivity) and Vpassive (i.e. passive pharyngeal tissue collapsibility).

Table 4*: Mixed Effects Model for Effect of Reboxetine plus Oxybutynin vs Placebo on, Vpassive, Vactive, Muscle Compensation, Arousal Threshold, and Loop Gain During NREM Sleep*

Variable	**Vpassive (%eupnea)**	**Vactive (%eupnea)**	**Muscle Compensation (%eupnea)**	**Arousal threshold (%eupnea)**	**Loop gain (unitless)**
Intercept (Baseline)	82 [58 to 106]	76 [46 to 107]	-57 [-115 to 1]	139 [107 to 171]	0.60 [0.47 to 0.74]
Placebo vs baseline	+6 [-12 to 23] P=0.52	+20 [0 to 41] P=0.049	+11 [-6 to 27] P=0.198	+5 [-16 to 25] P=0.645	-0.01 [-0.13 to 0.11] P=0.879
Reb-oxy vs baseline	+17 [-4 to 38] P=0.11	+35 [10 to 60] P=0.007	+40 [17 to 63] P<0.001	-23 [-43 to -2] P=0.033	-0.09 [-0.21 to 0.03] P=0.15
Reb-oxy vs placebo	+11 [-9 to 32] P=0.259	+15 [-9 to 39] P=0.219	+30 [7 to 53] P=0.012	-27 [-48 to -7] P=0.01	-0.08 [-0.21 to 0.04] P=0.192

Data are presented as mean [95%CI]. Values for Vpassive do not represent observed data but rather the underlying collapsibility derived from a sigmoidal transformation function, to handle the ceiling effects previously described for these types of data[16]. Values for Muscle Compensation were calculated from Vactive adjusting for Vpassive such that the effect shown is the additional effect on ventilation above Vpassive (thus representing pharyngeal compensation).

5.6 Predictors from patients' baseline characteristics

We found an inverse relationship between the change in AHI and baseline mean desaturation, expressed as the average difference between the highest and lowest saturation value during respiratory events; the lower the desaturation, the higher the AHI reduction, r=-0.68, p=0.004. It was also found that the lower the arousal threshold, the higher the AHI reduction, r=-0.56, p=0.024. There was also a direct relationship between baseline Vpassive and AHI reduction: the higher the Vpassive (better airway anatomy), the greater the AHI reduction, r=0.5, p=0.047.

5.7 Linear mixed model effect on primary outcome

Table 5 and 6 shown the **l**inear mixed effect model for AHI. The significant sequence effect suggests that there was a trend for an increased %reduction in AHI from baseline on placebo when the active treatment was administered first (sequence 1, carry-over effect). In order to explore this possibility, we analyzed the patients separately, based on treatment sequence. Figure 7 shows a significant difference between placebo and active treatment in the %reduction of AHI after dividing the patients according to treatment sequence (top graphs). Bottom graphs show a significant increase in %reduction from baseline on the placebo arm on sequence 1 vs sequence 0, but no significant difference between sequences in the reb-oxy arm. No period or sequence effects were found in the analysis of the other outcomes.

Table 5. *Linear mixed effect model for apnea hypopnea index (AHI, percent reduction from baseline)*

Fixed effects:	**Mean [95% CI]**	**P-value**
Placebo	11% [-2 to 24]	0.09
Reb-Oxy (*Change from Placebo)*	+48% [31.5 to 64]	<0.001

Table 6. *Linear mixed effect model for apnea hypopnea index (AHI, percent reduction from baseline) accounting for sequence and period*

Fixed effects:	**Mean [95% CI]**	**P-value**
Placebo	1% [-12 to 14]	0.87
Reb-Oxy (*Change from Placebo)*	+46.5% [31 to 62]	<0.001
Period	+10% [-6.5 to 25.5]	0.2
Sequence	+25% [8 to 41]	0.005

Periods in the model were represented by the following values: -0.5 for Visit 1, 0.5 for Visit 2. Sequences in the model were represented by the following values 0: Placebo first, Reb-oxy second; 1: Reb-oxy first, placebo second.

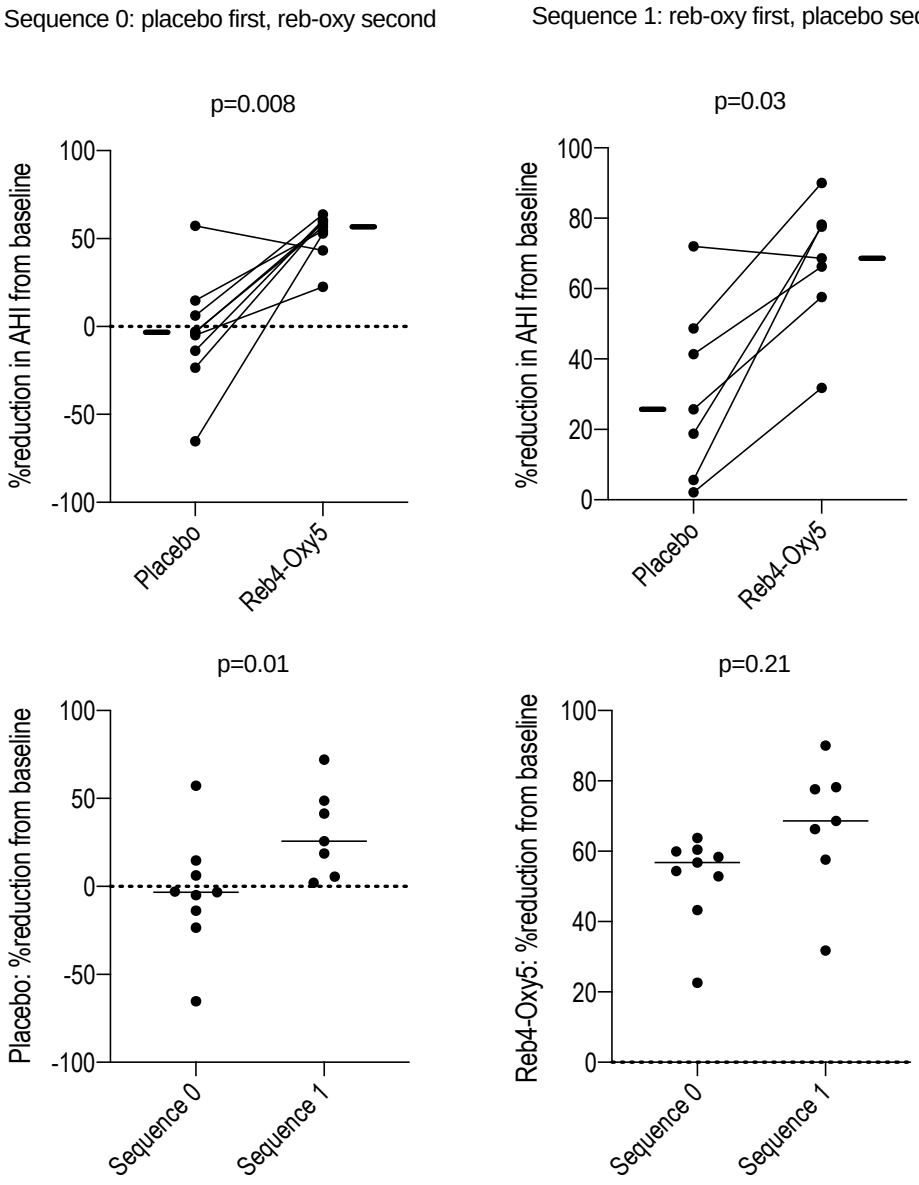

Figure 7. *Lines indicate medians; sequence 0: placebo first, then reb4-oxy5; sequence 1: reb4-oxy5 first, then placebo.*

5.8 Heart rate and blood pressure

Heart rate during the PSG increased from 65 [60-69] bpm at baseline to 69 [64-77] bpm on reb-oxy and to 66 [59-70] bpm on placebo (p=0.02) (Table 7). However, 24h heart rate measured during the 24h blood pressure evaluation was not different among treatment groups as shown in Table 7.

Reb-oxy did not significantly modify 24 h, daytime and night-time diastolic and systolic blood pressure (Table 8). Morning surge was not increased in reb-oxy versus placebo and blood pressure variability did not change during the day and the night between groups.

Neither in the time domain nor in the frequency one, reb-oxy administration was associated with any modification in heart rate variability (Table 7).

Table 7*: Obstructive Sleep Apnea Severity, main sleep characteristics and heart rate variability at baseline, on placebo and on drug combination (n = 16). Heart rate variability data calculated from nocturnal PSG with 1 channel ECG during N2 sleep at baseline, on placebo and on drug combination. P-values are calculated as the percentage change from baseline in placebo versus reb-oxy.*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
HR during full night PSG, bpm	65 [59.5 - 69]	65.6 [58.8 - 69.55]	69.35 [63.8 - 76.75]	0.02
RMSSD, ms^2	40.4 [17.5-51.6]	24.7 [17.6-43.1]	32.4 [25.4-51.6]	0.38
pNN50, ms^2	0.183 [0.01-0.32]	0.02 [0.001-0.18]	0.10 [0.03-0.35]	0.40
HF, ms^2	300.0 [90.7-748.4]	189.7 [85.6-527.2]	328.8 [181.5-887.9]	0.53
LF, ms^2	0.7 [0.4-0.8]	0.6 [0.5-0.7]	0.5 [0.4-0.6]	0.38
LF/HF, ms^2	2.4 [0.9-3.9]	1.3 [0.9-2.4]	1.1 [0.6-1.7]	0.46
VLF, ms^2	680.6 [341.1-2879.1]	504.4 [232.3-2095.3]	659.4 [434.4-885.6]	0.25

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; HR = heart rate; SD = standard deviation; RMSSD = Root Mean Square of the Successive Differences; pNN50 = the proportion of number of pairs of successive NN (R-R) intervals that differ by more than 50 ms; HF = high frequency; LF = low frequency; VLF = very low frequency. Data are presented as median (1^{st}-3^{rd} quartiles). % changes are expressed as the median of the group percentage change. P values compare placebo versus reb-oxy.

Table 8*: Ambulatory blood pressure data at baseline, on placebo and on drug combination (n = 16). P-values are calculated as the percentage change from baseline in placebo versus reb-oxy.*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
DBP 24h, mmHg	83.0 [76.3-87.8]	83.0 [75.3-85.7]	79.6 [75.6-89.5]	0.68
SBP 24h, mmHg	131.6 [122.1-140.3]	129.5 [121.0-133.1]	121.5 [115.4-140.9]	0.72
Day-time DBP, mmHg	86.8 [82.2-92.6]	85.78 [80.4-88.3]	82.18 [78.6-94.7]	0.86
Day-time SBP, mmHg	138.7 [129.9-145.1]	133.0 [125.1-138.1]	125.0 [117.3-144.3]	0.46
Nocturnal DBP, mmHg	71.8 [65.9-76.0]	70.7 [67.1-76.5]	69 [65.6-76.5]	0.50
Nocturnal SBP, mmHg	112.3 [106.1-124.2]	118.5 [108.6-126.2]	110.1 [107.6-132.3]	0.28
ABPM 24h HR, bpm	78.0 [71.7-90.4]	80.2 [71.5-87.6]	79.1 [69-86.6]	0.60
DBP Morning surge	20.0 [15.5-40.0]	15 [6.5-34.5]	20 [10-33]	0.60
SBP Morning Surge	48.0 [31.5-87.5]	33 [23.5-45.5]	32.0 [25.5-39.5]	0.38
DBP variability day	18.1 [11.5-20.6]	15.7 [12.7-18.3]	15.7 [11.7-19.1]	0.90
SBP variability day	17.9 [13.9-24.9]	16.9 [12.2-21.1]	17.2 [15.7-19.8]	0.79
DBP variability night	9.9 [7.9-13.2]	10.6 [8.4-13.7]	9.6 [9.3-10.9]	0.23
SBP variability night	12.1 [10.4-13.8]	13.2 [11.2-17.3]	12.3 [9.1-16.6]	0.08

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; DBP = diastolic blood pressure; SBP = systolic blood pressure; ABPM = ambulatory blood pressure monitoring.

5.9 Side Effects

The following side effects were reported during the study on the reb–oxy night: urinary hesitation (difficulties in initiating micturition in the morning n=7 males); dry mouth during the night and in the morning (n=10); sexual dysfunction (erectile dysfunction in the morning or decreased libido n=3 males); brief sensation of palpitation (n=1) and insomnia symptoms (difficulty initiating and maintaining sleep; n=1). On placebo, chest pain (n=1) and side pain (n=1) were observed. No participants experienced severe side effects or severe adverse events in either arm. No differences were found in terms of resting blood pressure, heart rate, or EKG among the visits. Side effects on reb-oxy and placebo are presented in Table 9.

***Table 9**: Adverse Events (AE) during the week on placebo and the week on reboxetine plus oxybutynin (reb-oxy). None reported severe AE. Comparisons were performed using a Chi-squared test (n = 16).*

	Placebo	**Reb-oxy**	**p-value**
Dry mouth	0	10	<0.01
Urinary hesitation	0	7	0.03
Sexual disfunction	0	3	0.69
Palpitation	0	1	0.31
Insomnia	0	1	0.31
Chest pain	1	0	0.31
Side pain	1	0	0.31
Headache	1	0	0.31
Cramps	1	0	0.31
Total n of patients reporting AE:	2	13	<0.01

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin;

Data are presented as number (percentage).

6 DISCUSSION

OSA is an underdiagnosed disease that determines intermittent hypoxia, sleep fragmentation and that deranges the sympathetic nervous system. Intermittent hypoxia might superimpose other respiratory conditions, worsening the hypoxic desaturation parameters and aggravating the outcome (2, 3). The definition of the exact hypoxic burden related to OSA has been recently recognize as even more relevant than the number of apneas and hypopneas in predicting cardiac outcomes (41). A prompt and accurate diagnosis is needed to prevent OSA consequences and to permit a precise quantification of OSA related desaturation severity parameters. Moreover, recent understanding of endotypic and phenotypic traits has permitted to define different subgroup of patients that may benefit from specific treatments, paving the way for a precision medicine approach for OSA (6, 80, 82, 84, 179)

After the recognition that muscle responsiveness is probably the most relevant pathophysiological trait responsible for OSA, many steps have been made to develop a pharmacological approach (159).

Our study provides experimental evidence that reboxetine plus oxybutynin administered before bedtime substantially reduces OSA severity (AHI) after 1-week of treatment. In addition to the AHI reduction, reb-oxy also exerted a significant effect on indices of hypoxemia, such as ODI and hypoxic burden. Reb-oxy also improved the performance on the vigilance testing. OSA alleviation was likely mediated by improved upper airway muscle activity and responsiveness, as suggested by the ~30% increase in muscle compensation on the drugs. Home pulse-oximetry recordings showed that reb-oxy was effective at improving nocturnal oxygen saturation as early as the first day of treatment, likely due to reduced OSA severity, and its efficacy was maintained through the 7^{th} day, as shown in the in-lab PSG. Moreover, this treatment was not associated with autonomic dysregulation, as the tested drug combination did not induce sympathetic overactivity as it could be expected by the reboxetine mechanism of action. Thus, one-week administration of reboxetine plus oxybutynin did not increase blood pressure during the day or the night and did not enhance cardiac sympathetic modulation as reflected by nighttime heart rate variability.

Reboxetine is highly selective for norepinephrine transporters and has low affinity with muscarinic, histamine-H1, and adrenergic α1 receptors (180). Reboxetine is prescribed for major depressive diseases,

dysthymia, and attention-deficit/hyperactivity disorder, its antidepressant effects being related to the sustained increase in norepinephrine levels in the central nervous system (181, 182). As a noradrenergic drug, reboxetine would be expected to increase sympathetic activity. Oxybutynin is an antispasmodic drug that inhibits the muscarinic action of acetylcholine on smooth muscle and is indicated for the treatment of symptoms of bladder instability in patients with neurogenic bladder.

The first study that demonstrated a great efficacy of pharmacological therapy on OSA severity was published in 2018 by Taranto-Montemurro and coauthors, who evaluated a combination of atomoxetine 80 mg and oxybutynin 5 mg (ato-oxy) in a single-night study (21). The ato-oxy combination reduced the AHI from 31/h on placebo to 8/h on active treatment ($p<0.0001$) by ~63% vs placebo in an unselected group of 20 patients over a single night (21). At the end of the trial, nine patients came back to perform additional sleep studies where the single agents (atomoxetine or oxybutynin) were administered in random order. Despite the drugs taken alone did not reduce the AHI compared to placebo, an accurate analysis of the endotypic traits (183) showed that atomoxetine, at the dose of 80mg improved pharyngeal collapsibility more than oxybutynin 5 mg, which, in its turn, showed mildly sedative properties offsetting the stimulant action of atomoxetine. The same therapy was administered to 6 patients for 1 week in an open label pilot trial: the consistent reduction of AHI on atomoxetine plus oxybutynin vs baseline suggested that the effect of these drugs could last beyond the first night (21).

Subsequently, Thomson et al. in Melbourne investigated if atomoxetine could be substituted with drugs with mixed profiles including both noradrenergic and serotonergic properties such as milnacipran and duloxetine (184), combined with oxybutynin. The trial compared the combinations to placebo and to oxybutynin taken alone over 1 night of treatment. Only the combination of duloxetine and oxybutynin improved upper airway collapsibility and reduced AHI in supine position compared to placebo, however its effect size appeared less promising compared to ato-oxy.

On the other hand, Aishah et al. in Sydney investigated the effects of combining atomoxetine with two antimuscarinics more selective than oxybutynin (185), which has broad affinity for all muscarinic receptor subtypes (M1-M5): solifenacin (M3 and M2 specific) and biperiden (M1 specific). The trial was a 3-arm

randomized crossover study in which 10 patients performed 3 in-lab PSGs one week apart on solifenacin 5mg or biperiden 1 mg plus atomoxetine 80mg or placebo. The new combinations had more modest effects on OSA severity and upper airway collapsibility compared to the combinations of ato-oxy in Taranto's trial or reb-oxy in ours. These findings were confirmed also by studies testing fesoterodine associated with atomoxetine (186, 187) and hyoscine butylbromide associated with reboxetine (23). Atomoxetine plus fesoterodine showed good efficacy in a subset of patients with mild upper airway collapsibility (6 out of 12), but overall there was no consistent reduction in AHI vs placebo in the 12 patients who took part to the trial (187).

Lim and coauthors demonstrated that reboxetine plus hyoscine butylbromide during sleep increased the activity of the tensor palatini muscle, a representative tonic upper airway muscle, and improved upper airway resistance in 10 healthy subjects (22). This combination of drugs also reduced REM sleep and increased N2 sleep compared to placebo, with no effect on sleep efficiency. Although we observed the same trend of REM reduction and increased N2 sleep, the differences in sleep structure between the nights of placebo and reb-oxy in our study were not significant. Due to the presence of moderate-to-severe OSA, our population probably had an altered sleep quality already, which was not further affected by drug intake. Moreover, we administered the medications for 7 days rather than 1 day, and it was previously shown that the negative effects of the noradrenergic reboxetine alone on sleep quality tend to disappear with prolonged therapy (188).

The same research group also evaluated the combined effects of reboxetine plus hyoscine butylbromide in a randomized controlled trial on 12 OSA subjects(23). The combination of these drugs reduced the AHI by approximately 35% compared to placebo.

Notably, all the antimuscarinics tested, except for oxybutynin, share a limited permeability to the blood brain barrier, which could partly explain their reduced efficacy when combined with norepinephrine reuptake inhibitors (24).

In our study, we performed a baseline PSG on the night before starting the drugs and tested again after 1 week of administration, rather than only acutely as performed in the previous described studies. Therefore, our study extends knowledge by providing evidence for effectiveness of the combination of noradrenergic and antimuscarinic agents administered over a full week. Moreover, we also evaluated subjective and objective

responses to the drugs administered. Although patients did not report subjective improvement in sleepiness as a group, patients with higher sleepiness, as detected by the Epworth Sleepiness scale at baseline, showed a clinically meaningful magnitude of improvement with the combination compared to placebo, and there was objective evidence of improvement in vigilance after 1 week of treatment with reb-oxy.

Regarding pathophysiological traits, our finding that reb-oxy improved muscle compensation (40% eupnea improvement on drugs vs baseline and 30% vs placebo) is consistent with previous single night effects of ato-oxy (improved compensation by approximately 29% eupnea). The reduction in arousal threshold we observed also support previous results; a lower arousal threshold may indicate a wake promoting effect of the noradrenergic agent, but alternatively may be an indirect consequence of lowered OSA improved nocturnal airflow.

Consistent with the atomoxetine and oxybutynin findings (21, 183), also the combination of reboxetine and hyoscine butylbromide reduced respiratory control instability (loop gain) by ~10–15% (189). Reductions in loop gain of this magnitude are less than other more direct interventions such as acetazolamide whereby 500 mg, twice daily over a week, reduces loop gain by ~40% (190), or more recently sulthiame (78). These improvements in loop gain with reb-oxy would be expected, at least in part, to contribute to the observed reductions in OSA severity. Like atomoxetine, loop gain reductions would be expected to be due to the noradrenergic agent reboxetine, rather than via an antimuscarinic effect (183). In addition, reboxetine and hyoscine butylbromide reduced the ventilatory response to arousal to a similar magnitude to that of 1 week of acetazolamide (500 mg twice daily) (74). Given the role that increased ventilatory responses to arousal can have on OSA pathogenesis, attenuation of this mechanism may also contribute to breathing stability and reductions in OSA severity (151). Similar to loop gain reductions, this effect is likely to be mediated via the noradrenergic properties of reboxetine. Indeed, 2.5 mg/kg of reboxetine administered in anaesthetised rats decreases the firing rate of noradrenergic neurons in the locus coeruleus by 66% (191). Most noradrenergic projections within the brain originate in the locus coeruleus (a major wake promoting nucleus), which provides input to multiple brain regions including the respiratory centres; more than 80% of these neurons increase their activity to CO_2 (192). Lesions to noradrenergic neurons in the locus coeruleus decrease the hypercapnic

ventilatory response during wakefulness and sleep (193). Thus, these findings indicate that noradrenergic neurons in the locus coeruleus are important modulating chemosensitive ventilatory responses. Indeed, in rat models, sustained noradrenergic reuptake blockade with reboxetine leads to major reductions noradrenergic firing activity, which may attenuate disproportionate ventilatory responses to fluctuations in CO_2 in people with OSA (191). Thus, reboxetine may stabilise breathing, contributing to therapeutic benefit in OSA via reductions in controller gain to reduce high loop gain and attenuate heightened ventilatory responses to arousal. Unlike ato-oxy and reboxetine-hyoscine, a reduction in loop gain was not observed in the current study. The reason may be due to a potential excessive effect on loop gain during the first night of treatment, or the different population we studied.

Regarding predictors, responders to ato-oxy(194) exhibited several signs of reduced collapsibility (lower AHI, higher Vpassive, and higher fraction of hypopneas over total events). Likewise, in the current study, several surrogates of milder collapsibility (higher Vpassive, lower arousal threshold, lesser mean desaturation) were associated with greater responses to reb-oxy, confirming the notion that pharmacological therapy for OSA may be most efficacious in patients with less severe pharyngeal compromise. The similar findings in the ato-oxy and the current reb-oxy studies is not surprising, since both norepinephrine reuptake inhibitors have a comparable receptor affinity profile, with some differences being that reboxetine has a longer plasma half-life than atomoxetine (~12 vs ~5 h, respectively) and that reboxetine's major path of elimination is CYP-3A4 P-450 isozymes(195), whereas atomoxetine's is CYP-2D6. It is therefore unlikely that reboxetine causes clinically significant interactions common to other antidepressants (196).

More recently, Altree and colleagues performed a randomized controlled trial to evaluate the efficacy of reboxetine alone and reboxetine plus oxybutynin (197) on OSA severity. A single 4 mg dose of reboxetine alone prior to sleep modestly reduced the AHI by 5.4 events/h [95% CI, -10.4 to -0.3], p=0.04, compared to placebo, while reboxetine plus oxybutynin compared to placebo did not significantly reduced OSA severity (4.2 events/h of difference [95% CI, -9.6 to 1.1]; p=0.11. Reboxetine as a single agent or when combined with oxybutynin also improves overnight oxygenation and snoring indices. These effects appear to be mediated largely through improvements in ventilatory control stability, as reboxetine and reboxetine-oxybutynin both

reduced loop gain and the ventilatory response to arousal versus placebo. Reb-oxy also largely improved muscle responsiveness as expected. Reboxetine with and without oxybutynin markedly reduces REM sleep which was replaced with stage 2 sleep without altering sleep efficiency. The differences between the results of Altree's study and the present study might be mostly due to patient's selection: first a presence of a less severe OSA, as participants in the Altree's study had higher overall sleep efficiency, proportionally more slow wave sleep and spent less time supine. In addition to the ~20 events/h lower baseline AHI in the Australian study compared to our, respiratory events were predominantly hypopnea driven and associated with cortical arousals rather than marked hypoxemia. Given the potential wake promoting effects of noradrenergic agents, these drugs may be less effective at resolving respiratory events purely associated with arousals versus more severe events associated with hypoxemia. Indeed, noradrenergic agents appear particularly effective at improving hypoxic burden, which was higher in our study compared to the one's from the Australian research team(197).

Concerning safety, similar studies using reboxetine plus hyoscine butylbromide (22), atomoxetine plus oxybutynin (21) and reboxetine plus oxybutynin (197) did not show major sides effects. Accordingly, we did not observe any severe major adverse effects, even after 7 days of reb-oxy administration.

As a noradrenergic drug, reboxetine would be expected to increase sympathetic activity. In fact, in previous studies on healthy volunteers, 4 mg of reboxetine has been associated with an increase in heart rate (198, 199). Oxybutynin determines inhibition of vagal activity on muscarinic cardiac receptors with an increase in heart rate (200). The single-night administration of atomoxetine plus oxybutynin in OSA patients led to a small (2.6 bpm) overnight increase in heart rate (21). Reboxetine plus oxybutynine and reboxetine alone significantly increased heart rate in a recent trial (83 ± 14 versus 69 ± 11) (197).

In our study, heart rate during the night only slightly increased (by 3.7 bpm) on reb-oxy versus placebo during the night. Moreover, the 24h heart rate evaluated during the ambulatory blood pressure monitoring was similar in the two groups. Previous studies in healthy subjects reported an increase in systolic and diastolic blood pressure after reboxetine administration (198, 199, 201), whereas neutral effects on blood pressure have been reported in long-term treatment with reboxetine (182, 202, 203). No differences in systolic and diastolic blood pressure were observed after the administration of reb-oxy in a recent trial (197). Accordingly, our

patients did not exhibit blood pressure's changes between placebo, confirming the neutral effect previously reported even in a population of OSA patients. Morning surge and blood pressure variability, additional markers of sympathetic activity, did not increase during reb-oxy administration, which, again, represents a reassuring clinical result considering the administration of a noradrenergic drug.

Heart rate variability and blood pressure evaluation are non-invasive reliable tools to assess sympathetic and parasympathetic cardiovascular modulation (204, 205). Using heart rate variability analysis, OSA patients were shown to be characterized by a predominance of sympathetic markers and by a reduction in parasympathetic indices (206). This is in line with the demonstration that sympathetic overactivity is a key mediator of adverse cardiovascular consequences in OSA (56). In fact, autonomic derangement and sympathovagal imbalance are associated with arrhythmias, which are often observed in patients with OSA, contributing to their cardiovascular mortality (207). Previous studies showed that reboxetine influences the autonomic nervous system by reducing the HF power of heart rate variability in healthy volunteers (199). Oxybutynin, in turn, showed an anti-cholinergic effect according to heart rate variability indexes (200). By contrast, our study points out that in OSA patients one week of reb-oxy did not increase sympathetic activity, as shown by no changes in a spectral index of sympathovagal balance, i.e., the LF/HF heart rate power ratio, and had no significant influence on indexes of parasympathetic cardiac control: RMSSD, pNN50, and the HF power of heart rate.

The absence of an expected increase in heart rate and blood pressure we observed, is likely to represent a positive consequence of nocturnal apneas reduction, as previously reported after long-term OSA treatment with CPAP (208). Thus, similarly to what happens with CPAP treatment, it might be supposed that the beneficial effect of drug combination on the severity of OSA would have mitigated the sympathetic hyperactivity induced by OSA events (208, 209). The reduction of the hypoxic burden, together with a reduced frequency of arousals from sleep determined by reb-oxy, might have determined the beneficial effect on the apnea-hypopnea-related sympathetic overflow (41, 210).

7 CONCLUSIONS

The hypoxic burden of OSA has been identified as responsible for the cardiovascular negative outcome of the disease, more than the number of episodes of apneas and hypopneas (41). Beside determining intermittent hypoxia per se, OSA also worsens the tonic hypoxia caused by other conditions such as chronic and acute respiratory diseases or altitude induced hypobaric hypoxia, and thus worsening their outcomes (2, 3).

Thanks to the recognition of pathophysiological traits responsible for OSA development, it has been possible to moved forward for alternative approaches to treat OSA and its related intermittent hypoxia and hypoxic burden. Among the others, a precision medicine approach targeting muscle responsiveness has been given the most effective results.

We demonstrated that repeated doses of the combination of noradrenergic and anti-muscarinic drugs are efficacious for the alleviation of OSA and its hypoxic consequences. Reboxetine plus oxybutynin administered before bedtime substantially reduce OSA severity (AHI) after 1-week of treatment. Specifically, over one week, reboxetine plus oxybutynin provided a 59% reduction in AHI, and halved OSA severity in 81% of individuals. In addition to the AHI reduction, reb-oxy also exerted a significant effect on indices of hypoxemia, such as ODI and hypoxic burden. Reb-oxy also improved the performance on the vigilance testing. OSA alleviation was likely mediated by improved upper airway muscle activity and responsiveness, as suggested by the ~30% increase in muscle compensation on the drugs. Home pulse-oximetry recordings showed that reb-oxy was effective at improving nocturnal oxygen saturation as early as the first day of treatment, likely due to reduced OSA severity, and its efficacy was maintained through the 7th day, as shown in the in-lab PSG. The combination of reboxetine plus oxybutynin did not increase blood pressure both during the day and during the night and did not increase the cardiac sympathetic modulation as reflected by heart rate variability during the night. While subjective sleepiness was not reduced in this population, objective psychomotor vigilance test showed promising signs of improvement without major safety issues. The current study showed for the first time that repeated doses of the combination of noradrenergic and anti-muscarinic drugs are efficacious for the alleviation of OSA, without determining clinically derangement in sympathetic activity.

Although our results report the efficacy of reb-oxy on OSA severity, our study is a proof-of-concept trial on a limited number of patients. While reb-oxy reduced AHI, hypoxic burden, and arousal index, their impact on subjective sleep quality was not statistically significant in this small trial. Larger and longer trials need to be performed to confirm the efficacy and the safety of these drugs in a broad range of individuals with OSA to proceed with the development of a pharmacological therapy for OSA patients based on a precision medicine approach. Moreover, future research might also evaluate the impact of OSA pharmacotherapy on the top of other conditions determining hypoxia and in other subgroups of comorbid patients.

Acknowledgements

First, I wish to thank Professor Gianfranco Parati for his guidance and encouragement throughout these years, and for setting an example with his work ethics.

A special thanks also to Professor Carolina Lombardi, for her unwavering support and for the great teamwork which made this and other projects successful. Together with Professor Gianfranco Parati, she has always been there for me ever since.

I would also like to extend my deepest gratitude to Dr Luigi Taranto for being a Friend and a Guide throughout these years. His constant support from afar cannot be overestimated.

My gratitude also goes to Professor Claudio Tantucci, Professor Stefania Redolfi, and Professor Douglas Bradley for their exceptional mentorship and for having moved with me the first steps of my career. This accomplishment would not have been possible without them.

Finally, a special thanks also to Mum and Dad for providing me with unfailing support since ever and for having encouraged my steps throughout my life.

8 REFERENCES

1. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. N Engl J Med. 2011;364(7):656-65.
2. Perger E, Baillieul S, Esteve F, Pichon A, Bilo G, Soranna D, et al. Nocturnal hypoxemia, blood pressure, vascular status and chronic mountain sickness in the highest city in the world. Annals of Medicine. 2022;54(1):1884-93.
3. Perger E, Soranna D, Pengo M, Meriggi P, Lombardi C, Parati G. Sleep-disordered Breathing among Hospitalized Patients with COVID-19. Am J Respir Crit Care Med. 2021;203(2):239-41.
4. Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Nieto FJ, et al. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. Am J Respir Crit Care Med. 2001;163(1):19-25.
5. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med. 2000;342(19):1378-84.
6. Perger E, Jutant EM, Redolfi S. Targeting volume overload and overnight rostral fluid shift: A new perspective to treat sleep apnea. Sleep Med Rev. 2018;42:160-70.
7. Weaver TE, Sawyer AM. Adherence to continuous positive airway pressure treatment for obstructive sleep apnoea: implications for future interventions. Indian J Med Res. 2010;131:245-58.
8. Eckert DJ, White DP, Jordan AS, Malhotra A, Wellman A. Defining Phenotypic Causes of Obstructive Sleep Apnea. Identification of Novel Therapeutic Targets. American Journal of Respiratory and Critical Care Medicine. 2013;188(8):996-1004.
9. Wellman A, Eckert DJ, Jordan AS, Edwards BA, Passaglia CL, Jackson AC, et al. A method for measuring and modeling the physiological traits causing obstructive sleep apnea. Journal of Applied Physiology. 2011;110(6):1627-37.
10. Wellman A, Edwards BA, Sands SA, Owens RL, Nemati S, Butler J, et al. A simplified method for determining phenotypic traits in patients with obstructive sleep apnea. Journal of Applied Physiology. 2013;114(7):911-22.
11. Younes M. Contributions of upper airway mechanics and control mechanisms to severity of obstructive apnea. Am J Respir Crit Care Med. 2003;168(6):645-58.
12. Gleadhill IC, Schwartz AR, Schubert N, Wise RA, Permutt S, Smith PL. Upper airway collapsibility in snorers and in patients with obstructive hypopnea and apnea. Am Rev Respir Dis. 1991;143(6):1300-3.
13. Loewen AHS, Ostrowski M, Laprairie J, Maturino F, Hanly PJ, Younes M. Response of genioglossus muscle to increasing chemical drive in sleeping obstructive apnea patients. Sleep. 2011;34(8):1061-73.
14. Younes M. Role of respiratory control mechanisms in the pathogenesis of obstructive sleep disorders. Journal of Applied Physiology. 2008;105(5):1389-405.
15. Wellman A, Jordan AS, Malhotra A, Fogel RB, Katz ES, Schory K, et al. Ventilatory control and airway anatomy in obstructive sleep apnea. Am J Respir Crit Care Med. 2004;170(11):1225-32.
16. Chan E, Steenland HW, Liu H, Horner RL. Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. Am J Respir Crit Care Med. 2006;174(11):1264-73.
17. Lai YY, Kodama T, Siegel JM. Changes in monoamine release in the ventral horn and hypoglossal nucleus linked to pontine inhibition of muscle tone: an in vivo microdialysis study. J Neurosci. 2001;21(18):7384-91.
18. Fenik VB, Davies RO, Kubin L. REM sleep-like atonia of hypoglossal (XII) motoneurons is caused by loss of noradrenergic and serotonergic inputs. Am J Respir Crit Care Med. 2005;172(10):1322-30.
19. Grace KP, Hughes SW, Horner RL. Identification of the mechanism mediating genioglossus muscle suppression in REM sleep. Am J Respir Crit Care Med. 2013;187(3):311-9.
20. Grace KP, Hughes SW, Shahabi S, Horner RL. K+ channel modulation causes genioglossus inhibition in REM sleep and is a strategy for reactivation. Respir Physiol Neurobiol. 2013;188(3):277-88.
21. Taranto-Montemurro L, Messineo L, Sands SA, Azarbarzin A, Marques M, Edwards BA, et al. The Combination of Atomoxetine and Oxybutynin Greatly Reduces Obstructive Sleep Apnea Severity. A

Randomized, Placebo-controlled, Double-Blind Crossover Trial. Am J Respir Crit Care Med. 2019;199(10):1267-76.
22. Lim R, Carberry JC, Wellman A, Grunstein R, Eckert DJ. Reboxetine and hyoscine butylbromide improve upper airway function during nonrapid eye movement and suppress rapid eye movement sleep in healthy individuals. Sleep. 2019;42(4).
23. Lim R, Messineo L, Grunstein R, Carberry J, Eckert D. Reboxetine and hyoscine butylbromide reduce obstructive sleep apnoea severity. Journal of Sleep Research. 2019;28(S1):e66_12912.
24. Chancellor M, Boone T. Anticholinergics for overactive bladder therapy: central nervous system effects. CNS Neurosci Ther. 2012;18(2):167-74.
25. León-Velarde F, Richalet JP. Respiratory control in residents at high altitude: physiology and pathophysiology. High Alt Med Biol. 2006;7(2):125-37.
26. Sun S, Oliver-Pickett C, Ping Y, Micco AJ, Droma T, Zamudio S, et al. Breathing and brain blood flow during sleep in patients with chronic mountain sickness. Journal of Applied Physiology. 1996;81(2):611-8.
27. Kryger M, Glas R, Jackson D, McCullough RE, Scoggin C, Grover RF, et al. Impaired oxygenation during sleep in excessive polycythemia of high altitude: improvement with respiratory stimulation. Sleep. 1978;1(1):3-17.
28. Spicuzza L, Casiraghi N, Gamboa A, Keyl C, Schneider A, Mori A, et al. Sleep-related hypoxaemia and excessive erythrocytosis in Andean high-altitude natives. European Respiratory Journal. 2004;23(1):41-6.
29. Julian CG, Vargas E, Gonzales M, Dávila RD, Ladenburger A, Reardon L, et al. Sleep-disordered breathing and oxidative stress in preclinical chronic mountain sickness (excessive erythrocytosis). Respir Physiol Neurobiol. 2013;186(2):188-96.
30. Guan W, Ga Q, Li R, Bai Z-Z, Wuren T, Wang J, et al. Sleep disturbances in long-term immigrants with chronic mountain sickness: a comparison with healthy immigrants at high altitude. Respir Physiol Neurobiol. 2015;206:4-10.
31. Torlasco C, Bilo G, Giuliano A, Soranna D, Ravaro S, Oliverio G, et al. Effects of acute exposure to moderate altitude on blood pressure and sleep breathing patterns. Int J Cardiol. 2020;301:173-9.
32. Lombardi C, Meriggi P, Agostoni P, Faini A, Bilo G, Revera M, et al. High-altitude hypoxia and periodic breathing during sleep: gender-related differences. J Sleep Res. 2013;22(3):322-30.
33. Pham LV, Meinzen C, Arias RS, Schwartz NG, Rattner A, Miele CH, et al. Cross-Sectional Comparison of Sleep-Disordered Breathing in Native Peruvian Highlanders and Lowlanders. High Alt Med Biol. 2017;18(1):11-9.
34. Villafuerte FC, Corante N. Chronic Mountain Sickness: Clinical Aspects, Etiology, Management, and Treatment. High Alt Med Biol. 2016;17(2):61-9.
35. Doutreleau S, Ulliel-Roche M, Hancco I, Bailly S, Oberholzer L, Robach P, et al. Cardiac remodelling in the highest city in the world: Effects of altitude and chronic mountain sickness. Eur J Prev Cardiol. 2022.
36. Enserink M. Hypoxia City. Science. 2019.
37. Richalet JP, Rivera M, Bouchet P, Chirinos E, Onnen I, Petitjean O, et al. Acetazolamide: a treatment for chronic mountain sickness. Am J Respir Crit Care Med. 2005;172(11):1427-33.
38. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
39. Eckert DJ, Sands SA. Hypoxia and Sleep-disordered Breathing: Friend or Foe? American Journal of Respiratory and Critical Care Medicine. 2022;205(8):869-72.
40. Gilmartin GS, Lynch M, Tamisier R, Weiss JW. Chronic intermittent hypoxia in humans during 28 nights results in blood pressure elevation and increased muscle sympathetic nerve activity. American Journal of Physiology-Heart and Circulatory Physiology. 2010;299(3):H925-H31.
41. Azarbarzin A, Sands SA, Stone KL, Taranto-Montemurro L, Messineo L, Terrill PI, et al. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. Eur Heart J. 2019;40(14):1149-57.

42. Azarbarzin A, Sands SA, Taranto-Montemurro L, Vena D, Sofer T, Kim SW, et al. The Sleep Apnea-Specific Hypoxic Burden Predicts Incident Heart Failure. Chest. 2020;158(2):739-50.
43. Mehra R, Azarbarzin A. Sleep Apnea–Specific Hypoxic Burden and Not the Sleepy Phenotype as a Novel Measure of Cardiovascular and Mortality Risk in a Clinical Cohort. American Thoracic Society; 2022. p. 12-3.
44. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. Lancet Respir Med. 2019;7(8):687-98.
45. Young T, Palta M, Dempsey J, Peppard PE, Nieto FJ, Hla KM. Burden of sleep apnea: rationale, design, and major findings of the Wisconsin Sleep Cohort study. WMJ. 2009;108(5):246-9.
46. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol. 2013;177(9):1006-14.
47. Heinzer R, Vat S, Marques-Vidal P, Marti-Soler H, Andries D, Tobback N, et al. Prevalence of sleep-disordered breathing in the general population: the HypnoLaus study. Lancet Respir Med. 2015;3(4):310-8.
48. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. Physiological reviews. 2010;90(1):47-112.
49. Sánchez-de-la-Torre M, Campos-Rodriguez F, Barbé F. Obstructive sleep apnoea and cardiovascular disease. Lancet Respir Med. 2013;1(1):61-72.
50. Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. JAMA. 2000;283(14):1829-36.
51. Hung J, Whitford EG, Parsons RW, Hillman DR. Association of sleep apnoea with myocardial infarction in men. Lancet. 1990;336(8710):261-4.
52. Gami AS, Howard DE, Olson EJ, Somers VK. Day-night pattern of sudden death in obstructive sleep apnea. N Engl J Med. 2005;352(12):1206-14.
53. Martínez-García MA, Campos-Rodríguez F, Catalán-Serra P, Soler-Cataluña JJ, Almeida-Gonzalez C, De la Cruz Morón I, et al. Cardiovascular mortality in obstructive sleep apnea in the elderly: role of long-term continuous positive airway pressure treatment: a prospective observational study. Am J Respir Crit Care Med. 2012;186(9):909-16.
54. Patel AR, Patel AR, Singh S, Singh S, Khawaja I. The Association Between Obstructive Sleep Apnea and Arrhythmias. Cureus. 2019;11(4):e4429.
55. Di Fusco SA, Pignalberi C, Santini L, Colivicchi F, Santini M. Arrhythmias and sleep apnea: physiopathologic link and clinical implications. J Interv Card Electrophysiol. 2020;57(3):387-97.
56. Javaheri S, Barbe F, Campos-Rodriguez F, Dempsey JA, Khayat R, Javaheri S, et al. Sleep Apnea: Types, Mechanisms, and Clinical Cardiovascular Consequences. Journal of the American College of Cardiology. 2017;69(7):841-58.
57. Bradley TD, Phillipson EA. Pathogenesis and pathophysiology of the obstructive sleep apnea syndrome. Med Clin North Am. 1985;69(6):1169-85.
58. Lyons OD, Bradley TD. Heart Failure and Sleep Apnea. Can J Cardiol. 2015;31(7):898-908.
59. Terán-Santos J, Jiménez-Gómez A, Cordero-Guevara J. The association between sleep apnea and the risk of traffic accidents. Cooperative Group Burgos-Santander. N Engl J Med. 1999;340(11):847-51.
60. Jennum P, Tønnesen P, Ibsen R, Kjellberg J. All-cause mortality from obstructive sleep apnea in male and female patients with and without continuous positive airway pressure treatment: a registry study with 10 years of follow-up. Nat Sci Sleep. 2015;7:43-50.
61. Weaver TE, Maislin G, Dinges DF, Bloxham T, George CF, Greenberg H, et al. Relationship between hours of CPAP use and achieving normal levels of sleepiness and daily functioning. Sleep. 2007;30(6):711-9.
62. Ramar K, Dort LC, Katz SG, Lettieri CJ, Harrod CG, Thomas SM, et al. Clinical Practice Guideline for the Treatment of Obstructive Sleep Apnea and Snoring with Oral Appliance Therapy: An Update for 2015. J Clin Sleep Med. 2015;11(7):773-827.

63. Carberry JC, Amatoury J, Eckert DJ. Personalized Management Approach for OSA. Chest. 2018;153(3):744-55.
64. Kuna ST, Reboussin DM, Strotmeyer ES, Millman RP, Zammit G, Walkup MP, et al. Effects of Weight Loss on Obstructive Sleep Apnea Severity. Ten-Year Results of the Sleep AHEAD Study. Am J Respir Crit Care Med. 2021;203(2):221-9.
65. Perger E, Blaise S, Vermorel C, Boge G, Pepin JL, Redolfi S, et al. Subjects with venous insufficiency have high risk of obstructive sleep apnea in relationship to fluid shift. Sleep Med. 2020;72:135-7.
66. Kribbs NB, Pack AI, Kline LR, Smith PL, Schwartz AR, Schubert NM, et al. Objective measurement of patterns of nasal CPAP use by patients with obstructive sleep apnea. Am Rev Respir Dis. 1993;147(4):887-95.
67. Engleman HM, Wild MR. Improving CPAP use by patients with the sleep apnoea/hypopnoea syndrome (SAHS). Sleep Med Rev. 2003;7(1):81-99.
68. Phillips CL, Grunstein RR, Darendeliler MA, Mihailidou AS, Srinivasan VK, Yee BJ, et al. Health outcomes of continuous positive airway pressure versus oral appliance treatment for obstructive sleep apnea: a randomized controlled trial. Am J Respir Crit Care Med. 2013;187(8):879-87.
69. Kim AM, Keenan BT, Jackson N, Chan EL, Staley B, Poptani H, et al. Tongue fat and its relationship to obstructive sleep apnea. Sleep. 2014;37(10):1639-48.
70. Jordan AS, White DP. Pharyngeal motor control and the pathogenesis of obstructive sleep apnea. Respir Physiol Neurobiol. 2008;160(1):1-7.
71. Horner RL, Hughes SW, Malhotra A. State-dependent and reflex drives to the upper airway: basic physiology with clinical implications. J Appl Physiol (1985). 2014;116(3):325-36.
72. Jordan AS, White DP, Lo Y-L, Wellman A, Eckert DJ, Yim-Yeh S, et al. Airway dilator muscle activity and lung volume during stable breathing in obstructive sleep apnea. Sleep. 2009;32(3):361-8.
73. Younes M, Ostrowski M, Atkar R, Laprairie J, Siemens A, Hanly P. Mechanisms of breathing instability in patients with obstructive sleep apnea. J Appl Physiol (1985). 2007;103(6):1929-41.
74. Edwards BA, Connolly JG, Campana LM, Sands SA, Trinder JA, White DP, et al. Acetazolamide attenuates the ventilatory response to arousal in patients with obstructive sleep apnea. Sleep. 2013;36(2):281-5.
75. Schwartz AR, Barnes M, Hillman D, Malhotra A, Kezirian E, Smith PL, et al. Acute upper airway responses to hypoglossal nerve stimulation during sleep in obstructive sleep apnea. American journal of respiratory and critical care medicine. 2012;185(4):420-6.
76. Perger E, Montemurro LT, Rosa D, Vicini S, Marconi M, Zanotti L, et al. Reboxetine plus Oxybutynin for Obstructed Sleep Apnea Treatment A 1-week Randomized, Placebo-controlled, Double-Blind Crossover Trial. Chest. 2021.
77. Eckert DJ, Malhotra A, Wellman A, White DP. Trazodone increases the respiratory arousal threshold in patients with obstructive sleep apnea and a low arousal threshold. Sleep. 2014;37(4):811-9.
78. Hedner J, Stenlöf K, Zou D, Hoff E, Hansen C, Kuhn K, et al. A Randomized Controlled Clinical Trial Exploring Safety and Tolerability of Sulthiame in Sleep Apnea. Am J Respir Crit Care Med. 2022;205(12):1461-9.
79. Op de Beeck S, Dieltjens M, Azarbarzin A, Willemen M, Verbraecken J, Braem MJ, et al. Mandibular Advancement Device Treatment Efficacy Is Associated with Polysomnographic Endotypes. Ann Am Thorac Soc. 2021;18(3):511-8.
80. Taranto-Montemurro L, Messineo L, Wellman A. Targeting Endotypic Traits with Medications for the Pharmacological Treatment of Obstructive Sleep Apnea. A Review of the Current Literature. J Clin Med. 2019;8(11).
81. Patil SP, Punjabi NM, Schneider H, O'Donnell CP, Smith PL, Schwartz AR. A simplified method for measuring critical pressures during sleep in the clinical setting. Am J Respir Crit Care Med. 2004;170(1):86-93.
82. Sands SA, Edwards BA, Terrill PI, Taranto-Montemurro L, Azarbarzin A, Marques M, et al. Phenotyping Pharyngeal Pathophysiology using Polysomnography in Patients with Obstructive Sleep Apnea. Am J Respir Crit Care Med. 2018;197(9):1187-97.

83. Sands SA, Terrill PI, Edwards BA, Taranto Montemurro L, Azarbarzin A, Marques M, et al. Quantifying the Arousal Threshold Using Polysomnography in Obstructive Sleep Apnea. Sleep. 2018;41(1).
84. Pini L, Magri R, Perger E, Levi G, Zambelli L, Giordani J, et al. Phenotyping OSAH patients during wakefulness. Sleep Breath. 2022.
85. Terrill PI, Edwards BA, Nemati S, Butler JP, Owens RL, Eckert DJ, et al. Quantifying the ventilatory control contribution to sleep apnoea using polysomnography. Eur Respir J. 2015;45(2):408-18.
86. Issa FG, Sullivan CE. Upper airway closing pressures in obstructive sleep apnea. Journal of Applied Physiology. 1984;57(2):520-7.
87. Gleadhill IC, Schwartz AR, Schubert N, Wise RA, Permutt S, Smith PL. Upper airway collapsibility in snorers and in patients with obstructive hypopnea and apnea. American Review of Respiratory Disease. 1991;143(6):1300-3.
88. Schwartz AR, Smith PL, Wise RA, Gold AR, Permutt S. Induction of upper airway occlusion in sleeping individuals with subatmospheric nasal pressure. Journal of Applied Physiology. 1988;64(2):535-42.
89. Smith PL, Wise RA, Gold AR, Schwartz AR, Permutt S. Upper airway pressure-flow relationships in obstructive sleep apnea. Journal of applied physiology. 1988;64(2):789-95.
90. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. Physiol Rev. 2010;90(1):47-112.
91. Sforza E, Bacon W, Weiss T, Thibault A, Petiau C, Krieger J. Upper airway collapsibility and cephalometric variables in patients with obstructive sleep apnea. Am J Respir Crit Care Med. 2000;161(2 Pt 1):347-52.
92. Chi L, Comyn FL, Mitra N, Reilly MP, Wan F, Maislin G, et al. Identification of craniofacial risk factors for obstructive sleep apnoea using three-dimensional MRI. Eur Respir J. 2011;38(2):348-58.
93. Mohsenin V. Gender differences in the expression of sleep-disordered breathing : role of upper airway dimensions. Chest. 2001;120(5):1442-7.
94. Kirkness JP, Schwartz AR, Schneider H, Punjabi NM, Maly JJ, Laffan AM, et al. Contribution of male sex, age, and obesity to mechanical instability of the upper airway during sleep. J Appl Physiol (1985). 2008;104(6):1618-24.
95. Malhotra A, Huang Y, Fogel RB, Pillar G, Edwards JK, Kikinis R, et al. The male predisposition to pharyngeal collapse: importance of airway length. Am J Respir Crit Care Med. 2002;166(10):1388-95.
96. Cakirer B, Hans MG, Graham G, Aylor J, Tishler PV, Redline S. The relationship between craniofacial morphology and obstructive sleep apnea in whites and in African-Americans. Am J Respir Crit Care Med. 2001;163(4):947-50.
97. Xu L, Keenan BT, Wiemken AS, Chi L, Staley B, Wang Z, et al. Differences in three-dimensional upper airway anatomy between Asian and European patients with obstructive sleep apnea. Sleep. 2020;43(5).
98. Schwab RJ, Pasirstein M, Pierson R, Mackley A, Hachadoorian R, Arens R, et al. Identification of upper airway anatomic risk factors for obstructive sleep apnea with volumetric magnetic resonance imaging. Am J Respir Crit Care Med. 2003;168(5):522-30.
99. Gozal D, Kheirandish-Gozal L. Sleep apnea in children--treatment considerations. Paediatr Respir Rev. 2006;7 Suppl 1:S58-61.
100. Redline S, Tishler PV, Tosteson TD, Williamson J, Kump K, Browner I, et al. The familial aggregation of obstructive sleep apnea. Am J Respir Crit Care Med. 1995;151(3 Pt 1):682-7.
101. Schwab RJ. Properties of tissues surrounding the upper airway. Sleep. 1996;19(10 Suppl):S170-4.
102. Hudgel DW, Patel SR, Ahasic AM, Bartlett SJ, Bessesen DH, Coaker MA, et al. The Role of Weight Management in the Treatment of Adult Obstructive Sleep Apnea. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;198(6):e70-e87.
103. White LH, Bradley TD. Role of nocturnal rostral fluid shift in the pathogenesis of obstructive and central sleep apnoea. The Journal of Physiology. 2013;591(5):1179-93.
104. Lin CH, Perger E, Lyons OD. Obstructive sleep apnea and chronic kidney disease. Curr Opin Pulm Med. 2018;24(6):549-54.

105. Stanchina ML, Malhotra A, Fogel RB, Trinder J, Edwards JK, Schory K, et al. The influence of lung volume on pharyngeal mechanics, collapsibility, and genioglossus muscle activation during sleep. Sleep. 2003;26(7):851-6.
106. Schwab RJ, Gupta KB, Gefter WB, Metzger LJ, Hoffman EA, Pack AI. Upper airway and soft tissue anatomy in normal subjects and patients with sleep-disordered breathing. Significance of the lateral pharyngeal walls. Am J Respir Crit Care Med. 1995;152(5 Pt 1):1673-89.
107. Orr JE, Schmickl CN, Edwards BA, DeYoung PN, Brena R, Sun XS, et al. Pathogenesis of obstructive sleep apnea in individuals with the COPD + OSA Overlap syndrome versus OSA alone. Physiol Rep. 2020;8(3):e14371.
108. Kairaitis K, Byth K, Parikh R, Stavrinou R, Wheatley JR, Amis TC. Tracheal traction effects on upper airway patency in rabbits: the role of tissue pressure. Sleep. 2007;30(2):179-86.
109. Kairaitis K, Stavrinou R, Parikh R, Wheatley JR, Amis TC. Mandibular advancement decreases pressures in the tissues surrounding the upper airway in rabbits. J Appl Physiol (1985). 2006;100(1):349-56.
110. Nicholas CL, Jordan AS, Heckel L, Worsnop C, Bei B, Saboisky JP, et al. Discharge Patterns of Human Tensor Palatini Motor Units During Sleep Onset. Sleep. 2012;35(5):699-707.
111. Basner RC, Ringler J, Schwartzstein RM, Weinberger SE, Weiss JW. Phasic electromyographic activity of the genioglossus increases in normals during slow-wave sleep. Respiration physiology. 1991;83(2):189-200.
112. Sands SA, Eckert DJ, Jordan AS, Edwards BA, Owens RL, Butler JP, et al. Enhanced upper-airway muscle responsiveness is a distinct feature of overweight/obese individuals without sleep apnea. Am J Respir Crit Care Med. 2014;190(8):930-7.
113. Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL. Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science. 1991;254(5032):726-9.
114. Long S, Duffin J. The neuronal determinants of respiratory rhythm. Prog Neurobiol. 1986;27(2):101-82.
115. Paintal AS. Mechanism of stimulation of type J pulmonary receptors. J Physiol. 1969;203(3):511-32.
116. Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. J Appl Physiol (1985). 2006;101(2):618-27.
117. Javaheri S, Kazemi H. Metabolic alkalosis and hypoventilation in humans. Am Rev Respir Dis. 1987;136(4):1011-6.
118. Biscoe TJ, Purves MJ, Sampson SR. The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded in vivo with intact circulation. J Physiol. 1970;208(1):121-31.
119. Schafer T. Respiratory pathophysiology: sleep-related breathing disorders. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2006;5:Doc01.
120. Von Leupoldt A, Vovk A, Bradley MM, Keil A, Lang PJ, Davenport PW. The impact of emotion on respiratory-related evoked potentials. Psychophysiology. 2010;47(3):579-86.
121. Jolley C, Luo Y, Steier J, Sylvester K, Man W, Rafferty G, et al. Neural respiratory drive and symptoms that limit exercise in chronic obstructive pulmonary disease. Lancet. 2015;385 Suppl 1:S51.
122. Khoo MC, Kronauer RE, Strohl KP, Slutsky AS. Factors inducing periodic breathing in humans: a general model. J Appl Physiol Respir Environ Exerc Physiol. 1982;53(3):644-59.
123. Ghazanshahi SD, Khoo MC. Estimation of chemoreflex loop gain using pseudorandom binary CO2 stimulation. IEEE Trans Biomed Eng. 1997;44(5):357-66.
124. Hudgel DW, Gordon EA, Thanakitcharu S, Bruce EN. Instability of ventilatory control in patients with obstructive sleep apnea. Am J Respir Crit Care Med. 1998;158(4):1142-9.
125. Sands SA, Owens RL. Congestive heart failure and central sleep apnea. Crit Care Clin. 2015;31(3):473-95.
126. Javaheri S. A mechanism of central sleep apnea in patients with heart failure. N Engl J Med. 1999;341(13):949-54.
127. Perger E, Inami T, Lyons OD, Alshaer H, Smith S, Floras JS, et al. Distinct Patterns of Hyperpnea During Cheyne-Stokes Respiration: Implication for Cardiac Function in Patients With Heart Failure. J Clin Sleep Med. 2017;13(11):1235-41.

128. Horner RL, Rivera MP, Kozar LF, Phillipson EA. The ventilatory response to arousal from sleep is not fully explained by differences in CO(2) levels between sleep and wakefulness. J Physiol. 2001;534(Pt 3):881-90.
129. Douglas NJ, White DP, Weil JV, Pickett CK, Zwillich CW. Hypercapnic ventilatory response in sleeping adults. Am Rev Respir Dis. 1982;126(5):758-62.
130. Messineo L, Taranto-Montemurro L, Azarbarzin A, Oliveira Marques MD, Calianese N, White DP, et al. Breath-holding as a means to estimate the loop gain contribution to obstructive sleep apnoea. J Physiol. 2018;596(17):4043-56.
131. White DP. Pathogenesis of obstructive and central sleep apnea. Am J Respir Crit Care Med. 2005;172(11):1363-70.
132. Dempsey JA. Crossing the apnoeic threshold: causes and consequences. Exp Physiol. 2005;90(1):13-24.
133. Gell LK, Vena D, Alex RM, Azarbarzin A, Calianese N, Hess LB, et al. Neural ventilatory drive decline as a predominant mechanism of obstructive sleep apnoea events. Thorax. 2022;77(7):707-16.
134. Messineo L, Eckert DJ. Obstructive sleep apnea phenotyping to understand pathophysiology and improve treatment and outcomes. In: Janes SM, editor. Encyclopedia of Respiratory Medicine. Second ed: Academic Press; 2022. p. 22-33.
135. Carberry JC, Jordan AS, White DP, Wellman A, Eckert DJ. Upper Airway Collapsibility (Pcrit) and Pharyngeal Dilator Muscle Activity are Sleep Stage Dependent. Sleep. 2016;39(3):511-21.
136. Messineo L, Taranto-Montemurro L, Azarbarzin A, Marques M, Calianese N, White DP, et al. Loop gain in REM versus non-REM sleep using CPAP manipulation: A pilot study. Respirology. 2019;24(8):805-8.
137. Messineo L, Eckert DJ, Taranto-Montemurro L, Vena D, Azarbarzin A, Hess LB, et al. Ventilatory Drive Withdrawal Rather Than Reduced Genioglossus Compensation as a Mechanism of Obstructive Sleep Apnea in REM Sleep. American journal of respiratory and critical care medicine. 2022;205(2):219-32.
138. Gleeson K, Zwillich CW, White DP. The influence of increasing ventilatory effort on arousal from sleep. Am Rev Respir Dis. 1990;142(2):295-300.
139. Phillipson EA, Sullivan CE. Arousal: the forgotten response to respiratory stimuli. Am Rev Respir Dis. 1978;118(5):807-9.
140. Younes M. Role of arousals in the pathogenesis of obstructive sleep apnea. American journal of respiratory and critical care medicine. 2004;169(5):623-33.
141. Rees K, Spence DP, Earis JE, Calverley PM. Arousal responses from apneic events during non-rapid-eye-movement sleep. Am J Respir Crit Care Med. 1995;152(3):1016-21.
142. Younes M, Loewen AH, Ostrowski M, Laprairie J, Maturino F, Hanly PJ. Genioglossus activity available via non-arousal mechanisms vs. that required for opening the airway in obstructive apnea patients. J Appl Physiol (1985). 2012;112(2):249-58.
143. Taylor KS, Murai H, Millar PJ, Haruki N, Kimmerly DS, Morris BL, et al. Arousal From Sleep and Sympathetic Excitation During Wakefulness. Hypertension. 2016;68(6):1467-74.
144. Azarbarzin A, Ostrowski M, Hanly P, Younes M. Relationship between arousal intensity and heart rate response to arousal. Sleep. 2014;37(4):645-53.
145. Butler MP, Emch JT, Rueschman M, Sands SA, Shea SA, Wellman A, et al. Apnea-Hypopnea Event Duration Predicts Mortality in Men and Women in the Sleep Heart Health Study. Am J Respir Crit Care Med. 2019;199(7):903-12.
146. Shahrbabaki SS, Linz D, Hartmann S, Redline S, Baumert M. Sleep arousal burden is associated with long-term all-cause and cardiovascular mortality in 8001 community-dwelling older men and women. Eur Heart J. 2021;42(21):2088-99.
147. Eckert DJ, White DP, Jordan AS, Malhotra A, Wellman A. Defining phenotypic causes of obstructive sleep apnea. Identification of novel therapeutic targets. American journal of respiratory and critical care medicine. 2013;188(8):996-1004.
148. Gray EL, McKenzie DK, Eckert DJ. Obstructive Sleep Apnea without Obesity Is Common and Difficult to Treat: Evidence for a Distinct Pathophysiological Phenotype. J Clin Sleep Med. 2017;13(1):81-8.

149. Dingli K, Fietze I, Assimakopoulos T, Quispe-Bravo S, Witt C, Douglas NJ. Arousability in sleep apnoea/hypopnoea syndrome patients. The European respiratory journal. 2002;20(3):733-40.
150. Borker PV, Reid M, Sofer T, Butler MP, Azarbarzin A, Wang H, et al. Non-REM Apnea and Hypopnea Duration Varies across Population Groups and Physiologic Traits. Am J Respir Crit Care Med. 2021;203(9):1173-82.
151. Jordan AS, Eckert DJ, Catcheside PG, McEvoy RD. Ventilatory response to brief arousal from non-rapid eye movement sleep is greater in men than in women. Am J Respir Crit Care Med. 2003;168(12):1512-9.
152. Amatoury J, Azarbarzin A, Younes M, Jordan AS, Wellman A, Eckert DJ. Arousal Intensity is a Distinct Pathophysiological Trait in Obstructive Sleep Apnea. Sleep. 2016;39(12):2091-100.
153. Carberry JC, Fisher LP, Grunstein RR, Gandevia SC, McKenzie DK, Butler JE, et al. Role of common hypnotics on the phenotypic causes of obstructive sleep apnoea: paradoxical effects of zolpidem. The European respiratory journal. 2017;50(6).
154. Edwards BA, Eckert DJ, McSharry DG, Sands SA, Desai A, Kehlmann G, et al. Clinical predictors of the respiratory arousal threshold in patients with obstructive sleep apnea. Am J Respir Crit Care Med. 2014;190(11):1293-300.
155. Tantucci C, Mehiri S, Duguet A, Similowski T, Arnulf I, Zelter M, et al. Application of negative expiratory pressure during expiration and activity of genioglossus in humans. J Appl Physiol (1985). 1998;84(3):1076-82.
156. Montemurro LT, Bettinzoli M, Corda L, Braghini A, Tantucci C. Relationship Between Critical Pressure and Volume Exhaled During Negative Pressure in Awake Subjects With Sleep-Disordered Breathing. Chest. 2010;137(6):1304-9.
157. Taranto-Montemurro L, Sands SA, Edwards BA, Azarbarzin A, Marques M, de Melo C, et al. Desipramine improves upper airway collapsibility and reduces OSA severity in patients with minimal muscle compensation. European Respiratory Journal. 2016;48(5):1340-50.
158. Vena D, Taranto-Montemurro L, Azarbarzin A, Op de Beeck S, Marques M, Vanderveken OM, et al. Clinical polysomnographic methods for estimating pharyngeal collapsibility in obstructive sleep apnea. Sleep. 2022;45(6).
159. Perger E, Taranto-Montemurro L. Upper airway muscles: influence on obstructive sleep apnoea pathophysiology and pharmacological and technical treatment options. Curr Opin Pulm Med. 2021.
160. Safiruddin F, Vanderveken OM, de Vries N, Maurer JT, Lee K, Ni Q, et al. Effect of upper-airway stimulation for obstructive sleep apnoea on airway dimensions. Eur Respir J. 2015;45(1):129-38.
161. Strollo PJ, Jr., Soose RJ, Maurer JT, de Vries N, Cornelius J, Froymovich O, et al. Upper-airway stimulation for obstructive sleep apnea. N Engl J Med. 2014;370(2):139-49.
162. Eastwood PR, Barnes M, MacKay SG, Wheatley JR, Hillman DR, Nguyên X-L, et al. Bilateral hypoglossal nerve stimulation for treatment of adult obstructive sleep apnoea. European Respiratory Journal. 2020;55(1):1901320.
163. Kent DT, Zealear D, Schwartz AR. Ansa Cervicalis Stimulation: A New Direction in Neurostimulation for OSA. Chest. 2021;159(3):1212-21.
164. Vanderveken OM, Maurer JT, Hohenhorst W, Hamans E, Lin H-S, Vroegop AV, et al. Evaluation of drug-induced sleep endoscopy as a patient selection tool for implanted upper airway stimulation for obstructive sleep apnea. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine. 2013;9(5):433-8.
165. Schwab RJ, Wang SH, Verbraecken J, Vanderveken OM, Van de Heyning P, Vos WG, et al. Anatomic predictors of response and mechanism of action of upper airway stimulation therapy in patients with obstructive sleep apnea. Sleep. 2018;41(4).
166. Boon M, Huntley C, Steffen A, Maurer JT, Sommer JU, Schwab R, et al. Upper Airway Stimulation for Obstructive Sleep Apnea: Results from the ADHERE Registry. Otolaryngol Head Neck Surg. 2018;159(2):379-85.
167. Withrow K, Evans S, Harwick J, Kezirian E, Strollo P. Upper Airway Stimulation Response in Older Adults with Moderate to Severe Obstructive Sleep Apnea. Otolaryngol Head Neck Surg. 2019;161(4):714-9.

168. Op de Beeck S, Wellman A, Dieltjens M, Strohl KP, Willemen M, Van de Heyning PH, et al. Endotypic Mechanisms of Successful Hypoglossal Nerve Stimulation for Obstructive Sleep Apnea. Am J Respir Crit Care Med. 2021;203(6):746-55.
169. Koka V, De Vito A, Roisman G, Petitjean M, Filograna Pignatelli GR, Padovani D, et al. Orofacial Myofunctional Therapy in Obstructive Sleep Apnea Syndrome: A Pathophysiological Perspective. Medicina (Kaunas). 2021;57(4).
170. de Felício CM, da Silva Dias FV, Trawitzki LVV. Obstructive sleep apnea: focus on myofunctional therapy. Nat Sci Sleep. 2018;10:271-86.
171. Chuang LC, Hwang YJ, Lian YC, Hervy-Auboiron M, Pirelli P, Huang YS, et al. Changes in craniofacial and airway morphology as well as quality of life after passive myofunctional therapy in children with obstructive sleep apnea: a comparative cohort study. Sleep Breath. 2019;23(4):1359-69.
172. O'Connor-Reina C, Ignacio Garcia JM, Rodriguez Ruiz E, Morillo Dominguez MDC, Ignacio Barrios V, Baptista Jardin P, et al. Myofunctional Therapy App for Severe Apnea-Hypopnea Sleep Obstructive Syndrome: Pilot Randomized Controlled Trial. JMIR Mhealth Uhealth. 2020;8(11):e23123-e.
173. AIFA. DITROPAN (oxibutinina cloridato). Riassunto delle caratteristiche del prodotto. first June 2000, update 2013.
174. AIFA. EDRONAX (reboxetina). Riassunto delle caratteristiche del prodotto. first may 1997, update 2007.
175. Sateia MJ. International classification of sleep disorders-third edition: highlights and modifications. Chest. 2014;146(5):1387-94.
176. Rosenberg RS, Hout SV. The American Academy of Sleep Medicine Inter-scorer Reliability Program: Respiratory Events. Journal of Clinical Sleep Medicine. 2014;10(04):447-54.
177. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991;14(6):540-5.
178. Yang F, Xu S, Basner M, Chai Y, Dinges DF, Rao H. 0227 Sleep Deprivation Enlarges Inter-stimulus Interval Effect On Vigilant Attention. Sleep. 2018;41(suppl_1):A88-A9.
179. Pengo MF, Soranna D, Giontella A, Perger E, Mattaliano P, Schwarz EI, et al. Obstructive sleep apnoea treatment and blood pressure: which phenotypes predict a response? A systematic review and meta-analysis. European Respiratory Journal. 2020;55(5):1901945.
180. Wong EH, Sonders MS, Amara SG, Tinholt PM, Piercey MF, Hoffmann WP, et al. Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. Biol Psychiatry. 2000;47(9):818-29.
181. Invernizzi RW, Parini S, Sacchetti G, Fracasso C, Caccia S, Annoni K, et al. Chronic treatment with reboxetine by osmotic pumps facilitates its effect on extracellular noradrenaline and may desensitize alpha(2)-adrenoceptors in the prefrontal cortex. Br J Pharmacol. 2001;132(1):183-8.
182. Fossa AA, Wisialowski TA, Cremers T, van der Hart M, Tseng E, Deng S, et al. Improved preclinical cardiovascular therapeutic indices with long-term inhibition of norepinephrine reuptake using reboxetine. Toxicol Appl Pharmacol. 2012;264(3):343-50.
183. Taranto-Montemurro L, Messineo L, Azarbarzin A, Vena D, Hess L, Calianese N, et al. Effects of the Combination of Atomoxetine and Oxybutynin on Obstructive Sleep Apnea Endotypic Traits. CHEST. 2020.
184. Thomson L, Landry S, Joosten SA, Wong A, Cheung T, Beatty C, et al. A Randomized, Placebo-Controlled, Double-Blind Crossover Trial Assessing the Combination of Noradrenergic/Serotonergic Reuptake Inhibitor and an Antimuscarinic Agent for the Treatment of Obstructive Sleep Apnea. ATS 2020. Contract No.: A4521.
185. Aishah A, Lim R, Sands SA, Taranto-Montemurro L, Wellman A, Carberry JC, et al. Different antimuscarinics when combined with atomoxetine have differential effects on obstructive sleep apnea severity. J Appl Physiol (1985). 2021;130(5):1373-82.
186. Taranto Montemurro L, Sands SA, Azarbarzin A, Calianese N, Hess L, Vena D, et al. The Combination of Atomoxetine and Fesoterodine Improves Obstructive Sleep Apnea Severity in Patients with Low Upper Airway Collapsibility. 2020. Contract No.: A4520.

187. Messineo L, Taranto-Montemurro L, Calianese N, Gell LK, Azarbarzin A, Labarca G, et al. Atomoxetine and fesoterodine combination improves obstructive sleep apnoea severity in patients with milder upper airway collapsibility. Respirology. 2022.
188. Ferini-Strambi L, Manconi M, Castronovo V, Riva L, Bianchi A. Effects of reboxetine on sleep and nocturnal cardiac autonomic activity in patients with dysthymia. J Psychopharmacol. 2004;18(3):417-22.
189. Lim R, Messineo L, Grunstein RR, Carberry JC, Eckert DJ. The noradrenergic agent reboxetine plus the antimuscarinic hyoscine butylbromide reduces sleep apnoea severity: a double-blind, placebo-controlled, randomised crossover trial. The Journal of Physiology. 2021;599(17):4183-95.
190. Edwards BA, Sands SA, Eckert DJ, White DP, Butler JP, Owens RL, et al. Acetazolamide improves loop gain but not the other physiological traits causing obstructive sleep apnoea. J Physiol. 2012;590(5):1199-211.
191. Szabo ST, Blier P. Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. Eur J Neurosci. 2001;13(11):2077-87.
192. Putnam RW, Filosa JA, Ritucci NA. Cellular mechanisms involved in CO2 and acid signaling in chemosensitive neurons. American Journal of Physiology-Cell Physiology. 2004;287(6):C1493-C526.
193. Biancardi V, Bícego KC, Almeida MC, Gargaglioni LH. Locus coeruleus noradrenergic neurons and CO2 drive to breathing. Pflugers Arch. 2008;455(6):1119-28.
194. Taranto-Montemurro L, Messineo L, Azarbarzin A, Vena D, Hess LB, Calianese NA, et al. Effects of the Combination of Atomoxetine and Oxybutynin on OSA Endotypic Traits. Chest. 2020;157(6):1626-36.
195. Hajós M, Fleishaker JC, Filipiak-Reisner JK, Brown MT, Wong EHF. The Selective Norepinephrine Reuptake Inhibitor Antidepressant Reboxetine: Pharmacological and Clinical Profile. CNS Drug Reviews. 2004;10(1):23-44.
196. Avenoso A, Facciola G, Scordo MG, Spina E. No effect of the new antidepressant reboxetine on CYP2D6 activity in healthy volunteers. Ther Drug Monit. 1999;21(5):577-9.
197. Altree TJ, Aishah A, Loffler KA, Grunstein RR, Eckert DJ. The norepinephrine reuptake inhibitor reboxetine alone reduces obstructive sleep apnea severity: a double blind, placebo controlled, randomized, cross-over trial. J Clin Sleep Med. 2022.
198. Schroeder C, Tank J, Boschmann M, Diedrich A, Sharma AM, Biaggioni I, et al. Selective norepinephrine reuptake inhibition as a human model of orthostatic intolerance. Circulation. 2002;105(3):347-53.
199. Penttilä J, Syvälahti E, Hinkka S, Kuusela T, Scheinin H. The effects of amitriptyline, citalopram and reboxetine on autonomic nervous system. Psychopharmacology. 2001;154(4):343-9.
200. Akbari E, Zare AH, Alipour A. The effect of oxybutynin on cardiac autonomic modulation in healthy subjects. Clin Auton Res. 2014;24(4):167-74.
201. Denolle T, Pellizzoni C, Jannuzzo MG, Poggesi I. Hemodynamic effects of reboxetine in healthy male volunteers. Clin Pharmacol Ther. 1999;66(3):282-7.
202. Riahi F, Tashakori A, Izadi-Mazidi S, Salehi-Veysi M. Effectiveness of reboxetine in treatment of outpatient children and adolescents with attention deficit-hyperactivity disorder with comorbid anxiety disorders. Iran J Psychiatry. 2013;8(4):195-200.
203. Agelink MW, Ullrich H, Baumann B, Strum S, Majewski T. Effects of reboxetine, a selective norepinephrine reuptake inhibitor, on sympathetic and parasympathetic outflow to the heart: preliminary data. Psychopharmacology (Berl). 2002;163(2):151-6.
204. Parati G, Mancia G, Di Rienzo M, Castiglioni P. Point: cardiovascular variability is/is not an index of autonomic control of circulation. J Appl Physiol (1985). 2006;101(2):676-8; discussion 81-2.
205. Electrophysiology TFotESoCtNASoP. Heart Rate Variability. Circulation. 1996;93(5):1043-65.
206. Sequeira VCC, Bandeira PM, Azevedo JCM. Heart rate variability in adults with obstructive sleep apnea: a systematic review. Sleep Sci. 2019;12(3):214-21.
207. May AM, Van Wagoner DR, Mehra R. OSA and Cardiac Arrhythmogenesis: Mechanistic Insights. Chest. 2017;151(1):225-41.

208. Bonsignore MR, Parati G, Insalaco G, Marrone O, Castiglioni P, Romano S, et al. Continuous positive airway pressure treatment improves baroreflex control of heart rate during sleep in severe obstructive sleep apnea syndrome. Am J Respir Crit Care Med. 2002;166(3):279-86.
209. Narkiewicz K, Kato M, Phillips BG, Pesek CA, Davison DE, Somers VK. Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. Circulation. 1999;100(23):2332-5.
210. Ferreira CB, Schoorlemmer GH, Rocha AA, Cravo SL. Increased sympathetic responses induced by chronic obstructive sleep apnea are caused by sleep fragmentation. Journal of Applied Physiology. 2020;129(1):163-72.

9 APPENDIX

Publications produced by the candidate during the PhD period related to this project:

- *Perger E, Montemurro LT, Rosa D, Vicini S, Marconi M, Zanotti L,* et al. **Reboxetine plus Oxybutynin for Obstructed Sleep Apnea Treatment A 1-week Randomized, Placebo-controlled, Double-Blind Crossover Trial**. Chest. 2021.

- *Perger E, Baillieul S, Esteve F, Pichon A, Bilo G, Soranna D,* et al. **Nocturnal hypoxemia, blood pressure, vascular status and chronic mountain sickness in the highest city in the world**. Annals of Medicine. 2022;54(1):1884-93.

- *Perger E, Soranna D, Pengo M, Meriggi P, Lombardi C, Parati G.* **Sleep-disordered Breathing among Hospitalized Patients with COVID-19.** Am J Respir Crit Care Med. 2021;203(2):239-41.

- *Perger E, Taranto-Montemurro L.* **Upper airway muscles: influence on obstructive sleep apnoea pathophysiology and pharmacological and technical treatment options**. Curr Opin Pulm Med. 2021; 27(6):505-513

- *Pini L, Magri R, Perger E, Levi G, Zambelli L, Giordani J*, et al. **Phenotyping OSAH patients during wakefulness.** Sleep Breath. 2022.

- *Pengo MF, Soranna D, Giontella A, Perger E, Mattaliano P, Schwarz EI*, et al. **Obstructive sleep apnoea treatment and blood pressure: which phenotypes predict a response? A systematic review and meta-analysis.** European Respiratory Journal. 2020;55(5):1901945.

- *Perger E, Castiglioni P, Faini A, Soranna D, Zambon A, Rosa D* et al. **Impact of Reboxetine plus Oxybutynin treatment for Obstructive Sleep Apnea on Cardiovascular Autonomic Modulation**. Submitted