THE EFFECTS OF REBOXETINE AND MORPHINE ON SLEEP AND BREATHING IN OBSTRUCTIVE SLEEP APNEA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any University; and
2. the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and
3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Dr Thomas J. Altree

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Abstract

Obstructive sleep apnea (OSA) and chronic obstructive pulmonary disease (COPD) are common respiratory diseases that cause significant symptom burden. OSA is characterized by repetitive pharyngeal airway narrowing and collapse, resulting in hypoxia, hypercapnia, and arousals during sleep. COPD is a small airways disease characterized by airflow obstruction and persistent respiratory symptoms, including breathlessness. Sleep disruption is common in both conditions, and currently available treatments are often incompletely effective.

Drugs initially developed for different purposes have shown therapeutic promise in both conditions. Antimuscarinics combined with noradrenergic reuptake inhibitors have been investigated as novel pharmacotherapy for OSA, but the optimal agents have yet to be determined. Morphine, originally developed for analgesia, was subsequently observed to relieve breathlessness, leading to its use in COPD. However, therapeutic responses vary, and the underlying effects on sleep, breathing and next day alertness in COPD are incompletely understood. In both conditions, clinical trials to investigate the effects of these potential pharmacotherapies are required.

Recent findings indicate that noradrenergic and muscarinic processes are important in pharyngeal muscle control, one of four key OSA endotypes. To date, reductions in OSA severity have only been detected when noradrenergic agents, such as reboxetine, are combined with an antimuscarinic. However, antimuscarinics cause significant side effects, and it is unclear if reboxetine alone is efficacious. Accordingly, in study one, I conducted a three-way, placebo-controlled, randomized trial, to determine if reboxetine alone reduces OSA severity. Reboxetine reduced OSA severity as measured by the apnea-hypopnea index (AHI). Reboxetine combined with the antimuscarinic oxybutynin did not cause additional reductions in AHI. Mechanistically, reboxetine improved pharyngeal collapsibility and respiratory control (loop gain), another important OSA endotype. These findings represent the first evidence that reboxetine alone reduces OSA severity, and provide insight into the role of noradrenergic agents on pharyngeal stability during sleep.

In COPD, breathlessness is common and can contribute to sleep disruption. Opioid analgesics, such as low-dose morphine, are included in international COPD guidelines for symptomatic relief of chronic breathlessness. Morphine is a central nervous system depressant, and as such may cause sedation and respiratory depression. Thus, there are significant safety concerns. The effects of morphine on sleep, breathing and next-day function have not been rigorously investigated in COPD.

My second project analyzed sleep questionnaire data from a placebo-controlled, randomized trial of low-dose morphine for breathlessness in ~150 people with COPD. After one week, there were no differences in perceived daytime sleepiness. This reassuring neutral effect persisted after four weeks. Additionally, participants who reported reduced breathlessness with morphine at four weeks also had improvements in sleep quality, raising a potential novel relationship between sleep and breathlessness which may be mediated by morphine.

In my third project, a placebo-controlled, randomized, cross-over trial, I aimed to objectively measure the sleep-related effects of morphine in COPD. After three daily 20mg doses, morphine did not affect sleep efficiency or the AHI, but reduced rapid eye movement sleep, respiratory rate, oxygenation and raised carbon dioxide levels during sleep. Despite these changes, there were no effects on next morning alertness or breathlessness.

Chapter One: Aims, Introduction and Literature Review

Parts of this literature review have been published[1-3]:

Altree TJ, Chung F, Chan MTV, Eckert DJ. Vulnerability to Postoperative Complications in Obstructive Sleep Apnea: Importance of Phenotypes. *Anesth Analg* 2021;132(5):1328-1337.

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Research Aims

The history of drug development includes several agents that were developed for one indication and were subsequently found to be effective for another. In some cases, advances in our understanding of disease pathophysiology have identified new targets for old drugs. In others, the effect of a repurposed drug can offer new insight into disease pathophysiology.

My PhD investigates the effects of repurposed pharmacotherapy during sleep in two common respiratory diseases, obstructive sleep apnea (OSA), and chronic obstructive pulmonary disease (COPD), through three separate research studies.

The combination of noradrenergic reuptake inhibitors and antimuscarinic drugs have recently shown promise as drug therapies for OSA. However, antimuscarinic agents cause significant side effects, and it is unclear if noradrenergic reuptake inhibitors such as reboxetine are effective on their own. Therefore, the aim of my first study is to determine if reboxetine is effective in treating

OSA in the absence of an antimuscarinic. I also aim to use novel non-invasive methods to assess the underlying mechanisms through which reboxetine improves OSA. I hypothesize that reboxetine alone will reduce OSA severity, and the predominant underlying mechanism by which it improves OSA will be by increasing upper airway dilator muscle tone during sleep.

The main aims of studies two and three are to determine the subjective (study two) and objective (study three) effects of low-dose morphine on sleep in breathless people with COPD. In study two, a secondary analysis of a large randomized controlled trial that assessed the effects of low-dose morphine on chronic breathlessness in COPD, I hypothesize that the relatively low doses of morphine studied, and the potential of morphine to improve sleep quality, will not impair alertness or increase daytime sleepiness. I will also explore potential associations between sleep quality and breathlessness. In study three, a randomized controlled trial of low-dose morphine during sleep, I hypothesize that morphine will increase sleep efficiency, but will reduce the respiratory rate, leading to reduced oxygenation and increased carbon dioxide levels. Study three will also objectively measure next-day alertness and several other exploratory outcomes, including blood morphine levels, sleep-disordered breathing, and responses to increased inspiratory resistive loads to breathing, to thoroughly assess the effects of low-dose morphine on sleep and breathing in people with COPD.

Obstructive Sleep Apnea

Overview and prevalence

Obstructive sleep apnea (OSA) is characterized by repetitive narrowing and partial or complete collapse of the pharyngeal airway, resulting in hypoxia, hypercapnia, and frequent arousals during sleep. Global estimates indicate that nearly one billion people have obstructive sleep apnea (OSA)[4]. Untreated OSA is associated with a range of adverse health outcomes including cardiovascular[5,6], neurocognitive[7,8], and metabolic disease[9]. Continuous positive airway pressure (CPAP) reduces the frequency of respiratory events during sleep and is currently the first-line treatment for severe OSA. However, many people find CPAP difficult to tolerate. Indeed, 46 to 83% of those prescribed CPAP are not adherent to therapy[10]. Other therapies such as mandibular

advancement splints have better adherence but variable and unpredictable efficacy[11]. Thus, there is an urgent need to develop new therapies to treat this highly prevalent chronic health condition.

OSA pathophysiology

OSA is a heterogeneous disease[12]. While all patients have a degree of upper airway anatomical impairment due to a narrow, crowded, or collapsible pharyngeal airway, the extent to which anatomical features contribute to OSA varies markedly from patient to patient. Indeed, more than two-thirds of people with OSA have additional "non-anatomical" traits or *endotypes* that contribute to disease pathogenesis[12]. These non-anatomical traits include reduced upper-airway dilator muscle function during sleep, instability of ventilatory control, and a low threshold for awakening (cortical arousal) to minor respiratory events during sleep. Although gold-standard methodology to quantify OSA endotypes are invasive and constrained to research settings[11,13], novel approaches such as automated signal processing analysis of standard polysomnography data and simple wakefulness tests offer promise as non-invasive, clinically deployable tools to estimate OSA endotypes[14-25].

The four key OSA endotypes

Impaired upper airway anatomy

The key determinant of OSA is a narrow, crowded, or collapsible upper airway[13]. Obesity is a major factor that contributes to reduced pharyngeal airspace. Adipose tissue deposition in the soft tissues of the neck and pharyngeal muscles crowds the upper airway[26]. The specific location of adipose tissue likely plays an important role in OSA development. Obese patients with OSA have more fat in the tongue compared to obese people without OSA[26]. Abdominal fat also plays an important role. Decreased resting lung volume caused by central adiposity reduces caudal traction on the upper airway structures, increasing pharyngeal collapsibility[27].

Craniofacial shape and size also impact pharyngeal airway cross-sectional area. Decreased mandible length and depth increase OSA risk in men[28]. Smaller upper airway bony dimensions may pose specific risk for upper airway collapse, particularly in those with Asian ethnicity[29,30]. An anatomically long upper airway (common in men) is more prone to collapse. Indeed, increased

upper airway length and inferior position of the hyoid bone are both associated with a diagnosis of OSA[31,32].

Body and head position can also influence upper airway collapsibility. Supine sleep position is associated with increased upper airway collapsibility compared to lateral[33]. Collapsibility increases with head flexion and reduces with extension[34]. Supine position also leads to rostral fluid shifts, with redistribution of fluid into various tissues, including those surrounding the upper airway. This increases pharyngeal tissue pressure, reduces cross-sectional area, and increases upper airway collapsibility[35].

A key physiological outcome of impaired upper airway anatomy is an increase in the luminal pressure at which upper airway collapse occurs. This measurement is known as the critical closing pressure (*Pcrit*). Severe anatomical compromise is designated where a Pcrit of >2cmH_2O is recorded during sleep. Conversely, circumstances where a negative pressure of less than -5cmH_2O is required to collapse the airway (i.e. a Pcrit of <-5cmH_2O) indicates an airway that is not prone to collapse[12]. Pcrit values close to atmospheric indicate intermediate anatomical compromise. Given that all patients with OSA have at least some degree of upper airway impairment, patients with OSA have, on average, a higher Pcrit than those without OSA[36]. However, due to the varying degree to which upper airway impairment and the non-anatomical traits contribute to the pathogenesis of OSA between individuals, there is considerable variability in Pcrit levels in people who have OSA ranging from -5 to +5cmH_2O and beyond[12,36].

Non-anatomical OSA endotypes

In addition to impaired upper airway anatomy, three specific non-anatomical endotypes play key roles in OSA pathogenesis. These non-anatomical traits have only recently been characterized[12].

Upper airway muscle responsiveness

The pharyngeal airway subserves multiple important functions. These include speech, swallowing, and breathing. There is complex interplay between multiple muscles within this non-rigid structure to facilitate these important functions. Without a rigid bony structure, airway patency is at risk and reliant on unimpaired function of pharyngeal muscles. These muscles increase their activity levels

and serve to dilate the upper airway in response to airway narrowing (or negative pharyngeal pressure). This process is termed “upper airway muscle responsiveness”.

Two key muscles involved in upper airway muscle responsiveness are genioglossus and tensor palatini. Genioglossus, the largest upper airway dilator, is located at the base of the tongue. Genioglossus has a phasic activation pattern with greater activity during inspiration versus expiration[37]. This serves to counteract airway narrowing from suction pressures generated during inspiration. Genioglossus activation is partly dependant on sleep state, and is also influenced by input from brainstem pattern generator neurons, pharyngeal airway pressure-sensitive mechanoreceptors, and chemical drive via hypoxia and hypercapnia[38-41].

In contrast to the phasic contraction of genioglossus, tensor palatini tends to display a tonic (i.e., constant) level of activation during quiet breathing[42]. Like genioglossus, tensor palatini activity is mediated by several factors that influence neural drive but is strongly influenced by the sleep state[41]. In experiments where upper airway resistance is minimized with continuous positive airway pressure (CPAP), tensor palatini activity markedly reduces at sleep onset but then remains relatively constant across sleep stages. Under the same conditions, genioglossus activity progressively diminishes from deeper slow-wave sleep, to lighter N2 sleep, to REM sleep[41,43].

The ability of the pharyngeal muscles to effectively increase muscle tone in response to respiratory stimuli (such as hypercapnia) during sleep is important in OSA pathogenesis. Over one third of OSA patients have impaired upper-airway dilator muscle responsiveness[12]. When exposed to airway narrowing during sleep, there is either no or very little muscle activation. A subset of patients with OSA have increased muscle activity in response to airway narrowing, yet the response is ineffective. Reasons for reduced “muscle effectiveness”, despite an appropriate increase in neural drive, include a dissociation between neural drive and dilator muscle response, impaired dilator muscle coordination, altered muscle mechanics (i.e. due to fat deposition), and increased fatiguability secondary to changes in muscle fibre type[44-46].

Given that patients with OSA do not experience airway obstruction awake, the interplay between the anatomical vulnerability, posture, and sleep-dependent reductions in upper airway muscle

responsiveness is a key concept in OSA pathogenesis. However, this interaction is not a dominant factor in every patient with OSA, where other non-anatomical processes play an important role.

Respiratory arousal threshold

Respiratory stimuli (i.e., hypoxia, hypercapnia, or respiratory loading) induce brief awakenings from sleep, known as cortical arousals. The degree of stimulus or respiratory effort required to induce a cortical arousal, measured as the nadir epiglottic or esophageal pressure just prior to cortical arousal, is known as the respiratory arousal threshold. The arousal threshold varies markedly between individuals[47,48]. Individuals who wake up very easily to minor levels of airway narrowing/respiratory effort (low arousal threshold) are susceptible to increased frequency of awakenings during sleep. At least one third of people with OSA have a low arousal threshold[12].

It was originally thought that an arousal was a protective response that was universally required for an obstructed airway to reopen through a state-related increase in upper-airway dilator muscle activity[49]. However, this notion has been challenged after the discovery that airflow can be restored in OSA in the absence of arousal via protective neuromuscular mechanisms[50]. Indeed, in some cases, arousals can exacerbate detrimental cyclical breathing patterns in OSA, as the instability of sleep onset, with its associated reduction in ventilatory drive, is propagated by recurrent arousals in those with low arousal thresholds[48].

Within an individual, arousal tends to occur at a relatively constant level of negative intrathoracic pressure. Approximately 30 to 50% of patients with OSA exhibit arousals in response to very small changes (0 to -15cmH_2O) in negative intrathoracic pressure. A low arousal threshold phenotype is likely to be important in the pathogenesis of OSA in most non-obese people with OSA[51].

There are three main pathways by which a low arousal threshold contributes to OSA. First, arousals prevent the progression of sleep into deeper stages where respiratory control is more stable[52]. Respiratory events occur less frequently in stage three ("deep") sleep[53]. Deep sleep is associated with a transient increase in the arousal threshold, and increased genioglossus muscle activity[41,54]. Frequent arousals, irrespective of cause, prevent the progression from lighter N1 and N2 sleep into deeper more stable N3 (slow wave) sleep[50]. Second, low arousal threshold reduces the opportunity

for upper-airway dilator muscle activation. As airway obstruction increases, there is a build-up of respiratory stimuli that increases drive to the pharyngeal dilator muscles. However, the arousal threshold is also sensitive to these inputs. People with a low arousal threshold typically experience arousal before the pharyngeal dilators receive sufficient drive to activate and re-establish adequate airway patency[55]. Third, arousals can trigger events that lead to ventilatory instability. Cortical arousals cause sudden increases in minute ventilation. Upon return to sleep, the increased ventilation can drive arterial carbon dioxide (CO_2) below the apnea threshold, resulting in central apnea and ventilatory control instability[48,56]. Nonetheless, while continual cortical arousals to minor airway narrowing/blood gas disturbances can perpetuate OSA severity, arousal also serves a vital protective role to rapidly restore airflow during more severe breathing disruptions[50]. Thus, in the anesthetized state, suppression of arousal mechanisms requires careful monitoring until consciousness and protective arousal responses are restored.

Loop gain

Ventilatory drive during sleep is highly dependent on blood CO_2 levels. The ventilatory response to fluctuations in CO_2 varies between individuals. In individuals with unstable or overly sensitive responses, OSA may occur[57]. In engineering, the sensitivity of a system controlled by feedback loops that modulate output is known as loop gain. Regarding ventilatory control, loop gain is defined as the ratio of the ventilatory response to a disturbance, e.g., a rise in arterial CO_2 tension. When the response is out of proportion to the stimulus, e.g., excessive hyperventilation that overcompensates for a small change in CO_2, loop gain is high. High loop gain systems are prone to oscillations and are inherently unstable, as they predispose to repetitive fluctuations of CO_2 levels between hyperventilation and the apnea threshold. Indeed, high loop gain contributes to OSA in several ways. First, ventilatory overshoot may cause rapid, large negative inspiratory pressures that increase suction forces within the pharyngeal airway in excess of levels to which the upper airway dilators can adequately respond[13]. Second, oscillations in ventilation can lower the drive to the upper airway dilators during periods of decreased ventilation. Thus, a mismatch between pharyngeal dilator muscle activity and upper airway resistance may occur, resulting in airway collapse[12]. Over one-third of patients with OSA have high loop gain. In OSA patients with only mild to moderately impaired upper airway anatomy, high loop gain plays an important role in disease pathogenesis[12,58].

Potential pharmacological approaches that target the upper airway dilator muscles

Neurotransmitters: possible treatment targets

Sleep-related reductions in pharyngeal muscle activity occur due to changes in neuromodulator inputs across sleep-wake states. Of the many pharyngeal dilator muscles, genioglossus, innervated by the hypoglossal nerve, plays a critical role in responding to respiratory stimuli during sleep. Genioglossus muscle activity is stimulated by endogenous noradrenergic, glutamatergic, and serotonergic inputs to the hypoglossal motor pool during wakefulness[59]. Withdrawal of these excitatory inputs leads to reduced genioglossus activity in sleep. The effect of individual neurotransmitters on genioglossus activity is also influenced by sleep stage. Recent animal studies highlight the critical role of noradrenergic and antimuscarinic processes in pharyngeal muscle control during sleep[60,61]. These studies indicate that loss of noradrenergic activity is the major mechanism responsible for sleep-related pharyngeal muscle hypotonia during non-rapid eye movement (NREM) sleep[60]. A lack of reduction in muscarinic activity further contributes to atonia during rapid eye movement (REM) sleep[61]. These findings suggest that medications targeting noradrenergic processes during NREM sleep and antimuscarinic processes during REM sleep may reduce OSA severity by augmenting pharyngeal dilator muscle activity.

The importance of these mechanisms in humans was confirmed by the recent findings of Taranto-Montemurro and colleagues where the selective noradrenaline reuptake inhibitor atomoxetine (80mg) combined with the antimuscarinic agent oxybutynin (5mg) reduced the AHI by ~60% and improved nadir overnight oxygen saturation from ~85% to the high 90's compared with placebo[62]. These beneficial effects were driven by a three-fold improvement in pharyngeal muscle responsiveness and a reduction in loop gain (improved respiratory control)[63]. The wake promoting effects of atomoxetine also modestly increased the propensity for awakening during respiratory events (lowered the respiratory arousal threshold)[63]. However, unlike the animal data, reductions in OSA severity did not occur when either atomoxetine or oxybutynin were administered alone[62]. An alternative combination of noradrenergic and antimuscarinic agents, reboxetine (4mg) and hyoscine butylbromide (20mg), improved upper airway stability during sleep in healthy adults[64], and reduced the AHI via increased tonic genioglossus muscle activity and reductions in loop gain in 12 people with OSA[65]. Unlike oxybutynin, hyoscine butylbromide minimally crosses the blood-

brain barrier[66]. Thus, the detected reductions in OSA severity with reboxetine and hyoscine butylbromide may have been predominantly driven by reboxetine. Reboxetine in combination with oxybutynin reduces OSA severity[67], however, the effects of reboxetine alone have not been investigated. Accordingly, the first project in this PhD will assess the effects of reboxetine alone and in combination with oxybutynin on OSA severity (primary outcome), OSA pathophysiological mechanisms, and effects on next day sleepiness and alertness (secondary outcomes).

OSA in the postoperative period

OSA is a common comorbidity in people undergoing surgical procedures, with rates estimated to range from 25% in those undergoing elective surgery, up to as high as 91% in those undergoing bariatric surgery[68,69]. OSA is an important risk factor for adverse postoperative outcomes including cardiac complications, opioid-induced ventilatory depression, and unplanned intensive care unit transfers[70-72]. Peak OSA worsening tends to occur on postoperative night 3, which coincides with the return of rapid-eye movement (REM) sleep (when OSA tends to be worse) that is suppressed in the first two nights after surgery[73]. By this time, many surgical patients have been discharged home, are unmonitored, and are not receiving any specific OSA treatment. Due to the risks of increased OSA severity in the days following upper airway surgery for OSA, it is recommended by society guidelines that CPAP users continue CPAP from night one postoperatively[74]. However, postoperative pain and swelling can render CPAP intolerable[1], and in the case of those undergoing upper airway surgery for OSA, many have opted for surgical treatment due to unwillingness or inability to use CPAP. If OSA pharmacotherapy is proven to be even partially effective, it could offer a new approach to OSA treatment in the postoperative setting.

The postoperative period involves exposure to risk factors that may worsen OSA severity. The effect of postoperative risk factors on OSA endotypes is however highly variable[2]. Few studies have directly assessed the impacts of risk factors on OSA endotypes in the postoperative setting, but hypotheses can be made based on detailed physiological studies conducted outside of surgical settings.

Upper airway collapsibility

Upper airway collapsibility is highly susceptible to postoperative risk factors. The use of CPAP is often impaired due to the presence of tubes at the nose or mouth (such as nasogastric tubes) that either prevent CPAP use or cause air leak. CPAP may be intolerable after upper airway surgery due to pain, swelling or hematomas. Indeed, CPAP compliance after surgery is only 45%[75]. Upper airway structures, in particular the epiglottis, are susceptible to dysfunction related to opioids and other central nervous system (CNS) depressants, which could affect CPAP tolerance although this has not been formally assessed[76].

Postoperative rostral fluid shifts in the supine position can increase upper airway collapsibility[77]. This is particularly relevant in post-surgical patients who have underlying fluid overload states (e.g., cardiac failure or renal impairment) or those who must remain supine for prolonged periods of time. Lower-limb compression devices for deep vein thrombosis prevention may even increase upper airway collapsibility via rostral fluid shifts, although this is not likely to be a major factor in most patients[78].

Postoperative opioid administration most likely worsens upper airway collapsibility, although there is conflicting evidence and the studies that have investigated relationships between opioids and upper airway collapsibility in the perioperative period have not used the gold-standard Pcrit method[79-81]. In one small, detailed physiology study that measured Pcrit in healthy individuals, naloxone infusion reducedairway collapsibility, suggesting a possible link between opioids and upper airway collapsibility, although in 21 healthy men with OSA, a single 40mg dose of slow-release morphine prior to sleep did not change Pcrit versus placebo[82,83]. Based on limited evidence, it seems likely that opioids, especially in high doses or when combined with other CNS depressants, may worsen postoperative upper airway stability.

Pharyngeal dilator muscles

Few studies have directly assessed dilator muscle responses to opioids. Animal studies demonstrate opioid-induced dilator muscle impairment[84,85], however the very-limited evidence in humans suggests that genioglossus muscle responsiveness in unaffected, at least to single doses of opioid[83]. The effects of opioids in repeated doses and in combination with other medications used

perioperatively has not been studied. Incomplete reversal of neuromuscular blocking agents has negative dilator muscle consequences. Partial neuromuscular blockade impairs genioglossus function, and residual blockade is a clearly defined risk factor for upper airway obstruction-related critical respiratory events in the postoperative setting[86,87].

The effects of noradrenergic agents alone or in combination with antimuscarinics have not been assessed in postoperative patients with OSA. However, given the promising results seen in recent OSA pharmacotherapy studies, these agents would be expected to improve OSA severity after surgery and hold promise as potential treatment alternatives in those unable to tolerate CPAP in the first few days after surgery. This background, combined with the publication/findings of chapter two, provides the rationale for a randomized clinical trial of reboxetine in people with OSA after head and neck surgery (currently underway- ClinicalTrials.gov NCT05978505). However, while complimentary, this project is beyond the scope of this PhD.

Loop gain

Morphine reduces the ventilatory response to CO_2[88,89]. In the setting of OSA, low doses of opioids do not worsen disease severity in those with higher awake CO_2 ventilatory recruitment thresholds[90], and reduce the ventilatory response to hypercapnia when arterial CO_2 tension rises during sleep[83]. However, OSA patients with lower daytime CO_2 ventilatory recruitment thresholds are at risk of increased hypoxia during sleep due to a single dose of morphine[90]. Thus, in postoperative patients with mild-to-moderate OSA and unstable respiratory control (i.e., high loop gain), opioids may not be of particular concern in terms of hypoxic events, but care must be taken in those patients who have lower loop gain or other characteristics that raise the risk of opioid-related adverse outcomes.

Supplemental oxygen therapy administered postoperatively in those with high loop gain endotypes would be expected to stabilize ventilatory control instability and thus improve OSA severity[18,91]. In patients with OSA (without knowing the underlying endotype contributions), postoperative oxygen supplementation improves AHI and oxygen parameters, but does increase the risk of carbon dioxide retention[92].

Arousal threshold

The CNS depressant properties of opioids pose a risk for postoperative ventilatory failure. However, if the predominant mechanism contributing to OSA in an individual is a low arousal threshold, then the CNS depressant effect of modest doses of opioids may prevent awakenings to minor respiratory stimuli via a raised arousal threshold and thus paradoxically lower OSA severity. This has not been formally assessed in postoperative patients, and there is clearly a risk of worsening hypoxia and OSA severity in postoperative patients with normal or high arousal thresholds, or if doses high enough to cause suppression of ventilation are administered.

Project one rationale

Reboxetine in combination with oxybutynin has been shown to reduce OSA severity. It is unclear if reboxetine as a single agent may also be effective in treating OSA. Therefore, in study one, I will conduct a three-way placebo controlled randomized controlled trial to assess the effects of reboxetine, reboxetine plus oxybutynin, and placebo on OSA severity over a single night. I hypothesize that reboxetine alone will reduce the AHI, and that the addition of oxybutynin will not cause major improvements above those caused by reboxetine alone.

Chronic Obstructive Pulmonary Disease

Overview and prevalence

Chronic obstructive pulmonary disease (COPD) is a common airways disease characterized by incompletely reversible airflow obstruction and persistent respiratory symptoms, such as breathlessness, cough, and excess sputum production[93]. COPD is usually caused by exposure to gases or noxious particles such as those present in cigarette smoke. COPD is a leading cause of morbidity and mortality worldwide, contributing to significant economic and social burden[94,95]. The prevalence of COPD in Australia is 7.5% in people aged ≥ 40 years, and 29.2% in those aged ≥ 75 years. COPD is a cause of significant extrapulmonary (systemic) effects such as weight loss, nutritional abnormalities and skeletal muscle dysfunction, and is associated with high rates of other chronic health conditions including cardiovascular disease, osteoporosis, depression, anxiety, diabetes mellitus, lung cancer, and sleep disorders including obstructive sleep apnea[96-101].

COPD and sleep

Sleep disturbance in COPD is common. As lung disease severity increases, there is an increase in self-reported nocturnal awakenings[102]. Independent of disease severity, sleep disturbance in COPD is associated with COPD exacerbations, emergency department visits, and overall survival[103,104]. Several factors are known to influence sleep quality in COPD, including changes in sleep architecture, persistence of COPD symptoms, and pathophysiological changes that occur in the supine position. The characteristic changes in sleep architecture seen in people with COPD include reductions in total sleep duration, sleep efficiency, amount of rapid eye movement (REM) and stage 3 (N3) sleep, and increased sleep latency, arousals, and wake after sleep onset (WASO)[105]. Nocturnal symptoms including cough, dyspnea, and sputum production can delay sleep onset and disturb sleep. Most people with COPD (~78%) experience nocturnal symptoms[106]. The supine position can worsen pulmonary ventilation/perfusion (V/Q) relationships, leading to nocturnal hypoxemia and hypercapnia[107]. COPD patients with dyspnea in the supine position (orthopnea) also tend to develop supine expiratory flow limitation and do not develop a supine-related increase in inspiratory capacity, as opposed to non-flow limited counterparts[108]. People with COPD and gas trapping (raised residual volume to total lung capacity ratio, RV/TLC) also tend to have higher loop gain during sleep, which could theoretically contribute to sleep disturbance via sleep-disordered breathing, although the links between lung volumes, COPD, and OSA endotypes have only been investigated in one small study to date[109]. Additionally, there is a negative association between the degree of gas trapping on computerized tomography and AHI, so the mechanical effects of hyperinflation on the upper airway are potentially protective in OSA[110]. Nevertheless, comorbid sleep disorders including insomnia and OSA are common in COPD populations. The prevalence of OSA among patients varies across studies but is likely to be similar to the general population in the case of mild COPD[111], and up to approximately 65% in those with moderate to severe OSA[112].

The use of slow-release morphine in COPD

Breathlessness is a common consequence of COPD. As such, reduction of dyspnea is a key target of COPD treatment[93]. However, despite the optimal use of inhaled medications and non-pharmacological therapies such as pulmonary rehabilitation, many patients with COPD continue to experience refractory, disabling breathlessness, termed the *chronic breathlessness syndrome*[113].

In 2019, the Therapeutic Goods Administration of Australia approved sustained-release oral morphine for the treatment of chronic breathlessness.

The mechanism of action of opioids in breathlessness

The mechanisms underlying breathlessness in COPD are complex and involve all the main components of control of breathing, including perception of breathing, central (efferent) respiratory activity, respiratory muscle function, ventilation, and gas exchange[114]. It is likely that the predominant mechanism by which opioids act to improve breathlessness is through their effect on *perception* of breathlessness, and potentially via reduced respiratory drive. In people with moderate to severe COPD, reversal of endogenous opioids with the opioid receptor antagonist naloxone leads to increased perceived breathlessness during resistive load breathing[115] and exercise[116]. The insula, dorsal anterior cingulate cortex, amygdala, and medial thalamus are all involved in processing the perception of breathlessness[117], and are all densely innervated with opioid receptors[118]. Direct opioid effects on the brainstem respiratory centres to hypoxia and hypercapnia reduce respiratory drive and likely reduce corollary discharge to the areas of the cortex involved in perception[119,120]. Opioids also appear to act on the frontal association cortex, an area of the brain involved in creating expectations and beliefs (psychological 'priors') based on previous experiences, such as anticipatory fear from previous unpleasant breathlessness, which moderate the perception of incoming sensory breathing information by interacting with the limbic system including the amygdala, hippocampus, and cingulate cortex[121]. Functional MRI (fMRI) in healthy volunteers indicates that the opioid remifentanil depresses anticipatory activity in the limbic system and reduces breathlessness unpleasantness[122]. However, there is likely a complex interaction between psychological priors and baseline anxiety or depression, as reduced affect reduces opioids' effect on breathlessness[123]. This finding may be a significant contributor to the heterogeneous responses to morphine that are observed in breathless people with COPD.

Heterogeneous responses to morphine

There is significant interindividual variability in response to morphine in breathless people with COPD. Several possible mechanisms explaining this heterogeneity have been proposed, although a common limitation of the few trials that have attempted to explain this issue is the inclusion of people with breathlessness of differing etiologies, rather than just COPD *per se*[124-126].

One possible reason is interindividual variability in single nucleotide polymorphisms (SNPs) related to opioid receptors, signaling, or pain modulation. An exploratory study of 2294 people over 18 years treated for pain related to cancer/cancer treatment found a significant association between increased breathlessness intensity and a SNP on the HTR3B gene (rs7103572 SNP), which is present in 8.4% of the population[126]. It is unknown if this SNP significantly relates to variability in COPD-only cohorts.

Interindividual differences in anxiety and depression levels may also contribute to morphine response. Evidence suggests that negative affect reduces the effect of morphine on breathlessness[123]. This observation is similar to the associations between negative affect and reduced opioid efficacy in chronic pain[127,128]. However, the current data on the potential links between mood and morphine response in COPD are limited, and larger studies that specifically address this link are required before firm conclusions can be made.

Younger age and higher baseline breathlessness intensity have been associated with positive response to opioids in chronic breathlessness. However these predictors were derived from a pooled analysis of four clinical trials involving both morphine and oxycodone, and differing etiologies of breathlessness rather than just COPD[124], and as such, it cannot currently be stated that these are definite predictive factors of morphine response in COPD. In a COPD-only cohort, a secondary analysis of the intervention arm of a randomized controlled trial showed that worse baseline breathlessness and higher body mass index were associated with clinically meaningful improvements in breathlessness with low-dose morphine[129]. However, to date, studies specifically designed to assess interindividual heterogeneity in breathlessness response to low-dose morphine have not been conducted, and as such, further studies assessing predictors of response specifically to low-dose morphine in COPD-only cohorts are required.

The effects of morphine on sleep

Morphine causes changes in sleep architecture and can alter ventilatory control mechanisms leading to ataxic breathing and central sleep apnea.

Sleep architecture

The effects of morphine on sleep architecture are mainly based on studies of healthy participants without significant comorbidities. In healthy individuals administered acute doses of intravenous morphine at a dose of 0.1mg/kg prior to sleep, slow wave and REM sleep stages are reduced[130]. Reduction in REM sleep is also seen after pre-sleep intramuscular administration of morphine at a dose of 30mg[131]. Oral morphine at a dose of 15mg prior to sleep causes reduced slow wave sleep as well as increased wake after sleep onset[132]. There are limited data on the changes to sleep architecture caused by morphine in specific disease states.

Respiratory rhythm

Opioids, including morphine, can induce a characteristic breathing pattern of irregular respiratory rate and variable tidal volume during sleep termed ataxic breathing[133]. The rhythm-generating parts of the brain, located in the pons and medulla, are sensitive to the effects of opioids[134]. The pre-Bötzinger complex, located in the ventrolateral medulla, is active during inspiration and is the main area responsible for respiratory rhythm generation[135]. Inspiratory drive generated within the pre-Bötzinger complex is inhibited by opioids[136]. The nearby retro-trapezoid and parafacial respiratory group, which is active during expiration and couples with the pre-Bötzinger complex to generate respiratory oscillations, is not sensitive to opioids[137]. Areas in the pons that influence breathing, such as the Kölliker-Fuse nucleus that moderates the transition from inspiration to expiration, are also affected by opioids[138]. In rats, opioids arterially perfused directly into the Kölliker-Fuse/parabrachial complex in the pons reduced the respiratory rate at low doses and caused apneas with brief periods of apneustic breathing at higher doses[139]. Thus, opioids including morphine affect respiratory rhythm generation during sleep.

Ventilatory chemoreflexes and the apneic threshold

Central sleep apnea (CSA) is a cessation of airflow without respiratory effort lasting greater than 10 seconds. It is commonly seen in the context of chronic, rather than acute opioid use[140]. The central ventilatory chemoreceptors (based in the brainstem, sensitive to changes in blood pH and $PaCO_2$) and peripheral chemoreceptors (located predominantly in the carotid bodies, sensitive to changes in PaO_2) input respiratory drive signals to the respiratory motor areas of the brainstem[141,142]. The exact mechanisms underlying opioid-induced CSA are not clear, but limited

evidence suggests that blunted hypercapnic and elevated hypoxic ventilatory responses play a role in developing ventilatory overshoot (hyperventilation) that cyclically drives $PaCO_2$ below the apneic threshold[143-145].

Morphine in sleep and COPD

At present, there are no objective data on the sleep-related effects of morphine in people with COPD. However, subjective data suggest that morphine may paradoxically improve sleep quality and perception of breathlessness in people with COPD. In a secondary analysis of data from 38 people with refractory breathlessness (33 of whom had COPD) taking oral sustained-release morphine 20mg daily for four days versus placebo, participants in the morphine arm described significantly less sleep disruption due to breathlessness, and were less likely to report poor sleep quality compared to placebo[146]. Those who experienced improved breathlessness during the four-day period were also more likely to report better sleep quality in the morphine arm. Although firm conclusions cannot be drawn from this study, the findings suggest that low-dose morphine in breathless people with COPD may improve sleep quality, possibly by reducing sleep fragmentation, and that improving sleep quality may improve perception of breathlessness. The percentage of participants with OSA/COPD overlap in this study was not known.

In a separate study of 21 men with OSA (none had COPD) that assessed OSA endotypes during a sleep study after a single oral dose of 40mg SR morphine, there were no significant changes in upper airway collapsibility, pharyngeal muscle responsiveness, or arousal threshold, but there were significant reductions in loop gain and the ventilatory response to hypercapnia[83]. In the context of COPD-OSA overlap, morphine could therefore potentially improve OSA severity via reductions in loop gain in COPD-OSA overlap syndrome, thus improving perceived sleep quality and next-day perception of breathlessness. However, morphine-induced CSA would be expected to lead to worse quality of sleep in other people with COPD, such as those without OSA and a high loop gain endotype. Therefore, the heterogeneity in breathlessness response to morphine may, at least to a degree, be associated with interindividual changes in sleep fragmentation and sleep disordered breathing in COPD patients prescribed oral morphine.

Project two rationale

The potential adverse effect profile of low-dose morphine on important outcomes including daytime sleepiness remains unclear[147-149]. Additionally, there is insufficient evidence to assess the potential relationship between sleep quality and perception of breathlessness. Project two analyzed data from a multisite, phase III, double-blind, parallel-arm placebo-controlled dose increment randomized trial of regular low-dose slow-release morphine for refractory breathlessness in people with COPD[150]. Specifically, I assessed participant responses to validated questionnaires on sleepiness and subjective sleep quality to investigate the effects of morphine on sleep in breathless people with COPD.

Project three rationale

There is currently no objective evidence on the effects of morphine on sleep or next day alertness in people with COPD, or the potential relationship between sleep and daytime breathlessness. Despite limited evidence supporting its effectiveness, low-dose slow-release morphine is prescribed in the outpatient setting for breathlessness[147]. Investigating the effects of clinically relevant doses of morphine during sleep in this group of people who are conceptually especially vulnerable to the potential adverse effects of opioids on breathing will provide important safety data and insight into the variables that contribute to the sensation of breathlessness in COPD.

I hypothesize that morphine will improve sleep efficiency through reductions in sleep fragmentation (arousals) and through reductions in ventilatory sensitivity (reduced loop gain). I also hypothesize that morphine will have no significant effect on next-day alertness, but will reduce the respiratory rate and cause increased transcutaneous CO_2 levels during sleep. It is expected that there will be heterogeneity in participant response to morphine (in terms of breathlessness and sleep parameters). Potential predictors of increased effectiveness of low-dose morphine on breathlessness will be explored, including baseline anxiety levels, age, BMI, and differences in morphine pharmacokinetics.

Chapter Two: The norepinephrine reuptake inhibitor reboxetine alone reduces obstructive sleep apnea severity: A double blind, placebo controlled, randomized, cross-over trial

This chapter has been published[151]:

Altree TJ, Aishah A, Loffler K, Grunstein RR, Eckert DJ. The norepinephrine reuptake inhibitor reboxetine alone reduces obstructive sleep apnea severity: a double blind, placebo controlled, randomized, cross-over trial. *Journal of Clinical Sleep Medicine* 2023;19(1):85-96

Abstract

Study Objectives: Recent findings indicate that noradrenergic and muscarinic processes are crucial for pharyngeal muscle control during sleep. However, to date, reductions in obstructive sleep apnea (OSA) severity have only been detected when noradrenergic agents are combined with an antimuscarinic. Accordingly, this study aimed to determine if reboxetine alone and combined with oxybutynin reduces OSA severity. The pathophysiological mechanisms underpinning the effects of these agents were also investigated via endotyping analysis.

Methods: 16 people (6 women) with OSA completed three polysomnograms (~1-week washout) according to a double-blind, placebo-controlled, three-way crossover design across two sites. Single doses of 4mg reboxetine, placebo, or 4mg reboxetine+5mg oxybutynin were administered before sleep (order randomized).

Results: Reboxetine reduced the apnea/hypopnea index (AHI-primary outcome) by 5.4 [95% CI -10.4 to -0.3] events/h, *P*=0.03 (-24±27% in men; -0.7±32% in women). Oxybutynin did not cause additional reductions in AHI. Reboxetine alone reduced the 4% oxygen desaturation index by (mean±SD) 5.2±7.2 events/h and reboxetine+oxybutynin by 5.1±10.6 events/h versus placebo, *P*=0.02. Nadir oxygen saturation also increased by 7±11% with reboxetine and 5±9% with reboxetine+oxybutynin versus placebo, *P*=0.01. Mechanistically, reboxetine and reboxetine+oxybutynin improved pharyngeal collapsibility and respiratory control (loop gain). Larger reductions in AHI with reboxetine in men were associated with higher baseline loop gain.

Conclusions: These findings show the first evidence that reboxetine alone reduces OSA severity. The data provide novel insight into the role of norepinephrine reuptake inhibitors on upper airway stability during sleep and are important to inform future pharmacotherapy development for OSA.

Introduction

Global estimates indicate that nearly one billion people have obstructive sleep apnea (OSA).[4,152] OSA is characterized by repetitive narrowing and partial or complete collapse of the pharyngeal airway, hypoxia, hypercapnia and frequent arousals during sleep. Untreated OSA is associated with a range of adverse health outcomes including cardiovascular,[5,6] neurocognitive,[7,8] and metabolic disease.[9] Continuous positive airway pressure (CPAP) is efficacious and is currently the first-line treatment for moderate-severe OSA. However, 46 to 83% of those prescribed CPAP are not adherent to therapy.[10] Other therapies such as mandibular advancement splints have better adherence but variable and unpredictable efficacy.[11] Thus, there is an urgent need to develop new therapies to treat this highly prevalent chronic health condition.

Sleep-dependent reductions in pharyngeal dilator muscle control combined with vulnerable upper airway anatomy are key contributors to OSA pathophysiology.[12] Recent animal studies highlight the critical role of noradrenergic and antimuscarinic processes in pharyngeal muscle control during sleep.[60,61] These studies indicate that loss of noradrenergic activity is the major mechanism responsible for sleep-related pharyngeal muscle hypotonia during non-rapid eye movement (NREM) sleep.[60] Muscarinic activity further contributes to atonia during rapid eye movement (REM) sleep.[61] These findings suggest that medications targeting noradrenergic processes during NREM sleep and antimuscarinic processes during REM sleep may reduce OSA severity by augmenting pharyngeal dilator muscle activity.

The importance of these mechanisms in humans was supported by the recent findings of Taranto-Montemurro and colleagues where the selective noradrenaline reuptake inhibitor atomoxetine (80mg) combined with the antimuscarinic agent oxybutynin (5mg) reduced the apnea/hypopnea index (AHI) by ~60% and improved nadir overnight oxygen saturation from ~85% to the high 90's compared with placebo.[62] These beneficial effects were driven by a three-fold improvement in pharyngeal muscle responsiveness and a reduction in loop gain (improved respiratory control).[63] The wake promoting effects of atomoxetine also modestly increased the propensity for awakening during respiratory events (lowered the respiratory arousal threshold).[63] However, unlike the animal data, reductions in OSA severity did not occur when either atomoxetine or oxybutynin were administered alone.[62] An alternative noradrenergic agent, reboxetine (4mg), combined with

oxybutynin (5mg) administered orally daily for seven days was recently shown to cause a median reduction in AHI of ~60% in sixteen people with severe OSA.[67] A single dose of reboxetine (4mg) combined with an alternative antimuscarinic, hyoscine butylbromide (20mg), improved upper airway stability during sleep in healthy adults,[64] and reduced the AHI via increased tonic genioglossus muscle activity and reductions in loop gain in 12 people with OSA.[153] However, hyoscine butylbromide minimally crosses the blood-brain barrier, so the reduction in OSA severity with reboxetine and hyoscine butylbromide may have been predominantly driven by reboxetine alone.[66] However, no studies have investigated the effects of reboxetine alone. Accordingly, this study aimed to determine the acute effects of a single pre-sleep dose of reboxetine alone (primary outcome) and in combination with oxybutynin on OSA severity, and on next day sleepiness and alertness (secondary outcomes). In addition, we also explored the effects of these agents on OSA pathophysiological mechanisms.

Methods

Participants

People with OSA (AHI ≥10 events/h confirmed via in-laboratory polysomnography within the past 12 months) aged between 18 and 65 years and not currently on OSA treatment were eligible to participate. Individuals were excluded if they used antidepressants, strong cytochrome P450 3A4 and 2D6 inhibitors, any medication known to influence breathing during sleep or daytime alertness (i.e. hypnotics, respiratory stimulants, antipsychotics, anxiolytics, psychostimulants), were pregnant, smoked >10 cigarettes per day (due to potential sleep disruption effects), had narcolepsy, a clinically significant mood disorder, cardiac disease including uncontrolled blood pressure, significant craniofacial malformation, epilepsy, schizophrenia, previous diagnosis of insomnia, history of benign prostatic hyperplasia or urinary retention, narrow angle glaucoma, or known allergy to reboxetine or oxybutynin. Current shift workers were also excluded. Participants were asked to abstain from alcohol on the days of the study and limit caffeine intake to a maximum of 400mg per day, and none in the three hours prior to bedtime. Participants were enrolled from sleep medicine clinics, a database of previous research participants and a clinical trial matching agency (HealthMatch). No participant had taken reboxetine previously. The study was approved by Bellberry Human Research Ethics Committee (2019-12-1081-A-1) and participants provided informed written consent prior to enrolment. The research was performed in accordance with

relevant guidelines and regulations including the Declaration of Helsinki and all local Human Research Ethics Committee requirements.

Protocol

Three overnight sleep studies were performed with an approximately 1-week washout between each visit according to a double-blind, randomized, placebo-controlled, three-way, crossover design (Figure 2.1). This was a multicentre study with two recruitment and data collection sites: 1) Adelaide Institute for Sleep Health, Flinders University, Adelaide, Australia and 2) the Woolcock Institute for Medical Research, Sydney, Australia. At each of the three visits, participants received oral reboxetine alone (4mg) or reboxetine (4mg) with oxybutynin (5mg) or placebo in randomized order immediately before bedtime. Study medications were prepared by Optima Ovest and were placed in identical capsules that could not be identified by study personnel or participants. The study pharmacist prepared the randomization code in blocks of 4. All participants, investigators, and outcome assessors were blinded to the treatment allocation. Bedtime was kept constant between study visits and participants were given an 8-hour sleep opportunity on each occasion. The predefined primary endpoint was the AHI (events/h sleep) using 3% desaturation criteria (AHI3). Secondary outcomes included other polysomnography outcomes such as sleep efficiency, the arousal index, measures of hypoxemia, snoring using a calibrated sound meter, AHI using the 4% desaturation criteria (AHI4) and markers of next day sleepiness and alertness. All data analyses were performed before unblinding of the intervention allocation. The protocol was prospectively registered on the Australian New Zealand Clinical Trials Registry (ACTRN12620000662965).

Measurements and equipment

Blood pressure and heart rate were measured three times each in the evening and the following morning during each visit. A standard clinical montage was used during overnight polysomnography including nasal flow, thermistor, respiratory bands, oximetry, chin and leg EMG, EEG and EOG (Grael 4K PSG:EEG, Compumedics, Abbotsford, Australia).[154] Participants completed a 30-min simulated driving task (AusEd Driving Simulator)[155] approximately 30 minutes after waking at each visit to assess next day alertness. Subjective sleepiness was measured

approximately one hour after waking using the Karolinska Sleepiness Scale (KSS)[156] and the Leeds Sleep Evaluation Questionnaire (LSEQ) was administered.[157]

Data analysis

Sleep staging, arousals and respiratory events were scored at each site using standard American Academy of Sleep Medicine guidelines[158] by an experienced sleep technologist blinded to the study intervention. Hypopneas were defined as a reduction in flow of 30% or more from baseline lasting at least 10 seconds, associated with either an arousal from sleep or an oxyhemoglobin desaturation ≥3% (AHI3) or ≥4% (AHI4).

OSA endotypic traits to explore pathophysiological mechanisms were quantified using a validated custom-designed algorithm from the polysomnography recordings (MATLAB; MathWorks).[16,17] Ventilation was estimated using the square root transform of the nasal pressure signal (tidal volume x respiratory frequency). This was integrated breath-by-breath to provide a time series of ventilation data that was normalized (mean ventilation =1.0, apnea= 0) for analysis as per the methodology described by Terrill et al and Sands et al.[16,17] The following traits were measured on each night during non-REM sleep in supine and lateral positions as a percentage of eupneic ventilation ($\dot{V}$eupnea):

- mean pharyngeal collapsibility ($\dot{V}$passive): the estimated average ventilation during sleep at eupneic drive when the pharyngeal muscles are relatively passive.[159] A higher value represents a less collapsible upper airway;
- nadir pharyngeal collapsibility ($\dot{V}passive_{min}$): the estimated ventilation when the pharyngeal muscles are at their most hypotonic level/the airway is most collapsible, quantified at the lowest estimated decile of ventilatory drive from the $\dot{V}$passive measures (analogous to the passive critical closing pressure of the upper airway).[160] A higher value represents a less collapsible airway at the point of highest likelihood of collapse;
- pharyngeal muscle recruitment ($\dot{V}$active): the estimated ventilation at maximum ventilatory drive. A higher value indicates increased muscle recruitment;
- pharyngeal muscle compensation ($\dot{V}$comp): the estimated change in ventilation that accompanies an increase in ventilatory drive, i.e., the ventilatory equivalent of the

active minus passive critical closing pressures measured as the difference between $\dot{V}$active and $\dot{V}$passive. A higher value represents greater muscle compensation;

- the ventilatory response to arousal (VRA): the estimated ventilatory overshoot during a transient cortical arousal from sleep. A higher value represents greater ventilatory overshoot and increased propensity for subsequent respiratory instability;
- ventilatory control stability (loop gain): LG_1, breathing response to a 1 cycle per minute reduction in ventilation and LGn, including circulatory delay effects. Higher values represent greater ventilatory control instability;
- respiratory arousal threshold: the estimated respiratory drive that causes an arousal from sleep. A higher value represents a larger fall in ventilation that can be sustained before an arousal from sleep occurs.

The hypoxic burden was also quantified using previously described methodology.[161]

Statistical Analysis

We performed a power analysis based on detection of a change in AHI of 9 events/h using an alpha of 0.05 and a power of 0.8. We determined the minimum number of participants required was 15. Note: that based on our previous reboxetine and hyoscine butylbromide study[153] we anticipated a larger effect size. However, we elected to use a more conservative effect size estimate in the current study. One-way repeated-measures analysis of variance (ANOVA) was used to test for differences in polysomnography parameters, OSA endotypes, and next-day measures of alertness and subjective sleep quality between reboxetine, placebo, and reboxetine+oxybutynin or one-way ANOVA on ranks for non-normally distributed data (according to a Shapiro-Wilk normality test). Where significant main effects were detected, pairwise comparisons were performed using Student-Newman-Keuls post-hoc test or Chi-square tests as appropriate. Post-hoc exploratory analyses to investigate potential sex differences in AHI responses, oxygen parameters, and OSA endotypes were performed using unpaired Students *t*-tests or Mann-Whitney Rank Sum Tests for non-normally distributed data. Polysomnography and endotype data were analyzed with SigmaPlot V14.5 (Systat Software, San Jose, CA, USA). All other analyses were performed using SPSS V25 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA). Statistical significance was inferred when $P<0.05$.

Results

Participants

Data collection for the study was undertaken from June to December 2020. Of 45 potential participants screened, 17 met the inclusion criteria. One was excluded after providing consent due to high blood pressure prior to drug administration on night 1 (Figure 2.1). Data were acquired in all the remaining 16 participants who commenced the study. Data collection was ceased when the prespecified sample size completed the study. On average, the 16 participants who completed all three nights were middle-aged, overweight to obese, had subclinical insomnia (according to Insomnia Severity Index scores collected on night one of the study), did not have significant daytime sleepiness, and had moderate-severe OSA (Table 2.1). Comorbidities and medication use were as expected for a cohort of people with OSA.

Table 2.1: Participant characteristics.

Sex	6 female, 10 male
Age (years)	49 ± 12
BMI (kg/m^2)	30.5 ± 4.7
Neck circumference (cm)	41 ± 4
Waist circumference (cm)	103 ± 12
Comorbidities, N (%)	
Hypertension	5 (31.25)
Hyperlipidemia	3 (18.75)
Type 2 Diabetes Mellitus	3 (18.75)
Hypothyroidism	1 (6.25)
Medications, n (%)	
Proton pump inhibitors	1 (6.25)
Statins	3 (18.75)
Antihypertensives	2 (12.5)
Oral hypoglycemics	1 (6.25)
Thyroid hormones	1 (6.25)
Epworth sleepiness scale (0-24 point scale)	5.5 (3.5 - 7.5)
Insomnia severity index	14.0 (8.0 - 16.5)

Key baseline polysomnography parameters	
AHI (events/h)	32 ± 14
Sleep efficiency (%)	81 (72 – 90)
Non-REM AHI (events/h)	31 ± 16
REM AHI (events/h)	35 ± 15
Nadir overnight oxygen saturation (%)	84 (79 – 89)

Definition of abbreviations: AHI: apnea/hypopnea index, BMI: body mass index, REM: rapid eye movement. Key baseline polysomnographic data were acquired from sleep studies performed prior to study enrolment. Data are presented as mean ± standard deviation or median (interquartile range) as appropriate, unless otherwise indicated.

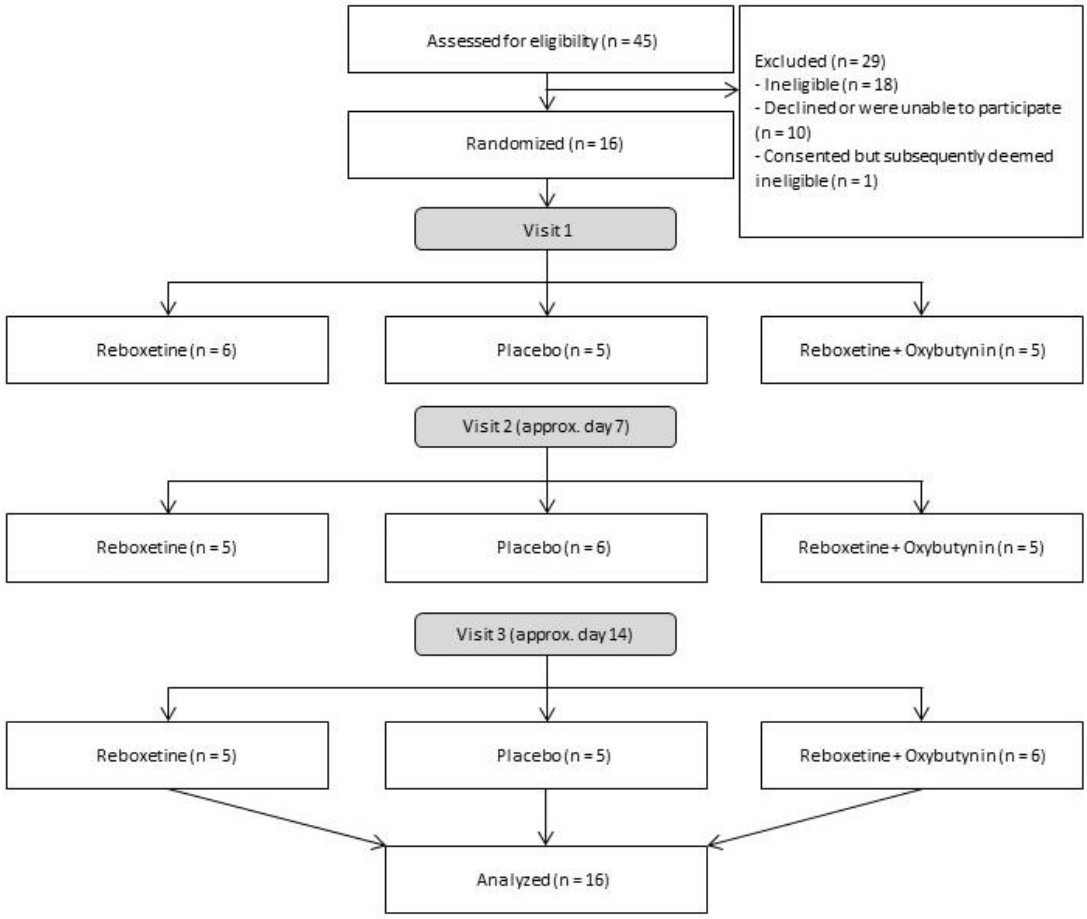

Figure 2.1: Consort flow diagram.

Enrolment and participant flow through the protocol and analysis for this double-blind, randomized, placebo-controlled three-way crossover study.

No serious adverse events were observed during the study. Seven participants reported mild-moderate adverse events related to reboxetine, five reported mild adverse events related to reboxetine+oxybutynin, and one reported a mild adverse event on placebo (Table 2.2). The adverse events recorded were known side effects of either reboxetine or oxybutynin and had no major impact on sleep efficiency (Table 2.3). No adverse event was serious enough to warrant unblinding of the allocation in any participant.

Table 2.2: Adverse events.

	Reboxetine	**Placebo**	**Reb-Oxy**
Total number of adverse events	10	1	9
Participants with ≥ 1 adverse event, n (%)	3 (18.75)	0	3 (18.75)
Total number of serious adverse events	0	0	0
Total number of moderate adverse events	2	0	0
Total number of mild adverse events	8	1	9
Total number of adverse events leading to participant withdrawal	0	0	0
Adverse events by System Organ Class:			
Gastrointestinal			
Abdominal pain	0	0	1
Constipation	1	0	0
Dyspepsia	1	0	0
Nausea	1	0	3
General			
Chills	2	0	1
Nervous System			
Dizziness	1	0	0
Dysgeusia	0	0	1
Headache	0	1	0

Paresthesia	1	0	1
Psychiatric			
Anxiety	1	0	0
Renal			
Urinary hesitancy	2	0	2

Definition of abbreviations: Reb-Oxy: reboxetine plus oxybutynin. Mild adverse event defined as "easily tolerated, causing minimal discomfort, not interfering with activities". Moderate adverse event defined as "sufficient discomfort to interfere with everyday activities".

Table 2.3: Polysomnography parameters.

	Reboxetine	**Placebo**	**Reb-Oxy**	***P* value**
Supine AHI (events/h)	43 ± 20	46 ± 15	42 ± 25	0.578
Supine sleep (%TST)	49 (27 - 80)	52 (31 - 94)	54 (35 - 71)	0.121
NREM AHI (events/h)	31 ± 15	35 ± 17	32 ± 17	0.253
Obstructive apnea index (events/h)	0 (0 - 3)	3 (0 - 10)	0 (0 - 3)	0.072
3% ODI (events/h)	8.9 (2.1 - 21.1)	13.1 (10.1 - 35.5)	13.1 (2.0 - 20.7)	0.029*^
Snoring index (snores/h)[#]	341 ± 179	469 ± 176	252 ± 177	0.001*^
Arousal index (events/h)	33 ± 13	32 ± 12	30 ± 12	0.609
Total sleep time (min)	376 ± 44	391 ± 51	400 ± 38	0.218
Sleep efficiency (%TIB)	79 ± 10	80 ± 10	85 ± 6	0.113
Wake after sleep onset (mins)	90 ± 35	83 ± 42	64 ± 28	0.105
Sleep stage (% of TST)				
N1	25 ± 17	21 ± 11	27 ± 14	0.185

N2	56 ± 16	45 ± 11	56 ± 14	0.003*^
N3	18 ± 8	21 ± 11	16 ± 7	0.100
REM	2 ± 2	13 ± 7	2 ± 4	<0.001*^
Morning measurements				
Heart rate (beats/min)	83 ± 14	69 ± 11	83 ± 13	<0.001*^
Systolic blood pressure (mm Hg)	134 ± 21	134 ± 15	134 ± 17	0.984
Diastolic blood pressure (mm Hg)	89 (83 - 96)	88 (79 - 91)	90 (83 - 97)	0.103

Definition of abbreviations: Reb-Oxy: reboxetine plus oxybutynin; AHI: apnea-hypopnea index; REM: rapid eye movement sleep; NREM: non-rapid eye movement sleep; ODI: oxygen desaturation index; TIB: time in bed; N1: stage 1 sleep; N2: stage 2 sleep; N3: stage 3 sleep; TST: total sleep time. Note AHI values refer to AHI scored using 3% desaturation criteria. Data are presented as mean ± standard deviation or median (interquartile range) as appropriate. *Reboxetine versus placebo pairwise comparison P <0.05. ^Reboxetine-Oxybutynin versus placebo pairwise comparison P <0.05. *[#]n=13. Three participants' snoring data were incomplete and therefore were not included in the analysis.*

Effects of reboxetine and reboxetine-oxybutynin on OSA severity and oxygenation (Figures 2.2 and 2.3).

There was an overall treatment effect on AHI3 (ANOVA P=0.049; Figure 2.2A). Reboxetine alone reduced the AHI3 by 5.4 events/h [95% CI, -10.4 to -0.3], P=0.04, (-8±9 events/h in men from a baseline of 39±18 events/h; -1±9 events/h in women from a baseline of 32±9 events/h) compared to placebo. AHI3 with reboxetine+oxybutynin compared to placebo was not significantly different (4.2 events/h [95% CI, -9.6 to 1.1]; P=0.11, -6±9 events/h in men; -2±12 events/h in women). There was also an overall treatment effect for AHI4 (ANOVA P=0.002; Figure 2.2B). Both reboxetine alone and reboxetine+oxybutynin reduced the AHI4 versus placebo (Figure 2.2B).

Nadir oxygen saturation increased by 7±11% (mean±SD) with reboxetine and 5±9% with reboxetine+oxybutynin versus placebo (Figure 2.3A, ANOVA P=0.013). Reboxetine and reboxetine+oxybutynin both reduced 4% oxygen desaturation index compared to placebo (Figure 2.3B, ANOVA P=0.018). Similarly, the hypoxic burden was reduced with treatment versus

placebo (Figure 2.3C, ANOVA *P*=0.049). Reboxetine and reboxetine+oxybutynin improved the 3% ODI and snoring index versus placebo (Table 2.3).

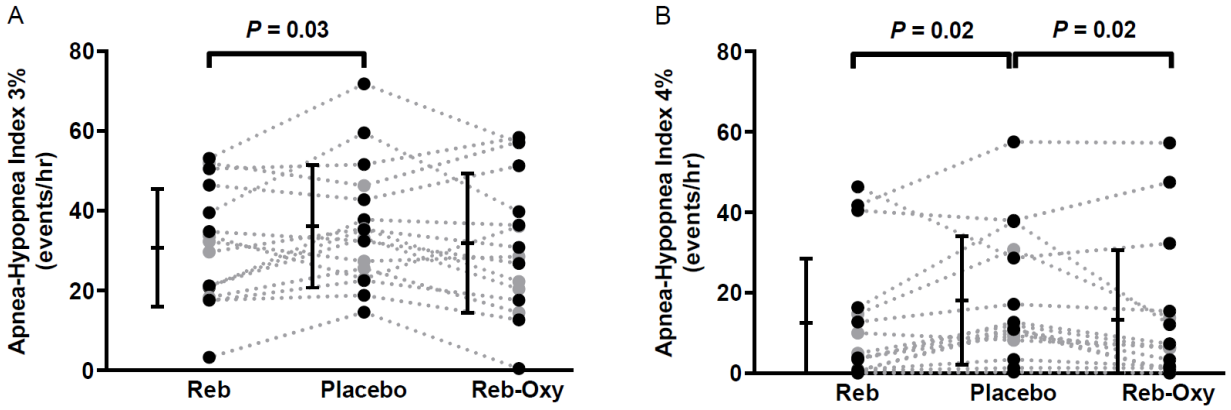

Figure 2.2: Effect of reboxetine (Reb) and reboxetine-oxybutynin combination (Reb-Oxy) on apnea-hypopnea index (AHI).

AHI using the 3% (A) and 4% desaturation criteria (B) are shown. Plots show mean and standard deviation (A) and median and interquartile range (B) plus individual values (gray circles indicate women, black circles indicate men). Significant pairwise comparisons P < 0.05 are indicated above the individual values.

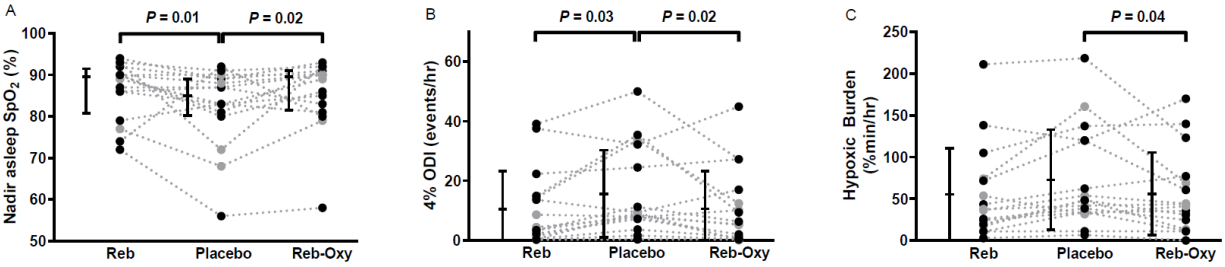

Figure 2.3: Effect of reboxetine (Reb) and reboxetine-oxybutynin combination (Reb-Oxy) on measures of overnight hypoxemia compared to placebo.

(A) Nadir O_2 saturation, (B) 4% oxygen desaturation index, (C) hypoxic burden. Plots show mean and standard deviation and individual values (gray circles indicate women, black circles indicate men). Significant pairwise comparisons P < 0.05 are indicated above the individual values.

Effects of reboxetine and reboxetine-oxybutynin on sleep parameters (Table 2.3).

Percent sleep time spent supine, sleep efficiency, wake after sleep onset, arousal index, NREM AHI, supine AHI and obstructive apnea index were not different between conditions. Reboxetine and reboxetine+oxybutynin reduced the proportion of REM sleep and increased stage N2 sleep,

with no changes in stages N1 or N3 sleep versus placebo. Reboxetine and reboxetine+oxybutynin increased morning heart rate by 14±11 and 14±8bpm compared to placebo, respectively. Despite the increased morning heart rate, there were no changes in morning systolic or diastolic blood pressure, and no participants experienced palpitations during the study.

OSA endotypes (Table 2.4 and Figure 2.4).

Reboxetine alone and in combination with oxybutynin improved pharyngeal collapsibility at the lowest decile of respiratory drive ($\dot{V}passive_{min}$) compared to placebo (median 7.7% [IQR 4.4 to 10.7] and 6.4% [IQR 2.7 to 6.4] respectively, both $P<0.001$). Reboxetine and reboxetine-oxybutynin both reduced LG_n and the ventilatory response to arousal versus placebo. Reboxetine+oxybutynin increased upper airway muscle compensation although reboxetine alone did not. Overall estimated pharyngeal collapsibility was not significantly different between conditions. Placebo night loop gain was higher in men versus women (0.44±0.09 vs. 0.35±0.06, $P=0.042$). The other OSA endotypes were not systematically different between men and women (e.g., $\dot{V}$passive 93 [86 to 95] vs. 94 [90 to 96]). AHI tended to improve with reboxetine in participants with high loop gain and high muscle compensation (Figure 2.4).

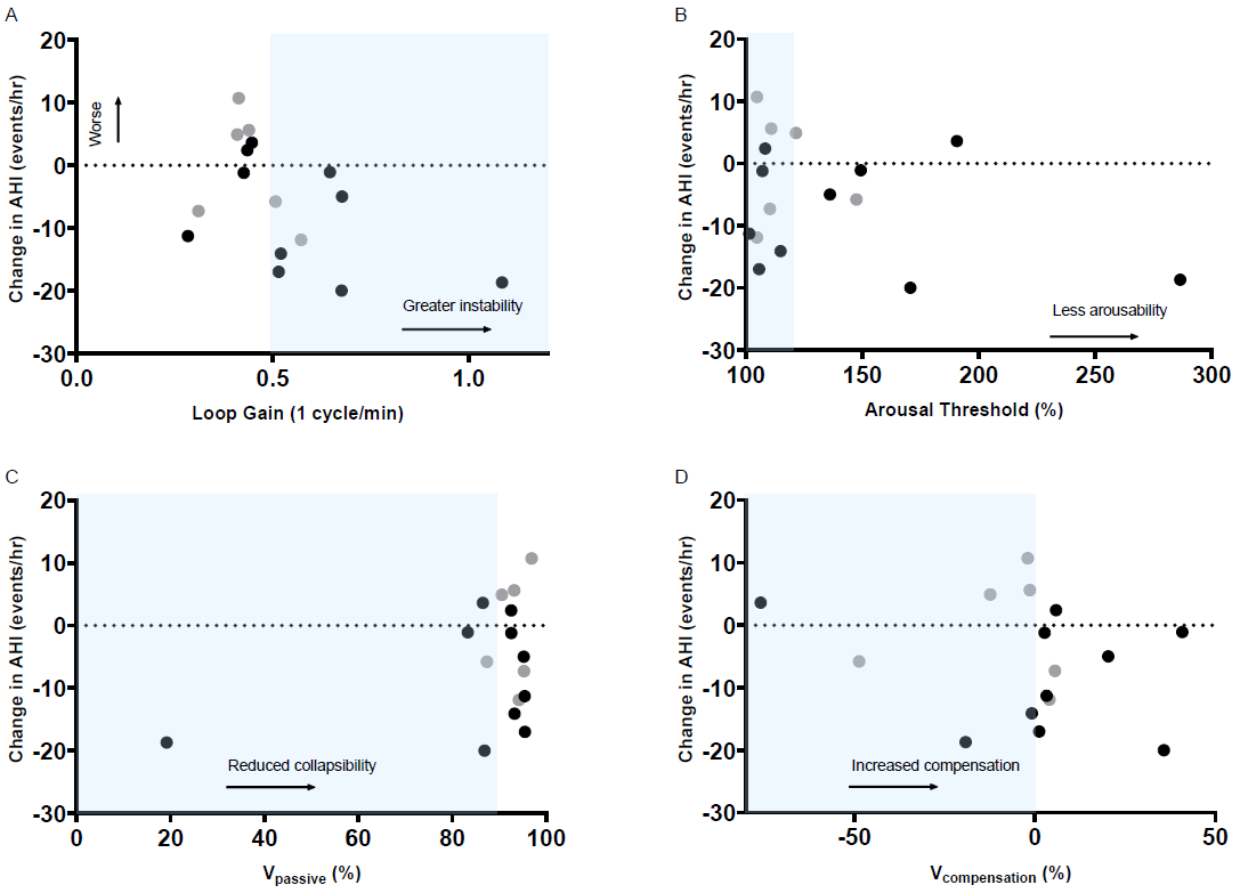

Figure 2.4: Change in AHI (events/h, 3% criteria) on reboxetine compared to baseline obstructive sleep apnea endotypes (as measured on placebo).

Change in AHI (events/h, 3% criteria) on reboxetine compared to baseline obstructive sleep apnea endotypes (as measured on placebo). (A) Loop gain (LG_1) representing ventilatory control hypersensitivity, (B) arousal threshold, (C) collapsibility (Vpassive) and (D) muscle compensation (Vcompensation) are presented as a percentage of eupneic levels. Refer to text for further details. Shading indicates unfavourable trait characteristics (i.e. high loop gain, low arousal threshold, collapsible pharyngeal airway and poor muscle compensation) as defined previously.[17,162-164] Gray circles indicate women, black circles indicate men. AHI: apnea-hypopnea index.

Table 2.4: OSA Endotypes.

	Reboxetine	**Placebo**	**Reb-Oxy**	***P* value**
*V*passive ($\%_{eupnea}$)	93 (89 - 95)	93 (87 - 95)	92 (89 - 95)	0.472
*V*passive$_{min}$ ($\%_{eupnea}$)	66 (57 - 74)	54 (43 - 66)	66 (54 - 76)	<0.001*^
*V*active ($\%_{eupnea}$)	96 (84 - 100)	96 (82 - 100)	98 (95 - 105)	0.075
*V*comp ($\%_{eupnea}$)	2 (-6 - 4)	2 (-10 - 6)	4 (2 - 9)	0.009^
VRA ($\%_{eupnea}$)	37 ± 20	49 ± 25	29 ± 13	<0.001*^
Loop gain$_n$ (dimensionless)	0.37 (0.31 - 0.41)	0.40 (0.35 - 0.46)	0.34 (0.32 - 0.43)	0.039*^
Loop gain$_1$ (dimensionless)	0.46 ± 0.16	0.52 ± 0.19	0.45 ± 0.10	0.097
Arousal threshold ($\%_{eupnea}$)	114 (107 - 134)	113 (106 - 149)	114 (109 - 127)	0.368

Definition of abbreviations: Reb-Oxy: reboxetine plus oxybutynin; V: ventilation; comp: compensation; VRA: ventilatory response to arousal.

Data are presented as mean ± standard deviation or median (interquartile range) as appropriate. *Reboxetine versus placebo pairwise comparison P <0.05. ^Reboxetine-Oxybutynin versus placebo pairwise comparison P <0.05.

Effects of reboxetine and reboxetine-oxybutynin on next-day alertness and subjective sleep quality (Table 2.5).

There were no differences in driving simulator performance measures between reboxetine, placebo, and reboxetine+oxybutynin conditions. There were also no differences in morning subjective sleepiness scores as measured by the Karolinska Sleepiness Scale. However, participants reported worse perceived sleep quality on reboxetine (mean difference in LSEQ

"Quality of Sleep" domain score, -3.46±5.97; *P*=0.04) and reboxetine+oxybutynin (-3.98±5.38; *P*=0.01) versus placebo.

Table 2.5: Measures of morning alertness.

	Reboxetine	**Placebo**	**Reb-Oxy**	***P* value**
AusEd Driving Simulator				
Steering deviation from median lane position (cm)	34.9 ± 13.0	36.6 ± 16.8	37.3 ± 13.2	0.436
Braking reaction time (seconds)	0.93 ± 0.17	0.93 ± 0.20	0.96 ± 0.14	0.523
Karolinska Sleepiness Scale				
Total score	5 ± 2	5 ± 2	5 ± 2	0.994
Leeds Sleep Evaluation Questionnaire				
GTS	11.90 ± 3.74	13.57 ± 7.21	10.92 ± 3.80	0.291
QOS	5.62 ± 2.78	9.08 ± 6.13	5.10 ± 3.46	0.014*^
AFS	10.04 ± 3.75	8.61 ± 3.84	10.93 ± 4.09	0.161
BFW	13.03 ± 4.33	10.98 ± 6.40	14.26 ± 4.90	0.252

Definition of abbreviations: Reb-Oxy: reboxetine plus oxybutynin; GTS: Getting To Sleep; QOS: Quality Of Sleep; AFS: Awake Following Sleep; BFW: Behaviour Following Wakening.

Data are presented as mean ± standard deviation or median (interquartile range) as appropriate. *Reboxetine versus placebo pairwise comparison *P* <0.05. ^Reboxetine-Oxybutynin versus placebo pairwise comparison *P* <0.05.

Discussion

The main finding from our study is that a single 4mg dose of reboxetine alone prior to sleep modestly reduces the AHI by an average of ~5 events/h of sleep. 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. In addition, reboxetine with and without oxybutynin markedly reduces REM sleep which is replaced with stage 2 sleep without altering sleep efficiency, does not change perceived next day sleepiness, alertness or blood pressure versus placebo but does increase morning heart rate and reduces

perceived sleep quality. These findings provide novel insight into the pathophysiological mechanisms by which reboxetine reduces OSA severity and its potential safety and tolerability profile to inform longer term trials.

Our study supports and extends recent upper airway physiology[64] and clinical findings from Lim and colleagues[153] with reboxetine plus hyoscine butylbromide and one week clinical findings from Perger et al[67] with reboxetine plus oxybutynin, and indicates that reboxetine alone can reduce OSA severity. However, the magnitude of the effect was less than the >15 event/h reductions in AHI seen in the recent Lim and colleagues[153] and Perger et al[67] studies. The reasons for these differences between studies are unclear but may relate to differences in participant characteristics and methodology. For example, while BMI, age, and perceived daytime sleepiness as measured by ESS were comparable between all three studies, the current participants had less severe OSA. Consistent with less severe OSA, participants in the current 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 current study compared to the two other recent reboxetine in OSA studies,[67,153] 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[62,67] which was comparatively small in the current study. Furthermore, the current study included both men and women rather than just men as per the Lim et al study[153] and ~90% men in the Perger et al study[67]. Indeed, in the current study, reductions in AHI with reboxetine occurred in men but not women. While this may indicate sex differences in response to reboxetine, as highlighted below, a more likely explanation is that the larger reductions in men are explained by higher loop gain values and sex differences in the ventilatory response to arousal.

Conversely, Taranto-Monetemurro and colleagues' recent findings with a different noradrenergic agent, atomoxetine, as a single agent did not reduce the AHI, but when combined with oxybutynin caused marked reductions in OSA severity.[62] The addition of oxybutynin to reboxetine in the current study did not yield additive improvements in AHI. This may also be due to differences in

participant characteristics (i.e., mostly men, more overweight, with greater upper airway collapsibility at baseline in the Taranto-Monetemurro and colleagues' study[63]), differences in noradrenergic potency between reboxetine versus atomoxetine or unique and currently incompletely understood interactions between atomoxetine and oxybutynin. As highlighted, recently published findings with one week of reboxetine plus oxybutynin also yielded larger reductions in OSA severity compared to the current study[67]. Possible differences in participant characteristics aside, this finding may suggest that a longer duration of administration could be required to achieve greater therapeutic efficacy.

Analyses of the effects of atomoxetine+oxybutynin on OSA endotypic traits found that the drug combination was most effective in patients with mild to moderate upper airway collapsibility and a predominance of hypopneas over apneas.[63] The median placebo night *V*passive ($\%_{eupnea}$) value in our study was 93%. This indicates that the current cohort generally did not have highly collapsible pharyngeal airways. Our findings therefore suggest that non-anatomical mechanisms such as improvements in respiratory control stability, which also occurred with atomoxetine+oxybutynin[63], atomoxetine with other antimuscarinics[165], and reboxetine with hyoscine butylbromide[153], contributed to the reduction in AHI with reboxetine in our study. Indeed, while the reported reductions in loop gain with noradrenergic and antimuscarinic agents of ~10-20% is less pronounced than with oxygen therapy and acetazolamide (~50%),[91,166] consistent with OSA endotyping concepts, the greatest reductions in OSA severity tended to occur in those with ventilatory control instability on placebo (high loop gain). These participants were mostly male. Given that male sex is associated with higher baseline loop gain[167] and an increased ventilatory response to arousal[168] as discussed below, these findings indicate that reboxetine reduces OSA, at least in part, via improvements in ventilatory control stability.

Sleep efficiency and wake after sleep onset tended to improve with the reboxetine+oxybutynin combination compared to reboxetine alone. These findings are consistent with a mild sedative effect with oxybutynin that attenuated the alerting effects of increased central nervous system norepinephrine levels from reboxetine. Anticholinergics are known to have mild sedative effects at low doses.[169] Indeed, atomoxetine has been shown to reduce the arousal threshold (i.e. easier to wake up), but the effect is offset by the addition of oxybutynin[63] and can be further offset with the

addition of the hypnotic zolpidem.[170] Our analysis showed no major differences in arousal threshold between reboxetine, placebo, and reboxetine+oxybutynin. Reboxetine and reboxetine+oxybutynin both improved nadir oxygen saturation and oxygen desaturation indices, indicating that the residual respiratory events were predominantly due to cortical arousals without major oxygen desaturations.

The reasons for reduced perceived sleep quality with the drug conditions versus placebo in the current single night study are likely driven by the excitatory noradrenergic properties of reboxetine as reflected by a shift towards lighter stages of sleep and potentially its mild side effects. While any reductions in perceived sleep quality are not favourable, the magnitude was mild. Indeed, overall objective sleep efficiency, next day perceived sleepiness and driving simulator performance were not different between conditions. Furthermore, subjective sleep quality was not different following one week of nightly reboxetine plus oxybutynin versus placebo in the recent Perger et al[67] study and psychomotor vigilance improved, presumably because of reduced OSA severity. This suggests that any perceived worsening in sleep quality with reboxetine may be transient. Indeed, most acute sleep architecture changes associated with reboxetine alone in people with persistent mild depression resolve over time[171] apart from reduced REM sleep which only partially returns. Thus, marked REM suppression as observed with reboxetine in the current study may only be partially restored over time. However, while the proportion of REM sleep was low at baseline, one week of nightly reboxetine plus oxybutynin in people with OSA did not significantly reduce REM sleep versus placebo in the recent Perger et al study[67]. Nonetheless, reduced REM sleep is common with most antidepressants.[64,172,173] However, it does not appear to cause major adverse outcomes in this context.

While REM was suppressed by reboxetine and reboxetine-oxybutynin, which may have, at least in part, contributed to the overall reduction in total AHI, this is unlikely to be the predominant mechanism of AHI reduction for several reasons. Firstly, for REM suppression to be the major mechanism, the REM AHI would be expected to be much higher than the NREM AHI at baseline. However, this was not the case. Thus, in the context of similar baseline REM and NREM AHI values, removal of REM sleep alone which was ~13% of total sleep time, and replacement with NREM would be expected to yield similar AHI values rather than an overall reduction in total AHI

as detected in the current study. Secondly, although there was no statistically significant reduction in NREM AHI with reboxetine versus placebo, the mean point estimate reduction in NREM AHI was of similar magnitude to the overall mean reduction in total AHI with reboxetine and reboxetine-oxybutynin. Furthermore, consistent with the NREM endotype changes detected in the current study, other recent noradrenergic and antimuscarinic combination therapy studies[62,67,153] have detected significant reductions in NREM AHI versus placebo indicating that total AHI reductions were not driven solely by REM suppression.

Reboxetine and reboxetine+oxybutynin both caused similar improvements in nadir pharyngeal collapsibility ($\dot{V}passive_{min}$). Based on these and previous findings,[63] it is likely that the changes were predominantly due to the noradrenergic effects of reboxetine. Although reboxetine was anticipated to reduce AHI primarily through improvements in upper airway dilator muscle activity,[64] estimates of dilator muscle compensation were not significantly different with reboxetine alone in the current study. However, the addition of oxybutynin with reboxetine increased pharyngeal muscle compensation during sleep in the current study, albeit to a much lesser extent than other recent combination therapy studies with noradrenergic and antimuscarinic agents.[62-64] Thus, as highlighted earlier, the beneficial effect on upper airway stability in the current study during the reboxetine conditions were likely driven primarily via improvements in ventilatory control stability.

In addition to overall respiratory control stability as quantified by loop gain, the ventilatory response to arousal is an important contributor to OSA pathogenesis.[168,174] Respiratory drive increases during partial airway obstruction, stimulating upper airway dilator muscle activity that eventually restores airway patency, at which point ventilation briefly exceeds baseline ventilation. If the restoration in airway patency is associated with a cortical arousal, the excessive ventilatory response may be sufficiently high to reduce respiratory drive and upper airway dilator muscle activity that feeds into a repetitive cycle of airway obstruction and arousals. On average, the ventilatory response to arousal is higher in men than women.[168] The carbonic anhydrase inhibitor acetazolamide and the serotonin-norepinephrine reuptake inhibitor venlafaxine reduce the ventilatory response to arousal[175,176] and in the case of acetazolamide reduces OSA severity.[166]

Thus, reductions in the ventilatory response to arousal with reboxetine may also contribute to breathing stability and the observed reductions in OSA severity.

Methodological considerations

While this study has several strengths including rigorous clinical trial design and provides both clinical and mechanistic insight, there are several limitations. First, the cohort was not selected based on individual endotypes. Thus, preselection based on endotype characterization may have yielded larger changes in OSA severity with reboxetine. However, despite predominately severe OSA as measured by the AHI, most participants had minimally collapsible upper airways at baseline which is typically associated with favourable therapeutic responses with similar drug combinations.[63] This may have been, at least in part, due to participants spending on average approximately 50% of the night lateral on each of the study nights which reduces upper airway collapsibility compared to the supine position.[177,178] Thus, it will be important to carefully control body position in future endotype studies. Second, detailed physiology quantification of OSA endotypes was not performed in the current study. However, the signal processing methodology that we used to estimate OSA endotypes is far less intrusive than the detailed physiology methodology and has recently been shown to have acceptable repeatability of measurement over time[179]. In addition, intervention studies aimed to modify one or more of the OSA endotypes, including previous OSA pharmacotherapy studies,[62,63,165,180,181] have consistently yielded quantifiable differences in endotypes versus placebo. Third, our study only assessed the effects of the medications over a single night. Thus, a longer duration study would be useful to determine if OSA severity is further decreased by reboxetine alone once the drug concentration reaches steady state, as recently published findings with combined reboxetine and oxybutynin suggest may be the case,[67] and if the adverse effects of reboxetine (with and without oxybutynin), including increased heart rate and reduced perceived sleep quality are clinically significant and persist or reduce over time. Based on previous findings from longer term studies in people who have not been screened for OSA, it would be expected that most of the acute changes in sleep architecture and elevated heart rate with reboxetine resolve within months.[171,172] Fourth, as highlighted, some of the characteristics of the current cohort including predominance of respiratory events associated with arousals rather than desaturations, subclinical insomnia, and minimal daytime sleepiness may not be ideally suited for noradrenergic pharmacotherapy. Thus, the current findings may not be

generalizable to all patients with OSA. Finally, we only studied a standard dose of reboxetine. Higher doses may have produced larger reductions in OSA severity. Thus, these unresolved clinically relevant questions require further investigation.

Conclusions

In this cohort with predominantly severe OSA with mostly arousal associated hypopneas, subclinical insomnia and minimal daytime sleepiness, a single dose of reboxetine alone modestly reduces the frequency of respiratory events and improves overnight oxygenation and snoring. These beneficial effects are likely driven largely by improvements in ventilatory control stability (reductions in loop gain and the ventilatory response to arousal). The addition of oxybutynin has mild sedative effects but does not produce additive benefit in reducing OSA severity on a single night despite modest improvements in pharyngeal muscle compensation. People with unstable ventilatory control (high loop gain endotype, mostly men in the current study) tend to respond most favourably to reboxetine. However, acutely, morning heart rate increases and perceived sleep quality decreases, although neither objective sleep quality, next day alertness or blood pressure change with a single dose of reboxetine. Thus, longer-term mechanistic and clinical studies to carefully study the effects of different doses of reboxetine and its efficacy, safety and tolerability profile in different patient populations that include both men and women are warranted. In summary, this study shows for the first time that reboxetine alone reduces OSA severity, provides new insight into the importance of noradrenergic mechanisms in OSA and will inform future pharmacotherapy investigations for OSA.

Chapter Three: Regular, low-dose, sustained-release morphine does not cause daytime sleepiness in people with chronic obstructive pulmonary disease: A secondary analysis of a randomized controlled trial

This chapter has been published:

Altree TJ, Toson B, Loffler KA, Ekström M, Currow DC, Eckert DJ. Low-Dose Morphine Does Not Cause Sleepiness in COPD: A Secondary Analysis of a Randomized Trial. *American Journal of Respiratory and Critical Care Medicine* 2024; Mar 13. doi: 10.1164/rccm.202310-1780OC. Online ahead of print.

Accompanied by an Editorial: Domnik NJ, Yaggi HK. Lessons About Low-Dose Morphine at the Intersection of Sleep and Breathlessness. Am J Respir Crit Care Med 2024: Jun 6. doi: 10.1164/rccm.202404-200682ED. Online ahead of print.

Abstract

Rationale: Regular, low-dose, sustained-release morphine is frequently prescribed for persistent breathlessness in chronic obstructive pulmonary disease (COPD). However, effects on daytime sleepiness, perceived sleep quality and daytime function have not been rigorously investigated.

Objectives: Determine the effects of regular, low-dose, sustained-release morphine on sleep parameters in COPD.

Methods: Pre-specified secondary analyses of validated sleep questionnaire data from a randomized trial of daily, low-dose, sustained -release morphine versus placebo over four weeks commencing at 8mg or 16mg/day with blinded up-titration over two weeks to a maximum of 32mg/day. Primary outcomes for these analyses were week-1 Epworth Sleepiness Scale (ESS) and Karolinska Sleepiness Scale (KSS) responses on morphine versus placebo. Secondary outcomes included Leeds Sleep Evaluation Questionnaire (LSEQ) scores (end of weeks 1 and 4), KSS and ESS beyond week-1 and associations between breathlessness, morphine, and questionnaire scores.

Measurements and main results: 156 people were randomized. Week-1 sleepiness scores were not different on morphine versus placebo (ΔESS [95%CI] versus placebo: 8mg group: -0.59 [-1.99, 0.81], p=0.41; 16mg group: -0.72 [-2.33, 0.9], p=0.38; ΔKSS versus placebo: 8mg group: 0.11 [-0.7, 0.9], p=0.78; 16mg group: -0.41 [-1.31, 0.49], p=0.37). This neutral effect persisted at later timepoints. In addition, participants who reported reduced breathlessness with morphine at 4 weeks also had improvement in LSEQ domain scores including perceived sleep quality and daytime function.

Conclusions: Regular, low-dose morphine does not worsen sleepiness when used for breathlessness in COPD. Individual improvements in breathlessness with morphine may be related to improvements in sleep.

Introduction

Breathlessness is a debilitating consequence of chronic obstructive pulmonary disease (COPD). Despite recommended non-pharmacological and pharmacological therapies, people with COPD frequently experience persistent disabling breathlessness[182]. While our recently published findings from the Breathlessness, Exertion and Morphine Sulfate (BEAMS) randomized trial did not indicate systematic improvement in worst breathlessness with morphine[147], clinical guidelines include the use of regular, low-dose morphine for symptomatic reduction of chronic breathlessness in COPD[93,183]. The potential adverse effect profile of regular, low-dose, sustained-release morphine on key outcomes such as daytime sleepiness and function in COPD remains unclear[147-149]. In addition, accurately predicting whether an individual will have therapeutic benefit in breathlessness or experience morphine-related harms remains an unresolved clinical challenge.

In addition to respiratory symptoms, people with COPD frequently experience poor sleep quality[184]. There are multiple potential contributors to poor sleep in COPD. These include nocturnal COPD symptoms, pathophysiological changes to ventilation during sleep when protective respiratory compensatory mechanisms diminish, and comorbid sleep disorders. Characteristic changes to sleep architecture in people with COPD include increased sleep fragmentation, less "deep" sleep (reduced stage 3 (N3) and rapid eye movement (REM) sleep), and reduced sleep duration[105]. Persistent symptoms such as cough, sputum production, and breathlessness contribute to sleep disturbance in over three-quarters of people with COPD[106]. Sleep

causes deterioration in respiratory mechanics in COPD that requires compensatory changes in inspiratory effort[185,186]. The supine position can worsen pulmonary ventilation/perfusion (V/Q) mismatch[107] and expiratory flow limitation[108], leading to nocturnal hypoxemia and hypercapnia. Comorbid sleep disorders including insomnia[187] and obstructive sleep apnea[112] are also very common in COPD.

Morphine could theoretically further perpetuate sleep problems in COPD, particularly those related to breathing, by its associated respiratory depressant effects[188]. Conversely, morphine may improve certain sleep disruptors (such as cough) in some individuals[189]. Morphine could also cause daytime drowsiness, and thus increase risk of harms from impaired attention-critical tasks such as driving[190]. Sleep quality may also alter the perception of daytime breathlessness in COPD, or vice versa[146]. The currently available evidence assessing this relationship, which is limited to one randomized, placebo-controlled, crossover trial that assessed the effects of 20mg oral sustained-release morphine over four days in 38 people with refractory breathlessness (30 male; 33 had COPD), reported that four days of low-dose sustained-release morphine caused improvements in subjective sleep quality, and that better perceived sleep was associated with decreased breathlessness during the day[146]. Daily breathlessness levels were recorded on a visual analog scale, and sleep was assessed by two simple, non-validated, daily questions designed by the team to assess sleep quality and sleep disruption from breathlessness[146].

The adverse and beneficial effects of morphine on subjective markers of sleep quality in a large cohort of people with COPD at variable doses and over a longer period have not been assessed. To improve understanding of the effects of regular, low-dose morphine on sleep in COPD, and the potential relationship between sleep and breathlessness, we assessed sleep questionnaire data from a multi-site, double-blind, randomized, placebo-controlled trial of people with COPD taking regular, low-dose sustained-release morphine for chronic breathlessness over four weeks[147]. Some of the results of this study have been previously reported in the form of an abstract[191].

Methods

Study design, interventions, and participants

This study analyzed sleep questionnaire data collected over four weeks from BEAMS, a multi-site, phase III, double-blind, parallel-arm, randomized, placebo-controlled trial of regular, low-dose, sustained-release morphine in adults with COPD and breathlessness[147]. The main trial was designed to assess the effects of placebo, 8mg, and 16mg oral morphine on breathlessness intensity and safety at one week (primary outcome). In addition, there was blinded up-titration (up to 32mg) at weeks two and three, including to some who were originally randomized to placebo, with accompanying assessment of symptoms, physical activity, functional capacity, health-related quality of life, and safety at varying time points including at 4 weeks[150]. The BEAMS study was conducted in accordance with the Declaration of Helsinki and the International Committee on Harmonisation Good Clinical Practice (ICH-GCP), reported according to CONSORT guidelines and was preregistered on clinicaltrials.gov (NCT02720822). See supplement for further information.

Outcomes

Responses to three sleep-related questionnaires were collected during the trial: the Epworth Sleepiness Scale (ESS) to assess daytime sleepiness[192]; Karolinska Sleepiness Scale (KSS) to assess current sleepiness[156]; and Leeds Sleep Evaluation Questionnaire (LSEQ) to assess four domains related to sleep: ability to fall asleep ("getting to sleep", GTS); sleep quality ("quality of sleep", QOS); ease awakening ("awake following sleep", AFS); and tiredness after wakening ("behaviour following wakening", BFW)[157]. ESS and LSEQ data were collected at baseline, week 1 and week 4. KSS data were collected weekly from baseline to week 3. Morphine dose had been stable for a minimum of one week prior to sleep questionnaire data collection at each assessment time point. The primary outcome of the current study was the effect of 8 and 16mg morphine on KSS and ESS at the end of week 1. The effects of morphine on week 1 LSEQ domain scores, and sleep questionnaire scores at week 3 (for KSS) and week 4 (ESS and LSEQ) were assessed as secondary outcomes. As exploratory outcomes, we also investigated the potential mediating effect of sleep on breathlessness within participants randomized to morphine at weeks 3 and 4. This included investigation of the potential relationship between subjective sleep markers (at weeks 3 and 4) and change in breathlessness from baseline and the potential for baseline LSEQ scores to predict breathlessness response at week 4. Breathlessness was measured using a numerical rating

scale (score range, 0 ["*no breathlessness*"] to 10 ["*worst possible breathlessness*"]) based on the previous 24 hours[147].

Statistical analysis

The effect of morphine dose on withdrawals per week was examined using Fisher's exact test. Outcomes from sleep-related measurements (KSS, ESS, and domains of the LSEQ) at week 1 were analyzed using linear regression with drug dose and corresponding baseline sleep measure as predictors. Robust variance estimator or splines were used in case of deviations from homoscedasticity or linearity assumptions.

The effects of morphine dose on the relationship between sleep-related measurements and breathlessness were analyzed using linear mixed models with the interaction between dose, study week (baseline and week 1) and sleep-related measurement. Morphine dose was dichotomised into any active morphine dose for week 3 and 4 versus baseline exploratory analyses to maintain statistical power. T-tests were used to test differences between drug dose at each study week and paired t-tests to compare outcomes between study weeks for each dose. For LSEQ domains subgroups (≤50, >50), one sample t-tests were employed to test whether change from baseline was different from zero. Analyses were carried out in Stata/SE 18.0.

Results

Participants

One hundred and fifty six participants were enrolled and randomized. Numbers of participants within each dose allocation from weeks 1 to 4 are presented in table E3.1 in the supplement. Characteristics at baseline were similar between the week 1 treatment groups (Table 3.1).

Table 3.1: Baseline characteristics of the participants.

	8 mg/day of morphine (n = 55)[a]	16 mg/day of morphine (n = 51)[a]	Placebo (n = 50)[a]
Age, median (IQR), y	73 (67 – 78)	73 (67 – 78)	72 (66 – 76)
Sex			
Male	28 (51)	25 (49)	28 (56)
Female	27 (49)	26 (51)	22 (44)

Body mass index, kg/m^2, median (IQR)[b]	26.1 (22.4-31.2)	27.0 (23.0-31.6)	25.9 (21.7-30.5)
Smoking status			
Former	43 (78)	43 (84)	38 (76)
Current	10 (18)	6 (12)	12 (24)
Never	2 (4)	2 (4)	0
Modified Medical Research Council breathlessness scale score[c]			
3[d]	49 (89)	38 (75)	34 (68)
4	6 (11)	13 (25)	16 (32)
Charlson Comorbidity Index[e]			
0	22 (40)	19 (37)	23 (46)
1-2	23 (42)	23 (45)	17 (34)
≥3	10 (18)	9 (18)	10 (20)
Other causes of breathlessness			
Had ≥1 other cause of breathlessness	28 (51)	24 (47)	21 (42)
Heart failure	12 (22)	12 (24)	5 (10)
Asthma	6 (11)	5 (10)	7 (14)
Restrictive lung disease	4 (7)	2 (4)	3 (6)
Thromboembolic cause	3 (6)	2 (4)	2 (4)
Bronchiectasis	2 (4)	1 (2)	1 (2)
Lung cancer or metastasis	1 (2)	1 (2)	2 (4)
Lung infection or inflammation	1 (2)	0	2 (4)
Other[f]	10 (18)	8 (16)	12 (24)
Supplemental oxygen therapy			
No	28 (51)	29 (56)[g]	33 (66)

Yes			
Continuous use [usual flow rate, median (IQR), L/min]	16 (29) [2.0 (2.0-2.0)]	10 (20)[g] [2.0 (1.5-2.5)]	9 (18) [2.0 (1.5-2.0)]
Only on exertion	3 (5)	7 (14)[g]	3 (6)
Only when needed	8 (15)	4 (8)[g]	5 (10)
Epworth Sleepiness Scale Score (mean±SD)			
	6.1±3.8	6.5±4.4	5.7±3.9

[a]Data are expressed as participants numbers and (%) unless otherwise indicated. [b]Calculated as weight in kilograms divided by height in meters squared. [c]Ordinal scale with scores that range from 0 to 4; the worst score is 4. [d]Corresponds to responses such as "I stop for breath after walking about 100 yards or after a few minutes on the level," "I am too breathless to leave the house," or "I am breathless when dressing." [e]Scores range from 0 to 37; higher scores indicate worse comorbidity. Based on the presence of 19 comorbidities. [f]Anemia, anxiety, arrhythmia, muscular and cardiovascular deconditioning, ischemic heart disease, being overweight or obese, pulmonary fibrosis, pulmonary hypertension, and valvular disease. [g]The denominator is 50 people. IQR: interquartile range; SD: standard deviation.

Rates and reasons for dropouts during the study are described in the primary paper[147]. There was no dose effect on the dropout rate (see supplement Table E3.1).

Sleep scores

Effects of morphine at 8 and 16mg/day on sleepiness at week 1 (primary outcome)

Overall, ESS and KSS scores were not different with morphine at 8mg or 16mg/day versus placebo after one week (Table 3.2, all p>0.05) (Figure 3.1).

Table 3.2: Effects of morphine (8mg and 16mg/day) compared to placebo on sleep scores after 1 week.

Questionnaire	Week 1 dose	Estimated marginal differences (95% CI)	p value
ESS	8 mg	0.49 (-0.55, 1.54)	0.354
	16 mg	0.3 (-0.77, 1.37)	0.584
KSS	8 mg	-0.06 (-0.83, 0.7)	0.869

	16 mg	0.55 (-0.24, 1.33)	0.17
LSEQ GTS	8 mg	5.51 (-0.09, 11.11)	0.054
	16 mg	5.7 (-0.05, 11.45)	0.052
LSEQ QOS	8 mg	1.48 (-6.35, 9.3)	0.71
	16 mg	4.11 (-3.91, 12.13)	0.313
LSEQ AFS	8 mg	4 (-3.1, 11.1)	0.267
	16 mg	-2.82 (-10.13, 4.49)	0.446
LSEQ BFW	8 mg	-4.68 (-11.83, 2.46)	0.197
	16 mg	-8.18 (-16.2, -0.15)	**0.046**

Analyses were performed using multiple linear regression including drug dosage and baseline sleep questionnaire score. Estimated marginal differences were estimated over a balanced population, at mean level of the baseline sleep questionnaire variable. Positive coefficient values denote improvements relative to change from baseline during placebo. Note: negative values in both morphine dose groups in the Leeds Sleep Evaluation Questionnaire (LSEQ) Behaviour Following Wake (BFW) domain reflect a relative reduction compared to placebo rather than worsening with morphine as BFW scores remained stable in both morphine groups from baseline, whereas there was an improvement in week one placebo LSEQ BFW domain scores. Refer to the text for further details. Bold values indicate $P < 0.05$. AFW: Awake Following Sleep domain; CI: confidence interval; ESS: Epworth Sleepiness Scale; GTS: Getting to Sleep domain; KSS: Karolinska Sleepiness Scale; QOS: Quality of Sleep domain.

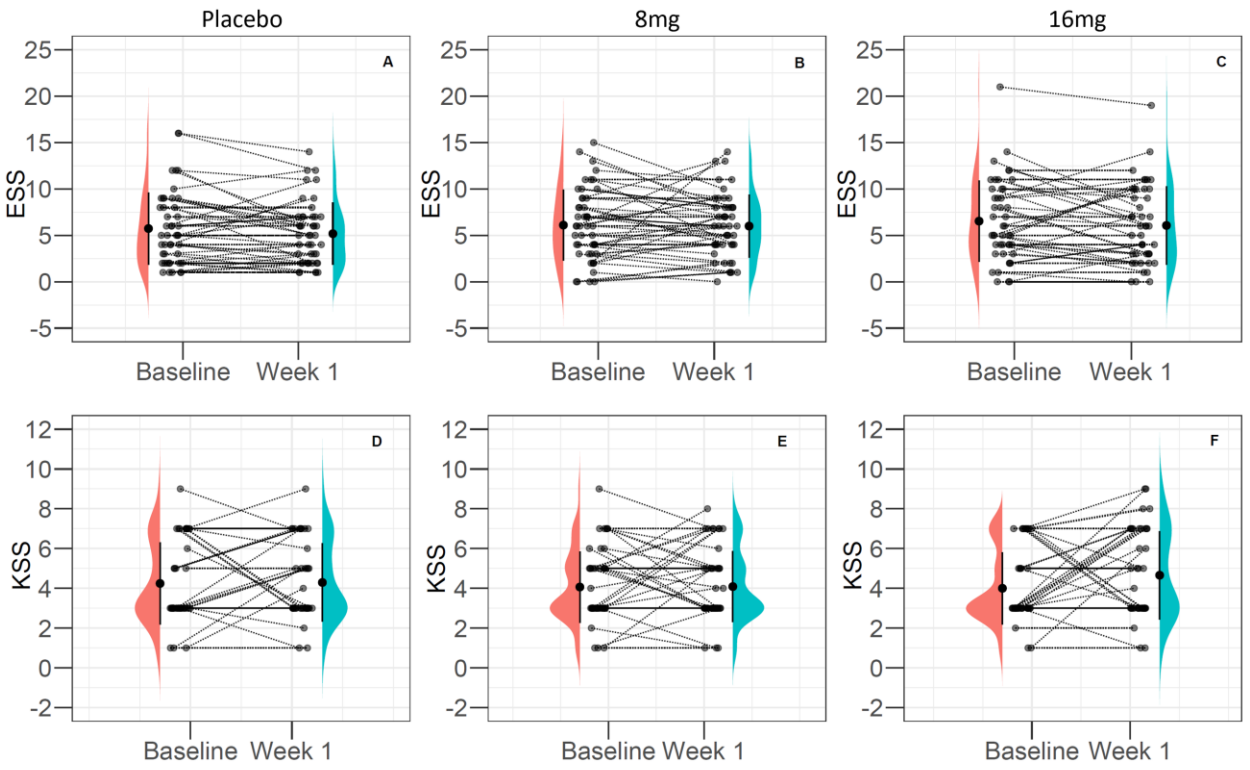

Figure 3.1: Epworth Sleepiness Score (ESS) Scale and Karolinska Sleepiness Scale (KSS) scores at baseline and week 1.

(A, B and C) ESS scores at baseline and week 1 on (A) placebo, (B) 8mg per day and (C) 16mg per day. (D, E and F) KSS scores at baseline and week 1 on (D) placebo, (E) 8mg per day and (F) 16 mg per day. Data show individual values with mean and standard deviation adjacent. The adjacent kernel density plots represent the distribution of values at baseline (pink) and one week (green) for KSS and ESS scores on placebo and the different morphine doses.

Effects of morphine at 8 and 16mg/day on the Leeds Sleep Evaluation Questionnaire at week 1

Overall Leeds Sleep Evaluation Questionnaire components were not different between morphine and placebo conditions at week 1 except BFW domain score. Specifically, the BFW scores increased from baseline to week one in the placebo condition (consistent with improvement) whereas BFW scores remained stable from baseline to end of week 1 in the 16mg/day morphine arm (Table 3.2, supplement Figure E3.1). There was a tendency for participants on the active morphine doses to report getting to sleep more easily (higher LSEQ GTS scores) ($p = 0.05$, Table 3.2).

Effects of morphine (doses grouped) at weeks 3 and 4 versus baseline (Table 3.3)

At week 3 and 4, when morphine doses were combined, there were no changes in ESS and KSS values from baseline. All LSEQ domain scores improved from baseline in the combined (8mg-32mg/day) morphine group at week 4 (Figure 3.2). An improvement from baseline in LSEQ GTS scores was also seen at week 4 in the placebo arm.

Table 3.3: Effects of morphine on sleep scores at weeks 3 and 4 compared to baseline.

	Placebo		**Morphine (8, 16, 24 & 32mg/day combined)**	
	Mean±SD	**p value**	**Mean±SD**	**p value**
ΔBaseline to week 3				
KSS	-0.56±2.19	0.4676	-0.10±2.45	0.7146
ΔBaseline to week 4				
ESS	-0.25±2.5	0.854	-0.67±3.14	0.124
LSEQ GTS	13.5±7.93	**0.042**	12.68±21.12	**<0.001**
LSEQ QOS	-2±11.23	0.745	18.27±23.98	**<0.001**
LSEQ AFS	2.5±12.56	0.717	6.82±22.77	**0.039**
LSEQ BFW	10.25±21.21	0.405	11.47±21.16	**<0.001**

Bold values indicate $P < 0.05$. Note: n ranges from 4 to 9 for placebo responses and 52 to 84 for combined morphine responses. There were no significant differences in the delta values from baseline to weeks 3 or 4 between conditions (placebo vs. morphine). However, caution interpreting these findings is warranted given the small sample sizes in the placebo arm. AFW: Awake Following Sleep domain; BFW: Behaviour Following Wake domain; ESS: Epworth Sleepiness Scale; GTS: Getting to Sleep domain; KSS: Karolinska Sleepiness Scale; LSEQ: Leeds Sleep Evaluation Questionnaire; QOS: Quality of Sleep domain; SD: standard deviation.

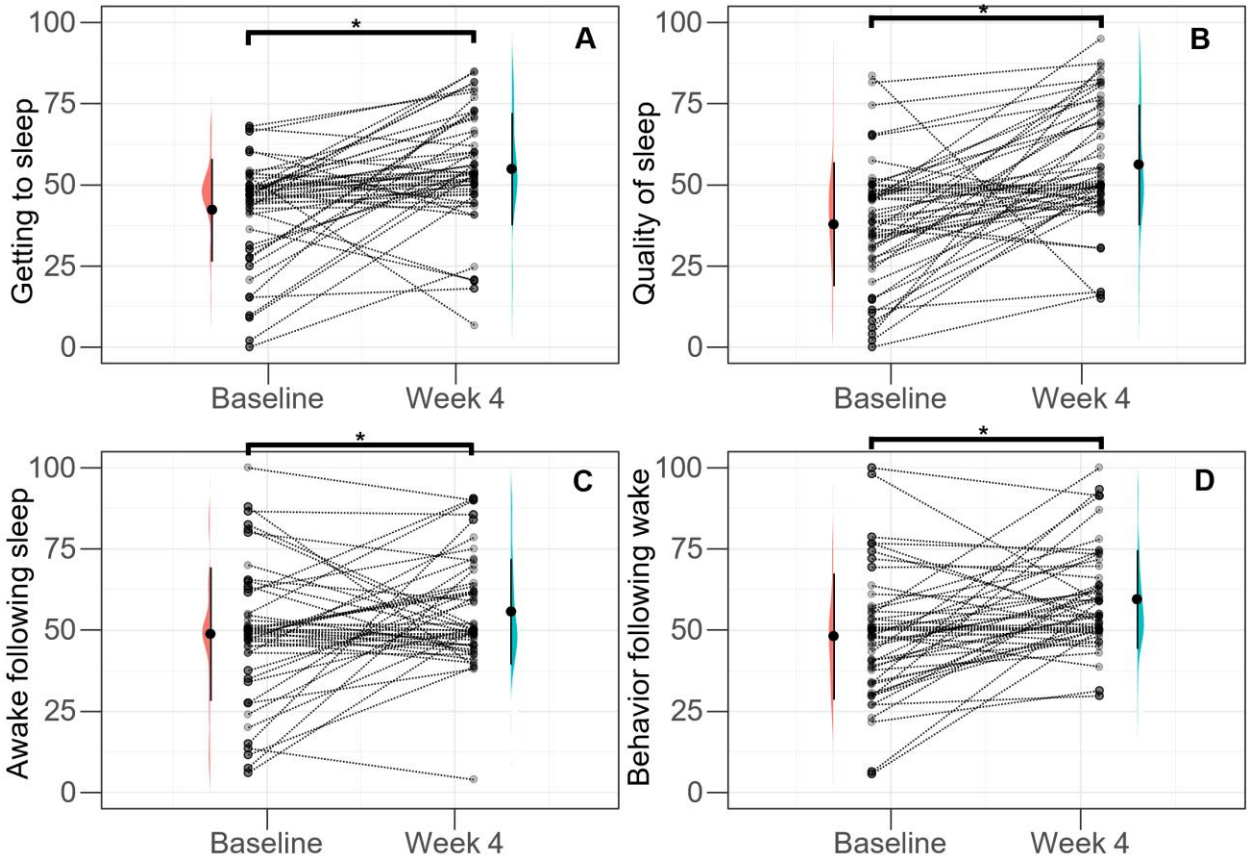

Figure 3.2: Leeds Sleep Evaluation Questionnaire (LSEQ) domain scores at baseline and week 4 on morphine (doses grouped).

Leeds Sleep Evaluation Questionnaire (LSEQ) domain scores at baseline and week 4 on morphine (doses grouped). (A) Getting to sleep (GTS); (B) Quality of sleep (QOS); (C) Awake following sleep (AFS); (D) Behavior following wake (BFW). Data show individual values (gray dots joined by lines for each individual) with mean and standard deviation adjacent. Darker gray color represents more overlapping data points. The adjacent kernel density plots represent the distribution of values at baseline (pink) and week four (green) for the individual LSEQ domain scores. *P < 0.05 for week 4 versus baseline comparison. Note that there was also a significant Getting to sleep domain improvement in the placebo group at week 4. Refer to Figure E3.2 in the supplement for a break-down of domain scores by morphine dose.

Effects of morphine on the relationship between sleep and breathlessness

Week one (8 and 16mg/day)

There was no significant interaction between morphine dose, sleep questionnaire responses, and breathlessness at baseline and week 1, except for ESS whereby participants with higher

breathlessness scores tended to be sleepier in some but not all conditions (see supplement for further detail).

Weeks 3 and 4 (doses grouped)

There were no relationships between the change in KSS and breathlessness scores from baseline to week 3, or between ESS and breathlessness scores from baseline to week 4 (see supplement for further detail). Conversely, in the combined morphine dose group (8-32mg/day) at week 4, improvements from baseline in each of the four sleep domains of the LSEQ were associated with improved breathlessness scores (Table 3.4, Figure 3.3). This was not the case in those who did not have an improvement in breathlessness from baseline to week 4 with morphine except for the AFS domain (Table 3.4).

Table 3.4: Change in breathlessness scores in those who did versus did not report improvement in Leeds Sleep Evaluation Questionnaire domains with morphine at week 4.

LSEQ Domain	**Group**	**n**	**Change in breathlessness from baseline (Mean [95% CI])**	**p-value of change in breathlessness from baseline**
GTS	improvement	27	-2.22 [-3.13 to -1.32]	**<0.0001**
	no improvement	11	-1.27 [-2.95 to 0.4]	0.12
QOS	improvement	33	-2 [-2.87 to -1.13]	**<0.0001**
	no improvement	5	-1.6 [-3.86 to 0.66]	0.12
AFS	improvement	18	-2.39 [-3.37 to -1.41]	**0.0001**
	no improvement	19	-1.63 [-2.93 to -0.33]	**0.02**
BFW	improvement	24	-2.5 [-3.47 to -1.54]	**<0.0001**
	no improvement	13	-1.08 [-2.48 to 0.33]	0.12

Bold values indicate P < 0.05. Breathlessness was measured using a numerical rating scale (score range, 0 ["*no breathlessness*"] to 10 ["*worst possible breathlessness*"]) based on the previous 24 hours. The minimal clinically important difference of this scale is one point[193]. AFW: Awake Following Sleep domain; BFW: Behaviour Following Wake domain; CI: confidence interval; GTS: Getting to Sleep domain; LSEQ: Leeds Sleep Evaluation Questionnaire; QOS: Quality of Sleep domain.

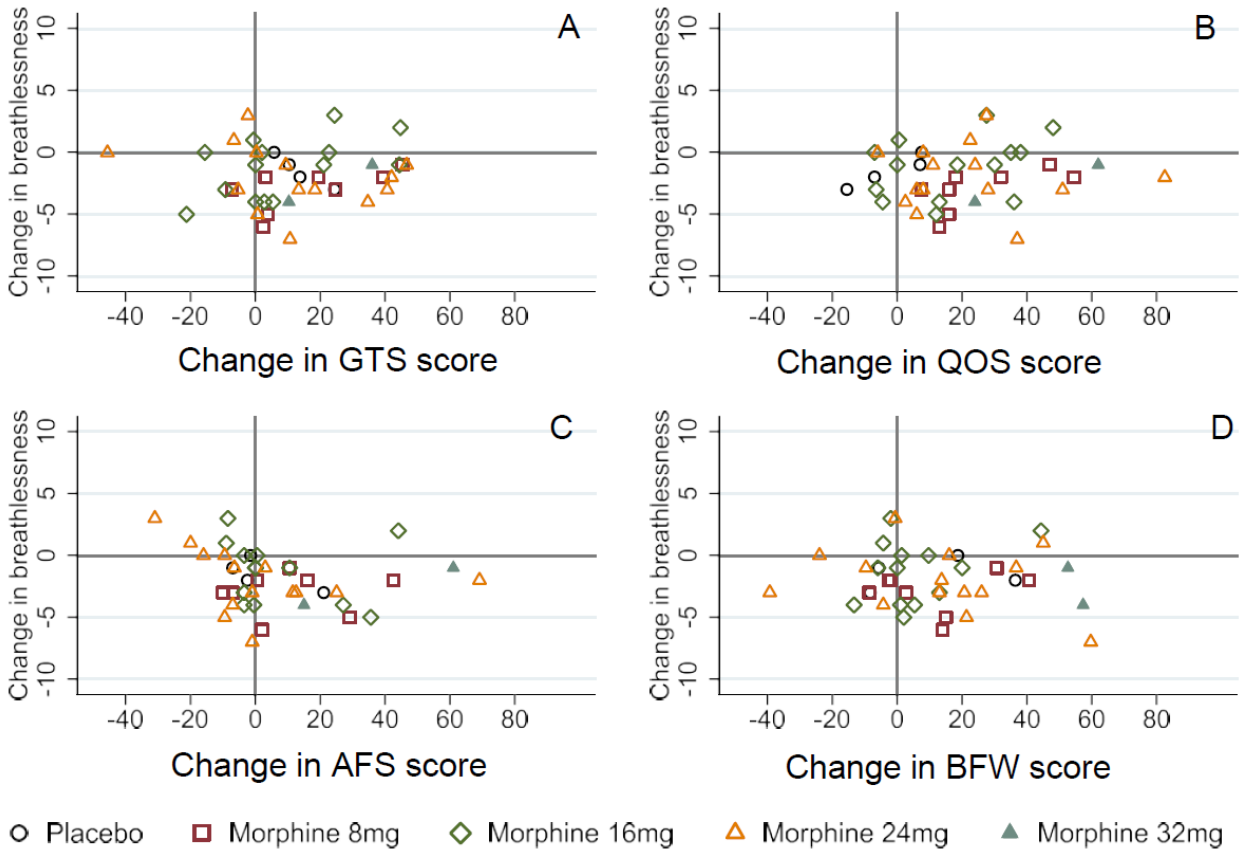

Figure 3.3: Change in Leeds Sleep Evaluation Questionnaire (LSEQ) domain scores plotted against change in breathlessness (worst breathlessness intensity in the past 24 hours) from baseline to week 4.
(A) Getting to sleep (GTS); (B) Quality of sleep (QOS); (C) Awake following sleep (AFS); (D) Behavior following wake (BFW).

Baseline sleep score as a predictor of breathlessness improvement with morphine

In the combined morphine dose group (8-32mg/day), participants with baseline GTS and BFW domains scores less than or equal to 50 (consistent with difficulty getting to sleep and feeling less alert/coordinated during the day) on the Leeds Sleep Evaluation Questionnaire had significant improvements in breathlessness at week 4 versus baseline (Figure 3.4 and supplement Table E3.3). This was not the case in those with GTS and BFW domains scores above 50 at baseline. Whether participants reported poor or good sleep quality (QOS) or relative ease or difficulty awakening (AFS) did not differentiate week 4 changes in breathlessness with morphine (Figure 3.4 and supplement Table E3.3).

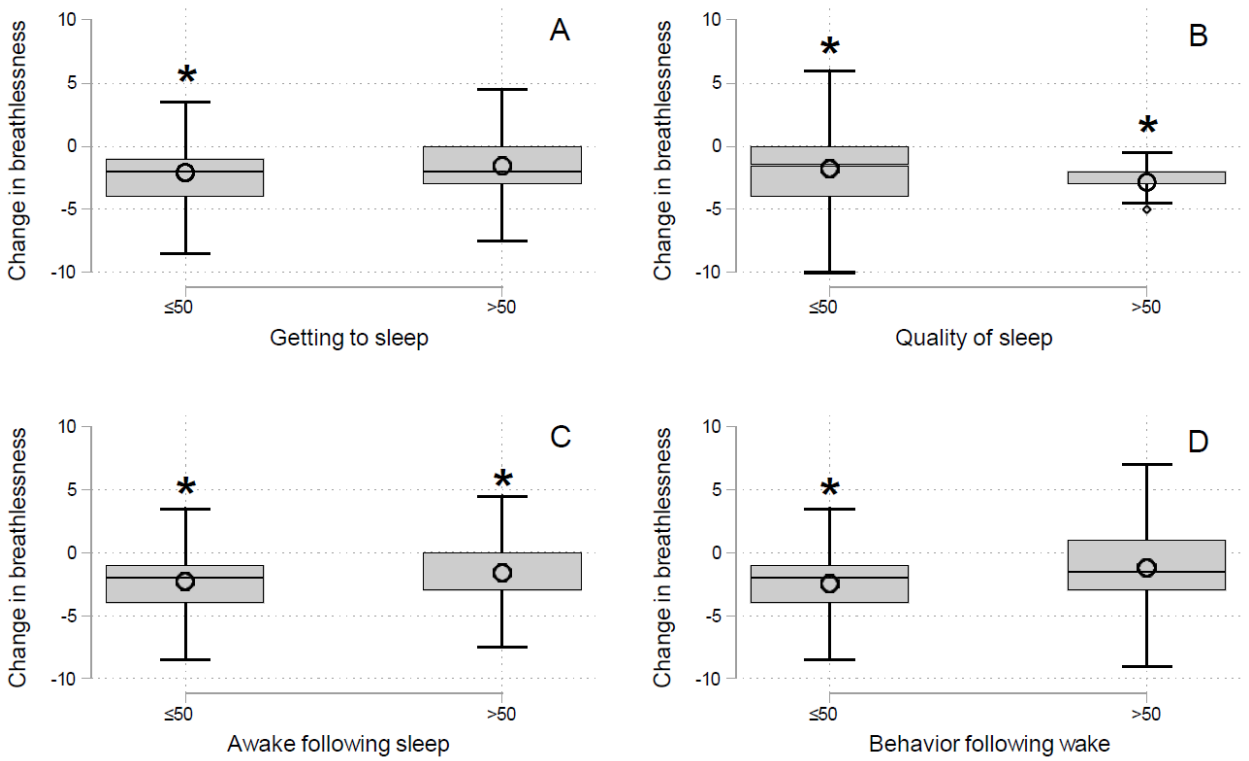

Figure 3.4: Change in breathlessness (worst breathlessness intensity in the past 24 hours) at week 4 in participants on morphine (doses grouped) in those with baseline Leeds Sleep Evaluation Questionnaire (LSEQ) domain scores ≤50 or >50.

Negative change in breathlessness scores indicate an improvement. *denotes P-value <0.05 for change in breathlessness compared to no change. (A) Getting to sleep; (B) Quality of sleep; (C) Awake following sleep; (D) Behavior following wake.

Discussion

Our results demonstrate that in people with COPD and breathlessness given regular, low-dose, sustained-release morphine daily for up to 4 weeks:

1) morphine does not worsen subjective daytime sleepiness, alertness, or sleep quality;

2) improvements in breathlessness are accompanied by improvements in subjective sleep quality; and

3) individuals with poor sleep quality at baseline may be more likely to experience a reduction in breathlessness with morphine.

These novel findings provide reassuring safety data and support the concept that there is a relationship between sleep quality and the perception of breathlessness.

Effects of morphine on sleep

Historically, there has been a reluctance amongst physicians to prescribe opioids for breathlessness due to safety concerns, especially regarding respiratory depression[194]. In the recently published Breathlessness, Exertion and Morphine Sulfate (BEAMS) trial[147], there were more serious treatment-emergent adverse events in the morphine arms during weeks 1 to 3, including two episodes of respiratory failure. However, the design of the study, with fewer participants on placebo throughout the study compared to active treatment, makes it challenging to definitively attribute respiratory failure to morphine rather than the underlying COPD. Other recent large randomized placebo-controlled trials of morphine for breathlessness have not shown increased rates of major adverse events[148], including daytime hypoventilation[149]. The current findings that regular, low-dose, sustained-release morphine does not cause daytime sleepiness, as measured using the ESS and KSS, provide further support for its safety profile in this context. Furthermore, in contrast to sedation-related concerns with morphine[194,195], participants reported *improved* ease of waking, alertness, balance, and coordination on the Leeds Sleep Evaluation Questionnaire by week 4. Additionally, they experienced greater ease falling asleep and better sleep quality. However, caution is warranted for the *getting to sleep* domain, as an improvement over time was also noted in the placebo arm from baseline to week 4. Subjective outcomes in sleep research can be influenced by placebo effects[196]. Given the up-titration study design with very small numbers in the placebo arm at week 4, the detected change in the *getting to sleep* domain from baseline to week 4 during placebo may be due to chance (Type 1 error).

Nonetheless, these findings for a range of sleep outcomes are consistent with the notion that low-dose, sustained-release morphine improves sleep quality in people with COPD. The mechanisms underlying these changes remain unclear. While acute (single night) administration of morphine at doses ranging from oral equivalents of approximately 15 to 21mg/day tend to disrupt sleep architecture in healthy people[130,132], there are no objective sleep data on the effects of low-dose morphine in people with COPD. Rather, the existing subjective data indicate that 4 days of acute low dose morphine in people with breathlessness, many of whom had COPD, improve perceived sleep quality[146]. Accordingly, in people with COPD taking low-dose sustained-release morphine, there may be no systematic acute impairment in sleep with morphine. This could be due to morphine-related reductions in respiratory sensation in at least some of the participants, which

may help minimise sleep disruption. Alternatively, the acute deleterious effects of morphine on sleep may resolve rapidly (i.e., within days). Indeed, in the prior Martins et al study[146], perceived measures of sleep were not impaired 4 days after initial dosing with morphine or after 1 week in the current study. Based on these findings, in addition to objective measures, both short (less than one week) and longer-term (more than one month) effects of morphine on sleep and alertness require further investigation.

While morphine did not systematically increase daytime sleepiness at week 1 (i.e., ESS scores), participants on 16mg/day at week 1 who reported higher ESS scores also had higher breathlessness scores. This was not the case in the 8mg/day group. Additionally, as reported in the BEAMS study, drowsiness measured on the Edmonton Symptom Assessment Scale was higher (although the mean score was still relatively low) in the 16mg/day group versus placebo, but not in the 8mg/day group[147]. Clinically, these findings suggest that a starting dose of 16mg/day may be too high for some. The reasons underlying these results are not clear. Possible mediating reasons may include opioid-induced sleep-disordered breathing, or reduction in respiratory drive below that required to meet ventilatory demand (both awake and during sleep), affecting both breathlessness and sleepiness. Monitoring for sleepiness or drowsiness could therefore help clinicians to balance the possible benefits and risks of morphine in COPD.

Effects of morphine on the relationship between sleep and breathlessness

The sleep questionnaire findings in the current study highlight a potential association between sleep and breathlessness. In the primary analysis of the BEAMS randomized trial[147], there was no systematic reduction in breathlessness with morphine at the week one primary outcome timepoint. However, consistent with prior observations[124], some individuals reported large improvements in breathlessness with morphine. It is unclear why some people experience reductions in breathlessness with morphine while others do not. The finding that up to 4 weeks of morphine improved participants' scores in all sleep domains of the Leeds Sleep Evaluation Questionnaire from baseline, which was associated with reductions in breathlessness, suggests that morphine may improve daytime perception of breathlessness through effects on sleep. While this possibility is intriguing and is consistent with the findings of a smaller trial that assessed acute (4-day) effects of morphine[146], it requires further investigation. Indeed, appropriately designed studies to investigate directionality are required as it is possible that morphine-related improvements in

breathlessness for some individuals could have led to accommodating improvements in sleep, vice versa or a combination of both potential mechanisms. In addition, caution is warranted when interpreting the *awake following sleep* data as there was also an improvement noted from baseline to week 4 during the placebo arm for this domain.

The mechanisms underlying breathlessness in COPD are complex and involve all the main components of control of breathing, including perception of breathing, central (efferent) respiratory activity, respiratory muscle function, ventilation, and gas exchange[114]. How morphine alleviates breathlessness is unclear, but it is likely that the predominant mechanism is through *perception* of breathlessness. In people with moderate to severe COPD, reversal of endogenous opioids with the opioid receptor antagonist naloxone leads to increased perceived breathlessness during resistive load breathing[115] and exercise[116]. The insula, dorsal anterior cingulate cortex, amygdala, and medial thalamus are all involved in processing perception of breathlessness[117], and are all densely innervated with opioid receptors[118]. To our knowledge, the effects of inadequate sleep on breathlessness in respiratory disease have not been assessed. However, there is evidence supporting this relationship in healthy participants. In sleep restricted or sleep deprived adults, perceived effort during exertion is higher than in well-rested controls, despite no clear differences in cardiopulmonary response to exercise[197,198]. In twenty healthy sleep-deprived men, inspiratory endurance was reduced by ~50% due to progressive reduction in the cortical contribution to respiratory motor output during inspiratory loaded breathing[199]. Additionally, the potential relationship between sleep and perception of breathlessness has parallels with the well-established bidirectional relationship between sleep deficiency and perception of pain, in which the opioid system plays a key mediating role[200]. Therefore, perception of breathlessness may also be influenced by sleep, which may be improved with regular, low-dose, sustained-release morphine in certain people with COPD[146]. This may be a direct effect of morphine on sleep or indirectly through reductions in nocturnal breathlessness. This novel association warrants future investigation with objective measurements of sleep.

Baseline sleep scores as a predictor of breathlessness improvement with morphine

While some people may experience clinically meaningful improvements in breathlessness, others do not experience any relief from breathlessness with morphine[124]. This heterogeneity means that

a substantial proportion of people with breathlessness are exposed to potential drug-related harm in the absence of clinical benefit. Accurately identifying which patients may experience overall benefit versus harm remains a major clinical challenge and a priority for the field. Younger age, higher body mass index, and worse breathlessness are characteristics that have been associated with clinically meaningful improvements in breathlessness with morphine[124,129]. There may also be genetic factors that influence responses to morphine[126]. However, these variables alone are not sufficient to adequately predict treatment response in breathless people with COPD. The current findings that baseline markers of poor sleep quality are associated with clinically meaningful improvements in breathlessness after four weeks of morphine suggest that baseline markers of poor sleep may help predict who will respond favourably to morphine.

Methodological considerations

While the current study is the largest of its type using a rigorous randomized clinical trial design, there are several limitations that need to be considered. Firstly, a key limitation of this study is the high number of dropouts beyond the primary endpoint at one week. This limits the per-dose power for the week 3 and 4 analyses. We attempted to overcome this limitation by combining morphine doses ranging from 8mg to 32mg/day and comparing outcomes with baseline. In addition, although there was no systematic dose effect on the dropout rate, given the high dropout rate overall beyond week 1, the potential for bias in the week 3 and 4 results in favour of morphine remains. Therefore, delineating specific dose effects beyond 1 week requires further investigation. Secondly, the design of blinded and randomized up-titration may have led to some participants receiving doses of morphine that were higher than necessary to reduce breathlessness. Accordingly, while there was no detected systematic difference in dropout rates with dose regime, some participants on higher than therapeutically optimal doses may have discontinued due to dose-related side effects. Similarly, the up-titration of dose over time also meant that the week 3 and 4 placebo-only groups were small, which limited the sample size for the placebo versus morphine comparisons. In addition, the cohort taking morphine at weeks 3 and 4 included a small proportion of participants who had taken placebo for the first one to two weeks. It is unclear if this may have influenced the results although this is perhaps unlikely given that the week 1 findings were similar to weeks 3 and 4. Thirdly, although sustained-release morphine does not appear to cause detrimental effects

on daytime sleepiness from one to four weeks, the effects over shorter and longer periods require further investigation.

Conclusions and summary

In conclusion, this multi-site, double-blind, randomized, placebo-controlled trial of regular, low-dose, sustained-release morphine in people with COPD and persistent breathlessness demonstrates that morphine does not increase daytime sleepiness. In addition, the findings of this study raise the possibility of a novel interaction between sleep quality and perception of breathlessness. Accordingly, sleep may be a key factor through which morphine moderates the sensation of chronic breathlessness in people with COPD. In addition, those with poor sleep quality may be more likely to experience a benefit in breathlessness with morphine. Further investigation into the potential links between sleep and breathlessness with objective measurements is required.

Chapter three supplement

Supplementary methods

Study design and interventions

At trial commencement, participants were randomly allocated using block randomization for each site in a 1:1:1 ratio to placebo, 8mg, or 16mg slow release oral morphine to be taken once daily. At the end of weeks one and two each participant was further randomized in a 1:1 ratio to add either 8mg slow-release morphine or placebo to their current dose. By week three, participants were on a range of doses from zero to 32mg slow-release morphine per day. All research staff, treating clinicians and participants were blinded to the treatment allocation. The protocol was approved by the Hunter New England Human Research Ethics Committee (HREC) (Reference No. 15/12/16/3.06) and New South Wales HREC (Reference No. HREC/15/HNE/502). The trial was prospectively registered on clinicaltrials.gov (NCT02720822). Informed written consent was obtained from all participants.

Participants

Inclusion criteria were age ≥18 years; physician-diagnosed COPD with a forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) ratio <0.7; persisting severe breathlessness defined as a modified Medical Research Council (mMRC) score of 3 or 4[201] despite optimal COPD treatment; *worst breathlessness* intensity ≥3 on a 0–10 numerical rating scale (NRS) during the last 24 hours before recruitment; ability to complete the assessments as judged by the investigator; stable COPD treatment during the previous week except 'as needed' medications. Exclusion criteria were any opioid use for breathlessness or regular opioid use ≥8mg morphine equivalent per day during the previous week; previous adverse reactions to the trial medication; documented central hypoventilation syndrome; pregnancy; marked hepatic or renal failure or signs of gastrointestinal obstruction.

Outcomes

The ESS assesses daytime sleepiness based on eight separate scenarios where respondents are asked to rate their usual chances of dozing or falling asleep. The KSS assesses sleepiness at the time of the test on a numerical rating scale. The LSEQ assesses four domains related to sleep:

ability to fall asleep ("getting to sleep", GTS); sleep quality ("quality of sleep", QOS); ease awakening ("awake following sleep", AFS); and tiredness after wakening ("behaviour following wakening", BFW).

The breathlessness data represent the worst breathlessness intensity in the previous 24 hours, measured using an 11 point (0-10) numerical rating scale. Higher scores equate to worse breathlessness.

Sample size and statistical power

Sample size calculations for the primary trial were based on the primary endpoint of breathlessness intensity at week 1 as previously reported[147]. Although caution is warranted given that this is a secondary analysis, based on our sample size of n=46 during placebo at the end of week 1 and n=49 in the morphine arm and a combined SD of 3.43 for the Epworth Sleepiness Scale (ESS), we estimate that we would be able to detect a difference of 1.99 in ESS if present with >80% power. This difference is equivalent to the minimal clinically important difference for the ESS[202].

Results

Randomization

A breakdown of the randomization allocation and timings is summarized in Table E3.1.

Effects of morphine on the relationship between sleep and change in breathlessness

There was no significant interaction between morphine dose, sleep questionnaire responses, and breathlessness (Table E3.2) except for ESS whereby participants with higher breathlessness scores at baseline in the placebo arm (but not the two morphine arms) reported higher ESS scores (Figure E3.3). This relationship remained in the placebo arm at week 1 and was also present at week 1 in the 16mg morphine arm (Figure E3.3).

There were no relationships between the change in KSS and breathlessness scores from baseline to week 3 or ESS and breathlessness scores from baseline to week 4 (Figure E3.4). Breathlessness scores were not different (p=0.81) in those who had an increase in KSS scores from baseline to week 3 in the combined morphine dose group (mean [95% CI] -1.78 [-2.71 to -0.85]) versus those who had a reduction in KSS scores during the same period (-1.62 [-2.54 to -0.69]).

At week 4, there was no association between improvement or no improvement in ESS scores versus baseline and breathlessness levels in the combined morphine group (mean change in breathlessness score [95% CI] -1.47 [-2.51 to -0.42] and -2.27 [-3.29 to -1.25] respectively). There was no significant difference in the change in week 4 breathlessness level between both groups ($p = 0.29$).

Table E3.1: Number of participants randomized to dose categories by week.

	Total number	Placebo	8mg/day	16mg/day	24mg/day	32mg/day	p-value
Week 1	156	50	55	51	-	-	0.50
Week 2	136	24	46	45	21	-	0.34
Week 3	120	12	31	38	30	9	0.66
Week 4	84	7	21	27	22	7	1.00

Fisher's exact test of association between morphine dose and withdrawals per week.

Table E3.2: Linear mixed model of breathlessness with three-way interaction between drug dose, study week and sleep scores.

Questionnaire	**Dose (mg/day)**	**Mixed model fixed effect coefficient (95% CI)**	**p-value**
KSS	8	-0.15 (-0.71 to 0.4)	0.86
	16	-0.05 (-0.61 to 0.51)	
LSEQ GTS	8	0.03 (-0.04 to 0.1)	0.61
	16	0.03 (-0.03 to 0.09)	
LSEQ QOS	8	0.03 (-0.03 to 0.08)	0.56
	16	0.02 (-0.03 to 0.07)	
LSEQ AFS	8	0.02 (-0.04 to 0.08)	0.67
	16	-0.01 (-0.06 to 0.05)	
LSEQ BFW	8	-0.01 (-0.07 to 0.05)	0.39
	16	-0.03 (-0.08 to 0.02)	

Three-way interaction between drug dose at week 1 (Placebo, 8mg, 16mg), study week (baseline, week 1), and sleep scores, with a random intercept for participant. P value is for the interaction term "Questionnaire

variable*dose*week", with placebo and baseline as the reference categories. KSS: Karolinska Sleepiness Scale; AFW: Awake Following Sleep domain; BFW: Behaviour Following Wake domain; CI: confidence interval; GTS: Getting to Sleep domain; LSEQ: Leeds Sleep Evaluation Questionnaire; QOS: Quality of Sleep domain.

Table E3.3: Change in breathlessness in morphine participants at week 4 based on baseline LSEQ domain scores ≤50 or >50.

LSEQ domain score at baseline	n	Change in breathlessness from baseline (Mean [95% CI])	P-value of change in breathlessness compared to no change
GTS >50	9	-1.56 [-3.8 to 0.69]	0.15
GTS ≤50	29	-2.07 [-2.91 to -1.23]	**<0.01**
QOS >50	6	-2.83 [-4.23 to -1.44]	**<0.01**
QOS ≤50	32	-1.78 [-2.68 to -0.89]	**<0.01**
AFS >50	15	-1.6 [-3.03 to -0.17]	**0.03**
AFS ≤50	22	-2.27 [-3.26 to -1.28]	**<0.01**
BFW >50	14	-1.21 [-2.72 to 0.29]	0.11
BFW ≤50	23	-2.48 [-3.4 to -1.56]	**<0.01**

Change in breathlessness in morphine participants at week 4 based on baseline Leeds Sleep Evaluation Questionnaire (LSEQ) domain scores ≤50 or >50. One sample t-test with null hypothesis being no change. Bold values indicate $P < 0.05$. AFW: Awake Following Sleep domain; BFW: Behaviour Following Wake domain; CI: confidence interval; GTS: Getting to Sleep domain; QOS: Quality of Sleep domain.

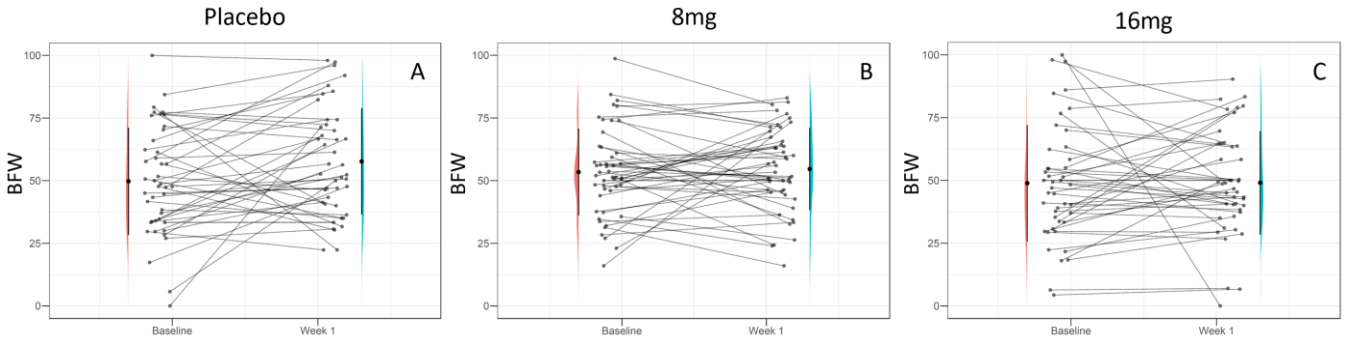

Figure E3.1: Leeds Sleep Evaluation Questionnaire Behaviour Following Wake (BFW) domain scores at baseline and Week 1.

(A) Placebo, (B) 8mg/day and (C) 16mg/day of morphine during week 1. Data show individual values with mean and standard deviation adjacent. The adjacent kernel density plots represent the distribution of values at baseline (pink) and one week (green) for BFW scores on placebo and the different morphine doses.

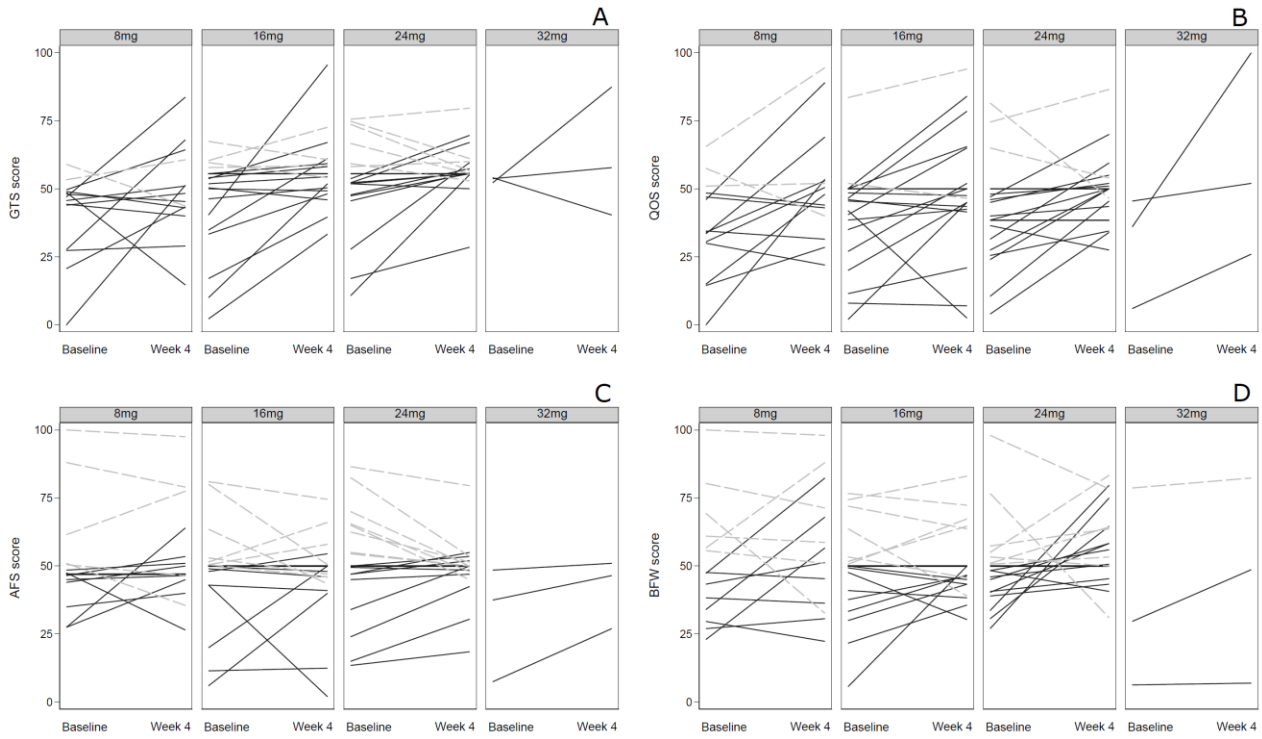

Figure E3.2: Leeds Sleep Evaluation Questionnaire (LSEQ) domain scores at baseline and week 4 on morphine per week 4 dose (8, 16, 24, or 32mg/day).

(A) Getting to Sleep (GTS); (B) Quality of Sleep (QOS); (C) Awake Following Sleep (AFS); (D) Behaviour Following Wake (BFW). Dotted lines indicate participants with baseline scores >50; solid lines indicate participants with baseline scores ≤50 (indicating poor sleep at baseline).

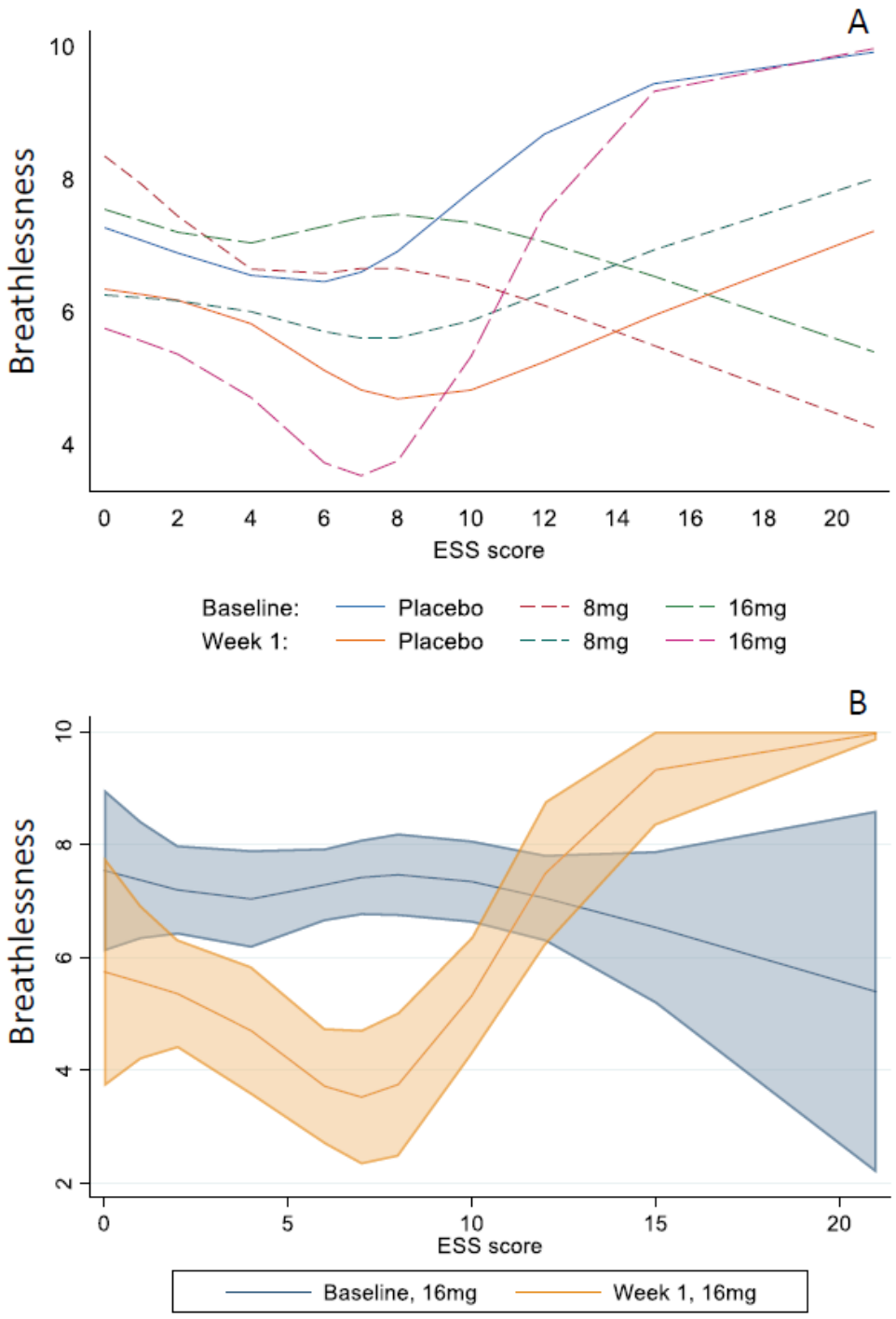

Figure E3.3: Estimated marginal means of worst breathlessness intensity in past 24 hours plotted against Epworth Sleepiness Scale (ESS) scores.

Placebo, 8mg/day and 16mg/day groups at baseline and week 1 (A), and at baseline and week 1 in the 16mg/day group only (B) with 95% confidence interval (shaded areas; blue: baseline; orange: week 1).

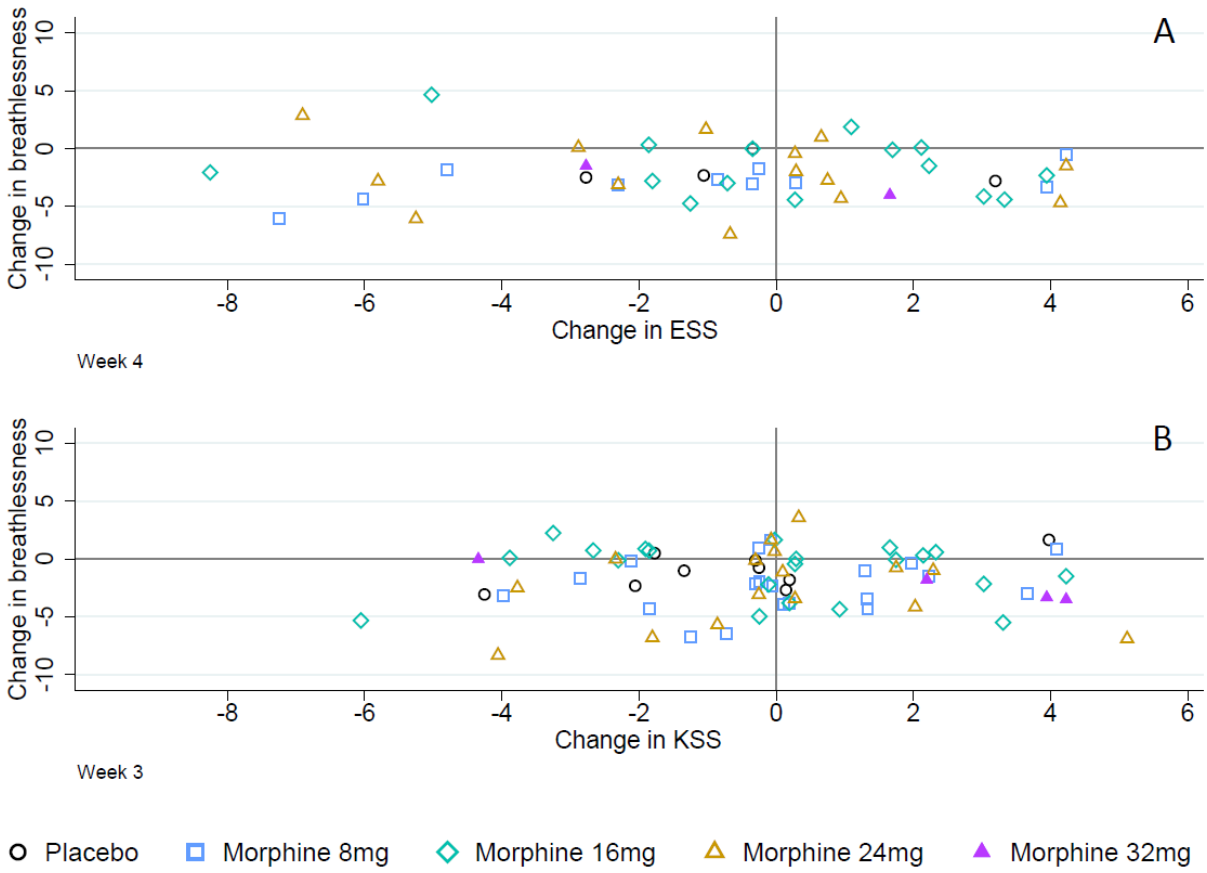

Figure E3.4: Change in Epworth Sleepiness Scale (ESS) and Karolinska Sleepiness Scale (KSS) scores plotted against change in worst breathlessness intensity in the past 24 hours.

(A) Baseline to week 4 ESS scores; (B) baseline to week 3 KSS scores.

Chapter Four: The effects of low-dose morphine on sleep and breathlessness in COPD: a randomized controlled trial

This chapter has been submitted for publication:

Altree TJ, Pinczel A, Toson B, Loffler KA, Hudson A, Zeng J, Proctor S, Naik G, Mukherjee S, Catcheside PG, Somogyi AA, Currow DC, Eckert DJ. The effects of low-dose morphine on sleep and breathlessness in COPD: A randomized controlled trial. Submitted March 2024.

Abstract

Rationale: Low-dose, sustained-release morphine may be prescribed to reduce chronic breathlessness in chronic obstructive pulmonary disease (COPD). Recent subjective findings suggest morphine may influence breathlessness through sleep-related mechanisms. However, concerns exist regarding opioid safety in COPD. The effects of morphine during sleep in COPD have not been objectively investigated.

Objectives: To objectively determine the effects of low-dose morphine on sleep. Outcomes included sleep efficiency (primary), sleep disordered breathing, oxygenation, transcutaneous carbon dioxide ($TcCO_2$), blood and physiology biomarkers, relationship between sleep and breathlessness, external resistive load responses, and driving simulator performance.

Methods: Randomized, double-blind, crossover trial of 20mg/day sustained-release morphine for three days (steady-state) versus placebo in nineteen breathless people with COPD (n=7 female). Physiology outcomes and pharmacokinetics were measured before and after in-laboratory overnight polysomnography with $TcCO_2$ monitoring.

Measurements and main results: Sleep efficiency was similar between placebo and morphine (66±17 vs. 67±19%, p=0.89). Morphine did not change the frequency of sleep-disordered breathing events, but reduced breathing frequency. Morphine reduced mean and nadir overnight oxygen saturation by [95%CI] 2 [-2.8 to -1.2] and 5 [-8 to -1]%, respectively. Mean $TcCO_2$ was 3.3 [1.6 to 5.1]mmHg higher during sleep versus placebo. Eight participants (42%) met American Academy of Sleep Medicine criteria for nocturnal hypoventilation with morphine versus four

(21%) on placebo, p=0.02. Morphine did not systematically reduce breathlessness or impair next day driving simulator performance.

Conclusions: Steady-state, low-dose morphine does not change sleep efficiency, sleep-disordered breathing frequency, or next day alertness but may cause hypoventilation during sleep, with the potential to be harmful.

Introduction

Chronic breathlessness is a distressing, debilitating consequence of chronic obstructive pulmonary disease (COPD)[113]. Reduction of breathlessness is therefore a key treatment goal[203]. However, many people with COPD continue to experience breathlessness despite optimal use of inhaled medications and non-pharmacological therapies such as pulmonary rehabilitation[182].

International guidelines recommend that regular, low-dose opioids can be used for symptomatic relief of persistent breathlessness in all people with COPD, not just those at the end of life[203]. However, safety concerns exist regarding the use of central respiratory depressants in people with advanced lung disease[194], and results from a recent large randomized controlled trial of the most studied drug, low-dose morphine, have raised doubts about efficacy[147].

During sleep, people with COPD experience pathophysiological changes that can further burden an already compromised respiratory system[107,108]. Opioids could potentially worsen impaired respiratory mechanics and breathing during sleep. Conversely, opioids could potentially improve COPD-related adverse impacts on sleep disturbance, by alleviating nocturnal COPD symptoms including breathlessness[102], facilitating more consolidated sleep and higher sleep efficiency[204]. Indeed, similar to the bi-directional relationship between improved sleep and reduced pain[200], in which the opioid system plays a key mediating role, opioid-related improvements in sleep may contribute to next day improvements in breathlessness without impairing daytime sleepiness or alertness in people with COPD[146,195,205]. However, the effects of opioids on these mechanisms and other factors that contribute to sleep impairment in COPD, including sleep disordered breathing, have not been objectively studied.

To address the lack of objective evidence on the effects of opioids during sleep in this potentially vulnerable cohort, we undertook a randomized, placebo-controlled controlled trial of low-dose morphine during sleep in people with COPD, with accompanying detailed respiratory physiology measurements to assess potential mechanisms. We hypothesized that morphine would improve sleep efficiency. Some of the results of this study have been previously reported in the form of an abstract[206].

Methods

Design

Placebo-controlled, randomized, double-blind crossover trial of oral sustained-release morphine sulfate 20mg/day. After screening, participants completed baseline testing before randomization to either morphine or placebo treatment first. The study drug was taken at 18:00hrs for three consecutive days, with a minimum four-day washout before beginning the next treatment (Figure 1). On the third night of each period, participants underwent an in-laboratory polysomnogram (PSG) followed by a morning driving simulator test[181]. Blood draws and inspiratory resistive load tests (six loads of varying resistance applied three times each, three breaths at a time in random order via nasal mask) with inspiratory pressure measurement[207] were performed before and after each PSG.

Outcomes

The primary outcome was sleep efficiency (% total sleep time) measured during the PSG versus placebo. Secondary outcomes included nocturnal oxygenation, transcutaneous carbon dioxide ($TcCO_2$), sleep disordered breathing, sleep architecture changes, and estimates of obstructive sleep apnea (OSA) endotypes from the PSG[17,208], morphine genotype (*OPRM1* rs1799971), plasma morphine and 3- and 6-glucuronide concentrations[90], resistive load test responses[207], driving simulator performance, and questionnaires that assessed symptoms, breathlessness, mood, and sleep. See the supplement for further details.

Participants provided informed written consent. The study was approved by the Southern Adelaide Clinical Human Research Ethics Committee (2021/HRE00178). The protocol and outcomes were pre-registered online (ACTRN12621000752864) and reported using the CONSORT framework[209].

Participants

Participants were eligible for inclusion if they met the following criteria: (1) aged ≥18 years; (2) had physician-diagnosed COPD with a post-bronchodilator forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) ratio <0.70; (3) had chronic breathlessness, defined as a modified Medical Research Council breathlessness score of ≥2 (i.e. "*I walk slower than people of the same age because of breathlessness, or have to stop for breath when walking at my own*

pace") at screening; (4) were taking optimal pharmacological COPD treatment as defined in the Global Initiative for COPD 2021 Report[93] and (5); scored ≥40 on the Australia-modified Karnofsky Performance Status scale (i.e., not in bed >50% of the time)[210].

See the supplement for the full list of exclusion criteria and information regarding participant recruitment.

Sample size

A recruitment target of 22 (allowing for an ~15% dropout rate, for a final sample size of 19) was calculated *a priori* to detect a 7% difference in sleep efficiency between conditions (SD=10)[211] with >80% power at an alpha level=0.05 (two-tailed paired t-test).

Randomization and blinding

All participants, investigators, and outcome assessors were blinded to treatment allocation order. See the supplement for further details.

Statistical Methods

Participant baseline characteristics were summarized using descriptive statistics. Continuous and count data were analyzed using generalized linear mixed models with treatment condition, period (first or second) and sequence as fixed effects and participant ID as a random effect, using either a normal (with restricted maximum likelihood estimation), Poisson or negative binomial distribution. Pearson and Spearman rank correlations were used to analyze associations between continuous variables. See the supplement for details.

Results

Participants

Seventy-five people were assessed for eligibility between September 2021 and October 2022 (Figure 4.1).

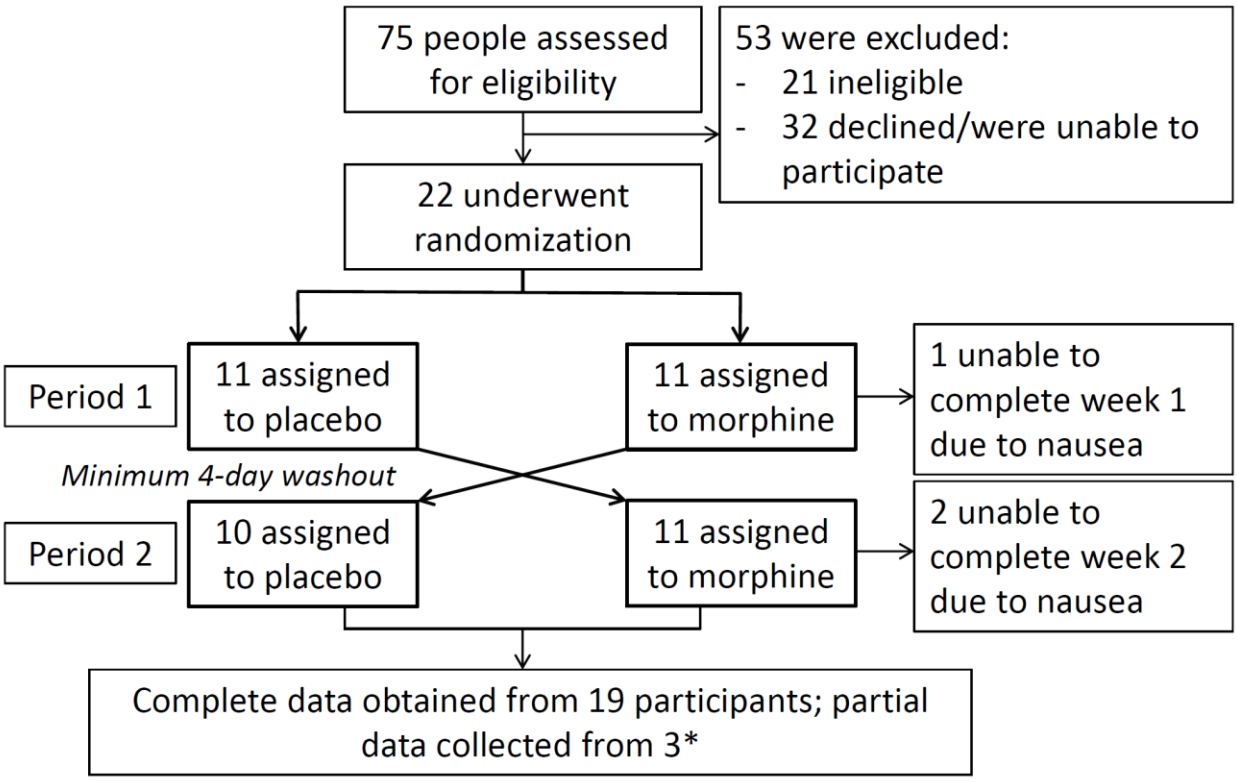

Figure 4.1: CONSORT flow diagram.

Participant recruitment, enrolment and flow through the protocol for this double-blind, randomized, placebo-controlled crossover study. *Data from all recruited participants (n=22) were included in the baseline characteristics, lung function and arterial blood gas results. Data from all participants who completed at least one period of the protocol (n=21) were included in the mixed model analyses.

Twenty-two participants underwent randomization. One participant dropped out due to nausea and vomiting before the week 1 PSG, and two dropped out due to nausea and vomiting prior to the week 2 PSG. Each drop out occurred in the morphine arm. Nineteen participants completed the protocol. Participant demographic and clinical characteristics are presented in Table 4.1 and Table E4.1 in the supplement. All participants were community-dwelling.

Table 4.1: Baseline characteristics.

Age (years)	71.1±7.2
Sex (F), %	10 (46%)
Height (cm)	170.1±11.3

Weight (kg)	76.5±23.7
BMI (kg/m^2)	26.1±6.2
Neck circumference (cm)	39.3±5.7
mMRC (median, IQR)	2 (2 to 3)
CAT	16.9±4.3
6 min. walk test (metres)	423±80
Insomnia severity index	9.1±5.7
Epworth sleepiness score	4.4±2.8
HADS	
Anxiety subscale	4±3.5
Depression subscale	3.6±2.4

Data presented as mean±SD unless otherwise stated for all enrolled participants (n=22). Three participants (14%) met criteria for clinical insomnia, one (5%) had excessive daytime sleepiness, four (18%) had at least mild anxiety and none had depression based on clinical cutoffs of ISI ≥15/28, ESS ≥10/24, HADS-Anxiety subscale ≥8/21, and HADS-Depression subscale ≥8/21 respectively. BMI: body mass index; CAT: Chronic Obstructive Pulmonary Disease Assessment Test; ESS: Epworth sleepiness score; HADS: Hospital anxiety and depression scale; ISI: Insomnia severity index; mMRC: Modified Medical Research Council Dyspnea Scale.

Participants were normocapnic at rest, with hyperinflation and moderately reduced diffusing capacity for carbon monoxide (Table 4.2).

Table 4.2: Baseline lung function and arterial blood gas results.

Arterial Blood Gas	
pH	7.4±0.03
PaO_2 (mmHg)	72.2±14.1
$PaCO_2$ (mmHg)	40.2±6.9
HCO_3^- (nmol/L)	25.7±3.7
Lung function	
Post-BD FEV1 (L)	1.67±0.6

Post-BD FEV1 (% predicted)	64.9±21.8
Post-BD FEV1/FVC	51.7±13.3
TLC (L)	6.6±1.7
TLC (% predicted)	106.7±22.2
FRC (L)	4.5±1.6
FRC (% predicted)	133.5±44.8
RV (L)	3.2±1.3
RV (% predicted)	139.5±60.2
RV/TLC	47.9±9.3
RV/TLC (% predicted)	126.4±34.9
DLCOcSB (% predicted)	56.3±16.4

Data presented as mean±SD for all enrolled participants (n=22). Arterial blood gas values were taken at rest on room air. BD: bronchodilator; DLCOcSB: single breath diffusing capacity of the lungs for carbon monoxide corrected for hemoglobin; FEV1: forced expiratory volume in 1 second; FRC: functional residual capacity; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity.

In-lab polysomnography

The primary endpoint of sleep efficiency was not different between placebo and morphine groups (mean±SE 66.4±4 vs. 67±4.2% respectively, mean difference [95% CI] 0.6 [-6.5 to 7.7]%, p=0.87, Figure 4.2).

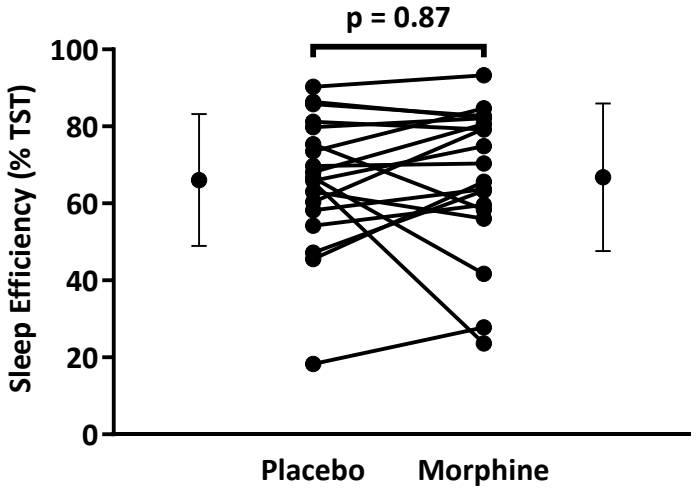

Figure 4.2: Sleep efficiency.

Sleep efficiency during PSG after administration of placebo or low-dose morphine for three days. PSG: polysomnography; TST: total sleep time.

Morphine caused significant changes to sleep architecture, including decreased REM and increased N2 sleep, fewer limb movements but more spontaneous arousals (all $p<0.05$, Table 4.3).

Table 4.3: Sleep parameters from polysomnography.

	Placebo	Morphine	Treatment effect [95% CI]
N1, %TST	22±4	25±4	4 [-1 to 9]
N2, %TST	42±3	49±3	6 [1 to 12]*
N3, %TST	18±3	14±3	-4 [-9 to 0]
REM, %TST	19±2	12±2	-6 [-11 to -2.2]**
PLM index, PLM/h	64±11	37±11	-27 [-48 to -7]**
PLM arousal index, PLM arousals/h	9±2	4±2	-5 [-8 to -2]**
Respiratory arousal index, events/h	18±4	20±4	1 [-5 to 7]
Spontaneous arousal index, events/h	7±2	12±2	5 [1 to 8]*
Total arousal index, events/h	37±4	37±4	1 [-5 to 7]
Sleep onset latency, min	50±13	36±13	-14 [-28 to 0]
Supine sleep, %TST	34±8	41±8	7 [-2 to 17]

TST, min	318±20	323±21	5 [-32 to 42]
WASO, min	111±16	124±17	14 [-18 to 45]

Data presented as estimated marginal mean ± standard error and average marginal effect with confidence intervals. These values were derived from linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect using restricted maximum likelihood estimation. N1, 2, 3: non-REM sleep stages 1, 2, and 3; PLM: periodic limb movement; REM: rapid eye movement sleep; TST: total sleep time; WASO: wake after sleep onset. **p<0.01 vs placebo, *p<0.05 vs. placebo.

Morphine had no significant effects on the frequency of sleep-disordered breathing events versus placebo (mean±SE apnea-hypopnea index (AHI) 28±7 vs. 25±7 events/hr respectively, mean difference [95% CI] 2 [-4 to 9] events/hr, p=0.46, Figure 4.3) or the magnitude of the accompanying oxygen desaturations (Table 4.4).

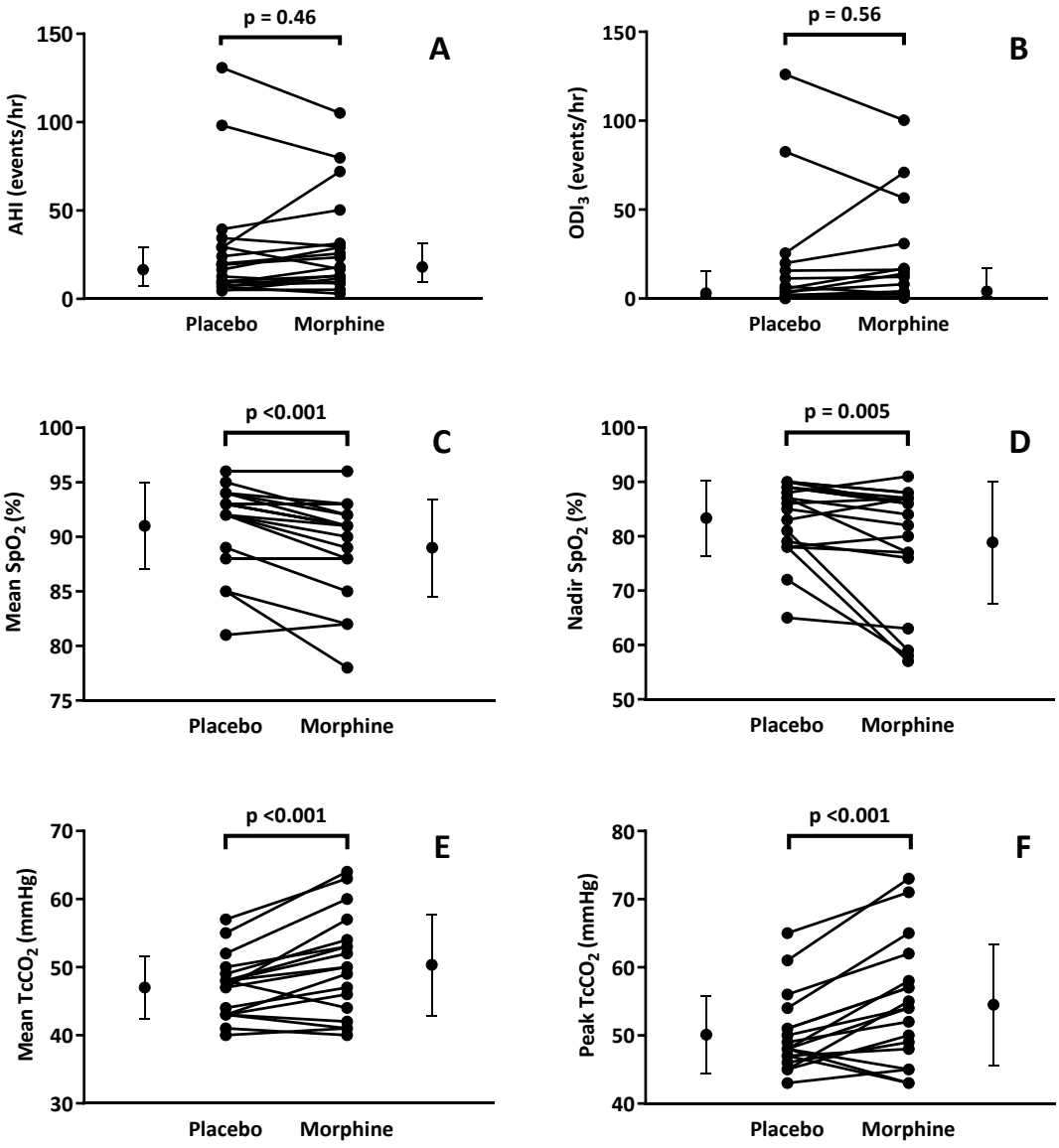

Figure 4.3: Respiratory events, oxygen saturation and transcutaneous carbon dioxide levels during sleep after three days of low-dose morphine or placebo.

A: apnea-hypopnea index; B: oxygen desaturation index (3%); C: mean oxygen saturation during sleep; D: minimum oxygen saturation during sleep, E: mean $TcCO_2$ during sleep; F: peak $TcCO_2$ during sleep. Note that the Y-axis scale differs for each panel. AHI: apnea-hypopnea index; ODI: oxygen desaturation index; SpO_2: oxygen saturation; $TcCO_2$: transcutaneous carbon dioxide.

Table 4.4: Respiratory parameters from polysomnography.

	Placebo	Morphine	Treatment effect [95% CI]
ODI_4, events/h	11.7±6	12.3±6	0.5 [-6.4 to 7.5]
Oxygen desaturation per event, %	3.8±0.4	4.1±0.4	0.3 [-0.2 to 0.8]
NREM AHI, events/h	24.6±7	28.2±7	3.5 [-3.5 to 10.5]
REM AHI, events/h	23.6±5.3	30.6±5.5	7.1 [-4.6 to 18.7]
Central apnea index, events/h	0.3±0.4	1.2±0.4	0.9 [-0.2 to 2]
Obstructive Apnea index, events/h	1.7±0.9	2.5±1	0.8 [-0.9 to 2.5]
Hypopnea index, events/h	23.1±5.7	23.8±5.7	0.8 [-2.9 to 4.5]
Awake oxygen saturation, %	93.3±0.8	91.6±0.8	-1.7 [-2.5 to -0.9]***
Mean NREM oxygen saturation, %	92.6±0.9	90.8±0.9	-1.8 [-2.5 to -1.1]***
Mean REM oxygen saturation, %	90.7±1.5	88.7±1.5	-2 [-3.4 to -0.6]**
Oxygen saturation <90%, mins	70±30	105±30	35 [6 to 63]*
Awake $TcCO_2$, mmHg	42.8±1.6	46±1.6	3.2 [1.6 to 4.8]***
Asleep respiratory rate, breaths/min	17.8±0.7	16.6±0.7	-1.2 [-2.3 to -0.2]*
NREM respiratory rate, breaths/min	17.5±0.7	16.3±0.7	-1.2 [-2.1 to -0.2]*
REM respiratory rate, breaths/min	19.3±0.8	18.4±0.8	-1 [-2 to 0.03]
Hypoxic burden (%min/h)	44.5±16	45.9±16.6	1.4 [-23.1 to 26]

Data presented as estimated marginal mean ± standard error and average marginal effect with confidence intervals. These values were derived from linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect using restricted maximum likelihood estimation. Awake variables were measured during quiet breathing in the supine position immediately prior to sleep. AHI: apnea-hypopnea index; NREM: non-rapid eye movement sleep; REM: rapid eye movement sleep; ODI: oxygen desaturation index; $TcCO_2$: transcutaneous carbon dioxide. ***p<0.001 vs. placebo, **p<0.01 vs. placebo, *p<0.05 vs. placebo.

Twelve (65%) participants on placebo and fourteen (74%) on the morphine had an AHI ≥10 events/hr.

Nocturnal oxygen saturation, respiratory rate, and transcutaneous carbon dioxide levels

Although morphine did not change the frequency of sleep disordered breathing events, morphine reduced oxygen saturation during wake (Table 4.4) and during sleep, by a mean [95% CI] of 2 [-3 to -1]%, p<0.001, and nadir asleep oxygen saturation by 5 [-8 to -1]%, p=0.005 (Figure

4.3).$TcCO_2$ levels during both wake and sleep were increased with morphine (Table 4.4, Figure 4.3), with mean and peak $TcCO_2$ levels 3.3 [1.6 to 5.1] and 4.4 [2.2 to 6.7] mmHg higher during sleep versus placebo, respectively (both $p<0.001$, Figure 4.3). At rest, the awake respiratory rate was lower on morphine versus placebo (mean±SE 17.6±0.7 versus 19±0.7 breaths/min respectively, p=0.01, mean difference [95% CI] -1.4 [-2.4 to 0.3] breaths/min). The respiratory rate during sleep was also lower on morphine (Table 4.4).

The number of participants meeting American Academy of Sleep Medicine criteria for nocturnal hypoventilation[158] was 4 (21%) on placebo and 8 (42%) on morphine, p=0.02.

OSA endotypes

There were no significant differences in OSA endotypes between morphine and placebo conditions or in a sub-analysis of endotypes in those with an AHI ≥10 events/hr (Table E4.2).

Breathlessness

Morphine did not improve any measure of subjective breathlessness quantified in the evening prior to the night 3 PSG nor the following morning (Table E4.3). Breathlessness was less on day 4 compared to pre-treatment baseline in both placebo and morphine conditions (placebo baseline to day 4 reduction 15±6 [4 to 26], $p<0.01$, morphine reduction 23±6 [11 to 34], $p<0.01$). However, the change was not statistically different with morphine versus placebo (-8±8, p=0.3 [-23 to 7]). There was no relationship between breathlessness and sleep efficiency (Figure E4.4). There were also no significant differences between conditions in Borg score or breathing patterns overall during inspiratory loaded breathing either before or after the in-laboratory sleep study (Figure 4.4, Table E4.4).

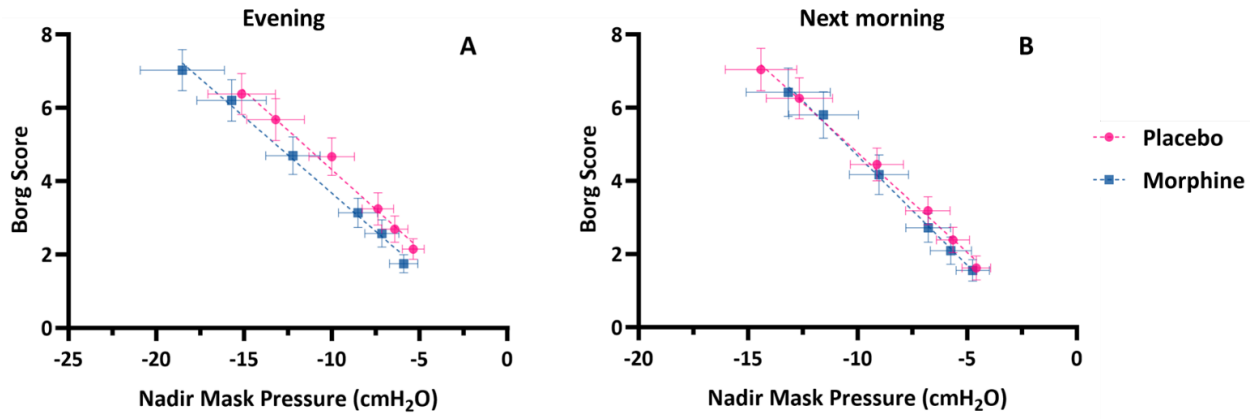

Figure 4.4: Respiratory load magnitude test results.

Respiratory load magnitude test results on the third night of low-dose morphine or placebo administration immediately prior to polysomnography (A) and the following morning (B). Six inspiratory loads of varying resistance were applied three times each for three breaths at a time in random order. Mean Borg score of difficulty breathing and nadir mask pressure were recorded for each resistance.

Morphine genotype and metabolism

There was wide inter-individual variability in plasma morphine and morphine-3 and 6-glucuronide concentrations in the evening prior to the PSG and the subsequent morning (Figure E4.5). There were no associations between morphine or metabolite concentrations and sleep efficiency (Figure E4.6).

Different responses to morphine were observed depending on the variant of the μ-opioid receptor gene, *OPRM1*. Specifically, those with the A118G single nucleotide polymorphism (5/19 participants) tended to have poorer sleep efficiency and less total sleep time on placebo (Figure E4.7). However, these individuals tended to have greater improvements in sleep efficiency, total sleep time, and accompanying increased time with SpO_2 below 90% (T90) on morphine versus placebo compared to the A118A homozygotes (Figure E4.7). There were no other systematic differences in the key study parameters including breathlessness responses between 118G carriers compared with AA homozygotes.

Driving simulator performance

Results from the 30-minute driving simulator performed in the morning after the PSG were not different between morphine and placebo. Driving simulator variables are presented in Table E4.5 and Figure 4.5.

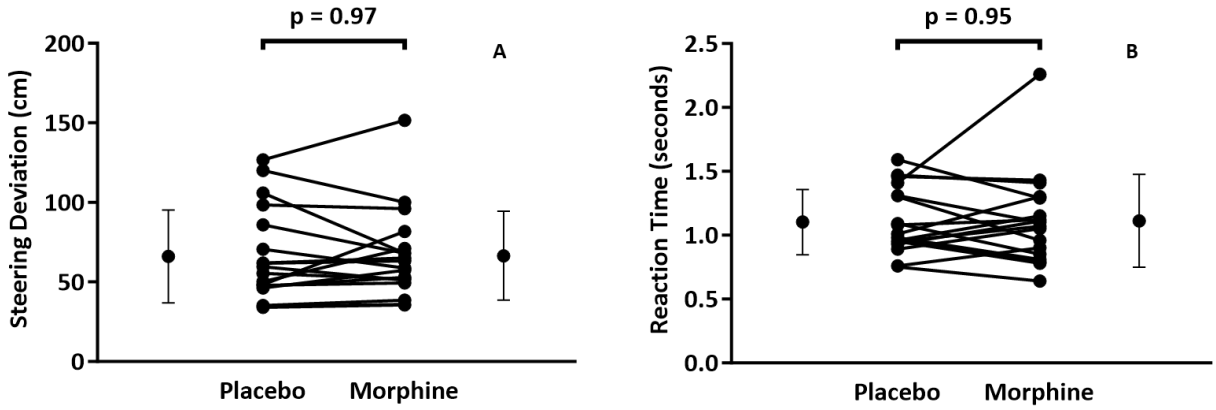

Figure 4.5: Driving simulator performance.

Morning driving simulator performance after three nights of low-dose morphine or placebo. A: mean steering deviation from median lane position, cm; B: mean reaction time, seconds.

Questionnaires assessing COPD symptoms, mood, and sleep

There were no significant differences in any COPD symptom, mood, or sleep questionnaire responses between placebo and morphine conditions (Table E4.6).

Predictors of improvement

No baseline variables that were considered potential predictors of breathlessness response to low-dose morphine (age; body mass index; breathlessness; COPD Assessment Test, Hospital Anxiety and Depression Scale, and Insomnia Severity Index scores; sleep efficiency (measured during the placebo condition); FEV_1; FVC; and diffusing capacity) were predictive of response.

Adverse events

No serious adverse events were recorded during the study. There was a 5.5 [1.6 to 18.5] times higher rate of adverse events reported with morphine. The most frequently reported adverse

event was nausea, with 6 (27%) instances during the morphine condition and none with placebo. Adverse events are listed according to treatment condition in table E4.7.

Discussion

In this randomized controlled trial of people with COPD and chronic breathlessness, regular low-dose sustained-release morphine caused nocturnal hypoventilation and hypoxia, in addition to detrimental sleep architecture changes including reduced REM sleep. However, low-dose morphine did not change sleep disordered breathing event frequency, next day alertness, or breathlessness. These objective findings demonstrate that people with COPD who take low-dose morphine for chronic breathlessness are exposed to potentially harmful effects on the respiratory system during sleep, without consistent relief of symptoms.

In-lab polysomnography

Despite the known sedative effects of opioids, three consecutive daily doses of 20mg sustained-release morphine did not improve sleep efficiency, sleep onset latency, or reduce total arousals. Rather, there were adverse changes to sleep architecture, including reduced REM sleep and increased spontaneous arousals. Reduced REM sleep has been observed in healthy people administered intravenous morphine (two 0.1mg/kg boluses during sleep)[130], and a single dose of 100mg oral tramadol[212]. This may be a dose-related effect, as neither single doses of 50mg oral tramadol[212] nor 15mg oral sustained-release morphine[132] reduced REM sleep. Increased N2 sleep is a consistent finding in all these studies[130,132,212] and was also observed in the current study.

Despite increased $TcCO_2$ and reduced oxygen saturation levels with morphine, we did not detect any major changes in sleep disordered breathing or the depth of desaturations associated with respiratory events. OSA is a common comorbidity in COPD. Indeed, 65% of the study participants had an AHI>10 events/hr despite excluding known severe OSA. The combination of OSA and COPD, known as *overlap syndrome*, is associated with increased risk of hospitalization and death[112,213]. However, the effects of opioids on the underlying pathophysiology of overlap syndrome are unclear. In general, people with OSA have increased ventilatory responses to hypercapnia/high loop gain[12,145], while the opposite is true in COPD[214]. In men with OSA given a single dose of 40mg sustained-release morphine, OSA was no worse overall, and there was a

positive correlation between a reduced ventilatory response to hypercapnia and improved OSA severity[90]. Despite current uncertainty regarding the pathophysiology of OSA in COPD, it appears that a dose of 20mg sustained-release morphine does not systematically increase the frequency of sleep-disordered breathing events in people with COPD. However, low-dose morphine does promote respiratory depression overall. The similarities in OSA endotype values between placebo and morphine nights supports this notion.

Periodic limb movements (PLMs) were reduced by morphine. A PLM index of >5 events/hr is considered abnormal[215], so the level recorded in this study is very high. The prevalence of PLMs in COPD is unknown, but they are closely associated with restless legs syndrome, which responds to oxycodone-naloxone therapy, with which this finding is consistent[216].

Nocturnal oxygen saturation, respiratory rate, and $TcCO_2$ levels

Sleep-related non-apneic oxygen desaturation is a well-known phenomenon in COPD[217]. Desaturations during sleep, especially during REM, are clinically significant in COPD because they are associated with more rapid progression to chronic respiratory failure and death[218,219]. Thus, while the effect size of morphine on oxygen saturation during sleep in the current study was relatively modest (2-5%), any reduction in the context of an already impaired respiratory system, as in the case of COPD, is of concern. We hypothesize that the reduction observed in the respiratory rate during sleep, leading to alveolar hypoventilation, was the main contributing factor. Diminished ventilatory response to blood gas disturbances due to morphine may have also played a role[83,90,119].

Perhaps even more concerning than the reductions in oxygen saturation were the relatively large increases in mean and peak transcutaneous CO_2 levels during sleep, and the doubling of the number of participants meeting AASM criteria for nocturnal hypoventilation. Hypercapnia in COPD is associated with higher mortality rates[220]. Hypoventilation tends to be more pronounced during REM sleep[221]. If opioid-related acute reductions in REM sleep stabilize and reverse over time, as appears to be the case[222], then nocturnal hypoventilation would be expected to increase even further above the levels seen in the current study. Likewise, in people with more severe COPD than the current cohort, we expect that the effects of morphine on ventilation during sleep would

be even more pronounced than observed here. Conversely, in people with more severe COPD, it is plausible that morphine-related reductions in respiratory rate could lower the degree of dynamic hyperinflation during sleep[185] and thus paradoxically improve nocturnal ventilation. However, this hypothesis has not been objectively tested, and the degree of hyperinflation would have to be high, given that the current cohort already had a relatively elevated mean functional residual capacity, and worse nocturnal hypoventilation with morphine despite a significantly reduced respiratory rate.

Opioid receptor genotype may also influence the effects of morphine on sleep and breathing. In people with OSA but without COPD, A118G single nucleotide polymorphism carriers tended to have the greatest improvements in T90 with morphine, which was related to reductions in chemosensitivity/respiratory control instability[90]. Contrary to these results, G carriers in the current study tended to have greater improvements in sleep with low-dose morphine, but at the expense of greater exposure to time asleep with an oxygen level below 90%. These findings require further careful investigation in larger samples.

Breathlessness

Morphine did not cause any systematic overall improvement in subjective breathlessness. This finding is consistent with the results of a recent large randomized controlled trial of low-dose morphine in approximately 150 people with COPD that found no improvements in breathlessness after one week[147]. Likewise, we observed no differences during inspiratory respiratory load magnitude testing between placebo and morphine. Therefore, although low-dose morphine reduced the respiratory rate at rest, 20mg morphine did not systematically blunt the inspiratory pressure generated when an acute respiratory stimulus was introduced. Thus, low-dose morphine does not appear to alter respiratory sensation, a result consistent with the effects of respiratory load testing in people with OSA administered 40mg of sustained-release morphine[207].

Additionally, there was no clear relationship between sleep efficiency and breathlessness. A recent analysis of sleep data from the BEAMS trial[205] suggested that morphine-related improvements in sleep quality may have influenced perception of breathlessness in those who experienced an improvement in breathlessness with low-dose morphine. The relationship between sleep,

breathlessness and morphine could not definitively be assessed in that study, as the number of participants in the placebo group was too low to make meaningful comparisons, and the sleep variables were measured subjectively. Based on the objective measures in the current study, morphine does not appear to systematically influence sleep efficiency, and changes in sleep efficiency does not appear to influence breathlessness. However, previous studies have noted large interindividual variability in the effects of low-dose morphine on breathlessness[125,126]. Accordingly, it may be that in some individuals, perception of breathlessness is mediated by morphine-related improvements during sleep that this study was not large enough to detect.

Morphine metabolism

The plasma morphine and metabolite concentrations demonstrate that even in a small group of individuals, there is wide variability in morphine metabolism. This finding is consistent with the wide ranges of plasma morphine and metabolite concentrations seen in men with OSA[90] and adults with COPD[223] prescribed oral morphine up to 40mg. Of note, in the latter study, there was no relationship between plasma morphine or metabolite concentrations and breathlessness during constant-load cardiopulmonary exercise testing. In the current study, there was no association between plasma morphine or metabolite concentrations and sleep efficiency. Based on the lack of improvement in breathlessness with low-dose morphine, no clear relationship between breathlessness and sleep efficiency, and no association between plasma drug/metabolite concentrations and sleep efficiency in the current study, the concept of a consistent relationship between breathlessness and sleep that is mediated by low-dose morphine is not supported.

Driving performance

The driving simulator results provide reassuring objective data that morning alertness is not impaired by low-dose morphine. In people with chronic breathlessness who drive, driving is an important component of identity and independence, and facilitates social connection[224]. These results provide important safety data and align with results of a recent large randomized controlled trial of low-dose morphine in COPD that showed no impairments in subjective measures of sleepiness and alertness over four weeks[205]. The Karolinska Sleepiness Scale results in the current study are also consistent with the notion that low-dose morphine does not cause sleepiness in chronically breathless people with COPD.

Predictors of benefit

Although low-dose morphine does not consistently relieve breathlessness in COPD, some individuals do experience clinically meaningful improvements[124]. Accurately identifying which patients are likely to benefit from low-dose morphine is an important aim for the field, so that patients unlikely to derive any benefit from low-dose morphine are not needlessly exposed to potential harm. Younger age, higher body mass, and worse breathlessness have been identified as potential predictors of beneficial response to morphine[124,129]. We assessed these and other factors, including COPD symptom burden, anxiety and depression levels, sleep efficiency, degree of insomnia, and baseline measures of lung function, but did not identify any clear predictors of beneficial response. The small size of the study cohort, and the lack of overall treatment effect on breathlessness may have limited the ability to assess predictive variables.

Methodological considerations

Although this study has several strengths including rigorous clinical trial design and the use of objective measures to investigate previously unanswered questions, there are some methodological limitations to note. Overall, the level of breathlessness was probably lower than the threshold at which most clinicians would prescribe low-dose morphine. Thus, the effects of morphine on outcomes reported in this study may not reflect real-world effects in people with more severe COPD, which, at least in terms of oxygenation and ventilation during sleep, would likely be worse. Similarly, although breathlessness was not the primary outcome of the study, measurement of breathlessness in the morning after the in-laboratory PSG may not have allowed sufficient time for participants to experience meaningful exertion and may have thus limited the ability of this study to investigate links between sleep and breathlessness. Furthermore, although participants reached pharmacokinetic steady-state, they may not have achieved peak therapeutic efficacy[225]. Thus, given the rapid tolerance to sedation/daytime sleepiness[205], investigation of the effects over a longer period is warranted. The measurement of sleep in the sleep laboratory rather than in participants' homes may also have altered the effects of morphine on sleep efficiency and potential relationships with breathlessness. However, not measuring sleep in the laboratory would have limited the ability to accurately measure other important sleep-related parameters. The starting dose of 20mg per day was potentially too high for some participants, leading to adverse events

such as nausea, which may not have occurred at lower doses. Medication side effects may also have led participants to believe that they were on the active drug rather than placebo, which could have potentially influenced subjective outcomes.

Conclusions

Low-dose, sustained-release morphine did not improve sleep efficiency, change sleep disordered breathing event frequency, or impair next day driving simulator performance in breathless people with COPD. However, morphine caused adverse changes to sleep architecture, reduced breathing frequency during sleep, and impaired blood gases. These results should not impact clinical decisions about the use of opioids in people with breathlessness in the terminal stage of COPD when management of symptoms takes precedence over other factors. However, in people with COPD and chronic breathlessness who are not in the terminal stage of the illness, low-dose morphine may cause harmful effects without any clear benefit. Accurately identifying which patients with breathlessness will respond to low-dose morphine remains a research priority.

Chapter four supplement

Participants

Participants were recruited from local advertisement and respiratory clinics including the Flinders Medical Centre respiratory clinics, the Southern Adelaide Local Health Network lung function labs, and the Southern Adelaide Local Health Network pulmonary rehabilitation and airway clearance programs. Participants were instructed not to drive on the days that study medications were taken. Participants were excluded if they met any of the following criteria:

- Concurrent use of opioids, benzodiazepines, or MAO inhibitors or within 7 days of such therapy
- Body mass index >40kg/m^2
- Known history of severe obstructive sleep apnea (apnea-hypopnea index ≥ 30 events/hr)
- Uncontrolled nausea, vomiting, or gastrointestinal obstruction
- Calculated creatinine clearance <25mL/min
- Two or more hepatic enzymes ≥3 times the upper limit of normal
- International normalised ratio >1.2 in the absence of warfarin
- Unresolved cardiac or respiratory event in the past 7 days (excluding upper respiratory tract infections)
- Anemia for which a blood transfusion was indicated for breathlessness in the past 12 months
- Pregnant, or childbearing potential not using contraception
- Breastfeeding
- Wait list for lung transplantation
- Change in COPD medications in the past 7 days (except as needed medications)
- Use of home non-invasive ventilation (NIV)
- History of endotracheal intubation for respiratory failure
- History of severe COPD exacerbation requiring acute NIV
- History of opioid-related respiratory failure
- History of opioid dependence (as per the International Classification of Diseases and Health Problems (ICD-10) definition)

- History of alcohol dependence (as per the International Classification of Diseases and Health Problems (ICD-10 definition)
- History of falls
- Mild cognitive impairment or dementia
- Any condition that, in the investigator's opinion, would present an unreasonable risk to the participant, or which would interfere with their participation in the study or confound study interpretation

Data collection

Baseline testing:

Participants completed baseline testing approximately 7 days prior to receiving any study medication. Data collected at baseline included: height, weight, waist and neck circumference, blood pressure and pulse rate; lung function testing (including pre- and post-bronchodilator spirometry, diffusing capacity for carbon monoxide, and body plethysmography based on Global Lung Initiative reference values from 2012, 2017, and 2021 respectively); room-air arterial blood gas; 6-minute walk test; and validated questionnaires including the *Breathlessness now* score[226], COPD Assessment Test[227], modified Medical Research Council score[228], Epworth Sleepiness Scale[192], Insomnia Severity Index[229], and the Hospital Anxiety and Depression Scale[230]. Participants also completed a 5 minute acclimatisation drive on the AusEd driving simulator. After baseline testing, participants were provided with a bottle containing the study medication for Period 1 (morphine or placebo depending on randomization allocation) and a bottle of aperients (see *Interventions* section below for further details).

Data collection during the morphine and placebo conditions (Figure E4.1):

Each morning after waking, participants completed a brief questionnaire at home to assess whether breathlessness disturbed their sleep (yes or no) and how they rated the previous night's sleep (very good, quite good, or poor or no sleep) based on the questionnaire reported previously[146].

On the third day, participants attended the Adelaide Institute for Sleep Health laboratory for an overnight polysomnogram (PSG) at approximately 19:00hrs. A standard clinical montage according to American Academy of Sleep Medicine version 2.4 guidelines[231] was used including

nasal flow, thermistor, respiratory bands, oximetry, chin and leg EMG, EEG and EOG (Grael 4K PSG:EEG, Compumedics, Abbotsford, Australia). Awake and asleep respiratory rates were derived from PSG data where periods of respiratory events and breathing artefact were excluded. A transcutaneous CO_2 ($TcCO_2$) monitor (SenTec Ag Digital Monitoring System, Therwil, Switzerland) was applied to the left forearm to monitor nocturnal CO_2 levels. The lights-out time was established according to each participant's habitual schedule and kept constant between the two in-lab PSGs. Each participant was given an 8 hour sleep opportunity during the PSG.

Approximately 60 minutes prior to the PSG, and again approximately 60 minutes after waking the following morning, inspiratory resistive load testing was performed. The test was conducted in the supine position. During testing, participants wore a nasal mask (Comfort Gel Blue, Philips Respironics, Murrysville, PA) connected to a carbon dioxide sensor (17630 CO_2 Analyzer, VacuMed, Ventura, CA), mask pressure sensor (CD19A, Validyne, Northridge, CA) and pneumotachograph (3700A, Hans Rudolph, Kansas City, MO). The pneumotachograph was attached to a T-shaped non-rebreathing valve (1410B, Hans Rudolph). The inspiratory end of the valve was attached to a 45cm flexible tube that was passed through a hole in the wall from the participant side to a control room, where the test operator sat. The operator end of the tube was attached to a three-way stopcock. Loads of six different resistances that generated mean inspiratory mask pressures ranging from -4.6±2.8 to -18.5±10.2 cmH_2O (Figure 4.4) were attached to the stopcock for three breaths at a time. Each load was tested 3 times during inspiration in random order, for a total of 18 loads of three breaths each. Expiration was not loaded. Participants were instructed to breathe through their nose for the duration of the test. They were given approximately six unloaded breaths to recover between each of the 18 loads. Before the test, participants were presented with the lowest (#1) and highest (#6) loads for acclimatisation. During the test, participants were instructed to indicate their perceived difficulty breathing by pointing to a score on an A3-sized 10-point Borg scale that was held in front of them after the third breath with each load. Encephalography (electrodes applied in the 10-20 system with additional Fz, Cz, and Oz electrodes) was also recorded during the test. Encephalography, mask pressure, CO_2, and airflow were collected via a CED 1401 analog-to-digital convertor and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Each test took approximately 20 minutes to complete.

Following evening respiratory loads testing, approximately 5mL of blood was taken via venepuncture. This was approximately 2 hours after medication ingestion. This sample was analyzed for morphine and morphine metabolite concentrations (plasma morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G)) and *OPRM1* genotype.

A 5mL sample for morphine metabolism was also taken the following morning immediately after the respiratory load magnitude test (approximately 90 minutes after waking). Morphine and morphine metabolite levels were assessed by liquid chromatography-tandem mass spectroscopy as follows: Morphine, morphine-3-glucuronide, morphine-6-glucuronide, and the deuterated compounds used as internal standards (morphine-d_3, morphine-3-glucuronide-d_3, morphine-6-glucuronide-d_3) were purchased from Cerilliant (Texas, USA). All solvents were of high-performance liquid chromatography grade. Protein precipitation was accomplished by adding 300 µL of methanol to a tube containing 10 µL of labelled internal standard, and 100 µL of standard, control or patient plasma sample. The resulting solution was vortexed at 10°C for 10 min, and centrifuged at 15,000×g for 10 min. The supernatant was transferred to a 96-well plate, evaporated to dryness using a vacuum centrifugal evaporator and resuspended in 100 µL of water containing 0.1% formic acid (v/v) and 5 mM ammonium formate (mobile phase A). An aliquot of 10 µL was injected for ultra-high-performance liquid chromatographic–tandem mass spectrometric analysis. Plasma concentrations were determined using a Nexera ultra-high-performance liquid chromatography system coupled to a liquid chromatography mass spectrometry LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Tokyo, Japan). Compounds were separated using a gradient on a Kinetex C18 column (100 × 2.1 mm, 1.7 µm) (Phenomenex, California, USA) maintained at 30°C. The mobile phase consisted of water containing 0.1% formic acid (v/v) (mobile phase A), and methanol containing 0.1% formic acid (v/v) (mobile phase B), with a flow rate of 0.4 ml/min. The validated calibration ranges for morphine, and morphine-3-glucuronide was 3–1000 ng/mL, and 1.5-500 ng/mL for morphine-6-glucuronide.

For genotyping, DNA was extracted from whole blood using Maxwell® RSC Blood DNA Purification kits on a Maxwell® RSC Magnetic Particle Processor (Promega Corporation, Sydney, NSW, Australia) according to the manufacturer's standard protocol. The concentration and purity

of isolated DNA was determined by spectrophotometric absorbance using a BioTek Synergy Mx Microplate Reader and Take3 plate (Agilent Technologies, Inc., Santa Clara, CA, USA). *OPRM1* rs1799971 genotype was determined by probe-based allelic discrimination PCR using forward and reverse custom primers and specific wildtype [A] and variant [G] probes (Sigma Aldrich Pty Ltd, Castle Hill, NSW, Australia). The assay included 1x iTaq Universal Probes Supermix (BioRad, Sydney, Australia), 150 nM each primer and probe, and 20 ng of DNA (20 uL total reaction volume) on a CFX96 real-time PCR system (BioRad). Thermocycling conditions were 95°C for 3 min then cycles of 95°C for 5 seconds, followed by 71°C for 30 seconds, with allelic discrimination performed using CFX Manager 3.0 software (BioRad) after 30 cycles. Sanger sequenced genotype controls and two no-template controls were included in each run.

All blood test analyses were performed at the laboratory of the Discipline of Pharmacology, The University of Adelaide.

Approximately 30 minutes after waking from the overnight PSG, participants completed a 30 minute simulated driving task (AusEd driving simulator)[155].

Finally, the following questionnaires were completed after the morning blood sample was taken, in addition to the daily questionnaire about breathlessness during sleep/sleep quality: Breathlessness Now, COPD Assessment Test, Hospital Anxiety and Depression Score, Karolinska Sleepiness Score, and the Leeds Sleep Evaluation Questionnaire.

After completing the morning questionnaires, participants returned home for a minimum four day washout period before returning to the laboratory to collect the medication for Period 2. The Period 2 protocol was identical to the Period 1 protocol, except that blood was not sent for genotype analysis in Period 2.

Interventions

To counter the risk of constipation with morphine, participants were provided with a container of docusate and sennoside B 50mg/8mg two tablets orally, maximum four tablets per day, to be taken if needed. These medications were dispensed at the start of the study, independent of the initial

study condition (morphine or placebo). If required, participants were prescribed 10mg oral metoclopramide by a study physician for as-needed use for nausea.

Randomization and blinding

Study medications were prepared by Optima Ovest pharmacy. Medications were placed in identical containers containing three identical capsules that could not be identified by study personnel or participants. The study pharmacist prepared the randomization code in blocks of four. All analyses were performed before unblinding of the intervention allocation.

Sleep disordered breathing endotype analysis

Obstructive sleep apnea (OSA) endotypes were quantified using a validated, custom-designed algorithm applied to the polysomnography recordings (MATLAB, MathWorks)[16,17]. Briefly, ventilation was estimated using the square root transform of the nasal pressure signal (tidal volume x respiratory frequency), which was integrated breath-by-breath to provide a time series of ventilation data normalized for analysis (mean ventilation = 1, apnea = 0). The traits were measured on each placebo and morphine night during non-REM sleep in supine and lateral positions. The traits are defined as follows:

- Mean pharyngeal collapsibility (Vpassive): the estimated average ventilation at eupneic drive when the pharyngeal muscles are in a passive state. A higher value represents a less collapsible airway. Measured as a percentage of eupneic ventilation.
- Nadir pharyngeal collapsibility ($Vpassive_{min}$): the estimated ventilation at the most hypotonic level of pharyngeal muscle activity i.e. when the airway is at its most collapsible, measured at the lowest Vpassive decile (equivalent to the passive critical closing pressure of the upper airway). A higher value represents a less collapsible airway at the point of higher likelihood of collapse.
- Pharyngeal muscle recruitment (Vactive): the estimated ventilation at maximum ventilatory drive. A higher value indicates increased muscle recruitment.
- Pharyngeal muscle compensation (Vcomp): the estimated change in ventilation that accompanies an increase in ventilatory drive, i.e., the ventilatory equivalent of the active minus passive critical closing pressures, measured as the difference between Vactive and

Vpassive. A higher value represents greater pharyngeal dilator muscle compensation, measured as a percentage of eupneic ventilation.

- Mean ventilatory response to arousal (VRA): the estimated ventilatory overshoot during transient arousals from sleep. A higher value represents greater overshoot/increased propensity to develop respiratory instability.
- Ventilatory control stability (loop gain): LG_1, breathing response to a 1 cycle/min reduction in ventilation, and LG_n, loop gain at the natural cycling frequency including circulatory delay effects. Higher values indicate greater ventilatory control instability.
- Arousal threshold: the estimated respiratory drive that causes a cortical arousal from sleep. A higher value represents a larger reduction in ventilation that can be tolerated before an arousal from sleep occurs, measured as a percentage of eupneic ventilation.

Statistical Methods

Participants' baseline characteristics were summarized using numbers and percentages for categorical variables and either mean and standard deviation (SD) or median and interquartile range for continuous variables, depending on the distribution. For continuous outcomes, differences between treatment conditions were analyzed using linear mixed models with treatment condition, period and sequence as fixed effects and participant ID as random effects, using restricted maximum likelihood estimation. For count data (e.g., number of crashes and number of adverse effects), generalized linear mixed models with treatment condition, period and sequence as fixed effects, and participant ID as a random effect were employed using either a Poisson or negative binomial distribution. Estimated marginal means, standard errors, average marginal effect for morphine versus placebo, confidence intervals, and p-values were presented. Resistive inspiratory load test results (i.e., Borg ratings of difficulty breathing) were analyzed using linear mixed model analyses, where mask pressure and condition interaction, period, and sequence were considered fixed effects, and participant ID was treated as a random effect using restricted maximum likelihood estimation. Morphine/metabolite concentrations were analyzed using Spearman rank correlation to account for the non-parametric distribution. The relationship between selected baseline variables and improvements in breathlessness with low-dose morphine was analyzed using Pearson correlation.

Supplementary Results

Questionnaires assessing sleep

Most participants did not report that breathlessness disrupted their sleep, independent of the study drug (Figure E2). When asked to answer "how was your sleep last night", most participants on placebo reported "quite good" sleep on nights 1 and 2, whereas most reported "poor or none" on night 3, which was spent in the sleep laboratory rather than at home (Figure E3). In the morphine arm, most reported "poor or none" on nights 1 and 3, with a majority reporting "quite good" sleep on night 2. There were 8 reports of at least one "very good" night of sleep in the participants taking morphine, compared to 2 in the placebo arm.

Table E4.1: Participants' regular inhaled medications and comorbidities

Inhaled medications		**n (%)**
	Long-acting muscarinic antagonist	16 (84%)
	Long-acting β-agonist	16 (84%)
	Inhaled corticosteroid	14 (74%)
Comorbidities		
	Osteoarthritis	4 (21%)
	Osteoporosis	1 (5%)
	Depression	4 (21%)
	Previous cancer	3 (16%)
	Hypercholesterolemia	4 (21%)
	Gastroesophageal reflux disease	4 (21%)
	Gout	2 (11%)
	Hypertension	9 (47%)
	Congestive cardiac failure	1 (5%)
	Type-2 diabetes mellitus	2 (11%)
	Peripheral vascular disease	2 (11%)
	Diverticulitis	2 (11%)
	Deep vein thrombosis	1 (5%)
	Bronchiectasis	1 (5%)
	Atrial fibrillation	1 (5%)
	Rheumatoid arthritis	2 (11%)

All comorbidities that could contribute to breathlessness were optimally managed prior to inclusion in the study. Comorbidities were defined based on clinical records and data obtained during screening.

Table E4.2. Obstructive sleep apnea endotypes

	Placebo	**Morphine**	**Treatment effect [95% CI]**	**p-value**
LG_1, 1 cycle/min	0.5±0.03	0.56±0.03	0.06 [-0.01 to 0.13]	0.068
LG_n, dimensionless	0.37±0.02	0.4±0.02	0.03 [-0.01 to 0.08]	0.14
Arousal threshold, % Veupnea	119.1±8.1	129.3±8.1	10.1 [-0.2 to 20.4]	0.054
Vpassive, % Veupnea	93.4±4.6	83.7±4.8	-9.7 [-19.9 to 0.5]	0.063
$Vpassive_{min}$	58.3±6.3	56.9±6.4	-1.4 [-9.8 to 7]	0.743
Vactive, % Veupnea	89.1±7.1	86.1±7.1	-3 [-9 to 3]	0.322
Vcomp, % Veupnea	-4.3±4.9	2.3 ±5	6.5 [-1.7 to 14.8]	0.121
VRA, % Veupnea	24.5±5	25.3±5	0.8 [-7.8 to 9.5]	0.85

Data are presented as estimated marginal mean ± standard error, average marginal effect with confidence intervals, and p-value. These values were derived from linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect using restricted maximum likelihood estimation. Loop gain (LG): estimated change in ventilatory drive in response to a ventilatory disturbance, presented as LG_1, breathing response to a 1 cycle per minute reduction in ventilation, and LG_n at the natural cycling frequency including circulatory delay effects); arousal threshold: the estimated respiratory drive that causes an arousal from sleep; Vpassive: the estimated ventilation (pharyngeal collapsibility) at normal/eupneic ventilatory drive; $Vpassive_{min}$: the estimated ventilation when the pharyngeal muscles are at their most hypotonic level; Vactive: the estimated ventilation at maximum ventilatory drive; Vcomp: the change in estimated ventilation that accompanies an increase in ventilatory drive, measured as the difference between Vactive and Vpassive; the ventilatory response to arousal (VRA): the estimated ventilatory overshoot during a transient arousal from sleep; % Veupnea: percentage of the eupneic level of ventilation. See text in the online supplement for further details. A sub-analysis of endotypes in people with obstructive sleep apnea (as defined by an apnea-hypopnea index ≥10 events/hr) also revealed no significant differences between placebo and morphine conditions.

Table E4.3. Subjective measures of breathlessness

	Placebo	Morphine	Treatment effect [95% CI]	p-value
Evening (prior to sleep study)				
Average breathlessness	31.8±3.8	32.1±4	0.3 [-6.9 to 7.5]	0.93
Current breathlessness	23.5±4.5	21.7±4.7	-1.8 [-13.7 to 10]	0.76
Worst breathlessness	44.2±5.9	45.8±6.1	1.6 [-11.4 to 14.7]	0.81
Morning (following sleep study)				
Average breathlessness	26±4.4	29.3±4.6	3.3 [-5 to 11.6]	0.44
Current breathlessness	20.6±4.1	19.4±4.2	-1.2 [-8.5 to 6]	0.74
Worst breathlessness	39.8±6.2	32.4±6.5	-7.4 [-22.7 to 7.9]	0.34

Data are presented as estimated marginal mean ± standard error, average marginal effect with confidence intervals, and p-value. These values were derived from linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect using restricted maximum likelihood estimation. Breathlessness was measured on a visual analog scale with scores between 0 (none) and 100 (worst or most intense). Average and worst breathlessness were based on the previous 24 hour time period.

Table E4.4. Average ventilatory parameters during resistive load testing

	Placebo	Morphine	Treatment effect [95% CI]	p-value
Evening				
Inspiratory time, s	2.5±0.2	2.4±0.2	-0.1 [-0.2 to 0.1]	0.51
Expiratory time, s	2.1±0.2	2.1±0.2	-0.03 [-0.2 to 0.2]	0.75
Respiratory rate, breaths/min	14.7±1.4	14.6±1.4	-0.1 [-1.1 to 0.9]	0.79
Tidal volume, L	1.8±0.7	1.8±0.7	0.01 [-0.1 to 0.2]	0.85
Minute volume, L/min	15.9±1.3	16.4±1.3	0.5 [-1.3 to 2.3]	0.58
Inspiratory flow, L/s	0.50±0.04	0.50±0.04	0.01 [-0.04 to 0.1]	0.63
Inspiratory fraction per breath	0.60±0.01	0.60±.01	-0.002 [-0.02 to 0.02]	0.87
Peak inspiratory flow, L/s	0.6±0.1	0.7±0.1	0.02 [-0.04 to 0.1]	0.46
Morning				
Inspiratory time, s	2.4±0.2	2.3±0.2	-0.1 [-0.3 to 0.04]	0.15
Expiratory time, s	2.1±0.2	2.1±0.2	0.04 [-0.3 to 0.3]	0.81
Respiratory rate, breaths/min	15.6±1.2	16.4±1.2	0.8 [-0.5 to 2]	0.24
Tidal volume, L	1±0.1	1.1±0.1	0.1 [-0.1 to 0.3]	0.24
Minute volume, L/min	15.7±1.5	16.4±1.5	0.7 [-3.3 to 4.6]	0.74
Inspiratory flow, L/s	0.4±0.1	0.5±0.1	0.1 [-0.1 to 0.2]	0.3
Inspiratory fraction per breath	0.60±0.01	0.50±0.01	-0.01 [-0.04 to 0.01]	0.3
Peak inspiratory flow, L/s	0.6±0.1	0.7±0.1	0.1 [-0.1 to 0.2]	0.28

Data are presented as estimated marginal mean ± standard error, average marginal effect with confidence intervals, and p-value. These values were derived from linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect, using restricted maximum likelihood estimation.

Table E4.5. Driving simulator results

	Placebo	**Morphine**	**Treatment effect [95% CI]**	**p-value**
Number of crashes	3.8±1.7	3.7±1.7	-0.2 [-1.6 to 1.3]	0.8
Reactions to an obstacle	8.1±0.6	8.4±0.7	0.4 [-1.4 to 2.2]	0.68
Speed deviation, km/h	7.3±2.3	7.0±2.4	-0.4 [-1.5 to 0.8]	0.52
Steering deviation from center of lane, cm	92±9.4	99.5±9.7	7.5 [-5.3 to 20.3]	0.25

Data were collected during a 30-minute drive on the AusEd driving simulator following the overnight PSG. Data are presented as estimated marginal mean ± standard error, average marginal effect with confidence intervals and p-value. These values were derived from generalized linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect. The analysis for the number of crashes utilized a Poisson distribution, while the remaining outcomes were based on a normal distribution.

Table E4.6. COPD symptom, mood, and sleep questionnaires

	Placebo	**Morphine**	**Treatment effect [95% CI]**	**p-value**
CAT	15±1	16±1	1 [-1 to 3]	0.35
HADS				
Anxiety subscale	4.3±0.8	4.7±0.8	0.5 [-0.3 to 1.2]	0.23
Depression subscale	3.4±0.5	3.7±0.5	0.3 [-0.6 to 1.2]	0.56
KSS	4.5±0.5	4.9±0.5	0.4 [-0.4 to 1.2]	0.32
LSEQ				
Getting to sleep	12.1±1	13.2±1	1.1 [-1.4 to 3.6]	0.39
Quality of sleep	7.1±0.8	8.7±0.9	1.7 [-0.6 to 3.9]	0.14
Awake following sleep	10.5±0.8	9.6±0.8	-0.9 [-3.1 to 1.3]	0.42
Behaviour following wake	14.7±1.3	14±1.3	-0.6 [-2 to 0.8]	0.37

Data are presented as estimated marginal mean ± standard error, average marginal effect with confidence intervals, and p-value. These values were derived from linear mixed model analyses, where treatment condition, period, and sequence were considered fixed effects, and participant ID was treated as a random effect, using restricted maximum likelihood estimation. CAT: COPD assessment test; COPD: chronic obstructive pulmonary disease; AHDS: hospital anxiety and depression scale; KSS: Karolinska sleepiness scale; LSEQ: Leeds sleep evaluation questionnaire.

Table E4.7. Adverse events

	Placebo	**Morphine**
Nausea	0	6
Vomiting	1	4
Paresthesia	0	1
Diaphoresis	0	1
Nightmare	1	0
Insomnia	0	2
Dizziness	0	1
Drowsiness	0	1
Constipation	0	2
Cough	1	0
Headache	1	1
Total AEs	4	19

No severe adverse events were recorded during the study. Three participants who experienced vomiting were unable to complete the study protocol. One placebo-related adverse event and fourteen morphine-related adverse events were graded as moderate in severity. AE: adverse event.

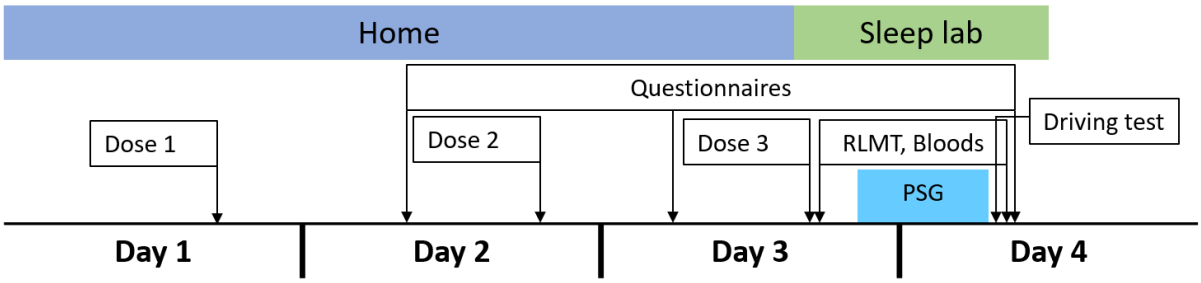

Figure E4.1. Timeline of data collection during each treatment period. Participants completed the protocol once for each treatment condition in blinded randomized order (i.e., with sequence placebo then morphine, or morphine then placebo), with a minimum 4-day washout between conditions. PSG: Polysomnogram; RLMT: respiratory load magnitude test.

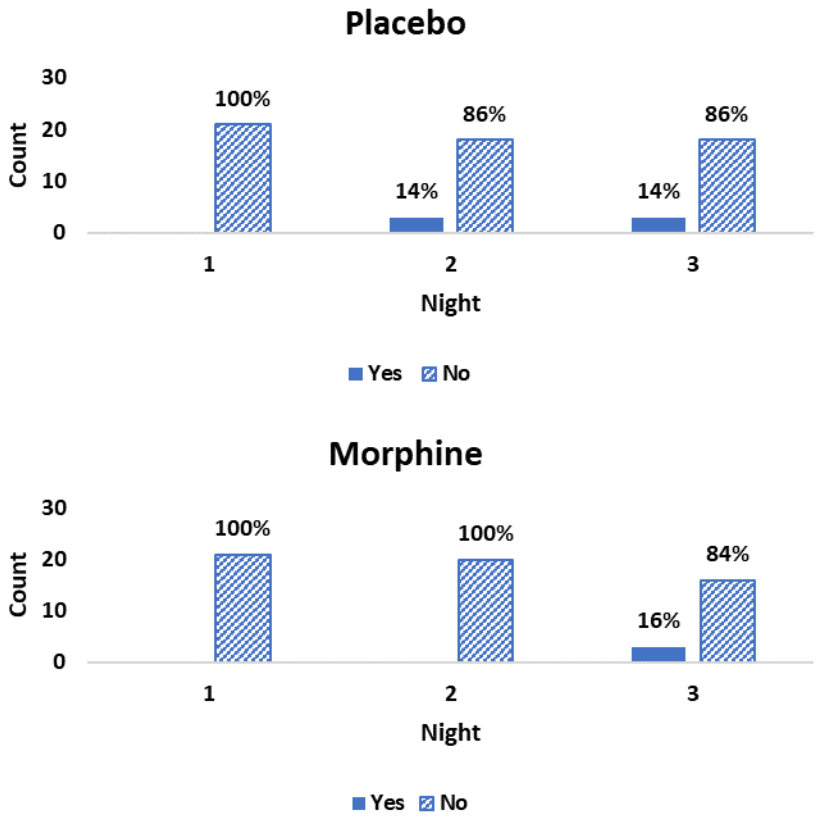

Figure E4.2. Count of participants who reported sleep disruption from breathlessness during the previous night's sleep, as measured in the participant diary each morning. Participants were asked to respond "yes" or "no" to the question "was your sleep disturbed by breathlessness?" Nights 1 and 2 were spent in participants' own homes, and night 3 was spent in the sleep laboratory.

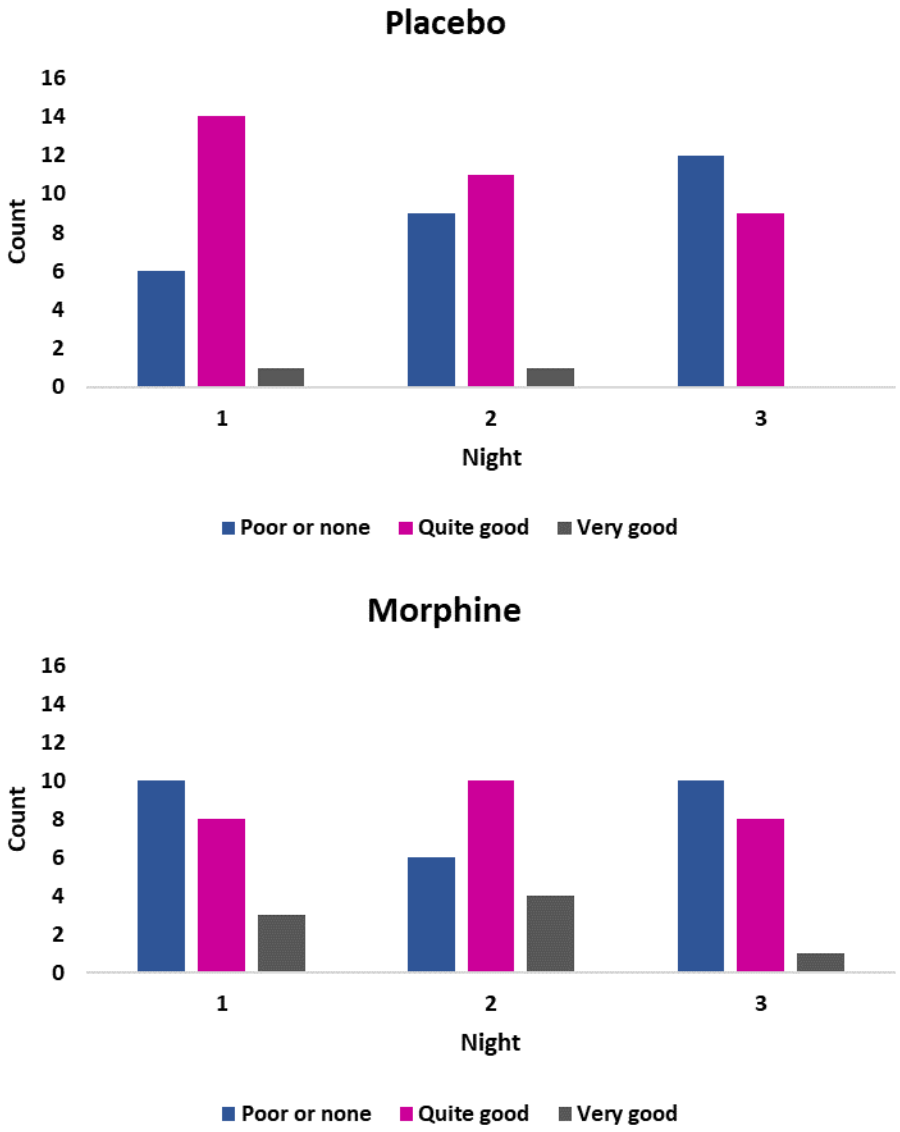

Figure E4.3. Count of nightly perceived sleep quality, as recorded in the participant diary each morning. Participants were asked to answer the question "how was your sleep last night" by selecting one of the following three options: poor or none; quite good; or very good. Nights 1 and 2 were spent in participants' own homes, and night 3 was spent in the sleep laboratory.

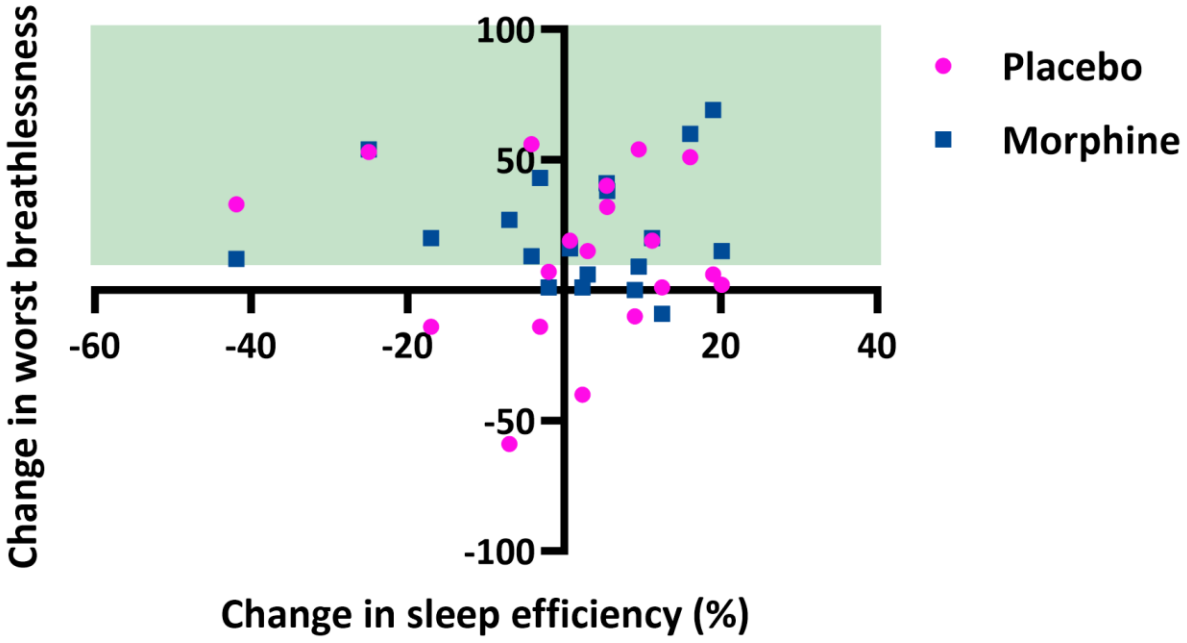

Figure E4.4. Change in breathlessness versus change in sleep efficiency. Change in sleep efficiency was measured as the difference between the morphine and placebo conditions. Change in breathlessness was measured as the difference between the worst breathlessness in the past 24 hours at pre-treatment baseline compared to the morning after the in-laboratory polysomnogram on a visual analog scale. Positive numbers in the figure indicate improvements in sleep efficiency and breathlessness respectively. The shaded area represents the minimal clinically important improvement in breathlessness[193]. Note: there was no difference in the slope of the relationship during placebo (0.0265, 95% CI [-0.765 to 0.818], p=0.948) or morphine (0.202, 95%CI [-0.589 to 0.9934], p=0.616) conditions (linear mixed model analysis which included baseline worst breathlessness, period [week] and sequence as fixed effects).

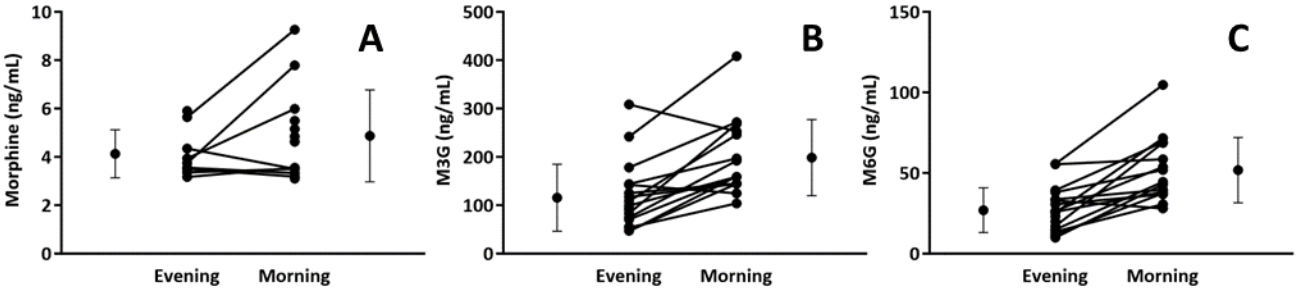

Figure E4.5. Plasma morphine and morphine metabolite levels. Samples taken in the evening (approximately two hours after medication ingestion) prior to the in-lab polysomnogram, and the following morning during morphine condition (i.e., no placebo data are included in these plots). A. Plasma morphine; B. Plasma morphine-3-glucuronide (M3G); C. Plasma morphine-6-glucuronide (M6G).

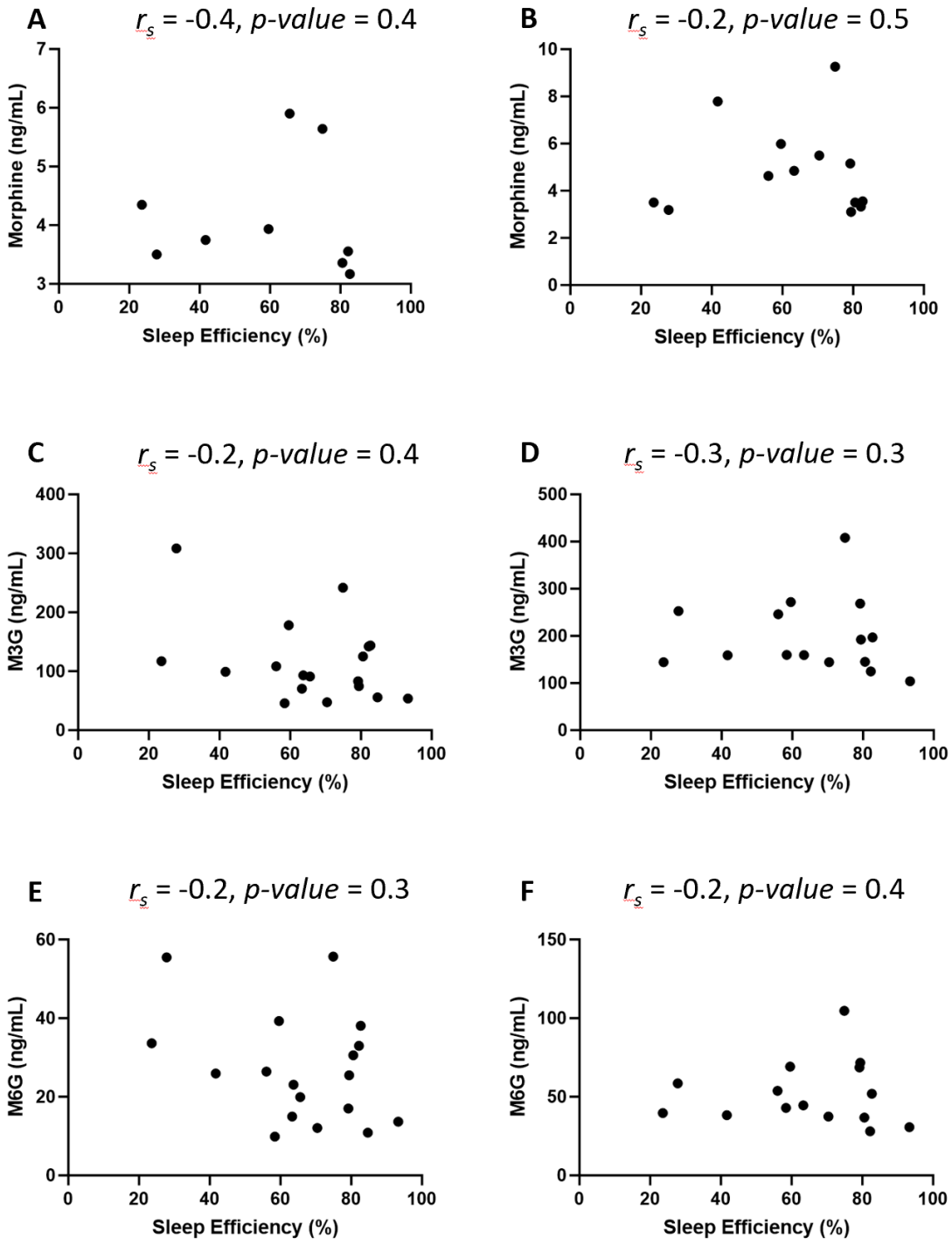

Figure E4.6. Plasma morphine and morphine metabolite levels versus sleep efficiency during the morphine condition.

A, C, and E: blood samples taken on night three prior to the in-lab PSG; B, D and F: blood samples taken after waking the following morning. Spearman test was used to test correlations. M3G: morphine-3-glucuronide; M6G: morphine-6-glucuronide (M6G); PSG: polysomnogram.

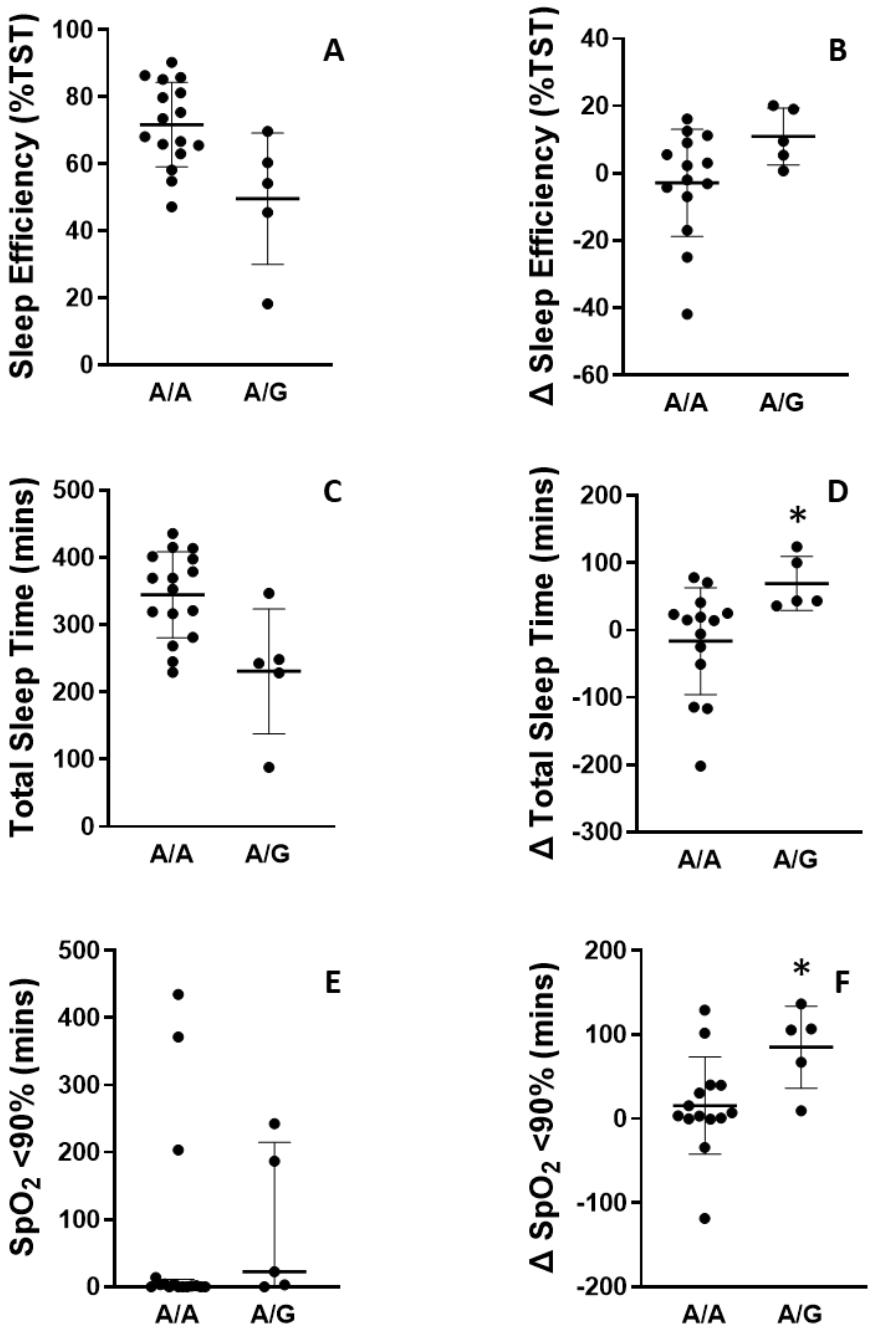

Figure E4.7. Sleep efficiency (% total sleep time), total sleep time (minutes), and sleep time spent below an estimated oxygen saturation level via pulse oximetry (SpO_2) of 90% separated by μ-opioid receptor (*OPRM1*) genotype (wild type, A/A, or variant, A/G). A, C, and E: data from the placebo condition. B, D, and F: data are change (Δ) on morphine compared to placebo condition. *indicates significant difference between A/A and A/G genotype via a two sample t-test. P values were as follows: A p=0.061, B p=0.085, C p=0.053, D p= 0.037, E p=0.822 and F p=0.029

Chapter Five: Thesis summary, conclusions, and future directions

Obstructive sleep apnea and chronic obstructive pulmonary disease are highly prevalent respiratory conditions that cause significant symptom burden. In both conditions, current treatment options often fail to adequately treat the disease and control symptoms. Recent advances in knowledge of OSA pathophysiology led to the notion that noradrenergic reuptake inhibitors and antimuscarinic drugs, two classes that were initially developed for different indications, may reduce OSA severity[60,61]. Similarly, observations that morphine, an analgesic, can improve breathlessness, led to studies that supported its use in refractory breathlessness in people with COPD[149,232]. However, there are major unanswered questions and safety concerns regarding the use of these repurposed drugs in OSA and COPD, especially relating to breathing during sleep and next day alertness. These knowledge gaps formed the basis of this thesis.

In **chapter two**, I performed a double-blind, randomized, controlled crossover trial of 16 people with OSA to investigate the effects of the noradrenaline reuptake inhibitor reboxetine at a dose of 4mg alone on OSA severity, compared to reboxetine combined with the antimuscarnic oxybutynin at a dose of 5mg, and placebo. Additionally, I used a novel signal processing method to investigate the effects of reboxetine on key OSA pathophysiological traits, to investigate how noradrenergic agents lead to improvements in OSA severity.

Reboxetine alone modestly reduced OSA severity as measured by the AHI and caused improvements in overnight oxygenation and snoring. These beneficial effects were likely driven largely by improvements in ventilatory control stability. The addition of oxybutynin led to mild sedative effects but did not produce additive reductions in OSA severity despite modest improvements in pharyngeal muscle compensation. People with unstable ventilatory control (high loop gain endotype, mostly men in the current study), responded most favourably to reboxetine. However, acutely, morning heart rate increased with reboxetine. Blood pressure was unchanged. Perceived sleep quality was reduced despite improvements in OSA severity, and REM sleep was also reduced. The long-term effects of REM suppression, and whether REM suppression persists over time or diminishes, are important questions raised by this study that require further investigation.

The results of this this randomized controlled trial provide new insight into the importance of noradrenergic mechanisms in OSA and the potential importance of endotype characterization to optimize pharmacotherapy strategies for OSA. The beneficial effects on AHI and oxygenation have also highlighted reboxetine as a potential treatment option for OSA in the days immediately following surgery, a period when standard OSA treatments such as CPAP are often poorly tolerated[75]. Untreated OSA is associated with worse postoperative outcomes[71,72], so the use of a simple daily tablet that improves OSA severity is potentially a highly effective and practice-changing novel approach. Based on the results of chapter two, my colleagues and I were awarded a grant from Flinders University to conduct a phase two randomized controlled trial to assess the feasibility of using reboxetine after head and neck surgery in people with OSA (ClinicalTrials.gov NCT05978505).

In **chapter three**, I analyzed sleep questionnaire and breathlessness data from a large, double-blind, randomized controlled trial of low-dose morphine for breathlessness in approximately 150 people with COPD. There is concern that the central nervous system depressant effects of morphine could potentially lead to adverse outcomes such as daytime drowsiness in people with COPD. However, to date, these effects have not been thoroughly investigated. Additionally, the mechanism by which morphine alleviates breathlessness is unclear. I hypothesized that morphine improves sleep quality, which mediates the perception of daytime breathlessness in much the same way that people with chronic pain experience worse pain in the setting of inadequate sleep.

My analysis demonstrated that morphine did not lead to impaired daytime sleepiness/alertness in breathless people with COPD, and improved subjective sleep quality. Additionally, the results supported the hypothesis that there is a relationship between sleep quality and daytime perception of breathlessness that may be mediated by low-dose morphine. I also showed that those with poor sleep quality at baseline appeared to be more likely to experience a beneficial breathlessness response to morphine. However, the subjective nature of the data limits firm conclusions being drawn about the secondary and exploratory outcomes. The effects on sleepiness and alertness are clinically reassuring and add to the evidence base for clinicians prescribing low-dose morphine for

breathlessness in COPD. The results of project two also highlighted that objective measures during sleep are required, which informed the design of project three (chapter four).

In **chapter four**, I thus used objective measures to further investigate the effects of low-dose morphine during sleep via a placebo controlled, double-blind randomized controlled crossover trial of three consecutive daily doses of 20mg sustained-release morphine in 19 people with COPD and chronic breathlessness. In this project, I sought to address the lack of objective evidence on the effects of low-dose morphine on the multiple pathophysiological changes that occur to the control of breathing during sleep in COPD. I hypothesized that low-dose morphine would improve sleep efficiency, but that it would also lower the respiratory rate during sleep, leading to hypoventilation and lower oxygenation. I also investigated links between sleep efficiency and breathlessness that were identified in chapter three and the associated manuscript[205], and assessed potential variables that might be predictive of breathlessness response to morphine. Additionally, to investigate the alertness findings of chapter three with objective measures, driving simulator performance was assessed.

Morphine did not improve sleep efficiency and reduced REM sleep. The respiratory rate was lowered, and there was evidence of hypoventilation based on a reduction in oxygenation and increased CO_2 levels. There were no significant effects on sleep disordered breathing event frequency. Breathlessness was not improved, and the results did not support the notion of a relationship between sleep efficiency and breathlessness. No clinically meaningful predictors of breathlessness response to low-dose morphine were identified. In support of the subjectively-based alertness and sleepiness results in chapter three, driving simulator performance was not impaired by three days of low-dose morphine. However, the absence of a relationship between sleep efficiency and breathlessness contrasted with the findings in chapter three. There are several reasons that may have led to these differing results. First, chapter four was conducted in the sleep laboratory, rather than the home, which may have influenced sleep duration and efficiency. Second, sleep duration and efficiency were objectively measured in chapter four, whereas only subjective measures of sleep quality were collected in chapter three. Third, the chapter three results assessing the potential relationship between sleep quality and breathlessness did not have a placebo comparator, and were therefore suggestive of a relationship, rather than being conclusive. Finally,

the baseline breathlessness severity of participants in chapter three was higher than participants studied in chapter four, which may have led to differences in sleep and response to morphine.

Chapter four demonstrated that low-dose morphine does not improve sleep efficiency when measured in the sleep laboratory, but causes adverse changes to sleep architecture, lowers the respiratory rate during sleep, and leads to worse blood gas disturbances during sleep. These are potentially harmful effects.

The results of chapters three and four raise other important questions that require further study. The overall efficacy of low-dose morphine as a treatment for chronic breathlessness remains unclear. It is likely that certain traits in people with COPD increase the likelihood of a beneficial response to morphine, but at present, there are insufficient data identifying robust predictors of response that are easy to determine in the clinic. Larger studies in well-characterised cohorts of people with COPD are required. The objective longer-term effects of low-dose opioids on sleep quality in people with chronic breathlessness also remain unclear. Research addressing this question would need to employ novel home-based objective measures of sleep over time. Whether the hypoventilation seen in chapter four leads to clinically significant harm, such as increased mortality, is also a priority for future research in people with COPD and chronic breathlessness.

Obstructive sleep apnea and chronic obstructive pulmonary disease are common, harmful respiratory conditions. Through the use of novel techniques and rigorous clinical trial design, this thesis addresses knowledge gaps in the pathophysiology and treatment of both diseases. It also identifies directions for future research. The results provide important new insight into the pharmacological management of obstructive sleep apnea, and the effects of low-dose morphine on sleep, breathing and daytime alertness in chronic obstructive pulmonary disease.

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Appendix A – Questionnaires used in this thesis

The following questionnaires are included below:

- Breathlessness Now Numerical Rating Scale
- Modified Medical Research Council Dyspnea Scale
- COPD Assessment Test
- Hospital Anxiety and Depression Scale
- Karolinska Sleepiness Scale
- Epworth Sleepiness Scale
- Leeds Sleep Evaluation Questionnaire
- Martins COPD Sleep Assessment Questions
- Australia-Modified Karnofsky Performance Status Scale
- Insomnia Severity Index

Breathlessness Now Numerical Rating Scale

How is your breathlessness right now?

None _____ **Worst possible**

How is your breathlessness right now?

None _____ **The most unpleasant I have ever felt**

Worst breathlessness intensity in the past 24 hours

None _____ **Worst possible**

Average breathlessness intensity in the past 24 hours

None _____ **Worst possible**

Best breathlessness intensity in the past 24 hours

None _____ **Worst possible**

Currow D et al. Thorax. 2020 Jan;75(1):50-56. doi: 10.1136/thoraxjnl-2019-213681. Epub 2019 Sep 26. Erratum in: Thorax. 2020 Jul;75(7):e5. PMID: 31558624.

Modified Medical Research Council (mMRC) Dyspnea Scale

Mahler DA, Wells CK. Chest. 1988 Mar;93(3):580-6. doi: 10.1378/chest.93.3.580. PMID: 3342669.

COPD Assessment Test

How is your COPD?

For each item below, place a mark (√) in the box that best describes your experience.

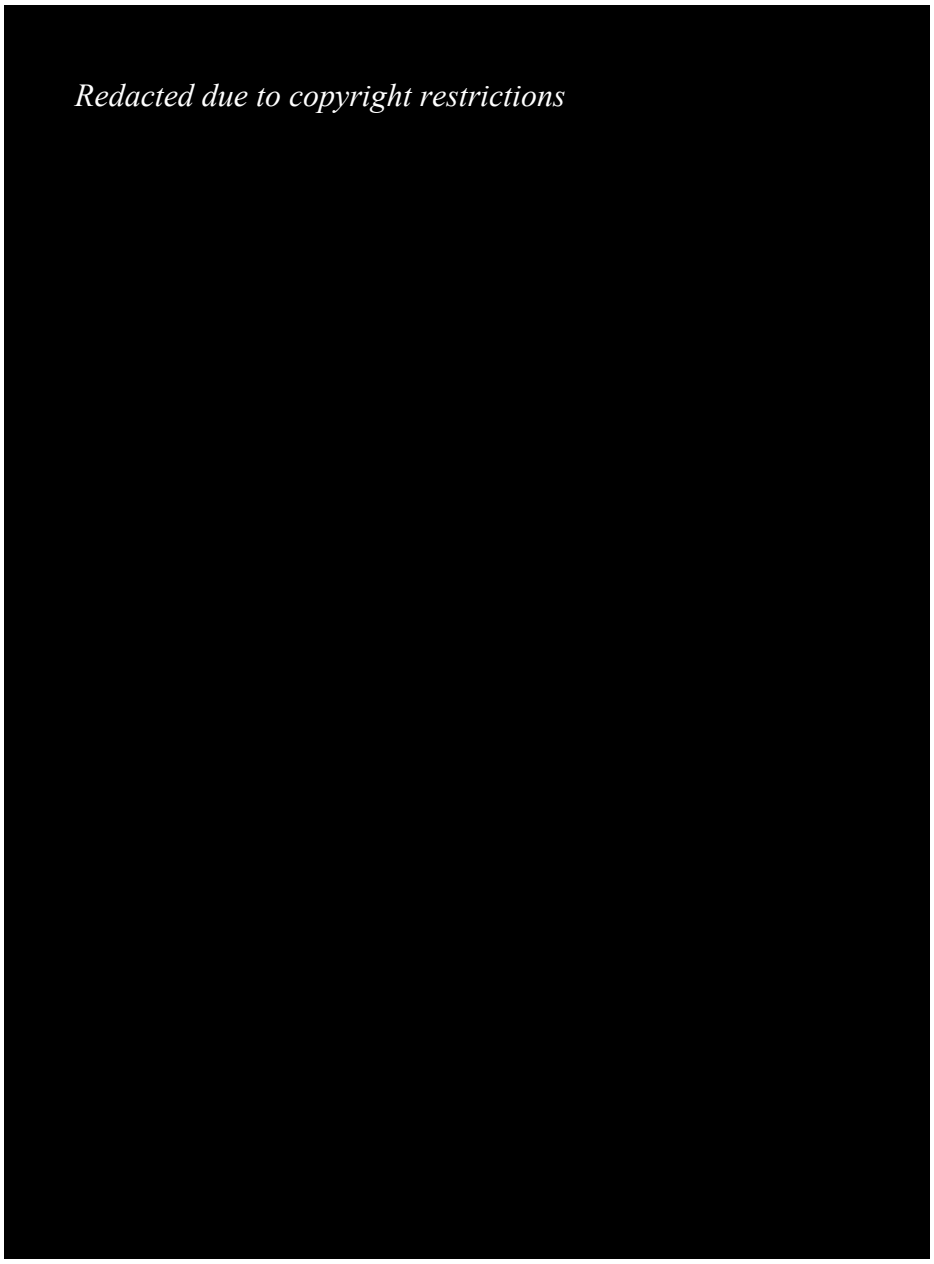
Example: I am very happy | 0 | √ 1 | 2 | 3 | 4 | 5 | I am very sad

			SCORE
I never cough	0 1 2 3 4 5	I cough all the time	☐
I have no phlegm (mucus) in my chest at all	0 1 2 3 4 5	My chest is completely full of phlegm (mucus)	☐
My chest does not feel tight at all	0 1 2 3 4 5	My chest feels very tight	☐
When I walk up a hill or one flight of stairs I am not breathless	0 1 2 3 4 5	When I walk up a hill or one flight of stairs I am very breathless	☐
I am not limited doing any activities at home	0 1 2 3 4 5	I am very limited doing activities at home	☐
I am confident leaving my home despite my lung condition	0 1 2 3 4 5	I am not at all confident leaving my home because of my lung condition	☐
I sleep soundly	0 1 2 3 4 5	I don't sleep soundly because of my lung condition	☐
I have lots of energy	0 1 2 3 4 5	I have no energy at all	☐

SCORE ☐☐

P. W. Jones et al. Eur Respir J 2009;34:648-654

Hospital Anxiety and Depression Scale



Zigmond AS, Snaith RP. Acta Psychiatr Scand. 1983 Jun;67(6):361-70. doi: 10.1111/j.1600-0447.1983.tb09716.x. PMID: 6880820.

Karolinska Sleepiness Scale

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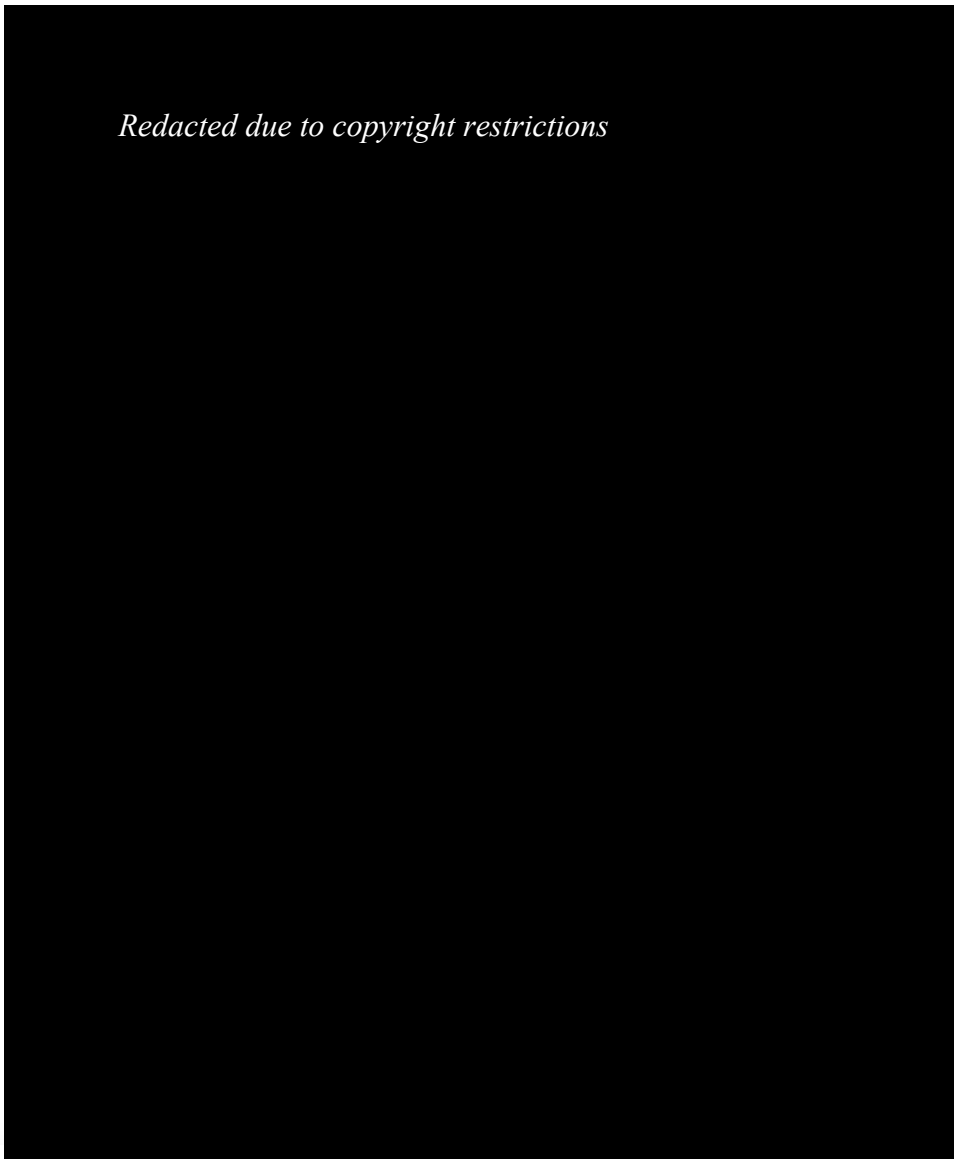
Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. Int J Neurosci 1990;52:29-37.

Epworth Sleepiness Scale



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

Leeds Sleep Evaluation Questionnaire

Hindmarch I. A 1,4-benzodiazepine, temazepam (K 3917), its effect on some psychological parameters of sleep and behaviour. Drug research 1975;25:1836-9.

Martins COPD Sleep Assessment Questions

Circle the answer for a. and b.

a. Was your sleep disturbed by your breathlessness?

Yes No

b. How was your sleep last night?

1. Very good
2. Quite good
3. Poor
4. No sleep

Martins RT, et al. Respirology. 2016 Feb;21(2):386-91. doi: 10.1111/resp.12681. Epub 2015 Nov 12. PMID: 26560987.

Australia-modified Karnofsky Performance Status (AKPS) scale

Abernethy AP, et al. BMC Palliat Care. 2005 Nov 12;4:7. doi: 10.1186/1472-684X-4-7. PMID: 16283937; PMCID: PMC1308820.

Insomnia Severity Index

Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. Sleep Med 2001;2:297-307.