Journal Pre-proof

Reboxetine plus Oxybutynin for Obstructed Sleep Apnea Treatment A 1-week Randomized, Placebo-controlled, Double-Blind Crossover Trial

Elisa Perger, Luigi Taranto Montemurro, Debora Rosa, Stefano Vicini, Mariapaola Marconi, Lucia Zanotti, Paolo Meriggi, Ali Azarbarzin, Scott A. Sands, Andrew Wellman, Carolina Lombardi, Gianfranco Parati

PII: S0012-3692(21)03861-7

DOI: https://doi.org/10.1016/j.chest.2021.08.080

Reference: CHEST 4607

To appear in: *CHEST*

Received Date: 19 March 2021

Revised Date: 10 August 2021

Accepted Date: 23 August 2021

Please cite this article as: Perger E, Montemurro LT, Rosa D, Vicini S, Marconi M, Zanotti L, Meriggi P, Azarbarzin A, Sands SA, Wellman A, Lombardi C, Parati G, Reboxetine plus Oxybutynin for Obstructed Sleep Apnea Treatment A 1-week Randomized, Placebo-controlled, Double-Blind Crossover Trial *CHEST* (2021), doi: https://doi.org/10.1016/j.chest.2021.08.080.

Reboxetine plus Oxybutynin for Obstructed Sleep Apnea Treatment

A 1-week Randomized, Placebo-controlled, Double-Blind Crossover Trial

Elisa Perger[1, 2], Luigi Taranto Montemurro[3], Debora Rosa[1], Stefano Vicini[1, 2], Mariapaola Marconi[1], Lucia Zanotti[1], Paolo Meriggi[4], Ali Azarbarzin[3], Scott A. Sands[3], Andrew Wellman[3], Carolina Lombardi[1, 2*], Gianfranco Parati[1, 2*].

From the [1]Istituto Auxologico Italiano, IRCCS, Sleep Disorders Center & Department of Cardiovascular, Neural and Metabolic Sciences, San Luca Hospital, Milan, Italy; [2]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; [3] Division of Sleep and Circadian Disorders, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [4]IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy

* these authors contributed with the same effort to the project

Corresponding Author: Elisa Perger

Sleep Disorders Center, San Luca Hospital

Istituto Auxologico Italiano IRCCS

Piazzale Brescia 20, 20149 Milan, Italy

Tel: +3902619112705

Email: elisaperger@hotmail.com

e.perger@auxologico.it

Funding: Apnimed Inc. (19 Ware Street, #3 Cambridge, MA 02138) financially supported the study.

Conflict of interest:

E.P, D.R, S.V, M.M, L.Z, P.M, C.L and G.P have no conflict of interest related to the current project.

L.T.M is Chief Scientific Officer at Apnimed and has a financial interest in Apnimed.

S.S. served as a consultant for Apnimed, Nox Medical, and Merck and reports grants from Apnimed, Prosomnus, and Dynaflex outside the current work.

A.A serves as consultant for Somnifix and Apnimed and reports grants from Somnifix

A.W. works as a consultant for Somnifix, Inspire, Apnimed, and Nox. He has a financial interest in Apnimed. This interest is monitored and managed by Brigham and Women's Hospital.

ABSTRACT

Background: The recent discovery that a combination of noradrenergic and antimuscarinic drugs improved upper airway muscle function during sleep and reduced obstructive sleep apnea (OSA) severity has revitalized interest in pharmacological therapies for OSA.

Research Question: Would 1-week of reboxetine plus oxybutynin be effective on OSA severity?

Study Design and Methods: We performed a randomized, placebo-controlled, double-blind, crossover trial comparing 4 mg reboxetine plus 5 mg oxybutynin (reb–oxy) to placebo in OSA subjects. After a baseline in-lab polysomnogram (PSG), patients performed PSGs after 7 nights of reb-oxy and 7

nights of placebo to compare apnea-hypopnea index (AHI, primary outcome). Response rate was based on the percentage of subjects with a ≥50% reduction in AHI from baseline. Secondary outcomes included Epworth Sleepiness Scale (ESS) and psychomotor vigilance test (PVT). Home oximetry evaluated overnight oxygen desaturation (ODI) throughout treatment.

Results: 16 subjects aged 57[51-61] years (median [interquartile range]) with body mass index 30[26-36] kg/m^2 completed the study. Reb-oxy lowered AHI from 49[35-57] events/h at baseline to 18[13-21] events/h (59% median reduction) compared with 39[29-48] events/h (6% median reduction) on placebo ($p<0.001$). Response rate for reb-oxy was 81% versus 13% for placebo $p<0.001$). Although ESS was not significantly lowered, PVT median reaction time decreased from 250[239-312] ms on baseline to 223[172-244] ms on reb-oxy versus 264[217-284] ms on placebo ($p<0.001$). Home oximetry illustrated acute and sustained improvement in ODI on reb-oxy versus placebo.

Interpretation: The administration of reboxetine-plus-oxybutynin greatly decreased OSA severity and increased vigilance. These results highlight potential possibilities for pharmacological treatment of OSA.

Clinical trial registration number: NCT04449133

Keywords: pharmacological treatment; anti-muscarinic and norepinephrine reuptake inhibitors; upper airway, vigilance, obstructive sleep apnea

Obstructive sleep apnea (OSA) is one of the most common sleep disorders and affects approximately 10-17% of the general population[1]. Due to repetitive collapse of the pharyngeal airway during sleep, OSA leads to intermittent oxygen desaturations, sleep fragmentation, excessive daytime sleepiness and cardiovascular impairment, and long-term OSA is associated with increased morbidity and mortality[2,3].

Although OSA is effectively alleviated with the use of continuous positive airway pressure (CPAP) and, for some individuals, oral appliances or multimodal approaches[4], these treatments are often poorly tolerated by patients and compliance is relatively low[5]. Efforts to develop pharmacological therapies for the treatment of OSA have accelerated over the last two decades, but currently no pharmacological intervention has been approved for clinical use.

One of the key pathophysiological traits in OSA is the loss of upper airway (UA) dilator muscle activity at sleep onset and the lack of reactivation (muscle compensation) during sleep in response to UA obstruction. Research in animals improved the understanding of the state-dependent neurotransmitters involved in pharyngeal muscle activation during sleep, providing evidence that both noradrenergic and antimuscarinic processes are involved. Specifically, the impairment of noradrenergic activity is now thought to play a key role in the sleep-related hypotonia of pharyngeal muscles mostly during Non-Rapid Eye Movement (NREM)[6-8] sleep, and muscarinic activity is primarily involved in Rapid Eye Movement (REM) muscle atonia[9,10].

Recently, it has been shown that a fixed dose combination of atomoxetine (a noradrenergic agent) and oxybutynin (an antimuscarinic compound)[11] reduced the frequency of obstructive events (AHI) by 63% and improved genioglossus responsiveness to negative pressure swings approximately 3-fold in 20 OSA patients over a single night of treatment. In a study on healthy individuals, a combination of reboxetine (a noradrenergic agent) and hyoscine butylbromide (an antimuscarinic drug) improved activity of the *tensor palatini* muscle and UA resistance during NREM sleep[12]. Preliminary data also suggested an improvement in OSA severity of approximately 35% after a single night of reboxetine plus hyoscine butylbromide[13]. Given that the putative mechanism of action of this combination is the stimulation of the hypoglossal motor pool in the brainstem, we considered that a combination of reboxetine plus oxybutynin might have greater therapeutic potential than reboxetine plus hyoscine, since oxybutynin can reach higher concentration in the brainstem parenchyma[14]. Since these processes

have been identified only recently, there has not yet been any attempt to stimulate the pharyngeal muscles with these drugs for longer than a single night.

Therefore, in this study we performed a randomized, placebo-controlled, double-blind, crossover trial to evaluate the efficacy of a combination of reboxetine and oxybutynin (reb-oxy) on the severity of OSA after 1-week of treatment. We hypothesized that reb-oxy would reduce AHI (primary outcome) and oxygen desaturation indices from baseline to a greater extent than with placebo. Moreover, we investigated the effects of this combination on OSA pathophysiological (or endotypic) traits[15,16], also exploring whether patients' baseline characteristics could predict the treatment success.

METHODS

Patients

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

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

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

The study was registered at ClinicalTrials.gov (NCT04449133).

Study Design

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

Study participants underwent further eligibility screening with a one-night in-lab baseline in-lab PSG (Embla, Reykjavik, Iceland), which served as the baseline for AHI and other PSG endpoints. Participants were eligible for randomization if AHI on baseline PSG was>15 events/hr. Eligible participants were then randomized equally to first receive 4 mg reboxetine plus 5 mg oxybutynin (reb–oxy) or matching placebo (2 capsules). Subjects started taking study drug at home the day after the baseline PSG immediately prior to bedtime for 7 days. A washout of 7-10 days preceded the switch to the other arm of the study. During the entire at-home period (6 nights on placebo and 6 nights on reb-oxy), the patients underwent full night pulse-oximetry testing (Nonin Medical Inc., 3150, Minnesota, USA). On the final night of dosing for each arm, participants performed an in-lab PSG to evaluate OSA severity. The predefined primary outcome variable was the change in AHI from baseline. Secondary outcomes were: response rate based on ≥50% reduction in AHI; proportion of participants with AHI<15/hour; change in subjective sleepiness with Epworth Sleepiness Scale (ESS), Psychomotor Vigilance Test (PVT), change from baseline in these PSG parameters: Oxygen Desaturation Index (ODI) at 3% threshold and hypoxic burden. Karolinska Sleepiness Scale (KSS), Patient Global Impression of OSA Severity (PGI-S), arousal index, periodic limb movement (PLM) index) and descriptive summary of nightly change with at-home pulse oximetry (ODI 4%) were also assessed.

Randomization and blinding

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

Data analyses and measurements of outcomes

Overnight PSG recordings and scoring were performed in accordance with the American Academy of Sleep Medicine (AASM) rules[17]. All studies were scored by the same specialized sleep clinician, blinded to treatment assignment, according to AASM criteria[18]. AHI, ODI 3%, arousal index, and PLM index were calculated from the PSG. The OSA specific hypoxic burden (respiratory event–related oxygen desaturation area under pre-event SpO_2 baseline curve, per hour) was also calculated[19,20]. ODI at 4% threshold level (ODI 4%) was collected during at-home pulse oximetry for each night of treatment. Adverse events were recorded at each visit.

Pathophysiological traits causing sleep apnea were (endotypes) estimated during NREM sleep using established automated methods and executed using custom software (Endo-Phenotyping Using

Polysomnography; MATLAB, Mathworks, Natick MA)[15,16,21]. For details please refer to supplement material.

The ESS questionnaire was taken to evaluate subjective somnolence over the preceding week of treatment[22] and the KSS was taken to measure the situational sleepiness in the late afternoon before the in-lab PSG. The PGI-S was used to rate the participants impression of disease severity. A validated 3-minutes PVT evaluated the sustained-attention and reaction-time by measuring the speed with which subjects responded to a visual stimulus[23,24]. The median reaction time (RT), the number of lapses (defined as RT > 500 ms, i.e. inability to respond in a timely fashion when a stimulus was present and the reciprocal RT as a measure of speed (1/RT) (lapses included) were studied. The above-mentioned evaluations together with respiratory rate, EKG and three measurements of blood pressure, were performed without coffee intake in the previous 3 hours and at the same time of the day before the PSG.

Statistical analysis

Individuals were enrolled until 16 completed baseline and both treatment nights. The study was powered to detect an AHI reduction with reboxetine plus oxybutynin (percent reduction from baseline) by 50+/-50 % more than placebo (alpha 5%, power 80%); SD of the effect was estimated from a previous trial[11].

Data are presented as median [interquartile range]. Continuous variables were compared using a two-tailed Wilcoxon matched-pairs signed-rank test. Categorical data were analyzed using Fisher's exact test. A linear mixed effect model for AHI (%reduction from baseline) accounting for treatments, period and sequence as fixed effects and subjects as random effects is included in the supplement material.

For the endotypic traits, the effect of the reb-oxy combination and placebo vs baseline were also modelled using a linear mixed effects model, with treatments as fixed effects and subjects as a random effect. See supplemental material for further details.

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

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

RESULTS

Subjects

Eighteen subjects were enrolled in the study and performed a baseline PSG night; all individuals were eligible for randomization based on AHI>15 events/hr (Consort diagram in Figure 1). One subject dropped out prior to starting the first treatment period (second wave of COVID-19 in Milan; active drug period). One subject dropped out at the end of the first treatment period (also active drug period) as the patient was unable to continue (personal problems).

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

The following side effects were reported during the study on the reb–oxy night: urinary hesitation (difficulties in initiating micturition in the morning n=7 males); dry mouth during the night and in the morning (n=10); sexual dysfunction (erectile dysfunction in the morning or decreased libido n=3

males); brief sensation of palpitation (n=1) and insomnia symptoms (difficulty initiating and maintaining sleep; n=1). On placebo, chest pain (n=1) and side pain (n=1) were observed (see supplemental material, table S1). No participants experienced severe side effects or severe adverse events in either arm. No differences were found in terms of resting blood pressure, heart rate, or EKG among the visits.

The results relative to primary and secondary outcomes were upheld when adjusted for sequence and period effects in a linear mixed effect model analysis (see supplemental material, table S2, S3 and Figure S1). A significant sequence effect was found in the analysis of AHI %reduction. Adjusted results showed a reduction of placebo effect, suggesting a possible mild carry-over effect on placebo when it was administered after reboxetine plus oxybutynin, see the supplement for the detailed model. Secondary outcomes such as hypoxic burden (HB) or PVT were not affected by period or sequence.

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

Reb–oxy reduced the AHI by a median of 26 events/h, or 59% (expressed as the median value of all reductions), compared with baseline and by 20 events/h, or 59% compared to placebo (Table 2; see Figure 2 for individual data). The vast majority of patients (81%) experienced a reduction in AHI > 50% on the treatment night, and 37% of the patients on reb-oxy had an AHI<15. Effects of the intervention on AHI specific to REM and NREM sleep stages, hypoxic burden, ODI, arousal index and sleep architecture are shown in Table 2. Reb-oxy significantly reduced hypoxic burden and ODI ($p<0.001$ and $p=0.021$, respectively). Considering that an hypoxic burden >53%min/h has been previously associated with higher cardiovascular-related mortality[19], reb-oxy reduced the hypoxic burden below this threshold in the 69% of our sample. Individual data on hypoxic burden are reported in Figure 3A and 3B. Reb-oxy significantly reduced the number of arousals compared to baseline and placebo, and sleep architecture was unchanged with the exception of a trend for reduced REM sleep

and increased N2 on reb-oxy compared to placebo. No difference in periodic leg movements were observed among the three nights.

Effect of reb-oxy on ODI at home

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

Effect of reb-oxy on subjective questionnaires and vigilance

Reb-oxy did not significantly improve subjective indices related to sleepiness, impression of disease severity or vigilance when considering group data (Table 3). Regarding subjective sleepiness, 4/5 patients with ESS>6 at baseline experienced improvement in the score from 11 [3 to 12.5] to 6 [1.5 to 6.5], although this did not reach statistical significance ($p=0.19$). PGI-S improved on reb-oxy compared to baseline, but again this difference did not reach statistical significance ($p=0.087$). Despite KSS revealing no change in subjective alertness between treatments, PVT as RT and 1\RT performance significantly improved on reb-oxy compared to placebo, as shown in Figure 4.

Effect of reb-oxy on pathophysiological traits

Group data from the mixed effects model of endotypic traits at baseline, on placebo, and on reb-oxy are shown in Table 4. Compared to placebo, reb-oxy increased muscle compensation by 30% of normal/eupneic ventilatory drive (eupnea), supporting the effect of this combination on UA muscle responsiveness. However, reb-oxy reduced the arousal threshold by 27% of eupnea, i.e. patients woke

more easily on active treatment. Vactive was increased on reb-oxy by 20% of eupnea compared to baseline but not compared to placebo. No changes were found in loop gain (i.e. ventilatory control sensitivity) and Vpassive (i.e. passive pharyngeal tissue collapsibility).

Predictors from patients' baseline characteristics

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

DISCUSSION

This study provides experimental evidence that reboxetine plus oxybutynin administered before bedtime substantially reduces OSA severity (AHI) after 1-week of treatment. In addition to the AHI reduction, reb-oxy also exerted a significant effect on indices of hypoxemia, such as ODI and hypoxic burden. Reb-oxy also improved the performance on the vigilance testing. OSA alleviation was likely mediated by improved UA muscle activity and responsiveness, as suggested by the ~30% increase in muscle compensation on the drugs. Home pulse-oximetry recordings showed that reb-oxy was effective at improving nocturnal oxygen saturation as early as the first day of treatment, likely due to reduced OSA severity, and its efficacy was maintained through the 7th day, as shown in the in-lab PSG.

Reboxetine is a norepinephrine reuptake inhibitor approved outside the United States for the treatment of major depression. Oxybutynin is an antispasmodic drug that inhibits the muscarinic action of acetylcholine on smooth muscle and is indicated for the treatment of symptoms of bladder instability in patients with neurogenic bladder.

To date, only one published study demonstrated a significant improvement in OSA severity through pharmacological therapy with noradrenergic and antimuscarinic agents[11]. In their project, Taranto-Montemurro and coauthors evaluated a combination of atomoxetine 80 mg and oxybutynin 5 mg (ato-oxy) in a single-night study[11]. The ato-oxy combination reduced the AHI from 31/h on placebo to 8/h on active treatment ($p<0.0001$)[11] In our study, we performed a baseline PSG on the night before starting the drugs and tested again after 1 week of administration, rather than only acutely. Therefore, our study extends knowledge by providing evidence for effectiveness of the combination of noradrenergic and antimuscarinic agents administered over a full week. Moreover, we also evaluated subjective and objective responses to the drugs administered. Our population presented low ESS, which might have influenced the reb-oxy effect on sleepiness. Although patients did not report subjective improvement in sleepiness as a group, patients with higher ESS at baseline showed a clinically

meaningful improvement with the combination compared to placebo, and there was evidence of improvement in vigilance (PVT reaction time and speed) after 1 week of treatment with reb-oxy. However, our study did not show a difference in PVT lapses, suggesting the absence of clinically significant baseline impairment of vigilance in our study population. The improvements in the PVT tests may be due both to an improvement of OSA and to the stimulant effects of reboxetine.

Regarding the pathophysiology traits, our finding that reb-oxy improved muscle compensation (40% eupnea improvement on drugs vs baseline and 30% vs placebo) is consistent with previous single night effects of ato-oxy (improved compensation by approximately 29% eupnea). The reduction in arousal threshold we observed is consistent with prior studies. It has indeed been shown in previous experiments that the arousal threshold can be modified by treatment: while the exposure to intermittent hypoxia increases the arousal threshold[25], treatment with CPAP reduces the arousal threshold in patients with OSA[26]. Other potential explanations include the possibility of a stimulant effect from reboxetine or a bias related to the methodology used to measure the arousal threshold[21]: spontaneous arousals occuring during mild flow limitation, may have contributed to a low arousal threshold score.

Unlike ato-oxy, a reduction in loop gain was not observed in the current study. Regarding predictors, responders to ato-oxy[27] exhibited several signs of reduced collapsibility (lower AHI, higher Vpassive, and higher fraction of hypopneas over total events). Likewise, in the current study, several surrogates of milder collapsibility (higher Vpassive, lower arousal threshold, lesser mean desaturation) were associated with greater responses to reb-oxy, confirming the notion that pharmacological therapy for OSA may be most efficacious in patients with less severe pharyngeal compromise. The similar findings in the ato-oxy and the current reb-oxy studies is not surprising, since both norepinephrine reuptake inhibitors have a comparable receptor affinity profile, with some differences being that reboxetine has a longer plasma half-life than atomoxetine (~12 vs ~5 h, respectively) and that reboxetine's major path of elimination is CYP-3A4 P-450 isozymes[28], whereas atomoxetine's is CYP-

2D6. It is therefore unlikely that reboxetine causes clinically significant interactions common to other antidepressants[29].

It has been recently shown by Lim and coauthors that reboxetine plus hyoscine butylbromide (an antimuscarinic drug) during sleep increased the activity of the tensor palatini muscle, a representative tonic UA muscle, and improved UA resistance in 10 healthy subjects[12]. This combination of drugs also reduced REM sleep and increased N2 sleep compared to placebo, with no effect on sleep efficiency. Although we observed the same trend of REM reduction and increased N2 sleep, the differences in sleep stage distribution between the nights of placebo and reb-oxy in our study were not significant. Due to the presence of moderate-to-severe OSA, our population probably had an altered sleep quality already, which was not further affected by drug intake. Moreover, we administered the medications for 7 days rather than 1 day, and it was previously shown that the negative effects of reboxetine alone on sleep quality tend to disappear with prolonged therapy[30].

The combined effects of reboxetine plus hyoscine butylbromide was also evaluated in a randomized controlled trial in 12 OSA subjects[13]. The combination of these drugs reduced the AHI by approximately 35%, increased the proportion of N2 sleep compared to placebo, and reduced REM sleep. Compared to oxybutynin, hyoscine butylbromide has a low permeability for the blood-brain barrier, possibly resulting in less efficacy[14]. Concerning safety, previous studies using reboxetine plus hyoscine butylbromide[12] and atomoxetine plus oxybutynin[11] did not show major sides effects. Accordingly, we did not observe any severe major adverse effects, even after 7 days of reb-oxy administration.

The true reb-oxy efficacy vs placebo might be underestimated in our study because of the crossover design. We found that the effect observed for placebo was greater when it was administered after reb-oxy (sequence effect, see Supplemental material), suggesting that longer washout period should be considered in future crossover trials.

Although our results report the efficacy of reb-oxy on OSA severity, our study is a proof-of-concept trial on a limited number of subjects. Larger and longer trials need to be performed to confirm the efficacy and the safety of these drugs in a broad range of subjects with OSA. Moreover, the safety of reboxetine plus oxybutynin in subjects with cardiac comorbidities also needs to be carefully studied. Considering the short duration of the trial, and to reduce participation burden, we decided to avoid a second baseline PSG. While reb-oxy reduced AHI, hypoxic burden, and arousal index, their impact on subjective sleep quality was not statistically significant in this small trial. Given that the combination showed a trend for reduction in sleepiness in patients with an ESS>6, future studies in sleepier patients should be considered.

Interpretation

The current study showed for the first time that repeated doses of the combination of noradrenergic and anti-muscarinic drugs is efficacious for the alleviation of OSA. Specifically, over one week, reboxetine plus oxybutynin provided a 59% reduction in AHI, and halved OSA severity in 81% of individuals. Acute effects exhibited on the first night were sustained at the end of the week. While subjective sleepiness was not reduced in this population, objective psychomotor vigilance test showed promising signs of improvement without major safety issues. These results provide strong pilot data for the design of larger and longer studies testing these drugs as a pharmacological therapy for OSA patients.

Author contributions:

E.P, L.TM, M.M, C.L, L.Z. and G.P. contributed to study design, E.P., D.R., S.V., L.Z., M.M. contributed to study execution. E.P, L.TM, P.M., S.S., A.A., contributed to data analysis. E.P. and L.TM contributed to drafting of the manuscript S.S., A.A, A.W. and P.M. contributed to revision and G.P. and C.L. contributed to final drafting of the manuscript

All the Authors have seen and approved the manuscript.

Take-Home Point:

Study Question: A combination of reboxetine plus oxybutynine will be effective in reducing OSA severity?

Results: Reboxetine plus oxybutynin reduced the apnea-hypopnea index by 59% from baseline together with an increase in oxygen saturation during the night. Moreover, reb-oxy improved sustained vigilance compared to placebo and did not cause severe adverse events.

Interpretation: Continuous positive airway pressure is still the most common treatment for OSA patients, and while it is often effective, many patients find it intolerable and therefore remain untreated. Our results provide strong evidence supporting OSA pharmacological therapy.

Figure 1: Consolidated Standards of Reporting Trials diagram of the clinical trial.

Figure 2: Individual data showing the effect of reboxetine plus oxybutynin (reb–oxy) on (A) total apnea–hypopnea index (AHI), during NREM (B) or REM (C) sleep stages. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles. (D) Group data showing percentage of apnea–hypopnea index (AHI) changes from baseline on placebo and on reb-oxy.

Figure 3: Effect of reboxetine plus oxybutynin (reb–oxy) on desaturation index: (A) hypoxic burden as individual data. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles. (B) Group data showing percentage of hypoxic burden changes from baseline on placebo and on reb-oxy are shown in panel. (C) Analysis of repeated measures of ODI 4% obtained during at-home pulse oximetry during placebo (grey squares) and during reboxetine plus oxybutynin (reb-oxy) weeks (black dots). Data were compared using a mixed effect model including treatment, time and time x treatment interaction as fixed effects and subjects as a random effect. Only

treatment effect was significantly associated with ODI4% (dependent variable). P value for day-by-day multiple comparison between placebo and reb-oxy arms are adjusted using Sidak method.

Figure 4: Effect of reboxetine plus oxybutynin (reb–oxy) on Psychomotor Vigilance Test (PVT) reaction time.

Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles.

REFERENCES

1. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. *Am J Epidemiol.* 2013;177(9):1006-1014.
2. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med.* 2000;342(19):1378-1384.
3. Shahar E, Whitney CW, Redline S, et al. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med.* 2001;163(1):19-25.
4. Perger E, Jutant EM, Redolfi S. Targeting volume overload and overnight rostral fluid shift: A new perspective to treat sleep apnea. *Sleep Med Rev.* 2018;42:160-170.
5. Weaver TE, Sawyer AM. Adherence to continuous positive airway pressure treatment for obstructive sleep apnoea: implications for future interventions. *Indian J Med Res.* 2010;131:245-258.
6. Chan E, Steenland HW, Liu H, Horner RL. Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. *Am J Respir Crit Care Med.* 2006;174(11):1264-1273.
7. Lai YY, Kodama T, Siegel JM. Changes in monoamine release in the ventral horn and hypoglossal nucleus linked to pontine inhibition of muscle tone: an in vivo microdialysis study. *J Neurosci.* 2001;21(18):7384-7391.
8. Fenik VB, Davies RO, Kubin L. REM sleep-like atonia of hypoglossal (XII) motoneurons is caused by loss of noradrenergic and serotonergic inputs. *Am J Respir Crit Care Med.* 2005;172(10):1322-1330.
9. Grace KP, Hughes SW, Horner RL. Identification of the mechanism mediating genioglossus muscle suppression in REM sleep. *Am J Respir Crit Care Med.* 2013;187(3):311-319.
10. Grace KP, Hughes SW, Shahabi S, Horner RL. K+ channel modulation causes genioglossus inhibition in REM sleep and is a strategy for reactivation. *Respir Physiol Neurobiol.* 2013;188(3):277-288.

11. Taranto-Montemurro L, Messineo L, Sands SA, et al. The Combination of Atomoxetine and Oxybutynin Greatly Reduces Obstructive Sleep Apnea Severity. A Randomized, Placebo-controlled, Double-Blind Crossover Trial. *Am J Respir Crit Care Med.* 2019;199(10):1267-1276.
12. Lim R, Carberry JC, Wellman A, Grunstein R, Eckert DJ. Reboxetine and hyoscine butylbromide improve upper airway function during nonrapid eye movement and suppress rapid eye movement sleep in healthy individuals. *Sleep.* 2019;42(4).
13. Lim R, Messineo L, Grunstein R, Carberry J, Eckert D. Reboxetine and hyoscine butylbromide reduce obstructive sleep apnoea severity. *J Physiol.* 2021; in press doi: 10.1113/JP281912.
14. Chancellor M, Boone T. Anticholinergics for overactive bladder therapy: central nervous system effects. *CNS Neurosci Ther.* 2012;18(2):167-174.
15. Terrill PI, Edwards BA, Nemati S, et al. Quantifying the ventilatory control contribution to sleep apnoea using polysomnography. *Eur Respir J.* 2015;45(2):408-418.
16. Sands SA, Edwards BA, Terrill PI, et al. Phenotyping Pharyngeal Pathophysiology using Polysomnography in Patients with Obstructive Sleep Apnea. *Am J Respir Crit Care Med.* 2018;197(9):1187-1197.
17. Sateia MJ. International classification of sleep disorders-third edition: highlights and modifications. *Chest.* 2014;146(5):1387-1394.
18. Rosenberg RS, Hout SV. The American Academy of Sleep Medicine Inter-scorer Reliability Program: Respiratory Events. *Journal of Clinical Sleep Medicine.* 2014;10(04):447-454.
19. Azarbarzin A, Sands SA, Stone KL, et al. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. *Eur Heart J.* 2019;40(14):1149-1157.
20. Azarbarzin A, Sands SA, Taranto-Montemurro L, et al. The Sleep Apnea-Specific Hypoxic Burden Predicts Incident Heart Failure. *Chest.* 2020;158(2):739-750.
21. Sands SA, Terrill PI, Edwards BA, et al. Quantifying the Arousal Threshold Using Polysomnography in Obstructive Sleep Apnea. *Sleep.* 2018;41(1).
22. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep.* 1991;14(6):540-545.
23. Yang F, Xu S, Basner M, Chai Y, Dinges DF, Rao H. 0227 Sleep Deprivation Enlarges Inter-stimulus Interval Effect On Vigilant Attention. *Sleep.* 2018;41(suppl_1):A88-A89.
24. Brunet JF, Dagenais D, Therrien M, Gartenberg D, Forest G. Validation of sleep-2-Peak: A smartphone application that can detect fatigue-related changes in reaction times during sleep deprivation. *Behav Res Methods.* 2017;49(4):1460-1469.
25. Alex RM, Panza GS, Hakim H, et al. Exposure to mild intermittent hypoxia increases loop gain and the arousal threshold in participants with obstructive sleep apnoea. *J Physiol.* 2019;597(14):3697-3711.
26. Loewen A, Ostrowski M, Laprairie J, et al. Determinants of Ventilatory Instability in Obstructive Sleep Apnea: Inherent or Acquired? *Sleep.* 2009;32(10):1355-1365.
27. Taranto-Montemurro L, Messineo L, Azarbarzin A, et al. Effects of the Combination of Atomoxetine and Oxybutynin on OSA Endotypic Traits. *Chest.* 2020;157(6):1626-1636.
28. Hajós M, Fleishaker JC, Filipiak-Reisner JK, Brown MT, Wong EHF. The Selective Norepinephrine Reuptake Inhibitor Antidepressant Reboxetine: Pharmacological and Clinical Profile. *CNS Drug Reviews.* 2004;10(1):23-44.
29. Avenoso A, Facciola G, Scordo MG, Spina E. No effect of the new antidepressant reboxetine on CYP2D6 activity in healthy volunteers. *Ther Drug Monit.* 1999;21(5):577-579.

30. Ferini-Strambi L, Manconi M, Castronovo V, Riva L, Bianchi A. Effects of reboxetine on sleep and nocturnal cardiac autonomic activity in patients with dysthymia. *J Psychopharmacol*. 2004;18(3):417-422.

Table 1: General characteristics of the population studied

CHARACTERISTICS	VALUES
Age, years	57 [51-61]
Male, N (%)	14 (87.5)
Height, cm	180 [171-184]
Weight, Kg	94 [77-105]
BMI, Kg/m^2	30 [26-36]
Waist circumference, cm	116 [103-123]
Neck circumference, cm	43 [39-46]
Mallampati score (1 / 2 / 3 / 4)	1 (6.3) / 10 (62.5) / 4 (25) / 1 (6.3)
Tonsils score (1/ 2 / 3 / 4)	15 (93.7) / 1 (6.3) / 0 / 0
Smoke	8 (50)
Previous OSA treatment, n (%)	
C-PAP	5 (31.2)
Comorbidities, N (%)	
Hypertension	7 (44)
Diabetes	1 (6.3)
Dyslipidemia	7 (44)
Hypothyroidism	3 (18.8)
Rheumatoid arthritis	1 (5.6)
Medications, N (%)	
ACE-I/ARB	6 (35)
CCB	1 (6.3)
Diuretics	1 (6.3)
Antilipidemics	4 (25)
Antidiabetics	1 (6.3)
Antithrombotics	2 (12.6)

Definition of abbreviations: BMI = body mass index; OSA = obstructive sleep apnea; CPAP = continuous positive airway pressure; ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CCB = calcium channel blocker

Data are expressed as number (%), median [interquartile range] unless otherwise specified

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

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
AHI total, events/h	48.7 [34.8 to 56.6]	38.7 [29.0 to 47.8]	18.0 [12.5 to 21.4]	<0.001
%change from baseline		5.9 [-4.5 to 37.5]	59.2 [53.3 to 68.1]	<0.001
AHI supine, events/h	60.4 [52.7 to 81.9]	56.3 [44.9 to 76.0]	33.7 [25.3 to 48.1]	<0.001
%change from baseline		7.0 [0.4 to 27.2]	51.1 [30.9 to 64.3]	<0.001
Proportions of patients with AHI reduction>50% from baseline		13%	81%	<0.001
Proportion of patients with AHI<15 events/h		6%	37%	0.080
Hypoxic burden, %min/h	90.8 [69.5 to 154]	75.5 [68.1 to 168.0]	39.7 [25.4 to 55.3]	<0.001
%change from baseline		7.7 [-17.3 to 44.5]	61.5 [38.2 to 72.5]	<0.001
ODI 3%, events/h	42.7 [32.3 to 53.0]	36.8 [23.8 to 43.2]	31.4 [19.1 to 37.7]	0.021
%change from baseline		11.1 [-4.6 to 25.3]	29.0 [13.3 to 42.6]	0.025
ODI 4%, events/h	34.8 [23.9 to 43.9]	30.1 [17.4 to 40.0]	20.1 [13.3 to 28.2]	0.001
%change from baseline		7.7 [-7.7 to 38.2]	38.5 [21.1 to 49.7]	0.016
Arousal index, events/h	30.6 [20.7 to 47.7]	26.6 [14.1 to 34.7]	10.7 [7.6 to 16.8]	0.003
Total Sleep time, min	329.5 [301.0 to 368.8]	323.5 [274.4 to 351.4]	321.8 [283.0 to 362.9]	0.376
Sleep efficiency, %TIB	71.2 [59.9 to 76.2]	71.7 [60.8 to 83.5]	69.7 [64.0 to 73.3]	0.504
N1, %TST	3.7 [2.4 to 7.3]	3.5 [2.8 to 4.5]	5.4 [2.7 to 9.9]	0.102
N2, %TST	63.5 [55.3 to 68.1]	62.9 [58.5 to 68.7]	68.0 [58.4 to 75.8]	0.051
N3, %TST	16.2 [10.9 to 22.1]	17.4 [9.5 to 26.3]	15.9 [6.8 to 23.0]	0.117
REM, %TST	18.1 [13.8 to 21.4]	16.2 [13.2 to 17.9]	10.2 [5.1 to 15.5]	0.057
PLM index, events/h	0.0 [0.0 to 2.8]	0.0 [0.0 to 2.8]	0.5 [0.0 to 2.8]	0.457
Heart Rate, bpm	78 [71 to 90]	82 [72 to 93]	79 [69 to 87]	0.700
Systolic blood pressure, mmHg	133 [124 to 145]	126 [118 to 135]	120 [115 to 138]	0.234
Diastolic blood pressure, mmHg	82 [75 to 89]	84 [75 to 92]	80 [73 to 88]	0.065

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; AHI = apnea–hypopnea index; ODI = oxygen desaturation index; TIB = time in bed; N1-2-3 = non-REM stage 1-2-3; TST = total sleep time; REM = rapid eye movements sleep; PLM = periodic legs movements.

Data are presented as median (interquartile range).

% changes are expressed as the median of the group percentage change.

P values compare placebo versus red-oxy.

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

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

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; ESS = Epworth Sleepiness Scale; KSS = Karolinska Sleepiness Scale; PGI-S = Patient Global Impression of OSA; RT: reaction time; Severity; PVT = Psychomotor Vigilance Test.

Data are presented as median [interquartile range].

% changes are expressed as the median of the group percentage change.

P values compare placebo versus reb-oxy.

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

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

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

Figure 2

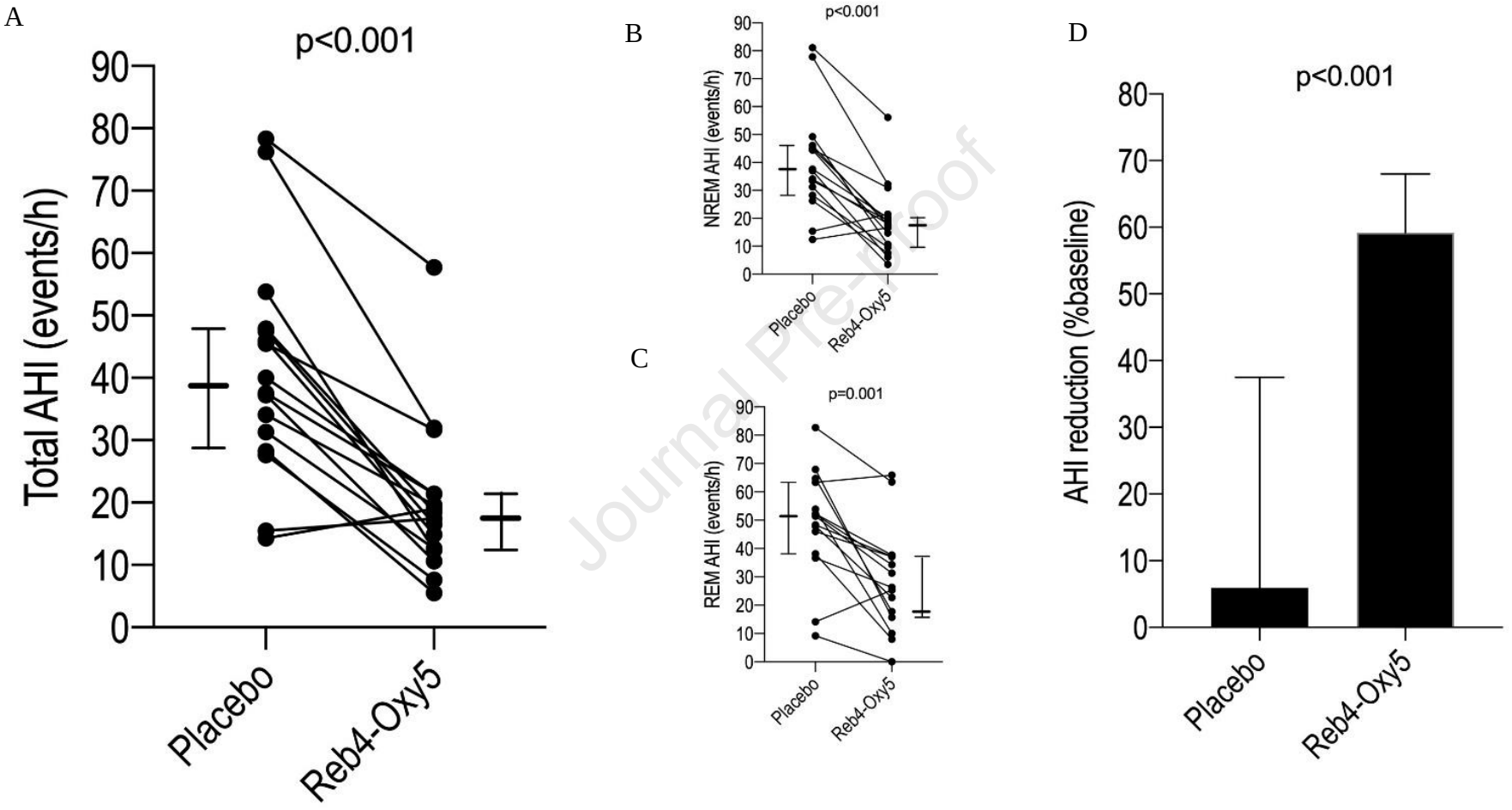


Figure 1

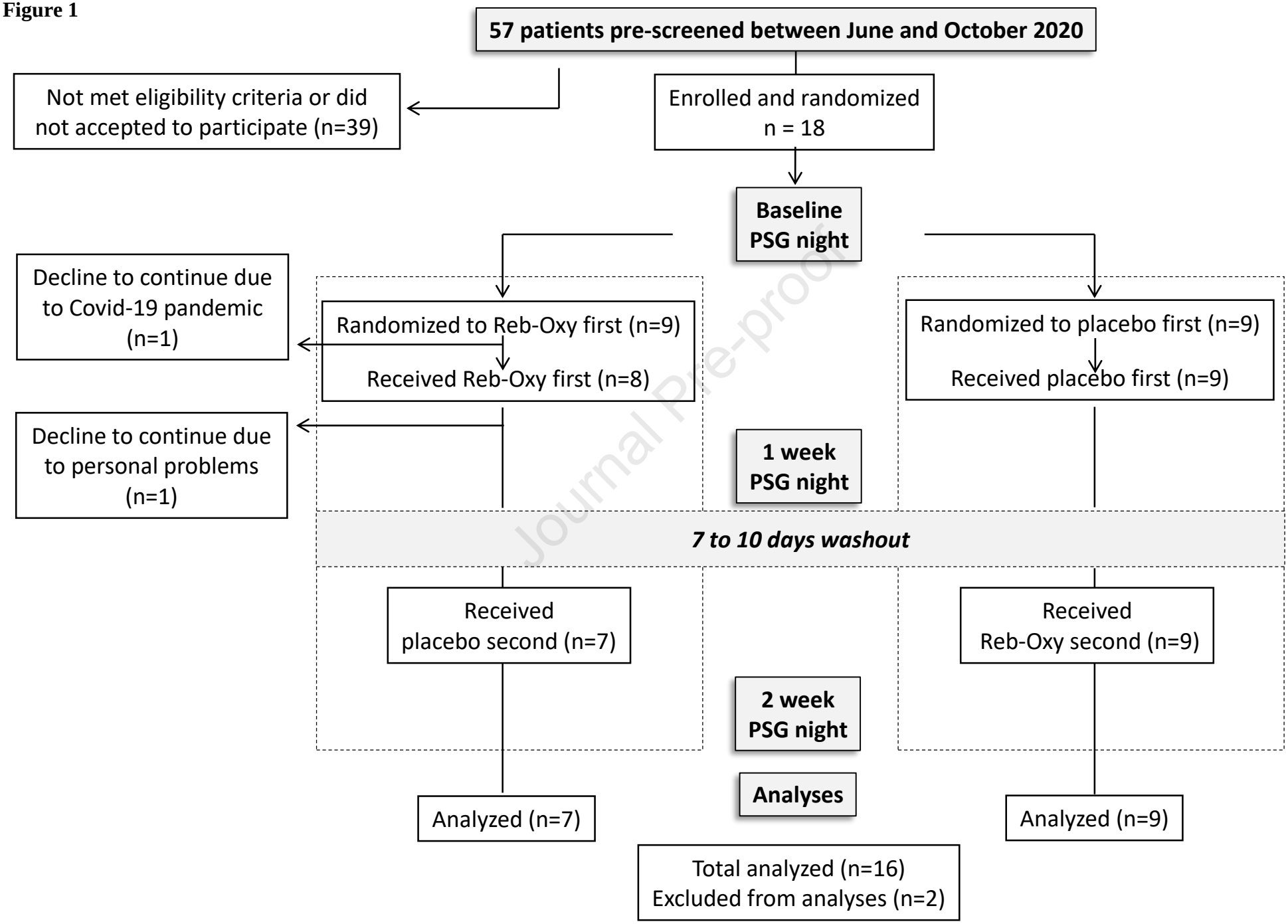


Figure 3

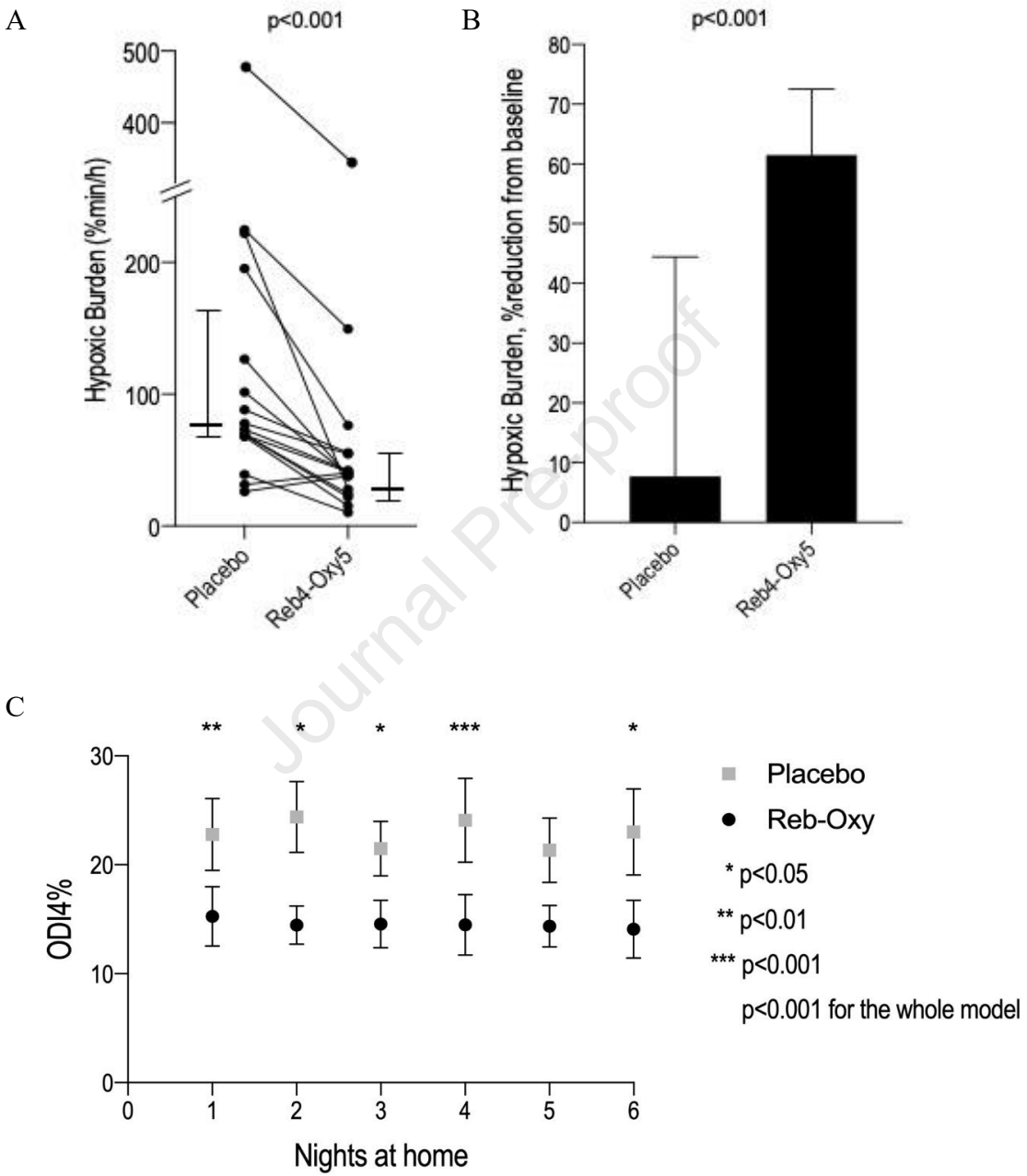
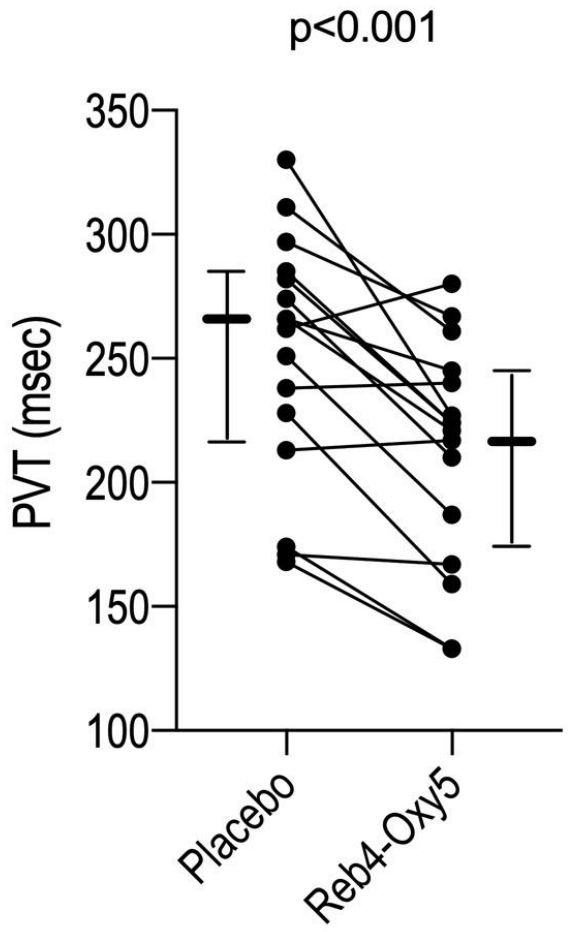


Figure 4



Abbreviation list

AASM = American Academy of Sleep Medicine

AHI = apnea-hypopnea index

AIFA = Agenzia Italiana del Farmaco

BMI = body mass index

CPAP = continuous positive airway pressure

ESS = Epworth Sleepiness Scale

KSS = Karolinska Sleepiness Scale

NREM = Non-Rapid Eye Movement

ODI = oxygen desaturation index

OSA = Obstructive sleep apnea

PGI-S = Patient Global Impression of OSA Severity

PLM = periodic limb movement

PSG = polysomnography

PVT = psychomotor vigilance test

Reb-oxy= reboxetine and oxybutynin

REM = Rapid Eye Movement

SpO2 = Oxygen Saturation

UA = upper airway