Sede Amministrativa: Università degli Studi di Padova
Dipartimento di Medicina (DIMED)

INTERNATIONAL PhD DEGREE IN ARTERIAL HYPERTENSION
AND VASCULAR BIOLOGY (XXVIII CICLO)

STUDIES ON ROLE OF HAEMODYNAMIC AND AUTONOMOUS NERVOUS SYSTEM CHANGES IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA

Coordinatore e Supervisore: Ch.mo Prof. Gian Paolo Rossi

Dottorando: Dott. Martino Pengo

INDEX

Abstract

Obstructive sleep apnea syndrome (OSAS) is a well established risk factor for hypertension and is associated with an enhanced sympathetic activity. In this series of studies we described the pathophysiological changes of blood pressure and autonomous nervous system activity in patients with OSAS.

We demonstrated that CPAP can modulate the autonomous nervous activity by reducing the sympathetic discharge as seen by a reduction of blood pressure variability.

In a cohort of 78 patients (76.9%male, 49%hypertensive) a 2 week autoset-CPAP treatment determined a reduction of systolic BPV (5.3±4.9 vs 4.2±3.4 mmHg, p=0.047) and pulse rate (78.0±14.5 vs 75.5±15.8 bpm, p=0.032).

An effect on blood pressure, pulse rate and blood pressure variability depends also on CPAP adherence and, particularly in obese subjects, on pressure's loading effect on the respiratory system. We studied 16 patients (13males, 47(10)years, BMI 38.5(5.8)kg/m^2). Blood pressure was measured continuously with a Finapres device at increasing CPAP pressure levels. BP and BPV increased with incremental CPAP (systolic BP r=0.960, p<0.001, diastolic BP r=0.961, p<0.001; systolic BPV r=0.662,p=0.026; diastolic BPV r=0.886,p<0.001) and abdominal muscles activity, a surrogate marker of neuro respiratory drive, correlated with both systolic (r=0.464,p=0.032) and diastolic BP (r=0.747,p=0.009).

As the activity of the autonomous nervous system is mediated by the catecholaminergic system we hypothesized that the activity of Catechol-O-methyltransferase (COMT), one of the major mammalian enzymes involved in the metabolic degradation of catecholamines, measured during drug induced sleep endoscopy (DISE), was altered during intermittent hypoxia in patients with OSAS.

We recruited 6 patients with OSAS who underwent DISE. During the procedure 7 blood samples were drown for the analysis of COMT activity and catecholamines concentration before, during and after an obstructive event. COMT analysis showed an increase of the enzyme activity after sedation. This was more marked when propofol-induced central apnea occurred compared to baseline (181.2 (12.8) pmol/min/mg vs 99.4 (11.1) pmol/min/mg, p<0.05).

In this preliminary analysis we showed that the catecholaminergic system is stimulated during DISE after apneic events. This might be due to an altered activity of COMT during hypoxia therefore suggesting a novel pathophysiological pathway responsible of hypertension in patients with OSAS.

Riassunto

La sindrome delle apnee ostruttive nel sonno (OSAS) e' un fattore di rischio per lo sviluppo di ipertensione ed e' associata ad un'attivazione del sistema nervoso simpatico. In questa serie di studi abbiamo dimostrato le alterazioni emodinamiche e del sistema nervoso autonomo in pazienti con OSAS.

La terapia con pressione continua (CPAP) e' in grado di modulare l'attività del sistema nervoso autonomo riducendo la variabilità pressoria (VP), un noto marker dell'attività simpatica.

Sono stati reclutati 78 pazienti (76.9% maschi, 49% ipertesi) sottoposti ad un ciclo di CPAP per due settimane. Al termine si e' osservata una riduzione della VP sistolica (5.3±4.9 vs 4.2±3.4 mmHg, p=0.047) e della frequenza cardiaca (78.0±14.5 vs 75.5±15.8 bpm, p=0.032).

Tali effetti della terapia con CPAP non possono prescindere dalla aderenza alla terapia stessa oltre che da altri fattori come la pressione erogata, in particolare in soggetti obesi.

Abbiamo studiato 16 pazienti (13 maschi, eta' media 47 anni, BMI 38.5 kg/m^2). La pressione arteriosa (PA) e' stata misurata in maniera continuativa con dispositivo Finapres a livelli crescenti di pressione nelle vie aeree. La PA e la VP aumentavano con l'aumentare della pressione della CPAP (PA sistolica r=0.960, p<0.001, diastolica r=0.961, p<0.001; VP sistolica r=0.662, p=0.026; diastolica r=0.886, p<0.001) e la attività dei muscoli respiratori accessori correlava con la PA sistolica e diastolica (r=0.464, p=0.032 e r=0.747, p=0.009 rispettivamente).

Poiché l'attività del sistema nervoso autonomo e' mediata dalle catecolamine abbiamo studiato l'attività della Catecol-O-metil-transferasi (COMT), uno dei più importanti enzimi coinvolti nella degradazione delle catecolamine, in soggetti con OSAS sottoposti ad endoscopia in sedazione (DISE) ipotizzando che l'attività enzimatica fosse alterata durante ipossia intermittente.

Abbiamo arruolato 6 pazienti ipertesi con OSAS di grado moderato che sono stati sottoposti a DISE durante la quale sono stati eseguiti 7 prelievi ematici seriati per il dosaggio delle catecolamine e dell'attività di COMT prima durante e dopo una apnea ostruttiva.

Dei pazienti arruolati 5 erano di sesso maschile, l'eta media era 48 anni e l'indice di massa corporea medio era di 27.71 (3.42) kg/m2. L'analisi della COMT ha mostrato un aumento dopo la sedazione in particolare in concomitanza dell'apnea centrale indotta dall'anestetico

(181.2 (12.8) pmol/min/mg vs 99.4 (11.1) pmol/min/mg, $p<0.05$).

Nell'analisi preliminare di questo studio il sistema catecolaminergico appare stimolato durante DISE dopo un'apnea. Questo e' verosimilmente dovuto all'alterata attività dell'enzima durante ipossia il che suggerisce un nuovo meccanismo fisiopatologico per lo sviluppo di ipertensione nei pazienti con OSAS.

Background

Obstructive sleep apnoea syndrome (OSAS) is a sleep disorder characterised by recurrent episodes of upper airway obstruction during sleep and associated with recurrent cycles of desaturation and re-oxygenation, sympathetic over-activity and intra-thoracic pressure changes, leading to fragmentation of sleep and consequent daytime fatigue and sleepiness. [1]

Of all sleep-disordered breathing (SDB) OSAS is by far the most prevalent and should be considered as a syndrome giving the multiplicity and diversity of the symptoms complained by the affected patients, which include nocturnal chocking, snoring, and unrefreshing sleep, alongside the breathing abnormalities seen during sleep such as obstructive apnoeas (OSAS), oxygen desaturations and arousals from sleep. Sleep apnea severity is usually assessed by the apnea-hypopnoea index (AHI), which is the number of complete (apneas) or incomplete (hypopneas) obstructive events per hour of sleep.

OSAS is usually distinguished on the basis of occurrence of oxygen desaturations from upper-airway resistance syndrome (UARS) in which there are neither apneas/hypopnoeas nor oxygen desaturations in spite of a disturbed sleep due to arousals secondary to increased work of breathing to overcome the increased airway resistance. [2]

Epidemiology

The prevalence of OSAS varies according to different factors such as age, gender, body mass index (BMI) and most of all severity of the disease and its related symptoms. Indeed, according to the Wisconsin Sleep Cohort, one of the most important prospective population-based studies on the natural history of sleep disordered breathing, the prevalence of OSAS is about 24% in men and 9% in women aged 30-60 years of age when using an AHI greater or equal than 5 events/hour as a diagnostic criteria.[3]

The prevalence of OSAS is slightly lower by combining sleep disturbances with associated excessive daytime somnolence, being approximately 3% to 7% in adult men and 2% to 5% in adult women.[4]

As mentioned above, OSAS has a tight association with obesity. In fact, the prevalence of

OSAS is approximately 40% in moderately overweight men and raises up to 90% in morbidly obese men (BMI > 40 kg/m^2).[5]

Moreover, when data from the Wisconsin sleep cohort study were re-examined with adjustment for overweight and obesity, a marked increase in the prevalence of OSAS was observed (34% of men and 17.4% of women between the ages of 30-70 had an AHI ≥5). [6]

These data reflect the worsening modern pandemic of obesity in western society which, as a consequence, make the prevalence of OSAS likely to further increase in the next decades.[7]

Indeed, obesity is not only the strongest risk factor for OSAS, but may also have an important role on the pathogenesis of obesity therefore inducing a vicious circle.

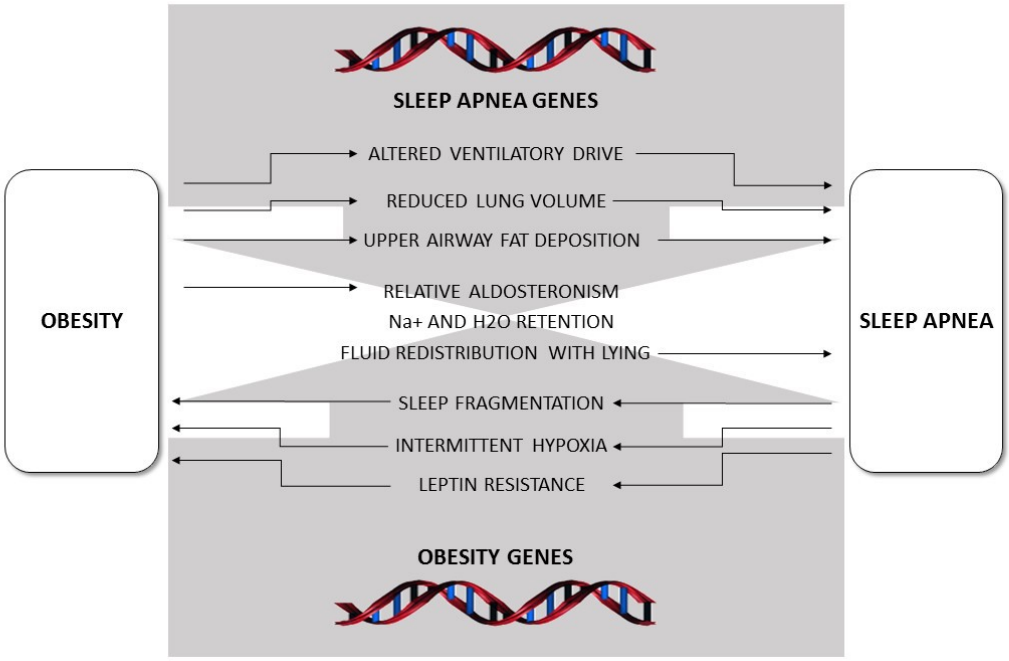

Figure 1: determinants of sleep-disordered breathing and obesity.[8]

Obesity alters the normal upper airway mechanics and contributes to the pathophysiology of OSAS in a number of ways (Figure 1): by the para-pharyngeal fat deposition which results in a reduction in caliber and a change in shape of the upper airway promoting collapsibility,[9] by the reduction in lung volumes, especially functional residual capacity, contributing to increased upper airway resistance,[10] and by enhancing leptin

resistance.[11]

Leptin is a hormone produced by adipocytes depending on their triglyceride content which is involved in the suppression of appetite. It also acts on the central respiratory centres to stimulate ventilation and its deficiency has been associated with hypoventilation. Indeed, in mice lacking the gene encoding for leptin, a marked obesity was observed together with an impaired hypercapnic ventilatory response during both wakefulness and sleep.[12] Obesity-induced leptin resistance can alter the response to hypercapnia leading to worsening of the latter and impairment of arousal from sleep during apneas.

However, these important patho-physiological findings were confirmed by several clinical studies such as the Sleep Heart Health Study, a multi-centre observational cohort study of cardiovascular correlates of OSAS in middle-aged and older Americans.[13]

In this study a weight gain of 10 kilograms over a 5-year period conferred a 5.2- and 2.5-fold raise in the likelihood of increasing the AHI by 15 events per hour in men and women respectively.

OSAS is more frequent in men compared to women [14], which is not only due to the greater prevalence of obesity among males, but also to several other factors and in particular hormonal changes. In fact, OSAS prevalence is higher in postmenopausal than pre-menopausal women and and hormone replacement therapy in post-menopausal women may protect against the development of OSAS. [15]

Age is surely one of the other OSAS determinants: the prevalence of OSAS increases with age in adults and this age-related increase may be attributable to parapharyngeal fat deposition, lengthening of the soft palate and changes in other anatomic parapharyngeal structures. [16]

Genetic aspects

It has been shown that up to 40% of the risk of developing OSAS is genetically determined.[17]

In fact, the prevalence of OSAS in first-degree relatives of patients with OSAS ranges from 22-84% with an odds ratio (OR) of a first-degree relative having OSAS ranging from 2 to

46. [18]

Anatomic risk factors for OSAS, such as obesity and upper airway soft tissue structure, demonstrate familial aggregation.

Interestingly, a case-control study in a Scottish cohort identified a strong familial component to OSAS and suggested that differences in facial structure were more important than obesity in this regard.[19]

The volume of lateral pharyngeal wall, tongue and total upper airway soft tissue have a significant level of heritability after adjusting for sex, age, ethnic background, craniofacial properties and neck fat deposition.

A number of candidate gene associations have been investigated in OSAS including apolipoprotein E4 (ApoE4), tumour necrosis factor (TNF), and angiotensin-converting enzyme (ACE), but only one a TNF alpha polymorphism (TNFA rs1800629) was significantly associated with OSAS under an allele frequency model.[20]

However, the literature on this topic is rapidly growing and giving that the vast majority of the associations have been published in that last 5 years, it is likely that future studies will be able to identify more genes associated with sleep-disordered breathing.

Pathophysiology

The pathogenesis of sleep-disordered breathing is rather complex as it involves multiple mechanisms, which alter the patency of the upper airway.

The upper airway can be divided into three compartments: (I) the naso-pharynx (epi-pharynx) whose function is mainly respiratory; (II) the oro-pharynx which has respiratory, deglutitory and reflex functions; and (III) the laryngo-pharynx (hypo-pharynx) whose functions include speech, swallow and respiratory function.

There are static and dynamic factors that contribute to the upper-airway patency. Among the formers we can find surface adhesive forces which for instance promote contact of the tongue with the mucosa of the oral cavity during nasal breathing.[21] Mouth opening potentially destabilizes the airway by freeing the mucosal attachments of the tongue and soft palate and allowing these now freely moving structures to relocate posteriorly and compromise the pharyngeal airway. Another important static element is the position of neck and jaw: the latter in particular can narrow the upper-airway by moving the tongue

backwards. This is frequently seen when patients are sleeping in the supine position as gravity plays an additional role as well.[22]

Lastly, another important static factor is the tracheal tug: increasing lung volume causes a caudal displacement of the intra thoracic trachea that, in turn, generates caudally directed forces on the upper airway.[23]

Dynamic factors include the upstream resistance in the nasal airway and pharynx conditions as mucosal congestion or nasal polyps can increase nasal airway resistance during inspiration producing a more-negative inspiratory swing in pharyngeal intraluminal pressure and consequently a reduction of pharyngeal cross-sectional area.

Another dynamic factor is the Bernoulli effect, which is defined by the conversion of energy from static to kinetic caused by an increase in the velocity of airflow when cross-sectional airway area decreases. This causes a decreased pharyngeal intraluminal pressure leading to narrowing of the pharynx during inspiration.

During sleep the impact of static and dynamic forces varies as the neuro muscular drive drops, and makes the muscles more collapsible, therefore further reducing the airway patency.[24]

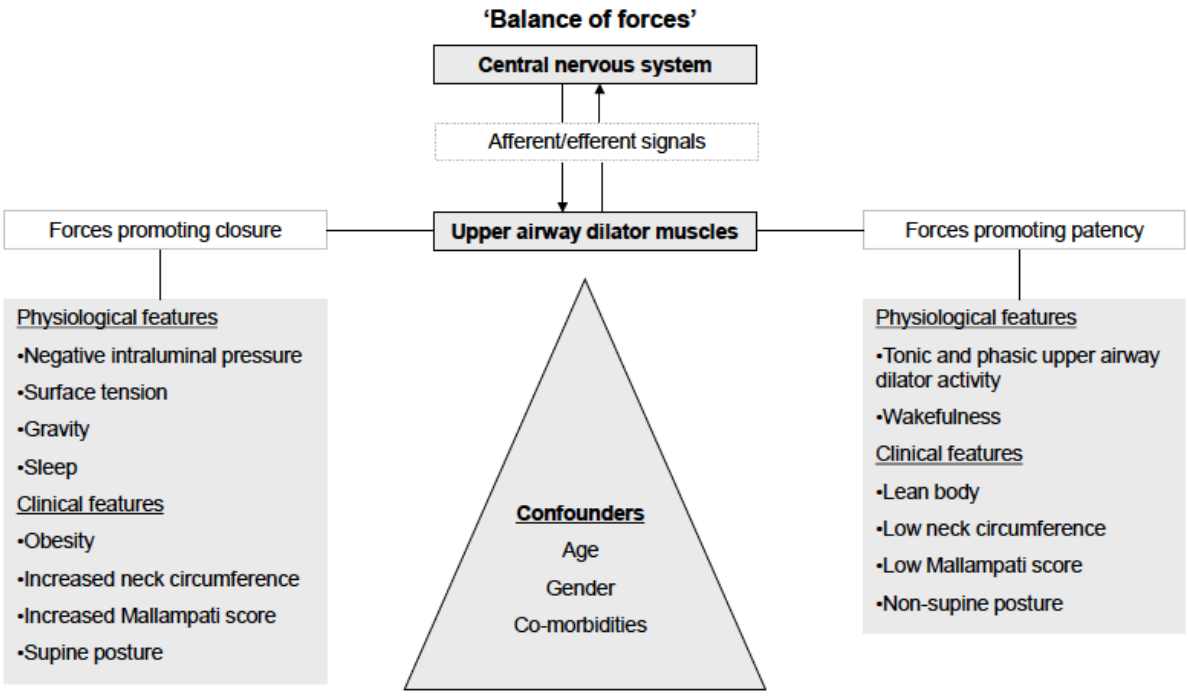

Figure 2: Simplified model of the 'balance of forces', factors that promote closure or patency of the upper airway.[25]

Other than sleep, confounders such as age and gender do affect static and dynamic factors. To make the picture even more complex, in pathologic conditions such as hypervolemic status heart failure [26] or obesity,[27] oedema and fat distribution can further increase the size of soft tissue structures surrounding the upper-airway.

To summarise the pathophysiology of OSAS there are forces that promote patency and closure of the upper airway. Both are influenced by physiological and pathological mechanisms and in patients with OSAS the so called “balance of forces” favours obstruction, therefore causing repetitive upper-airway occlusion during sleep.(Figure 2)

Clinical manifestations

The clinical features of OSAS can be divided into daytime and nighttime symptoms. (Table 1)

Table 1: clinical features of OSAS.

Daytime features	Nighttime features
Excessive daytime sleepiness	Witnessed apneas
Unrefreshing sleep	Choking at night
Morning headaches	Snoring
Dry mouth	Sleep fragmentation
Decreased concentration	Nocturia
Memory loss	
Decreased libido	
Irritability	

The nocturnal (sleep-related) symptoms and signs are more specific for OSAS than those expressed during diurnal wakefulness such as excessive daytime sleepiness, which is usually the result of abnormal sleep regardless of the cause. The OSAS patient might report symptoms of tiredness, fatigue, or drowsiness, rather than overt daytime sleepiness, and nighttime features are more often complained by the bed partner.[28]

Snoring is one of the major features of OSAS as almost all patients with OSAS do snore, at least according to their partners. In addition, complaint of snoring often precedes the complaint of daytime sleepiness, and the intensity increases with weight gain and bedtime

alcohol intake.[29]Among all daytime symptoms, daytime sleepiness or fatigue seem to be the most common complaints in patients with OSAS.[30]

The manifestations of sleepiness can have subtle consequences (midafternoon drowsiness during a group meeting leading to an occasional nap), severe consequences (falling asleep while eating or talking), or catastrophic consequences (falling asleep while driving).

Causes of sleepiness in OSAS patients are multifactorial. Nocturnal sleep disruption may be secondary to repetitive abnormal breathing events leading to arousals or complete awakenings in the middle of night or to early morning awakenings, with subsequent complaints of insomnia and reduced total sleep time.

Diagnosis

The diagnosis of OSAS does include a comprehensive sleep evaluation which consist of a sleep oriented history and physical examination, and findings identified by sleep testing.[31]

The first step is usually determining patients at high risk of OSAS (Table 2)

Table 2: risk factors for OSAS.

Obesity (BMI>35 kg/m2)
Congestive heart failure
Atrial fibrillation
Difficult to treat/resistant hypertension
Stroke
Type 2 diabetes
Nocturnal arrhythmias
Pulmonary hypertension
High risk driving population
Preoperative for bariatric surgery

These patients should undergo a sleep assessment in order to investigate the presence of signs or symptoms of OSAS previously described. In particular, more attention should be paid to excessive daytime sleepiness, its severity should be assessed by the Epworth Sleepiness Scale.[32]

Table 3: The Epworth Sleepiness Scale.

Situation	**Chance of dozing**
Sitting and reading	0.....1.....2.....3
Watching TV	0.....1.....2.....3
Sitting inactive in a public place (e.g a theater or a meeting)	0.....1.....2.....3
As a passenger in a car for an hour without a break	0.....1.....2.....3
Lying down to rest in the afternoon when circumstances permit	0.....1.....2.....3
Sitting and talking to someone	0.....1.....2.....3
Sitting quietly after a lunch without alcohol	0.....1.....2.....3
In a car, while stopped for a few minutes in traffic	0.....1.....2.....3

The Epworth Sleepiness Scale (ESS) is a simple, self-administered, 8-questions questionnaire, which allows patients to rate their degree of daytime sleepiness. (Table 3)
The ESS asks people to rate, on a 4-point scale (0 – 3), their usual chances of dozing off or falling asleep in 8 different situations or activities that most people engage in as part of their daily lives. The total ESS score is the sum of 8 item-scores and can range between 0 and 24. The higher the score, the higher the person's level of daytime sleepiness.
The physical examination can be crucial as it can allow to identify some potential removable causes of the disease. Particular attention should be paid to the presence of obesity, signs of upper airway narrowing, or the presence of other disorders that can contribute to the development or the consequences of OSAS. Features to be evaluated that may suggest the presence of OSAS are summarised in Table 4. [33]

Table 4: Physical examination findings suggesting OSAS.

increased neck circumference (> 43 cm in men, > 37 cm in women)
body mass index (BMI) ≥ 30 kg/m2
modified Mallampati score of 3 or 4
retrognathia
lateral peritonsillar narrowing
macroglossia
tonsillar hypertrophy
elongated/enlarged uvula
high arched/narrow hard palate
nasal abnormalities (polyps, deviation, valve abnormalities, turbinate hypertrophy)

Following history and physical examination patients should be assessed with objective testing as the severity of OSAS must be established in order to make appropriate decisions for treatment.[34]

The gold standard for diagnosing OSAS is full in laboratory polysomnography (PSG) which involves the measurements of several parameters overnight such as: electroencephalogram (EEG), electro-oculogram (EOG), chin electromyogram, airflow, oxygen saturation, respiratory effort, and electrocardiogram (ECG) or heart rate (additional recommended parameters include body position and leg EMG derivations). This test allows the clinician to understand not only the severity of OSAS, as determined by the apnea + hypopnea index (AHI) or respiratory disturbance index (RDI), but also the degree of sleep fragmentation, the presence of other sleep disorders that often co exist such as periodic limb movements, and the overall sleep architecture. (Figure 3)

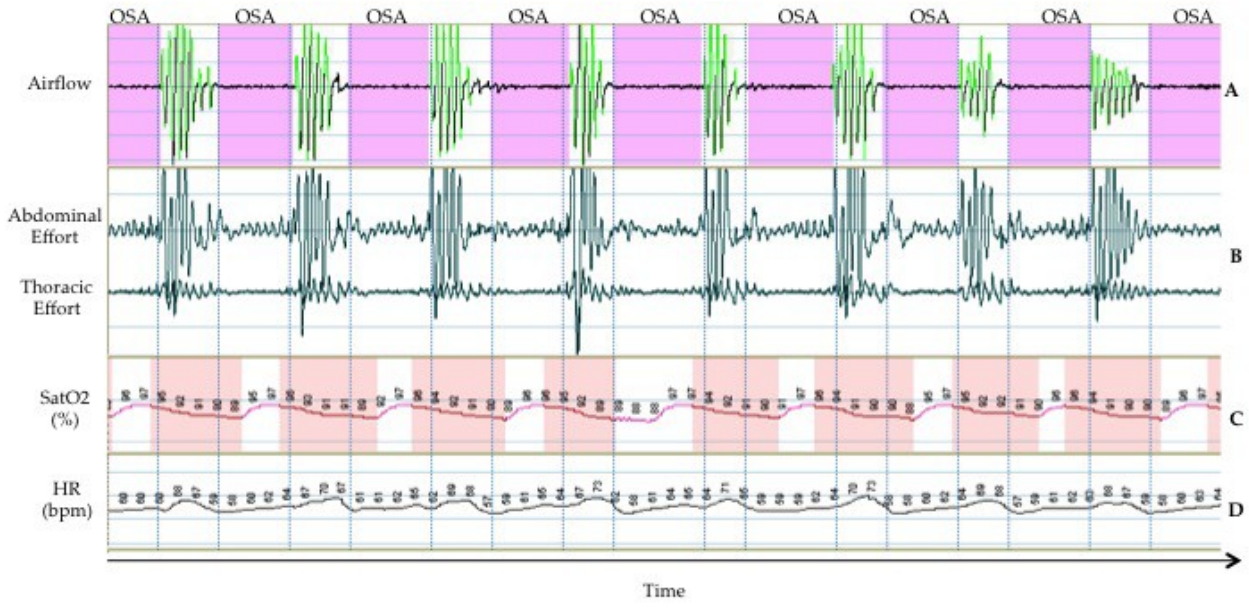

Figure 3: Printout of a PSG trace of a patient with severe OSAS. The violet boxes represent apneas, defined by the absence of flow for at least 10 seconds, and the corresponding pink boxes the oxygen desaturations.

The diagnosis of OSAS is confirmed if the number of obstructive events (apneas, hypopneas + respiratory event related arousals) on PSG is greater than 15 events/hr or greater than 5/hour in a patient who reports any of the following: unintentional sleep

episodes during wakefulness; daytime sleepiness; unrefreshing sleep; fatigue; insomnia; waking up breath holding, gasping, or choking; or the bed partner describing loud snoring, breathing interruptions, or both during the patient's sleep.[35] OSAS severity is defined as mild for RDI ≥ 5 and < 15, moderate for RDI ≥ 15 and ≤ 30, and severe for RDI > 30/hr. [31]
Other accepted tools to diagnose OSAS are home testing with portable monitors (PM) however, they should be performed only in conjunction with a comprehensive sleep evaluation.[31] A PM should, at a minimum, record airflow, respiratory effort, and blood oxygenation.

Treatment

Treatment options should be discussed in the context of the severity of the patient's OSAS, their risk factors, any associated conditions, and the patient's expectations. The first step should always be patient's education. The patient should be made aware of the impact of weight loss, sleep position, alcohol avoidance, risk factor modification, and medication effects. Weight loss in particular should be the first treatment option in obese patients as it has been demonstrated that a reduction in body weight is accompanied with a reduction of the AHI.[13]
The best treatment option for patients with moderate severe OSAS is positive airway pressure (PAP). First described by Sullivan in 1981,[36] PAP provides pneumatic splinting of the airway and is effective in reducing the AHI.[37]
PAP may be delivered in continuous (CPAP), bilevel (BPAP), or autotitrating (APAP) modes and it can be applied through a nasal, oral, or oronasal interface during sleep.
CPAP has been proven to reduce OSAS symptoms and in particular daytime sleepiness, improve quality of life and ameliorate haemodynamic parameters such as blood pressure and heart rate. [34]
Another treatment option, although less effective, are custom made oral appliances (OA) that may improve upper airway patency during sleep by enlarging the upper airway and/or by decreasing upper airway collapsibility (e.g., improving upper airway muscle tone).[38]
Mandibular repositioning appliances (MRA) cover the upper and lower teeth and hold the

mandible in an advanced position with respect to the resting position. Tongue retaining devices (TRD) hold only the tongue in a forward position with respect to the resting position, without mandibular repositioning. OA are indicated in patients with mild disease or in those who did not tolerate CPAP. Of utmost importance, CPAP adherence can be very poor, as shown by several prospective studies.[39]

Lastly, in the recent years different research groups proposed electrical stimulation as a mean to treat OSAS by activating the dilator muscles of the upper airway in order to maintain it patent while asleep. [25] More specifically electrical stimulation through the hypoglossal nerve stimulation leads to a significant reduction in the apnoea-hypopnoea index and the oxygen desaturation index as demonstrated by the STAr trial recently published. [40] However, there are similar results published from feasibility studies for transcutaneous electrical stimulation.[41]

OSAS and hypertension

The relationship between OSAS and hypertension has been extensively studied and although many studies had some methodological limitations, recently some large population based studies have consistently shown that significant associations of polysomnographically determined OSAS and hypertension, defined by blood pressure thresholds or use of antihypertensive medication, remain after adjustment for potential confounding factors. (Table 5)

Collectively, the relationship between OSAS and hypertension has been assessed with state-of-the-art measurements in more than 9,000 men and women from the general population, and results have been consistent.

Table 5: Association of polysomnographically determined sleep-disordered breathing and hypertension in four population studies. CI=confidence interval, AHI=apnea hypopnea index. Odds ratios are all adjusted for age, sex, body mass index (BMI), neck circumference, alcohol intake, and cigarette smoking.

STUDY DESIGN	Odds Ratio* for Hypertension (95% CI)					
	N	AHI CATEGORY				
		<1.0	1 TO 4.9	5 TO 14.9	15 TO 30	≥30
1. Wisconsin Sleep Cohort Study,[42] state employees, ages 30 to 65 years, prospective, 4-8 years follow-up	709	1.0	1.2 (1.1-1.8)	2.0 (1.3-3.2)	2.9 (1.5-5.6)	—
2. Sleep Heart Health Study,[43] multicenter, ages 40-97 years						
a. Cross-sectional[44]	6132	1.0	1.1 (0.9-1.3)	1.2 (1.0-1.4)	1.3 (1.9-1.6)	1.4 (1.0-1.8)
b. Prospective, 2- and 5-year followup[43]	2470	—	1.0	0.9 (0.7-1.2)	1.1 (0.8-1.5)	1.5 (0.9-2.5)
3. Southern Pennsylvania, population sample via random-digit dialing, ages 20-100 years, cross-sectional[45]	1741	1.0	—	2.3 (1.4-3.6)	6.9 (2.0-26.4)	—
4. Vitoria-Gasteiz, Spain, random census sample, ages 30-70 years, cross-sectional[46]	552	1.0	2.5 (1.1-5.8)	1.3 (0.5-4.1)	2.3 (0.9-5.7)	

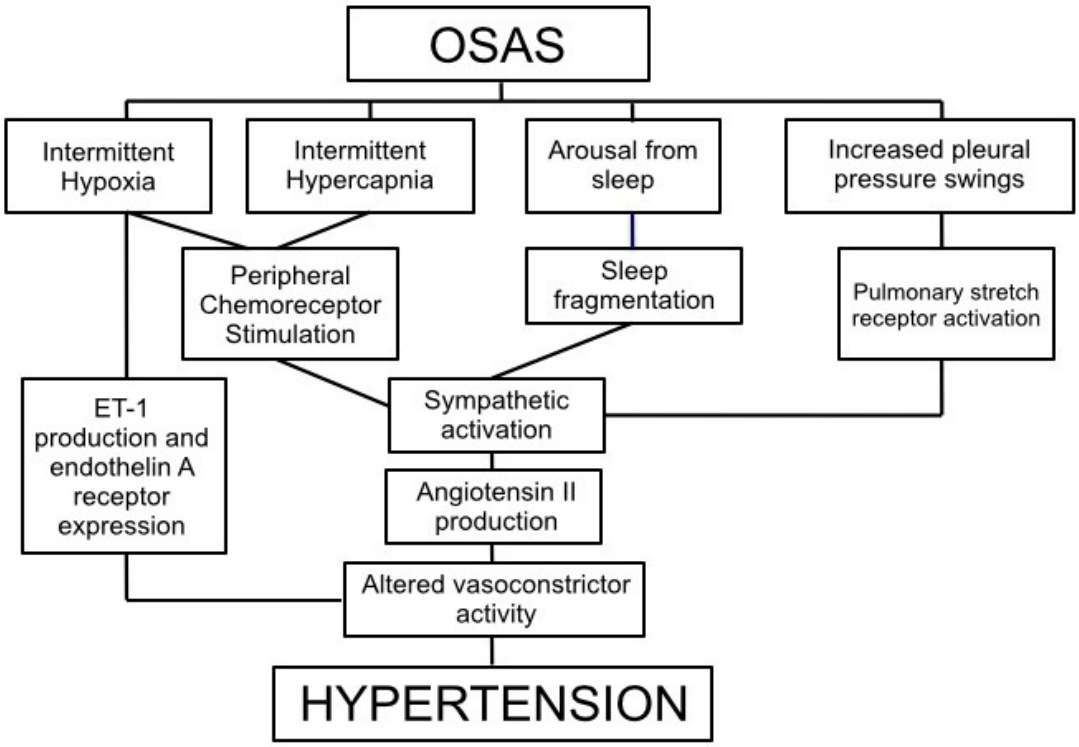

Figure 4: Pathophysiological mechanisms that link OSAS to the development of hypertension.

Whilst the epidemiological data suggest a causal relationship between OSAS and hypertension, a number of studies that have highlighted different pathophysiological mechanisms summarised in Figure 4.

Intermittent hypoxia, particularly if associated with episodes of intermittent re-oxygenation, is one of the main mechanisms as it promotes the production of reactive oxygen species (ROS), activates systemic inflammation, and ultimately impairs endothelial function.

Endothelial dysfunction can be defined as an imbalance between vasorelaxation and vasoconstriction substances produced by the endothelium.[47] Previous studies have demonstrated the strong association between endothelial dysfunction and OSAS even in cases of mild disease.[48]

Nitric oxide (NO), the most important vasodilatory molecule synthesized by the endothelium, is reduced in patients with OSAS.[49]

Moreover, repetitive cycles of hypoxia/reoxygenation stimulate the release of endothelin 1, one of the most important vasoconstricting peptides. [50]

The impact of CPAP on blood pressure

Recent studies have revealed that optimal CPAP control reduces BP, with more marked effects in patients with resistant hypertension implying a role of the autonomic nervous system in relation to changes in blood pressure.[51]

This reinforces the relationship between OSAS and hypertension as CPAP is the best available treatment for this disease which is able to improve symptoms but also to restore the haemodynamic balance.

Whilst the role of CPAP on blood pressure is well established, less is known about its effect on blood pressure variability (BPV) giving that patients with OSAS exhibit also high BPV.[52]

BPV is a marker of the autonomic nervous system and an independent predictor of cardiovascular morbidity and mortality.[53] It is defined as the fluctuation of BP between different measurements over a defined time interval.[54] Visit-to-visit BPV comparison is related to an increased risk of cardiovascular events.[55]

We aimed to investigate how CPAP treatment may modify the risk of increased sympathetic activation, through its impact on BP and BPV, while comparing normotensives and hypertensive patients with obstructive sleep apnoea.

Patients with a diagnosis of OSA, and who had been referred to a tertiary sleep centre (Sleep Disorder Centre, Guy's & St Thomas' Hospitals, London, UK), were enrolled between, June 2013 to December 2013.

OSA was diagnosed with a 4% oxygen desaturation index (ODI) > 5/hour combined with excessive daytime sleepiness (ESS>10). The study was approved by the local institution's ethical board (2014/3081) and all patients gave written informed consent.

Hypertension was defined according to the current guidelines,[56] In order to investigate any differential impact of CPAP, OSA patients where divided in 2 subgroups: patients with a known diagnosis of hypertension or a BP greater that 140/90 mmHg on three occasions were included in the hypertensive subgroup, patients with BP <140/90 mmHg on three occasions were included in the normotensive subgroup.

Following baseline recording of demographic data and BP measurements patients underwent nocturnal pulse oximetry and were provided with an APAP device for home use. At 2-week follow-up compliance data were obtained and and BP was measured again, BPV was calculated.

At baseline, participant's age, sex, height, weight, body mass index (BMI) and Epworth sleepiness scale (ESS), as a measure of daytime sleepiness,[32] were recorded.

At baseline and at 2-week follow-up, three blood pressure measurements each were taken, with 1-minute intervals, using an automatic sphygmomanometer (Mindray VS-800, Medical International Limited, Shenzhen, China). Patients were rested (>5 minutes), and seated in an upright position.

The average of pulse rate, systolic and diastolic BP was calculated from these readings. Systolic and diastolic range was calculated as the difference between the maximum and minimum BP values.

Systolic and diastolic BPV was calculated as the standard deviation (SD) of the 3 measurements.

All data were compared between baseline and 2-week follow-up (following APAP treatment), this included delta (difference in) systolic and diastolic BP range, means/average BP and SD; in addition to delta pulse.

Following baseline measurements, patients were issued with an auto-titrating continuous

positive airway pressure device (APAP, S8/S9, ResMed Ltd, Sydney, Australia) for 2 weeks of home use.

The following compliance and treatment indices were downloaded, and calculated: average daily APAP usage (h), days of APAP usage >4 hours per day (n), days of APAP usage <4 hours per day (n), percentage of days of APAP usage > 4 hours per day (%), air leak (L/min) and the 95th percentile of APAP to control respiratory events (cmH_2O).

The primary outcome parameter was change in BPV between baseline measurements and at 2-weeks of treatment. Secondary outcomes included change in pulse rate, change in absolute BP values, and comparison of these parameters in patients with and without hypertension. Lastly, we compared patients with good CPAP compliance, defined as CPAP usage greater than 4 hours per night for at least 70% of the total days, and sub-optimal CPAP compliance to determine whether BPV changes were dependent on CPAP usage.

Data were analysed using SPSS statistics 21 (IBM, New York, NY, USA) and tested for a normal distribution using the Shapiro-Wilk normality test. Normally distributed data are presented as mean (SD), and analysed with paired and unpaired t-tests. Non-normally distributed and are presented as median (IQR), and analysed with the Wilcoxon Rank sum test when paired, and the Mann-Whitney U test when unpaired.

Correlation analysis was used to describe correlations between continuous independent and dependent variables.

Where bivariate analyses revealed significant correlations, logistic regression analyses were employed to identify independent correlations with the BPV parameters. BPV was inserted as the dependent variable in the multivariate analysis with age, gender, BMI, APAP compliance as independent variables. A sub-analysis compared BPV in patients with optimal and sub-optimal APAP compliance. Non-normally distributed data were log-transformed prior to regression analysis and comparison. A level of significance was defined as $p<0.05$. To understand the significance of the difference found on BPV pre and post treatment we performed a power calculation giving a significance level (adjusted for sidedness) of 0.025, the total number of patients and the obtained difference in means of 1.2 mmHg. The calculated power was 99 percent that the study would detect a difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.200 times the standard deviation.

We recruited a total of 78 participants with OSA: 76.9% males, BMI 36.2±6.9 kg/m^2, age 49.0±12.9 years. At baseline, the systolic and diastolic BP was 130.9±15.5 mmHg and

82.7±10.4 mmHg, respectively. Oximetry data confirmed severe sleep-disordered breathing with a mean 4%ODI of 27.4±19.9 x $hour^{-1}$ and a mean 3%ODI of 33.4±20.3 x $hour^{-1}$, whilst average oxygen saturation was 93.9±3.2, and baseline Epworth sleepiness scale was 11.3±7.6 points (table 6).

Thirty-eight participants had hypertension, 17 (45%) were untreated, 13 (34%) were treated with angiotensin receptor blockers, 3 (8%) with beta blockers, 12 (32%) with calcium channel blockers, 5 (13%) with diuretics and 1 (3%) patient with alpha-adrenergic blockers.

The hypertensive group (n=38) was older with mean age 51 (16.5) vs 43 (13) years, p=0.008, and had a higher BMI 37.3 (8.0) vs 32.8 (8.9) kg/m^2, p=0.019, than the normotensive OSA group (n=40). No other baseline differences existed between the two groups (table 7).

Table 6: BP parameters pre and post 2 weeks of APAP treatment in all individuals (n=78; hypertensive and normotensive participants with OSA, BP=blood pressure, APAP=autoset continuous positive airway pressure, OSA=obstructive sleep apnoea, SD=standard deviation, ODI=oxygen desaturation index). Data presented as median (IQ range) unless stated otherwise.

	Pre-APAP	Post-APAP	p-value
Systolic BP(mmHg)	130.9 (15.5)	129.9 (14.9)	0.486
Diastolic BP(mmHg)	82.7 (10.4)	82.2 (10.5)	0.615
Systolic SD (mmHg)	5.3 (4.9)	4.2 (3.4)	0.049
Diastolic SD (mmHg)	2.0 (2.1)	2.3 (2.0)	0.410
Systolic Range (mmHg)	10.0 (8.8)	8.0 (6.8)	0.04
Diastolic Range (mmHg)	4.0 (4.0)	4.0 (4.0)	0.483
Pulse rate (bpm)	78.0 (14.5)	75.5(15.8)	0.033
Nocturnal Pulse rate (bpm)	70.0 (8.8)	69.7 (8.7)	0.416
Pulse rise index (h^{-1})	39.0 (20.4)	41.3 (19.6)	0.703
Nocturnal oxygen sat (%)	93.9 (3.2)	94.3 (3.1)	0.993
3% ODI (events/hour)	26.8 (28.4)	35.6 (29.7)	0.841
4% ODI (events/hour)	20.6 (27.0)	28.3 (26.3)	0.675
Epworth Sleepiness Scale (points)	11.0 (10.8)	7.5 (8.0)	<0.001

Table 7: Baseline characteristics and OSA severity of patients with and without hypertension. (OSA=obstructive sleep apnoea, ESS=Epworth sleepiness scale, BMI=body mass index, ODI=oxygen desaturation index). Data displayed as median and interquartile range; and group differences are analysed with the Mann-Whitney U test, unless indicated otherwise.

Parameter	HTN (n=38)	NTN (n=40)	P-value
Sex (% male)	71.0%	82.5%	0.367
Age (y)	51.0 (16.5)	43.0 (13.0)	0.008
BMI (kg/m^2)	37.3 (8.0)	32.9 (8.9)	0.019
ESS (pts)	12.0 (10.0)	10.0 (11.25)	0.960
3% ODI (ev/h)	33.2 (31.9)	22.0 (19.2)	0.121
4% ODI (ev/h)	25.1 (29.6)	17.9 (16.5)	0.114
Mean Oxygen Saturation (%)	93.5 (3.6)	94.2 (2.9)	0.162

All 78 patients were seen at 2 weeks for follow up. No drop-outs were recorded. For all patients, there was a significant reduction in symptoms, as measured by the ESS from baseline 11.0±10.8 to follow up 7.5 ±8.0 points ($p<0.001$). The 95th percentile APAP was 13.3±5.0 cmH_2O and air leaks were acceptable (at 95th percentile 0.30±0.60 L/sec). APAP was used for 14.0±0.0 days and the daily APAP usage was 3.0±2.3 hours. 41.0 % of patients used APAP for more than 4 hours per day and the total hours of APAP usage over 14 days was 43.3±31.4 hours.

There were no differences in compliance data when comparing the hypertensive and normotensive groups.

Blood pressure variability as expressed by systolic BP standard deviation (5.3±4.9 to 4.2±3.4, $p=0.049$), pulse rate (78.0±14.5 to 75.5±15.8, $p=0.033$) and systolic BP range (10.0±8.8 to 8.0±6.8, $p=0.040$) decreased compared to baseline. There was no difference in absolute BP (systolic and diastolic), diastolic SD or diastolic range (table 6).

A significant change in pulse rate was observed in the hypertensive OSA cohort ($p=0.026$), but not within the normotensive cohort ($p=0.471$). A sub-analysis of patients did not show any impact of compliance on changes of BP or BPV in the two groups but revealed a greater change in the pulse rate (0.02±8.92 vs -4.44±6.72 bpm, $p<0.05$) in the group with higher APAP compliance.

We found that delta mean systolic BP was associated with increased APAP pressure. ($r=0.293$, $p=0.009$) and a delta BPV (systolic standard deviation) was inversely associated with an increased APAP 95th percentile leak ($r=-0.237$, $p=0.048$). A decrease in pulse rate (delta pulse), after compared to before APAP treatment, was correlated with: an increased number of days where APAP was used for >4hours ($r=-0.408$, $p<0.0001$), a decrease in

the number of days where APAP was not used (r=0.334, p=0.003), an increased total hours used (r=-0.355, p=0.002), and an increased average daily usage (r=-0.352, p=0.002). Regression analyses did not find any one of these APAP compliance measures to independently predict a delta pulse.

When comparing patients with optimal and suboptimal CPAP compliance, there was no difference in the change of the BP, but a reduction in the pulse rate in patients with optimal compliance: -5.50±(8.25) *vs* -0.50 (10.50) bpm; p<0.05).

In this study, two-weeks of continuous positive airway pressure (APAP) treatment have a beneficial effect on BPV in patients with OSA. This effect seems to occur through modulation of blood pressure variability in the hypertensive patients, most likely due to an improved sympathetic-vagal balance.

These results indicate that short-term treatment of OSA with CPAP treatment has a favourable impact also on BPV.

As seen in this study there are many determinants which can impact on blood pressure and blood pressure variability in patients with OSAS treated with CPAP. As discussed previously CPAP treatment can not only treat apneas and therefore reduce the increased sympathetic activation responsible for high blood pressure. Indeed CPAP can also cause sleep disruption, reduced sleep efficiency and increased sleep arousals if the treatment is not well managed and in particular if the CPAP pressure is not correctly titrated, if the are too many leaks or if either the mask or the pressure is creating discomfort rather than allowing a good sleep. Also compliance plays an inevitable huge role as roughly a quarter of patients on CPAP stop its usage within weeks[57] and at one-year follow up less than half of the patients continue with regular use.[58]

This is of interest because symptoms amelioration and potential benefit on blood pressure depend on tolerance and adherence to treatment.

In obese subjects in particular, the load on respiratory system is greater than in lean subjects as CPAP pressure is greater which consequently increases the potential leaks causing sense of breathlessness and imparied breathing at night.

Neural respiratory drive (NRD), as measured by the electromyogram (EMG) of the diaphragm or using a surrogate marker, the parasternal EMG,[59] reflects the load on the respiratory system and is closely associated with breathlessness.[60]

We therefore performed a study in obese patients with OSAS that were studied in supine posture while awake to determine the point at which CPAP maximally offloads the

respiratory system, as determined by the lowest level of NRD and breathlessness. We hypothesized that the effective nocturnal CPAP level differs from that required to maximally offload the respiratory system while awake which could results in breathlessness, impaired blood pressure control and affect long-term compliance.

Obese subjects (>18years) with a body-mass index (BMI) above >25kg/m^2 and confirmed OSA were recruited from the Sleep Disorders Centre at Guy's & St Thomas' NHS Foundation Trust, London, UK. The study was approved by the local Research Ethics Committee and informed written consent was obtained from each patient.

Patients were fully evaluated by an expert sleep physician and were included if they had either an apnoea-hypopnea index (AHI) >15/h, or if they were symptomatic (Epworth Sleepiness Scale >10 points) and had and AHI >5/h. Anthropometric measurements included age, sex, height, weight, body mass index (BMI), neck, waist and hip circumference, Mallampati score, and smoking history. The Epworth sleepiness scale was administered before the overnight polysomnography on CPAP titration. Continuous BP was measured with a beat-to-beat BP monitor (Finapres).

CPAP during the titration night was determined by expert sleep technicians, as defined by the 95th percentile of CPAP level that was required to avoid upper airway occlusion (CPAP, *sleep*). The next morning, the surface electromyogram of the parasternal muscles (EMG_{para}) and abdominal muscles ($EMG_{abdomen}$) was measured, as previously described.[61] For recording of the surface EMG_{para}, electrodes were placed on each side of the sternum 3 cm from the midline in the second intercostal space; for the surface $EMG_{abdomen}$, one electrode was placed in the middle of a vertical line connecting the lower rib cage with the anterior superior iliac spine, with the subject standing. The other electrode was placed approximately 4–5cm anterior to that location using surface electrodes (Kendall Arbo, Tyco Healthcare Neustadt, Germany). Patients were then asked to rest on a bed to measure baseline breathing and titrate pressures, while awake.

Prior to any measurements, patients were asked to lie on a bed in a standardised supine position (legs straight and uncrossed, hands by their side with their palms facing down, and head/shoulders supported using a pillow so that the patient felt no effort to maintain head and neck posture) for five minutes. The surface EMG (EMG_{para}; $EMG_{abdomen}$) was then recorded for three minutes at baseline (without interface). A further three minutes were recorded with a CPAP mask on (ResMed Mirage Quattro full face mask, ResMed Ltd, Oxfordshire, UK), open to air. After that, the surface EMG signal was recorded while the

mask was attached to a CPAP machine (CPAP S8, Resmed Ltd, Oxfordshire, UK) and CPAP levels were changed stepwise from 4cmH_2O to 20cmH_2O, using increments of +2cmH_2O, every level was recorded for at least 3 minutes. At the end of each level, patients were asked to score their perceived degree of breathlessness on a modified Borg Scale (mBorg) for dyspnoea (from '0' to '10' points, with higher numbers indicating more breathlessness). The protocol finished either when patients had reached a CPAP of 20cmH_2O for >3minutes or when they felt that the pressures were unpleasant or caused breathlessness.

The EMG data were amplified and band-pass filtered between 20Hz and 1kHz (Yinghui Medical Equipment Scientific Ltd, Guangzhou, China), saved and analyzed using LabChart (Version 7.3.7, ADInstruments, Colorado Springs, CO, USA). Raw EMG signals were converted to root-mean-square (RMS), and all of the RMS of EMG was normalized to baseline RMS of the EMG recorded when the patients were breathing at rest, without mask, in supine posture; the results were expressed as percent of baseline activity (%EMG, *baseline*). The mean value of RMS (time constant 100ms) in the final minute of each pressure level (0-20cmH_2O) was calculated to define NRD. Following daytime CPAP titration, the pressure that led to the lowest level of NRD, as defined by the surface EMG_{para}, was marked (CPAP, *awake*); this value was compared with CPAP, *sleep*.

Data were statistically analysed using SPSS (SPSS, Chicago, IL, USA). Following testing for normality, Borg scale and surface EMG during CPAP titration in individual cases were expressed as mean ± SD. A one-way ANOVA was applied when Borg scale and surface EMG were compared to baseline and maximal pressures, respectively. CPAP, *sleep* and CPAP, *awake* were compared using paired t-test, post hoc corrections were used for multiple comparisons. Statistical level of significance was defined with a p-value <0.05.

15 patients with a confirmed diagnosis of OSA were studied. The patients were middle-aged and predominantly male (80%), and obese, with one patient being in the overweight category (number 4). Two patients had mild (number 3 and 15) and all others had moderate-severe obstructive sleep apnoea. Patients had relatively preserved lung function, as expressed by the age, gender and height adjusted percent predicted (Table 8). Baseline BP was 131.0(10.2)/85.1(9.1) mmHg.

Table 8: Demographics of patients. The group was predominantly male, middle-aged, all but two patients suffered with moderate-to-severe obstructive sleep apnoea. F:M female and male participants.

Subject (n)	Age (years)	Gender (F:M)	BMI (kg/m^2)	AHI (h^{-1})	FEV1 (L/ %pred)	FVC (L/ %pred)	FEV1/FVC (%)
1	34	M	32.6	28	3.49 / 81	4.66 / 89	75
2	38	M	37.2	23	4.13 / 102	5.09 / 103	81
3	54	F	49.7	10.4	1.43 / 70	1.59 / 65	90
4	57	M	27.9	51.2	3.64 / 106	4.13 / 96	88
5	54	M	40.4	77.4	3.09 / 86	3.80 / 84	81
6	54	M	42.6	18.7	3.00 / 83	3.85 / 85	78
7	49	M	36.7	22.6	2.77 / 76	3.53 / 79	79
8	48	M	38.7	20	2.35 / 62	3.79 / 80	62
9	37	F	37.3	52	1.88 / 83	2.68 / 101	70
10	53	M	30.8	40	3.16 / 99	3.60 / 91	88
11	62	M	41.5	16.3	2.45 / 75	3.62 / 86	68
12	46	F	36.4	20	1.53 / 63	2.25 / 80	68
13	60	M	44.7	24.7	3.13 / 93	4.01 / 93	78
14	31	M	44.9	69.4	4.28 / 90	5.49 / 95	78
15	37	M	42.1	9	3.57 / 92	4.50 / 95	79
Mean	**47.6**	**3:12**	**38.9**	**32.2**	**2.9 / 84.1**	**3.8 / 88.1**	**77.5**
SD	**9.9**		**5.8**	**21.1**	**0.9 / 13.3**	**1.0 / 9.8**	**8.0**

All but one patient completed the study protocol until they had reached a CPAP level of 20cmH_2O; subject number 2 stopped at 14cmH_2O due to discomfort. Except for subject number 13, we observed in all patients that increasing levels of CPAP offloaded the respiratory system resulting either in a clear reduction of EMG_{para} from baseline and/or a substantial increase once the pressure levels for optimal chest inflation, as defined in the method section as CPAP, *awake*, had been exceeded. The effect of CPAP on $EMG_{abdomen}$ was variable, but there was a clear tendency to recruit abdominal muscles with higher pressures in 9/15 participants.

The optimal level of chest inflation, as defined in the method section as CPAP, *awake*, achieved a reduction in EMG_{para} to 70.2±17.7% of baseline activity (p=0.026), which was lower than at the beginning and at the end of the protocol (EMG_{para} at CPAP of 20cmH_2O was 103.3±49.3 % from baseline). $EMG_{abdomen}$ was significantly lower at CPAP, *awake* (93.8±9.3% of baseline activity, p<0.001) when compared to the EMG at the beginning of the protocol (CPAP of 0cmH_2O), at higher CPAP levels (20cmH_2O) $EMG_{abdomen}$ increased and became highly variable, with a coefficient of variation as high as 70%. When CPAP pressures were increased above 6cmH_2O patients slowly started to feel more breathless

(Figure 5) and with CPAP approaching 20cmH_2O the Borg scale was significantly higher than at the beginning of the protocol, although there was a high variability with a coefficient of variation of up to 95%. The comparison of CPAP*, sleep* vs CPAP, *awake* revealed significant higher nocturnal pressures (p=0.026) and associated Borg scores (p<0.05). In 7/15 patients Borg scores increased when comparing mBorg at CPAP, *awake* vs CPAP, *sleep* (subjects number 2-5, 9, 13-14), in the remaining 8/15 patients mBorg scores remained either the same or fell slightly (subject number 15).

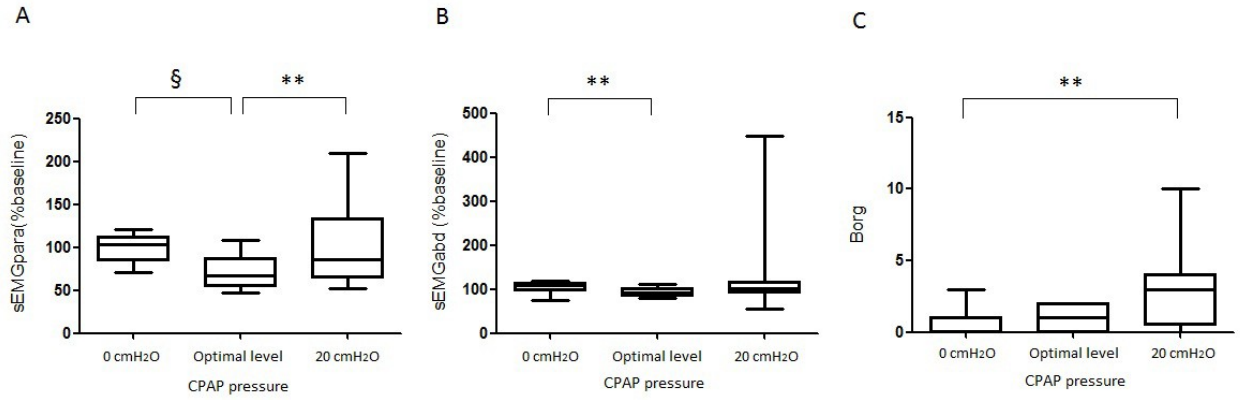

Figure 5: Box Whisker Plots for the whole cohort indicating the A) EMGpara and B) mBorg score at 0cmH_2O, CPAP, awake and maximal pressures of 20cmH_2O. * p<0.05, ** p<0.01

BP (figure 6) and BPV increased with incremental CPAP (systolic BP r=0.960,p<0.001, diastolic BP r=0.961,p<0.001; systolic BPV r=0.662,p=0.026; diastolic BPV r=0.886,p<0.001). $sEMG_{abdo}$ correlated with both systolic (r=0.464,p=0.032) and diastolic BP (r=0.747,p=0.009).

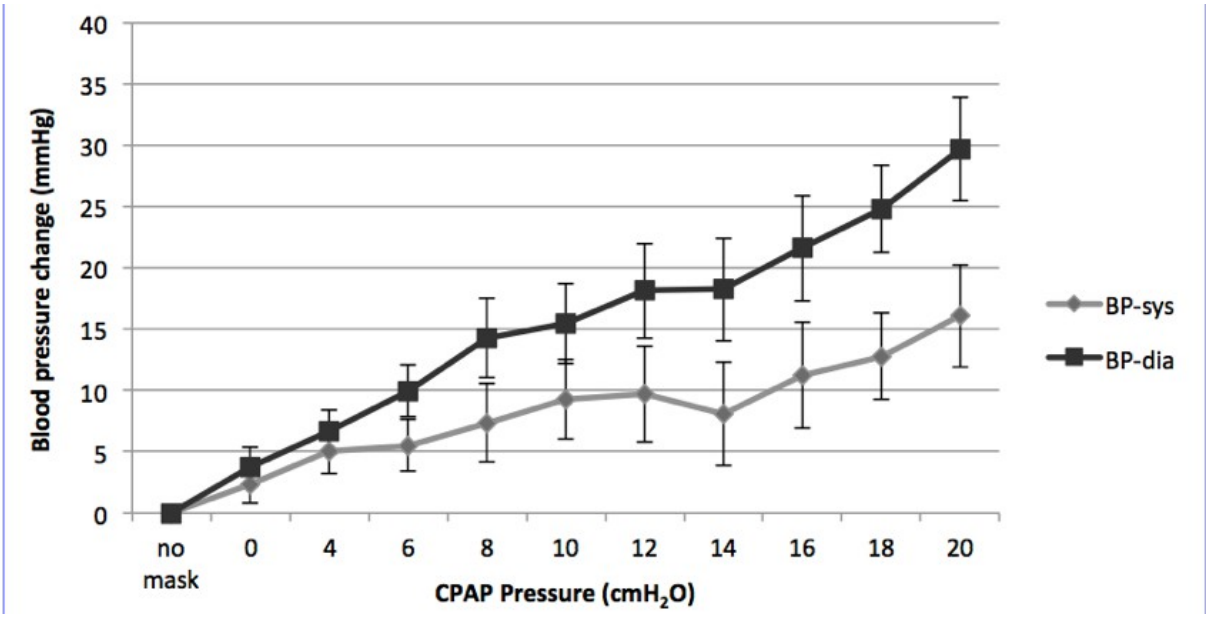

Figure 6: Relationship of incremental CPAP titration and blood pressure change (mean, SEM) from baseline. BP systolic (grey), BP diastolic (black)

Abdominal muscle recruitment ($sEMG_{abdo}$) increased with higher CPAP (r=0.782, p=0.004); there was no correlation between $sEMG_{para}$ and CPAP (r=-0.110,p=0.748). CPAP level was associated with blood pressure changes irrespective of muscle recruitment BPV (table 2). Abdominal EMG was associated with some of the blood pressure variables, irrespective of parasternal muscle recruitment (table 2).

This is the first study that systematically measured NRD and breathlessness during CPAP titration in obese patients with OSA while awake and related these findings to the effective CPAP level (CPAP, sleep) required to maintain airway patency when asleep. CPAP effectively offloads the respiratory system in obese subjects, when awake, and reduced NRD by 30% during optimal chest inflation. Breathlessness is of no concern when NRD is reduced. However, when CPAP was increased to higher pressures, NRD, as reflected by activity of the parasternal intercostal and expiratory muscles, increased and patients became breathless. Moreover, CPAP led to an increase of both BP and BPV and this was associated with EMG changes at different pressure.

This is of clinical interest giving that patients on CPAP are likely to stop the treatment the longer the intervention. More interestingly, measuring EMG activity can help understanding who is going to benefit more in terms of BP and BPV reduction.

OSAS and the autonomous nervous system

OSAS is known to be related to a dysregulation of the autonomic nervous system (ANS) and of the catecholaminergic metabolism although the pathophysiology of these alterations is not fully understood.

The repetitive episodes of complete or partial obstruction of the upper airway during sleep lead to oxygen desaturations which trigger the so called “oxygen-conserving reflex” that restores normoxia by promoting hyperventilation.[62]

This process generates a hyperactivation of the sympathetic nervous system that mediates vasoconstriction to redistribute oxygenated blood flow to vital organs. At the same time the parasympathetic nervous system is activated with consequent bradycardia

in order to reduce myocardial oxygen demand.[63] Once normal breathing resumes, venous return and cardiac output increase, but the increased cardiac output is delivered into a highly constricted peripheral vasculature, resulting in increase of BP at the end of each apnoea.

This physiologic response becomes pathological when the enhanced sympatho-excitation is sustained over years, as is the case in OSAS. This phenomenon may be responsible of the “non-dipping” BP profile at night, which has been associated with an increased risk of cardiovascular events.

These hemodynamic and autonomic changes are not confined to nighttime: Elmasry and colleagues showed that, in a population-based sample of hypertensive males (n = 116), OSAS was associated with increased urinary concentrations of extraneuronal metabolites of CAs, when compared to subjects without OSAS. This association was independent of major confounding factors (age, BMI and severity of hypertension), suggesting an increased sympatho-adrenal activity.[64]

Treatment with CPAP for 4 weeks only has also been associated with a reduction in urine normetanephrine excretion,[65] however data emerging from other studies and clinical trials have revealed a substantial variability regarding CPAP treatment and its effects on CAs levels.[66] This is presumably due to the complexity of the cathecolamine metabolism.

Catechol-O-methyltransferase (COMT) is one of the major mammalian enzymes involved in the metabolic degradation of catecholamines. (Figure 7)

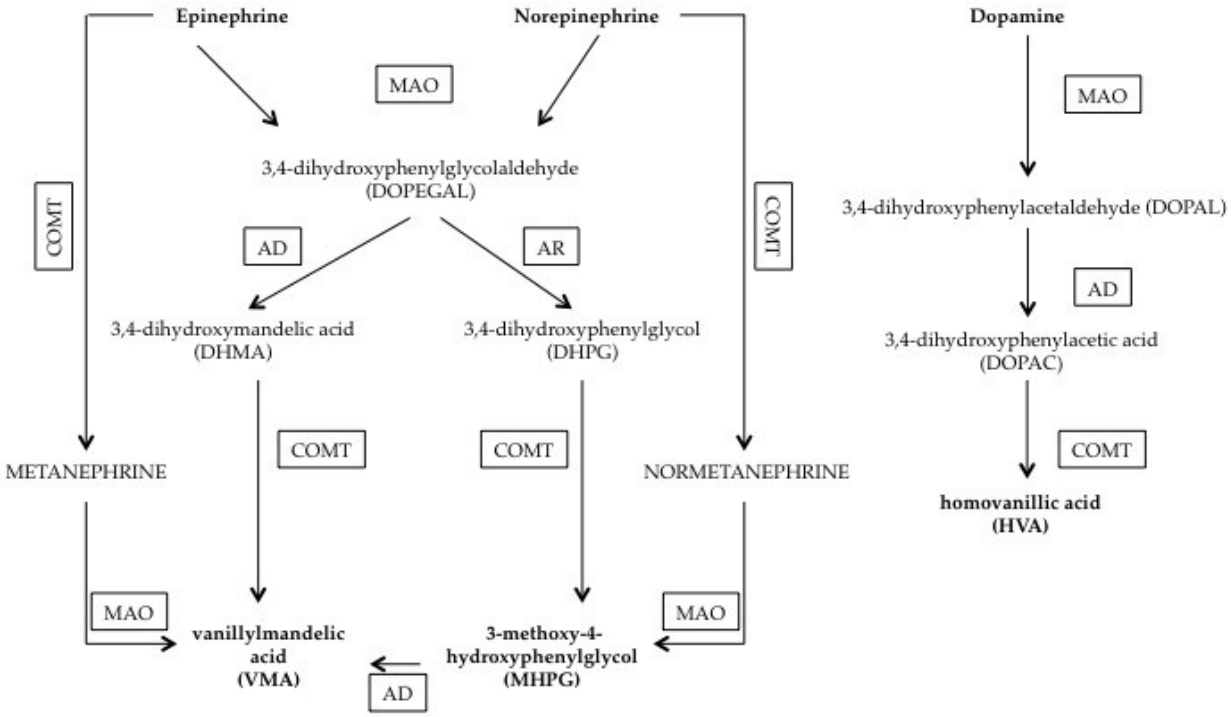

Figure 7: Enzymes involved in catecholamines metabolism.

COMT was discovered by the Nobel prize Julius Axelrod in 1957 [67] who showed that this enzyme introduces a methyl group to the catecholamine, which is donated by S-adenosyl methionine, and this process is mediated by Mg2+ as a catalyst of electron exchange and inhibited by Ca2+. Any compound, either endogenous or exogenous, having a catechol structure, like catecholestrogens and catechol-containing flavonoids, are substrates of COMT.

However, giving that COMT metabolises also dopamine, it has been extensively studied for the treatment of Parkinson disease. Less is known about the role of COMT in catecholamine metabolism with regards to the development of hypertension and other cardiovascular disorders.

COMT gene is located on chromosome 22, it is transcribed into 2 different mRNAs (1.3kb and 1.5kb). This is due to the presence of 2 different primers: P1 allows the transcription and translation of the soluble form of the enzyme (S-COMT) whilst P2 allows the transcription and translation of both the soluble form and the membrane bound form of the enzyme (MB-COMT). [68](Figure 8)

There are many different polymorphisms of COMT gene: one of the most important ones is the VAL158MET that is due to a G-to-A transition at codon 158 of the COMT gene,

resulting in a valine-to-methionine (V158M) substitution.[69] This genetic polymorphism results in a 3- to 4-fold reduction in COMT activity in red blood cells and hepatocytes which results in a reduced methabolisation of catecholamines. This polymorphism doesn't modify the kinetic characteristics of the enzyme as it doesn't involve the catalytic domain.[70] Interestingly, this polymorphism was associated with individual variation in COMT thermal instability. This is presumably due to a greater tendency to oxidation, as it is reverted by adding dithiothreitol, a small-molecule redox reagent.

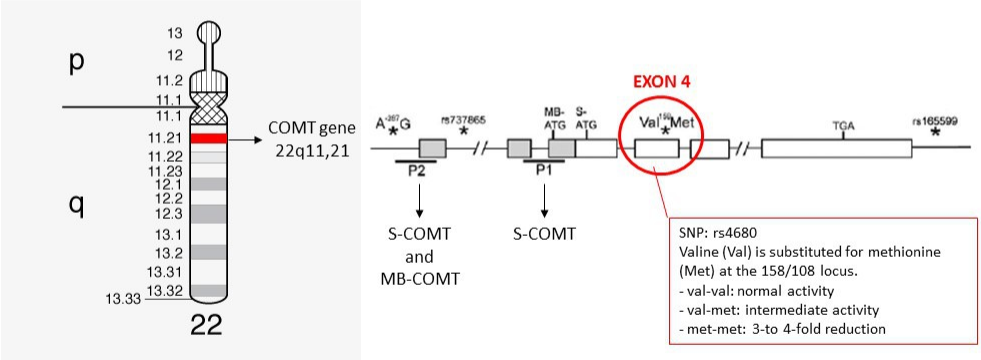

Figure 8: COMT location on chromosome 22 and the transcription into 2 isoforms of the enzyme.

The world population allele frequency is 37% for the mutated allele and 63% for the wild type however, in the European population the frequency can reach 50%. [71]
There are also other important single nucleotide polymorphisms (SNP) such as Rs6269-A/G, Rs4633-C/T (His62His) and Rs4818-C/G (Leu136Leu).[72] These have been grouped into 3 major haplotypes (table 9).

Table 9: COMT major SNP and the different haplotypes.

Activity	SNP			
	rs6269	rs4148	rs4680	rs4633
high	+	+	+	-
medium	-	-	-	+
low	-	-	+	-

A study has analysed the kinetics of COMT and found that it is dependent on the isoform and the substrate: MB-COMT has a greater affinity but a lower O-methylation capacity of

catecholamines when compared with S-COMT. In fact, S-COMT V_{max} is greater than MB-COMT whilst K_m is lower.[73]

These differences show that in cases of low substrate concentration MB-COMT activity will be greater whilst in cases of high substrate concentration, MB-COMT contribution to O-methylation will be less significant compared to S-COMT. During in vitro studies, usually very high substrate concentrations are used compared to physiologic catecholamine concentrations, leading to an underestimation of MB-COMT contribution to catecholamine metabolism.

COMT is widely present in many different organs and tissues in humans. (Figure 9)

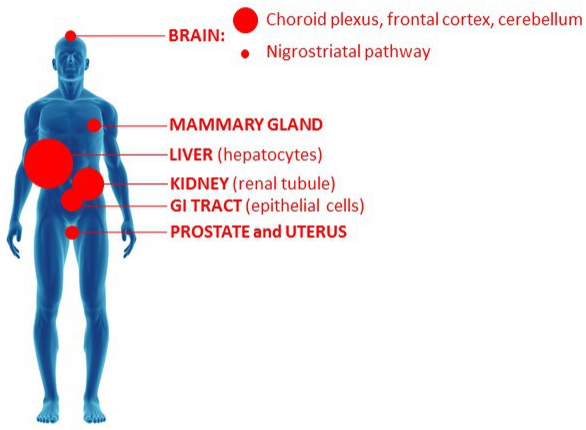

Figure 9: distribution of COMT enzyme in humans.

Giving the high interest in neurological disorders it has been extensively studied in the central nervous system however, when compared with other organs (e.g. peripheral tissues), mRNA levels, COMT quantity and activity are rather low. Also, COMT is more prevalent in the choroid plexus, frontal cortex and cerebellum whilst in the nigrostriatal pathway is less present.[74]

The highest COMT activity has been seen in the liver where is 2-3 fold greater than in kidneys. [75]COMT activity has been seen also in spleen, beta and delta pancreatic cells, uterus, mammary glands, prostate and chromaffin cells of adrenal glands.

Sub-cellular localisation has been extensively studied in cerebral and cerebellar cortex:

COMT has been found in neurons, dendrites and astrocytes mainly at the pre synaptic level. [76]

Interestingly COMT activity seems to be greater in men than in women. [77]

Also, it varies with age: when measured in the liver, COMT activity increases up to 10 times from birth up to 50 years and then starts declining after 60 years of age.[78]

Giving the importance of COMT in the metabolism of catecholamines it is of interest also in the pathophysiology of arterial hypertension. Catecholamines regulate blood pressure via a central and peripheral pathway.

In particular the arterial baroreflex, one of the body's homeostatic mechanisms that helps to maintain blood pressure at nearly constant levels, is mediated by the sympathetic nervous system.

The baroreflex provides a rapid negative feedback loop in which an elevated blood pressure reflexively causes the heart rate to decrease and also causes blood pressure to decrease. Similarly, decreased blood pressure decreases baroreflex activation and causes heart rate to increase and to restore blood pressure levels.

This important reflex acts also peripherally by activating alpha adrenergic receptors which mediate vasoconstriction.[79]

Imai and colleagues [80] demonstrated that hypertensive rats had a blunted response to noradrenaline release secondary to hypotension stimulus.

After the administration of S-Adenosyl methionine a marked hypotension and increased levels of normetanephrine were observed, due to the activation of COMT.

Tsunoda and colleagues [81] showed that MB-COMT levels were reduced in hepatocytes of hypertensive rats but this finding has not been replicated in the kidney, confirming that there is a significant difference of COMT activity and quantity in different organs and tissues.

To conclude, the role of COMT in the development of hypertension is still debated. Giving the complexity of catecholamine metabolism and the different variable involved (mutations, isoforms, possible inhibitors) it is important to better understand whether the inhibition of COMT is a predisposing factor to the development of hypertension.

More importantly, it is crucial to understand the role of intermittent hypoxia, one of the main features of OSAS, as a possible triggering factor of COMT activity.

OSAS and catecholamine metabolism

We tried to demonstrate that during apneas patients with OSAS exhibit an activation of the catecholaminergic system. We also wanted to understand whether COMT activity was triggered by hypoxia and to what extent.
Lastly we wanted to demonstrate that during sleep apneas patients with OSAS also exhibit an activation of the endothelin and renin angiotensin aldosterone system, an increase of the acute inflammatory indices and derangement of insulin secretion, which are all associated to an increased cardiovascular risk.
We enrolled patients referred to either the Hypertension clinic or the ENT clinic services (both Hospital of Padua, Italy) for assessment of suspected OSAS.

All patients were provided with a patient information sheet and informed written consent was obtained prior to enrolment. This study was approved by the hospital ethics committee. (Number 3217/AO/14)

Patients of both genders (age 18 ≥ and ≤ 70 years) were eligible if they had:

- Symptoms and/or signs suggestive of OSAS (except craniofacial dysmorphism and oropharyngeal abnormalities) such as obesity (BMI > 29 kg/m^2), neck circumference (> 43 cm in men and > 41 cm in women), daytime sleepiness, impaired concentration, morning headaches, nocturnal chocking;
- Overnight monitoring demonstrating an AHI > 5 events/hour during sleep.
- Patients with clinical evidence suggestive of OSAS and for which a DISE was needed to better investigate the site of obstruction.
- Patients able to provide written informed consent.

Exclusion criteria were:

- Secondary hypertension;
- Treatment with beta-blockers;
- Decompensated diabetes mellitus type I and II;
- Pregnancy;
- Life expectancy < 2 years
- Hemodynamic instability severe enough to require intravenous positive inotropic agents;

- Comorbidity conditions limiting the execution of DISE;
- Acute coronary syndromes (NSTEMI or STEMI) within 4 weeks;
- Unwillingness or inability to complete follow up;
- Patient with any serious medical condition, which in the opinion of the investigator, may adversely affect the safety of the participant;
- Known history of unresolved drug use or alcohol dependency;
- Current enrolment in another investigational drug or device trial.

All patients were screened to exclude a secondary hypertension diagnosis within the previous 5 years by means of plasma aldosterone and renin after an appropriate wash-out,[82] urinary 24 hours catecholamines and metanephrines; moreover, when appropriate patients underwent renal artery duplex, and adrenal CT or renal arteries CT angiography.
All subjects enrolled in the study underwent a full medical assessment where past medical history and current medications were recorded. Also physical examination was performed and antrophometric data were recorded. Excessive daytime sleepiness was evaluated through the Epworth Sleepiness Scale (ESS). [32]Before being booked for DISE all patients underwent an attended cardio respiratory monitoring (CRM) to confirm the diagnosis of OSAS, an ambulatory blood pressure monitoring (ABPM) and blood tests. (Figure 10)

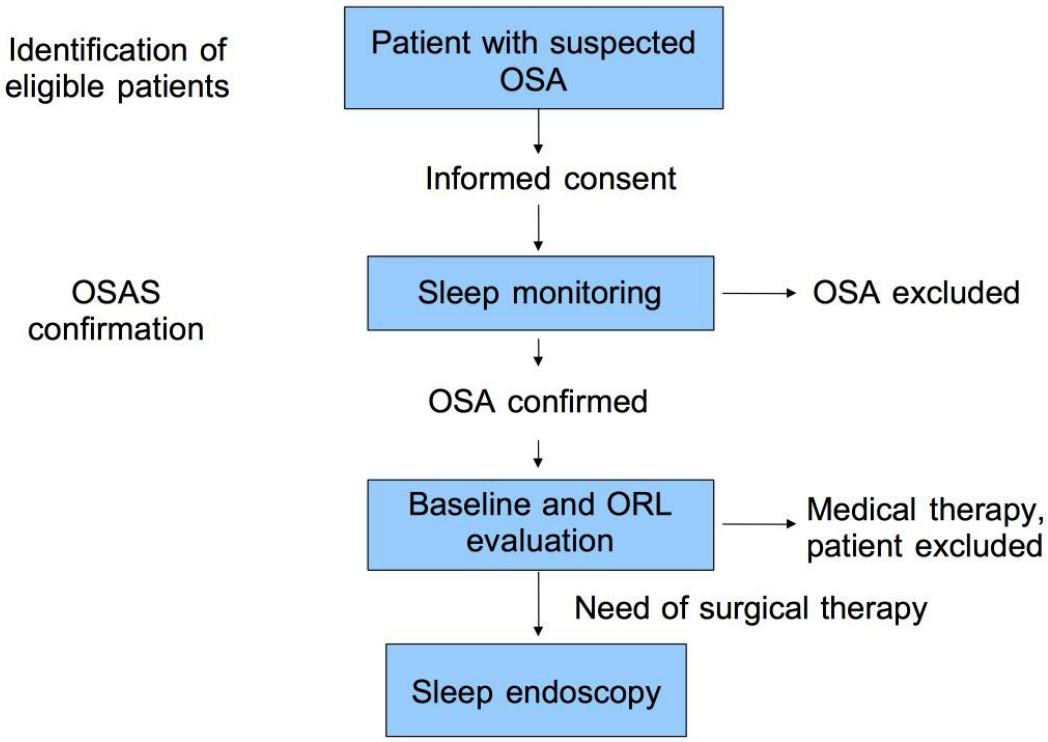

Figure 10: flow chart of the study.

Cardiorespiratory monitoring (CRM) was performed with an Embletta X 100 (Rembla, Natus, Pleasanton, CA 94566 USA) which has previously been validated for patients with suspected OSAS.[83]

Measurements obtained included nasal pressure, airflow by thermistor, respiratory effort by impedance plethysmography, sonography, and finger pulse oximetry. The Embletta devices were programmed to automatically begin recording at 22:00 and stop at 06:00. Based on sleep logs and out of centre sleep testing (OCST) actigraphy (an intrinsic component of the Embletta system), the scoring technologist modified the analysis start time and end time, resulting in a TRT that attempted to approximate total sleep time (TST) as closely as possible. Respiratory events (apneas and hypopneas) were scored according to the American Academy of Sleep Medicine (AASM) Scoring Manual.[84]

In particular, an apnea was defined if there was a drop in the peak signal excursion by ≥ 90% of pre-event baseline and the duration of the ≥ 90% drop in sensor signal was ≥ 10 seconds. A hypopnea was defined when the peak signal excursions dropped by ≥ 30% of pre-event baseline and the duration of the ≥ 30% drop in signal excursions was ≥ 10 seconds, and there was ≥ 3% oxygen desaturation from pre-event baseline or the event was associated with an arousal.

The apnea-hypopnea index (AHI) was calculated as the total number of apneas plus hypopneas divided by the hours of sleep. OSAS was defined when the AHI was greater than 5 events per hour. Patients with significant central sleep apnea (> 5% of events) were excluded.

Ambulatory blood pressure monitoring (ABPM) was performed using Spacelabs 90207 (SpaceLabs, Washington, USA). The between measurement intervals were 15 min (daytime) and 20 min (night-time). During each recording, subjects were required to attend at their usual daily activities, only refraining from unusual physical exercise or behavioral challenges. Patients were also asked to mark on a diary their main activities, including the time of meals, bed rest or sleep and awakening times. Only recordings rated as of sufficient quality, i.e. including at least 70% of valid readings over the 24 h and at least two valid readings per hour during daytime and one valid reading per hour during nighttime, were considered for the final analysis. Day and night periods were defined and corrected according to what reported by the patient in the diary. The average daytime period was finally identified as the interval from 0800 h to 2300 h and the night period as the interval from 2300 h to 0800 h. All procedures were performed according to current guidelines.[85]

From each recording we calculated: the average 24-h, day and night systolic (SBP) and diastolic (DBP) blood pressure; the degree of nocturnal BP fall and the standard deviations (SD) of the mean of all individual readings over the different time periods considered.

Based on the degree of nocturnal BP fall, subjects were classified as dippers (BP fall ≥ 10% and <20% of daytime average BP) or non-dippers (fall<10%), separately for SBP and for DBP. A subgroup of dippers with BP fall ≥ 20% were identified as extreme dippers.

The blood tests performed included: full blood count, C-reactive protein (CRP), serum creatinine, blood urea nitrogen (BUN), serum ions (Na+, K+, Ca2+), glycaemia, HbA1c, lipid profile, plasma catecholamines, PTH e vitamin D, fibrinogen, prothrombin time (PT) and partial thromboplastin time (PTT), thyroid stimulating hormone (TSH), thyroxine (FT4), serum cortisol, adreno-corticotropic hormone (ACTH), plasma aldosterone concentration (PAC), plasma renin activity (PRA).

Also further metabolic and endothelial markers were studied: oxidized low density lipoproteins (oLDL), endothelin 1 (ET-1), pro ET1, interleukin 2 (IL2), interleukin 6 (IL6), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), metalloproteinase, nitrotyrosine, myeloperoxidase (MPO), neutrophil gelatinase-associated lipocalin (NGAL).

Also urinary tests were performed: urinary albumin excretion (UAE), urinary catecholamine

and metanephrine, 24h-urinary ions (Na+, K+, PO43-, Ca2+) and urinary isoprostanes.
Drug induced sleep endoscopy (DISE) was performed by an experienced otolaryngologist in a semi dark and silent operating room with the patient lying supine in a hospital bed. The whole procedure was performed according to the current guidelines.[86]

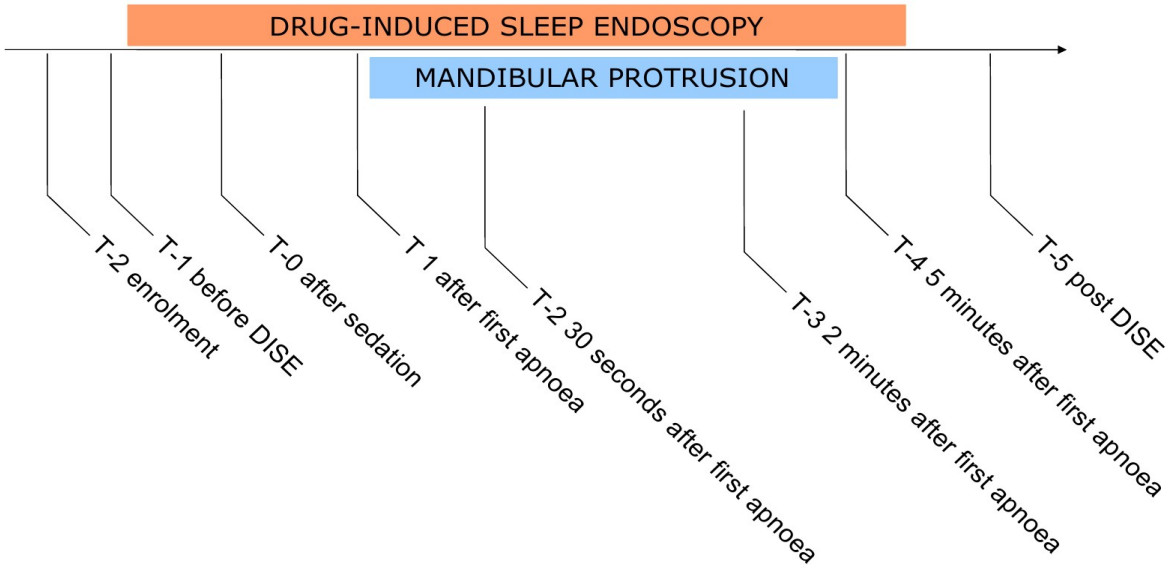

Figure 11: Timing of blood tests performed before during and after DISE.

Drug-induced sleep was achieved with intravenous administration of propofol with a target-controlled infusion rate after an initial loading dose.
The electrocardiogram, blood pressure, and oxygen saturation were continuously monitored by both the surgeon and the anesthesiologist. A flexible endoscope was used to sequentially observe the nasal cavity, nasopharynx, velum, oropharynx, tongue base, and epiglottis.
During DISE, blood tests for cathecolamines and ET1 were performed as per Figure 11.
In particular after propofol infusion T0 blood test was performed. Afterwards, at the occurrence of the first obstructive apnea, T1 was performed and then airway was kept patent through mandibular protrusion manouver performed by either the surgeon or an expert nurse. Then T2, T3 and T4 were performed when the patient was breathing normally without any upper airway obstruction. Oxygen saturation (including minimum SpO2 after the first apnea), heart rate and blood pressure were recorded throughout the procedure.

Blood samples were collected using a syringe through a previously placed intravenous line.

Samples were stored in Vacutainers containing EDTA as anticoagulant. Within 1 hour, samples were centrifuged (4 °C, 2,500 rpm, 15 min). Afterwards plasma was transferred in 1,5 mL tubes and stored at -20°C. Buffy coat (500 µL) was also stored at -20°C. Lastly, red blood cells (400 µL) were transferred in a 1,5 mL tube, 200 µL of RIPA buffer was added and vortex briefly. When lysis was complete, tubes were left on ice and eventually stored at -80°C.

COMT activity was performed with the Nissinen and Mannisto method [87] using 3,4-Dihydroxybenzylamine (DHBA) which is a catecholamine analog as a substrate, to obtain vanillic and isovanillic acid. These metabolites were then analysed and quantified with High Performance Liquid Chromatography (HPLC).

Buffer solution (400 µL) contained: phosphate buffer preparation (pH 7,4 250 mM) to keep pH levels within the normal range in order for the enzyme to remain active, $MgCl_2$ 6.25 mM as a catalyst of electron exchange, SAM 2,5 mM as a methyl group donor and DHBA 0,5 mM as a substrate. The enzyme reaction was started by adding 200 µL of the red blood cell lysis solution to the buffer solution and incubated for 45 minutes at 37°C. Tubes were then placed on ice and $HClO_4$ 4M (150 µL) was added to obtain protein precipitation.

Tubes were then centrifuged at 10,000 rpm for 15 minutes at 4°C and supernatant was collected and transferred to new 1,5 mL tubes and stored at -80°C for further quantification of vanillic acid by HPLC method.

In the HPLC analysis, the reaction products (vanillic and isovanillic acid) in the supernatant were analyzed by HPLC with electrochemical detection (ECD). The chromatographic system consisted of a solvent delivery system CLC300 (Chromsystems), a programmable autosampler CLC200 (Chromsystems) and a ECD CLC100 (Chromsystems). A Discovery® C18 HPLC column (15cm x 4.6 mm, 5µm, SUPELCO) at 25°C was used as the analytical column. A glassy carbon working electrode was set at 0.90 V versus an Ag/AgCl reference electrode. The sensitivity was set at 20 nA. The mobile phase consists of the buffer disodium hydrogen phosphate (0.1 M) and EDTA (0.15 mM) methanol (80:20, v/v), the pH was adjusted to 3.2 with phosphoric acid. The flow rate was 0.8 ml/min. The injection volume of the standard solution and the supernatant was 20 µL. COMT activity was expressed as pmol of vanillic acid /min/mg prot. The analytical performance of the method developed met the requirements of the FDA for bioanalytical method validation (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidan

ces/ucm070107.pdf. Accessed 2010)

Protein measurement was performed using Quantum Protein kit (Euroclone, Milan, Italy) which is a formulation based on bicinchoninic acid (BCA) allowing a rapid identification of protein content.

The BCA assay relies on the formation of a Cu2+-protein complex under alkaline conditions, followed by reduction of the Cu2+ to Cu1+. The amount of reduction is proportional to the protein present. The absorbance at 562 nm is then recorded and the protein concentration is determined by comparison to a standard curve.

The assay has been performed diluting the lysis solution 1:500. 100 µL of the diluted lysis solution was then transferred to a new 10 mL tube.

50 parts of reagent A were added to 1 part of reagent B provided in the Quantum Protein kit. 2 mL of the mixture of reagents were then transferred to the tube containing 100 µL of lysis solution and were incubated at 37°C for 30 minutes. Absorbance at 562 nm was measured with a spectrophotometer uv visible.

All the samples used for calibration curve have been managed similarly. Calibration curve have been performed with a standard albumin solution 1mg/mL, progressively diluted in order to get different concentrations (0,05-0,1-0,25-0,5 e 1 mg/mL).

All measurements have been performed twice.

At a two-sided 0.05 significance level a total of 12 patients will be required to have a 90 percent probability to detect an increase of endothelin-1 after DISE completion compared to 5 minutes before its start if the true difference is 4.0 pg/ml. This is based on the assumption that the within-patient standard deviation of endothelin-1 is 3.75 pg/ml (Paired t – test). [88]

Statistical analysis was performed using the SPSS statistical analysis programme (SPSS 17.0). Data are reported as median and interquartile range (IQR) as non-normally distributed.

COMT activity between different time points has been compared using Wilcoxon Signed-Rank test. A $p < 0.05$ was considered to be statistically significant.

Statistical analysis was performed using SPSS (version 23 for Mac, IBM, Italy).

Thus far we screened 15 patients, 6 of which met all inclusion criteria and underwent DISE.

There was only one female and the median age was 48 years. They were almost all

overweight with a waist hip ratio of 1 (0.10). Only 2 patients out of 6 had symptoms of excessive daytime sleepiness. Physical cardio pulmonary examination was unremarkable in all subjects but 2, who had a systolic bruit. One patient had mild leg swelling with no signs of decompensated heart failure or deep vein thrombosis. Two patients had severe visual airway occlusion as represented by a Mallampati score of 4. Main characteristics are shown in Table 10.

Table 10: Demographic and anthropometric characteristics of the enrolled patients. Data in the last column are presented in median and inter quartile range (IQR) for continuous variables. ESS=Epworth Sleepiness Scale. UoM=Unit of measure.

Patient ID	Age	Sex	BMI	Neck size	Waist	Hip	Waist-hip ratio	ESS	Mallampati
UoM	years		Kg/m^2	cm	cm	cm		points	
001	48	F	40.83		124	140	0.89	11	4
002	45	M	29.89	45	103	101	1.02	8	1
003	48	M	22.59	35.2	90	89	1.01	13	2
004	48	M	25.38	37	86	100	0.86	9	4
005	73	M	27.64	43.5	103	102	1.01	7	2
006	48	M	27.78	27.8	107	107	1	1	2
MEDIAN	48		27.71	37	103	101.5	1	8.5	
IQR	0		3.42	8.3	12.75	5.5	0.1	3.2	

Past medical history showed that all patients had hypertension either untreated (1 subject) or on antihypertensive medications (2 patients on angiotensin receptor blockers, 3 on ACE inhibitors, 2 on diuretics and 2 on calcium channel blockers). Two out of six patients had a prior diagnosis of coronary artery disease, 1 had dyslipidemia but no history of stroke, atrial fibrillation or myocardial infarction was reported. Only one was a former smoker whilst 3 were ex smokers. Of note, one subject had been diagnosed with restless leg syndrome but was not on any medication.

Family medical history was positive for hypertension in 5 out of 6 subjects and no clear features of secondary hypertension was noticed on physical examination.

Laboratory blood tests revealed that full blood count was normal among all patients, 2 patients had an elevated fasting glucose but only in one patient this was accompanied by an elevated HbA1c. One patient had an impaired renal function and 4 patients out of 6 had low vitamin D levels. (Table 11)

Table 11: Laboratory data of the enrolled patients. Data in the last column are presented

in median and inter quartile range (IQR) for continuous variables. e-GFR was calculated with the MDRD formula. UoM=Unit of measure.

Patient ID	Hb	Creatinine	e-GFR	HbA1c	glucose	Chol. tot	Na	K	Vit. D
UoM	g/dL	umol/L	ml/min/1.73m^2	mmol/mol	mmol/L	Mg/dL	mmol/L	mmol/L	nmol/L
001	13.3	48	127	50	6.6	156	139	3.2	62
002	16.3	84	91	40	5.1	234	137	4.2	53
003	13.7	82	92	33	5.1	210	141	4.3	88
004	16.2	67	117	34	4.4	127	140	4.3	56
005	15.8	90	76	36	6	164	141	3.8	81
006	13.1	121	59	34	5.2	176	139	4.8	44
MEDIAN	14.7	83	91.5	35	5.15	170	139.5	4.25	59
IQR	2.7	17.5	31	5	0.7	43.5	1.75	0.4	22.5

Hormonal laboratory tests showed normal thyroid function among all patients. Two patients had slightly increased parathyroid hormone levels and this was presumably secondary to low vitamin D levels in both patients and impaired renal function in one patient. (Table 12)

Table 12: Hormonal data of the enrolled patients. Data in the last column are presented in median and inter quartile range (IQR) for continuous variables. UoM=Unit of measure. DRA= direct renin activity, PAC= plasma aldosterone concentration, TSH=thyroid stimulating hormone, T4=thyroxine, PTH= parathormone, ACTH= Adreno Cortico Tropic Hormone.

Patient ID	DRA	PAC	TSH	T4	PTH	Cortisol	ACTH
UoM	mIU/L	pmol/L	mIU/L	pmol/L	ng/L	nmol/L	ng/L
001	350.5	383	1.12	14.65	19.3	271	20
002	811	201	1.37	17.54	33	252	11
003	47.3	116	1.16	15.66	17.8	250	17
004	12.1	67.5	1.08	15.59	15.6	46	6
005	25.2	246	1.16	19.4	18.7	315	27
006	30.2	265	1.8	18.13	33.5	428	23
MEDIAN	38.75	223.5	1.16	16.6	18.9	261.5	18.5
IQR	248.25	123	0.18	2.37	11.5	53.5	9.75

Three patients had increased renin activity but this was not associated with increased aldosterone concentrations. Cortisol levels, measured at 8 in the morning, and ACTH levels were within normal limits in all patients. Blood pressure profiles are shown in table 9. Four patient had high office blood pressure, however 24 hours blood pressure profile was within normal limits for 5 patients out of 6. Of note 4 patients had a non dipping blood pressure pattern.

Table 13: Office and ambulatory blood pressure data of the enrolled patients. Data in the last column are presented in median and inter quartile range (IQR) for continuous variables. All variables were measured in millimetres of mercury (mmHg)

	OFFICE		AMBULATORY					
Patient ID	SBP	DBP	SBP (24h)	DBP (24h)	SBP (day)	DBP (day)	SBP (night)	DBP (night)
001	151	91	120	69	124	73	103	52
002	129	91	125	83	128	87	117	72
003	122	81	118	76	120	77	114	72
004	143	91	119	75	121	78	112	67
005	159	87	141	79	140	79	142	79
006	117	72	121	77	125	80	108	67
MEDIAN	136.3	89	120.5	76.5	124.5	78.5	113	69.5
IQR	25.5	8.5	4.75	3.25	5.5	2.5	7.25	5.75

Polysomnographic data confirmed obstructive sleep apnea in all subjects: only one had severe OSAS, the majority had mild-severe OSAS. (Table 14).

Table 14: Polysomnographic data of the studied patients. Data in the last column are presented in either median and inter quartile range (IQR) for continuous variables. UoM=Unit of measure.

Patient ID	TRT	AHI	Mean SpO2	N obs. apneas	N hypopneas
UoM	min	Events/hour	%		
001	316.7	21	94.1	30	82
002	444.1	22	94.7	47	119
003	414.1	9.3	94.9	3	61
004	447.6	30	92	112	123
005	404.8	29.1	93.4	127	69
006	431.2	13.5	96.8	67	30
MEDIAN	422.6	21.5	94.4	57	75.5
IQR	33.7	11.9	1.2	66.5	46.7

Urinary data did not show relevant abnormalities: sodium 24 h secretion was increased in 2 subjects and calcium secretion was within normal limits. Catecholamines secretion was also normal even when adrenaline and noradrenaline urinary secretion was considered. (Table 15)

Twenty-four-hour urinary albumin excretion (UAE) was normal in all subjects not suggesting renal damage.

Table 15: Urinary data of the enrolled patients. Data in the last column are presented in either median and inter quartile range (IQR) for continuous variables. UoM=Unit of measure.

Patient ID	Methanephrine	Methanephrine 24h	Normetanephrine	Normetanephrine 24h
UoM	nmol/L	nmol/24h	nmol/L	nmol/24h
001	0.1	0.18	0.14	0.25
002	0.08	0.09	0.27	0.39
003	0.06	0.13	0.1	0.22
004	0.11	0.24	0.08	0.18
005	0.03	0.08	0.13	0.33
006	0.09	0.22	0.11	0.26
MEDIAN	0.08	0.16	0.12	0.25
IQR	0.03	0.11	0.03	0.08

DISE was completed in all subjects without any complications.

Propofol was used for sedation either as boluses (4 subjects) or given via a continuous infusion after a loading dose (2 subjects). The median propofol dose per procedure was 150 (75) mg and the number of boluses ranged between 2 and 3. In three patients sevoflurane was employed as additional anaesthetic and in 2 patients fentanil was used for analgesia. Blood pressure at the start of the procedure was 155/77.5 (17.5/8.5) mmHg and at the end was 120/67.5 (22.5/12.5) mmHg.

Four patients had a propofol-induced central apnoea at the beginning of the procedure. One patient did not have any obstructive apnea during the procedure but only an hypopnea with a lowest oxygen saturation of 87%.

After propofol administration, COMT activity reached a peak at 134.6 [87.7 – 141.2] pmol/min/mg and a second peak was observed 2 minutes after the obstructive apneic event. (Figure 12)

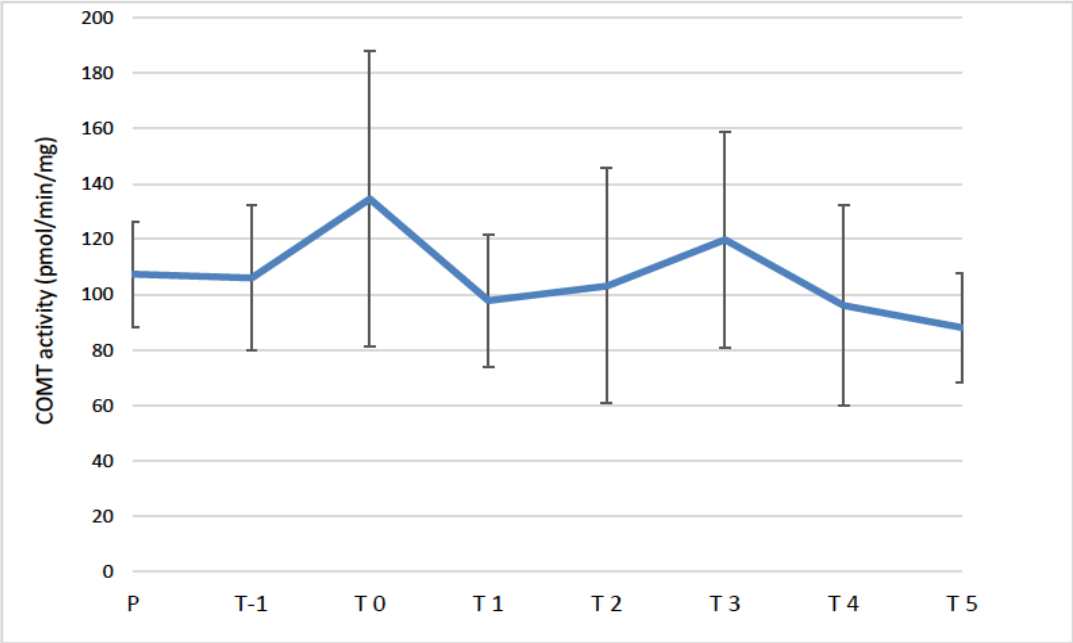

Figure 12: COMT activity during sleep endoscopy in the different stages for all subjects. Data are expressed as median and IQR.

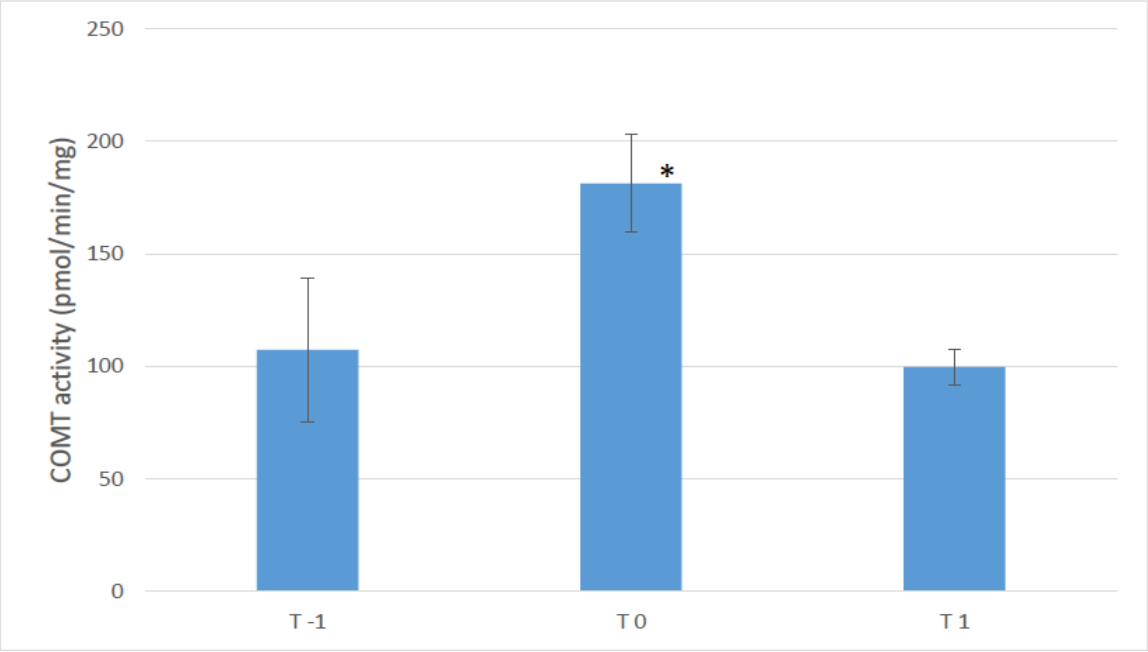

Figure 13: COMT activity during sleep endoscopy in subjects with the propofol induced central sleep apnea. The star stands for a significant reduction ($p<0.05$) between T 0 and T 1. Data are expressed as median and IQR.

No statistical significance was seen when we compared COMT activity for the different

time points, however, when we considered only the four subjects who had a central apnea after propofol administration the peak was more marked. Indeed COMT activity at T0 was 181.2 (12.8) pmol/min/mg and at T1 99.4 (11.1) pmol/min/mg ($p<0.05$) (Figure 13)

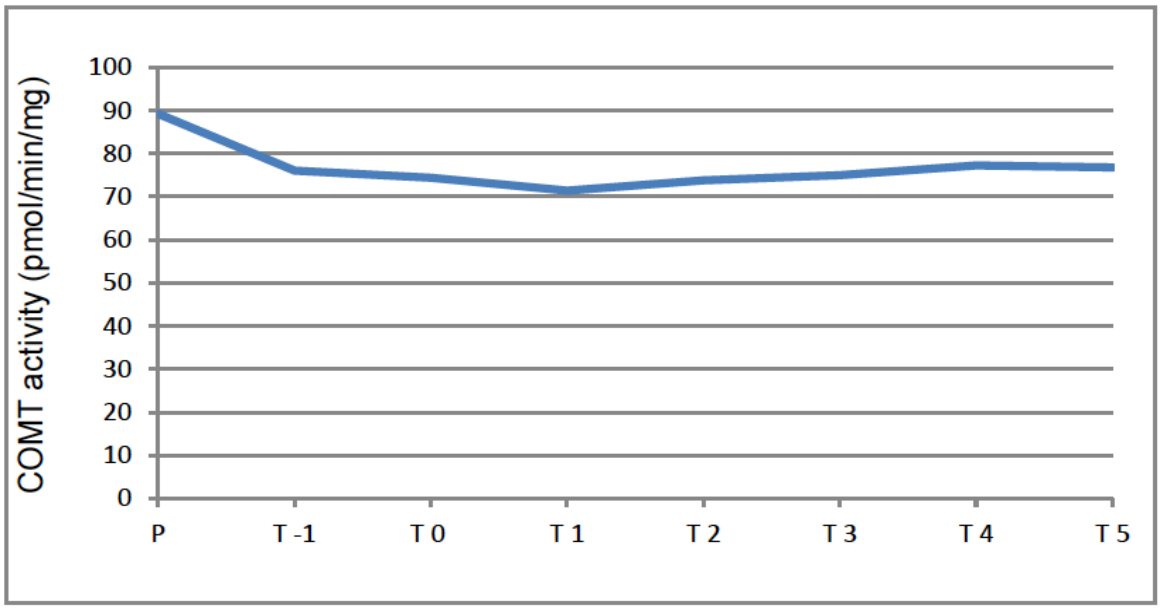

Figure 14: average COMT activity during sleep endoscopy in a subject with no relevant desaturations during the procedure.

Furthermore, in one patient who did not have relevant apnoeas during the procedure no changes of COMT activity were seen throughout (Figure 14).
COMT activity seemed to be influenced by age as the oldest patient (73 years old) had the lowest activity when measured at baseline before the procedure (55.6 pmol/min/mg).
In one patient without the propofol-induced central sleep apnea and with an obstructive event reaching a minimum oxygen saturation of 88% a peak of COMT activity was seen after the first apnea.

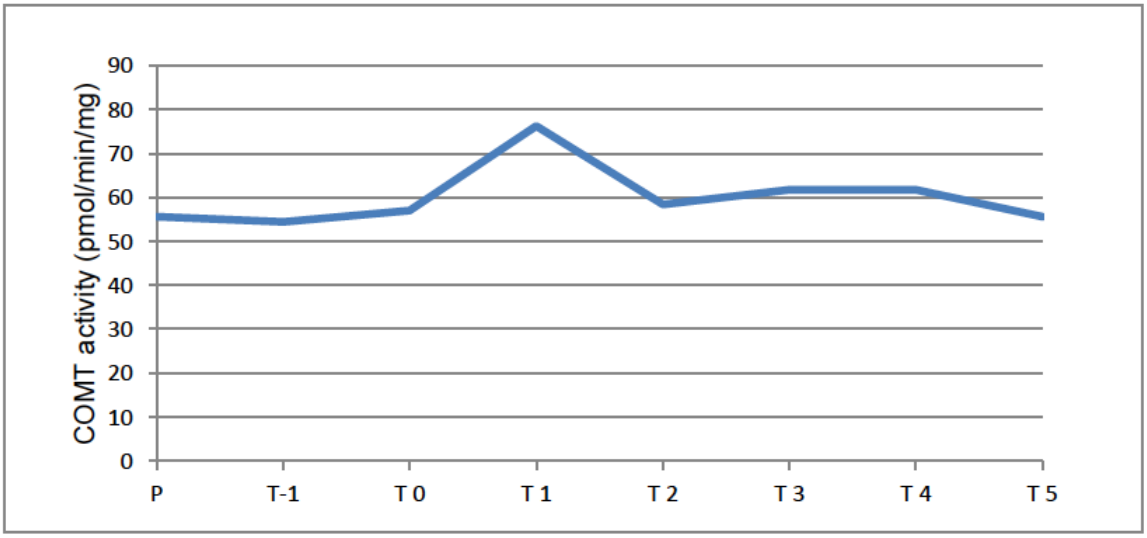

Figure 15: COMT activity in a subject without the central event induced by propofol but with an obstructive even reaching a minimum oxygen saturation of 88%.

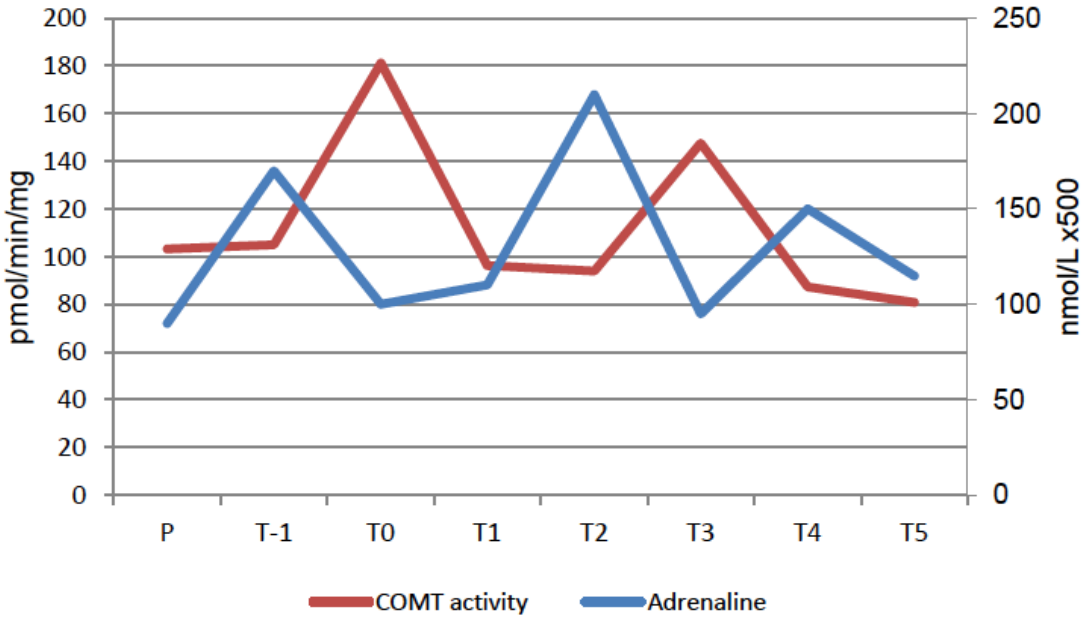

Figure 16: average COMT activity and adrenaline concentration during sleep endoscopy in a subject with an initial central sleep apnea propofol-induced.

COMT activity returned to baseline values after 30 seconds from the obstructive event. (Figure 15) In one patient we analysed adrenaline concentration at each time-point. Figure 16 shows that a higher COMT activity corresponds to lower adrenaline levels.

In order to understand whether propofol itself could exert an inhibiting or stimulating effect on COMT activity we incubated red blood cells of a healthy subject (age 24 years, BMI 25.1 kg/m2) with propofol. COMT activity was analysed at baseline, after 5, 10 minutes

and one hour. The experiment was repeated twice to correct for analytic errors.

The results are showed in figure 17. A slight decrease of COMT activity was seen throughout. However, when considering the time frame of 5 minutes, the change was less relevant.

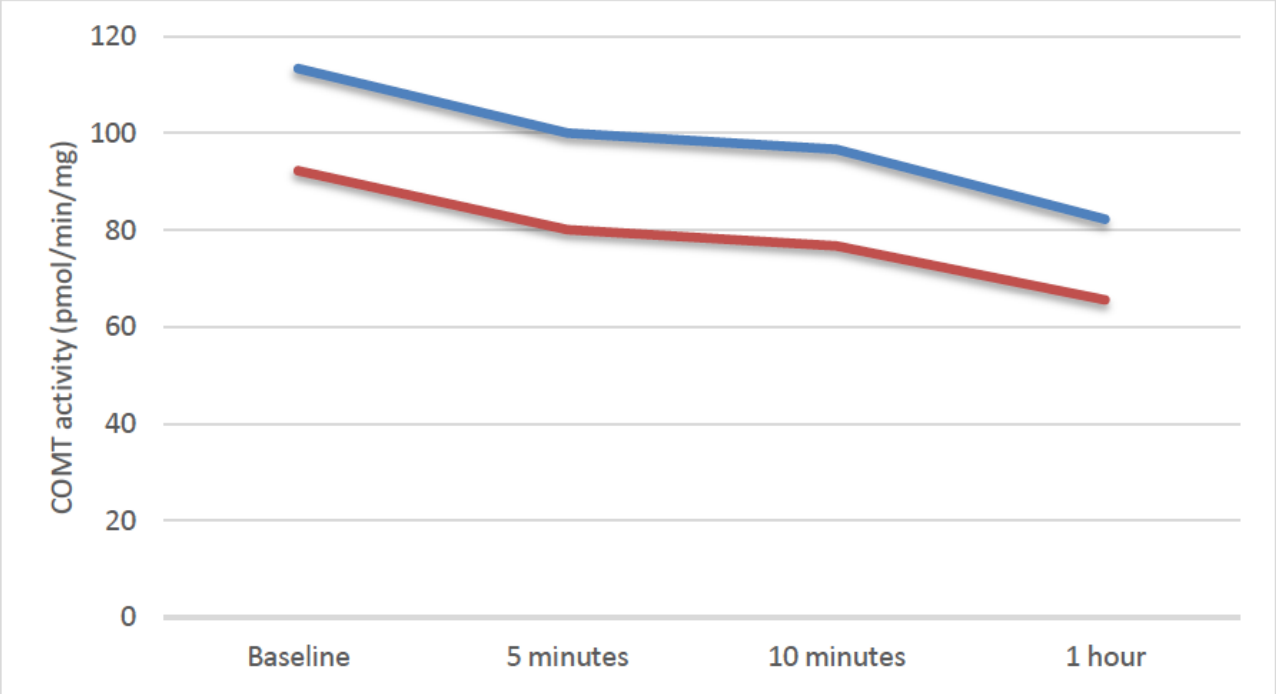

Figure 17: COMT activity in red blood cells of a healthy subject incubated with propofol. Red and blue lines are representing the same experiment repeated twice.

We further wanted to understand the type of COMT enzyme in red blood cells as we know that the kinetics of COMT is dependent on the isoform and the substrate: MB-COMT has a greater affinity but a lower O-methylation capacity of catecholamines when compared with S-COMT.[73]

These differences in activity between the two isoforms show that in cases of low substrate concentration MB-COMT activity will be greater whilst in cases of high substrate concentration, MB-COMT contribution to O-methylation will be less significant compared to S-COMT.

We therefore performed Western blot on red blood cell lysis solution of the first three patients enrolled. (Figure 18)

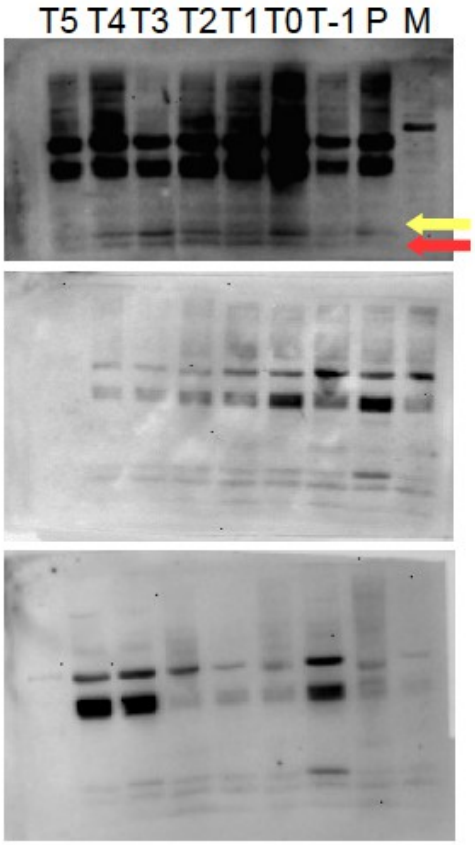

Figure 18: Western blot for patient 001, 002 and 003. Arrow represents the two bands corresponding to the two COMT isoforms (yellow arrow MB-COMT, red arrow S-COMT). M=marker.

Other than the 2 bands corresponding to the two isoforms (26 e 30kDa, MB and S-COMT respectively), there were two more bands visible with a molecular weight of 50-60 kDa.
We initially hypothesised, giving that the additional bands had an increased molecular weight by 2-fold, that this could be a dimer because there are seven cysteine residues in human S-COMT which can then create disulfide bridges.
This hypothesis needs to confirmed by further experiments, however studying COMT activity in red blood cells is rather complex because of haemoglobin interferences but most of all because COMT quantity is lower when compared with other cells (eg. Hepatocytes).
Moreover, we did not see 50-60 kDa bands when performing western blot in haepatocytes and macrophages (data not shown). This is intriguing as dimers could be a sort of reservoir of the enzyme which can be activated in conditions of high demand.
COMT dimers have been already observed both in humans and rats,[83] however their role is

still controversial.

An analysis of the dimers by gel-permeation chromatography revealed an equilibrium with monomers that could be accelerated by increased temperature. The activation energy for the monomer–dimer transition must therefore be rather small, which also explains the large variations in dimer content that was observed for different protein preparations.[89]

The preliminary data of our study on the first 6 subjects showed that the cathecolaminergic system is stimulated during DISE in patients with OSAS.

COMT activity is increased after propofol administration more markedly if a central apnea occurs, conversely a less relevant increase in COMT activity was seen after the first obstructive event. COMT activity does not seem to be influenced by the anaesthetic agent used and an increase in COMT activity is associated with lower plasma adrenaline levels.

Discussion

This series of studies aimed to better understand the changes of the autonomous nervous system and blood pressure in patients with OSAS. This is of crucial importance giving that not all patients with OSAS develop hypertension and cardiovascular complications and, similarly, not all patients benefit from CPAP also in terms of blood pressure and sympathetic hyperactivity correction.

The World Health Organization states that cardiovascular diseases are the first cause of death globally and that most of them can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies.[90]

Obesity is one of the most serious public health challenges of the 21st century as the worldwide prevalence has nearly doubled between 1980 and 2008.[91]

As obesity is associated with OSAS the same trend of increasing prevalence has been demonstrated for the latter.

In fact, the prevalence of OSA was 4% of middle aged men and 2% of middle aged women in the United States in the early 1990's,[3] but with the obesity epidemic the prevalence now reached 10% for 30-49 year-old men and at 3% for 30-49 year-old women in the US.[6]

Thus, OSAS represents other than a sleep disorder a huge public health issue and understanding the pathophysiology behind its association with hypertension and cardiovascular events is of crucial importance.

In this series of studies we aimed to understand firstly the haemodynamic changes that occur in patients with OSAS and, most of all, if treatment with CPAP can be of help and to what extent.

Whilst the association of OSAS and hypertension has been extensively proved, much more complex is demonstrating that CPAP treatment exerts a beneficial effect not only on symptoms, but also on high blood pressure.

The latter has a prognostic value on cardiovascular events and this should be considered when assessing patients with OSA and hypertension.[92]

There is evidence showing that assessing BPV is important when determining the overall cardiovascular risk of a patient with hypertension. Rothwell et al have demonstrated that

systolic BPV and maximal systolic BP are strong predictors of stroke, independent of the mean systolic BP.[53] An increased residual variability of the systolic BP in patients with treated hypertension is associated with a high risk of vascular events; patients with OSA are subjected to repeated surges of sympathetic nervous system activity[93] which explains their higher BPV.[52]

The observed decrease in BPV in patients with OSA treated with APAP is accompanied by a decrease in the pulse rate suggesting a beneficial effect of CPAP on the overall sympathetic activation. Heart rate, represented by its surrogate pulse rate, can also predict long-term BP changes in patients with OSA treated with CPAP and it could be evaluated in prospective studies of patients with sleep-disordered breathing who are treated with CPAP in clinical sleep services.

Supporting the findings that APAP might reduce BP parameters, systolic BP changes was associated with an increased APAP pressure. Air leaks are associated with low CPAP compliance, and indeed we found increased APAP leak reduced the effect of APAP treatment on BPV. This suggests that mask leak could influence compliance and have an indirect haemodynamic effect with a detrimental impact on BPV.

When we compared subgroups with optimal and suboptimal compliance the decreases in BPV parameters did not reach statistical significance, but this might be due to the small sample size. However, optimal CPAP compliance was associated with a greater reduction in pulse rate, suggesting a greater impact on sympathetic nervous system activation.

One of the main issues with CPAP is the poor adherence. In fact, depending on the definition of compliance and the system used approximately a quarter of patients is non-compliant after 6 weeks and more than half after 1-year.[94]

Patient discomfort plays an important role in patients' adherence and, particularly in obese subjects, higher CPAP levels can increase the work of breathing, induce hyperventilation and impair lung mechanics.

In fact, obese subjects breathe at low lung volumes.[61] Employing CPAP to inflate the chest, to stent the airway, abolishes intrinsic positive end-expiratory pressure (PEEPi) in supine posture and shift the tidal volume to a more-favourable part on the pressure-volume (PV) curve helping to improve compliance of the respiratory system, reducing the work of breathing and NRD.[95]

However, when pressures are increased so that the chest is inflated beyond a normal level of functional residual capacity, the work of breathing increases again and this is reflected in an increased EMG_{para}. This is associated with a raised sympathetic activation, which is paralleled by an increased BP and BPV.

As a consequence, patients who have their effective CPAP level at pressures higher than what is well tolerated when awake will experience discomfort and breathlessness. It is not unlikely that patients who try to fall asleep, or wake up at night, feeling breathless because of the high CPAP level will stop using the device in the long term. To address this problem, a lower level of CPAP, potentially sub-therapeutic, might prove more beneficial than an optimal CPAP setting with lower compliance. Furthermore, it would be of interest to understand whether CPAP adherence and blood pressure control could be improved by adjusting pressures according to the level of breathlessness and/or NRD. The fact that we found different physiological responses with CPAP titration also raises the question on what defines different phenotypes; the measurement of oesophageal and gastric pressures could aid to understand what determines different responses.

As described previously, modulations of the autonomous nervous system are common in OSAS.[96] Dimsdale et al. measured catecholamines in plasma and urine and found that 24-hours urinary norepinephrine levels were significantly higher in patients with OSAS than in patients without OSAS (58.2 ng vs. 40.2 ng, $p < 0.002$), both during the day and at night. [97]

In fact, in OSAS patients, the repetitive apnoeic events lead to intermittent hypoxia (IH) and CO_2 retention, both of which can augment sympathetic nervous activity (SNA) via stimulation of central and peripheral chemoreceptors.[98]

Triggered by the reduction in blood pressure and stroke volume during obstructive apnoeic events carotid sinus baroreceptors also stimulate SNA.

Indeed, the baroreflex provides a negative feedback loop in which elevated blood pressure inhibits sympathetic outflow with ensuing decreased heart rate and blood pressure. On the other hand, decreased blood pressure blunts baroreflex mechanisms, causing heart rate increases, and blood pressure to rise.[99]

A large amount of studies confirmed an increase in catecholamine levels in patients with OSAS and showed also that CPAP treatment might effectively reduce catecholamine levels.[65] This make it plausible to exploit catecholamine levels in the blood or urine as a

potentially viable biomarker of OSAS severity that can be used to monitor patients' responses to treatment.[66]

Our hypothesis generating study speculates that COMT plays an important role in metabolising catecholamines and in particular that its activity could be modulated by intermittent hypoxia.

We showed that central apnea and the consequent oxygen desaturation is associated with an increased activity of the enzyme which may well be related to a physiologic response to preserve the blood oxygenation homeostasis.

The carotid body is a small chemosensory organ located at the bifurcation of the internal and external carotid arteries that senses arterial oxygen partial pressure. Intermittent hypoxia triggers signalling from the carotid body that activates the sympathetic nervous system, leading to increased catecholamine secretion, which increases arterial tone, with ensuing hypertension. [100]

This process is presumably mediated by the Hypoxia Inducible Factor (HIF) as exposure of *Hif1a+/–* mice and their wild type littermates to intermittent hypoxia for 10 days resulted in marked increases in systolic and diastolic blood pressures and a significant elevation in plasma norepinephrine concentration in the WT mice, whereas their *Hif1a+/–* littermates are unaffected.[101]

If from a purely pathophysiological perspective, our study sheds some light on the biochemical mechanisms that cause an increase in catecholamine levels in patients with OSAS, from the clinical perspective it could help clinicians understanding which patients with OSAS are more prone to develop hypertension, are at higher risk of developing cardiovascular events, and therefore might benefit more the most from treatment.

Current guidelines suggest that a treatment is highly recommended for patients with moderate or severe sleep-disordered breathing and symptoms of excessive daytime sleepiness.[37]

However, for patients with mild or moderate disease, in particular with no relevant daytime symptoms, there is no clear recommendation. This is of interest as the proportion of patients with mild-moderate disease is relatively high and is at risk of cardiovascular events. Although trials on patients with mild or moderate disease are lacking, Marin and colleagues published prospective observational data on 403 patients with untreated mild to moderate OSAS (AHI between 5 and 30) compared to 264 controls, and demonstrated a

tendency toward increased fatal and non-fatal cardiovascular events in the patient group at 9 years of follow-up.[102]

Thus, measuring COMT activity might identify OSAS patients with an impaired catecholamine metabolism and presumably at risk of developing hypertension.

This approach has potential advantages compared to catecholamines use as a biomarker in that their role for diagnosis of OSAS is not firmly established, most likely because of the large amount of confounding factors and associated morbidities that frequently accompany OSAS and can independently lead to changes in catecholamine levels. Indeed, confounding factors such as age, hypertension, obesity, and the use of anti-hypertensive medications are not easy to control for, thereby increasing the difficulty to interpret at the individual patient level the real impact of OSAS on catecholamine levels.

One of the findings of this study is that a drug induced central apnea caused marked changes in catecholamines and COMT activity. This is of interest because uncertainty still exists as to whether we need to treat patients with central sleep apnea (CSA) to prevent cardiovascular morbidity and mortality.

Central sleep apneas and hypopneas arise from complete or partial reductions in central respiratory drive during sleep that lead, respectively, to complete or partial cessation of airflow for at least 10 seconds. Central apneas occur when $PaCO_2$ falls below the threshold required to stimulate breathing. A CSA disorder is present when central apneas and hypopneas occur cyclically. CSA is often seen in the setting of heart failure (HF) due to a tendency to hyperventilate so that PaCO2 repeatedly drops below the apnea threshold, usually after acute increases in ventilation following arousals from sleep.[77]

Our findings are consistent with the work by Javaheri where among HF patients, CSA was associated with greater sympathetic activation while awake than in those without sleep apnea.[78] However, unlike OSAS, no inspiratory efforts or negative intrathoracic pressure is generated during central apneas so that they do not cause as great an increase in left ventricular afterload.

The purpose of this and future research is to understand what are the determinants that modulate COMT activity and to what extent. As previously discussed, COMT activity seems to be greater in men than in women.[60] Our findings confirm what is previously known about the effect of age: COMT activity increases up to 10 times from birth up to 50 years and then starts declining after 60 years of age.[78]

As we noticed a significant increase of COMT activity after propofol administration we had

to dismiss the possibility that the drug itself could alter the kinetic of the enzyme.

Propofol is the most important intravenous general anaesthetic in current clinical use. It acts by potentiating GABA-A (γ-aminobutyric acid type A) receptors. The binding site has been only recently discovered and is located within the β subunit at the interface between the transmembrane domains and the extracellular domain.[105]

Of all the induction agents, propofol has the most profound cardiovascular depressant effects. It causes the largest reduction in mean arterial pressure through a balanced venous and arterial vasodilation, resulting in a reduction in both cardiac preload and afterload. It also blocks the normal baroreceptor reflex-mediated tachycardia which would typically accompany these changes, resulting in only small increases in heart rate.

Propofol also causes profound respiratory depression, consistently producing apnea at induction doses. At lower doses, such as those used for sedation, minute ventilation is reduced, with decreases in tidal volume being larger than decreases in respiratory rate. The ventilatory response to hypoxia and hypercapnia is reduced as well. [106]

In our study we did not show a relevant inhibitory effect of propofol on COMT although, when incubating red blood cells with propofol a low inhibitory effect was seen after one hour.

In our study we could demonstrate an effect of hypoxia on COMT activity, which, as far as we are aware of, has never been reported in human beings.

In fact, Garvey and colleagues studied rats maintained under normal (normoxic) and high-altitude, 3800 m, 13% PO2 (hypoxic) conditions. They showed that COMT activity at high altitude was increased on the last days of gestation but depressed after birth, compared to control levels. [107]

However, intermittent hypoxia involves brief episodes of hypoxia followed by longer periods of reoxygenation, whilst continuous hypoxia does not involve such fluctuations. Although HIF-1 activity seems to be induced in both conditions, the mechanisms regulating its activity under conditions of chronic hypoxia or intermittent hypoxia may differ.[108]

Thus, further studies are needed to understand the role of HIF in intermittent hypoxia with regards to the development of hypertension in patients with OSAS.

Conclusion

In this series of studies we described pathophysiological changes of blood pressure and autonomous nervous system in patients with OSAS.

We demonstrated that CPAP treatment can not only treat OSAS symptoms and reduce blood pressure, but can also modulate the autonomous nervous activity by reducing the sympathetic discharge as seen by a reduction of blood pressure variability.

This can be achieved only if compliance is optimal. Also, other CPAP related factors such as leaks and CPAP pressure might have a huge impact on the aforementioned parameters. In fact, a higher pressure can overload the respiratory system leading to patient discomfort, breathlessness, increased sympathetic discharge, and increased blood pressure and blood pressure variability.

As the activity of the autonomous nervous system is mediated by the catecholaminergic system we also showed it is stimulated during apnoeic events as seen during drug induced sleep apnea. This might be due to an altered activity of COMT, one of the most important enzymes involved in catecholamine metabolism, therefore suggesting a novel pathophysiological pathway responsible of hypertension in patients with OSAS.

References

1. McNicholas WT, Bonsigore MR, Bonsignore MR, Management Committee of EU COST ACTION B26. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. *Eur Respir J* 2007;**29**:156–78

2. Stoohs R, Guilleminault C. Obstructive sleep apnea syndrome or abnormal upper airway resistance during sleep? *J Clin Neurophysiol Off Publ Am Electroencephalogr Soc* 1990;**7**:83–92

3. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993;**328**:1230–5

4. Punjabi NM. The epidemiology of adult obstructive sleep apnea. *Proc Am Thorac Soc* 2008;**5**:136–43

5. Rajala R, Partinen M, Sane T, Pelkonen R, Huikuri K, Seppäläinen AM. Obstructive sleep apnoea syndrome in morbidly obese patients. *J Intern Med* 1991;**230**:125–9

6. 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**:1006–14

7. Steier J, Martin A, Harris J, Jarrold I, Pugh D, Williams A. Predicted relative prevalence estimates for obstructive sleep apnoea and the associated healthcare provision across the UK. *Thorax* 2014;**69**:390–2

8. Patel SR. Shared genetic risk factors for obstructive sleep apnea and obesity. *J Appl Physiol Bethesda Md 1985* 2005;**99**:1600–6

9. Schwartz AR, Patil SP, Squier S, Schneider H, Kirkness JP, Smith PL. Obesity and upper airway control during sleep. *J Appl Physiol Bethesda Md 1985* 2010;**108**:430–5

10. Steier J, Lunt A, Hart N, Polkey MI, Moxham J. Observational study of the effect of obesity on lung volumes. *Thorax* 2014;**69**:752–9

11. Atwood CW. Sleep-related hypoventilation: the evolving role of leptin. *Chest* 2005;**128**:1079–81

12. Münzberg H, Morrison CD. Structure, production and signaling of leptin. *Metabolism* 2015;**64**:13–23

13. Newman AB, Foster G, Givelber R, Nieto FJ, Redline S, Young T. Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study. *Arch Intern Med* 2005;**165**:2408–13

14. Young T, Evans L, Finn L, Palta M. Estimation of the clinically diagnosed proportion of sleep apnea syndrome in middle-aged men and women. *Sleep* 1997;**20**:705–6

15. Shahar E, Redline S, Young T, et al. Hormone replacement therapy and sleep-disordered breathing. *Am J Respir Crit Care Med* 2003;**167**:1186–92

16. Malhotra A, Huang Y, Fogel R, et al. Aging influences on pharyngeal anatomy and physiology: the predisposition to pharyngeal collapse. *Am J Med* 2006;**119**:72.e9–14

17. Kent BD, Ryan S, McNicholas WT. The genetics of obstructive sleep apnoea. *Curr Opin Pulm Med* 2010;**16**:536–42

18. Redline S, Tishler PV. The genetics of sleep apnea. *Sleep Med Rev* 2000;**4**:583–602

19. Mathur R, Douglas NJ. Family studies in patients with the sleep apnea-hypopnea syndrome. *Ann Intern Med* 1995;**122**:174–8

20. Varvarigou V, Dahabreh IJ, Malhotra A, Kales SN. A review of genetic association studies of obstructive sleep apnea: field synopsis and meta-analysis. *Sleep* 2011;**34**:1461–8

21. Kirkness JP, Madronio M, Stavrinou R, Wheatley JR, Amis TC. Relationship between surface tension of upper airway lining liquid and upper airway collapsibility during sleep in obstructive sleep apnea hypopnea syndrome. *J Appl Physiol Bethesda Md 1985* 2003;**95**:1761–6

22. Isono S, Tanaka A, Tagaito Y, Ishikawa T, Nishino T. Influences of head positions and bite opening on collapsibility of the passive pharynx. *J Appl Physiol Bethesda Md 1985* 2004;**97**:339–46

23. Stanchina ML, Malhotra A, Fogel RB, et al. The influence of lung volume on pharyngeal mechanics, collapsibility, and genioglossus muscle activation during sleep. *Sleep* 2003;**26**:851–6

24. Knudson RJ, Knudson DE. Effect of muscle constriction on flow-limiting collapse of isolated canine trachea. *J Appl Physiol* 1975;**38**:125–31

25. Pengo MF, Steier J. Emerging technology: electrical stimulation in obstructive sleep apnoea. *J Thorac Dis* 2015;**7**:1286–97

26. Ferrier K, Campbell A, Yee B, et al. Sleep-disordered breathing occurs frequently in stable outpatients with congestive heart failure. *Chest* 2005;**128**:2116–22

27. Stauffer JL, Buick MK, Bixler EO, et al. Morphology of the uvula in obstructive sleep apnea. *Am Rev Respir Dis* 1989;**140**:724–8

28. Kales A, Cadieux RJ, Bixler EO, et al. Severe obstructive sleep apnea--I: Onset, clinical course, and characteristics. *J Chronic Dis* 1985;**38**:419–25

29. Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndromes. *Annu Rev Med* 1976;**27**:465–84

30. Hoffstein V, Szalai JP. Predictive value of clinical features in diagnosing obstructive sleep apnea. *Sleep* 1993;**16**:118–22

31. Epstein LJ, Kristo D, Strollo PJ, et al. Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. *J Clin Sleep Med JCSM Off Publ Am Acad Sleep Med* 2009;**5**:263–76

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

33. Friedman M, Tanyeri H, La Rosa M, et al. Clinical predictors of obstructive sleep apnea. *The Laryngoscope* 1999;**109**:1901–7

34. Kushida CA, Morgenthaler TI, Littner MR, et al. Practice parameters for the treatment of snoring and Obstructive Sleep Apnea with oral appliances: an update for 2005. *Sleep* 2006;**29**:240–3

35. American Academy of Sleep Medicine. Diagnostic and coding manual: American Academy of Sleep Medicine. International classification of sleep disorders. 2nd Edition. Westchester, IL; 2005.

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

37. Gay P, Weaver T, Loube D, et al. Evaluation of positive airway pressure treatment for sleep related breathing disorders in adults. *Sleep* 2006;**29**:381–401

38. Ferguson KA, Cartwright R, Rogers R, Schmidt-Nowara W. Oral appliances for snoring and obstructive sleep apnea: a review. *Sleep* 2006;**29**:244–62

39. Weaver TE, Grunstein RR. Adherence to continuous positive airway pressure therapy: the challenge to effective treatment. *Proc Am Thorac Soc* 2008;**5**:173–8

40. Strollo PJ, Soose RJ, Maurer JT, et al. Upper-airway stimulation for obstructive sleep apnea. *N Engl J Med* 2014;**370**:139–49

41. Steier J, Seymour J, Rafferty GF, et al. Continuous transcutaneous submental electrical stimulation in obstructive sleep apnea: a feasibility study. *Chest* 2011;**140**:998–1007

42. 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**:1378–84

43. O'Connor GT, Caffo B, Newman AB, et al. Prospective study of sleep-disordered breathing and hypertension: the Sleep Heart Health Study. *Am J Respir Crit Care Med* 2009;**179**:1159–64

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

45. Bixler EO, Vgontzas AN, Lin HM, et al. Association of hypertension and sleep-disordered breathing. *Arch Intern Med* 2000;**160**:2289–95

46. Durán J, Esnaola S, Rubio R, Iztueta A. Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. *Am J Respir Crit Care Med* 2001;**163**:685–9

47. Deanfield J, Donald A, Ferri C, et al. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;**23**:7–17

48. Duchna H-W, Stoohs R, Guilleminault C, Christine Anspach M, Schultze-Werninghaus G, Orth M. Vascular endothelial dysfunction in patients with mild obstructive sleep apnea syndrome. *Wien Med Wochenschr 1946* 2006;**156**:596–604

49. Carlson JT, Rångemark C, Hedner JA. Attenuated endothelium-dependent vascular relaxation in patients with sleep apnoea. *J Hypertens* 1996;**14**:577–84

50. Kanagy NL, Walker BR, Nelin LD. Role of endothelin in intermittent hypoxia-induced hypertension. *Hypertension* 2001;**37**:511–5

51. Bratton DJ, Gaisl T, Wons AM, Kohler M. CPAP vs Mandibular Advancement Devices and Blood Pressure in Patients With Obstructive Sleep Apnea: A Systematic Review and Meta-analysis. *JAMA* 2015;**314**:2280–93

52. Leroy M, Van Surell C, Pilliere R, et al. Short-term variability of blood pressure during sleep in snorers with or without apnea. *Hypertension* 1996;**28**:937–43

53. Rothwell PM, Howard SC, Dolan E, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet Lond Engl* 2010;**375**:895–905

54. Parati G, Ochoa JE, Lombardi C, Bilo G. Assessment and management of blood-pressure variability. *Nat Rev Cardiol* 2013;**10**:143–55

55. Eguchi K, Hoshide S, Schwartz JE, Shimada K, Kario K. Visit-to-visit and ambulatory blood pressure variability as predictors of incident cardiovascular events in patients with hypertension. *Am J Hypertens* 2012;**25**:962–8

56. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013;**31**:1281–357

57. Collard P, Pieters T, Aubert G, Delguste P, Rodenstein DO. Compliance with nasal CPAP in obstructive sleep apnea patients. *Sleep Med Rev* 1997;**1**:33–44

58. Chai-Coetzer CL, Luo Y-M, Antic NA, et al. Predictors of long-term adherence to continuous positive airway pressure therapy in patients with obstructive sleep apnea and cardiovascular disease in the SAVE study. *Sleep* 2013;**36**:1929–37

59. Reilly CC, Ward K, Jolley CJ, et al. Neural respiratory drive, pulmonary mechanics and breathlessness in patients with cystic fibrosis. *Thorax* 2011;**66**:240–6

60. Jolley CJ, Luo YM, Steier J, Rafferty GF, Polkey MI, Moxham J. Neural respiratory drive and breathlessness in COPD. *Eur Respir J* 2015;**45**:355–64

61. Steier J, Lunt A, Hart N, Polkey MI, Moxham J. Observational study of the effect of obesity on lung volumes. *Thorax* 2014;**69**:752–9

62. Tilkian AG, Guilleminault C, Schroeder JS, Lehrman KL, Simmons FB, Dement WC. Hemodynamics in sleep-induced apnea. Studies during wakefulness and sleep. *Ann Intern Med* 1976;**85**:714–9

63. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med* 1993;**328**:303–7

64. Elmasry A, Lindberg E, Hedner J, Janson C, Boman G. Obstructive sleep apnoea and urine catecholamines in hypertensive males: a population-based study. *Eur Respir J* 2002;**19**:511–7

65. Kohler M, Pepperell JCT, Casadei B, et al. CPAP and measures of cardiovascular risk in males with OSAS. *Eur Respir J* 2008;**32**:1488–96

66. Comondore VR, Cheema R, Fox J, et al. The impact of CPAP on cardiovascular biomarkers in minimally symptomatic patients with obstructive sleep apnea: a pilot feasibility randomized crossover trial. *Lung* 2009;**187**:17–22

67. Axelrod J, Tomchick R. Enzymatic O-methylation of epinephrine and other catechols. *J Biol Chem* 1958;**233**:702–5

68. Tenhunen J, Salminen M, Lundström K, Kiviluoto T, Savolainen R, Ulmanen I. Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. *Eur J Biochem FEBS* 1994;**223**:1049–59

69. Weinshilboum RM, Raymond FA. Inheritance of low erythrocyte catechol-o-methyltransferase activity in man. *Am J Hum Genet* 1977;**29**:125–35

70. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996;**6**:243–50

71. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JAG. COMT gene and risk for Parkinson's disease: a systematic review and meta-analysis. *Pharmacogenet Genomics* 2014;**24**:331–9

72. Nackley AG, Shabalina SA, Tchivileva IE, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 2006;**314**:1930–3

73. Lotta T, Vidgren J, Tilgmann C, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry (Mosc)* 1995;**34**:4202–10

74. Axelrod J, Albers W, Clemente CD. Distribution of Catechol-O-Methyl Transferase in the Nervous System and Other Tissues. *J Neurochem* 1959;**5**:68–72

75. Tsunoda M, Takezawa K, Masuda M, Imai K. Rat liver and kidney catechol-O-

methyltransferase activity measured by high-performance liquid chromatography with fluorescence detection. *Biomed Chromatogr* 2002;**16**:536–41

76. Karhunen T, Tilgmann C, Ulmanen I, Panul P. Catechol-O-methyltransferase (COMT) in rat brain: immunoelectron microscopic study with an antiserum against rat recombinant COMT protein. *Neurosci Lett* 1995;**187**:57–60

77. Boudíková B, Szumlanski C, Maidak B, Weinshilboum R. Human liver catechol-O-methyltransferase pharmacogenetics. *Clin Pharmacol Ther* 1990;**48**:381–9

78. Guldberg HC, Marsden CA. Catechol-O-methyl transferase: pharmacological aspects and physiological role. *Pharmacol Rev* 1975;**27**:135–206

79. Goldstein DS. Arterial baroreflex sensitivity, plasma catecholamines, and pressor responsiveness in essential hypertension. *Circulation* 1983;**68**:234–40

80. Imai K, Tsunoda M, Takezawa K, et al. Attenuation of methylation of catecholamines and treatment with a coenzyme of catechol-O-methyltransferase in spontaneously hypertensive rats (SHR). *Proc Jpn Acad Ser B* 1999;**75**:224–7

81. Tsunoda M, Tenhunen J, Tilgmann C, Arai H, Imai K. Reduced membrane-bound catechol-O-methyltransferase in the liver of spontaneously hypertensive rats. *Hypertens Res Off J Jpn Soc Hypertens* 2003;**26**:923–7

82. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol* 2006;**48**:2293–300

83. Ng SSS, Chan T-O, To K-W, et al. Validation of Embletta portable diagnostic system for identifying patients with suspected obstructive sleep apnoea syndrome (OSAS). *Respirol Carlton Vic* 2010;**15**:336–42

84. Berry RB, Budhiraja R, Gottlieb DJ, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med JCSM Off Publ Am Acad Sleep Med* 2012;**8**:597–619

85. O'Brien E, Asmar R, Beilin L, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens* 2003;**21**:821–48

86. De Vito A, Carrasco Llatas M, Vanni A, et al. European position paper on drug-induced sedation endoscopy (DISE). *Sleep Breath Schlaf Atm* 2014;**18**:453–65

87. Nissinen E, Männistö PT. Biochemistry and pharmacology of catechol-O-methyltransferase inhibitors. *Int Rev Neurobiol* 2010;**95**:73–118

88. Phillips BG, Narkiewicz K, Pesek CA, Haynes WG, Dyken ME, Somers VK. Effects of obstructive sleep apnea on endothelin-1 and blood pressure. *J Hypertens* 1999;**17**:61–6

89. Ehler A, Benz J, Schlatter D, Rudolph MG. Mapping the conformational space accessible to catechol-O-methyltransferase. *Acta Crystallogr D Biol Crystallogr* 2014;**70**:2163–74

90. WHO | Cardiovascular diseases (CVDs) [Internet]. WHO. [cited 2016 Jan 30]. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/

91. WHO | Obesity [Internet]. WHO. [cited 2016 Jan 30]. Available from: http://www.who.int/gho/ncd/risk_factors/obesity_text/en/

92. Johansson JK, Niiranen TJ, Puukka PJ, Jula AM. Prognostic value of the variability in home-measured blood pressure and heart rate: the Finn-Home Study. *Hypertension* 2012;**59**:212–8

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

94. Collard P, Pieters T, Aubert G, Delguste P, Rodenstein DO. Compliance with nasal CPAP in obstructive sleep apnea patients. *Sleep Med Rev* 1997;**1**:33–44

95. Steier J, Jolley CJ, Seymour J, Roughton M, Polkey MI, Moxham J. Neural respiratory drive in obesity. *Thorax* 2009;**64**:719–25

96. Bisogni V, Pengo MF, Maiolino G, Rossi GP. The sympathetic nervous system and catecholamines metabolism in obstructive sleep apnoea. *J Thorac Dis* [Internet] 2015 [cited 2016 Jan 22];**3** Available from: http://www.jthoracdis.com/article/view/5947

97. Dimsdale JE, Coy T, Ziegler MG, Ancoli-Israel S, Clausen J. The effect of sleep apnea on plasma and urinary catecholamines. *Sleep* 1995;**18**:377–81

98. Somers VK, Mark AL, Zavala DC, Abboud FM. Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *J Appl Physiol Bethesda Md 1985* 1989;**67**:2101–6

99. Golbidi S, Badran M, Ayas N, Laher I. Cardiovascular consequences of sleep apnea. *Lung* 2012;**190**:113–32

100. Lesske J, Fletcher EC, Bao G, Unger T. Hypertension caused by chronic intermittent hypoxia--influence of chemoreceptors and sympathetic nervous system. *J Hypertens* 1997;**15**:1593–603

101. Peng Y-J, Yuan G, Ramakrishnan D, et al. Heterozygous HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol* 2006;**577**:705–16

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

103. Bradley TD, Phillipson EA. Central sleep apnea. *Clin Chest Med* 1992;**13**:493–505

104. Javaheri S. A mechanism of central sleep apnea in patients with heart failure. *N Engl J Med* 1999;**341**:949–54

105. Yip GMS, Chen Z-W, Edge CJ, et al. A propofol binding site on mammalian GABAA receptors identified by photolabeling. *Nat Chem Biol* 2013;**9**:715–20

106. Goodchild CS, Serrao JM. Cardiovascular effects of propofol in the anaesthetized dog. *Br J Anaesth* 1989;**63**:87–92

107. Garvey DJ, Vaccari A, Timiras PS. Developmental profiles of catecholaminergic enzymes in adrenals of perinatal rats: effect of a hypoxic environment. *Horm Metab Res Horm Stoffwechselforschung Horm Métabolisme* 1980;**12**:318–22

*108. Yuan G, Nanduri J, Khan S, Semenza GL, Prabhakar NR. Induction of HIF-1alpha expression by intermittent hypoxia: involvement of NADPH oxidase, Ca2+ signaling, prolyl hydroxylases, and mTOR. J Cell Physiol 2008;**217**:674–85*