

Applying metabolomics in diagnosing and monitoring Obstructive Sleep Apnoea Hypopnoea Syndrome

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Submitted to Swansea University in fulfilment of the requirements for the
Degree of Doctor of Medicine

Swansea University, 2024

Summary

Obstructive sleep apnoea hypopnoea syndrome (OSAHS) is associated with adverse cardiometabolic risk and a chronic inflammatory metabolic state. Current sleep tests are limited by waiting times, are labour intensive, and have ongoing controversies regarding the optimal physiological parameter(s).

Metabolomics is the study of multiple chemical processes simultaneously of the small molecule substrates, intermediates, and products of cell metabolism. It provides a chemical fingerprint of an organism at a precise timepoint. Metabolomics has mainly been applied to plasma in OSAHS, with inconsistent methods and results and remains far from clinical application.

By applying untargeted metabolomic profiling on the plasma, serum, and urine of consecutive attenders to our sleep service, I demonstrated a panel of 5 biologically plausible urinary metabolites that could distinguish between those with OSAHS and their non-OSAHS counterparts with an AUC of 0.77 (0.52-0.93). Furthermore, I found a single urinary metabolite, octadecanamide, could differentiate groups with an AUC 0.86 (0.76-0.93), 84% sensitivity, and 78% specificity.

Furthermore, the same patients profiled for OSAHS were followed-up and re-sampled after a period of treatment with CPAP to assess the effect of CPAP on their circulating metabolomic profile. I found significant down-regulation in again biologically plausible metabolites following treatment with CPAP ($p < 0.05$), with 2-anilino-6-cyclohexylmethoxypurine differentiating between pre- and post-CPAP groups with an AUC of 0.93 [0.858-0.971].

Metabolomics in urine offers a promising and non-invasive way to differentiate OSAHS from non-OSAHS, whilst identifying pathways activated by chronic intermittent hypoxia, oxidative stress, and inflammation. Furthermore, correlation of these key metabolites with the known cardiometabolic consequences of OSAHS could potentially highlight those at an increased risk of future adverse complications and provide areas for future targeted treatments that CPAP is unable to impact currently.

Declarations and Statements

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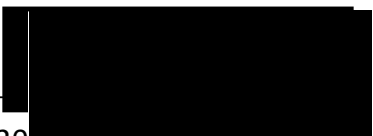
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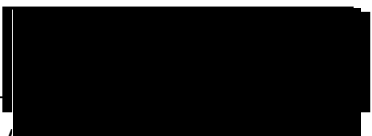
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Publications and Presentations

Publications

- 1) Under submission – ‘Urinary metabolomic profiling in Obstructive Sleep Apnoea Hypopnoea Syndrome’. Thorax 2024

Abstracts

- 1) British Thoracic Society: ‘The effect of continuous positive airway pressure on the urine metabolomic profile in those with Obstructive Sleep Apnoea Hypopnoea Syndrome’ 2024. Spoken presentation.
- 2) European Respiratory Society abstract: ‘Plasma metabolomics identifies obstructive sleep apnoea hypopnoea syndrome’
https://erj.ersjournals.com/content/58/suppl_65/PA2097 September 2021
- 3) British Sleep Society abstract: ‘The effect of continuous positive airway pressure (CPAP) on key plasma metabolites identifying obstructive sleep apnoea hypopnoea syndrome (OSAHS)’
https://bmjopenrespres.bmj.com/content/8/Suppl_1/A6 November 2021

Presentations non-abstracted

- 1) Survey of Sleep Services in Wales. Welsh Thoracic Society Meeting (Cardiff, Oct 2022)
- 2) ‘The potential of metabolomics in diagnosing and monitoring obstructive sleep apnoea syndrome’. Welsh Thoracic Society Meeting, Cardiff, June 2022
- 3) ‘Update on plasma metabolomics and OSA’. Researchers in Wales Study Day Presentation, The Beacon, Llanelli, May 2022
- 4) ‘Metabolomics and OSA’. Novel technologies study day, Prince Phillip Hospital, Llanelli, May 2021

Awards

- 5) Royal College of Physicians – Turner-Warwick Prize for Wales 2021
- 6) ‘Kim Harrison Award for Best Presentation’ at Welsh Thoracic Society Annual Meeting 2022

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Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisor Professor Keir Lewis for his continual support and guidance, as well as his motivation and enthusiasm during my postgraduate study. Thank you. I also had the offer of support from Professor Conlan to whom I would also like to thank.

I would like to acknowledge the 'Novel Technologies in Diagnosis and Monitoring Lung Disease' (ISRCTN 16657101) research group for providing the platform for this research to be carried out.

I also extend thanks to Professor Luis Mur and his PhD students at Aberystwyth University for their support and guidance in the study of metabolomics, and Priya Sai-Giridhar, Advanced Biochemical Scientist at Prince Phillip Hospital, and her team, for their help with the practical aspects of this research.

I would also like to thank our senior sleep physiologist Catrin Lewis and her team, as well as Sarah Bowen, Research Assistant, who all helped with the clinical and logistical aspects of this study. I would also like to thank every single patient who kindly agreed to participate to allow this research to be possible.

Last and most importantly, I would like to thank my wife, children, and parents for their unwavering support.

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Abbreviations

AA	Arachidonic acid
AASM	American Academy of Sleep Medicine
ACE	Angiotensin-converting enzyme
AF	Atrial fibrillation
AG	Arachidonoylglycerols
AHI	Apnoea-hypopnoea index
ARB	Angiotensin receptor blocker
ATP	Adenosine triphosphate
AUC	Area under the ROC curve
BMI	Body mass index
BP	Blood pressure
BTS	British Thoracic Society
CH	Chronic hypoxia
CI	Confidence interval
CIH	Chronic intermittent hypoxia
COPD	Chronic Obstructive Pulmonary Disease
COVID	Coronavirus disease
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CT	Computed tomography
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DVLA	Driver and Vehicle Licensing Agency
ECG	Electrocardiogram
EDS	Excessive daytime sleepiness
EDTA	Ethylenediamine tetraacetic acid
EEG	Electroencephalogram
EMG	Electromyogram
ESS	Epworth Sleepiness Scale

FC	Fold change
FDR	False discovery rate
FHX	Family history
GC	Gas chromatography
HF	Heart failure
HIV	Human immunodeficiency virus
HNS	Hypoglossal nerve stimulation
HRMS	High-resolution mass spectrometry
HSAT	Home sleep apnoea test
HTN	Hypertension
IH	Intermittent hypoxia
IHD	Ischaemic heart disease
LAUP	Laser-assisted uvuloplasty
LC	Liquid chromatography
LV	Left ventricle
MAD	Mandibular advancement device
MRI	Magnetic resonance imaging
MS	Mass spectrometry
NFKB	Nuclear factor kappa B
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMR	Nuclear magnetic resonance
NREM	Non-rapid eye movement
ODI	Oxygen desaturation index
OHS	Obesity hypoventilation syndrome
OR	Odds ratio
OSA	Obstructive sleep apnoea
OSAHS	Obstructive sleep apnoea hypopnoea syndrome
OSAS	Obstructive sleep apnoea syndrome
PAP	Positive airway pressure
PC	Principal component
PCA	Principal component analysis

PCI	Percutaneous coronary intervention
PIS-ICF	Patient information sheet - informed consent form
PLSDA	Partial least-squares discriminant analysis
PPP	Pentose phosphate pathway
PSG	Polysomnography
PVI	Pulmonary vein isolation
QALY	Quality-adjusted life year
QOL	Quality of life
RCT	Randomised control trial
REM	Rapid eye movement
RIP	Respiratory inductance plethysmography
ROC	Receiver operating characteristic
ROS	Oxygen-free radicals
RPM	Revolutions per minute
SAVE	Sleep Apnoea Cardiovascular Endpoints
SD	Standard deviation
SDB	Sleep-disordered breathing
SF	Sleep fragmentation
SHBG	Sex hormone-binding globulin
SP	Sleepy participants
SST	Serum separator tube
TNF	Tumour necrosis factor
UA	Upper airway
UARS	Upper airway resistance syndrome
UHPLC	Ultra-high-performance liquid chromatography
UK	United Kingdom
UPPP	Uvulopalatopharyngoplasty
USA	United States of America
VCAM	Vascular cell adhesion molecules
VEGF	Vascular endothelial growth factor
VIP	Variable importance projection

1 Introduction

“Laugh and the world laughs with you, snore and you sleep alone.”

Anthony Burgess

1.1 Sleep – what is it?

Sleep is a condition of body and mind that typically recurs for several hours every night, and is a phenomenon observed in all animals in some form, usually as being a vulnerable state that is incompatible with behaviours that nourish and propagate, which suggests that it likely has some evolutionary relevance.[1] During sleep the nervous system is relatively inactive, the eyes close, the postural muscles are relaxed, and the consciousness is practically suspended.[2] It is an essential function which encompasses up to a third of our lives, and it is as important to our health and wellbeing as eating, drinking, and breathing.[3] The exact benefits of sleep remain slightly elusive, with sleep being viewed as a quiet resting restorative state with many physiological changes in ventilation and respiration. Sleep provides a vital life-sustaining function with a powerful internal drive to do it, with the symptom of tiredness to the state of sleep as akin as hunger is to eating.

Sleep can be considered as a state of adaptive inactivity that lies on a continuum of dormant states that ranges from hibernation to continuous activity. Hibernation is when an organism spends much the winter in a state of dormancy, where they adopt a prolonged period of torpor to survive cold conditions.[4] Torpor refers to a regulated state of reduced metabolism and is the most effective means of energy conservation available to mammals and birds.[4] It allows the normal physiology in an organism's body including heart rate, breathing, and energy consumption to slow down and appears to have evolved to effectively compensate for environmental stresses such as cold weather and scarcity of food.[5] The length of torpor in animals can vary hugely, with some animals such as hummingbirds adopting this state on a daily basis, with hedgehogs and bears adopting more prolonged periods. During hibernation, a mammal's body temperature reduces significantly, cortical electroencephalogram (EEG) activity largely ceases, neuronal activity through the brain diminishes, and

responsiveness and energy consumption are greatly reduced, with full arousal from hibernation possibly taking an hour or more.[1]

Sleep is a biologic process that is essential for life and optimal health, and plays a critical role in development, energy conservation, brain waste clearance, modulation of immune responses, cognition, performance, vigilance, disease, and psychological state.[6] For obvious ethical reasons, sleep deprivation studies in human are limited, but the vital importance of sleep to good health was dramatically demonstrated in rats by Rechtschaffen et al. who showed that total sleep deprivation resulted in the death of all rats within 2 to 3 weeks, with a tirade of health disorders leading to their untimely fate.[7] In humans, sleep scientists often refer to Randy Gardner when citing cases of extreme sleep deprivation, known as 'The Boy Who Stayed Awake for Eleven Days', who infamously stayed awake for 11 days and 24 minutes (264.4 hours) at the age of 17 in December 1963/January 1964.[8] Despite initially feeling only mood changes associated with tiredness from the initial sleep deprivation, Gardner later experienced problems with concentration, short term memory loss, paranoia, and hallucinations, and by the eleventh day when asked questions about himself, proceeded to get his age and race wrong.[8]

The wide-ranging effects of sleep disruption are often interrelated and bidirectional, with short-term consequences from studies of the sleep deprived and insomniacs showing increased autonomic sympathetic activation, somatic problems, reduced quality of life, emotional distress, mood disorders, and cognitive, memory and performance deficits.[9] The longer-term consequences of sleep disruption include a higher risk of hypertension, cardiovascular disease, dyslipidaemia, metabolic syndrome, and diabetes mellitus, all of which are well-documented co-morbidities associated with obstructive sleep apnoea hypopnoea syndrome (OSAHS).[9, 10]

Disrupted sleep is a pervasive problem, and even today the physical state of sleep and sleep-disordered breathing (SDB) remains a quagmire of a knowledge base, with physiologists and physicians alike still learning the full restorative function and benefits of a clinical state that we spend a third of our lives in. Compared to most medical specialities that were established in the mid-1800s, sleep medicine is still in its early years, with its own diagnostic procedures and therapeutic strategies only being established once key findings in neurophysiology and basic sleep research allowed a

breakthrough in the understanding of the sleeping brain, the majority of which happening in the latter half of the 20th century.[11]

The awareness and understanding of sleep disorders has significantly progressed in recent decades, aided by sleep medicine being formally recognised as a medical subspecialty by the American Medical Association in 1994. However, given the detrimental impact of poor sleep, it is vital that healthcare professionals can identify and treat the underlying aetiology, despite the ongoing debate in the medical community with regards to what exactly defines poor sleep and excessive tiredness due to their profound subjectivity. The widespread impact of sleep disorders including SDB on an individual's physical, mental, and emotional health cannot be underestimated, with a need for further extensive research in many areas.

2 Obstructive sleep apnoea: definition, history, epidemiology, pathophysiology, and management

"Sleep those little slices of death - how I loathe them."

Edgar Allan Poe

2.1 Definition and Overview

The obstructive sleep apnoea hypopnoea syndrome (OSAHS) is defined as the co-existence of excessive daytime sleepiness with irregular breathing at night.[12] It encompasses both obstructive sleep apnoea (OSA) and upper airway resistance syndrome (UARS) which are two distinct but related clinical entities in the spectrum of sleep-disordered breathing (SDB).[13] It is a heterogenous condition which is a commonly under-recognised cause of excessive daytime sleepiness and is characterised by repetitive episodes of complete or partial upper pharyngeal airway collapse during sleep.[12-14] OSAHS is independently linked with the development and poor control of numerous cardiovascular and metabolic conditions including hypertension, coronary artery disease, arrhythmias, stroke, heart failure, type 2 diabetes mellitus, and non-alcoholic fatty liver disease (independent of obesity) leading to substantial morbidity and mortality.[15]

In OSA, the airway may obstruct completely with no respiratory airflow (apnoea) or partially causing hypoventilation (hypopnoea).[16] Apnoeas can either be 'central', in which there is no respiratory effort, or 'obstructive', in which respiratory effort is present but airflow is decreased due to a loss of patency of the upper airway.[17] Conventionally, an apnoea is defined as the absence of airflow at the nose and mouth for at least 10 seconds, and a hypopnoea is a major reduction (>50%) in airflow also for 10 seconds.[12] To restore their airway patency during an apnoeic or hypopnoeic event, patients experience sleep fragmentation and recurrent awakenings to increase muscle tone.

A sleep study of sufficient duration and technical standard is required to confirm the clinical suspicion of SDB and to assess its severity. Polysomnography (PSG), a type of study, is a multi-parameter study of sleep and a diagnostic tool in sleep medicine.

The test result is called a polysomnogram, also abbreviated PSG. The name is derived from Greek and Latin roots: the Greek *polus* (for "many, much", indicating many channels), the Latin *somnus* ("sleep"), and the Greek *graphein* ("to write").

Type I polysomnography is a sleep study performed overnight while being continuously monitored by a credentialed technologist. It records the physiological changes that occur during sleep, usually at night, though some laboratories can accommodate shift workers and people with circadian rhythm sleep disorders who sleep at other times. The PSG monitors many body functions, including brain activity (EEG), eye movements, muscle activity or skeletal muscle activation (EMG), and heart rhythm (ECG). However, this process is expensive and often deemed unnecessary as despite the quantity of neurophysiological signals obtained during an overnight PSG, most of the data collected is ignored and treatment decisions rely heavily on measures of breathing -the apnoea-hypopnoea index (AHI). Figure 2.1 shows a polysomnogram demonstrating the physiological effects of OSA.[18]

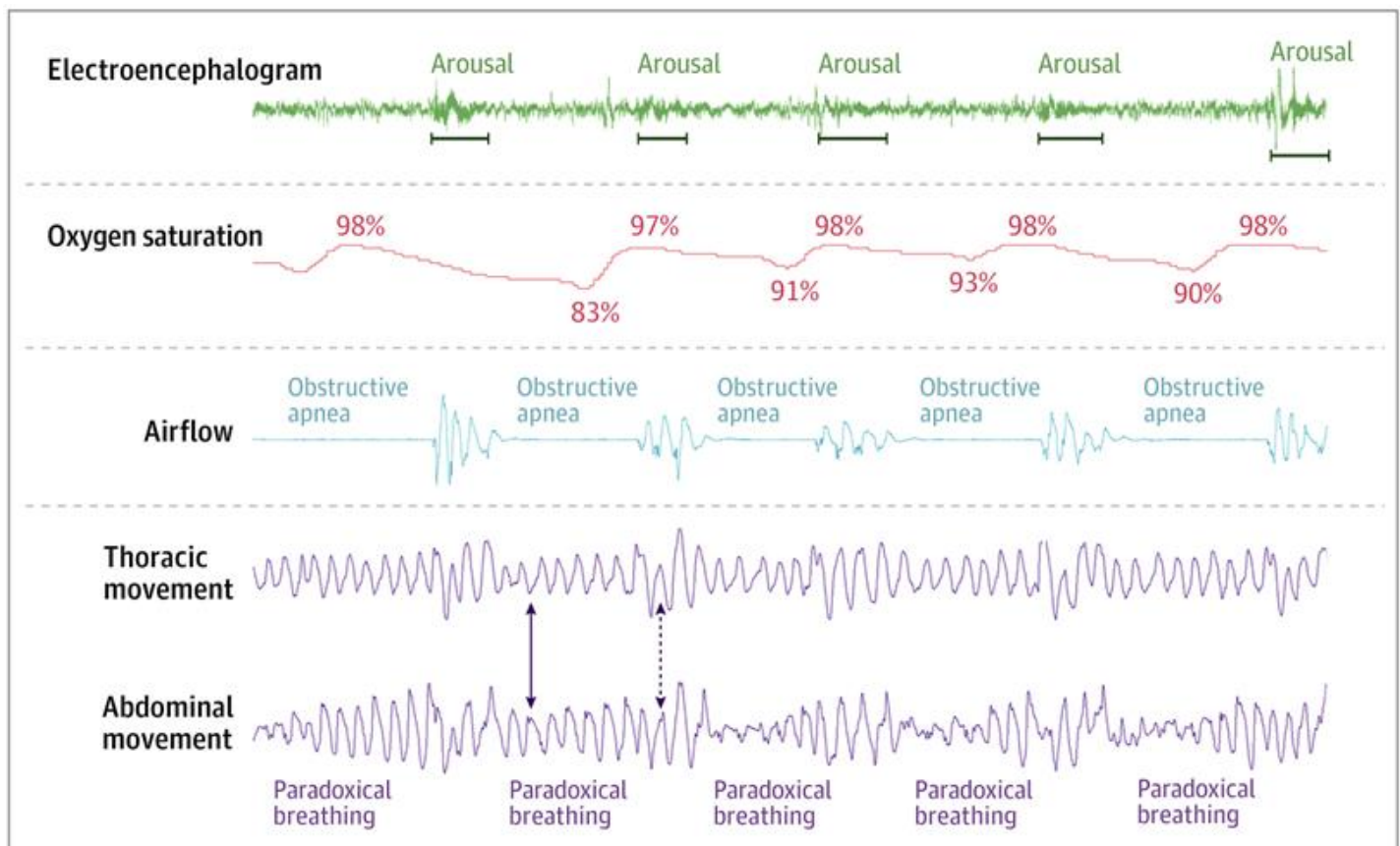


Figure 2.1: Polysomnogram demonstrating the physiological effects of OSA.

Reproduced with permission from *Diagnosis and Management of Obstructive Sleep Apnea: A Review*. JAMA, 2020. 323(14): p. 1389-1400.

An acceptable, more widely used diagnostic alternative is a home sleep test, which is usually a limited channel sleep polygraphy study allowing monitoring of multiple (poly) signals, namely snoring, body movement, heart rate, oronasal flow, and chest wall movement, allowing adequate detection of apnoea and hypopnoeas, whilst being able to distinguish between obstructive and central events. These are usually performed in the patient's own home overnight so are cheaper and probably more realistic in a natural setting. They are increasingly accepted as an alternative diagnostic method and are the main diagnostic tool for the UK NHS. However, home monitors cannot discern between sleep and wakefulness, and are not recommended in patients with comorbidities such as congestive heart failure, chronic lung disease, or neurological conditions as they have not yet been verified in these populations.[19, 20]

Following a sleep study, the apnoea-hypopnoea index is calculated, which is the summation of the total apnoeas and hypopnoeas per hour. Currently, the AHI is then used to stratify the severity of OSAHS into:

- normal (<5 events per hour)
- mild (5-≤15 events per hour)
- moderate (15-<30 events per hour)
- severe (≥30 events per hour).[14]

The British Thoracic Society (BTS) and National Institute for Health and Care Excellence (NICE) defines OSA based upon AHI (normal, mild, moderate, severe) following overnight polysomnography or polygraphy that may or may not be causing symptoms. OSAHS is diagnosed following sleep studies and having appropriate symptoms principally excessive daytime sleeping.[21]

For this thesis I will apply the NICE/BTS definitions of OSAHS for diagnosis and severity.[21]

2.2 History of OSAHS

Obstructive sleep apnoea (OSA) has been observed since ancient times, and there are records of its cardinal symptoms such as heavy snoring and apnoeas found in documents dating back to antiquity. The Greek god of fertility, Dionysus, surrounded himself with women, the Maenads, who used thyrsus sticks (a long stick wrapped in ivy or vine leaves and tipped with a pine cone) to gently poke the god back in to breathing when he snored and stopped breathing.[22] Other notable examples were the Ptolemy dynasty that ruled Egypt for almost 300 years (305 BC to 30 BC) who had symptoms associated with OSA with family members being recorded as hugely obese, to more recent prominent historical figures such as Emperor Napoleon Bonaparte and President Theodore Roosevelt who both had the co-existence of intrusive snoring and obesity.[23-25] There is an argument that the development of likely sleep-disordered breathing alongside obesity in Napoleon's latter years contributed to significant daytime drowsiness which ultimately may have led to a waning in his genius that had up to that point helped him to be a successful military tactician and adept politician (figure 2.2).[25]

There are anecdotes that that during the American Revolutionary War in 1775-1783, cannonballs were strapped to the backs of snoring soldiers to prevent them from sleeping on their backs (which can worsen OSAHS) with the aim of preventing weakening of troop morale through sleeplessness caused by loud snoring, and to avoid betraying the location of the sleeping soldiers to the enemy.[26]

William Wadd, Surgical Extraordinary to King George III, observed in the early 1800s that in the obese "respiration is performed imperfectly, or with difficulty", and that obese people "could fall asleep at any time".[27] Intriguingly, the famous novelist Charles Dickens devised characters suffering from loud snoring, excessive sleepiness, plethoric faces, and obesity notably in 'Joe the "fat boy"' from his 'The Posthumous Papers of the Pickwick Club' (figure 2.2).[28] Later the term 'Pickwickian Syndrome' was adopted by the world renowned physician and Professor of Medicine at Oxford University, Sir William Osler, to describe obesity hypoventilation syndrome; a more severe form of sleep-disordered breathing.[28, 29]

In 1956, Burwell et al. published their classic description of the Pickwickian syndrome, with medical professionals now believing that alveolar hypoventilation secondary to obesity was the cause of the excessive daytime somnolence observed in those with SDB. Furthermore, electrical measuring and recording devices were available in the first half of the 20th century which provided the tools for the study of the pathophysiology and diagnosis of the OSA, but unfortunately, they continued to concentrate on the patient's obesity, rather than on any disordered breathing during sleep.

In 1965, the first polysomnogram was performed by Gastaut et al. on a patient showing "typical Pickwickian syndrome" alongside arterial gastometry, with polygraphic monitoring carried out both day and night.[30] They identified hypopnoeic and apnoeic events during sleep, and noted the patient remained symptomatic during the daytime despite blood oxyhaemoglobin saturations and alveolar CO₂ concentrations remaining entirely normal. These results caused a seismic paradigm shift in the understanding of sleep and hypoventilation as they had found that obesity was not the cause of the syndrome, but merely a risk factor, and that hypercapnia was not the only reason for daytime somnolence, with the importance of sleep fragmentation and quality of sleep needing to be accounted for.[30-32]



Figure 2.2: An 1821 drawing done only two months before Napoleon's death—note his considerable girth (left) and Joe 'The Fat Boy', asleep again, from Charles Dickens' Posthumous Papers of the Pickwick Club

2.3 Epidemiology

The prevalence of OSAHS is increasing and is likely to continue to do so considering the obesity pandemic, alongside a rising awareness of both its signs and symptoms but also the long-term complications if left untreated. In 1993, Young et. al published a report on the prevalence of OSA in the general population in the New England Journal of Medicine which remains one of the most cited papers of all sleep-related research articles published since 1965.[33] Their Sleep Cohort Study performed overnight polysomnography and symptom questionnaires on a random sample of 602 employed men and women who were originally part of the Wisconsin Sleep Cohort Study, a longitudinal study of the natural history of cardiopulmonary disorders of sleep established in 1988.[34] It was a population-based prospective study using overnight polysomnography to investigate the epidemiological features of SDB on a random sample of state employees in Wisconsin. In the first stage, all employees 30 to 60 years of age who worked for three large state agencies were surveyed about their sleep patterns and other characteristics using six survey questions, answered by five-point frequency scale and “do not know” category. Using these mailed questionnaires, participants were split into habitual (almost every night or every night) or non-habitual snorers, with all those who admitted to habitual snoring, and 25% of those who denied habitual snoring recruited for overnight polysomnography. Following a participation rate of just 43% for sleep studies, with the most common reason for declining being the inconvenience of sleeping away from home, they estimated that the prevalence of sleep-disordered breathing, defined as an apnoea-hypopnoea index of 5 or higher, was 9 % for women and 24% for men, with 2% of men and 4% of women having symptoms of hypersomnolence congruent with OSAHS.[34] In 2013, Peppard et. al using statistical modelling and once again using data from the Wisconsin Sleep Cohort Study suggested prevalence rates of at least 14% of men and 5% of women now having an AHI ≥ 5 with symptoms of daytime sleepiness i.e. OSAHS.[35]

When solely looking at evidence of sleep-disordered breathing, recent polysomnographic data from the population-based CoLaus/PsyCoLaus cohort study estimated that 23% of women and nearly 50% of men have moderate to severe OSA, defined as an apnoea-hypopnoea index of 15 or more per hour.[36]

The CoLaus/PsyCoLaus cohort study was to investigate the prevalence of cardiovascular risk factors and psychiatric disorders on 6733 participants chosen at random to identify genetic determinants and mechanisms involved in their association.[37]

In 2019, using publicly available data and by contacting key opinion leaders, and based on the published literature and by matching countries based on population demographics where specific data were not available, Benjafield et al. estimated that almost 1 billion people worldwide aged between 30–69 years have mild OSA (AHI of five or more events per hour). This figure is likely to underestimate the global prevalence of OSA giving that the analysis focussed only on adults aged 30–69 years, whilst also using a more conservative AHI scoring criteria (AASM 2012 criteria) which I will discuss further in chapter 2.7. Furthermore, they estimate that 425 million persons have at least moderate disease (AHI 15 events or more), and that the prevalence of OSA could exceed 50% in some countries.[38] However, they had no specific requirement for how obstructive sleep apnoea symptoms were evaluated or reported, and so symptoms were not included in their study. Figure 2.3 outlines the top ten countries with the highest estimated numbers of individuals with OSA, with the highest number of affected individuals with an AHI ≥ 15 being in China, followed by the USA, Brazil, and India.[38]

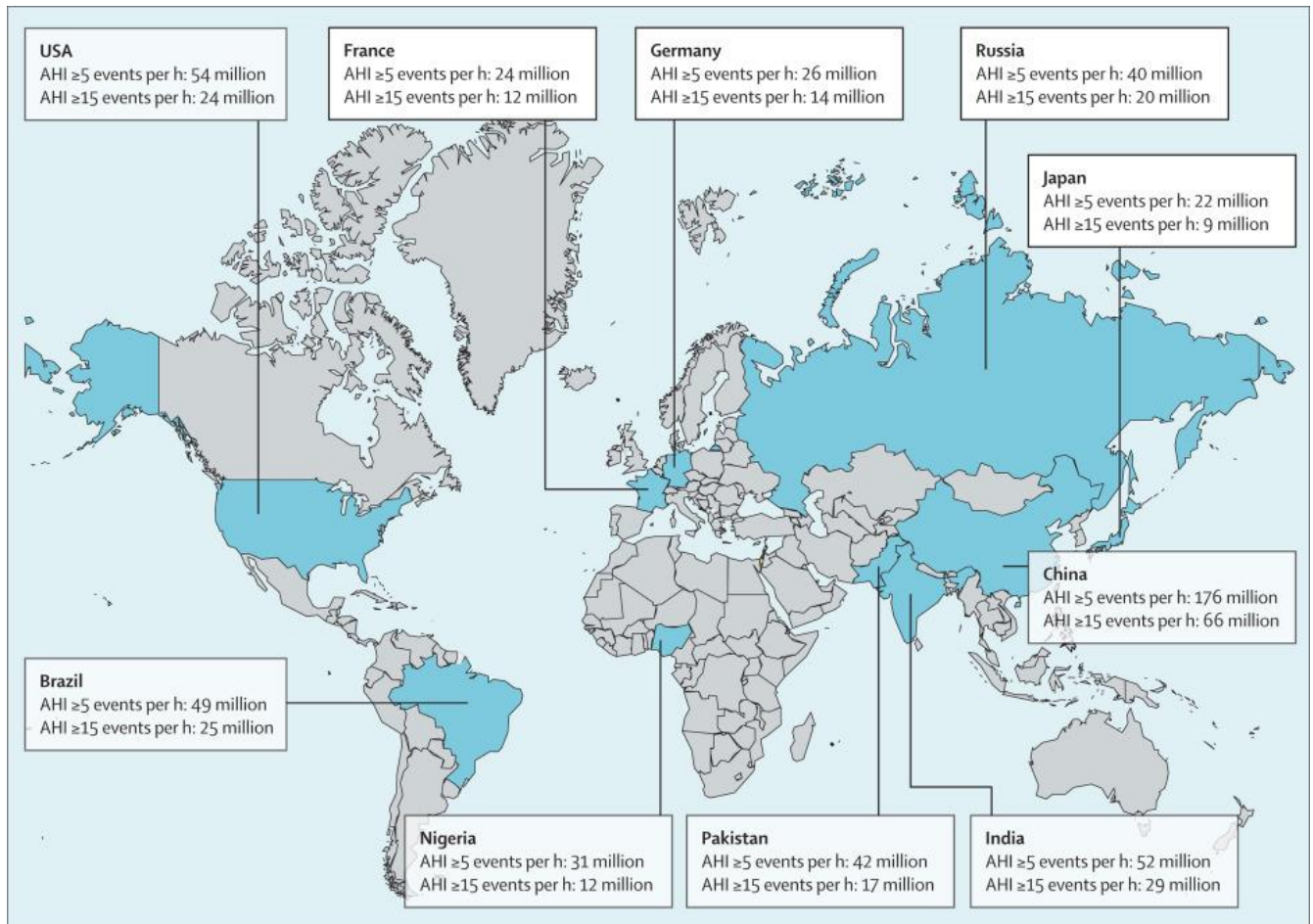


Figure 2.3: Top ten countries with the highest estimated numbers of individuals with OSA based on the American Academy of Sleep Medicine 2012 Criteria.

Reproduced with permission from *Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis*. *Lancet Respir Med*, 2019. 7(8): p. 687-698

With regards to the UK, there is uncertainty about the number of adults who have OSAHS, as it is estimated that up to 85% of sufferers remain undiagnosed and therefore untreated.[39] In 2014, it has been estimated that 1.5 million adults have OSA, and that only 330,000 of these were formally diagnosed and treated. However more recent studies estimate that 5% of the UK population now have undiagnosed OSAHS which would equate to 3.35 million people in 2021.[21, 40]

These prevalence figures are comparable to other major chronic conditions e.g. with type two diabetes mellitus (just under 4 million prevalence in 2019) and chronic obstructive pulmonary disease (estimates 3 million UK people in 2018). It is likely that the burden of OSAHS and its associated co-morbidities and increased all-cause mortality are yet to be felt.[41, 42]

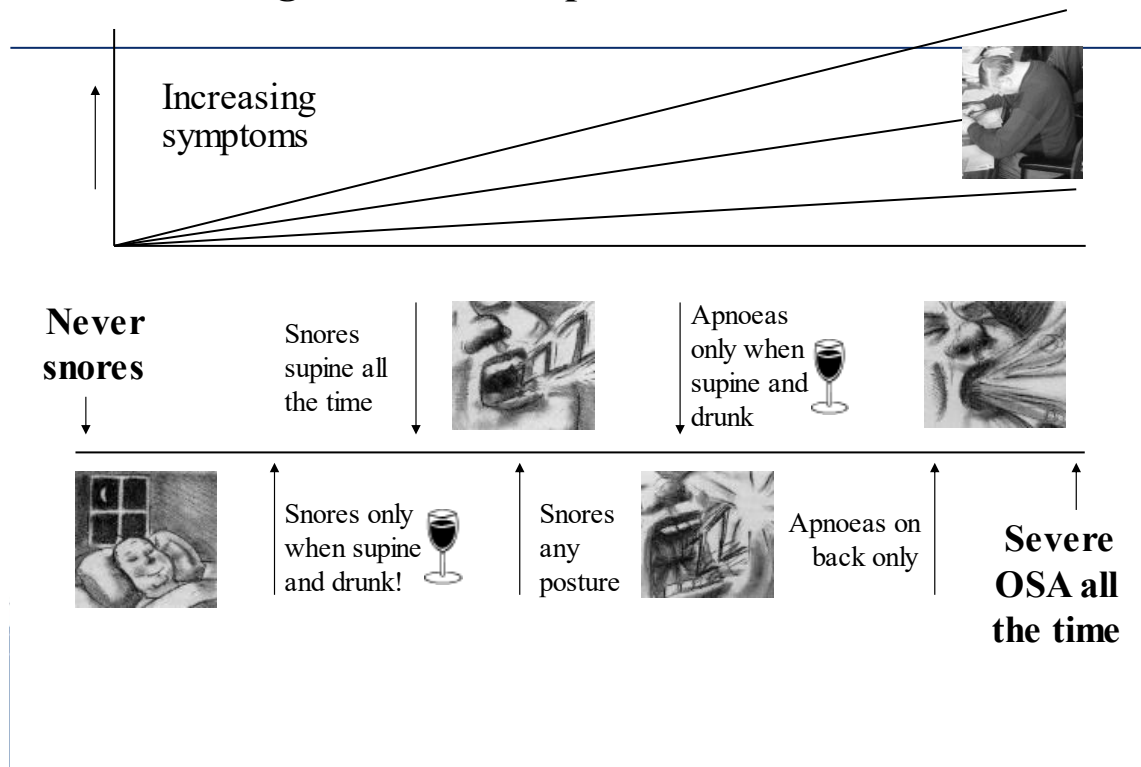
2.4 Pathophysiology of OSAHS

2.4.1 The Spectrum of OSAHS

OSA is part of a spectrum, with snoring at one end, and repetitive complete obstruction throughout the night with symptoms of daytime tiredness and cardiometabolic complications at the other (figure 2.4). It is a chronic condition characterised by recurrent upper airway collapse leading to pauses in breathing during sleep from the obstruction (figure 2.5).[43] This leads to intermittent hypoxaemia, mild hypercapnia, sympathetic activation, large swings in intrathoracic pressure, arousals and hence sleep fragmentation, with research to date suggesting that it is the intermittent hypoxia (IH) and sleep fragmentation (SF) that are the key determinants in the cardiometabolic complications of OSA.[44]

Normal sleep is divided into non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep accounts for 75% to 80% of total sleep time, and REM sleep accounts for the remaining 20% to 25%.[45] These two categories of sleep comprise a 90 minute cycle, and normal adults repeat this cycle four to six times during the night. The cycle becomes longer and more frequent over the course of sleep. Under physiological conditions, blood pressure, heart rate, and sympathetic activation decrease during the non-REM phase of sleep, while they increase during awakening. These physiological traits have a circadian rhythm and a synchrony with both molecular and metabolic responses, and disturbances in the circadian rhythm and frequent arousals secondary to upper airway obstruction leads to a deleterious physiological state.

Snoring and OSA are part of a continuum



Aetiology OSAHS: Flash MRI images of an obstructive apnoea

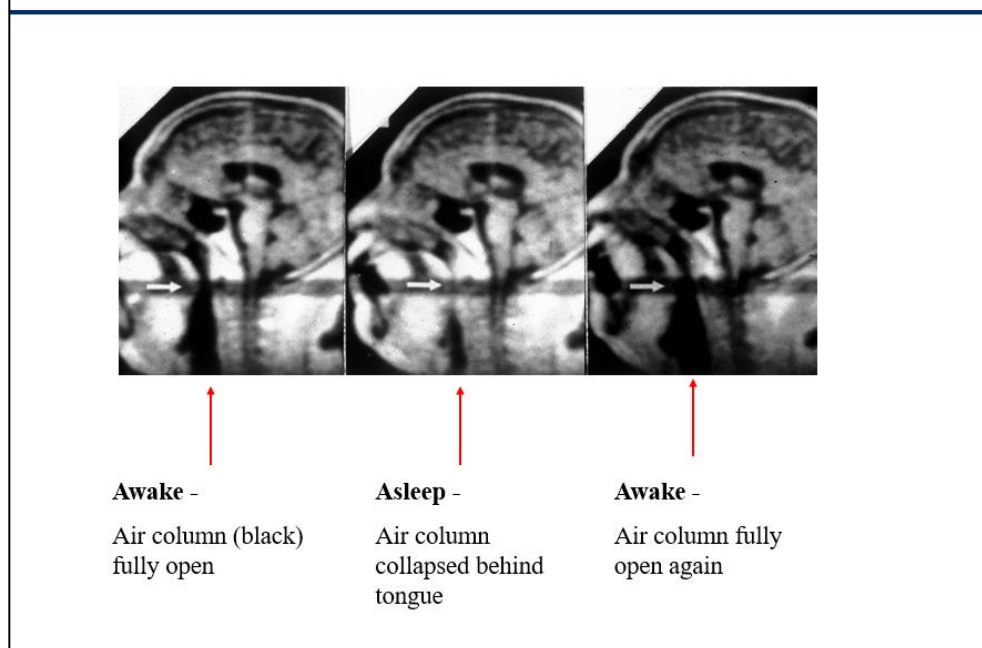


Figure 2.4: The continuum of sleep-disordered breathing and Flash MRI images of an obstructive apnoea. Adapted from BTS Educational Slide Sets 2001

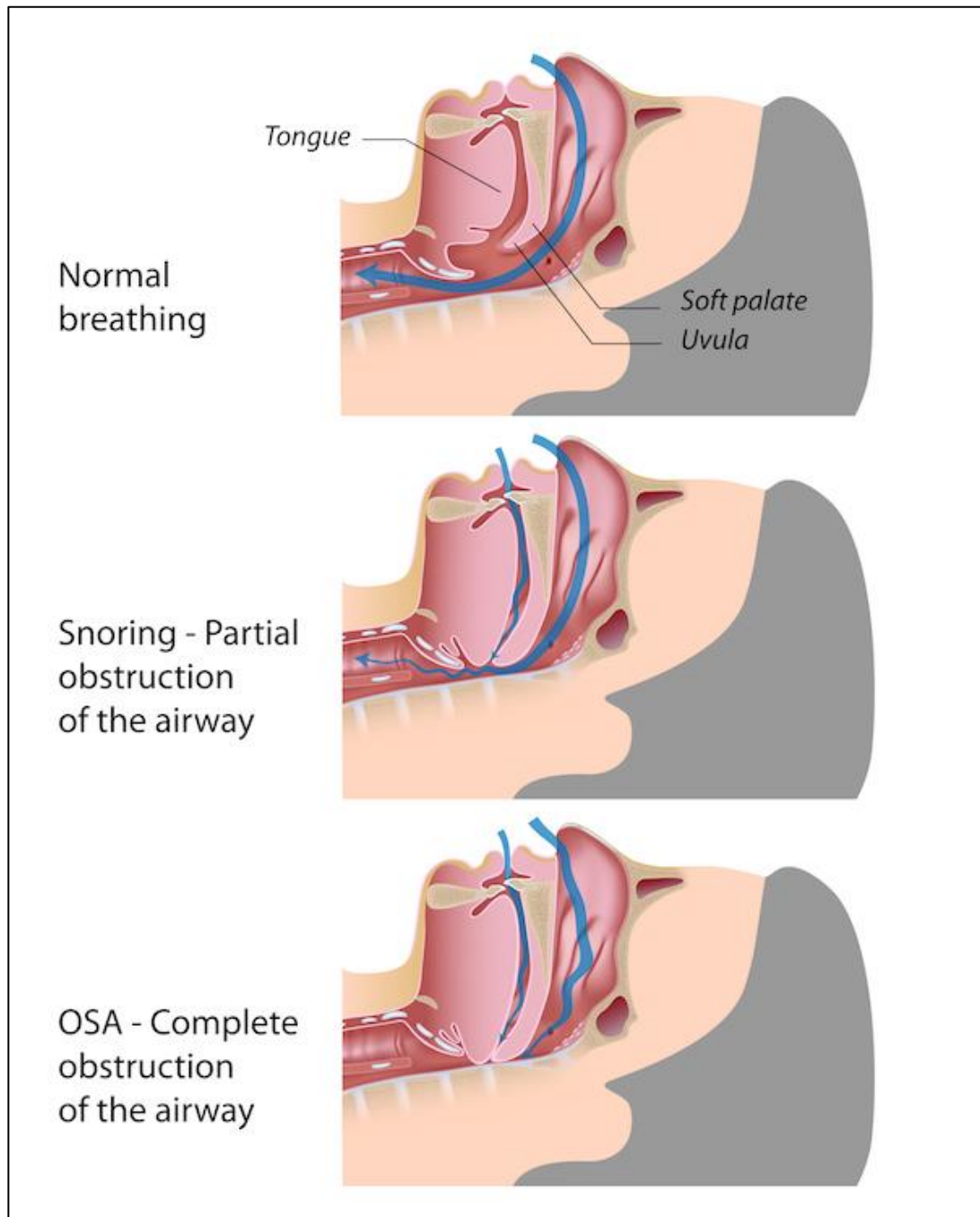


Figure 2.5: Upper airway collapse as seen in snoring and OSA

The pathophysiology of OSA is complex and stems from the interplay between anatomical and non-anatomical factors. The key abnormality of OSA is upper airway collapsibility characterised by the periodic blockage of the upper airway (UA) during sleep, as the negative pressure generated during inspiration overcomes the force exerted by the UA dilator muscles to maintain patency. The UA dilating muscles, contracting in a phasic manner that precede each inspiration, work to counteract the negative pressure generated in the UA during inspiration. This delicate balance can be compromised by any factor that escalates this negative pressure or diminishes the effectiveness of UA dilating muscle contractions, thereby leading to an increased risk of UA obstruction.[46]

Basic observation from the earliest observers of SDB allowed clinicians to establish that the impasse was as high as the pharynx, as during obstructive events, the diaphragm and respiratory muscles were still seen to be functioning, but no air was getting in to the lungs.[47] In 1999, Barsh L. interestingly postulates that during Hominid evolution to an upright stance to achieve a bipedal form of locomotion, the face has retracted under the cranium due to the enlarging and increasing brain power. Consequently, the lower jaw could no longer then fully accommodate the tongue, which is achieved by all quadrupeds (animals with four feet), forcing it backwards and curving it downwards to from the anterior aspect of the oropharynx.[46] This evolutionary disadvantage has been magnified because of mankind's recent access to increased food over the last 100 years without adequate time to evolve our airways and cardiovascular systems to what is essentially a food-toxic environment causing obesity in many.

The multifunctional upper airway, its mechanical properties, and the muscles that control its shape and size are complex.[48] Precise coordination of the upper airway muscles is essential for safe breathing and swallowing, and sleep and its associated supine posture place additional demands on it.[48] Furthermore, upper pharyngeal dilatory muscle activity decreases with sleep onset, namely the genioglossus and tensor palatini, and both tonic and phasic dilator activity is further reduced during rapid eye movement (REM) sleep. Consequently, the pharynx is most vulnerable to collapse at end expiration due to the loss of the neural tone of the pharyngeal dilators, which is further compounded by the loss of the positive intraluminal pressure.[49]

Furthermore, Onal et al and Dempsey et al have proposed an alternative neural ventilatory drive-dependent model of pharyngeal obstruction in OSA, whereby a decrease in neural ventilatory drive, which would lead to a reduction in dilator muscle tone, would consequently lead to an obstruction, particularly at low ventilatory drive levels.[50, 51] This is in contrast to the already discussed 'classic model' of OSA which suggests that apnoeas and hypopnoeas occur as a result of a loss of pharyngeal dilator muscle tone with sleep-onset, which actually elicits a rise in neural ventilatory drive to facilitate ventilatory recovery. This was further demonstrated by Gell et al who noted that ventilatory drive fell (rather than rose) when they observed ventilation via flow through an oronasal mask and pneumotach alongside ventilatory drive via intra-oesophageal diaphragm EMG in 50 OSA patients (AHI 5-91 events/hour).[52]

Narrowing of the upper airway, either due to obesity, anatomical deficiency, neck fat accumulation, and/or rostral fluid shifts in the supine posture have traditionally been implicated as key determinants in the pathogenesis of OSA. However, OSA exists in the absence of obesity and structural inadequacies of the upper airway, something of which we have developed a better understanding of in recent years.

Even in a largely phenotypically homogenous patient group (overweight, middle-aged males), early research showed clear between-patient differences in breathing and neuromuscular responses during similar desaturations when airway closure events.[47] Aside from anatomical inadequacies and the efficacy of the upper airway dilator muscles, further physiological phenotypes have been established in recent years. Current literature suggests there are at least four underlying mechanisms that contribute to OSA.

1. Upper airways anatomy
2. Poor pharyngeal muscle responsiveness
3. Respiratory control instability (high loop gain) i.e. a ventilatory control system overreaction perpetuating periodic breathing
4. Low arousal threshold i.e. wakes up too easily from obstructive event

The relative contributions of these four mechanisms in an individual form their endotype, and it is estimated that up to 70% of patients have one or more non-anatomical mechanisms playing a critical role in their OSA pathogenesis.[53]

2.4.2 Common Risk Factors

The most important risk factors for OSAHS include obesity, male sex, and aging, however the physiological mechanisms underlying these risk factors is not clearly understood. Epidemiological and causal studies are often confounded by the presence of obesity, as well as the heterogeneity in their cohorts due to variability in sleep patterns, shift patterns, alcohol consumption, polypharmacy issues, and smoking etc. Table 2.1 shows the common risk factors associated with OSA and their odds ratios.[18]

.

Table 2.1: Common risk factors for OSAHS

Risk factors	Odds ratio
Weight • Overweight vs normal weight • Obese vs normal weight	2.3-3.4 4.0-10.5
Male sex (vs female)	1.7-3.0
Age (per 10-year increment)	1.4-3.2
Postmenopausal state	2.8-4.3
Enlarged upper airway soft tissues (e.g. tonsils, adenoids, tongue)	Unknown
Craniofacial abnormalities (e.g. retrognathia)	Unknown

Obesity

Obesity and OSA share multiple pathophysiological mechanisms, including insulin resistance, hyperleptinemia, endothelial dysfunction, and sympathetic overactivity.[54] Multiple studies using a variety of imaging techniques have consistently shown that the static cross-sectional area of the pharyngeal airway in people with OSA is smaller when compared to those without the condition.[55]

Obesity is a major cause of a narrow pharyngeal airway. The relationship between obesity and OSA appears bidirectional, with approximately 60-70% of individuals being overweight, and about 58% of moderate to severe OSA cases being attributed to excess weight.[56] Obesity can crowd the upper airway via deposition of adipose tissue in the regions surrounding the airway, including within the tongue, soft tissues of the neck, and pharyngeal muscles. As a result, obese patients have a more crowded airway which narrows the UA diameter meaning a higher propensity for airway collapse. Recent MRI studies have shown that many people with OSA have increased fat also within their tongues compared to their obese non-OSA counterparts confirming location of fat deposition is important, with Pack et al. demonstrating via MRI volumetric analysis that the volume of the lateral pharyngeal walls ($p < 0.0001$), tongue ($p < 0.0001$), and total oropharyngeal soft tissue ($p < 0.0001$) was significantly larger in subjects with sleep apnoea than in normal subjects.[57]

We know that central adiposity can restrict diaphragmatic and ribcage movements, reduce chest wall compliance and lung volume, impair respiratory stability, and increase susceptibility to OSA. This is particularly important when we know that men store excess weight around the abdomen more than women, which highlights the importance of gender in its pathophysiology.[58]

Gender

Higher prevalence of OSA in men compared to women has been consistently reported in clinic-based and community-based studies, with the latter showing that the male to female ratio for OSA is in the range of 2:1 or 3:1. [59]

Pharyngeal cross-sectional area is similar in men and women when normalised for body size, but men have shown a greater upper airway collapsibility than women when measured by the critical collapsing pressure.[59] Hormonal differences between the

sexes have been thought to account for the increased prevalence of OSA in men, as the prevalence is equal between the sexes pre-puberty, and the differences in airway length only become apparent in the post-pubertal period.[60] Furthermore, the menopause is a well-documented risk factor for the development of OSA, where the prevalence of OSA/OSAHS doubles, during which the pharyngeal airway becomes longer in postmenopausal women as compared with premenopausal women, and it is at this point the prevalence differences between sexes becomes more equal.[61]

Hypotestosteronaemia is associated with obesity, in particular abdominal fat, and studies have shown that men with OSA have lower testosterone levels compared to age-matched subjects without OSA.[62] This may be due to adipose tissue expressing aromatase, which converts testosterone to 17 β -estradiol when in an inflamed and insulin-resistant state.[63] Furthermore, obesity reduces the levels of sex hormone-binding globulin (SHBG), which carries testosterone in the bloodstream, due to obesity-associated hyperinsulinemia.[64] Although obesity leads to a lower testosterone level, low testosterone may also promote obesity. A bidirectional relationship between obesity and low testosterone underpins this association, as indicated by the hypogonadal-obesity cycle and weight loss leading to increased testosterone levels.[62] However it has also been reported that the AHI increased in hypogonadal men following testosterone administration. This may be due to the acute administration of testosterone increasing the ventilatory response to hypoxia and hypercarbia, subsequently enhancing ventilatory instability and increasing predisposition to OSA.[65] Consequently, testosterone replacement therapy is used cautiously in obese SDB patients.

Despite these anatomical and physiological differences between sexes, there is concern that the distinct clinical presentation and sex-specific differences in symptoms may lead to underdiagnosis of SDB in females and account for the differences in prevalence. Women are less likely to report snoring or witnessed apnoeas and are more likely to complain of daytime fatigue and asthenia, rather than fulminant daytime somnolence.[66] Furthermore, compared to males, females have lower AHIs and shorter apnoeic episodes, and report sleepiness at relatively low levels of AHI.[67, 68] Consequently, the AHI alone may not be sufficient for categorising the overall severity of OSAHS in females. Furthermore, the rising awareness of OSAHS and obesity

amongst primary care practitioners who are acutely aware of the classical phenotype of an OSAHS patient (middle-aged, obese, male) could have possibly led to a lower threshold for these to be referred onwards to a secondary sleep service than a female.

Age

The prevalence of SDB increases with age and is estimated to be two-fold greater in those over the age of 65 years, compared to a working population of between 30-39 years of age.[69] The increase in sleep-related obstructive events may be due to a generalised age-related decrease in the size of the upper airway lumen in older people. Furthermore, ageing leads to lengthening of the pharyngeal airway, and the descent of the hyoid bone.[70] Interestingly, older people with OSA typically have a lower body mass index (BMI) and neck circumference, compared with younger patients with similar disease severity.[61, 70] Muscle tone and function weakens with age. We also know that ageing leads to an increased collapsibility of the upper airway independent of BMI, which is likely contributed to by a decreased response of the genioglossus muscle (an upper airway muscle) to negative pressure in older age. Moreover, the respiratory arousal-response to hypoxia is also reduced in older people compared to their younger counterparts.[61, 70]

Other risk factors

It is likely that alongside the variable upper airway narrowing secondary to anatomical factors, impairment in one or more of the non-anatomical traits can cause or influence its severity. Advances in physiological testing have led to greater awareness of factors such as impaired upper airway muscle function during sleep, unstable respiratory control, and a low respiratory arousal threshold. These are all likely to play a contributory role.[55]

Ethanol ingestion increases the frequency and duration of apnoeas through a combination of a reduction in upper airway dilating muscle activity and a depressant effect on the reticular activating system, which impairs respiratory-related central neural drive and arousal. Similar effects have been noted with medications such as benzodiazepines.[71] Smokers are three times more likely to have SDB, than non-smokers, and it is hypothesised that is due to upper airway inflammation, fluid retention in the upper airway, and the relaxation of the upper airway muscles and

increased arousal threshold induced by nicotine.[72] We know of no studies on the effects of stopping smoking and OSAHS, and it is possible that the improvement in any upper airway oedema would be limited by the likely weight gain during a quit attempt.

2.4.3 OSA, intermittent hypoxia and sleep fragmentation

OSA is a model of intermittent hypoxia characterised by cycles of hypoxia and re-oxygenation of short duration (15-120 seconds) occurring during sleep often for many years. This repetitive nocturnal hypoxaemia is associated with activation of a number of neural, humoral, thrombotic, metabolic, and inflammatory disease mechanisms, all of which may interact to potentiate the development of cardiac and vascular disease.[73] Chronic hypoxia (CH), as seen in chronic lung diseases such as chronic obstructive pulmonary disease (COPD), has well-defined protective mechanisms to attempt and overcome the initial hypoxic insult. The sustained cellular hypoxia seen in CH leads to the activation of hypoxia-inducible factor-1 (HIF-1), resulting in increased expression of a number of gene encoding proteins such as erythropoietin (EPO), vascular endothelial growth factor (VEGF), and inducible nitric oxide synthase, which mediate an adaptive response to the sustained hypoxia.[74]

Depending on the frequency of cycles, the depth of the hypoxic period, and the duration of the exposure, IH can induce both beneficial and detrimental effects. For example, short exposures to low-frequency IH with mild hypoxia improves endurance performance of athletes, facilitates high-altitude acclimatisation, and may be cardio and neuroprotective through pre and post-conditioning effects.[75-79] However, the typical pattern of IH in OSA with repetitive short cycles of IH with deeper desaturations and prolonged exposure, termed chronic intermittent hypoxia (CIH), leads to the formation of increased levels of reactive oxygen species (ROS) and numerous pathophysiological consequences, without the same compensatory mechanisms that occur in CH. Various clinical studies demonstrate the superiority of markers defining the severity of IH in predicting adverse cardiovascular outcomes over the traditional AHI.[80]

The chronic intermittent hypoxia (CIH) seen in OSA promotes a systemic inflammatory state, increasing the expression and concentration of several inflammatory stress markers, increasing vascular cell adhesion molecules such as VCAM-1, and consequently causing metabolic dysregulation, endothelial dysfunction, insulin resistance, and a hypercoagulable state.[81-83] Both animal and human models of chronic intermittent hypoxaemia appear to show a significant role in the pathogenesis

of OSA comorbidity, including hypertension, cardiovascular events, diabetes, neurocognitive impairment, and cancer.[84, 85] CIH increases the expression of oxygen-free radicals (ROS) which are keys in the proinflammatory response, which contribute to the generation of adhesion molecules and activation of leukocytes.[86, 87] In OSA, inflammatory factors such as nuclear factor kappa B (NFkB) are selectively activated at a systemic level rather than HIF-1, the transcriptional regulator of the adaptive pathway, leading to increased expression of tumour necrosis factor (TNF- α), interleukins 1, 6, and 8, C-reactive protein, intracellular and vascular cell adhesion molecules, E selectin, CD15, and inducible nitric oxide synthase (iNOS).[88, 89] Furthermore, in IH, the extent of hypoxia is not sufficient to allow HIF-1 stabilisation, and possibly through mitochondrial stress, results in the activation of this NFkB pathway.[83] The increase in ROS is a similar phenomenon observed in myocardial ischaemia followed by reperfusion, where xanthine oxidase generates free radicals in the presence of oxygen contributing to oxidative stress.[90] Furthermore, all these mechanisms may interact with each other, thus potentiating their pathophysiological implications. Chronic exposure to CIH can deplete the carotid bodies of nitric oxide, enhance the sensitivity of the peripheral chemoreceptor reflex, and increase pulmonary vascular resistance.[91] Endothelial dysfunction is frequently recognised in an OSA patient, often before any clinical manifestation of cardiovascular disease.[92]

Both oxidative stress and inflammation are fundamental mechanisms in various pathologies seen in OSA, inducing vascular dysfunction, transcriptional reprogramming, inflammation, and innate and adaptive immune activation.

2.5 OSA and Cardiovascular Disease

OSA and cardiovascular diseases (CVD) commonly co-exist with observational studies showing OSA is independently associated with CVD, and that untreated OSA is associated with an increased risk of cardiac events, including sudden cardiac death. [93-95] OSA is significantly common among patients with established hypertension (>30%), heart failure (40%), coronary artery disease, and arrhythmias (30%). [94, 96-100] In fact, moderate or obstructive SDB can be detected in a third or more of patients with primary hypertension and in up to 80% of individuals with drug-resistant hypertension. [101] Furthermore, OSA is also associated with various conditions that increase the risk of CVD themselves, such as atherosclerosis and hyperlipidaemia. [102, 103] Figure 2.7 shows the prevalence of OSA in CVD. [103]

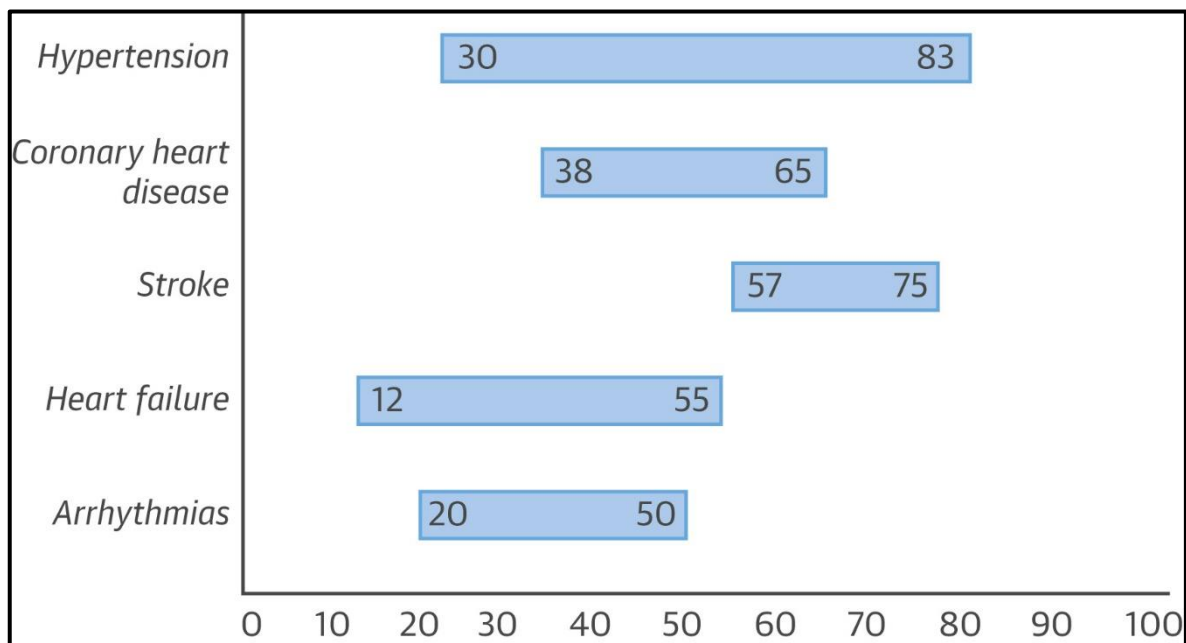


Figure 2.6: Prevalence (%) of OSA in CVD.

Reproduced with permission from Javaheri, S., et al., *Sleep Apnea: Types, mechanisms, and Clinical Cardiovascular Consequences*. Journal of the American College of Cardiology, 2017. 69(7): p. 841-858.

On a more superficial physiological level, we know that upper airway obstruction secondary to pharyngeal occlusions triggers a significant compensatory response in the cardiovascular system. The fall in intrathoracic pressure due to a reduction or cessation in airflow and inspiring against a closed glottis, results in increased blood flow into the thorax (mainly from lower limb veins); this causes significant haemodynamic changes by increasing right ventricular filling and atrial dilation and putting acute stress on the left ventricle of the heart as well as increasing the sympathetic tone.[104] This phenomenon is enhanced by the vasoconstrictor response after an apnoea due to an increase in the vagal reflex. In addition, an apnoea also simulates an adrenergic discharge from the central nervous system, which is a sympathetic stimulus causing systemic vasoconstriction and changes in heart rate.[105] Hypoxaemia can occur if the apnoea is long enough, which can further increase the vagal tone, and any subsequent hypercapnia stimulates the chemoreceptors and causes sympathetic excitation and the release of neural norepinephrine.[105] Interestingly, higher levels of sympathetic tone persist during the wakefulness period in patients with OSA. All these mechanical -autonomic interactions, caused after each obstructive event, acutely predispose the patient to cardiac ischaemia and atrial and ventricular arrhythmias. [106]

2.5.1 Hypertension

Hypertension remains the leading cause of death globally, accounting for 10.4 million deaths per year. [107] It affects a quarter of the adult population, and remains a leading cause of cardiovascular mortality, with half of all strokes and ischaemic heart disease being attributable to high blood pressure (BP).[108, 109]

Healthy sleep onset is usually accompanied by a physiological reduction or dipping of the blood pressure reaching about 10-20% of the values recorded during daytime. The pathophysiology of systemic hypertension in OSAHS is complex with intermittent hypoxaemia, the renin-angiotensin system, chemoreceptor stimulation, and sympathetic activation appearing to be associated with raised blood pressure in these patients.[110] It is believed that the IH associated with SDB causes increased sympathetic tone whilst impairing baroreflex gain, which is confounded by stimulation of the renin-angiotensin-aldosterone system causing alterations in arterial vasoconstriction and vasodilation.[111, 112] In fact, animal studies have shown that sympathetic activity is the most likely mechanism which may lead to elevated blood pressure, with Brooks et al demonstrating by inducing OSA in canines that acute transient rises in nocturnal blood pressure resulted in sustained daytime hypertension.[113]

There is evidence that OSAHS is a stronger independent risk factor for hypertension than any other cardiovascular disease state and, moreover, that treatment with CPAP can reduce blood pressure.[114, 115] In the clinical series of Grunstein et al., in which subjects were recruited from general practices, the AHI was associated with hypertension independently of age, obesity, and other confounding variables.[116, 117] A cross sectional analysis of 6132 subjects from The Sleep Heart Health Study reported that the odds ratio for hypertension after adjusting for demographics and anthropometric variables (including BMI, neck circumference, and waist-to-hip ratio), as well as for alcohol intake and smoking, was 1.37 (95% CI 1.03 TO 1.83), when comparing subjects with an AHI >30 events per hour with those with an AHI <1.5 events per hour.[118] Furthermore, the Wisconsin prospective sleep cohort of 709 subjects showed a dose-response relationship between sleep-disordered breathing at baseline and the presence of hypertension four years later, with 'mild-moderate' (AHI

5-14.9) and 'moderate-severe' ($AHI \geq 15$) SDB having adjusted odds ratios of 2.03 (95% CI 1.29-3.17) and 2.89 (95% CI 1.46 to 5.64) respectively for developing hypertension over the next 4 years, compared with subjects without SDB.[119] More recently, the meta-analysis by Pengo et al. identified younger age (<60 years), uncontrolled BP at baseline, and severe OSA-related oxygen desaturations (minimum arterial oxygen saturation measured by pulse oximetry <77% at baseline) as positive predictors of a favourable BP response to OSA treatment.[120]

In the study of Lavie et al., a total of 2677 adults with suspected OSA had inpatient nocturnal PSG alongside twice daily blood pressure measurements. It found that blood pressure and the number of patients with hypertension increased linearly with severity of sleep apnoea, showing that apnoea was a significant predictor of both systolic and diastolic blood pressure after adjustment for age, body mass index, and sex.[121] Multiple logistic regression further showed that each additional apnoeic event per hour of sleep increased the odds of hypertension by approximately 1%, whereas each 10% decrease in nocturnal oxygen saturation increased the odds by 13%.[121] Understandably, SDB is now an important part of both the European and American guidelines as an identifiable and treatable cause of secondary hypertension, however treating patients without tiredness would be a deviation from current sleep-disordered breathing guidelines[122, 123]

2.5.2 Heart failure

Heart failure (HF) affects up to 23 million people worldwide and SDB is highly prevalent in patients with HF, with studies indicating a prevalence between 50% and 70%.[124] Notably, CSA accounts for approximately two thirds of the sleep apnoea observed in this population, while OSA comprises a significant minority. While CSA and OSA coexist in a significant proportion of cases, one acts as the dominant phenotype in the majority of patients.[124] In 2006, Javaheri found that 49% of patients with a left ventricular ejection fraction of <45% had severe SDB (defined as an AHI >44/hour), with 37% having CSA and 12% OSA, with or without daytime tiredness.[125] The changes in intrathoracic pressure during an episode of mechanical obstruction to airflow causes an increase in the left ventricular transmural pressure, which increases afterload. This drop in intrathoracic pressure subsequently increases venous return, causing right ventricular distention with a consequent leftward shift of the interventricular septum and consequent decreased LV filling. This resulting decreased LV filling and increased afterload results in a reduced stroke volume, and in combination with the known blood pressure and heart rate fluctuations and increased sympathetic nervous system activity, results in a mismatch between myocardial oxygen demand and supply, chronically exposing the individual to left ventricular hypertrophy, enlargement, and heart failure.[89]

2.5.3 Ischaemic Heart Disease

Atherosclerosis, a disease of the large arteries, is the primary cause of ischaemic heart disease and stroke. It is a chronic, progressive inflammatory disease characterised by the accumulation of lipids and fibrous elements in the large arteries. The mechanism leads to the formation of atherosclerotic plaques involving complex interactions between systemic inflammation, endothelial dysfunction, and haemostatic mechanisms including platelets and coagulation pathways.[126] A cross-sectional analysis of 6424 participants from 'The Sleep Heart Healthy Study' demonstrated a strong association between OSA and coronary heart disease with an odds ratio of 1.27 (95% CI 0.99-1.62) and 0.92 (95% CI 0.71-1.20) in those with an AHI>11 and those with AHI<4.5 respectively.[127] A subsequent Swedish longitudinal prospective cohort study followed up 182 middle-aged men with or without OSA over a 7 year period, with all participants free of hypertension or other CVD, pulmonary disease, diabetes mellitus, psychiatric disorder, and alcohol dependency found that OSA at baseline was a significant predictor of future incidence of CVD (OR 4.9; 95% CI 1.8-13.6) independent of age, BMI, blood pressure and smoking status, with multiple regression analysis showing a significant risk reduction for CVD incidence (OR 0.1; 95% CI, 0.0-0.7) following effective treatment with CPAP (OR 0.1; 95% CI, 0.0-0.7). [128]

Interestingly, Computed tomography (CT) in patients with OSA has revealed larger coronary plaque burdens and a larger lipid core compared to patients without OSA.[129] A retrospective analysis of 81 patients, 49 with OSA (mean AHI 42.2, BMI 34.4) and 32 without OSA (AHI 7.5, BMI 34.4), who had undergone multidetector-row helical computed tomography scanning, found that the frequency of noncalcified/mixed plaques is much higher in patients with OSA than in non-OSA with an odds ratio of 7.0 (1.9-26.5; $P < 0.05$) after adjustment for other risk factors such as age, sex, race, hypercholesterolemia, and history of smoking. Furthermore, patients with OSA also had more severe stenosis and a higher number of vessels involved (85.7% having multiple vessels involved in OSA vs 34.5% non-OSA) with an adjusted OR for having multiple vessels of 8.5 (95% CI: 2.3-30.8). In addition, 22.5% of patients with OSA had $\geq 70\%$ stenosis of the highlighted vessel, compared to only 6% of those without.

Intravascular ultrasound of coronary vasculature has also suggested that patients with OSA have a larger coronary atherosclerotic plaque volume than patients without OSA, with no significant difference between groups in terms of anthropometric parameters, lipid and glycaemic profiles or smoking habits.[130] Furthermore a retrospective analysis of optical frequency-domain imaging (OFDI) performed during percutaneous coronary intervention (PCI) for coronary artery plaques has suggested that OSA is associated with unstable plaque characteristics, theoretically increasing the risk of thrombotic events such as myocardial infarction, and that more intensive medical management for stabilisation of coronary atherosclerotic plaque is needed in patients with OSA.[131] However, the sample size of this retrospective, cross-sectional study conducted at a single medical centre was small (n=70), and by the author's own admission, there is an inherent discrepancy between characteristics assessed by OFDI and actual histopathological findings.

2.5.4 Arrhythmias

Cardiac rhythm disorders (arrhythmias) are common among patients with OSA than the background population, and it is postulated that arrhythmias can be precipitated by both the acute pathophysiological changes seen in OSA such as swings in intrathoracic pressure and surges in sympathetic activity, as well as the more chronic issues of IH with the formation of ROS causing more systemic widespread inflammation. Compared with those without sleep-disordered breathing and adjusting for age, sex, body mass index, and prevalent coronary heart disease, individuals with sleep-disordered breathing had four times the odds of atrial fibrillation (odds ratio [OR], 4.02; 95% confidence interval [CI], 1.03-15.74), three times the odds of non-sustained ventricular tachycardia (OR, 3.40; 95% CI, 1.03-11.20), and almost twice the odds of complex ventricular ectopy (OR, 1.74; 95% CI, 1.11-2.74).[132]

In 1983, Guilleminault et al. performed 24-hour Holter electrocardiography (ECG) and simultaneously recorded a polygraph during late afternoon or nocturnal sleep on 400 patients with OSAHS. Of the 400 patients, 48% had documented cardiac arrhythmias, including sustained ventricular tachycardia in 2%, sinus arrest in 11%, second-degree atrioventricular block in 8%, and frequent premature ventricular contractions in 19%. This compares to a prevalence of 1.5% to 5% of arrhythmias in the general population.[133] The mean number of apnoeic events, age, weight and lowest oxygen saturation during sleep were not significantly different in those with arrhythmias. Remarkably, a tracheostomy, a significant surgical treatment modality pre-dating CPAP, abolished the SDB and eradicated these arrhythmias in the lucky (or unlucky) 50 patients.[134]

Atrial fibrillation (AF) is widely reported in OSAHS, with atrial distension and cardiac chamber remodelling being the anatomical substrates of its pathogenesis.[106] Individuals with sleep apnoea have 2- to 4-fold higher odds of having AF than those without sleep apnoea, even after adjustment for potential confounders, with the incidence of AF in patients with SDB being strongly predicted by obesity, nocturnal desaturation in subjects <65 years old, and by heart failure in older subjects.[132, 135] OSA has also been recognized as a risk factor for the beginning and progression of AF

and reduces the effectiveness of antiarrhythmic drugs, electric cardioversion (EC), and catheter ablation (CA) in AF.[136]

As with non OSAHS people, embolic stroke and thromboembolism are the main complications if AF is left untreated or inadequately managed. Furthermore, this disorganised electrical activity in the atria results in ineffective ventricular failure, reducing the cardiac output by as much as 20%, and a large meta-analysis in 2016 (data from 64 studies with 1,009,501 participants) found that AF in all individuals was associated with a 46% increase in all-cause mortality (relative risk 1.46, 95% CI 1.39 to 1.54), and an 88% higher risk of sudden cardiac death (relative risk 1.88, 95% CI 1.36 to 2.60).[137] Among the outcomes examined, the highest absolute risk increase was for heart failure (relative risk 4.99, 95%CI 3.04 to 8.22), with the associations between atrial fibrillation and included outcomes being broadly consistent across subgroups and in sensitivity analyses.[137]

A meta-analysis of 1087 patients from seven prospective cohort studies by Shukla et al. found that the use of CPAP is associated with a 42% relative risk reduction in AF recurrence in patients with OSA independent of medical or catheter ablation therapy, and is consistent across patient groups with OSA.[138] The impact of CPAP on AF was so profound that the authors advocate for active screening for undiagnosed OSA in patients with AF when OSA is clinically suspected. The use of CPAP offers to be the third potential treatment option for AF (in addition to medical therapy and PVI), and although results from RCTs may be warranted to understand the true extent of its efficacy, its use and adherence needs to be promoted aggressively in these patient groups.

2.6 Clinical Presentation

The symptoms of OSA usually begin insidiously and are often present for years before the patient is referred for evaluation.[139] Most patients with OSA complain of daytime sleepiness, or their bed partner reports loud snoring, gasping, choking, snoring, or interruptions in breathing while sleeping.[140] Table 2.2 is a list of the common daytime and nocturnal symptoms seen in OSAHS.[18]

Table 2.2: Common daytime and nocturnal symptoms of OSAHS

Clinical symptoms and signs	Prevalence (%)
Excessive sleepiness/unrefreshing sleep	73-90
Snoring most nights	50-60
Apnoeas, choking, or gasping during sleep	10-15
Nocturia (2 or more times per night)	30
Morning headache	12-18

A history of snoring and witnessed apnoeas with symptoms of sleep fragmentation, such as excessive daytime sleepiness, is suggestive of OSAHS. However snoring is very common in the general population and affects up to 45% of men and 28% of women.[34, 141] It is also the most frequent symptom of OSAHS, occurring in up to 90% of patients.[142] However, because of its high prevalence in the general population, it is not specific and so is a poor predictor in itself for OSAHS. Conversely, the absence of snoring makes OSAHS less likely but does not exclude it because at least 6% of patients with OSAHS with tiredness and raised AHIs on sleep study do not report snoring.[143]

Excessive daytime sleepiness is caused by fragmented sleep related to frequent arousals, and like snoring, is a poor discriminator of the patient with OSAHS, as 30 to 50% of the general population without OSAHS report moderate to severe daytime sleepiness. [34, 144] A variety of conditions similarly present with excessive daytime sleepiness, which generally can be distinguished from OSAHS via clinical assessment and appropriate investigations including sleep studies.

It is important to remember that the symptom of tiredness can have many causes e.g., medical, psychological, environmental, and is often multifactorial, and so it is imperative that all patients with sleepiness are screened for other causes including for lifestyle factors (bedtime routine, sleep patterns, alcohol, and caffeine misuse etc) and especially prescribed (and non-prescribed e.g. over-the-counter and illicit) drugs that can cause tiredness/sleepiness.

2.7 Diagnosing OSAHS

OSAHS is a heterogenous and complex disease with the diagnosis requiring screening questionnaires and symptom review (subjective), an overnight sleep study (objective), and then clinical review of the case by an experienced sleep practitioner.

2.7.1 Subjective

Questionnaires to assess medical history, sleepiness, and quality of life (QOL) are valuable tools to define the functional impact of sleep-related breathing events in patients with SDB. Assessment scales are used to help with the identification of OSAHS and OHS and enable healthcare professionals to assess patients in a standardised way to help ensure only those most likely to have one of these conditions are referred onward to a sleep clinic for further investigation and diagnosis.

Introduced in 1991, the Epworth Sleepiness Scale (ESS) is the most widely used tool to measure sleepiness. It is a self-administered eight-item questionnaire, which provides a measure of the general level of sleepiness by asking people to rate their usual chances of dozing off or falling asleep in 8 different situations on a 4-point scale (0-3). The maximum score is 24, with a score of 10 or more suggesting excessive sleepiness.[145]

The ESS is intended to assess for sleepiness rather than to diagnose OSAHS, and is poor to modest both for sensitivity (45%) and specificity (81%) with a cut-off point of 8/24 in diagnosing impactful sleepiness and possible OSAHS.[146] However, assessment of sleepiness is vital for determining treatment and especially assessing response to treatment and the ESS is the standard tool for doing so.

During each visit to our sleep service, we ask the patient to complete our locally devised questionnaire which combines day and night-time symptoms and ask them to report both on the frequency and severity of them. The questionnaire also incorporates the ESS, as well as specifically asking about occupation, co-morbidities, medications, and tiredness whilst driving (Appendix 1).

2.7.2 Objective

Sleep studies performed in the home can quantify the apnoea-hypopnoea index, which is required to diagnose OSA.[19] The AHI is then used to stratify the severity of OSAHS in to normal (<5 events per hour), mild (>5-14 events per hour), moderate (15-29 events per hour), and severe (>30 events per hour).[14]

Our sleep unit uses the American Academy of Sleep Medicine (AASM) 2020 scoring manual when analysing a limited channel sleep study Apnoea-Link™ (ResMed, San Diego, USA), which includes the following parameters.[147]

- Sleep studies should be ≥ 4 hours in duration.
- Any periods of artefact or periods where the patient is awake should be removed from the study.
- Small periods of movement (where patient is assumed to be asleep) should not be removed from the study so as not to overestimate AHI.
- SpO2 desaturations should be marked if there is a >3% oxygen desaturation from pre-event baseline.
- Mark an apnoea if there is at least a >90% reduction from pre-event baseline in airflow for at least 10 seconds. An Apnoea can be marked without a desaturation however to do so, you need either an oronasal cannula or respiratory inductance plethysmography (RIP) showing reduction in effort/paradoxical breathing.
- Score as an obstructive apnoea if it meets above criteria and is associated with continued/increased effort throughout its duration. RIP flow will show paradoxical breathing.
- Score as a central apnoea if it meets above criteria and is associated with absent respiratory effort for the duration of the event.
- Score as a mixed apnoea if there is a reduction in airflow of >90% and is associated with absent respiratory effort at the beginning of the event, followed by respiratory effort resuming for the second half of the event. There is no evidence regarding duration of mixed apnoeas so it does not need to be a specific duration.
- Hypopnoeas should be marked if there is either:

- A 50% reduction in nasal airflow from pre-event baseline for at least 10 seconds with a >3% SpO2 desaturation OR
- There is a 30% reduction in nasal airflow from pre-event baseline for at least 10 seconds with a >4% SpO2 desaturation.

Additional points:

- Any periods where there is no SpO2 data, the nasal flow/RIP flow should be excluded, or the AHI would be underestimated. Therefore, if there is no SpO2 data for the study's entirety, the study must be repeated.
- In the absence of nasal flow only, you can analyse the SpO2 data as with an overnight oximetry. However, if the oximetry data is negative for OSA, it is recommended to repeat the study.

2.7.3 Clinical review

Following their overnight sleep study, those that have a positive respiratory polygraphy ($AHI \geq 15$) with symptoms consistent with OSAHS such as excessive daytime somnolence, sleep fragmentation, poor sleep quality, waking unrefreshed etc would then usually be started directly on treatment with a continuous positive airways pressure (CPAP) device. In more nuanced cases where the AHI falls between 5 and 15 which is considered to be a mildly positive test result, clinical discretion through discussion at a weekly sleep multi-disciplinary meeting (doctors and physiologists) is applied in deciding whether patients should proceed for a trial of treatment, focus on lifestyle measures only such as weight loss and/or a reduction in sedating drugs, and/or be followed-up within the next 12 months.

Individuals with a negative sleep study ($AHI < 5$) are usually discharged directly from the service after a consultant review of the referring history and sleep study, with their symptoms of sleepiness shown not be directly attributable to SDB at this time.

Those patients with milder disease who are unable or unwilling to use CPAP are referred for a bespoke mandibular advancement device (MAD) through a regional orthodontist service, as a second line treatment.

Patients with features of OHS (profound prolonged periods of hypoxia) and a daytime $PaCO_2 > 7.5 \text{ kPa}$ are started on bi-level, non-invasive ventilation.

2.8 Management of OSA/OSAHS

Despite the remarkably high prevalence of SDB in the general population, and OSA being a heterogenous disorder, the current available treatment modalities are limited.

2.8.1 Conservative management

The most important intervention for OSA in obese individuals is weight loss. Weight loss results in changes in pharyngeal anatomy and function that decrease airway collapsibility and airflow resistance. The critical upper airway pressure at which inspiratory airflow ceases is increased by weight loss and this correlates directly with the attenuation of OSA. Even modest reductions in weight are likely to result in significant improvements in OSA.[54]

Excess weight is the main modifiable risk factor in OSA, and RCTs have shown that weight loss and physical activity improve sleep-disordered breathing in OSA. Weight loss, including that from bariatric surgery, has also been shown to improve overall health and metabolic parameters, reduce BP, and probably decrease daytime sleepiness.[148] In the Wisconsin Sleep Cohort, 690 subjects (mean age at baseline, 46 years; 56% male) were evaluated twice at 4-year intervals, which found that a 10% weight gain predicted a 32% increase in AHI, whereas a 10% weight loss was associated with a 26% reduction in AHI.[149] It is also postulated that obesity worsens the magnitude of IH associated with obstructive events, which could accelerate the development of cardiovascular co-morbidities.[150]

The mortality and morbidity directly associated with obesity have been well documented and despite a worsening obesity epidemic for many years, there are now an increasing number of drugs being approved for the pharmacological management of obesity in Europe. However, the importance of behavioural therapy and weight management programmes with or without pharmacological treatments remain the mainstay of treatment. Bariatric surgery is an effective method to obtain long-term weight loss, with several different surgical procedures including laparoscopic adjustable gastric banding and Roux-en Y gastric bypass being used in the management of morbid obesity.[151] The effectiveness of bariatric surgery is demonstrated immaterial of the exact surgical technique used, with the weight loss, improvement in metabolic complications, and reduction in inflammatory status, all leading to an improvement in OSA.[151] In 2009, a meta-analysis by Greenburg et al. identified twelve studies representing 342 patients undergoing bariatric surgery and found that a 32% reduction in BMI from 55.3 (95% CI 53.5-57.1) to 37.7 (36.6-38.9)kg/m was

associated with an AHI reduction of 71%.[152] However, the mean AHI index after surgical weight loss was consistent with moderate OSA, and therefore patients should not expect a cure of their OSA after surgical weight loss. Nevertheless, weight loss (immaterial of mechanism) and exercise should be recommended to all patients with OSA who are overweight or obese.

An internal survey suggested less than 5% of our patients could lose enough weight and keep the weight off for more than 6 months, to allow a negative sleep study and return of CPAP machine. (personal communication from our sleep service-unpublished).

Immaterial of their sleep study result, all patients need to be advised regarding the risk of obesity, alcohol consumption, and the importance of smoking cessation, with support needing to be offered at every opportunity.

Furthermore, medication reviews should be carried out to reduce or stop any medications that could worsen tiredness and/or sleep disordered breathing. There is particular focus on drugs that have inhibitory effects on the central nervous system e.g. opiates, benzodiazepines, certain antidepressants and most recently pregabalin and gabapentin.[153]

Patients should also be counselled on the importance of sleep hygiene despite a questionable evidence base for this, with the emphasis on forming regular sleep patterns to aid sleep initiation and maintenance.

2.8.2 Positive Airway Pressure

Elimination of apnoeas and hypopnoeas with positive airway pressure (PAP) is the first-line treatment for OSA, reducing daytime sleepiness and improving quality of life.[154] PAP is the mainstay of therapy for adults with OSA and involves maintaining upper airway patency regardless of the exact pathophysiology. Continuous positive airway pressure (CPAP), the most common PAP device used, helps maintain a positive intraluminal pressure which exceeds the surrounding pressure, thus maintaining airway patency, and stabilising the upper airway through increased end-expiratory lung volume (see figure 2.8).[14]

Prior to the non-invasive treatment of SDB with a CPAP machine, surgical intervention was the most common treatment option. Potential surgery would involve removal of the part of the UA thought to be compromising patency during sleep, with some patients being left with a permanent tracheostomy if small volume surgery was inadequate. It wasn't until 1980, Dr Colin Sullivan, first tested the idea of positive pressure applied through the nasal airway after being approached by a "perfect patient", who was strongly advised tracheostomy for his severe OSAHS, but his family refused. Instead, they opted for a trial of one of Sullivan's pressure machines, which anecdotally had only been used on dogs previously, with the machine allegedly comprising of parts from a vacuum cleaner, which had its airflow reversed, connected to tubing and a face mask.[155] The patient apparently woke up the next morning reporting feeling aware and alert for the first time in many years, and in 1981 Sullivan et al. released their ground-breaking results of CPAP in SDB in 5 patients in the Lancet journal.[156]

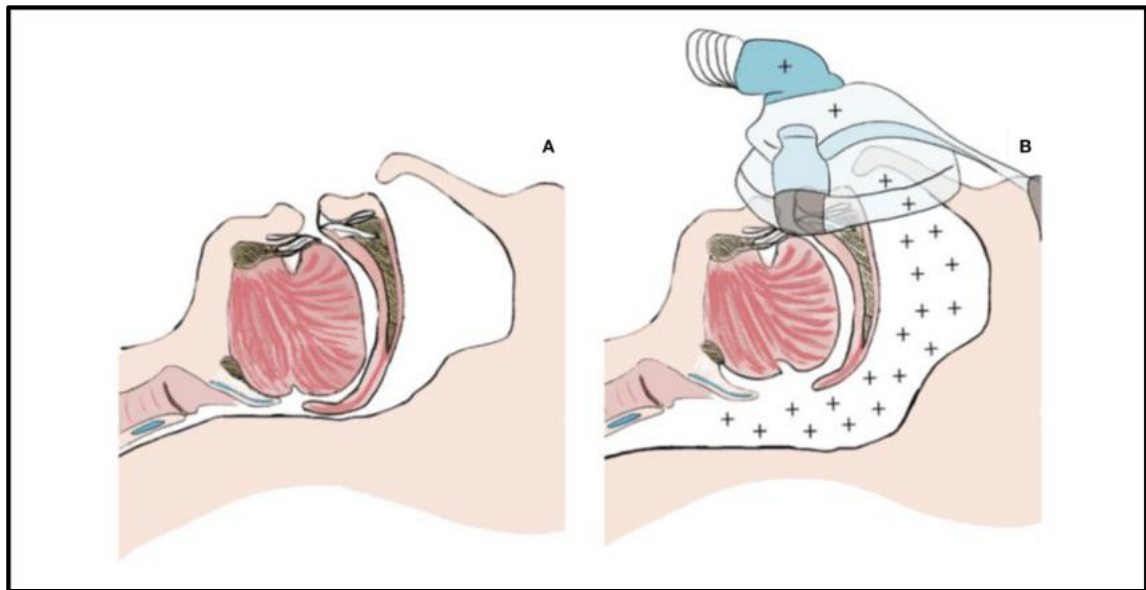


Figure 2.7: (A) Collapsed airway and (B) Open airway with a continuous positive airway pressure (CPAP).

Reproduced from Kazemeini, E., et al., *Critical to Know Pcrit: A Review on Pharyngeal Critical Closing Pressure in Obstructive Sleep Apnea*. Frontiers in Neurology, 2022.

CPAP is by far the most effective and cost-effective treatment for OSAHS and OHS. This led to the latest NICE guidelines [NG202] published in August 2021, not advocating for further research in to alternative treatment options in its 9 key recommendations for future research.[21] A detailed cost effectiveness model of using CPAP in the management of severe OSAHS compared to no treatment estimated that 57% of untreated patients are expected to be alive at the end of 14 years compared with 72% of patients treated with CPAP.[157] It projected that these untreated patients are expected to cost the NHS £10,645 (95% CI £7988 to £14,098) per patient over 14 years due to the risks of leaving the condition untreated e.g. cardiovascular risks, road traffic accidents etc. compared with £9672 (95% CI £8057 to £12,860) per CPAP-treated patient. This indicated that the use of CPAP over 14 years is estimated to afford the NHS a cost-effective technology since, after 2 years of treatment with CPAP, the cost per QALY gained is <£10 000 and, and after 13 years of treatment, CPAP becomes the dominant treatment (.i.e. more effective than no treatment for less cost). These findings are concordant with those from four other studies, all of which found CPAP to be a cost-effective treatment. Further sensitivity analyses showed that the relative cost-effectiveness of CPAP is sensitive to continuation rates. Thus, any intervention that improves long-term take-up rates such as better patient education is worth employing.

The modern-day CPAP device consists of a unit that generates airflow which is directed to the airway via a mask, of which there are two types: fixed and auto-titrating. Fixed CPAP devices deliver air at constant pressure throughout the night, and the person will continue to receive this pressure until a further titration study is performed, and auto-titrating, where pressures are continually adjusted throughout the night, with the aim of improving comfort and ultimately adherence. The price of CPAP devices currently ranges from £250 to £550, with each device having an estimated lifespan of 7 years, and requiring new consumables (e.g. new mask) at least annually.[158]

For CPAP treatment to be fully effective, the person must wear their device for the majority of time when they go to sleep, with nonadherence generally being defined as using their devices for less than an average of four hours per night or less than 70% of nights (i.e. less than five nights each week).[159] Patients are subsequently advised to use their devices for a minimum of four hours per night at least five nights per week,

with this four-hour cut-off derived from being used in the previous CPAP studies showing an improvement or normalisation in daytime sleepiness, quality of life, CVD, and diabetes.[160-162] As a result, it has subsequently been adopted by the Driver and Vehicle Licensing Agency (DVLA), as well as insurers for quantification and objective purposes.

There is high quality evidence from randomised trials and meta-analyses that in most adults, including the elderly, PAP therapy reduces the frequency of respiratory events during sleep, decreases daytime sleepiness, improves systemic blood pressure, lowers the risk of road traffic accidents, and improves quality of life.[156, 159, 163-166] One of the most dramatic immediate effects of any medical treatment is the ability of a CPAP device to reverse obstructive respiratory events and improve daytime tiredness, with patients anecdotally and in our sleep clinics, describing the improvement as an emergence from a “daytime fog” and being able to live a productive and healthy life-almost immediately. In 2006, a meta-analysis of 22 randomised trials (1160 patients) by Giles et al. showed nocturnal CPAP significantly improved sleepiness, cognitive function, mood, and quality of life compared to controls (sham CPAP, placebo tablets, or conservative management).[164] This was superseded by a 2019 meta-analysis by the AASM, which found that CPAP had a significant impact on OSA severity (-23 events per hour; 95% CI -29 to -18 events/hour), ESS score (-2.4 points; 95% CI -2.8 to -1.9 points), nocturnal systolic BP (-4.2 mmHg; 95% CI -6.0 to -2.5mmg), diastolic BP (-2.3mmHg; 95% CI -3.7 to -0.9), and 24 hour mean BP (-2.6 mmHg, 95% CI -3.4 to -1.4 mmHg compared with no therapy).[165]

Furthermore, two interventional (randomised controlled) studies showed a significant fall in blood pressure in patients receiving therapeutic CPAP (set at a pressure that abolished obstructive events) but no change in blood pressure with “placebo” CPAP set at sub-therapeutic pressure settings.[115, 167] In the randomised parallel study by Pepperell et al. of 118 men with OSAHS (Epworth score >9, and a >4% oxygen desaturation index of >10 per h), they found that one month of therapeutic nasal CPAP reduced mean arterial ambulatory blood pressure by 2.5 mmHg (SE 0.8), whereas subtherapeutic nasal-CPAP increased blood pressure by 0.8 mmHg (0.7) (difference -3.3 [95% ci -5.3 to -1.3]; p=0.0013) (figure 2.10).[115] The improvement was seen in

both systolic and diastolic blood pressure, with the benefit correlating strongly with the improvement in sleepiness as with OSA severity.

A randomised placebo-controlled trial by Ziegler et. al on the effects of CPAP treatment on the sympathetic nervous system measured through various indices including heart rate and urinary norepinephrine showed that sympathetic overactivity (e.g. urinary norepinephrine concentrations) is higher in those with OSA, and can be reduced by CPAP therapy.[168]

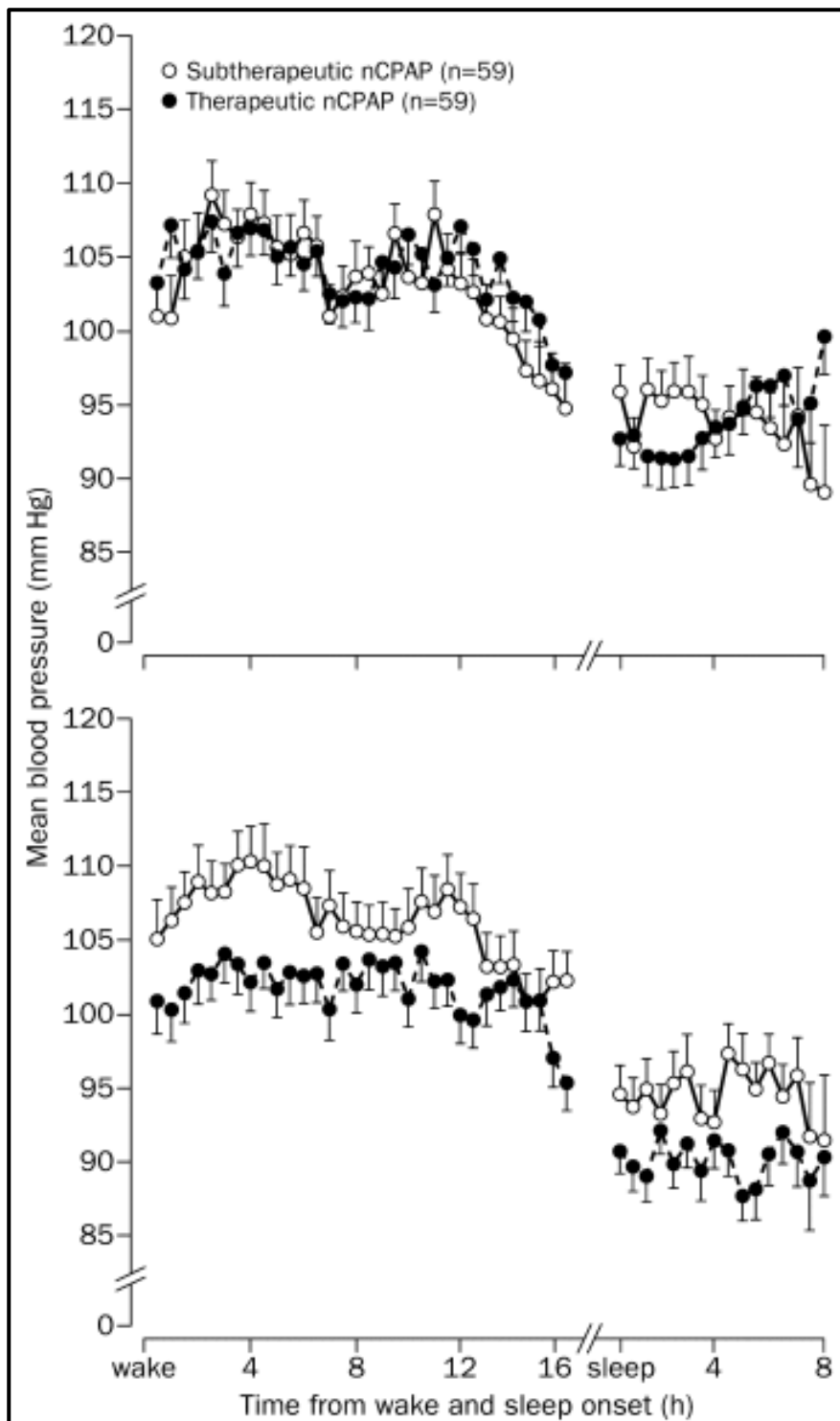


Figure 2.8: Mean ambulatory blood pressure profile before (top) and after (bottom) therapeutic nasal CPAP.

Reproduced with permission from Pepperell et al., *Ambulatory blood pressure after therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised parallel trial*. Lancet, 2002.

2.8.3 Mandibular Advancement Devices

MADs have emerged as the main alternative to CPAP for OSAHS, and for patients with mild or moderate OSAHS who are nonadherent to CPAP therapy.[21] Often, an oral appliance is a reasonable alternative especially in non-severe disease and those with severe nasal blockage. MADs aim to increase the upper airway size by pulling forward the lower jaw (and tongue) and thus reduce obstructive respiratory events, however these devices have a variable clinical response due to inter-individual variations in the UA anatomy and function.[169] There is increasing evidence that MADs constitute an effective therapeutic option for OSA despite its lower efficacy in reducing SDB than CPAP. This is due to their higher compliance rates but definitive RCTs are still lacking.[170]

However, the use of MADs will not be discussed extensively in this thesis as they are not routinely used in our sleep clinics.

2.8.4 Surgical management

Due to the complex, often multi-level upper airway obstruction associated with OSA, surgical interventions have historically been limited to just a few patients who have focal anatomical abnormalities. Consequently, current guidelines advise CPAP as first-line treatment in those with OSAHS, and surgery only to be offered as a primary treatment option in clear-cut cases e.g. non-obese, young, enlarged tonsils, or as a salvage in those with usually severe OSAHS who are unable to tolerate or non-compliant with CPAP.[21]

Newer surgical treatments centred on genioglossus function are currently the mainstay of surgical options and research, with hypoglossal nerve stimulation (HNS) having already showed a sustained and significant improvement in AHI, quality of life and sleepiness.[171] HNS works by delivering an electrical current to the hypoglossal nerve which contracts the genioglossus muscle (the major muscle responsible for tongue protrusion, and all other intrinsic muscles of the tongue).[172, 173] Using general anaesthesia, a neurostimulator is implanted in an infraclavicular subcutaneous pocket and a stimulating lead is placed on the main trunk of the hypoglossal nerve.[173] The neurostimulator delivers electrical pulses to the hypoglossal nerve, with some devices having the ability to be synchronised with respiration.

HNS is a viable second option for those who have moderate or severe OSA with no evidence of complete concentric collapse of the soft palate (as demonstrated on drug-induced sleep endoscopy) who fail or cannot tolerate CPAP treatment. [171] A recent meta-analysis of 350 patients from 12 studies by Costantino et al. indicated the surgical success rates at 55-75%, a reduction of the apnoea hypopnea index (AHI) of 18 events/h, and a reduction in the Epworth Sleepiness Scale (ESS) of 2.9-5.3.[171] [174] Despite the clear benefits, there is still not a substantial quantity of quality evidence, and this treatment modality is significantly more time and cost consuming than CPAP due to the need for a surgical procedure for stimulator placement, multiple follow-up visits, and titration polysomnography. Therefore, the decision to utilize HNS as a treatment for OSA should be based on robust discussions between the patient and an interdisciplinary healthcare team including sleep medicine, and otolaryngology providers.

Other surgical options include tracheostomy, mandibular osteotomy with genioglossus or inferior border advancement, uvulopalatopharyngoplasty (UPPP), laser-assisted uvuloplasty (LAUP), reduction glossectomy, internal and external nasal reconstruction, tonsillectomy and adenoidectomy, and advancement of the upper and lower jaws.[175]

Any potential benefits of these intrusive and potentially dangerous surgeries need to be weighed very carefully against the risk of immediate and long-term side effects.[169] Surgery is therefore an evolving and adaptable intervention, able to address site-specific anatomical considerations in appropriately and carefully selected patients i.e. young, non-obese individuals with minimal other risk factors for their SDB. It is felt that people with a BMI of 35 kg/m² or above are less likely to benefit from upper airway surgery because they are more likely to have multi-level upper airway obstruction, and that the perioperative risk of complications such as higher intubation rates, hypercapnia, significant oxygen desaturation events, cardiac arrhythmias, and the need for monitoring on an intensive care unit, are clear barriers to surgical interventions that do not yet have a strong evidence base.[176]

2.8.5 Pharmacological management

There have been a range of pharmacological studies examining the potential of various drugs on ventilatory drive, arousal threshold, and activation of the upper airway muscles. Although modest-moderate therapeutic effects have been reported in several drug trials, conventional drug therapies explored in OSA have generally produced disappointing results and there is a shortage of pharmacological treatment alternatives in this disorder. It is generally accepted that there remains no pharmacological treatment that can be uniformly recommended as first line treatment of OSAHS at the time of writing.[177, 178]

As already discussed, we are still yet to understand the many mechanisms that contribute to sleep-disordered breathing, and so any future development of new pharmacological approaches to OSAHS awaits a greater understanding of the various endotypes as well as the central neuronal mechanisms and various neurotransmitters involved in the modulation of motor output to the upper airway dilator muscles. There have been positive outcomes in some clinical trials which have identified several pharmacological agents that show a convincing reduction in sleep-disordered breathing in both the short and intermediate term.[177] However, the effects of these are not universal and so such drugs may only be particularly useful in certain endotypes of OSA and not in others.

One of the most promising studies in recent years was the randomised, placebo-controlled, double blinded crossover trial by Taranto-Montemurro et al. which demonstrated that a combination of atomoxetine (norepinephrine reuptake inhibitor) and oxybutynin (antimuscarinic) taken orally at bedtime can reduce OSA severity.[179] The combination was able to improve the AHI by 63% (34–86%), from 28.5 (10.9–51.6) events/hour to 7.5 (2.4–18.6) events/hour ($P < 0.001$), compared with placebo, with the mechanism thought to be increased genioglossus activity. However, neither atomoxetine nor oxybutynin reduced the AHI when administered separately, and despite an improvement in AHI, a large proportion still had an AHI of 10 or more following treatment. Furthermore, these drugs have potentially very toxic side effects, with atomoxetine being contraindicated in patients with severe cardiovascular

conditions, such as heart failure or cardiomyopathies, and oxybutynin being known to cause severe, dose-dependent, anticholinergic side effects.

Despite barriers to finding a curative drug, there have been other developments which particularly focus on the complications OSAHS can cause when left untreated i.e. daytime somnolence and various forms of cardiovascular/metabolic disease. One of the most extensively studied compounds so far is the central nervous stimulant Modafinil. This has been shown to significantly improve alertness and subjective and objective daytime sleepiness, in those who report residual tiredness despite good compliance with CPAP in two randomised, double blind, placebo controlled trials.[180] Despite not being a curative treatment, it has potential as an adjunctive symptomatic therapy.

Finally, there is also an increased awareness of the pathophysiological complications of obesity and its bidirectional relationship with OSA, and so pharmacological targeting of obesity may not only provide positive metabolic effects with wider health benefits seen in weight loss, but may also be a way to eliminate anatomic upper airway embarrassment seen in obese OSA patients.

3 Current clinical dilemmas in OSAHS

“It is a common experience that a problem difficult at night is resolved in the morning after the committee of sleep has worked on it.”

John Steinbeck

There is a quagmire of uncertainty in OSAHS which presents itself at the time of referral or first consultation with our sleep service, which seeps through to our current diagnostic and management pathways, and onwards to prognostication with regards to cardiovascular risk stratification and the public health risk (driving) that one might pose.

As an example of the enormity of the challenges in SDB, one could argue that even the definition of OSAHS is compromised. The clinical definition of OSAHS requires the combination of both a raised AHI and daytime symptoms, particularly excessive daytime sleepiness. This definition is hindered from the outset due to the difficulty in defining excessive daytime somnolence and its associated symptoms e.g., cognitive impairment (subjective). We also know there is a poor correlation between symptoms and overnight sleep studies, with the natural concern being are we using the right parameter to quantify SDB (objective), and there is clearly a paucity of evidence in highlighting which patients should be treated. We know the crude tool of CPAP is key in improving tiredness, however we have been unable to identify those at high risk of end organ dysfunction or worsening of other co-morbidities e.g. hypertension and cardiovascular disease, who could possibly benefit from treatment. I will explore these issues in further detail in this chapter and explain why we need a paradigm shift.

3.1 Subjective – what is tiredness?

Defining and quantifying tiredness is a challenge. It is important to remember that snoring is very common in the general population and affects up to 45% of men and 28% of women, and that at any one time, 30-50% of the general population without OSAHS report moderate to severe daytime sleepiness. [34, 141, 144] Although the ESS has advantages of being brief and simple to carry out, it focusses on people's likelihood to fall asleep. Many people, especially in certain occupations may feel very tired but are trained or have learned not to fall asleep. Therefore, they are tired with the syndrome but have normal or low Epworth Scores. Tiredness varies from day-to-day, and subjects can over or underscore. At least 2 questions involve being a driver or passenger of a car which will not be relevant to some people resulting in a lower score. The whole score is subjective, is open to recall and report bias, and patients may convey symptoms of fatigue, rather than pure excessive daytime sleepiness. Furthermore, the questionnaire was originally validated in white middle-aged US population, and where subjects were more likely to be active and autonomous, but it is now routinely used in a variety of populations and different ethnicities.[181] The correlation of ESS with other objective measures of sleepiness such as the 'Mean Sleep Latency Test' is inconsistent.[182] Moreover, only approximately 40% of persons with severe OSAHS have an ESS greater than 10, and there is concern it may underestimate sleepiness severity especially in older subjects.[183, 184] It has also been found that patients with heart failure have less subjective daytime sleepiness despite a significantly reduced sleep time, and that the absence of subjective sleepiness is not a reliable means of ruling out OSA.[185] Moreover, there are gender differences in the presentation of OSA, where women may emphasise fatigue and lack of energy in describing their symptoms.[186]

3.2 Objective - diagnostics

While the AHI remains a widely used measure to quantify OSA severity clinically and for research purposes (including this thesis), it has several limitations. The AHI gives us an idea of the frequency of respiratory events during sleep but does not always reflect the extent of resultant oxygen desaturation (ODI or 4% dip-rate) and its associated hypoxic burden or severity of inflammatory state. For example, a patient with very long apnoeas/hypopnoeas may experience a substantial degree and prolonged periods of hypoxaemia but will have a relatively low AHI, whereas conversely, another patient may have more frequent events and therefore a much higher AHI, but minimal exposure to severe hypoxaemia. For example, when you compare 2 patients with an AHI of 60 events/hr, we don't take into the account that one patient is having apnoeas lasting up to 30 seconds, compared to their counterpart's 12 seconds. As a result, the effects of hypoxaemia and its adverse effects on the body may be underestimated by solely referring to the AHI.

Furthermore, we struggle to differentiate the importance of a patient predominantly having hypopnoeas rather than apnoeas, as the evidence of the consequences of this is lacking, but one would assume that a cessation of airflow as seen in apnoeas, would have a far greater physiological insult than a reduction in airflow (hypopnoea).

We also know that there is significant night-to-night variation in OSA severity and therefore AHI, with variations of at least 10 AHI events/hr found in 15% to 65% of patients.[187, 188] The worry therefore is that a single isolated study possibly under or over-estimates the AHI, leading to inappropriate management plans including no treatment modality being initiated. The addition of a second night's respiratory polygraphy has the potential to reduce the likelihood of missed diagnoses, however the cost and logistical implications of this are unattractive and therefore not routinely done in practice.[189]

AHI as a measure of OSA is also limited by the inclusion of arousal in the definition, making PSG the optimum modality for assessment. It could be the frequency and degree of arousals that determine the inflammatory state as well as just tiredness.

3.3 CPAP. Problem solved?

Despite the ability of a CPAP device to reduce apnoeas and hypopnoeas, stabilise blood gases, reduce sleep fragmentation, and improve symptoms of sleepiness, it is estimated that almost half of patients (ranging from 28% to 83%) are nonadherent as defined by less than an average of four hours use per night.[190] The prevalence when the definition also includes less than 70% of nights is unknown. Reasons for not adhering to CPAP treatment include poor mask fit, pressure intolerance, and more commonly, upper airways symptoms such as nasal dryness, nasal bleeding and throat irritation.[158] Interventions such as additional behavioural support, clinic appointments and different masks have had limited effect, possibly increasing CPAP usage by around 1 hour per night. Consequently, humidification devices are now commonly offered in conjunction with CPAP devices to reduce their side effects.

Overall, the average duration of CPAP use is approximately five to six hours per nights, with the benefits felt within days to weeks of starting CPAP therapy.[191] Similarly, the impact of nonadherence is typically felt within days to weeks of stopping CPAP therapy. Evaluating the physiological effects of OSA and CPAP by recruiting untreated patients in RCTs is time-consuming and expensive, and so in 2011, Kohler et al. randomly allocated 41 patients with OSA who had been using CPAP successfully for at least one year without subjective sleepiness to either continue with their current CPAP, or CPAP withdrawal (CPAP device set at subtherapeutic level).[192] They found that CPAP withdrawal led to a recurrence of obstructive respiratory events, increased diurnal blood pressure, elevated morning heart rate, and subjective daytime tiredness; all occurring within two weeks of withdrawal. Furthermore, in 2022, Pepin et. al identified that CPAP adherence was associated with a reduction in all-cause mortality compared to CPAP termination (HR 0.61; 95% CI 0.57-0.65) in a cohort of patients' propensity matched for demographics and co-morbidities.[193]

Predicting poor usage can be done but is imprecise as nonadherence is multifactorial, with studies showing that the decision to continue with CPAP occurs during the first week of therapy. However, there remains no single factor that has been consistently identified to be a predictor of long-term adherence.[194, 195] Patients in our sleep service who are suitable for treatment with a CPAP device have a 1:1 induction session

with our sleep physiologist explaining their diagnosis, short and long-term impacts of treatment with CPAP, and an in-depth demonstration on how to set up their CPAP device at home. They are then asked to return for another face-to-face review 2 days later to assess machine usage, troubleshoot any early issues, optimise adherence, encourage engagement, and reinforce the importance of nightly CPAP use going forward. After this second visit, they are booked in to see a doctor (either face-to-face or a virtual appointment) around 4-6 weeks later. Here they discuss ongoing symptoms, CPAP usage (benefits and side-effects), occupational and driving risk and are then advised to notify the DVLA.

The current goals of OSA therapy are to resolve signs and symptoms, improve sleep quality, and normalise the AHI and oxygen saturation levels. However, many cardiovascular diseases are independently associated with SDB, with the mechanisms behind these associations being biologically plausible, even if the back-and-forth cause-and-effect are still being elucidated. Furthermore, many risk factors are involved in the development of CVD including hypertension, insulin resistance, genetics, smoking, obesity, systemic inflammation, and age, and since several of these risk factors are also shared by patients with SDB, it remains difficult to study their respective role independently.

Despite these caveats, well-designed randomised controlled (intervention) trials have now established that OSAHS is an independent and treatable risk factor for at least hypertension, and further research into diagnostics and treatment modalities are needed in the management of this very complex disease state.

The 2019 American Academy of Sleep Medicine meta-analysis curiously found that CPAP had no impact on the frequency of incident CVD events, overall glycaemic control, or left ventricular ejection fraction, despite it significantly reducing OSA severity. Although multiple epidemiologic studies have supported a causal association between OSA and CVD, randomised controlled trials that have analysed the effect of CPAP in patients with OSA have sometimes struggled to consistently demonstrate improvements in cardiovascular outcomes following treatment. In 2012, a RCT by Barbe et al. failed to demonstrate a statistically significant reduction in the incidence of hypertension or cardiovascular events in 725 consecutive non-sleepy OSA patients ($AHI \geq 20$ and $ESS \leq 10$) when prescribed CPAP compared to no treatment over 4 years.

However, by the authors own admission, their study had limited power to detect a significant difference given the heterogeneity in disease severity and variable use of CPAP.

The effect of CPAP on cardiovascular risk was again found to be modest in the Sleep Apnoea Cardiovascular Endpoints (SAVE) RCT, which randomised 2717 patients with moderate to severe OSA and established cardiovascular disease to either CPAP or usual care. They found no benefit of CPAP on the composite endpoint of death from cardiovascular causes, myocardial infarction, CVA, hospitalisation for unstable angina, heart failure, or transient ischaemic attack. However, mean nightly CPAP usage only consisted of 3.3 hours/night and the cohort again did not report significant sleepiness with an average ESS score of 7.3 ± 3.6 . Severely sleepy individuals (ESS score >15) and patients with severe hypoxaemia (oxyhaemoglobin saturations <80% for >10% of sleep-study time) were excluded from the study, thus potentially limiting the sample to a less severe group. It is possible that low CPAP adherence (in minimally motivated /symptomatic people) led to this neutral result, and the significance of sleep fragmentation and subsequent excessive daytime somnolence is underestimated in the manifestation of its co-morbidities.

4 Metabolomics

“I love sleep. My life has the tendency to fall apart when I’m awake, you know?”

Ernest Hemingway

4.1 Definitions

Metabolomics is an analytical profiling technique of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms, and can be broadly regarded as the measurement of the thousands of metabolites present in a biological system at any given time or under specific conditions.[196] It is a powerful method for revealing novel or unanticipated metabolites and metabolic pathways, allowing minute metabolic activity to be measured, and form the chemical fingerprint of an organism at a precise timepoint. Within a clinical context, metabolomics could provide information that otherwise require multiple conventional tests to arrive at, allowing hypothesis generation and testing.

Just as genomics is the study of DNA and genetic information, and proteomics aims to capture the entire protein products of the human genome, metabolomics attempts to capture smaller biochemical compounds, including simple amino acids and related amines, as well as lipids, carbohydrates, nucleotides, toxins, other intermediary metabolites, and just about any other chemical with a molecular weight of <1500 Daltons (Da).[197] Furthermore, whereas the genetic sequence is largely static from birth, metabolomic measures are relatively dynamic, representing cellular activity and the effects of extrinsic exposures, including dietary intake, physical fitness, microbiota variation, and toxicant exposures. Omics characterise and quantify pools of biological molecules to explore their roles, relationships, and actions in human cells. Figure 4.1 highlights the potential of metabolomics in hypothesis generation and testing.[198]

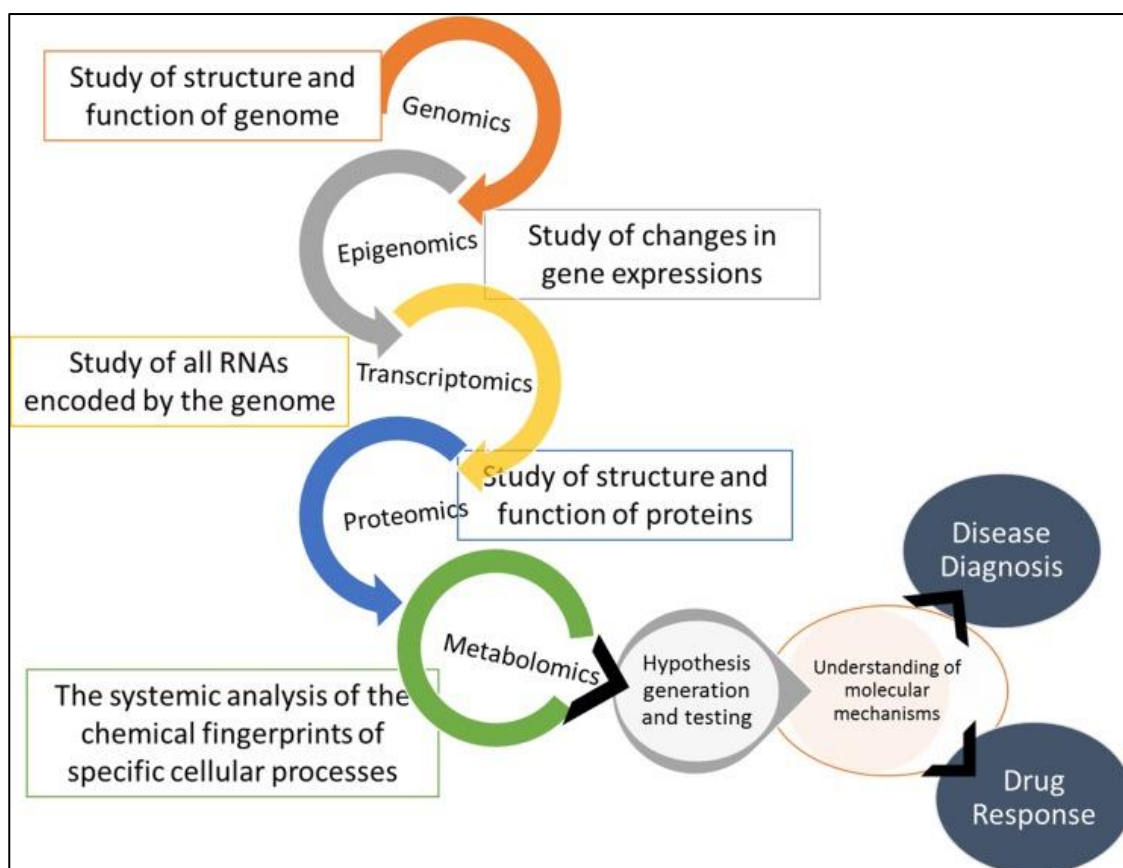


Figure 4.1: Metabolomics in hypothesis generation and testing.

Reproduced with permission from Shah, N.J., S. Sureshkumar, and D.G. Shewade, *Metabolomics: A Tool Ahead for Understanding Molecular Mechanisms of Drugs and Diseases*. Indian J Clin Biochem, 2015. **30**(3): p. 247-54.

However, unlike the genome and proteome, the metabolome itself is not easily defined, and humans are estimated to consist of 40,000 metabolites at any specific point in time.[197]

The main methodologies that are used for metabolite recovery and identification are untargeted and targeted metabolomics i.e., in a pattern discovery manner or targeted manner. Untargeted metabolomic analysis allows for an assessment of the metabolites extracted from a sample and can reveal novel and unexpected perturbations, with its primary advantage being that it offers an unbiased means to examine the relationship between interconnected metabolites from multiple pathways. Targeted metabolomic analysis allows the measurement of concentrations of a predefined set of metabolites, where prior knowledge of expected concentration ranges of the metabolite of interest have been established so that accurate quantification can be gained.[199] Figure 4.2 shows the relationships of the genome, transcriptome, proteome, and metabolome.[200]

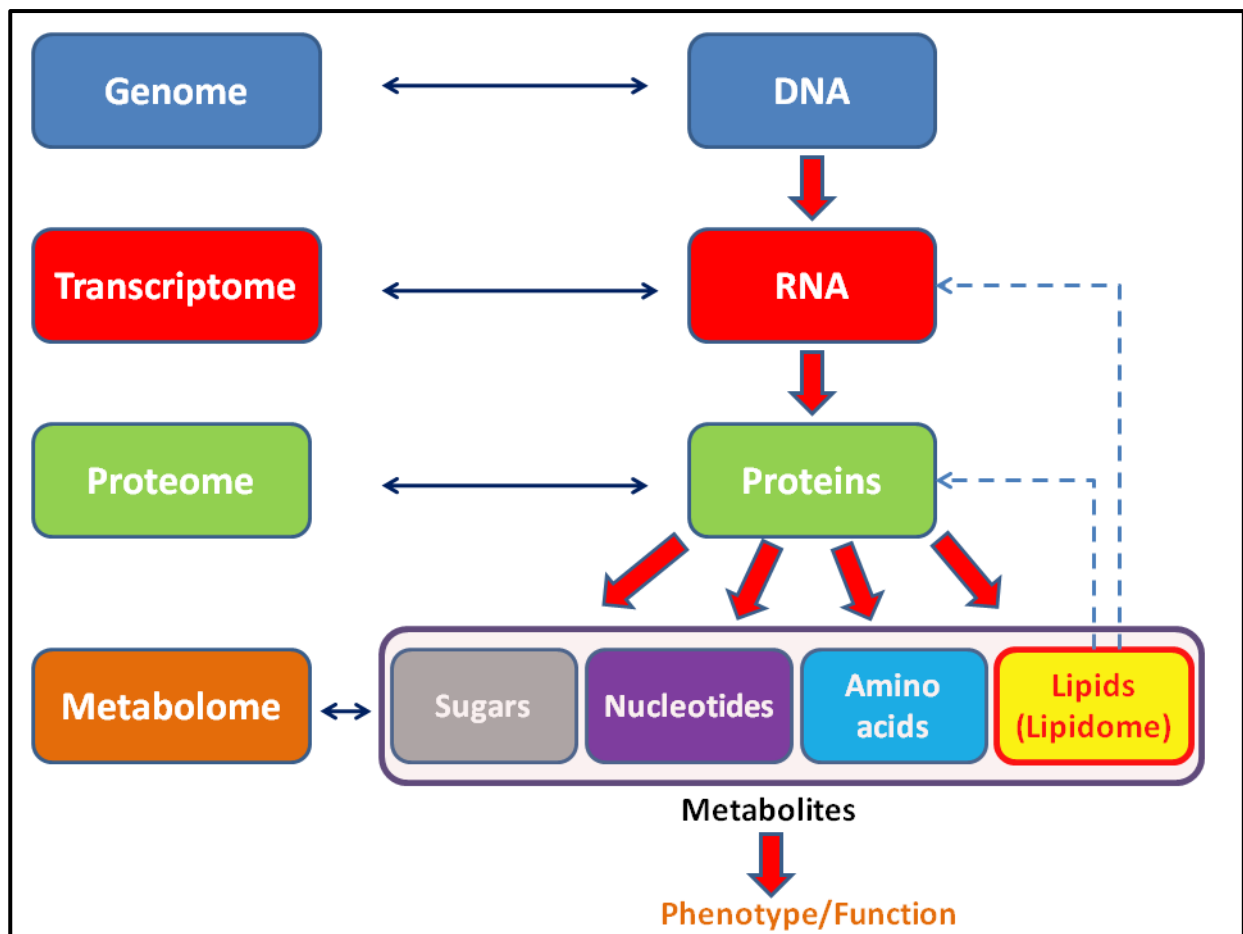


Figure 4.2: General schema showing the relationships of the genome, transcriptome, proteome, and metabolome

Metabolites are the substrates and products of metabolism that drive essential cellular functions, such as energy production and storage, signal transduction and apoptosis, and because they are downstream of genetic variation and transcriptional changes, they can provide instantaneous snapshots of the state of a cell or organism.[199] However, our environment and microflora both contribute significantly to the metabolome, and so the human metabolome consists of endogenous and exogenous (food, drinks, drugs etc.) compounds which makes characterisation difficult. [197]

Metabolomics is a powerful approach because metabolites and their concentrations directly reflect the underlying biochemical activity and state of cells/tissues. It allows profiling of much larger numbers of metabolites that cannot be covered by standard laboratory techniques, and as a result have been increasingly applied to biomarker discovery, with results to date demonstrating both feasibility and flexibility across physiological, interventional, and epidemiological human studies.[201] Furthermore, there are ongoing significant advancements in the analytical technology, meaning continuous potential for further discovery, and so within a clinical context, metabolomics can provide information that otherwise requires multiple conventional tests to arrive at similar conclusions, and its use in translational research appears to be increasing quicker than all the other -omics based approaches.[202]

Urine and blood serum or plasma are the most used biological fluids in metabolomic-based studies for the simple reasons that they both contain hundreds to thousands of detectable metabolites, and can be obtained non- or minimally invasively.[203] A number of other fluids such as cerebrospinal fluid, bile, seminal fluid, amniotic fluid, synovial fluid, gut aspirate and saliva have also been studied.[36–38]

4.2 Metabolomic analysis

Endogenous metabolites span a variety of compound classes, with significant differences in size and polarity, across a wide range of concentrations.[201] Consequently, no single analytical method is able to accommodate the chemical diversity of the entire metabolome, and a range of technical approaches have been used for the analysis of metabolites in biospecimens, including nuclear magnetic resonance (NMR) and mass spectrometry (MS), with the latter coupled to an array of separation techniques including gas chromatography (GC) and liquid chromatography (LC).[204]. Both MS and NMR spectroscopy can be used for metabolomics fingerprinting and profiling, each with their own advantages and limitations.

NMR spectroscopy is a quantitative method based on exciting atomic nuclei with radio waves, with the differences in the resulting resonance frequencies reflecting atomic structure and chemical bonds, and thus chemical characteristics.[205] The technique requires little sample preparation, is non-destructive, which allows for subsequent structural analyses. However, it can detect only highly abundant analytes, lacks the sensitivity of MS, with the identification of individual metabolites based on chemical shift signals that cause sample clustering in multivariate analysis being challenging in complex mixtures.[206]

In comparison, MS is a destructive process where samples are ionised by bombarding them with electrons and relies upon the formation of gas phase ions, which are subsequently separated by their mass/charge ratio (m/z) using electric or magnetic fields.[206] However, MS is an ideal analytical modality for metabolomics research as it combines a sensitivity and selectivity platform, and allows easier identification of the metabolite(s) of interest. For the remainder of this thesis, we will focus on MS only.

Essentially a mass spectrometer (figure 4.4) carries out 3 functions[207]:

- 1) The ionisation source – molecules are converted to gas-phase ions so that they can be moved about and manipulated by external electric and magnetic fields.
- 2) The mass analyser – differentiates the ions according to their mass/charge ratio (m/z)

- 3) The ion detection system - measures the relative abundances of ions and their relative masses; the information being represented as a mass spectrum.

MASS SPECTROMETRY

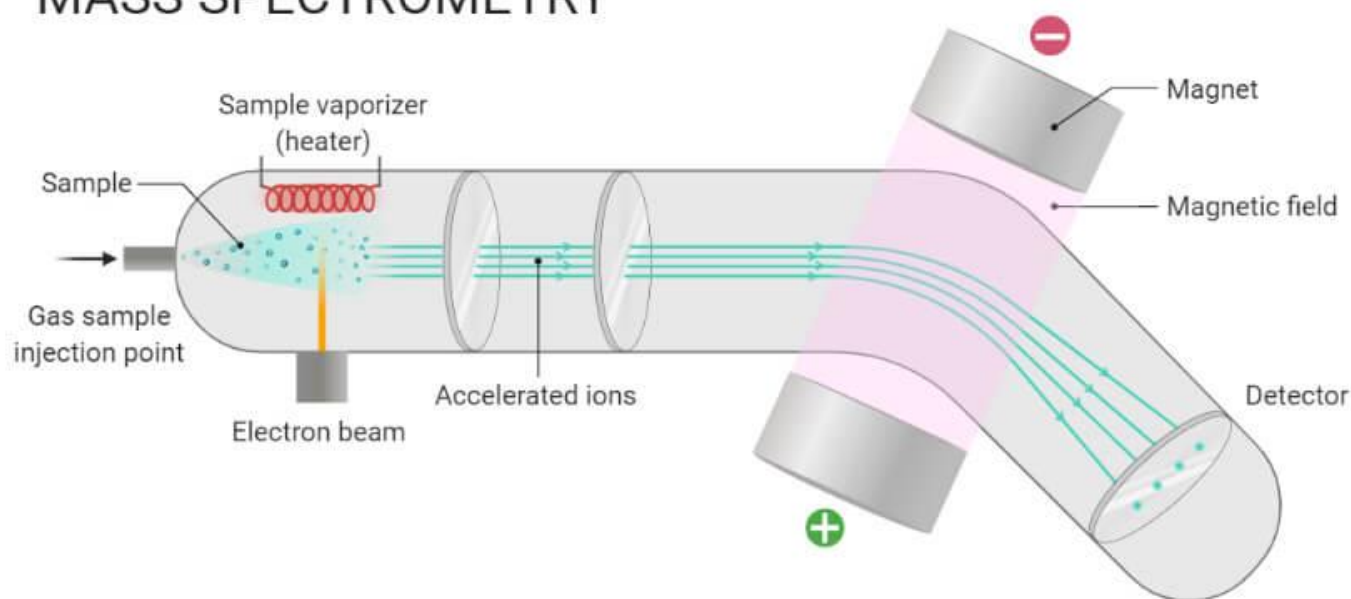


Figure 4.3: A mass spectrometer (created with BioRender.com)

In general, the workflow for MS metabolomics remains largely consistent and is represented in figure 4.5.[208] The range of m/z that are generated by MS can be detected using a range of technologies, the most common being 'Time of Flight' (ToF) or 'Orbitrap'. [209] Following MS metabolomic analysis, an individual experienced in MS needs to extract biological relevant data from the vast amount of acquired data so that you are typically left with an annotated feature matrix. A feature is a typically a peak or signal that represents a chemical compound. This data is subsequently analysed and compared against available mass spectral databases to predict the molecular identity of the constituents. The quality of the data obtained can be improved further by using tandem or 'hyphenated' approaches, where samples are pre-fractionated using gas or liquid chromatography (hence GC-MS or LC-MS). [210] A range of univariate and multivariate statistical approaches can then be performed at this stage, which also focus upon data pre- and post-processing tasks such as signal extraction/peak detection and noise reduction (removal of spurious signal detection).

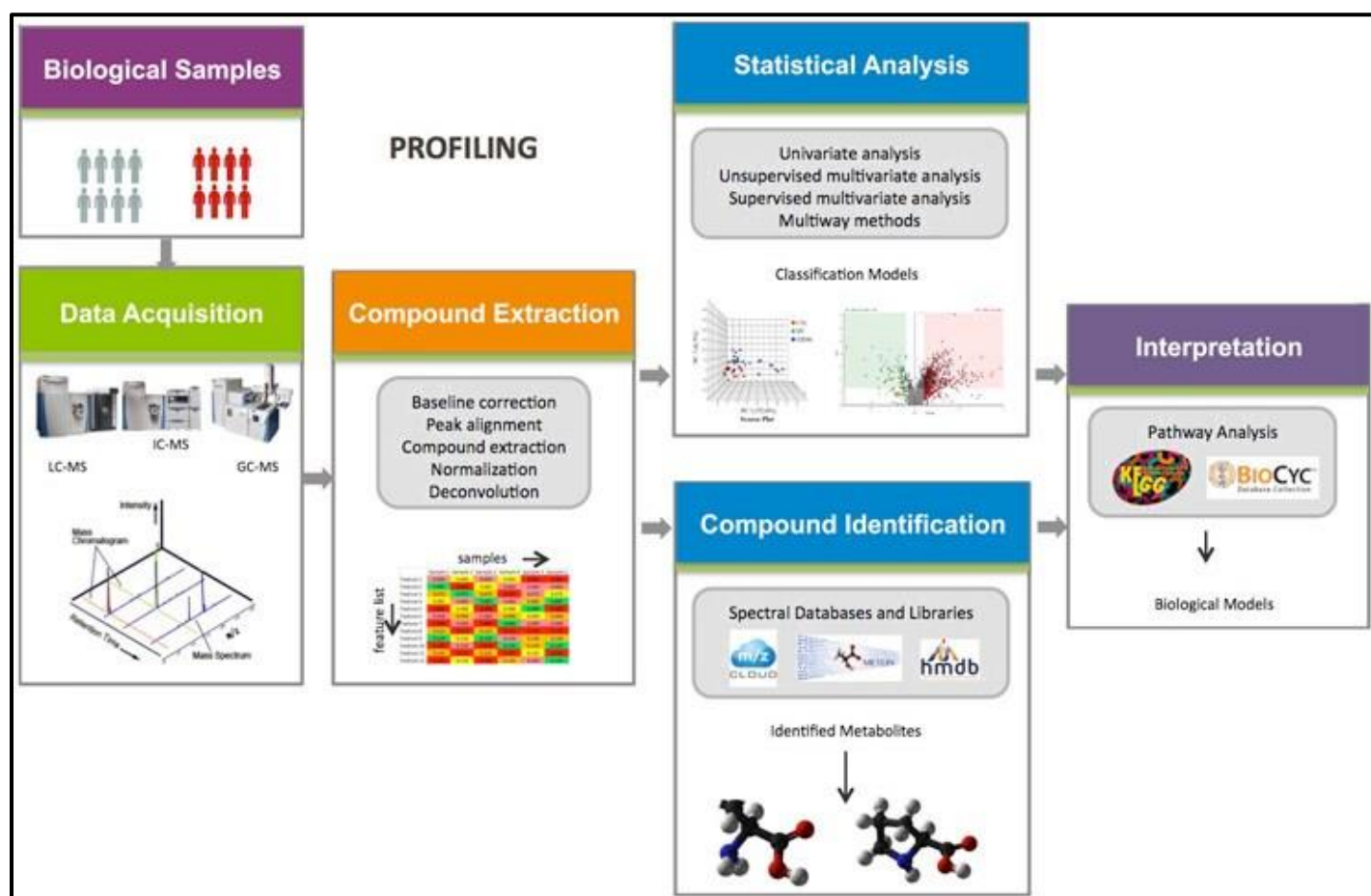


Figure 4.4: Untargeted metabolomics workflows.
Reproduced from ThermoFisher Scientific. *Untargeted MS workflows*. 2024

4.3 A brief history of metabolomic research and applications

It was as early as the 1940's when Roger Williams introduced the concept of a 'metabolic profile' that was reflected in their biological fluids. He demonstrated this using paper chromatography to show that individuals with schizophrenia have characteristic metabolic patterns in their urine and saliva. We then had to wait for the relevant technological advancements in the early 1970s when Arthur Robinson and Linus Pauling conceived the core idea that the quantitative and qualitative pattern of metabolites in biologic fluids reflected the functional status of the complex system from where they were derived, and in the same year, Horning et al. first used the term metabolic profiling to describe the output of a gas chromatogram from a patient sample.[211, 212] It was around the same time that NMR technology began to be used to detect metabolites in raw biological samples, and by 1984 the technology had advanced to the point where Nicholson et al. were able to provide a 'NMR fingerprint' using blood and urine samples from fasting normal and diabetic subjects.[213]

At the time of writing, metabolomics has been applied successfully to numerous in vitro and in vivo studies. Researchers have found novel metabolic pathway disruption in many disease types including cardiovascular disease, diabetes, inborn errors of metabolism, alcohol-induced liver injury and various cancers.[214-220] However, it is important to emphasise that despite there being a burgeoning number of potential metabolomic applications that could disrupt clinical practice in the near future, there are little, if any, that are currently used in clinical practice.

Although there are admittedly limited clinical applications for metabolomics currently, it is a rapidly evolving analytical tool which has already demonstrated substantial potential for new discovery and insight. Brindle et al. have utilised metabolomics to identify individuals with stenosis of all three coronary vessels to be distinguished from subjects with angiographically normal coronary arteries with a specificity of >90%.[221] Furthermore, Makinen et al. have been able to metabolically phenotype individuals with Type 1 Diabetes Mellitus who are likely to develop nephropathy, and pharmacometabolomics has allowed a greater understanding in the mechanism of some of our most commonest drugs including aspirin, paracetamol, statins and citalopram.[198, 222]

4.4 Metabolomics and OSA/OSAHS

The potential of metabolomic profiling in OSA/OSAHS patients has been increasingly explored in recent years, with samples of plasma, urine, saliva, tonsillar tissue, adipose tissue, and even cerebrospinal fluid being analysed.[36–38] However, results have remained inconsistent, with clinical numbers being small, and results remaining translational and far from a Point-of-Care clinical test.

Table 4.1 summarises the main metabolomic findings in the literature.

Table 4.1: A summary of metabolomics in OSA/OSAHS

Reference	Sample	Number of Subjects	Expressed metabolites
Fletcher et al. 1987[223]	Urine	8 severe OSA and 5 HTN and obese non OSA patients	Epinephrine (E), Norepinephrine (NE), Metanephrine (MN), Normetanephrine (NMN).
Gislason et al. 1992[224]	Cerebrospinal fluid	15 OSA and 18 healthy controls, 12 patients with suspected neurological disease	5-hydroxyindoleacetic acid (5-HIAA, serotonin metabolite), Homovanillic acid (HVA, DA metabolite), 3-methoxy-4-hydroxyphenyl glycol (MHPG)
Paci et al. 2000[225]	Plasma	10 male OSA (8 normotensive and 2 untreated HTN) and 11 controls	Norepinephrine (NE), Epinephrine (E), Dopamine (DA), Endogenous digitalis-like factor (EDLF)
Stanke Labesque et al. 2009[226]	Urine	40 non obese OSA and 20 controls	Leukotriene E(4) (U-LTE (4)), 11-dehydroTXB2
O'Driscoll et al. 2011[227]	Urine	70 snorers and 26 controls	Epinephrine (E), Norepinephrine (NE), Dopamine (DA), Noradrenaline, Adrenaline
Engeli et al. 2012[228]	Plasma	29 OSA, 26 OSA type	Anandamide; (AEA), Arachidonoylglycerols; (AG), Oleoyl ethanolamide; (OEA), Arachidonic acid

		II diabetes, 21 controls	(AA), increase in the total monounsaturated fatty acids (MUFA)
Barcelò et al. 2012[229]	Saliva	119 OSAS and 35 controls	Gamma glutamyltransferase (GGT), Fetuin-A
Ferrarini et al. 2013[230]	Plasma	18 OSA severe and 15 OSA non severe	Phosphatidylcholine (PC), Phosphoserine (PS), Lysophosphatidylsophosphatidylcholines (LPC), Lysophosphatidylethanolamine (LPE), LPA, PE methyl-hydroperoxy- octadecatrienoate, PGF2-alpha diethyl amide Pipecolic acid, Arg, Phe, His
Kawai et al. 2013[231]	Saliva	20 male OSA	Phosphatidylcholine (PC)
Ezzedini et al. 2013[232]	Tonsillar tissue	114 paediatrics OSA and 92 recurrent tonsillitis	Palmitoleic acid, Oleic acid, Stearic acid
Xu et al. 2016[233]	Urine and plasma	60 OSA, 30 simple snorers and 30 controls	2-hydroxy-3-methylbutyric acid, 3,4- dihydroxybutyric acid, 3- hydroxybutyric acid, 4- hydroxypentenoic acid, cytidine 5'- diphosphocholine, ethanolamine, myo- inositol, 2,3-dihydroxypropanoic acid, arabinose, arabitol, cellobiose, maltose, threitol, alanine, isoleucine, serine, threoninyl-methionine, trimethylamine N-oxide, valine, 5- hydroxyindoleacetic acid, lactic acid, glycochenodeoxycholate-3-sulfate, putrescine, 4-hydroxybutyric acid,

			vanillic acid, hypoxanthine, inosine, xanthine
Cho et al. 2017 [234]	Urine	48 male attenders; 14 controls, 34 OSA	31 acylcarnitines, 21 amino acids, 15 biogenic amines, 13 glycerophospholipids, 5 sphingolipids, and 1 sum of hexoses
Lebkuchen et al. 2018[235]	Plasma	37 OSA and 16 controls	Deoxy sugar; 2,6-diphenyl-1,7-dihydrodipyrrolo[2,3-b:3',2'-e]pyridine; 9-hexadecenoic acid (Z), Arachidonic acid (AA), 5,5'-bipthalide, L-glutamine, Glycerophosphoethanolamines (PE), Monoacylglycerophosphocholines (lyso-phosphocholines) (LPC), sphingomyelin (SM), diacylglycerols (DAG), glycerophosphocholines (PC), glycerophosphates (PA), Glutamic acid, Methyl cysteine, Serine
Pinilla et al. 2022[236]	Serum	64 non-OSA and 142 OSAHS	glycerophospholipids (cardiolipin (CL), phosphatidylcholine (PC), phosphatidylethanolamine (PE)), sterols (bile acids), and fatty acid derivatives (oxylipids)

The earliest study of metabolomics in OSA was by Fletcher et al. in 1987 who examined diurnal variation in urinary catecholamines in 8 subjects with severe OSA before and after tracheostomy, alongside 5 obese hypertensive subjects without OSA who acted as controls.[223] Three urine specimens were collected over a 24-hour period (morning, evening, and asleep) both pre- and post-operatively, and it was found that norepinephrine and normetanephrine were significantly higher in apnoeic patients pre-tracheostomy compared to controls ($p < 0.01$) and compared with their own values post-tracheostomy ($p < 0.03$, $p < 0.05$). Interestingly there was no significant diurnal variation in urinary catecholamines pre or post tracheostomy in the OSA group or in the non-apnoea group. In normal healthy individuals it is usual for blood pressure to fall during sleeping hours, and as previously mentioned (see pathophysiology chapter), obstructive respiratory events result in increased sympathetic activity and therefore increased blood pressure. It is therefore biologically plausible that the lack of any significant diurnal variation in catecholamines in both the pre and post tracheostomy OSA group is evidence of a hangover of the ongoing increased sympathetic activity during the daytime often seen in patients with OSA. However, to confound matters further, the authors do not comment on urinary catheterisation during the study, and therefore one must assume that participants were woken for urine samples to be collected, which would naturally cause sympathetic surges.

The sympathetic overdrive seen in OSA was further evidenced by Paci et al. in 2000 who also found higher levels of plasma norepinephrine in 8 male normotensive OSA patients (AHI 70 ± 6 events/hr) and 2 untreated hypertensive OSA patients, compared to the 11 age-matched healthy male controls.[225] Similar findings of increased sympathetic activity as measured by overnight urinary catecholamines was further demonstrated by O'Driscoll et al. in 2011, this time in children (age range 3-12 years), with their subsequent multivariate analysis showing AHI and its severity as a significant independent predictor of overnight urinary noradrenaline and adrenaline levels ($p = 0.001$ and $p < 0.05$ respectively).[227]

One of the largest studies of urinary metabolite levels was by Xu et al. in 2016 who performed untargeted MS metabolomic analysis on 120 subjects consisting of 30 controls (ESS 2.30 ± 2.51 ; AHI 0.62 ± 0.60), 30 'sleepy snorers' (SS) (ESS 6.77 ± 6.00 ; AHI 1.98 ± 1.35), and 60 with OSA (ESS 7.97 ± 5.64 ; with AHI 34.40 ± 24.33).[233] There

were no statistical differences in age, sex, or BMI between the groups. The researchers identified 21 metabolites that distinguished the SS group from normal subjects, and 31 metabolites that differentiated the OSA group from the normal subjects. ROC analysis revealed that the combination of 4-hydroxypentenoic acid, arabinose, glycochenodeoxycholate-3-sulfate, isoleucine, serine, and xanthine could distinguish OSA from those without OSA with a sensitivity of 75% and specificity of 78%. The area under ROC curve (AUC) was 0.835 (95% CI 0.757-0.897). These discriminating metabolites are also biologically plausible i.e. branch chain amino acids such as isoleucine can cause mitochondrial dysfunction which is a predictive factor for oxidative stress, and elevated levels of these amino acids are reported to be a predictor of diabetes mellitus.[237, 238] Furthermore, the higher levels of arabinose in the SS and OSA groups could be indicative of a glucose metabolic disorder, and the presence of xanthine could reflect excessive oxidative stress and nuclear and mitochondrial dysfunction. However, a significant limiting factor of this study was that those with significant cardiac, pulmonary, or renal disease were excluded.

One of the largest studies of metabolomics in adults in OSA came from Barcelo et al. in 2012 who studied the plasma level of fetuin-A in 119 patients with OSAHS (AHI 40.1 ± 20.0) defined by full PSG against 35 controls (AHI 5.0 ± 2.1).[229] Groups had similar age, sex, BMI and all patients were assessed for EDS by a self-reported ESS however the control cohort's ESS was not defined. Fetuin-A is known to be an important inhibitor of vascular calcification and a potent anti-inflammatory cytokine mainly synthesised in the liver, participating in macrophage deactivation and inhibition of apoptosis of vascular smooth muscle cells.[229] It is considered to be a mediator that links chronic inflammation to CVD, and also as a risk factor for metabolic syndrome. No participant had any other known chronic disease (pulmonary, liver, thyroid dysfunction, rheumatoid arthritis, chronic renal failure and/or psychiatric disorders), but compared with control subjects, Fetuin-A levels were significantly lower in patients with OSAS than controls ($p=0.015$) and were significantly related to the severity of the AHI ($p=0.006$). This inverse relationship between Fetuin-A levels and OSAS, alongside lower levels of the anti-inflammatory Fetuin-A in those with OSAS with significant CVD than without, would suggest that this multifunctional glycoprotein could be one of the

contributing factors for the development of cardiovascular complications in OSAS patients.

Cho et al. performed a targeted metabolomic approach focusing on the urinary metabolites related to inflammation or metabolic perturbations: e.g., oxidative stress, membrane disruption, energy metabolism alteration, dyslipidaemia, and insulin sensitivity/resistance in those with OSAHS in 2017.[234] They found that acylcarnitines, biogenic amines, glycerophospholipids, and sphingolipids distinguished between OSA groups and non-OSA ($p < 0.05$ compared to control), with further targeted metabolomic analysis after adjusting for age and BMI, finding a combination of acylcarnitines, biogenic amines sphingolipids correlating with AHI, and their key metabolite hydroxytetradecenoylcarnitine [C14:1-OH], an acylcarnitine, being able to distinguish between OSA and non-OSA with an AUC of 0.746. However, they excluded females, those with diabetes mellitus, hyperlipidaemias, and chronic hypoxia-related diseases, and their cohorts were of a relatively young age ($33.9 \text{ years} \pm 10.7$ for controls and 40.5 ± 8.4 for severe OSA).

In 2018, Lebkuchen et al. utilised a combination of metabolomic analytical platforms including ultra-performance LC coupled to tandem quadrupole MS, and GC coupled to quadrupole on the plasma of 37 men with OSA (defined by $\text{AHI} \geq 15$ events/hr) and 16 controls matched for age, gender, fat composition via bioimpedance analysis, and BMI.[235] They identified 6 metabolites, with glutamic acid ($p = 0.023$), deoxy sugar ($p = 0.021$, AUC 0.667 CR 0.883 - 0.451) and arachidonic acid ($p = 0.036$, AUC 0.596 CR 0.758 - 0.435) having the strongest association with the presence of OSA. These metabolites are involved in glucose and inflammatory pathways, with deoxy sugar, a monosaccharide glucose analogue, produced by the action of glucose oxidase. Glucose oxidase is a subset of oxidoreductase enzymes that catalyses the transfer of electrons from an oxidant to a reductant, and with the knowledge of the activation of ROS secondary to CIH, this finding is certainly biologically relevant. They also found that combining a high NOSAS score (Neck circumference, Obesity, Snoring, Age, Sex Score; a 5-point screening scale), with these metabolites resulted in an AUC of 0.911 (95% CI 0.987 – 0.843). However, the study is not generalisable to a typical OSAHS population as it excluded females, those with alcohol excess (not defined explicitly), participants on any medication, and those with any known pulmonary, CVD, or kidney disease.

They also did not attempt to quantify a subjective level of sleepiness or other symptomology beyond snoring.

The largest metabolomic analysis in OSAHS was an observational-prospective-longitudinal study on 206 consecutive attenders with suspected OSAHS by Pinilla et al. in 2022 (published whilst this thesis was being written). [236] All individuals underwent overnight full PSG (where an AHI of 15 or more defined OSAHS) and untargeted plasma metabolomic and lipidomic profiling was applied using LC-MS. After adjusting for age, sex, and BMI, they identified a plasma profile of 33 metabolites which consisted mainly of glycerophospholipids and bile acids. Machine learning analyses identified 4 metabolites that could differentiate those with OSAHS (AHI 41.0 (26.8;62.4), ESS 11.0 (7.0-15.0)) from those without OSAHS (AHI 9.26 (5.18;11.9), ESS 10.0 (6.50;13.5)) with an accuracy of 0.98 (95% CI 0.95–0.99).

3 of the 4 metabolites forming this specific plasma fingerprint were identified as 25-cinnamoyl-vulgaroside, 33-hydroxyfurohyperforin, and 15,16-DiHODE, with 602.4646@11.712 (exact mass@retention time) metabolite remaining unknown. The identification of glycerophospholipids and bile acids is biologically relevant and suggests that the metabolic impact of OSA systemically is mainly mediated by a dysregulation in glycerophospholipid metabolism and the biosynthesis of primary bile acids.

Pinilla et al. also evaluated the impact of CPAP on the metabolomic panel and found a significant modulation of 5 of the 33 plasma metabolites previously altered by OSA in their untargeted analysis (but not the 4 key differentiating metabolites). The OSAHS patients treated with CPAP had a mean CPAP usage of 5.21 (2.84) hr/night, with 69% of treated patients being deemed good compliers i.e. CPAP use ≥ 4 hr/night. This result suggests that CPAP seems to have an effect on the metabolic plasma profile of individuals with OSAHS, but not the ones that differentiate between OSAHS and non-OSAHS. This suggests that CPAP does have a significant impact on the pro-inflammatory metabolic profile seen in those with OSAHS, however does not fully reverse the metabolomic insult.

5 Aims of thesis

“Sleep is the best meditation.”

Dalai Lama

5.1 Why we need a biomarker

A biomarker is defined as “a natural occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease can be identified”. [239]

Within a clinical context, metabolomics can provide information that otherwise requires multiple conventional tests to arrive at similar conclusions. For example, circulating biomarkers that have been successfully incorporated into practice include diagnostic markers of acute pathology e.g. troponin in myocardial infarction, and B-type natriuretic peptide for decompensated congestive heart failure. [201] The discovery of these biomarkers required the ability to differentiate between subtle clinical phenotypes, both in acute illness/at presentation and over time, and also account for wide range of confounding variables. [201]

Considering only direct benefits, it was estimated that the NHS would save £55 million in 2014 if all people with moderate to severe OSA were diagnosed and adequately treated, and this has likely increased further since. [39, 240] These potential direct cost-savings incorporate the estimated reduction in road traffic accidents, cardiovascular events and strokes, and their positive consequences for patients’ length and quality of life. The indirect benefits from less time off-work and more productivity were not included. Furthermore, relative to the current estimated rate of OSA patients treated across the UK (22% of OSA patients), a further £28 million can be saved annually by just increasing diagnosis and treatment rates to 45% of the target population with appropriate symptoms. [39, 240]

It is therefore likely that a biomarker; a reliable, non-expensive, and non-invasive modality, could potentially overhaul current OSAHS diagnostic and treatment pathways. OSA and its associated syndrome OSAHS, is uniquely suited for such metabolomic profiling and is desperate for potential metabolite biomarker discovery.

The majority of the metabolomic studies already discussed have primarily been interested in revealing a metabolic phenotype of relevance, rather than identifying a panel of metabolites that could be used to differentiate the healthy from the pathological i.e., a biomarker. Furthermore, very few have evaluated any change in the same metabolomic fingerprints after CPAP treatment.

Non-invasive methods of specimen provision are clearly more favourable and beneficial to the patient and healthcare systems, and the speed at which they can be utilised can certainly aid diagnostic and treatment pathways and reduce any logistical barriers e.g., an overnight 10-hour sleep study versus an instantaneous blood/urine result. The benefits of just this single example as a diagnostic tool would reduce the need for hardware, manpower, night-to-night variability, travel time and subsequent carbon footprint (as respiratory polygraph needs to be returned the next morning).

Furthermore, a biomarker could help identify asymptomatic individuals who are at a higher risk of end-organ dysfunction that would benefit from treatment. It could further be used as a surrogate marker of CPAP usage and compliance, whilst informing long-term risk stratification for cardiovascular disease and the metabolic syndrome. Any newly found metabolites and biomarker of relevance would also provide a greater understanding of the circulating metabolome and the changes it undergoes during an apnoeic event and offer other targets for pharmacological agents to treat the associated co-morbid risk.

5.2 The discovery of a biomarker

Using untargeted metabolomic profiling alongside clinical phenotyping and stratification according to the AHI (≥ 15 events/hour), we will ask the following questions.

- 1) Is there a panel of metabolites that can distinguish between those with OSAHS and those with symptoms of excessive daytime tiredness but a negative sleep study (Sleepy Participants (SP))?
- 2) What is the effect of CPAP treatment on the circulating metabolomic profile in those with OSAHS, and do the key metabolites that can differentiate between SP and OSAHS, if any, change with a trial of CPAP?

6 Materials and Methods

“Tired minds don't plan well. Sleep first, plan later.”

Walter Reisch

6.1 Study Design

This was an observational prospective and longitudinal study under a larger umbrella study ‘Novel Technologies in Diagnosis and Monitoring Lung Disease’ (ISRCTN 16657101).

Following loco-regional ethics and local research and development permissions (REC: 16/WA/0036), all enrolled subjects provided informed written consent to participate.

This project adhered to the principles of good clinical practice as outlined in the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95), and was conducted in compliance with the protocol, the Declaration of Helsinki (South Africa, 1996), the Research Governance Framework for Health and Social Care and incorporating the Human Tissue Act, and the Data Protection Act.

1) Inclusion criteria

Patients referred by their general practitioners (GPs) or other specialities with a possible diagnosis of OSAHS

2) Exclusion criteria

- a) no excessive sleepiness on screening questionnaires **and** an Epworth Sleepiness Score ≤ 10
- b) inability/unwillingness to sign informed consent
- c) life expectancy less than 6 months
- d) significant illness which in the opinion of the principal investigator puts them or researchers taking biological samples at risk (e.g., suspected infectious disease such as tuberculosis, hepatitis B or C, SARS-CoV-2, or HIV)

3) Participant recruitment process

The sleep unit at Prince Phillip Hospital, Llanelli, is one of the largest sleep services in Wales, with over 3000 patients on CPAP, and covers the whole geographical area of Hywel Dda Health Board. It has 4 sleep physiologists, one consultant supported by a registrar (myself), and has a weekly clinic and weekly multi-disciplinary team meeting.

All patients referred to the sleep service would be triaged by the Consultant in Respiratory Medicine with a special interest in OSAHS and SDB following e-referrals or written referrals from mainly primary care, but also secondary care. We included subjects referred to our sleep unit for suspected OSA/OSAHS who had appropriate symptoms to warrant an overnight home sleep apnoea test (HSAT) i.e. ResMed ApnoeaLink™.

Patients were recruited between September 2020 and December 2021. This was an exploratory trial, using untargeted metabolomic profiling, and so no power calculation was used. However, we did set a recruitment target of at least 80 participants and continued recruiting until at least 40 of these required a trial of CPAP.

We were unsure whether this number would yield statistically significant results, however due to logistical (single-centre, one investigator), time (only two year recruitment period due to maximum lead investigator was authorised to time out of respiratory specialty training by Health Education and Improvement Wales (HEIW)), and funding constraints, it was hoped this number would at least reveal clinically important trends and inform other power calculations if needed.

Where possible, subjects were posted a Participant Information Sheet-Informed Consent Form (PIS-ICF) (Appendix 2) with their hospital invites for their overnight HSAT between 2-10 days prior to their scheduled attendance. Those who attended but had not received a PIS prior to their attendance could be approached and given a PIS whilst they were waiting for their HSAT to be issued. Consent could be provided at any time providing the patient volunteered to participate. Participants were able to indicate which samples they were willing to allow to be used as part of the study on the consent form.

All participants had a confirmation of recruitment (Appendix 3) and small credit-card-sized sticker applied to their medical records stating that they were part of the study. This reduced the chance of being re-consented, and also alerted us if they were admitted to hospital.

4) Study procedures

To avoid additional visits with their attendant cost, clinical data and sampling was collected during their routine clinical appointments wherever possible.

At the initial visit, general physical and anthropometric parameters were recorded including blood pressure, heart rate, oxygen saturations, and a detailed clinical history including sociodemographic characteristics, medical history, medication use, smoking history, and alcohol consumption was obtained. Data was recorded on a standard clinical information sheet (Appendix 4), and urine, plasma and serum samples were collected.

All patients were required to complete our in-house sleep questionnaires which evaluated levels of daytime somnolence and other symptoms associated with OSA/OSAHS, as well as the ESS so we could measure subjective sleepiness (Appendix 1).

Clinical data was semi-anonymised and recorded on a case report form. Each participant was given a unique study number which was recorded in the trial site file. The unique study number was transferred to the password protected database controlled by the research team at Aberystwyth University, ensuring that participants were unable to be identified directly. No directly identifiable data was included on the case report form or was transferred outside the NHS or clinical research team.

All patients who satisfied the eligibility criteria underwent a level 3 HSAT (ResMed ApnoeaLink™) that recorded respiratory excursion, nasal flow, heart rate, and oxygen saturations, with the results from all sleep studies manually reviewed by our trained sleep physiologists using the criteria outlined in the previous chapter 'Diagnosing OSAHS – Objective'.

An overnight sleep test was deemed inadequate if it was less than 4 hours in duration, or if any of the sensors/channels became detached from patient for whatever reason. All physiological sensors that came into contact with a participant underwent a process of robust cleansing and disinfection prior to its reuse.

Based on current UK guidelines from the National Institute for Health and Care Excellence, patients with an AHI of ≥ 5 events/hour were considered for a trial of CPAP only if symptoms were significant and affecting quality of life and usual daytime activities based on clinical discretion. For those with an AHI of ≥ 15 events/hour, a lower threshold of symptomatic burden was applied, and CPAP offered as part of routine practice unless clear contraindications.

Those who had an AHI of ≤ 5 events per hour, called 'sleepy participants', were sent a letter of reassurance and where appropriate were advised on lifestyle (weight loss, sleep hygiene, medication review or screening blood tests). Very occasionally, a repeat sleep study would be requested based on the history and clinical discretion of the investigators.

5) Follow-up/further visits

The consent form allowed patients to specify whether they were willing to have repeat samples and interviews at a later date. This was for a maximum of one occasion and, where participants consented to these further procedures, they were usually timed to coincide with their routine clinical appointment e.g., routine review in sleep-clinic. Some participants were given the option to attend outside clinical appointments if it was more convenient for them.

All patients treated with CPAP (Phillips Respironics DreamStation Auto CPAP) were offered a physiology follow-up 2 days after initiation for an early machine download, support, and troubleshooting, as per standard local practice. They were then asked to return for a clinical review to check symptoms, a CPAP machine download (check AHI, mask leak and usage), and blood and urine sampling between 6-10 weeks later. They were also asked to complete a further sleep questionnaire identical to the one from their index visit, and this data was collected on the follow-up clinical information sheet (Appendix 5).

6.2 Sampling processing

Non-fasting venous blood samples were obtained at various times of the day based on clinical appointment time. Plasma was collected in EDTA (ethylene diamine tetra acetic acid) anticoagulant tubes (Vacurette, Greiner Bio-One, Kremsmünster, Austria), and serum in SST (serum separator tubes) (Vacurette, Greiner Bio-One, Kremsmünster, Austria) using standard aseptic venepuncture techniques (21g 'green' needle (BD Vacutainer)) from the antecubital fossa with the patient in a sitting position. In the event of difficult venous access, a smaller 23g needle was used (BD Vacutainer), and/or another site on the upper limb was used.

SST blood samples underwent 8 inversions post-collection, and then sat at room temperature for at least 30 minutes to allow clotting, EDTA samples also underwent 8 inversions, however, could proceed to centrifugation immediately.

Both SST and EDTA vacutainers were taken to the onsite laboratory within 10 minutes of sampling and centrifuged for 10 minutes at 3407 RCF/1300 revolutions per minute (RPM) (Heraeus Labofuge 400R Centrifuge, Thermo Scientific, USA). Being careful not to disrupt the separation, a bulb pipette was used to transfer the separation to a 5ml cryovial, until 0.5cm of sample was left in the vacutainer. The separations were then immediately stored at -80 degrees centigrade in the R&D freezers on hospital site.

Spot urine samples were collected directly into a 50ml falcon tube and required no processing. They were also taken to the on-site laboratory within 10 minutes and placed directly into the freezer.

The frozen samples were then transported in batches on dry ice at a later date to be analysed in Aberystwyth University. They were stored at -80C when not being analysed.

The recruitment guidelines and sample processing are outlined in Appendix 6.

6.3 Metabolomic processing and identification

Samples were defrosted on ice, vortexed and centrifuged before a 300 µL aliquot was mixed with 300 µL of MeOH (Fisher, UK). The samples were then stored at -20 °C until ready for metabolomic analysis. From this mix, 20 µL was transferred to a 2 mL glass vial with a glass insert (Crawford Scientific Ltd) with 80 µL of 70% MeOH. Sample injection order was randomised to reduce batch effects. For each sample, 20 µL were injected into a flow of 60 µL per minute in methanol/water (70/30 % v/v), using a Surveyor flow system, into a Q Exactive plus mass analyser instrument with an ultra-high-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific®, Bremen, Germany) for high-throughput flow-infusion electrospray high-resolution mass spectrometry (FIE-HRMS). Data acquisition for each serum sample was carried out using alternating positive and negative ionization modes, in four different scan ranges (15–110, 100–220, 210–510, 500–1200 m/z) with an acquisition time of 2 min.

According to previously published work, differentially expressed features, defined by exact mass and retention time, were searched against the Human Metabolome Database (HMDB) (accuracy <30 ppm). [241] Potential identities were confirmed by comparison of the exact mass, retention time and MS/MS spectra fragmentation pattern of the class representative internal standards, when available, with public databases.[242]

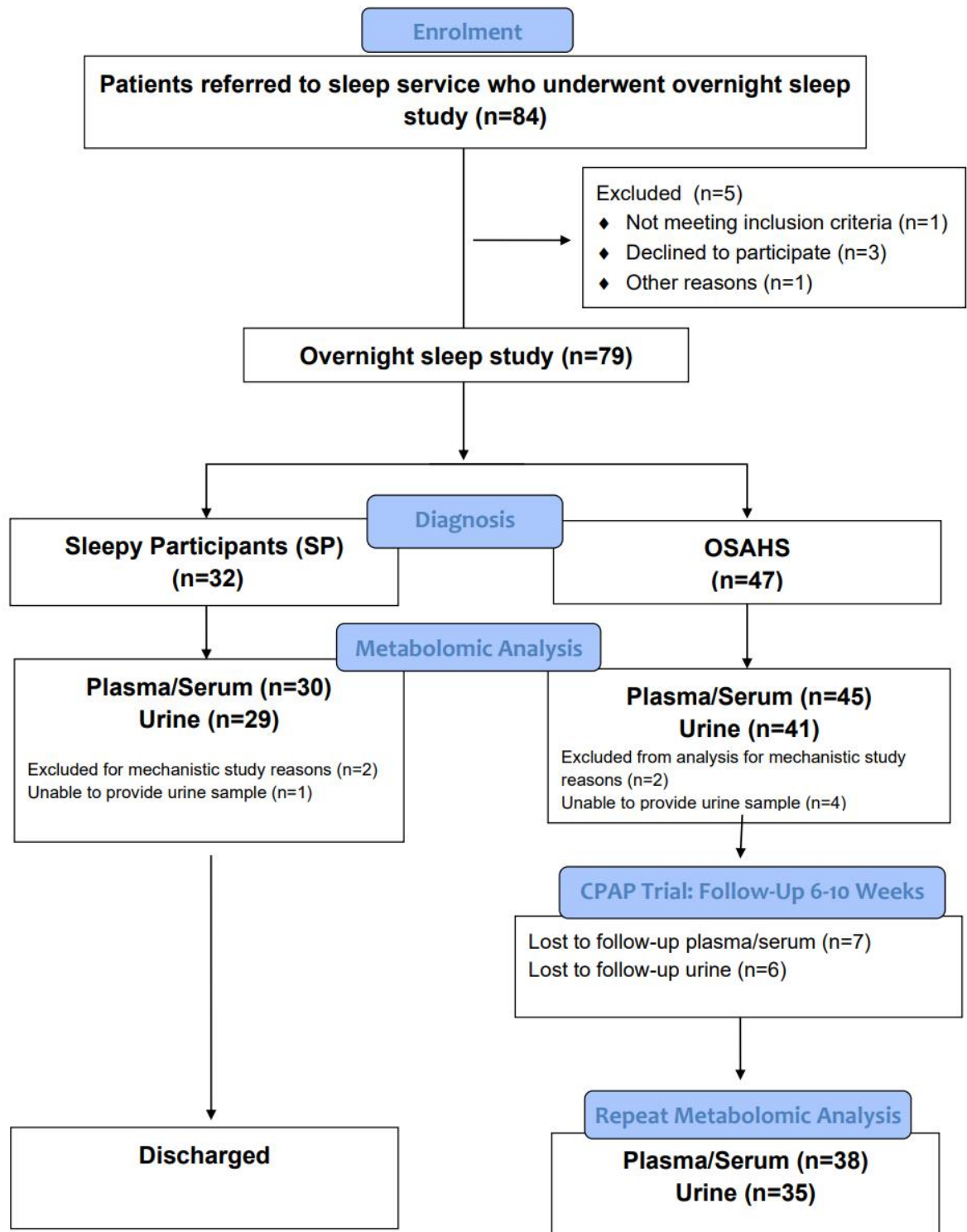
6.4 Statistical Analysis

Principal component analyses (PCA) and partial least squares discriminant analysis (PLS-DA) analyses were performed using the MetaboAnalyst 5.0 platform [62]. Raw data were log-transformed and Pareto-scaled before analysis, and outputs were Bonferroni-corrected for multiple comparisons (false discovery rate). Major sources of variation were identified using t-tests or variable importance projection (VIP) scores. Metabolites were tentatively identified using the DIMEdb (dimedb.ivers.aber.ac.uk) and HMDB (<https://hmdb.ca/>) databases based on predicted masses m/z ratios and likely ionisation forms. All isotopes/adducts were considered when deriving the identities for individual m/z . In MetaboAnalyst 5.0 pathway analysis (integrating enrichment analysis and pathway topology analysis) incorporating the mummichog algorithm, was used for biochemical pathway enrichment analysis.

Data are presented as the mean [SD] for quantitative variables and n (%) for qualitative variables. Chi-squared test was used for categorical variables, and the Mann-Whitney U test used for numeric data.

7 Results

Consort flow diagram of the study



7.1 Urine metabolomic analysis

7.1.1 Study Population

A total of 70 patients with available clinical data and urine samples were included in the metabolomic profiling analysis, of which 41 were OSAHS, and 29 were non-OAHS ('sleepy participants' (SP)).

The study population was mainly middle-aged, obese, and male (table 7.1). Patients with OSAHS were slightly older, had a higher BMI ($p=0.01$), and had a higher prevalence of type 2 diabetes mellitus. However, the SP had a higher prevalence of hypertension ($p=0.52$) and a higher proportion taking opioid medications ($p=0.01$). The OSAHS group also had a statistically significant number of participants taking beta-blockers ($p=0.03$).

Table 7.1: Clinical and limited channel sleep characteristics of the urine study population at baseline

	All (n=70)	SP (n=29)	OSAHS (n=41)	p-value
Demographic variables				
<i>Age (years)</i>	51.0 [11.5]	50.3 [13.2]	51.5 [10.2]	0.86
<i>Sex, male</i>	47 (67.1%)	18 (62.1%)	29 (70.7%)	0.45
<i>BMI (kg/m²)</i>	36.7 [8.5]	33.7 [8.2]	38.9 [8.2]	0.01
<i>Current smoker (y)</i>	8 (11.4%)	4 (13.8%)	4 (9.8%)	0.49
<i>ESS</i>	13.4 [5.7]	11.9 [6.3]	14.5 [5.0]	0.09
HSAT parameters				
<i>AHI (events/hour)</i>	32.4 [29.6]	5.6 [3.9]	51.3 [24.7]	<0.001
<i>ODI (events/hour)</i>	30.9 [26.7]	6.9 [4.1]	47.9 [22.5]	<0.001
<i>TSat90</i>	25.3 [25.3]	7.7 [16.0]	37.7 [23.2]	<0.001
<i>Mean oxygen saturations (%)</i>	91.5 [3.1]	93.3 [1.4]	90.1 [3.3]	<0.001
<i>Minimum oxygen saturations (%)</i>	75.6 [11.3]	83.5 [4.9]	70.0 [11.3]	<0.001
Co-morbidities				
<i>HTN</i>	33 (47.1%)	15 (51.7%)	18 (43.9%)	0.52
<i>DM</i>	10 (14.3%)	3 (10.3%)	7 (17.1%)	0.43
<i>COPD</i>	4 (5.7%)	2 (6.9%)	2 (4.9%)	0.72
<i>IHD</i>	2 (2.9%)	0 (0.0%)	2 (4.9%)	0.23
<i>FHX</i>	6 (8.6%)	2 (6.9%)	4 (9.8%)	0.67
Medication history				
<i>ACE/ARB</i>	25 (35.7%)	11 (37.9%)	14 (34.1%)	0.75
<i>Beta-blockers</i>	6 (8.6%)	0 (0.0%)	6 (14.6%)	0.03
<i>Calcium channel blockers</i>	19 (27.1%)	8 (27.6%)	11 (26.8%)	0.94
<i>Diuretics</i>	8 (11.4%)	3 (10.3%)	5 (12.2%)	0.81
<i>Lipid lowering</i>	19 (27.1%)	8 (27.6%)	11 (26.8%)	0.94
<i>Anti-coagulants</i>	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
<i>Opioids</i>	16 (22.9%)	11 (37.9%)	5 (12.2%)	0.01

Data are presented as the mean [SD] for quantitative variables and n (%) for qualitative variables. y = yes, ESS = Epworth sleepiness score, AHI = Apnoea Hypopnoea Index, ODI = oxygen desaturation index, HTN = hypertension, DM = diabetes mellitus, COPD = chronic obstructive pulmonary disease, IHD = ischaemic heart disease, FHX = family history of OSAHS, ACE/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, TSat90 = time with SaO₂ < 90%. Chi-squared test was used for categorical variables, and the Mann-Whitney U test used for numeric data.

7.1.2 Metabolomic signatures associated with OSAHS in urine

The first aim of this study was to analyse global metabolomic differences between patients with OSAHS compared to their SP counterparts. Following an untargeted metabolomic profiling approach based on high resolution mass spectrometry (HR-MS), data were obtained from samples following negative and positive ionisation. After quality control and signal correction, 1617 metabolites were detected in the urine and included in the subsequent analysis.

To evaluate global metabolomic variation between both groups, multivariate analyses using partial least squares discriminant analysis (PLS -DA; MetaboAnalyst 5.0), were performed (Figure 7.1). The 2D space generated by PLS-DA shows the distribution of analytes from both groups, with each point representing a sample, coloured according to the group it belongs. Considering the metabolomic shifts, the results show that the metabolite profiles of the OSAHS cohort is distinctly separated from the control group. This indicates that the OSAHS group had metabolomic changes not seen in their SP counterparts.

The significant sources of variation between the groups shown on the PLS-DA were identified based on VIP scores (Figure 7.2). The VIP value provides a measure of the importance of each metabolite in the separation between different sample groups, with higher VIP values indicating greater importance. Out of the 1617 metabolites, 15 had VIP scores of significance, with VIP score of 1.0 being a typical cutoff point of having a significant impact on the separation between groups. Prolyl-Glutamine and Octadecanamide had VIP scores in excess of 3.

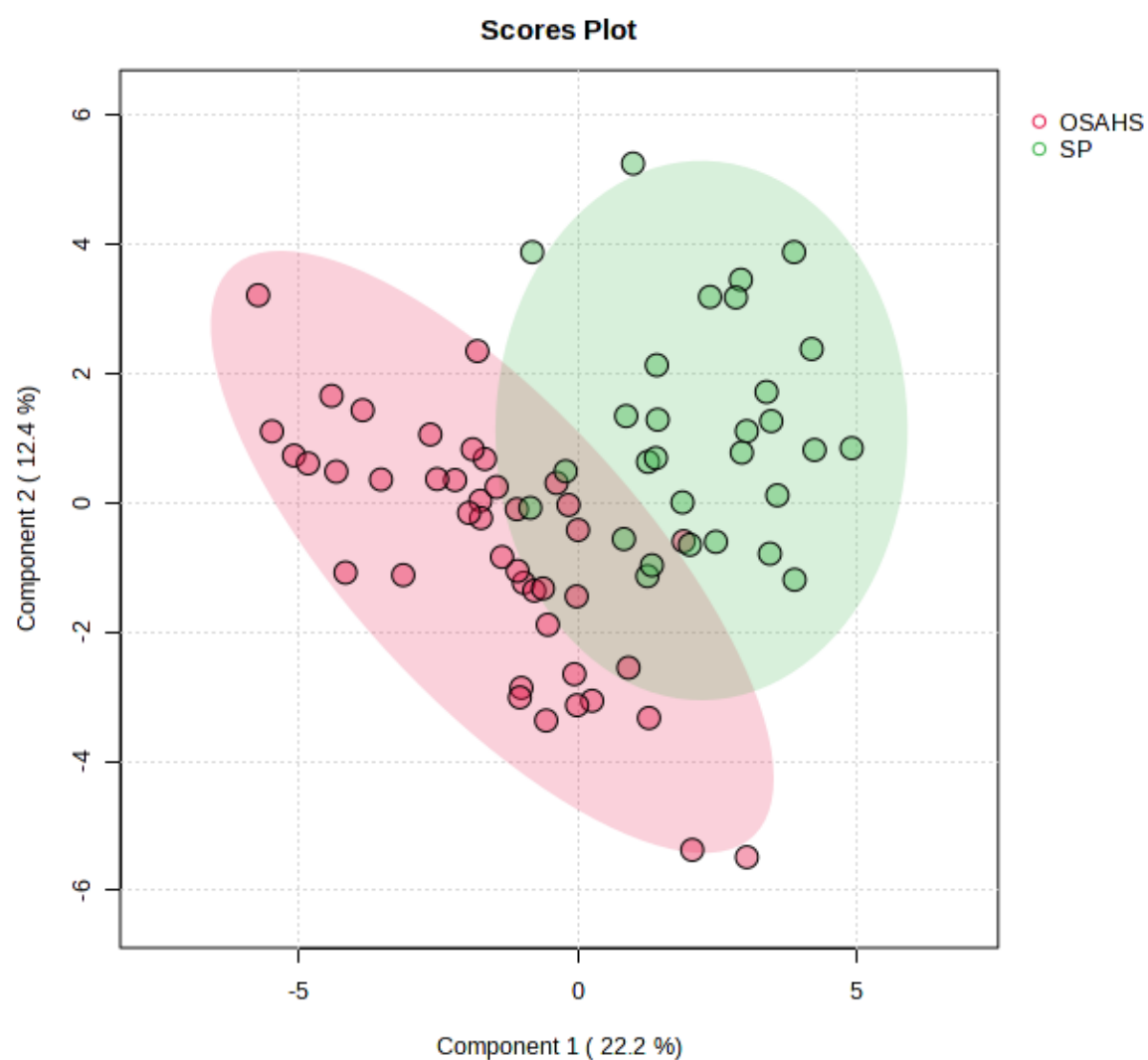


Figure 7.1 : Partial least squares discriminant analysis of urine metabolomic profile in OSAHS and SP. Each data point is one sample, ellipses = 95% CI. 70 samples, PC1=22.2%, PC2=12.4%

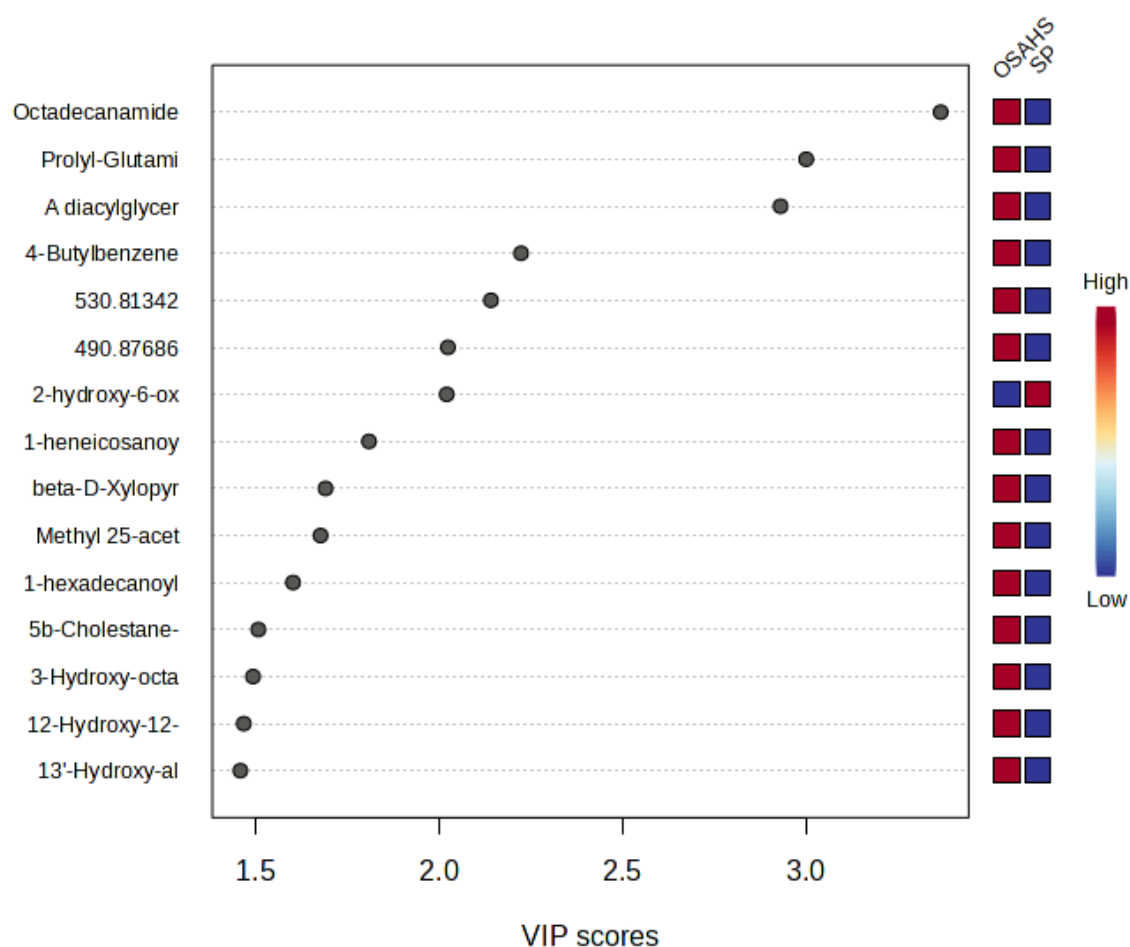


Figure 7.2: Variable importance in projection (VIP) scores for the top metabolites in urine contributing to variation in metabolic profiles of OSAHS vs SP. The vertical coordinate in the graph indicates a metabolite, and the horizontal coordinate indicates the VIP value. The relative abundance of metabolites in each group (OSAHS and SP) is indicated by the coloured scale from blue to red representing the low and high, respectively.

The metabolomic data were also subjected to univariate t tests, then filtered by statistical significance ($P < 0.01$), corrected for false discovery rate. The top 20 metabolites are highlighted in table 7.2 where their fold changes ($\log_2FC$) (OSAHS/SP) are indicated alongside their area under the curve values (AUC) as determined by receiver operating characteristic (ROC) curve analyses. The 20 metabolites are displayed on figure 7.3 on a hierarchical clustering model (heat map) and dendrogram. This shows the relationships between the metabolites and the clinical groups. To ease viewing the targeted metabolites are also displayed using boxplots where their relative levels are shown in figure 7.4. All metabolites were significantly increased in OSAHS with the exception of DL-arabinose. Table 7.3 shows the enhanced biochemical pathways based on metabolites which differentiate between OSA and SP groups, based on VIP scores and t-tests.

Table 7.2: Potential identities of the significantly differentially expressed metabolites between OSA and SP in urine

Name	AUC	T-tests	Log2 FC	Pattern of dysregulation (OSAHS vs SP)
Octadecanamide	0.85509	1.0029E-8	1.6859	Up
Prolyl-Glutamine	0.83703	8.055E-9	1.8846	Up
A diacylglycerol (40:7)	0.79803	1.1251E-7	1.3306	Up
beta-D-Xylopyranosyl-(1->5)-alpha-L-arabinofuranosyl-(1->5)-L-arabinose	0.77545	4.1554E-4	2.016	Up
12-Hydroxy-12-octadecanoylcarnitine	0.77011	3.2457E-4	1.1726	Up
1-heneicosanoyl-glycero-3-phosphate	0.76437	2.741E-4	1.4656	Up
13'-Hydroxy-alpha-tocopherol	0.74589	3.2569E-4	1.2451	Up
DL-Arabinose	0.74302	0.0019656	-0.51556	Down
Methyl 25-acetoxy-3beta-hydroxy-cholest-5-en-19-oate	0.7381	0.011155	4.2907	Up
1-hexadecanoyl-2-(5-hydroxy-7-carboxy-6E-heptenoyl)-sn-glycero-3-phosphate	0.73194	3.5665E-4	1.3351	Up
457.24551	0.73153	0.0012752	0.50263	Up
350.14709	0.72332	0.0013151	1.1738	Up
490.87686	0.71757	3.1984E-4	0.53674	Up
531.28271	0.71757	0.0021005	0.45485	Up
3-Hydroxy-octadecenoylcarnitine	0.71141	5.9956E-4	1.1394	Up
2-Hydroxy-22-methyltetracosanoic acid	0.70731	0.0015348	1.15	Up
508.68359	0.7069	9.7448E-4	0.88729	Up
11-HDoHE	0.70567	0.0027358	1.8033	Up
5b-Cholestane-3a,7a,12a,24,25-pentol	0.69745	0.0045743	3.9469	Up
2-hydroxy-6-oxohepta-2,4-dienoic acid	0.69704	0.0021326	-0.71632	Up

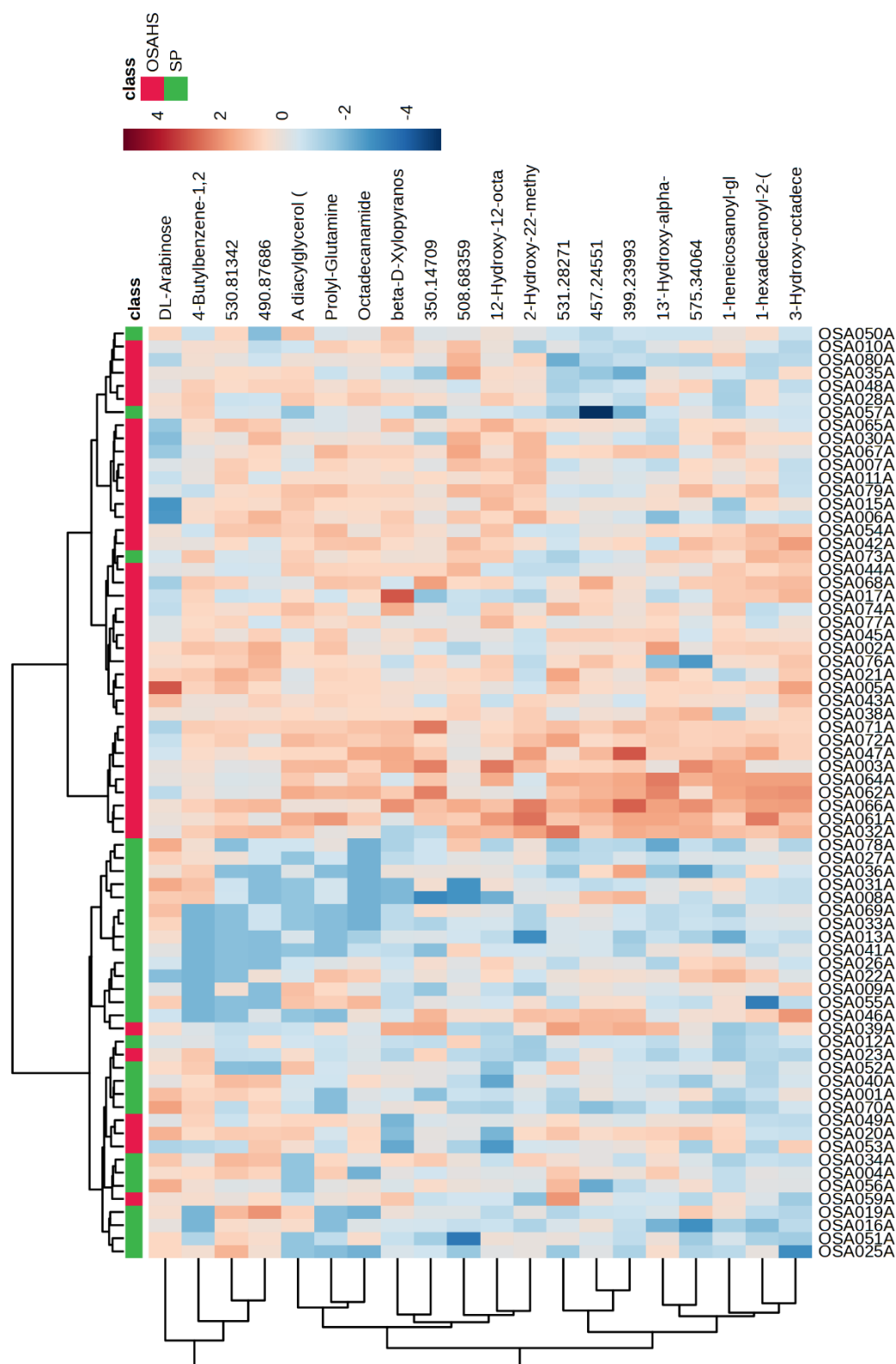
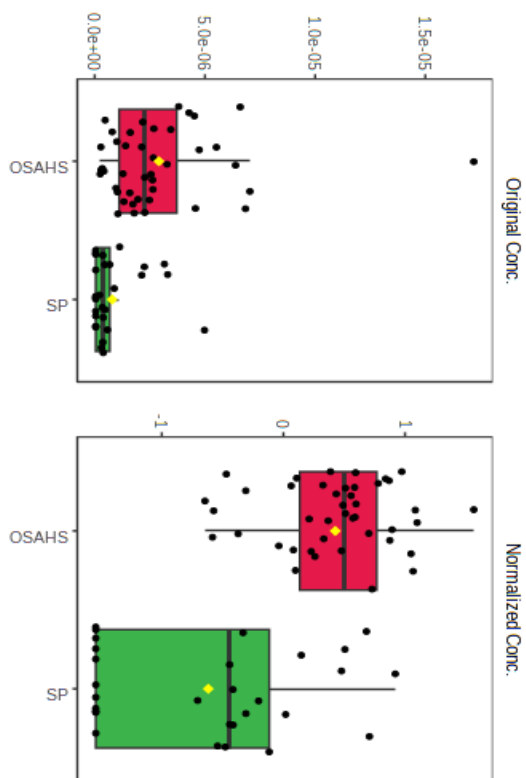
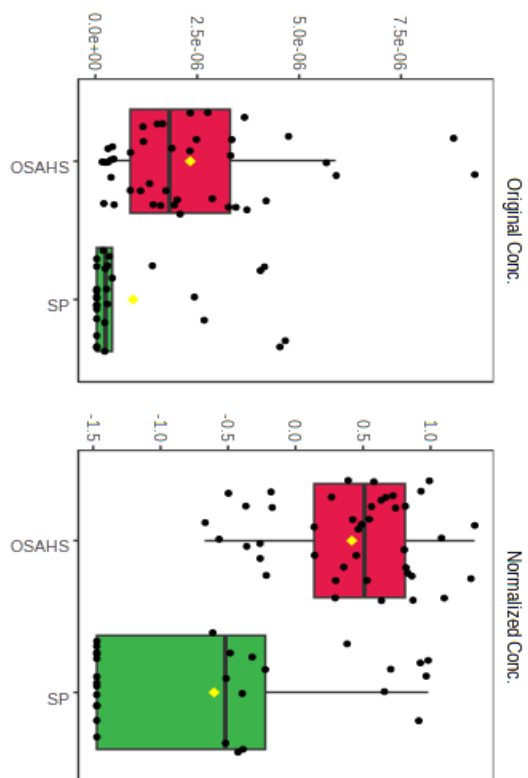


Figure 7.3: Hierarchical clustering model of the top 20 metabolites found in urine. Each row represents a urine sample, and each column represents the expression profile of a metabolite across age groups. OSAHS patients appear in red, and SP in green. The colour scale illustrates the relative expression level of each molecule in each patient and ranges from blue to red, indicating relatively low to high expression, respectively; blue colour represents a downregulation, and red colour upregulation.

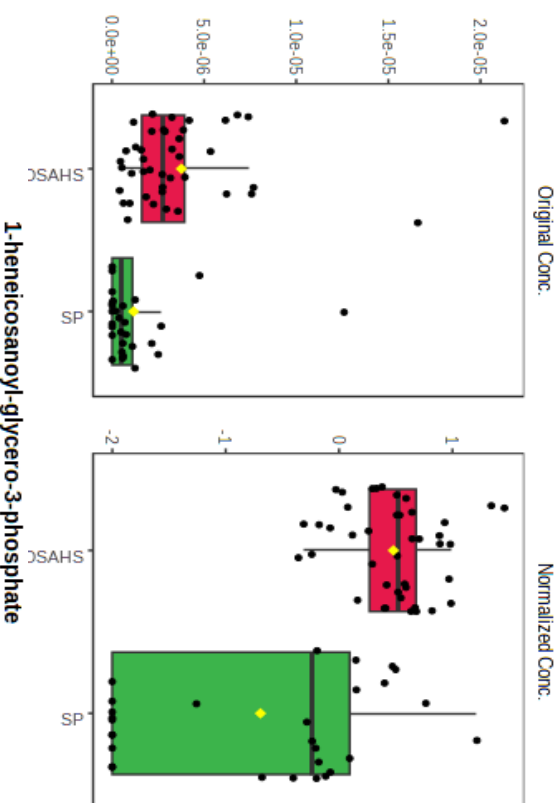
Prolyl-Glutamine



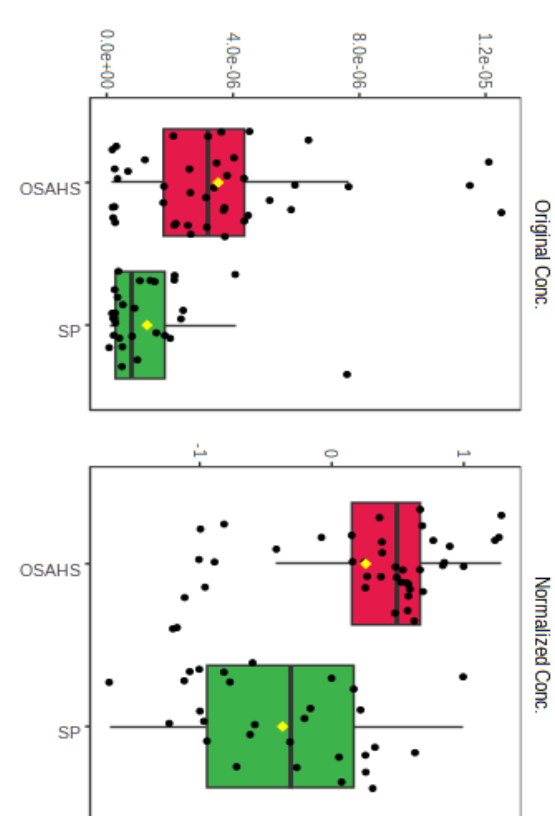
A diacylglycerol (40:7)



Octadecanamide



1-heneicosanoyl-glycerol-3-phosphate



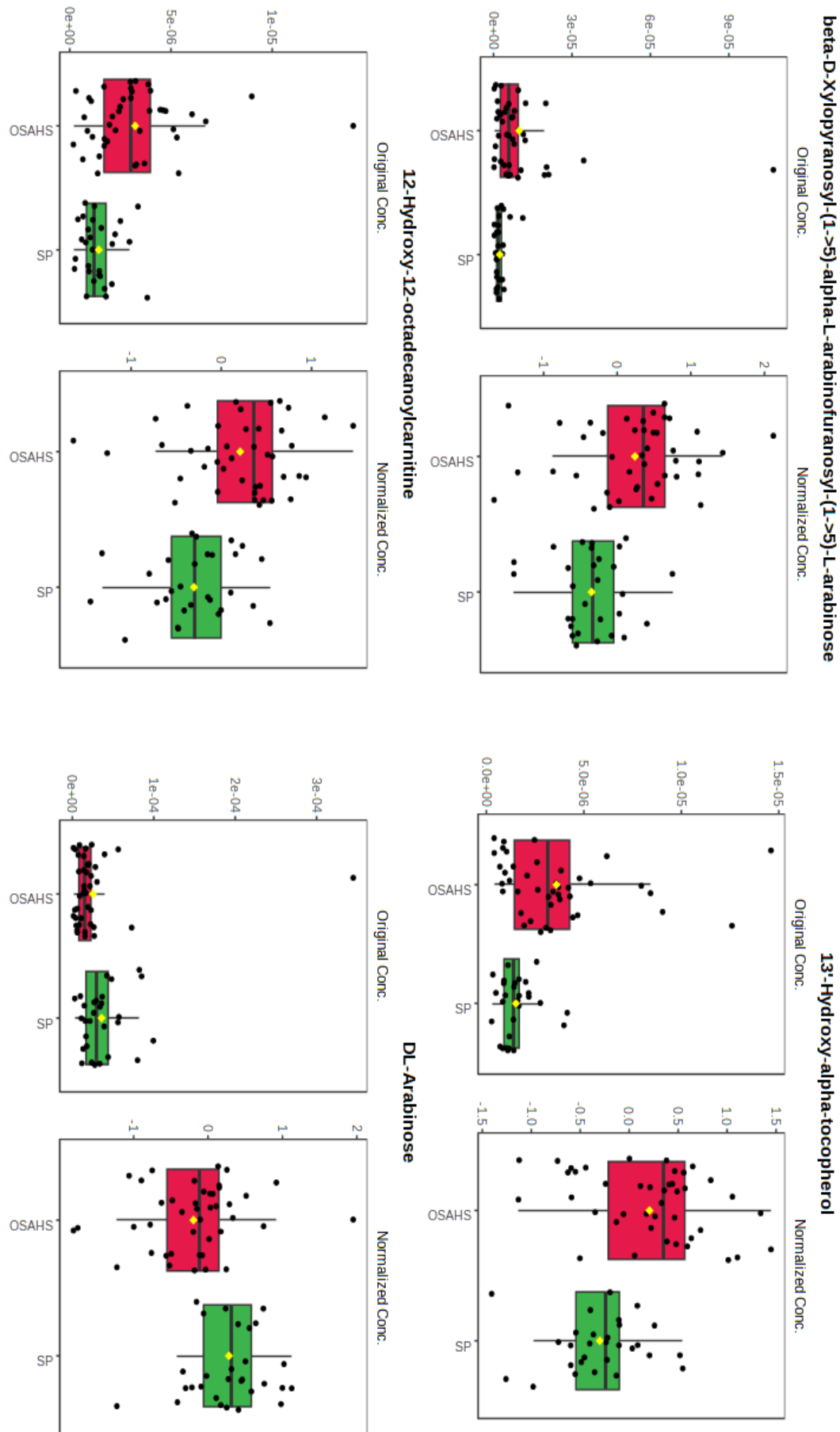


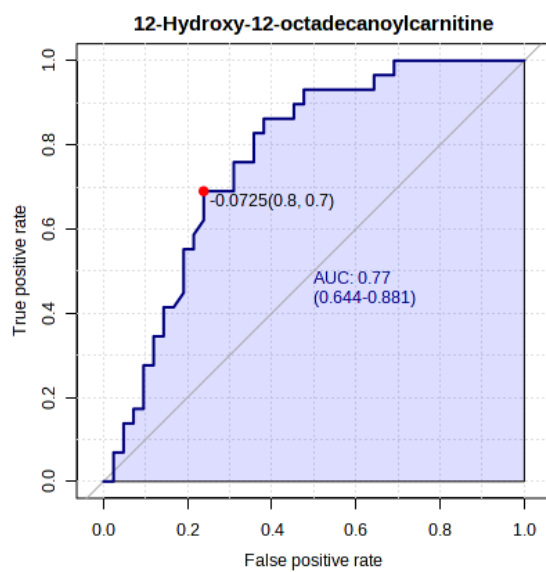
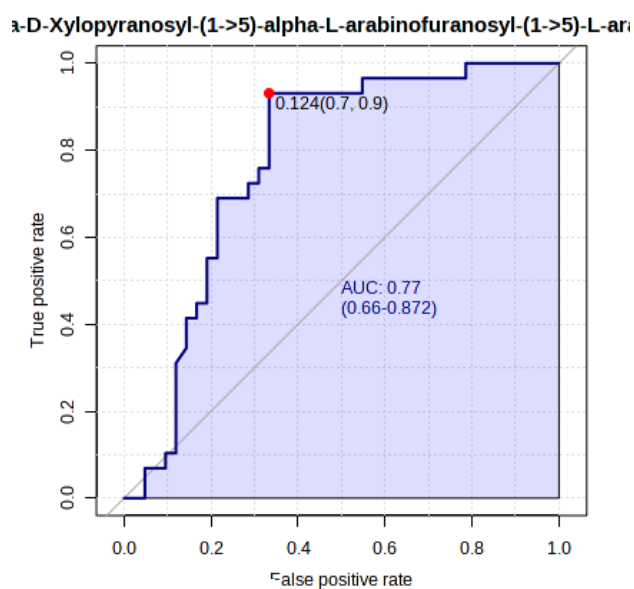
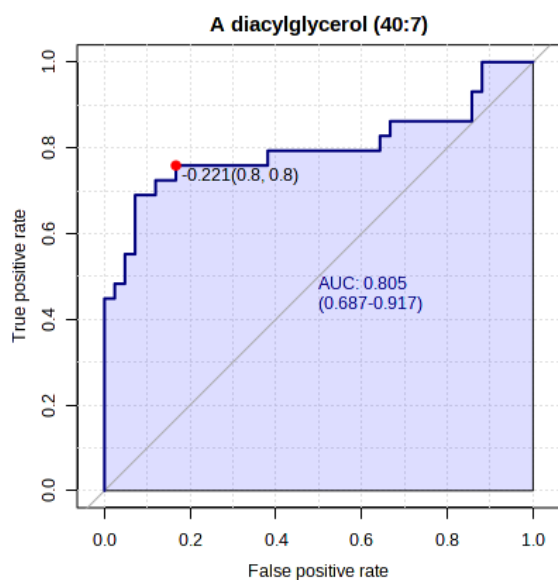
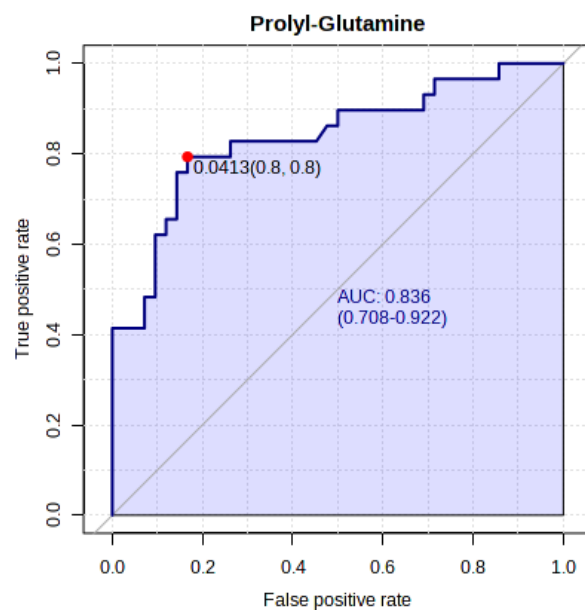
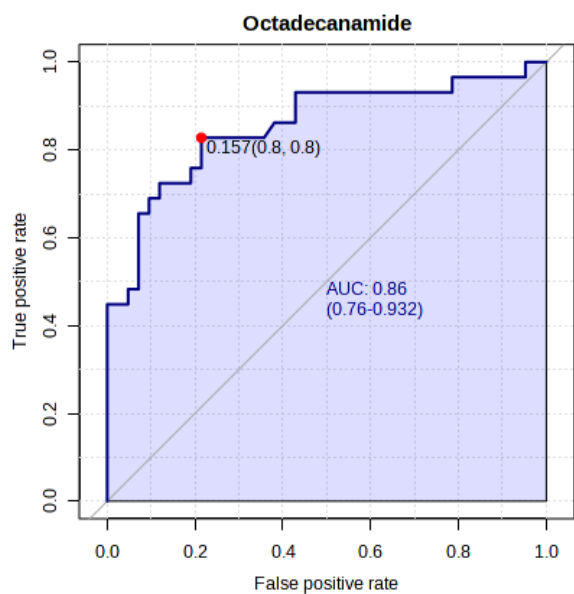
Figure 7.4 :Up/down regulation of top 8 key metabolites in urine. Each boxplot shows the range of values of the metabolite in OSAHS and SP, with the median value represented by a horizontal line within the box.

Table 7.3: Enhanced biochemical pathways based on metabolites which differentiate between OSA and SP groups in urine, based on VIP scores and t-tests

Pathway Name	Total	Hits (all)	Hits (sig.)	Expected	P(FDR)
Vitamin E metabolism	54	38	15	5.0563	0.025523
Carnitine shuttle	72	25	10	6.7418	0.028334
Bile acid biosynthesis	82	51	17	7.6781	0.029459
De novo fatty acid biosynthesis	106	17	6	9.9254	0.042666
Electron transport chain	7	2	2	0.65545	0.043986
Fatty Acid Metabolism	63	10	4	5.899	0.046405
Vitamin D3 (cholecalciferol) metabolism	16	14	5	1.4982	0.046613

To determine the ability of these potentially key differential metabolites to differentiate between OSAHS and SP, we generated subject operating characteristic curves (ROC curves) on the top 5 metabolites and calculated the area under the curve (AUC) for each. The horizontal coordinates in the graph indicate the negative positive class rate, i.e. the false positive rate (1-specificity) and the vertical coordinates indicate the true class rate, i.e. the true positive rate (sensitivity). The larger the AUC is, the higher the prediction accuracy, with the closer the curve is to the upper left corner (the smaller the X and the larger the Y), the higher the prediction accuracy. An $AUC < 0.5$ indicates that the test has no diagnostic value, AUC of 0.8–0.9 indicates that the test has good accuracy, and $AUC > 0.9$ indicates that the diagnostic test has high accuracy (figure 7.5).

We also combined these top 5 metabolites (octadecanamide, prolyl-glutamine, a diacylglycerol, beta-D-Xylopyranosyl-(1->5)-alpha-L-arabinofuranosyl-(1->5)-L-arabinose, and 12-Hydroxy-12-octadecanoylcarnitine) and found they could differentiate between the OSAHS and SP with an AUC of 0.779 (CI 0.524-0.928).



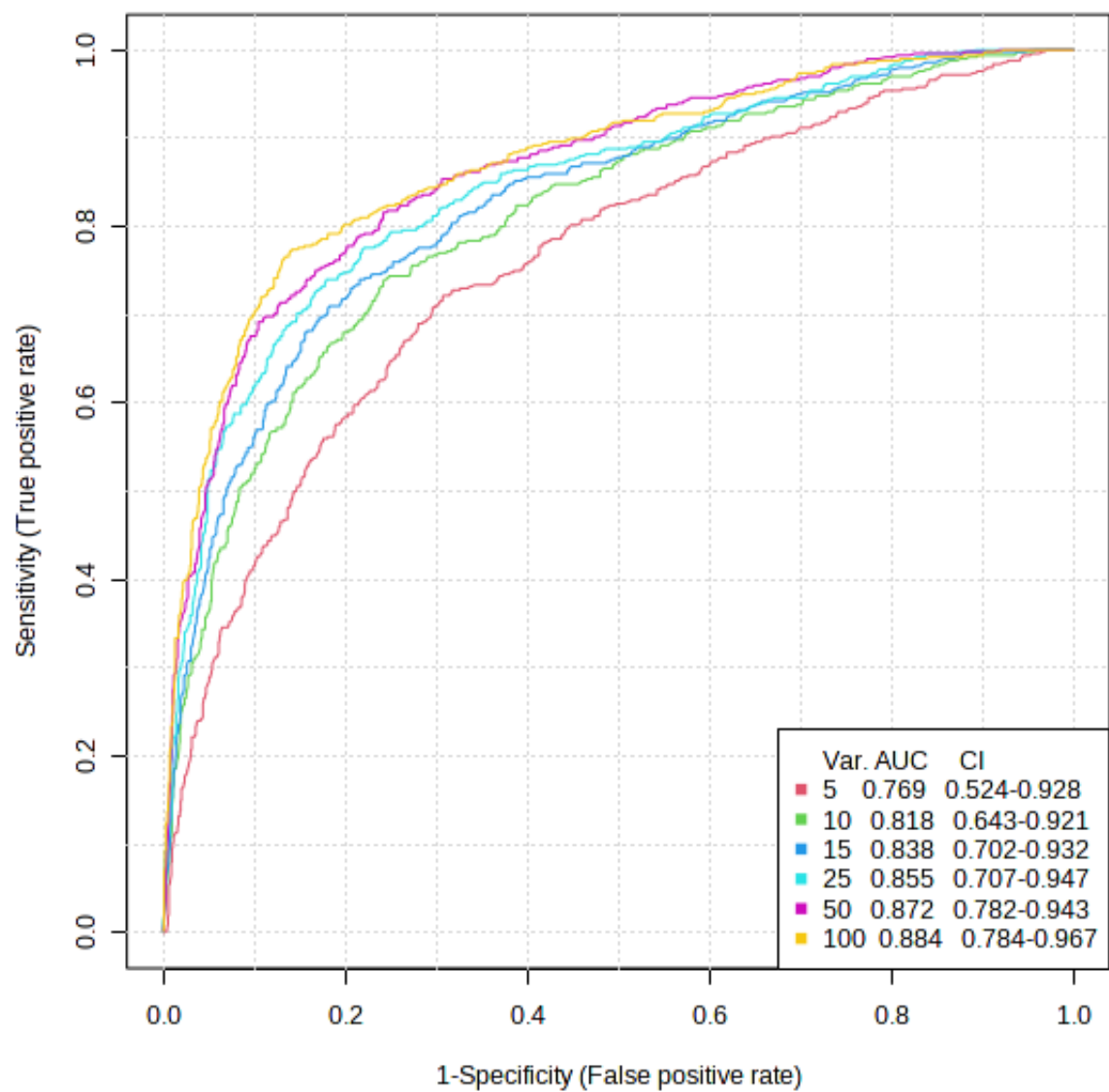


Figure 7.5: ROC curve graphs of top 5 urinary metabolites and combined AUC for distinguishing OSAHS from SP

7.2 Plasma and serum metabolomic analysis

7.2.1 Study Population

A total of 75 patients with available clinical data and plasma and serum samples were included in the metabolomic profiling analysis, of which 45 were OSAHS and 30 were SP.

This study population was very similar to the urine population, again comprising of mainly middle-aged (51.2 years [11.5]), obese (BMI 37.3kg/m² [9.3]) men (table 7.4). Patients with OSAHS were once again slightly older ($p=0.95$), had a higher BMI ($p<0.01$), and had a higher prevalence of type 2 diabetes mellitus ($p=0.35$). The SP population again had a higher prevalence of hypertension ($p=0.63$) and a higher proportion taking opioid medications ($p=0.03$). There was once again a significant higher proportion of those with OSAHS taking beta-blockers ($p=0.02$).

Table 7.4: Clinical and limited channel sleep characteristics of the plasma and serum study population at baseline.

	All (n=75)	SP (n=30)	OSAHS (n=45)	p-value
Demographic variables				
<i>Age (years)</i>	51.2 [11.5]	50.7 [13.1]	51.6 [10.5]	0.95
<i>Sex, male</i>	51 (68%)	19 (63.0%)	32 (71.0%)	0.48
<i>BMI (kg/m²)</i>	37.3 [9.3]	33.7 [8.1]	39.7 [9.4]	<0.01
<i>Current smoker (y)</i>	9 (12.0%)	4 (13.3%)	5 (11.1%)	0.56
<i>ESS</i>	13.3 [5.6]	11.7 [6.3]	14.4 [4.8]	0.07
HSAT parameters				
<i>AHI (events/hour)</i>	32.8 [30.0]	5.9 [4.0]	50.8 [24.0]	<0.001
<i>ODI (events/hour)</i>	31.3 [26.1]	7.1 [4.2]	47.3 [21.8]	<0.001
<i>TSat90</i>	26.4 [26.0]	7.8 [15.7]	38.9 [24.1]	<0.001
<i>Mean oxygen saturations (%)</i>	91.4 [3.0]	93.3 [1.4]	90.1 [3.2]	<0.001
<i>Minimum oxygen saturations (%)</i>	75 [11.4]	83.4 [4.8]	69.4 [11.1]	<0.001
Co-morbidities				
<i>HTN</i>	35 (47%)	15 (50%)	20 (44.0%)	0.63
<i>DM</i>	11 (14.7%)	3 (10%)	8 (17.8%)	0.35
<i>COPD</i>	4 (5.3%)	2 (6.7%)	2 (4.4%)	0.67
<i>IHD</i>	3 (4.0%)	0 (0.0%)	3 (6.7%)	0.15
<i>FHX</i>	7 (9.3%)	2 (6.7%)	5 (11.1%)	0.52
Medication history				
<i>ACE/ARB</i>	28 (37.3%)	11 (36.7%)	17 (37.8%)	0.92
<i>Beta-blockers</i>	7 (9.3%)	0 (0.0%)	7 (15.6%)	0.02
<i>Calcium channel blockers</i>	19 (25.3%)	8 (26.7%)	11 (24.4%)	0.82
<i>Diuretics</i>	9 (12.0%)	3 (10%)	6 (13.3%)	0.66
<i>Lipid lowering</i>	20 (26.7%)	8 (26.7%)	12 (26.7%)	0.78
<i>Anti-coagulants</i>	5 (6.7%)	0 (0.0%)	5 (11.1%)	NA
<i>Opioids</i>	17 (22.7%)	12 (40%)	5 (11.1%)	0.03

Data are presented as the mean [SD] for quantitative variables and n (%) for qualitative variables. y = yes, ESS = Epworth sleepiness score, AHI = Apnoea Hypopnoea Index, ODI = oxygen desaturation index, HTN = hypertension, DM = diabetes mellitus, COPD = chronic obstructive pulmonary disease, IHD = ischaemic heart disease, FHX = family history of OSAHS, ACE/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, TSat90 = time with SaO₂ < 90%. Chi-squared test was used for categorical variables, and the Mann-Whitney U test used for numeric data.

7.2.2 Metabolomic signatures associated with OSAHS in plasma and serum

We applied the same process as in urine to the plasma and serum samples. After quality control and signal correction, 3697 metabolites were detected in the plasma and 3549 metabolites in the serum which were included in the subsequent analysis.

PLSDA did show separation between the OSAHS and SP groups in the plasma samples (figure 7.6), and 8 metabolites were identified with a VIP score of greater than 1.0 (figure 7.8). The most significant metabolites based on VIP scores were GlcCer(d18:1/12:0 and N(6)-L-Homocysteiny-L-lysine. There was minimal separation on partial least squares discriminant analysis (PLSDA) in the serum samples (figure 7.7), and consequently no VIP scores of significance were generated.

Based on univariate analysis and filtering by statistical significance, 14 metabolites found in plasma are highlighted in table 7.5 alongside their $-\log_{10}p$ (OSAHS /SP) and AUC values. Similarly, the 8 metabolites found in serum are found in table 7.6.

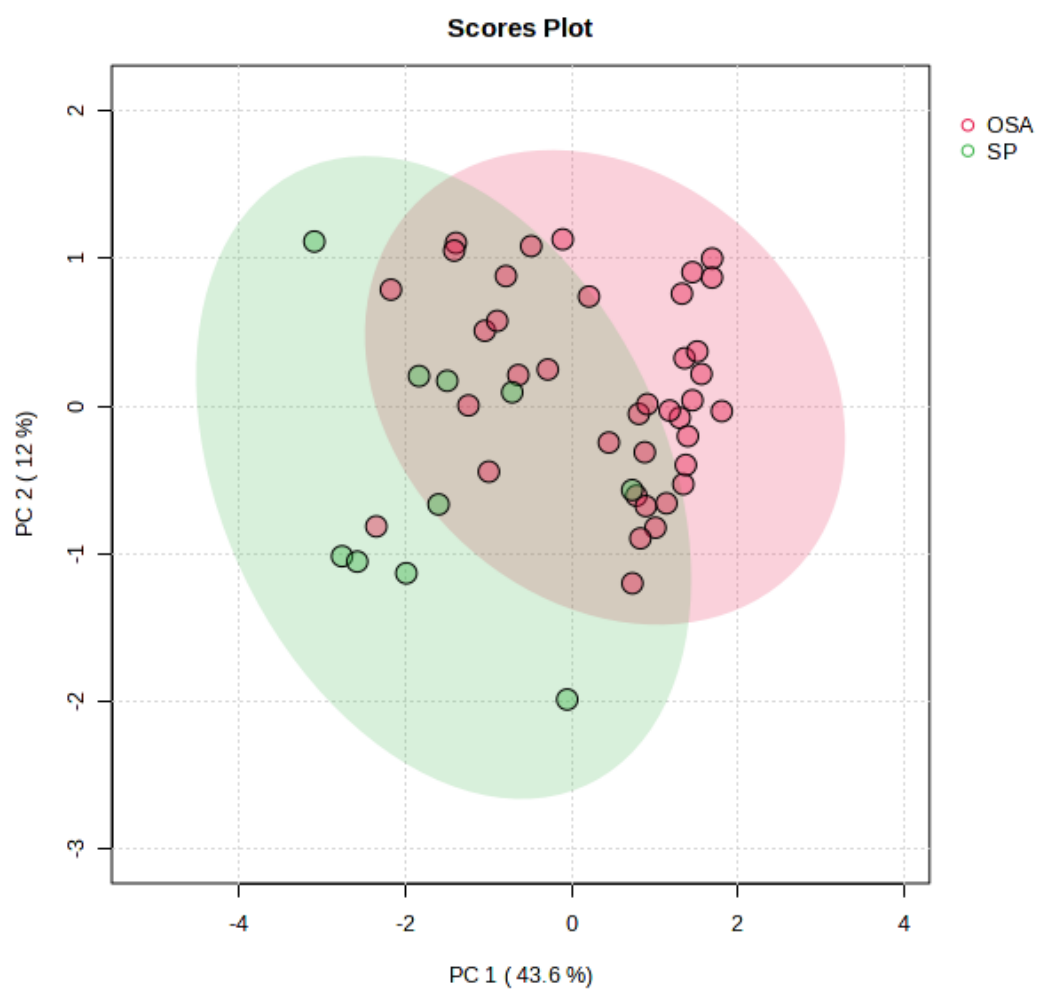


Figure 7.6 : Partial least squares discriminant analysis of plasma metabolomic profile in OSAHS and SP. Each data point is one sample, ellipses = 95% CI. 75 samples, PC1=43.6%, PC2=12.0%

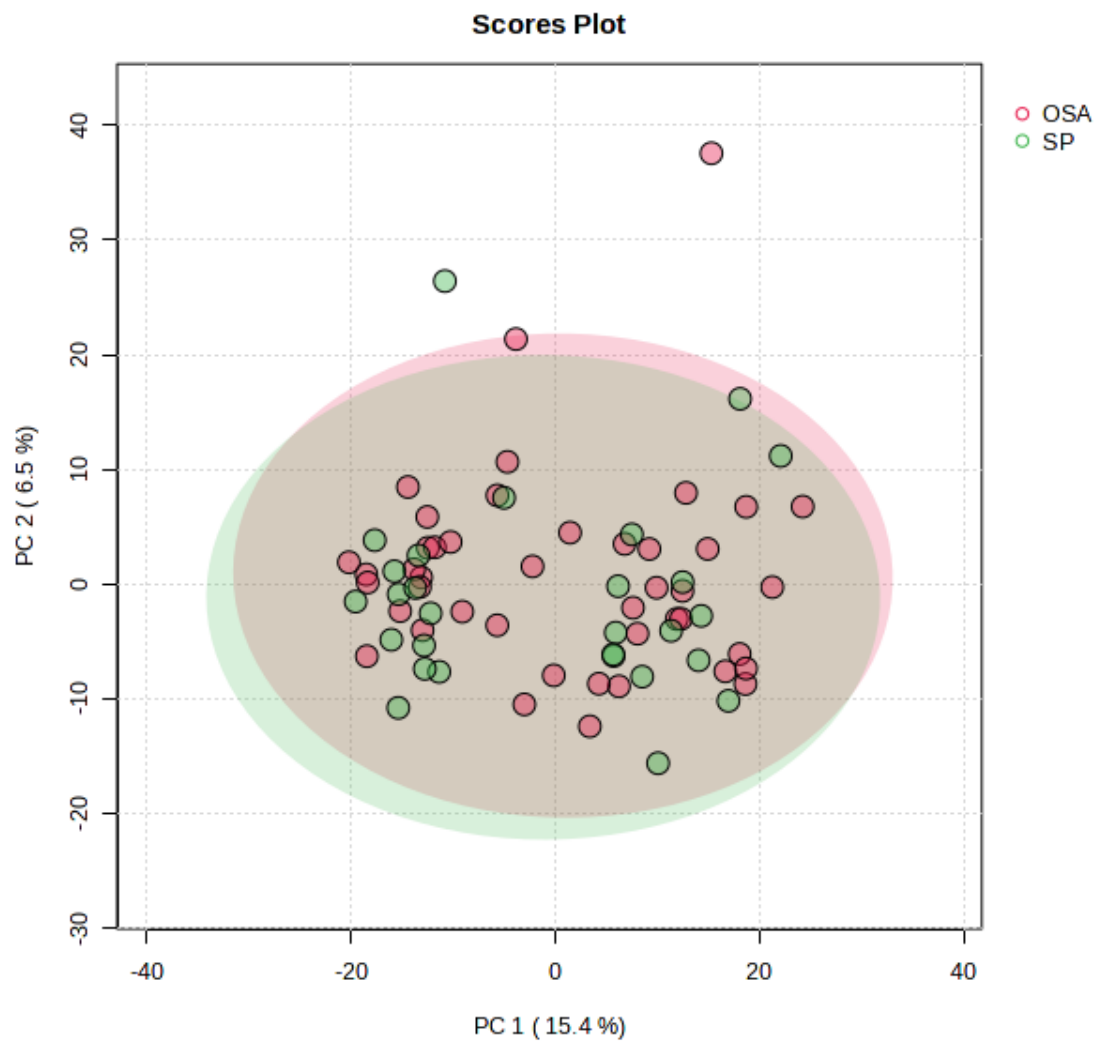


Figure 7.7 : Partial least squares discriminant analysis of serum metabolomic profile in OSAHS and SP. Each data point is one sample, ellipses = 95% CI. 75 samples, PC1=15.4%, PC2=6.5%

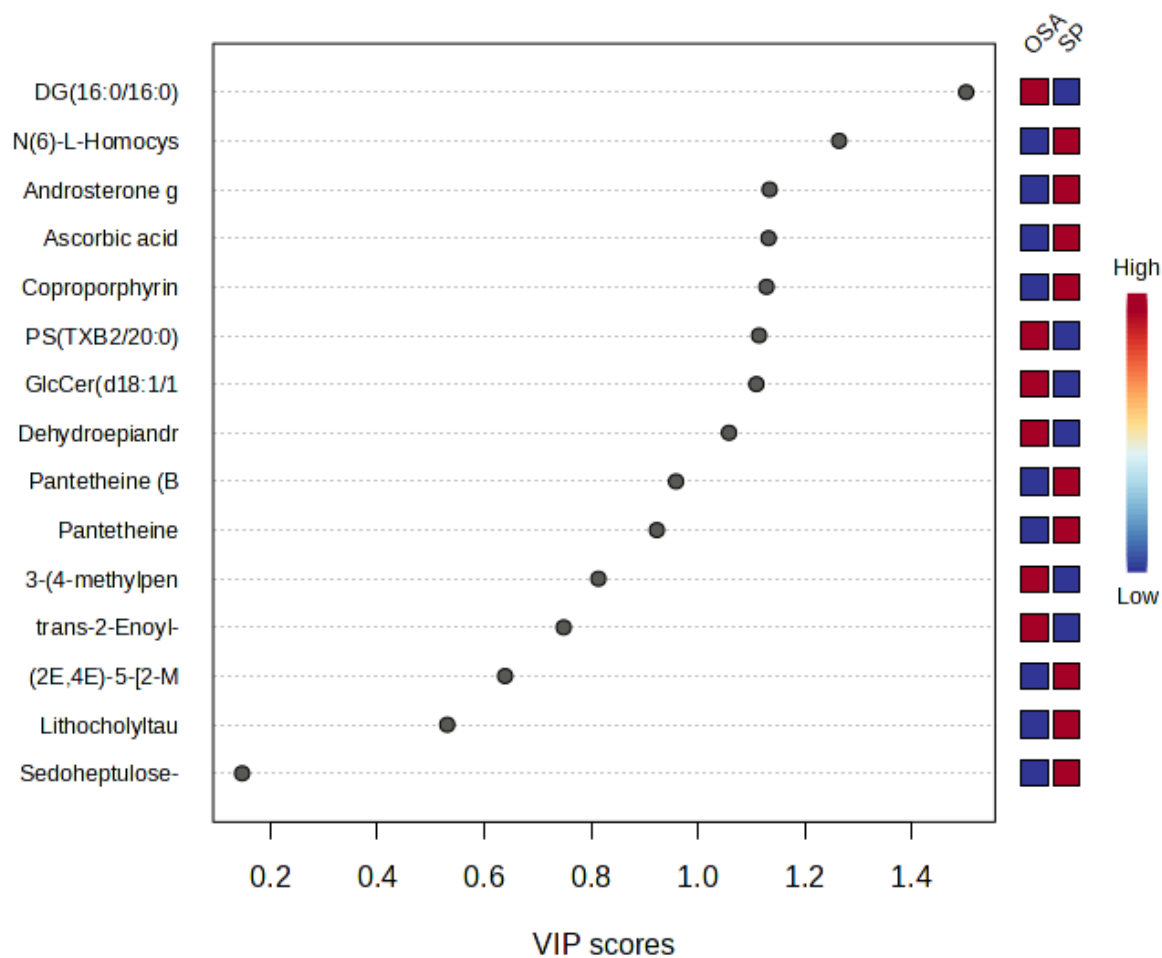


Figure 7.8: Variable importance in projection (VIP) scores for the top metabolites in plasma contributing to variation in metabolic profiles of OSAHS vs SP

Table 7.5: Potential identities of the significantly differentially expressed metabolites between OSA and SP in plasma

Name/m.z	t-stat	T-tests	-Log10(p)	Pattern of dysregulation (OSAHS vs SP)
GlcCer(d18:1/12:0)	4.1219	1.5526E-4	3.8089	Up
PS(TXB2/20:0)	3.6985	5.7701E-4	3.2388	Up
DG(16:0/16:0)	3.3703	0.0015284	2.8158	Up
Coproporphyrinogen	-3.3503	0.0016196	2.7906	Down
3-(4-methylpent-3-en-1-yl)pent-2-enedioyl-CoA	3.3498	0.0016222	2.7899	Up
N(6)-L-Homocysteinyl-L-lysine	-3.3269	0.0017334	2.7611	Down
Pantetheine (B5)	-3.2369	0.0022431	2.6491	Down
Androsterone glucuronide	-2.89	0.0058605	2.2321	Down
Dehydroepiandrosterone sulfate	2.7473	0.0085503	2.068	Up
Ascorbic acid	-2.5816	0.013086	1.8832	Down
trans-2-Enoyl-OPC4-CoA	2.2191	0.031451	1.5024	Up
(2E,4E)-5-[2-Methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene-6-yl)cyclopropyl]-3-methyl-2,4-pentadienoic acid	-2.1118	0.040164	1.3962	Down
Pantetheine (B5)	-3.2369	0.0022431	2.6491	Down
Androsterone glucuronide	-2.89	0.0058605	2.2321	Down

Table 7.6: Potential identities of the significantly differentially expressed metabolites between OSA and SP in serum

Name/m.z	t-stat	T-tests	-Log10(p)	Pattern of dysregulation (OSAHS vs SP)
922.35712	-5.1155	2.1412E-6	5.6693	Down
14beta-14,15-dihydrocalonysterone	-5.008	3.2765E-6	5.4846	Down
592.63147	-4.7178	1.0122E-5	4.9947	Down
856.40753	-4.2781	5.2386E-5	4.2808	Down
384.8331	-3.8915	2.0678E-4	3.6845	Down
Cer(t15:0(14Me)/23:0(2OH[R]))	3.8823	2.1344E-4	3.6707	Up
DG(18:2(9Z,12Z)/0:0/18:2(9Z,12Z))	3.8766	2.1772E-4	3.6621	Up
Am-PE(O-16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	2.84	0.0057346	2.2415	Up

7.3 The effect of CPAP on the global urine metabolomic profile

7.3.1 Study Population

The second aim of this study was to analyse the effect of CPAP usage on global metabolomic profile in those with OSAHS.

A total of 35 patients with available clinical data and urine samples were included in this metabolomic profiling analysis. The study population was again very similar to previous comprising mainly middle-aged, obese, men (table 7.7). All had at least moderate OSAHS based on AHI (≥ 15 events/hour), with a mean AHI of 53.4 [25.0] events/hour. CPAP usage was optimal over the mean 58.3 [10.4] nights, with the participants using their machines 93.0% of all available nights, for an average of 6 hours 6 minutes (366.3 [103.1] minutes) a night. This usage led to a reduction in their baseline AHI to 7.8 [7.5] events/hour.

Table 7.7: Clinical, limited channel sleep characteristics, and CPAP adherence in the urine population

	Urine (n=35)
Demographic variables	
Age (years)	51.9 [10.9]
Sex, male	24 (68.6%)
BMI (kg/m²)	39.3 [8.4]
ESS	14.9 [5.1]
HSAT parameters	
AHI	53.4 [25.0]
ODI	49.7 [22.1]
TSat90	38.9 [23.9]
Mean oxygen saturations	90.0 [3.4]
Minimum oxygen saturations	69.3 [11.5]
CPAP download	
Days on CPAP	58.3 [10.4]
% days total usage	93.0 [10.7]
% days > 4 hours	81.7 [20.6]
Average usage all nights (mins)	366.3 [103.1]
Residual AHI (events/hour)	7.8 [7.5]

Data are presented as the mean [SD] for quantitative variables and n (%) for qualitative variables. ESS = Epworth Sleepiness Score, AHI = Apnoea Hypopnoea Index, ODI = oxygen desaturation index, TSat90 = time with SaO₂ < 90%

7.3.2 Urine metabolomic signatures in those with OSAHS following treatment with CPAP

The same laboratory processes described in study one were applied to this cohort, and after quality control and signal correction, the metabolites detected in the urine of those who had undergone treatment with CPAP, were compared with the 1617 metabolites found in their urine samples at baseline in study one.

PLSDA showed significant separation in the metabolomic profile of those with OSAHS following treatment with CPAP (Figure 7.9). A range of metabolites were identified with VIP scores greater than 1.0, and figure 7.10 shows the top 15 metabolites according to their VIP score. 2-anilino-6-cyclohexylmethoxypurine, N-Undecanoylglycine, and Dodecenoic acid had VIP scores of greater than 8, and 5-Phenylvaleric acid had a VIP score in excess of 10.

Based on univariate analysis and filtering by statistical significance, the top 13 metabolites in urine are highlighted in table 7.8 alongside their fold changes ($\log_2FC$) (OSAHS /SP) and AUC values. The top 10 metabolites are displayed on figure 7.11 on a heat map and dendrogram showing the relationships between the metabolites and the clinical groups. These targeted metabolites are also displayed using boxplots where their relative levels are shown (figure 7.12). All metabolites were significantly decreased following CPAP treatment, with the exception of 2,3-Methyleneglutaric acid.

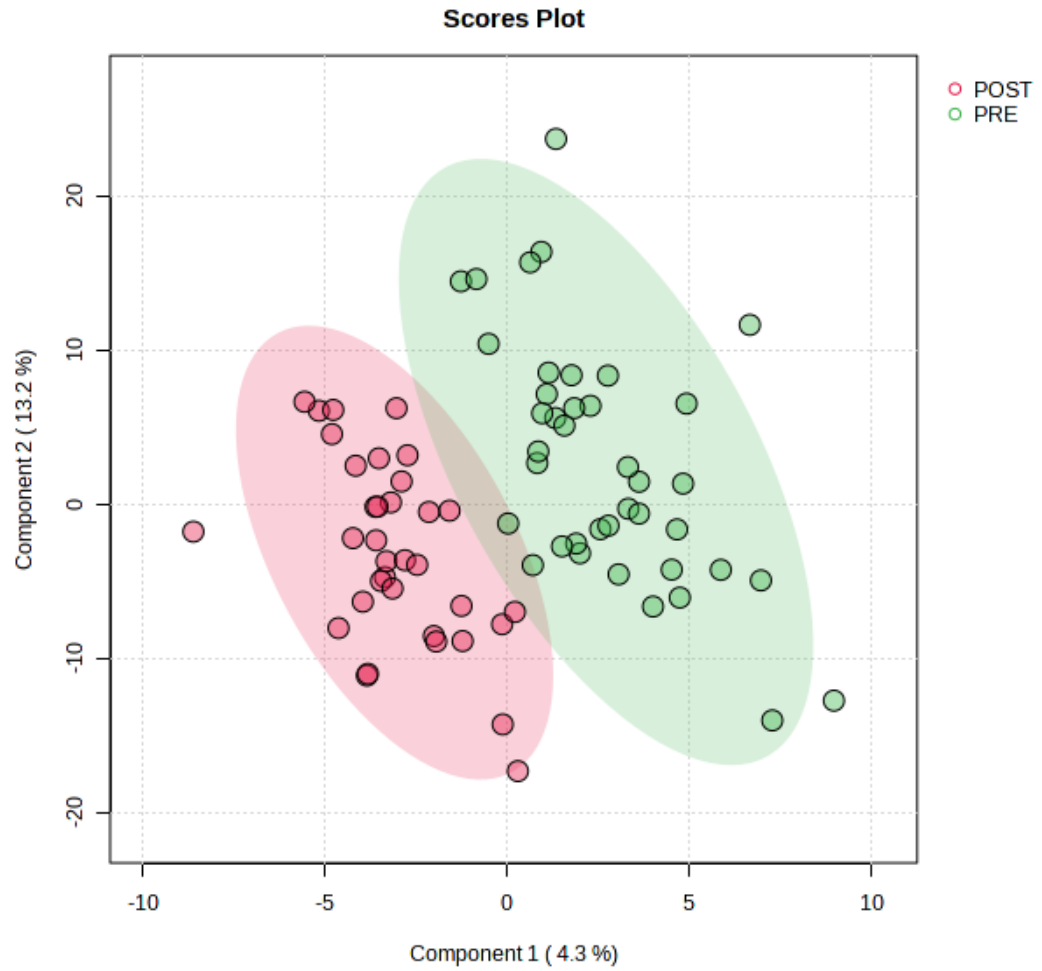


Figure 7.9: Partial least squares discriminant analysis of urine metabolomic profile following CPAP treatment. Each data point is one sample, ellipses = 95% CI. 70 samples, PC1=4.3%, PC2=13.2%

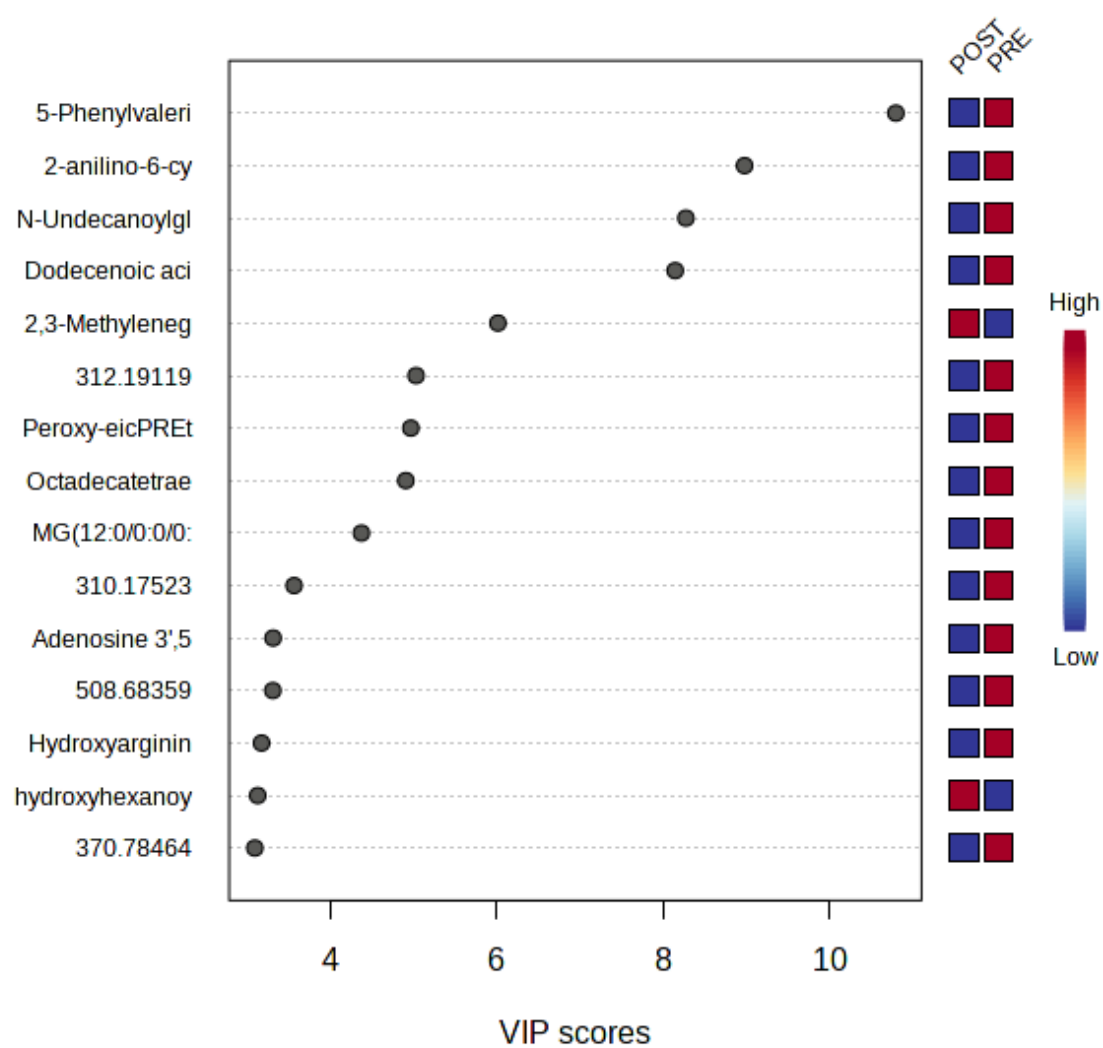


Figure 7.10: VIP scores for the top metabolites in urine contributing to the variation in metabolic profiles following treatment with CPAP

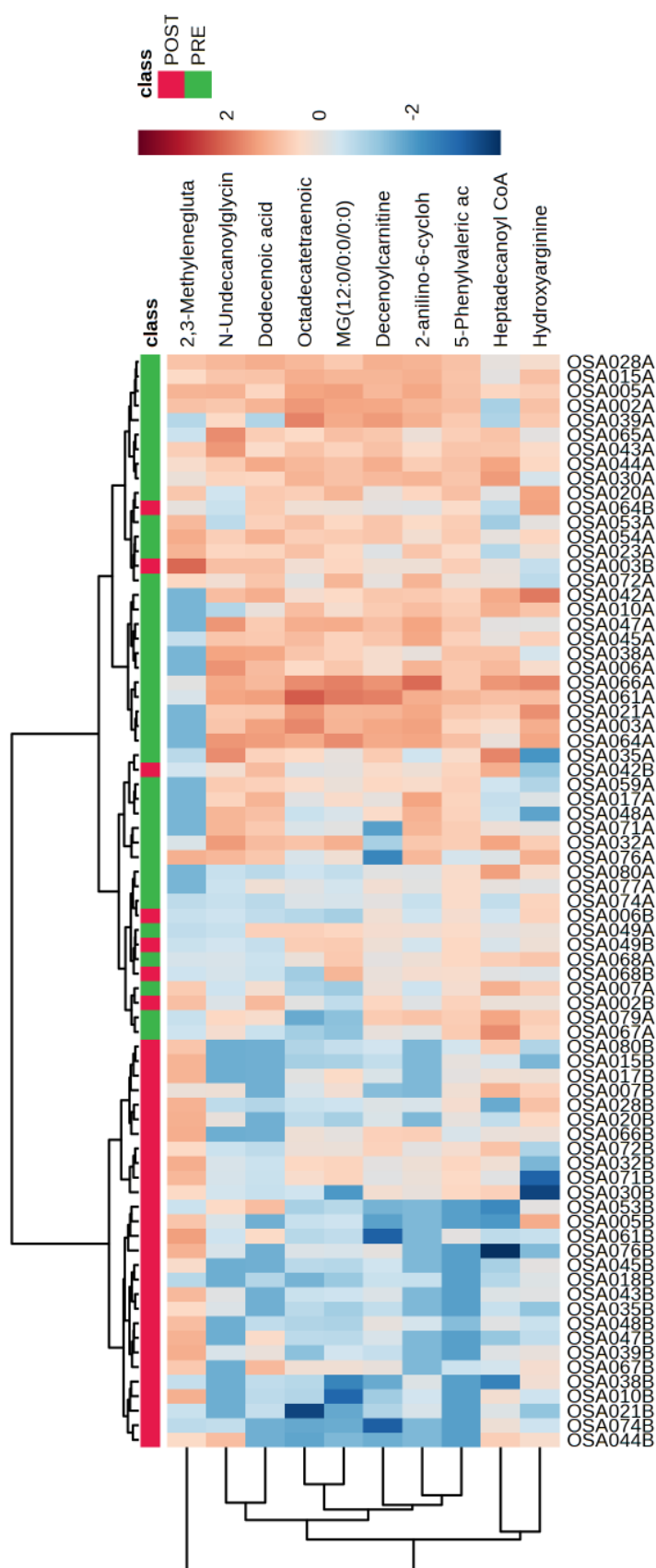


Figure 7.11: Hierarchical clustering model of the top 10 metabolites in urine following treatment with CPAP

Table 7.8: Potential identities of the significantly differentially expressed metabolites in urine following treatment with CPAP

Name	AUC	t-tests	Log2 FC	Pattern of dysregulation (Pre vs Post-CPAP)
2-anilino-6-cyclohexylmethoxypurine	0.9264	1.76E-14	-3.2781	Down
5-Phenylvaleric acid	0.925	1.67E-10	-2.4488	Down
Octadecatetraenoic acid	0.8561	3.10E-08	-1.4445	Down
N-Undecanoylglycine	0.8511	3.17E-10	-3.0039	Down
Decenoylcarnitine	0.8443	5.05E-06	-1.5956	Down
Dodecenoic acid	0.8439	5.57E-10	-2.0124	Down
MG(12:0/0:0/0:0)	0.8371	1.82E-07	-1.0894	Down
2,3-Methyleneglutaric acid	0.7625	2.11E-05	1.7389	Up
(2E,4Z)-decadienoylcarnitine	0.7607	0.002629	-1.4493	Down
Hydroxyarginine	0.7464	4.75E-04	-0.8163	Down
Heptadecanoyl CoA	0.715	4.29E-04	-0.9231	Down
Peroxy-eicPREtetraenoate	0.6914	4.85E-04	-0.2365	Down
Adenosine 3',5'-diphosphate	0.6771	0.006274	-1.2022	Down

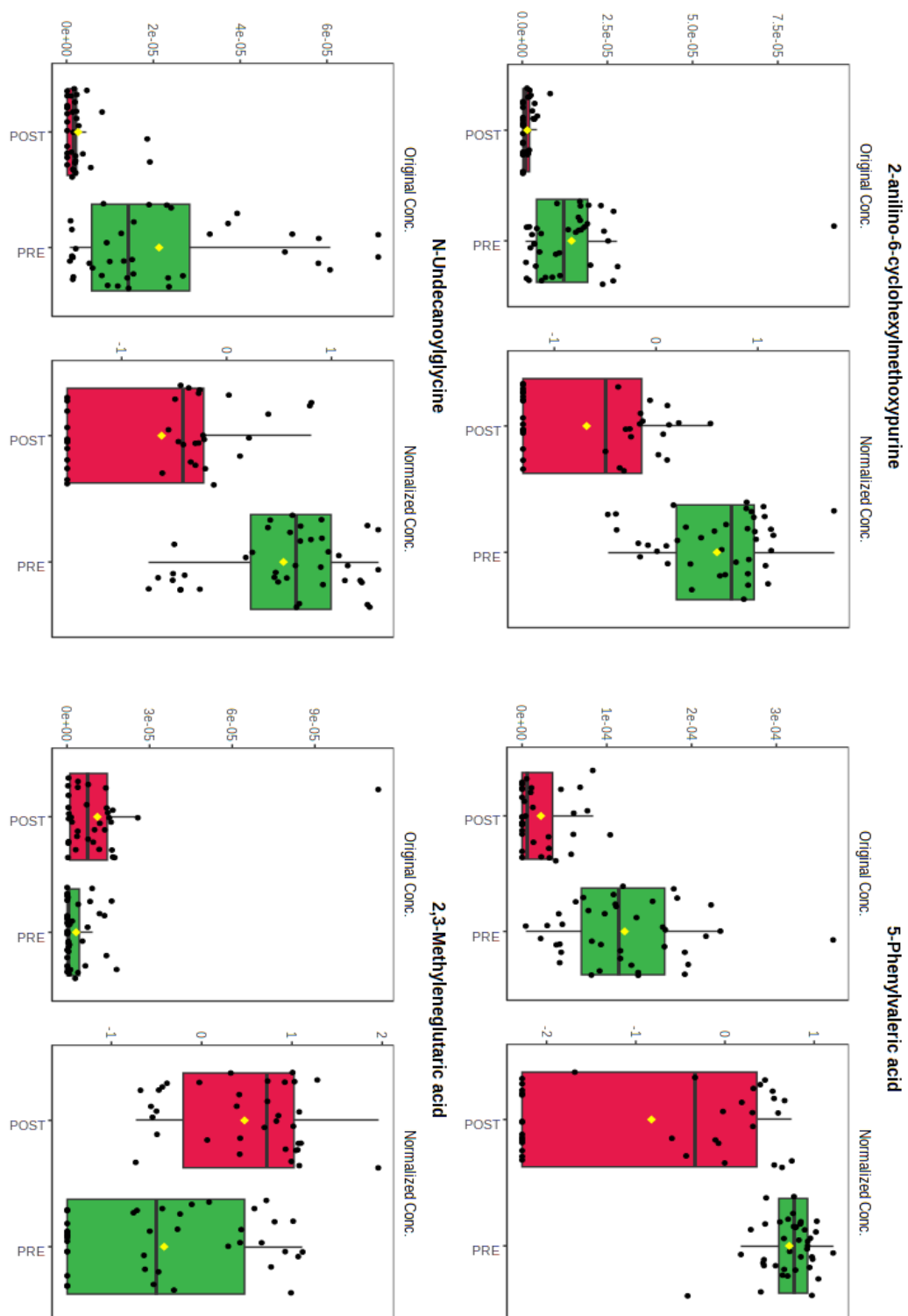


Figure 7.12 :Up/down regulation of key urinary metabolites in those with OSAHS following treatment with CPAP

7.4 The effect of CPAP on the global plasma and serum metabolomic profile

7.4.1 Study Population

A total of 38 patients with available clinical data and plasma and serum samples were included in this metabolomic profiling analysis. The study population has remained consistent, again comprising mainly middle-aged, obese, men (table 7.9). All had at least moderate OSAHS based on AHI (≥ 15 events/hour), with a mean AHI of 52.6 [24.5] events/hour. CPAP usage was optimal over the mean 57.9 [10.4] nights, with the participants using their machines 93.0% of all available nights, for an average of 5 hours 59 minutes (358.9 [104.8] minutes) a night. This usage led to a reduction in their baseline AHI to a mean of 7.4 [7.3] events/hour.

Table 7.9: Clinical, limited channel sleep characteristics, and CPAP adherence in the plasma and serum population

	Plasma/Serum (n=38)
Demographic variables	
Age (years)	51.8 [11.2]
Sex, male	26 (68.4%)
BMI (kg/m²)	39.8 [9.4]
ESS	14.8 [5.0]
HSAT parameters	
AHI	52.6 [24.5]
ODI	48.6 [21.6]
TSat90	39.1 [24.0]
Mean oxygen saturations	90.0 [3.3]
Minimum oxygen saturations	69.0 [11.3]
CPAP download	
Days on CPAP	57.9 [10.4]
% days total usage	93.0 [10.4]
% days > 4 hours	80.3% [21.6]
Average usage all nights (mins)	358.9 [104.8]
Residual AHI (events/hour)	7.4 [7.3]

Data are presented as the mean [SD] for quantitative variables and n (%) for qualitative variables. ESS = Epworth Sleepiness Score, AHI = Apnoea Hypopnoea Index, ODI = oxygen desaturation index, TSat90 = time with SaO₂ < 90%

7.4.2 Plasma/Serum metabolomic signatures associated with OSAHS following treatment with CPAP

After quality control and signal correction, the metabolites detected in the plasma and serum samples following treatment with CPAP were compared with the 3697 metabolites found in their plasma samples and 3549 metabolites in their serum at baseline in study one.

PLSDA did show separation between the OSAHS and SP groups in the plasma samples, but not at the same level of significance as seen in the urine samples (figure 7.13). Only one metabolite, ascorbic acid, had a VIP score of 1.5 (figure 7.15). Similarly, to study one, there was minimal separation on PLSDA in the serum samples following treatment with CPAP (figure 7.14), and consequently no VIP scores of significance were generated.

Based on univariate analysis and filtering by statistical significance, 7 metabolites found in plasma following treatment with CPAP are highlighted in table 7.10 alongside their $-\log_{10}p$ (OSAHS /SP) and AUC values. The levels of these key metabolites all increase following treatment with CPAP, with the exception of GlcCer(d18:1/12:0) (key metabolite in study one), and 3-(4-methylpent-3-en-1-yl)pent-2-enedioyl-CoA. Similarly, the 6 key metabolites found in serum are found in table 7.11, however only two were able to be identified.

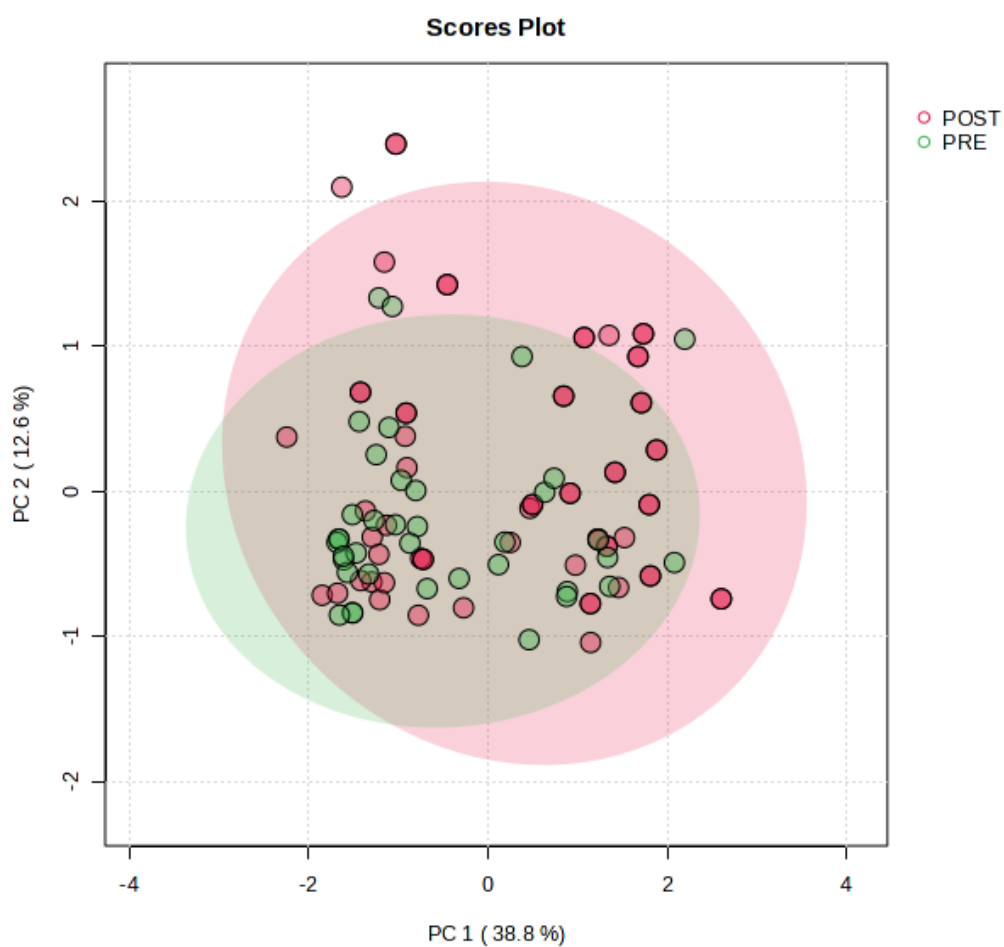


Figure 7.13: Partial least squares discriminant analysis of plasma metabolomic profile following CPAP treatment. Each data point is one sample, ellipses = 95% CI. 76 samples, PC1=38.8%, PC2=12.6%

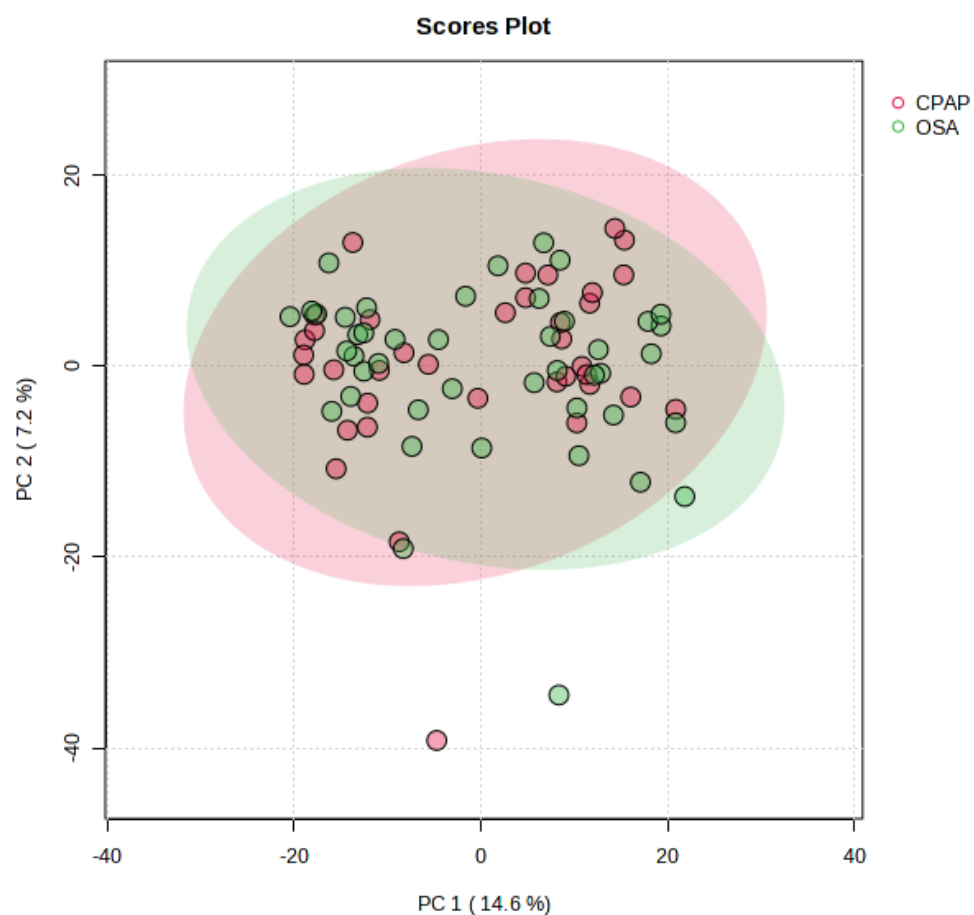


Figure 7.14: Partial least squares discriminant analysis of serum metabolomic profile following CPAP treatment. Each data point is one sample, ellipses = 95% CI. 76 samples, PC1=14.6%, PC2=7.2%

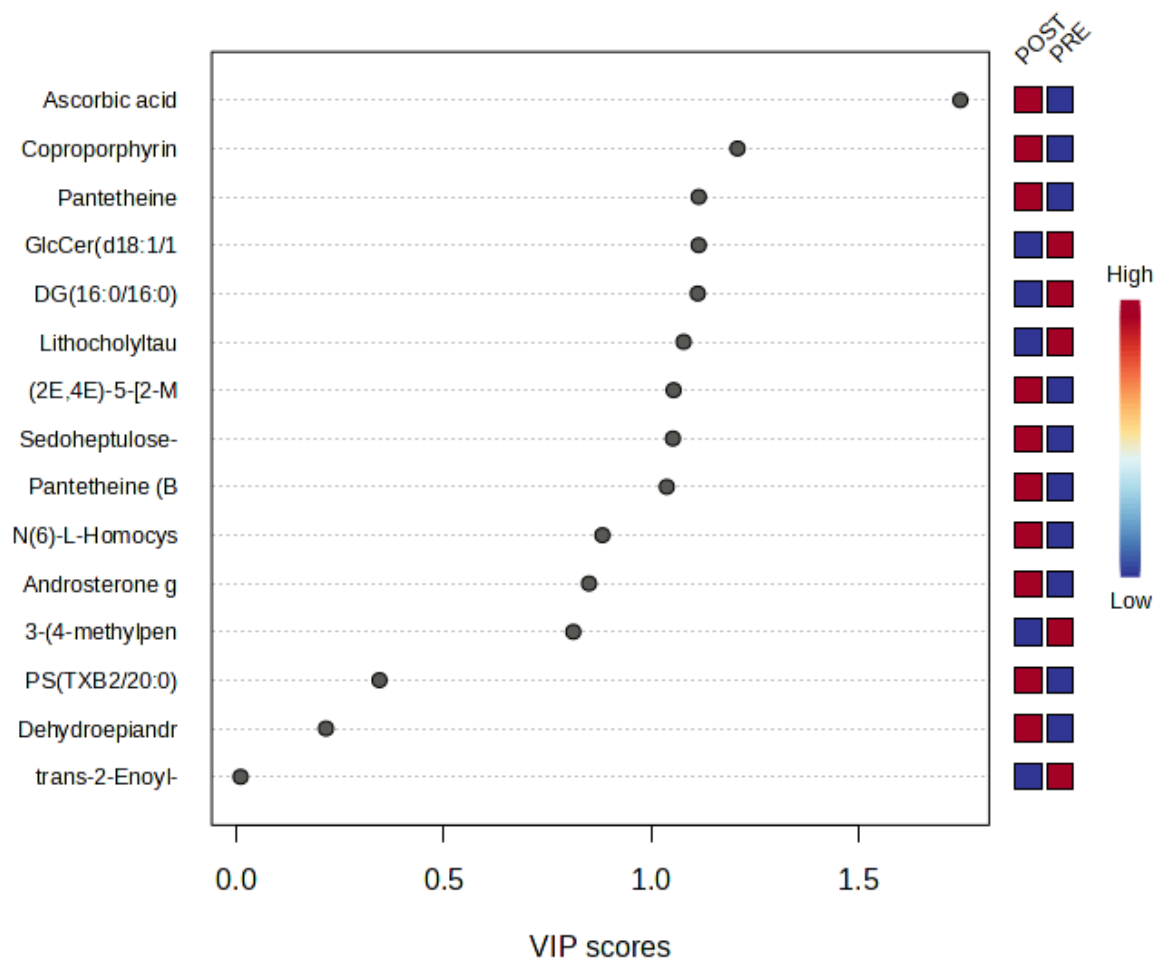


Figure 7.15: VIP scores for the top metabolites in plasma contributing to the variation in metabolic profiles following treatment with CPAP

Table 7.10: Potential identities of the significantly differentially expressed metabolites in plasma in those with OSAHS following treatment with CPAP

Name/m.z	t-stat	t-tests	-Log10(p)	Pattern of dysregulation (OSAHS vs SP)
Sedoheptulose-phosphate	3.0642	0.0027989	2.553	Up
Ascorbic acid	3.0102	0.0032973	2.4818	Up
(2E,4E)-5-[2-Methyl-2-(1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene-6-yl)cyclopropyl]-3-methyl-2,4-pentadienoic acid	2.8851	0.0047836	2.3202	Up
Coproporphyrinogen	2.7641	0.006786	2.1684	Up
GlcCer(d18:1/12:0)	-2.7159	0.0077758	2.1093	Down
Pantetheine (Vitamin B5)	2.6781	0.0086438	2.0633	Up
3-(4-methylpent-3-en-1-yl)pent-2-enedioyl-CoA	-2.3888	0.018762	1.7267	Down

Table 7.11: Potential identities of the significantly differentially expressed metabolites in serum in those with OSAHS following treatment with CPAP

Name/m.z	t-stat	T-tests	-Log10(p)	Pattern of dysregulation (OSAHS vs SP)
922.35712	-5.1155	2.1412E-6	5.6693	Down
14beta-14,15-dihydrocalonysterone	-5.008	3.2765E-6	5.4846	Down
592.63147	-4.7178	1.0122E-5	4.9947	Down
856.40753	-4.2781	5.2386E-5	4.2808	Down
384.8331	-3.8915	2.0678E-4	3.6845	Down
Cer(t15:0(14Me)/23:0(2OH[R]))	3.8823	2.1344E-4	3.6707	Up

7.4.3 The effect of CPAP on the key urinary metabolites from study one

We identified 5 urinary metabolites (octadecanamide, prolyl-glutamine, a diacylglycerol, beta-D-Xylopyranosyl-(1->5)-alpha-L-arabinofuranosyl-(1->5)-L-arabinose, and 12-Hydroxy-12-octadecanoylcarnitine) that could differentiate between the OSAHS and SP groups with an AUC of 0.779 (CI 0.524-0.928). (See chapter 7.1).

Using the same approach as outlined in chapter 7.3 where we evaluated the change in the global urine metabolomic profile following treatment with CPAP, we examined the effect of CPAP on these key metabolites.

Despite these key metabolites being able to differentiate between OSAHS and SP, there was no significant change following a trial of CPAP (figure 7.16). However, the metabolite 4-butylbenzene-1,2-diamine which had a VIP score in excess of 2 (see chapter 7.1), did significantly reduce following treatment with CPAP ($p < 0.05$). Figure 7.17 shows these targeted metabolites displayed using boxplots where their relative levels are shown.

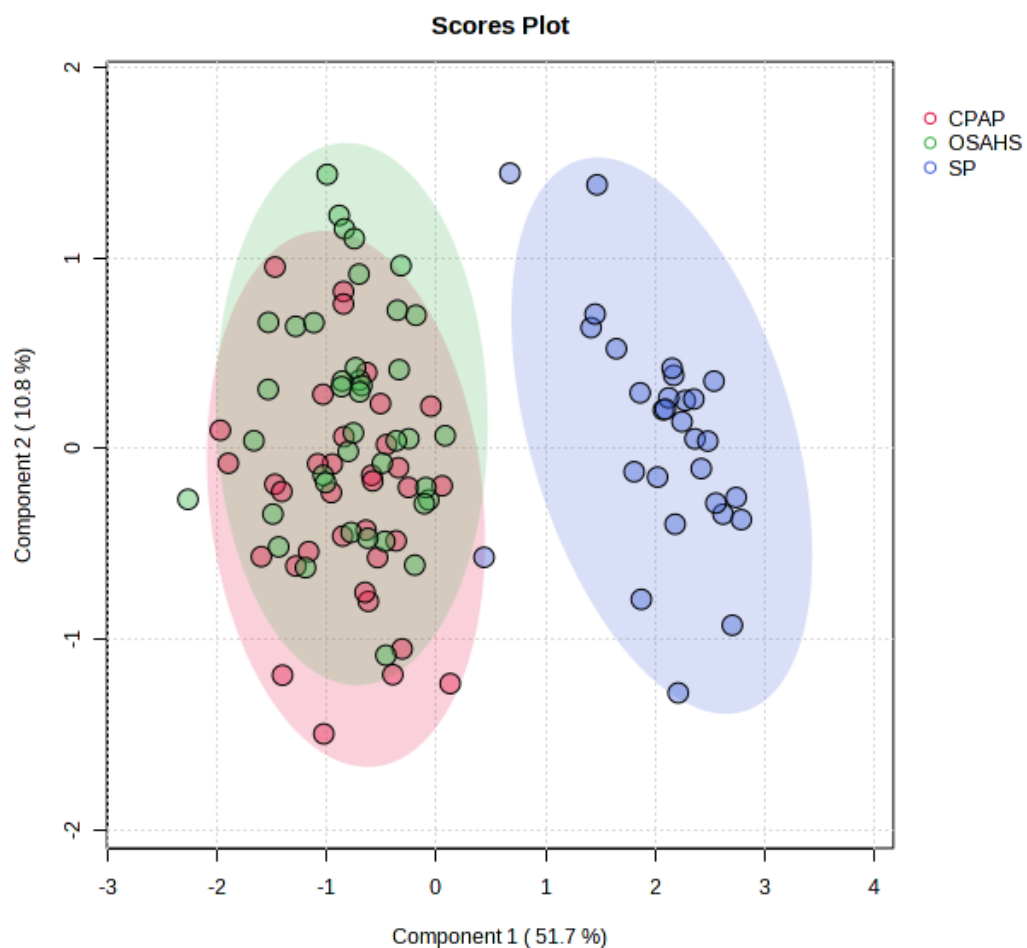


Figure 7.16: Partial least squares discriminant analysis of the top 5 urinary metabolites of SP and OSAHS, and those with OSAHS following treatment with CPAP. Each data point is one sample, ellipses = 95% CI. PC1=51.7%, PC2=10.8%

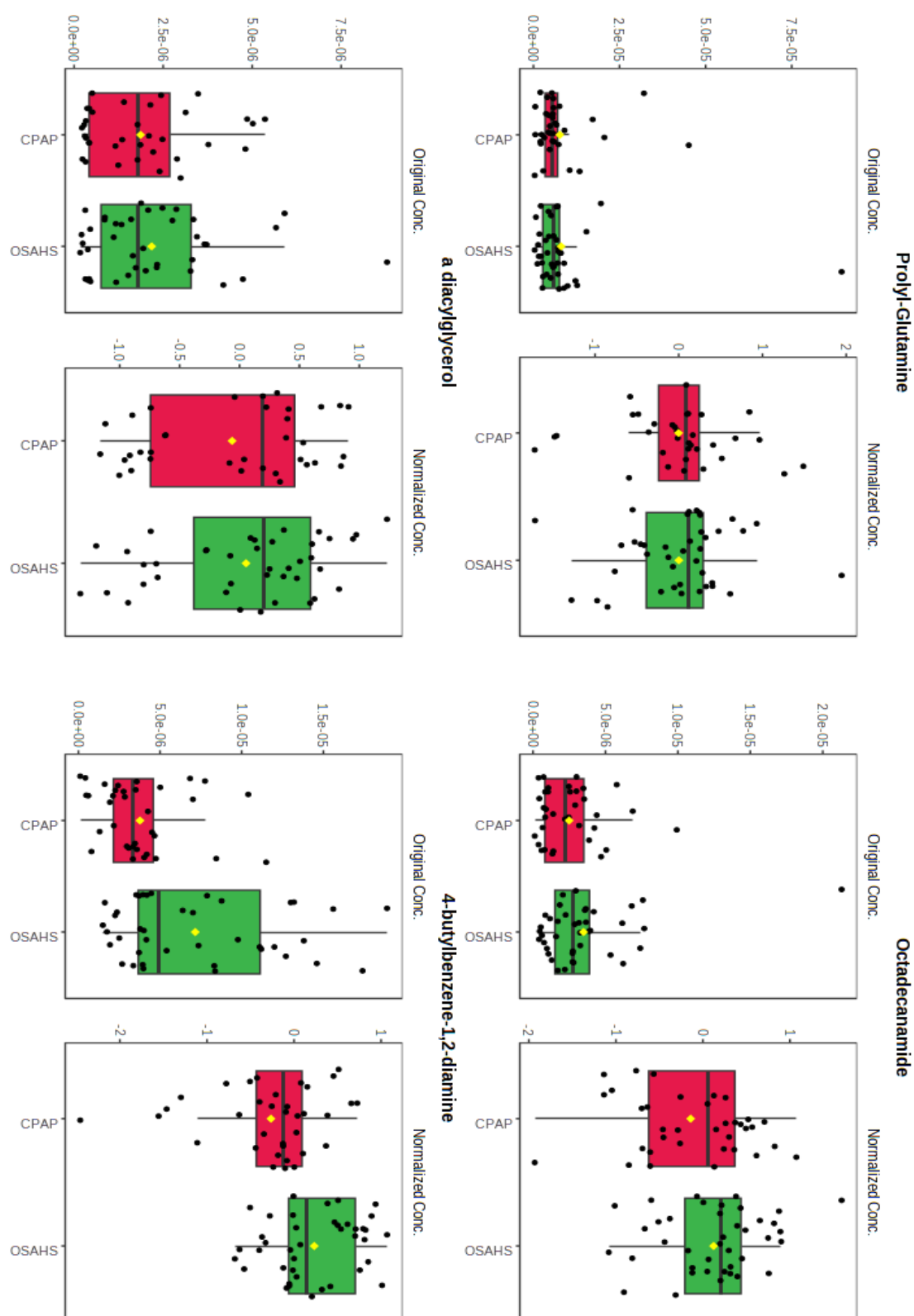


Figure 7.17: Up/down regulation of key differentiating urinary metabolites following treatment with CPAP.

8 Discussion

“Never waste any time you can spend sleeping.”

Frank H. Knight

8.1 Metabolomic profiling of OSAHS

The main difficulty in diagnosing and monitoring OSAHS is the lack of diagnostic and treatment modalities not being able to encompass the global burden this complex disease state can inflict. In these studies, the urine, plasma, and serum were analysed in those with OSAHS, and compared to their negative SP counterparts based on the currently extensively used diagnostic parameter AHI.

In the urine metabolomic analysis, we were able to show using multivariate analysis via principal component analysis and PLS-DA the differing metabolomic profiles in participants in OSAHS compared to SP. We identified 5 key metabolites (octadecanamide, prolyl-glutamine, a diacylglycerol, beta-D-Xylopyranosyl-(1->5)-alpha-L-arabinofuranosyl-(1->5)-L-arabinose, and 12-Hydroxy-12-octadecanoylcarnitine) that could differentiate between OSAHS and SP with an AUC of 0.779 (CI 0.524-0.928). We further identified 20 significant urine molecules ($p < 0.001$) that were differentially abundant in OSAHS compared to SP, with the top two metabolites, Prolyl-Glutamine and Octadecanamide showing significant up-regulation in those with OSAHS compared to their SP, having VIP scores in excess of 3. Furthermore, octadecanamide as a sole metabolite, could distinguish between OSAHS and SP better than any other metabolite or combination thereof, with an AUC of 0.86 [CI 0.76-0.93].

Octadecanamide, also known as stearamide or kemamide S, belongs to the class of organic compounds known as carboximide acids. It is functionally related to an octadecanoic acid (also known as stearic acid), which is a long-chain fatty acid.[243] Fatty acids are the simplest form of lipids, with lipids being the third macromolecule we consume in our diet in addition to carbohydrates and proteins. They are energy-rich carbon compounds that are synthesised in the cytoplasm of the liver and fat cells

and are regarded to be one of the main forms of energy storage. Stearic acid is a long-chain fatty acid abundant in the modern diet, however unlike most other saturated fats, it has an overall neutral effect on most cardiovascular risk factors and cholesterol levels. However, more relevantly, it acts as a signalling molecule affecting mitochondrial function and lipid oxidation.

Prolyl-Glutamine is a dipeptide composed of proline and glutamine and is an incomplete breakdown product of protein digestion or protein catabolism.[244] Protein catabolism is the breakdown of proteins into absorbable monomers for further degradation or reassembly. Large protein chains are disassembled to eventually leave free amino acids that can be taken up into the blood and transported to various cells around the body for further breakdown.[245] Cells can use those amino acids to construct vital proteins or as substrates for energy creation. Glutamine is the most abundant and versatile amino acid in the body and is of fundamental importance to intermediary metabolism and pH homeostasis. In almost every cell, glutamine can be used as a substrate for nucleotide synthesis, antioxidants, and numerous other vital physiological pathways involved in the maintenance of cellular integrity and function.[246]

12-Hydroxy-12-octadecanoylcarnitine is an acylcarnitine; an intermediate lipid metabolite that is generated by the esterification of a carnitine molecule. To be transported into mitochondria for subsequent β -oxidation, long-chain fatty acids are first converted to acylcarnitines by the enzyme carnitine *O*-palmitoyltransferase 1 which is part of the carnitine shuttle[247]. Carnitine shuttle activation occurs due to a need for fatty acid oxidation which is required for energy production. The carnitine shuttle represents a mechanism by which long-chain fatty acids, which are impermeable to the mitochondrial membranes, are transported into the mitochondrial matrix for the purpose β -oxidation and energy production. Acylcarnitines are therefore of upmost importance for fatty acid transportation, and can be regarded as markers of mitochondrial function due to the changes in their concentrations during dysfunction of mitochondrial β -oxidation.[248]

Both oxidative stress and inflammation are fundamental mechanisms in various pathologies seen in OSA, with their mechanisms tightly interconnected and having the ability to exacerbate each other, and our results reflect this. They are two of the most

prominent underlying mechanisms implicated in the cardiometabolic complications seen in those with OSAHS, with the former defined as an imbalance between pro-oxidant and anti-oxidant systems resulting in excessive production of ROS, with the latter being the body's response to a variety of external as well as internal insults including oxidative stress. We were further able to highlight these key physiological pathways to which these metabolomes belong based on their VIP scores and t-tests (Figure 8.1.1), with other pathways of significance including vitamin D/E metabolism and the electron transport chain.

The electron transport chain is a series of four protein complexes that couple redox reactions, creating an electrochemical gradient that leads to the creation of ATP (possibly from fatty acids) in a complete system named oxidative phosphorylation [249]. In a broader context, all vitamin E forms are potent antioxidants, and have anti-inflammatory effects on proinflammatory pathways. These vitamin E forms and their metabolites scavenge lipid peroxyl radicals, which are free radicals implicated in oxidative stress.[250] There was also significant enhancement in Vitamin D3 metabolism. Vitamin D has a significant role in bone and calcium metabolism, with its metabolites involved in calcium homeostasis, with a deficiency associated with a higher risk of CVD, T2DM, metabolic syndrome, and even COVID-19 (vitamin D was briefly trialled as a preventative and treatment of covid-19).[251]

The only metabolite to show significant down-regulation was DL -arabinose (log2FC - 0.51556). DL-arabinose is a monosaccharide and an aldopentose, which are involved in the pentose phosphate pathway (PPP). The PPP is a metabolic pathway that runs parallel to glycolysis, and has been demonstrated to be a major regulator for cellular reduction-oxidation (redox) homeostasis and biosynthesis.[252]

Our plasma metabolomic profiling did show separation between the OSAHS and SP groups, however not at the same significance as urine. 8 metabolites were identified with a VIP score of greater than 1.0, with GlcCer(d18:1/12:0 and N(6)-L-Homocysteinyl-L-lysine the most significant based on VIP scores. GlcCer(d18:1/12:0) is a glycosphingolipid, composed of ceramide and oligosaccharide, or oligoglycosylceramide, with one or more sialic acids (i.e. n-acetylneuraminic acid) linked on the sugar chain.[253] It is a component of the cell plasma membrane which modulates cell signal transduction events. Sphingolipids are believed to protect the cell

surface against harmful environmental factors by forming a mechanically stable and chemically resistant outer leaflet of the plasma membrane lipid bilayer, with ceramide having been proposed as a “coordinator” of the eukaryotic stress responses.[254] This is supported by the repeated findings that many inducers of stress response e.g. cytokines (TNF, nerve growth factor) and hypoxia/reperfusion insults, result in ceramide accumulation, usually as a result of activation of either sphingomyelinases or the *de novo* pathway of sphingolipid synthesis.[254]

There was minimal separation on PLSDA in the serum samples, and consequently no VIP scores of significance were generated.

8.2 The effect of CPAP on the metabolomic profile

The use of CPAP had a significant effect on the urine metabolomic profile of those with OSAHS, and significantly down-regulated metabolites, with 2-anilino-6-cyclohexylmethoxypurine and 5-Phenylvaleric acid having a VIP scores in excess of 8 and 10 respectively.

5-Phenylvaleric acid is a medium-chain fatty acid and a member of benzenes and is derived from valeric acid. 2-anilino-6-cyclohexylmethoxypurine is a hypoxanthine, and is formed during anaerobic cellular metabolic conditions from the degradation of adenosine (a metabolite of ATP).[255] The hypoxanthine oxidase then catalyses the conversion of hypoxanthine to superoxide and hydroxyl free radicals.

The key urine metabolite Octadecanamide, which was able to differentiate between OSAHS and SP with an AUC of 0.86, did not show any significant change following treatment with CPAP. However, the urine metabolite 4-butylbenzene-1,2-diamine which had a VIP score in excess of 2 as a potential discriminator between OSAHS and SP, did show significant down-regulation ($p < 0.05$) following CPAP.

PLSDA did show separation between the OSAHS and SP groups in the plasma samples, but not at the same level of significance as seen in the urine samples. Only one metabolite, ascorbic acid, also known as vitamin C, had a VIP score of 1.5, and was significantly up-regulated following treatment with CPAP ($P < 0.05$). Furthermore, GlcCer(d18:1/12:0, a key plasma metabolite and glycosphingolipid that is significantly up-regulated in OSAHS, showed significant down-regulation ($p < 0.05$) following treatment with CPAP.

Similar to the other studies, there was minimal separation on PLSDA in the serum samples following treatment with CPAP, and consequently no VIP scores of significance were generated.

8.3 How do the results compare

There is limited evidence for metabolomic profiling in OSA/OSAHS (see chapter 6.3), with only a handful of previous studies screening for potential OSA biomarkers. The selection of matched controls and exclusion of relevant and significant co-morbidities in previous studies affect the interpretation of the effects of SDB on the circulating metabolomic profile.

Our results indicate that a urinary metabolomic profile is better suited to profiling OSAHS and SP's than plasma and serum, and that these urine results are reported by others. Furthermore, we have identified a single metabolite, octadecanamide, a long chain fatty acid that acts as a signalling molecule affecting mitochondrial function and lipid oxidation, which can differentiate between SP and OSAHS with an AUC of 0.86, which has better performance than the majority of combined metabolomic profiles identified to date.

Xu et al. found that fatty acid biosynthesis, bile acid metabolism, and nucleic acid metabolism were higher in those with OSA compared to those without. Their panel of 6 urinary metabolites could distinguish OSA from those without OSA with a sensitivity of 75% and specificity of 78%, and an AUC of 0.835 (95% CI 0.757-0.897). Their discriminating metabolites were also biologically plausible in the context of mitochondrial dysfunction and oxidative stress, however, a significant limiting factor of Xu's study was that those with significant cardiac, pulmonary, or renal disease were excluded. They did not look at the impact of CPAP on these metabolites.

Our findings of acylcarnitines in the urine are consistent with the key finding by Cho et al. with their key metabolite, hydroxytetradecenoylcarnitine, an acylcarnitine, being able to distinguish between OSA and non-OSA with an AUC of 0.746. However, their subjects did not include any females, and excluded those with diabetes mellitus, hyperlipidaemia, and chronic hypoxia-related diseases.

Our plasma and serum analysis of the circulating metabolomic profile in those with OSAHS did not show such significant variation as seen in the urine samples and was minimal in serum. Our findings of up-regulation of glycosphingolipids in plasma in those with OSAHS is consistent with the key plasma metabolomic studies in the

literature; Lebkuchen et al. in 2018 and Pinilla et al. The untargeted plasma metabolomic profiling using LC-MS by Pinilla et al identified a plasma profile of 33 metabolites which consisted mainly of glycerophospholipids and bile acids after adjusting for age, sex and BMI. They identified 4 metabolites that could differentiate those with OSAHS (AHI 41.0 (26.8;62.4), ESS 11.0 (7.0-15.0)) from those without OSAHS (AHI 9.26 (5.18;11.9), ESS 10.0 (6.50;13.5)) with an AUC of 0.98 (95% CI 0.95–0.99). The identification of glycerophospholipids and bile acids is biologically relevant and suggests that the metabolic impact of OSA systemically is mainly mediated by a dysregulation in glycerophospholipid metabolism and the biosynthesis of primary bile acids.

Lebkuchen et al. utilised a combination of metabolomic analytical platforms including ultra-performance LC coupled to tandem quadrupole MS, and GC coupled to quadrupole on the plasma of 37 men with OSA (defined by AHI ≥ 15 events/hr) and 16 controls matched for age, gender, fat composition via bioimpedance analysis, and BMI.[235] They identified 6 metabolites, with glutamic acid ($p = 0.023$), deoxy sugar ($p = 0.021$, AUC 0.667 CR 0.883 - 0.451) and arachidonic acid ($p = 0.036$, AUC 0.596 CR 0.758 - 0.435) having the strongest association with the presence of OSA. These metabolites are involved in glucose and inflammatory pathways, with deoxy sugar, a monosaccharide glucose analogue, produced by the action of glucose oxidase. However, the study was not generalisable to a typical OSAHS population as it excluded females, those with alcohol excess (not defined explicitly), participants on any medication, and those with any known pulmonary, CVD, or kidney disease. They also did not attempt to quantify a subjective level of sleepiness or other symptomology beyond snoring.

In September 2024 and at the time of submission of this thesis, Wang et al found a panel of urinary metabolites that could distinguish healthy control participants from patients with mild-moderate OSAHS (AUC = 0.78) and patients with severe OSAHS (AUC = 0.78), but also in differentiating between patients with mild-moderate and severe OSAHS (AUC = 0.71).[256] The full text of the article was not accessible at this time, and so further comparisons cannot be made at the time of writing.

Following treatment with CPAP, we found a significant down-regulation in fatty acids and hypoxanthine in urine, but no significant differences in these same urinary metabolites that could differentiate between OSAHS and SP in chapter 7.1.2. To the best of our knowledge, only Pinilla has evaluated the impact of CPAP on the circulating metabolomic profile, and this was in plasma. Their OSAHS patients treated with CPAP had a mean CPAP usage of 5.21 (2.84) hours/night compared to 6 hours 6 minutes a night for our group, with 69% of their treated patients being deemed good compliers i.e. CPAP use ≥ 4 hr/night., compared to our 81.7%. Both studies suggest that CPAP has a significant effect on the metabolomic profile in individuals with OSAHS, but curiously, not the ones that differentiate between OSAHS and non-OSAHS. This suggests that CPAP does have a significant impact on the pro-inflammatory metabolic profile seen in those with OSAHS, however does not fully reverse the metabolomic insult, which raised two key hypotheses. Firstly, are these key differentiating metabolites up regulated for such a prolonged period in somebody with undiagnosed OSAHS (possibly years), and therefore a longer period of CPAP treatment is required to reverse this dysregulation, however Pinilla's cohort were treated for 6 months before repeat metabolomic analysis. Secondly, it is likely that there are other metabolomic pathways at play that we haven't been able to identify and correlate yet, and there means it is more likely that this sub-optimal correction of key differentiating metabolites between OSAHS and SP reflects the lack of understanding on what is happening in an individual with SDB on a metabolic level, and would make one question whether correlation with the AHI is the key to determining the severity of complications of OSAHS, and whether we should shift focus on to other parameters such as time spent $< 90\%$ oxygen saturation, number of arousals, or pulse rate/variation.

8.4 Strengths and limitations

This was a single-centred, observational prospective and longitudinal study carried out during the Covid-19 pandemic. It was an exploratory study, aiming to gain new insights, and generate new hypotheses alongside highlighting the potential of a biomarker in diagnosis and managing OSAHS.

One of the main strengths of this research is the evaluation of a moderate sized group of consecutive attenders with a minimal exclusion criterion allowing the more generalisability to real world clinical settings. The concept of recruiting consecutive attenders allowed us to evaluate the spectrum of SDB based on an AHI of above or below 15 events per hour, which reflects real-life day-to-day practice and cut-offs for most CPAP treatments.

For the sake of the mechanistic study, we removed four patients from both groups (two from SP, and two from OSAHS) during the metabolomic analysis to allow a clearer distinction between SP and OSAHS. The participants removed from the SP group had an AHI more than 15 events per hour, however, did not warrant treatment based on symptomatology and clinical review. The two removed from the OSAHS group conversely had an AHI of less than 15 events/hour (but did receive CPAP because they were highly symptomatic).

All patients underwent a HSAT (the recommended diagnostic modality recommended by NICE), and the longitudinal design of the study and the consequent evaluation of the effect of CPAP treatment on the urine, plasma, and serum metabolomic profiles helped to highlight the widespread cardiometabolic and physiological burden as demonstrated by the differing abundances of metabolites.

One limitation of this study is that the OSAHS and SP groups were not completely matched for potentially confounding variables that affect inflammatory/ metabolic processes: the OSAHS had a higher BMI, more were prescribed betablockers and less were prescribed opioids. The groups were otherwise similar.

Our samples were non-fasting and collected at different times of the day depending on the subject's routine appointment time with our sleep physiologists. The circulating metabolomic profile is likely to be affected by diet, hormones such as cortisol, and

environmental stressors. Ideally, we would have taken samples at the same time of day and perhaps only fasting, but this would have been quite onerous to patients who lived up to 20 miles from the hospital. We did not have resources for inpatient (overnight sleep studies). Ideally any clinically useful biomarker should allow flexibility in sample timing for it to be deployed at scale and even remotely in e.g. home testing.

Furthermore, based on timescales (self-funded MD and working as a full-time respiratory registrar), local logistics (working in alignment with routine appointment schedules and covid-19 pandemic), and ethical guidelines, we were unable to identify and recruit a tighter control group with symptoms but very normal sleep studies (e.g. all with $AHI \leq 5$). Such a group would have allowed us to compare the 'extremes' of metabolomic profiles to look for any mechanistic patterns.

A significant criticism of metabolomic profiling, which applies to all studies in the literature, is the lack of standardisation and subsequent variability in the pre-analytical phase and processing of biological specimens. Metabolomics can be significantly influenced by a range of pre-analytical factors, such as sample collection, pre-processing, aliquoting, transport, storage and thawing, and also between scientists in the same laboratory. [257] Furthermore, there is not yet a standardised, universal approach to metabolomic profiling itself with each study using different analytical platforms i.e. NMR spectroscopy vs MS. As the science develops there remain issues comparing data between laboratories due to the complexity and nuances of identifying metabolites e.g. variation in collision energies in MS. There have long been calls for a central reporting database where investigators can share their methodologies and results, and ultimately develop a standard operating procedure. [258] Some of these reasons may account for the disappointing finding.

We did not look for a dose response curve e.g. measuring levels of octadecanamide across a range of severity of AHI nor other biomarkers against the change in AHI with CPAP. The study groups are not large and there will be other confounders e.g. BMI, CPAP adherence/usage, and even the use of opioids, which will affect results.

9 Conclusion

“No day is so bad it can’t be fixed with a nap.”

Carrie Snow

9.1 General conclusions

We have established that OSAHS needs to be approached as a complex, heterogeneous, and variable chronic disease that requires long term, multidisciplinary care. We know that it is exceedingly common but often underrecognized, with its prevalence likely to increase due to the obesity pandemic which is further compounded by an ageing population. We know that current gold-standard diagnostic modalities are unable to truly reflect the hypoxic burden, oxidative stress, and proinflammatory state caused by OSA, and identifying in whom it would be appropriate to initiate treatment based on symptoms is a perennial challenge to the sleep physician. Furthermore, the predictors of end-organ damage and potentially fatal co-morbidities are likely to vary between individuals and even within individuals over time, and we have little evidence to guide us on this and so heavily rely on unilateral sole clinical acumen and predictions which certainly will vary from physician to physician.

We have found a urinary metabolite, octadecanamide, that can differentiate between SP and OSAHS with an AUC of 0.86. This could potentially be used as a (non-invasive) biomarker, avoiding the need for labour intensive and time-consuming sleep studies, or be used in conjunction with especially a borderline sleep study, helping identify the physiological parameter that we should be using when diagnosing and monitoring OSAHS. We also identified a panel of metabolites that could further help differentiate sleepy people from sleepy people with OSA and help understand the inflammatory and metabolic processes. However, the obvious benefit in clinical use would be in validating a single key metabolite which could then be used in the development of a lower cost point-of-care diagnostic tool.

Urine has several advantages over other types of biofluids for metabolomic analysis, such as blood or tissue. Urine is a non-invasive and easily accessible biofluid that is

relatively stable. In comparison to the continuously changing plasma or serum metabolomic profile by constant regulatory mechanisms, urine is collected in the bladder over a period of minutes to hours, negating the effects of short-lived changes, and providing a sample representing the metabolomic changes the body has undergone over a more prolonged period of time. Furthermore, urine contains a wide range of metabolites, including those produced by the kidneys, liver, and gut, and therefore provides more comprehensive picture of an individual's metabolic status.

9.2 Future directions

We have demonstrated the heterogeneity of SDB and highlighted the challenges in diagnosing and monitoring this complex condition. However, this pilot study shows metabolomic profiling of especially urine is feasible and indeed promising. The candidate biomarkers are biologically plausible and provide more information on the inflammatory and cellular pathways.

The proposed next step needs to be validation of our findings, using a targeted (not untargeted) approach with our key metabolite octadecanamide, and correlating this with the various physiological parameters on sleep studies from a bigger target population. If the biomarkers still appeared robust differentiators and there were 5 or less, then developing a portable point-of care test based on e.g. lateral flow technology, would not be insurmountable. The benefit of using a biomarker alongside a screening questionnaire like the ESS could also improve the pretest probability of OSAHS to interpret sleep studies, or depending on sensitivity and specificity, they could remove the need for time consuming, labour intensive and inconvenient sleep studies altogether.

Furthermore, various sampling modes for urinary bioanalysis exist, and in this study, we have used spot-sampling i.e. at the time of clinical appointment. 24-hour urinary sampling would likely provide a more accurate picture for mechanistic understanding but would not be feasible in clinical practice.

In addition, repeat measuring of the 5 key metabolites e.g. immediately on waking, after food, or fasting, would allow further insight in to how the metabolites are impacted by the circadian rhythm, diet and environment.

Alongside future validation, we are discussing with other groups ways to standardise metabolomic techniques and analysis to improve generalisability, and there is optimism with regards to the potential of metabolomics in diagnosing and monitoring medical disease, where we just need to be reassured by the significant advances that have already been made in other medical specialties.

Appendices

Appendix 1: Sleep-Disordered Breathing Questionnaire

SLEEP DISORDERED BREATHING QUESTIONNAIRE

Insert sticker

Date:

Alcohol intake(per week):

Smoker (Y / N):

No. Per day:

Who referred you? (ie GP, ENT doctor etc):

Please write your full medication list:

Please list all of your other illnesses:

Your current occupation:

What type of drivers licence do you hold? (non-driver, standard (cars), HGV, PSV, PPL):

What time do you usually - go to bed?..... wake up?.....

Do you? **Never** **1+ Monthly** **Weekly** **Daily**

**A
W
A
K
E**

Have severe sleepiness during the day?				
Have morning headaches?				
Have excessive sleepiness whilst driving?				
Have restless legs throughout the day that get worse in the evening?				

**S
L
E
E
P**

Snore?				
Stop breathing during sleep?				
Have choking sensation during sleep?				
Pass water at night?				
Have night sweats?				
Have sudden movements of arms/legs?				

EPWORTH SLEEPINESS SCALE

This scale assesses your level of sleepiness during the day.

How likely are you to doze off or fall asleep in the following situations, in contrast to just feeling tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you. Please tick one option only for each question.

SITUATION	CHANCE OF DOZING			
	Would never	Slight chance	Moderate chance	High chance
Sitting and reading				
Watching TV				
Sitting, inactive in a public place (eg cinema or a meeting)				
As a passenger in a car for half an hour without a break				
Lying down for a rest in the afternoon when circumstances Permit				
Sitting and talking to someone				
Sitting quietly after lunch without alcohol				
In a car, while stopped for a few minutes in traffic				

To be completed by clinic nurse or doctor only:

Height:

B/P:

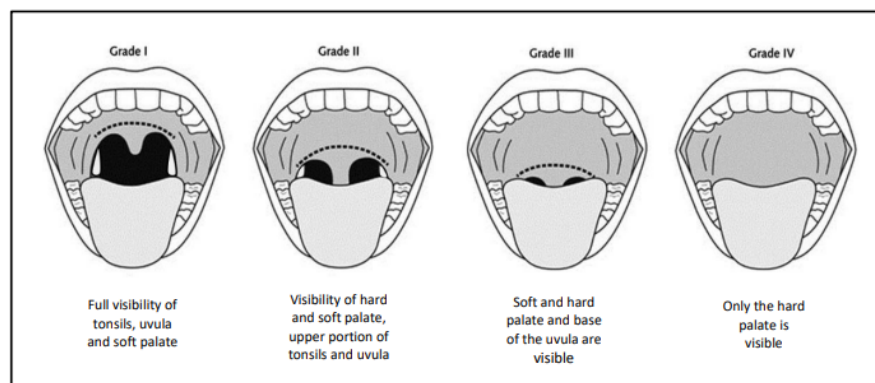
Weight:

Neck circumference:

BMI:

Abdominal girth (cms):

Epworth score:



Appendix 2: Patient Information Sheet – Informed Consent Form



Bwrdd Iechyd Prifysgol
Hywel Dda
University Health Board

PARTICIPANT INFORMATION SHEET – SLEEP APNOEA

Version 1, 12th August 2020

Research Study Title: Novel Technologies for Diagnosing and Monitoring Pulmonary Diseases ('Novel Technologies')

REC reference: 16/WA/0036

IRAS reference: 187325

You are being invited to take part in a research study. Before you make a decision it is important to explain why the research is being conducted and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Do not hesitate to ask us if there is anything that is not clear or if you would like additional information. Take time to decide whether or not you wish to take part in our study.

1. What is the purpose of the research study?

We believe that many lung/breathing conditions can change certain chemicals in people's blood, urine, saliva and sputum (phlegm). These chemicals or 'biomarkers' may be detectable even before a patient develops symptoms or we see changes on hospital scans and chest X-rays. We want to look at new tests including genetic testing in our research laboratories to try and work out which of these chemicals are different in people with lung/breathing conditions and if these chemicals change with treatment. This research programme, which is contributing to an educational study, may help to diagnose these type of conditions earlier, and improve our care in the near future.

2. Why have I been chosen to participate?

Your doctor is concerned that you might be suffering from a lung or breathing condition. We would like your help by asking you to provide occasional extra samples when you attend our hospital for routine tests and treatment.

3. Do I have to participate in this study?

It is up to you to decide whether you would like to take part or not. If you decide to take part, you will be given this Participant Information Sheet and we will ask you to sign the attached Informed Consent Form after discussing it with a researcher or doctor. You are free to withdraw from the study at any time without providing us with a reason. This will not affect your treatment.

4. What will happen to me if I agree to participate in the research?

Firstly, we will ask you some questions about your health. We will then ask you to provide a sample of your blood and urine, and may also ask you to provide us with sample of your saliva and/or sputum.

It is up to you to decide what sample(s) you want to provide us with, depending on how comfortable you feel.

After the first set of samples, you may be contacted again by letter to ask if you are willing to attend a follow up review where the researcher will again ask you questions about your health. If you are willing to return to the hospital, the same samples as before will be collected. This visit will be timed to coincide with routine appointments wherever possible but may occur at another time more convenient to you.

The samples will be stored in Hywel Dda University Health Board and sent to different research institutions who are working in partnership with the hospital doctors. These include research laboratories in Aberystwyth University and/or other institutions for specialised laboratory tests that

Version 1, 12th August 2020 *Sleep Apnoea*



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are not currently available in hospitals. The samples you provide will be kept and may also be used for new tests in the future if new technologies are invented.

If you give your consent, any of your samples that are left over will be transferred to a long term sample storage facility in Hywel Dda University Health Board (known as a Biobank), which stores various types of tissue and information for other research studies in the future. If you do not consent to your samples entering the Biobank, they will be disposed of with respect and dignity at the end of this study.

5. What will I have to do?

There are no lifestyle restrictions if you decide to take part in the study. We will want you to continue your normal activities and all of your usual health treatments and medications will be unchanged.

If you attend hospital at a later date, we may ask you again to provide samples to see if the 'biomarkers' have changed e.g. after treatment.

6. What are the side effects of participating in this study?

There are no known side effects from taking part in this study, but we will need 5 minutes of your time to answer the screening questions and then up to 30 minutes to collect your blood, urine, saliva, and sputum samples depending on the number of samples you want to provide.

7. What are the benefits associated with me taking part in this research study?

The results of the research will have no direct benefits to you but the results of the research may benefit patients with lung or breathing conditions in the future by moving towards earlier diagnosis and more personalised treatments. Unfortunately, we cannot pay you for participating in this study.

8. What will happen if something goes wrong?

If you feel that you have any reason to complain about any aspect of the way you have been approached in the hospital or further treated during the course of the study, the normal National Health Service complaints mechanisms are available to you.

9. Will my participation in this study be kept confidential?

All the collected information about you during the course of our research programme will be kept strictly confidential. Any information that leaves the hospital will be coded so you cannot be identified from it. In addition, we will not give any identifiable information to life insurance, private medical insurance companies or any other third parties.

10. How will we use information about you?

We will need to use information from your medical records for this research project. This information will include your NHS number, name and date of birth. People will use this information to do the research or to check your records to make sure that the research is being done properly. People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead. We will keep all information about you safe and secure. Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

11. What are your choices about how your information is used?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study as part of the Hywel Dda Biobank.

12. Where can you find out more about how your information is used?

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- our leaflet which you should receive with this information sheet.
- by asking one of the research team
- by sending an email to chris.tattersall@wales.nhs.uk

13. What will happen with the results generated by this research programme?

The findings from our study may be published in scientific journals and presented at conferences in a fully anonymised way. You are welcome to ask us for update reports of the research and we will also endeavour to send you a final study report.

14. Who is organising and funding this research?

This research is the result of a collaboration between Hywel Dda University Health Board and Aberystwyth University. It is funded by the European Social Fund, the Brazilian Government through the project 'Science without Borders', Tenovus Charity and ProTEM Services Ltd (a pharmaceutical company). The company has had no influence on the design of this research and will not keep samples but it jointly funds the PhD Students in Aberystwyth University.

15. Who has reviewed the study?

Our research study has undergone review by medical doctors and scientific researchers within Hywel Dda University Health Board and across Wales as well as an NHS Research Ethics Committee (WALES REC 7).

Contacts for further information:

For independent advice, please contact Mr Chris Tattersall, R&D Department, Withybush Hospital,

Tel: [REDACTED]

Email: [REDACTED]

If you have any further queries please do not hesitate to contact:

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University Health Board

INFORMED CONSENT FORM – SLEEP APNOEA

Version 1, 12th August 2020

Research Study Title: Novel Technologies for Diagnosing and Monitoring Pulmonary Diseases ('Novel Technologies')

Participant Identification Number: _____

N.B. Three copies will be made for: (1) Participant, (2) Researcher, (3) Hospital notes (if relevant).

Research Team Leads: Professor Keir Lewis/Professor Luis Mur

Contact Telephone Number: [REDACTED]

Read carefully the following statements and, if you agree, please INITIAL (do not tick) the adjacent boxes.

INITIAL BOX

1.	I confirm that I have read and understood the Participant Information Sheet – Sleep Apnoea (Version 1, 12 th August 2020) for the above research study. I have had the opportunity to ask questions, and I am happy with the answers given.	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3.	I understand that sections of any of my medical notes may be looked at by: responsible individuals from the Research and Biobank Team, regulatory authorities where it is relevant to my taking part in research and the sponsor's representatives in Hywel Dda University Health Board for monitoring the conduct of the study. I give permission for these individuals to have access to my records.	
4.	I agree to provide samples in addition to any needed for clinical care. Please initial the boxes next to the samples that you wish to provide for the research.	BLOOD
		URINE
		SALIVA
		SPUTUM
5.	I agree to take part in an ongoing study, where I may be asked to attend a follow up review and provide more samples.	
6.	I agree that any of my samples that are left over can be stored in the Hywel Dda University Health Board Biobank and my data can be accessed for future research.	
7.	If relevant, I understand and agree that a letter will be sent to my GP informing them about my participation in the research study.	
8.	I confirm that I have understood all the above mentioned aspects and I agree to take part in the 'Novel Technologies' research study.	

Full Name of Participant (PRINT)	Date:	Signature:
Full Name of person taking consent (PRINT)	Date:	Signature:

Version 1, 12th August 2020 Sleep Apnoea

Appendix 3: Confirmation of Recruitment

NOVEL TECHNOLOGIES FOR DIAGNOSING AND MONITORING PULMONARY DISEASES - SLEEP
APNOEA CI Prof K Lewis

Seen by Dr Scott O'Rourke.

Patient Information Sheet (PIS) and Informed Consent Form (ICF) SLEEP APNOEA v.... dated
given to patient and study discussed. All questions/queries answered and eligibility confirmed.
Patient happy to be in study.

CRF completed and samples of collected and processed as per
study guidelines.

Copy of signed PIS/ICF provided to patient, copy of signed PIS/ICF filed in notes and original signed
PIS/ICF filed in research file.

(No further follow up is needed) or (Patient agreed to be followed up on next appointment.)

Signature

Extension

Appendix 4: Baseline Clinical Information Datasheet



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CYMRU
NHS
WALES

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Hywel Dda
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CLINICAL INFORMATION DATASHEET: SLEEP APNOEA ONLY

Version 1: 12th August 2020

Novel Technologies for Diagnosing and Monitoring Pulmonary Diseases

ID Number:		Hospital name:	
Visit date:	Visit no:	Age:	M ☐ F ☐

Hypertension (on treatment)	Yes ☐ No ☐	Type 2 Diabetes	Yes ☐ No ☐	COPD	Yes ☐ No ☐
IHD	Yes ☐ No ☐	High cholesterol	Yes ☐ No ☐	Family history of OSA	Yes ☐ No ☐
Smoking History: Current ☐ Ex ☐ Never ☐ If ex-smoker, quit how long ago:		Smoking Pack Years		Alcohol units/week	
Snorer? Yes ☐ No ☐					
Sleepiness (whilst driving)? Yes ☐ No ☐ NA ☐					
Witnessed apnoea? Yes ☐ No ☐					
Epworth score:		Height (m)		BMI	
		Weight (kg)			
		Neck circum (cm)			
Blood Pressure (mmHg)		Heart Rate (bpm)			
Oxygen Saturations		FiO2			
Mallampati score					

Medical History:	1.	4.	7.
	2.	5.	8.
	3.	6.	9.

Version 1 dated 12th August 2020 *Sleep Apnoea*



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Drug History:	1.	4.	7.
	2.	5.	8.
	3.	6.	9.
	10.	11.	12.

Sleep Study:

AHI	ODI	Mean O2 Sats	Minimum O2 Sats	%Time Sats <90% (mins)

Diagnosis of obstructive sleep apnoea?	Yes ☐ No ☐
If yes, APAP commenced?	Yes ☐ No ☐
	If no, why?

Follow up:

Repeat Epworth:

Duration of APAP (days)	Average use/night (hours)	Residual AHI	APAP Pressure	% nights >4 hours use

Samples Collected:

Sample type	Consented to give:	Collected:	Time of collection:	Collected by (name):	Time placed in freezer:
	Please tick or cross each box as appropriate				
Urine					
Blood (SST)					(As serum)
Blood (EDTA)					(As plasma)
Sputum					
Saliva					
Comments:					

Signature:

Date:

.....

.....

Version 1 dated 12th August 2020 Sleep Apnoea

Appendix 5: Follow-Up Clinical Information Sheet



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FOLLOW-UP
CLINICAL INFORMATION DATASHEET: SLEEP APNOEA ONLY
Version 1: 28th January 2021
Novel Technologies for Diagnosing and Monitoring Pulmonary Diseases

ID Number:		Hospital name:
Visit date:	Visit no:	

Epsworth score on APAP:	Height (m)	BMI
	Weight (kg)	
	O2 Sats:	
Heart Rate (bpm)	Blood Pressure (mmHg)	

APAP Machine Download	
Date started	
Number of days since starting APAP	
AHI	
%usage > 4 hours	
Average usage/night (minutes)	
Other:	

Samples Collected:

Sample type	Consented to give:	Collected:	Time of collection:	Collected by (name):	Time placed in freezer:
	<i>Please tick or cross each box as appropriate</i>				
Urine					
Blood (SST)					(As serum)
Blood (EDTA)					(As plasma)

Signature:

Date:

.....

.....

Version 1 dated 28th January 2021 Follow- Up Sleep Apnoea

Appendix 6: Recruitment Guidelines and Sample Processing

LOCAL GUIDELINES - NOVEL TECHNOLOGIES RECRUITMENT AT PPH, LLANELLI

OBSTRUCTIVE SLEEP APNOEA PATIENTS ONLY

- 1 Study IDs begin with the letters OSA. Sample bottle cryolabels with the same study ID number should also be used. Two sets of cryolabels are included to cover a follow-up visit (with a prefix of A to indicate first visit and B to indicate second visit eg OSA001A). Unused visit labels should be retained with the study participant's CRF, following the first visit.
- 2 Consent packs will be prepared at CRC and contain everything required to collect samples. Do not use any other types of tubes, vacutainer systems or any other materials in the collection of samples. Invitation letters and consent paperwork (Patient Information Sheet/Informed Consent Forms, Case Record Form) is held on the shared drive and should be printed only as required to ensure current versions are always used.
- 3 Once a patient has consented to participate, the CRF should be completed and samples should be collected and labelled as detailed in the quick reference guide below, with details included in the CRF prior to processing. Accurate timings for collection and storage of samples must be recorded.
- 4 An ID card (which patients can show if they attend PPH in future) and a copy of the completed PIS/ICF should be given to the patient. If photocopying facilities are unavailable, the patient should be advised that a copy of the PIS/ICF will be posted.
- 5 Following consent, patient notes should be updated with a copy of signed PIS/ICF included and a research study participant sticker attached to the inside page of the notes; and a GP letter should be issued. The patient enrolment log held with the site file should be completed. The patient screening log held with the site file should also be completed with details of all patients invited to participate.
- 6 Participants may be seen for a follow-up visit, approximately six weeks after initial recruitment. Although informed consent should be confirmed at these visits, participants **should not** be allocated a new study ID or asked to complete a new ICF. At this visit, a second set of samples should be collected and a second CRF completed.

NOVEL TECHNOLOGIES FOR DIAGNOSING AND MONITORING PULMONARY DISEASES - Specimen Collection – Quick Reference Guide					
Sample	Collection Label	Freezer Label	Collection container	Freezer container	Processing
Sputum	Sputum 50ml Study ID		50ml falcon tube		No processing required.
Saliva	Saliva 15ml Study ID		15ml falcon tube		<i>Samples should be frozen as soon as possible and kept for no longer than 20 minutes at room temperature. Where available, samples may be kept on wet ice for an extended period of 30 minutes.</i>
Urine	Urine 50ml Study ID		50ml falcon tube		
Blood (Serum)	Yellow top Study ID	Serum 5ml Study ID	Yellow top (SST) vacutainer	5ml cryovial	
					8 inversions immediately post-collection then sit at room temp for at least 30 mins to allow clotting¹ - Set centrifuge to 22-25°C. Centrifuge sample for 10 mins at 3407 RCF/1300 RPM - Being careful not to disrupt the separation, use a bulb pipette, transfer to 5ml cryovial, until 0.5cm of sample is left in the vacutainer. - Discard vacutainer and bulb pipette in clinical waste.
Blood (Plasma)	Purple top Study ID	Plasma 5ml Study ID	Purple top (EDTA) vacutainer	5ml cryovial	8 inversions immediately post-collection then centrifuge immediately² - Set centrifuge to 22-25°C. Centrifuge sample for 10 mins at 3407 RCF/1300 RPM - Being careful not to disrupt the separation, use a bulb pipette, transfer to 5ml cryovial, until 0.5cm of sample is left in the vacutainer. - Discard vacutainer and bulb pipette in clinical waste.

NB. Vacutainer tubes that do not have an appropriate amount of blood should not be processed. If processed, please indicate on the CRF.

After centrifugation please indicate on the CRF if haemolysis occurred in any of the samples.

¹ The SST tube requires a clotting time of 30 minutes.

² The K₂EDTA tube should be centrifuged as soon as possible. However, a timeframe of maximum 30-60 minutes of standing at room temperature before centrifugation will still produce a quality sample.

Recruitment guidelines PPH OBSTRUCTIVE SLEEP APNOEA ONLY- version 1 dated 21st August 2020

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