

**Nocturnal tissue hypoxia and associated cardiovascular
risk factors in obstructive sleep apnoea syndrome
assessed by overnight change in urine uric acid and
metanephrine excretion in a New Zealand population**

Sujata Hemmady

A thesis submitted to Auckland University of Technology
in partial fulfillment of the requirement for the degree of
Master in Medical Laboratory Science (MMLS)

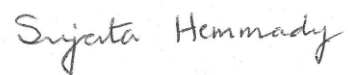
2014

School of Applied Science

Primary Supervisor: Mark Duxbury

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

A handwritten signature in black ink, reading "Sujata Hemmady". The signature is written in a cursive style with a horizontal line underneath it.

Sujata Hemmady

Abstract

Obstructive sleep apnoea syndrome (OSA) is caused by obstruction of the upper airway and is characterized by repetitive pauses in breathing during sleep, despite the effort to breathe. An apnoea is a period of time during which breathing stops or is markedly reduced, associated with reduced blood oxygen saturation (SaO_2) level, called hypoxia. An individual with sleep apnoea is rarely aware of having difficulty in breathing, even upon awakening. If left untreated, it may cause high blood pressure, stroke and congestive heart failure. A hypopnoea is a decrease in breathing that is not as severe as an apnoea but is also associated with a low level of blood oxygen. The severity of OSA is measured by the apnoea-hypopnoea index (AHI/h) during sleep studies conducted at the “Sleep Laboratory” of Auckland City Hospital.

Some studies suggest the pattern of overnight change in urinary uric acid to creatinine ratio significantly differ between OSA and healthy subjects and the use of overnight continuous positive airway pressure (CPAP) reverses the pattern. Low blood oxygen levels may hamper efficient formation of adenosine triphosphate (ATP), leading to increased release of the purine catabolic end product, uric acid. Thus, elevated uric acid in body fluids may be an indicator of tissue hypoxia. To the researcher's knowledge, there have not been similar studies conducted in New Zealand which has high prevalence of gout, obesity and OSA. If delta urine uric acid over creatinine can prove to clearly distinguish normal from significant OSA, then with these tests' easy availability and low cost compared with the relatively scarce availability of overnight sleep clinic assessment slots in Auckland, there is potential for these tests to be used for screening of OSA, prioritising the potentially higher yield referrals for confirmatory sleep studies.

Therefore the current study investigated the diagnostic utility of urinary uric acid as a marker in identifying the possible relationship between hypoxia and

sympathetic activity in a New Zealand population. Ninety subjects from the “Sleep Laboratory” participated in this study over six months. During the sleep study, each participant contributed urine samples before going to bed and in the morning after the sleep study. An additional sample was required if CPAP applied during duration of the sleep study. The urine samples of all the participants were tested for uric acid and metanephrine at LabPLUS, Auckland City Hospital. The results of these biochemical tests were compared to the AHI/h and other data obtained from the sleep study.

Although the severity of OSA as measured by the AHI/h index correlated significantly with conventional risk factors associated with OSA such as Body Mass Index and neck circumference there was no significant correlation between change in urinary uric acid/creatinine ratio and AHI/h. Likewise there was no significant correlation between urinary metanephrine measured in the present study and AHI/h. Thus, at least in the population studied, measurements of changes in urinary uric acid and/or urinary metanephrine do not have diagnostic value in predicting severity of sleep apnoea.

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I wish to thank the following ethics committees for the time they took to review my study to grant approval:

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- Maori Research Review Committee, Ethics Application Number A+5125, approved on 2 September 2011.
- The Auckland DHB Research Review Committee (ADHB-RRC), Ethics Application Number A+5125, approved on 6 September 2011.
- Auckland University of Technology Ethics Committee (AUTEC), Ethics Application Number 11/258, approved on 15 September 2011.

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Abbreviations

ACE: Angiotensin receptor

ACH: Auckland City Hospital

ADHB-RRC: Auckland district health board research review committee

AHI/H: Apnoea-hypopnoea index

AI: Arousal index

ANOVA: Analysis of variation ATP: Adenosine triphosphate

AUTEC: Auckland university of technology ethics committee

BMI: Body mass index

CI: Confidence interval

COMT: Catecholamine-O-methyltransferase

COPD: Chronic obstructive pulmonary disease

CPAP: Continuous positive airway pressure

Cr: Creatinine

ECG: Electrocardiogram

EEG: Electro encephalograph

eGFR: Estimated glomerular filtration rate

ESS: Epworth sleepiness scale

FAS: Fatigue assessment score

GP: General practitioner

HDCA: Health and disability commissioner act

HPLC: High performance liquid chromatography

MHBA: Methoxyhydroxybenzylamine

MRRC: Maori review research committee

NREM: Non rapid eye movement

OSA: Obstructive sleep apnoea syndrome

PaO₂: Partial pressure of oxygen in arterial blood

PSG: Polysomnography

REM: Rapid eye movement

ROS: Reactive oxygen species

RPM: Revolutions per minute

SaO₂: Oxygen saturation

SD: Standard deviation

SNS: Sympathetic nervous system

UA: Uric acid

ΔUA:Cr: Delta uric acid to creatinine ratio

Chapter 1

Introduction

1.1. Obstructive Sleep Apnoea Syndrome

Sleep apnoea is defined as cessation of air flow at the nose and mouth for 10 seconds or longer, during sleep.

Obstructive sleep apnoea syndrome (OSA) is caused by obstruction of the upper airway (Figure 1) and is characterized by repetitive pauses in breathing during sleep, despite the effort to breathe. However, the underlying mechanisms are not entirely understood.

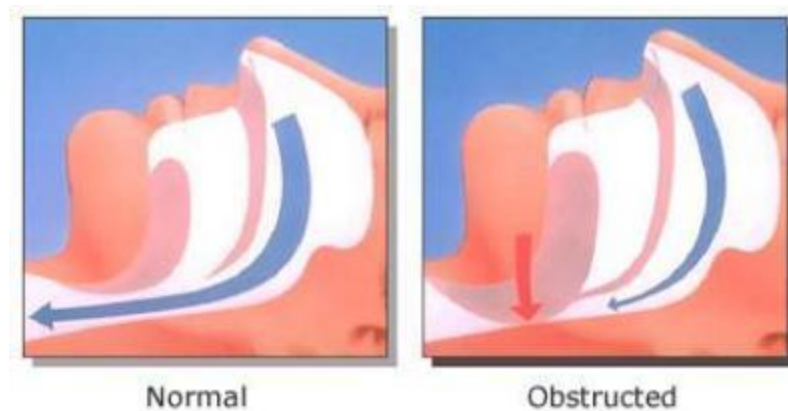


Figure 1: Sleep apnoea caused by obstructed airways (<http://www.amc.nl>)

OSA is characterised by cyclic changes in arterial oxygen saturation (SaO_2) and cardiovascular variables such as heart rate and blood pressure. Each episode of airway obstruction is usually followed by a marked decrease of arterial oxygen saturation (hypoxia), which rapidly normalizes after ventilation resumes. In some severe cases, breathing may stop (apnoea) or is markedly reduced (hypopnoea). The severity of OSA is measured by the apnoea-hypopnoea index (AHI/H) during sleep studies conducted at the “Sleep Laboratory” of Auckland City Hospital.

These repeated changes of oxygen saturation could be considered analogous to recurrent episodes of ischaemia-reperfusion injury, which causes damage after the restoration of blood flow to ischemic or hypoxic tissues. In normal subjects, transient reduction in oxygen saturation occurs during rapid eye movement (REM) sleep but in OSA, this occurs frequently during both REM and non-REM

sleep (Guilleminault & Dement, 1978). In patients with untreated OSA, episodes of hypoxia/re-oxygenation occur frequently during each hour of sleep and may happen every night for several decades resulting in the onset of cardiovascular complications.

In New Zealand, OSA is not uncommon. It is estimated that 4.4% Māori men, 4.1% non-Māori men, 2.0% Māori women, and 0.7% non-Māori women experience episodes of overnight hypoxia (Mihaere et al., 2009).

In recent years, OSA has emerged as an important risk factor for cardiovascular disease. Associations have been reported between sleep apnoea and systemic hypertension, pulmonary hypertension, ischemic heart disease, and stroke (Lavie et al., 2000).

This syndrome results in an inadequate supply of oxygen required for aerobic metabolism at a cellular level and effects the production of adenosine triphosphate (ATP), a compound mandatory for maintaining cellular homeostasis. Due to the impaired synthesis of ATP, purine nucleotide intermediates (adenosine, inosine, hypoxanthine and xanthine) and the purine catabolic end product, uric acid are released. Thus, elevated uric acid in body fluids could be indicative of tissue hypoxia (Van Den Berghe et al., 1989).

Hasday and Grum (1987) were the first to measure an overnight change of urinary uric acid secretion in OSA patients that had a significant correlation to the SaO₂ trends with specificity of 83% in patients with an AHI/h of over 15. They found a pattern of reduced uric acid:creatinine levels from the morning urine compared to pre-bed uric acid:creatinine (Δ UA:Cr) levels in healthy subjects; but, the opposite was true in patients with significant OSA. In patients that were given overnight continuous positive airway pressure (CPAP) application, a marked reduction in apnoea and an increase in SaO₂ was reported.

In a similar study performed in Italy, Braghiroli et al. (1993) concurred with these findings, reporting a positive $\Delta\text{UA}:\text{Cr}$ that co-related to a significant dip in SaO_2 in OSA patients. Out of a total of twenty patients, that had an AHI/h of over 15, fourteen reverted to an AHI/h of less than 10 after a week of CPAP application.

While both studies reported a marked increase in SaO_2 and $\Delta\text{UA}:\text{Cr}$ levels after CPAP application for patients with significant OSA, there seemed to be some conflicting results in the overnight change in urinary uric acid: creatinine ratio for all other subjects. However, the lack of a 'gold standard' definition for nocturnal tissue hypoxia in OSA has prompted this investigation in the diagnostic utility of urinary uric acid as a marker in identifying the possible relationship between hypoxia and urinary uric acid in a New Zealand population.

The homeostatic mechanism is regulated by the sympathetic nervous system (SNS) and mediates the neural and stress response of the body. The sympatho-adrenal response (fight-or-flight) activates sympathetic fibres that end in the adrenal medulla to secrete adrenaline and noradrenaline. Therefore, this response that acts primarily on the cardiovascular system is mediated directly via impulses transmitted through SNS and indirectly via catecholamines secreted from the adrenal medulla.

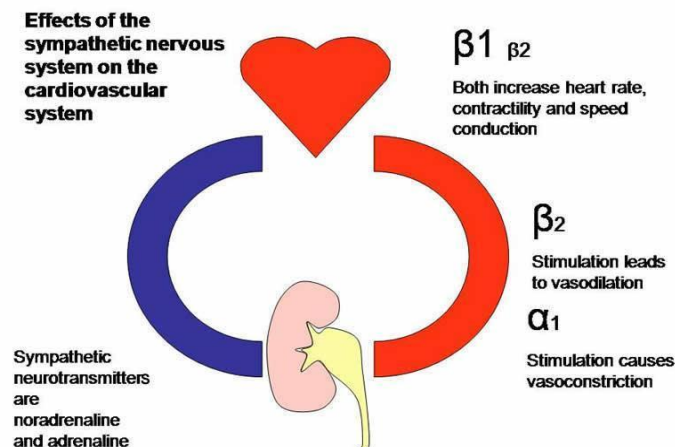


Figure 2: Effects of SNS on cardiovascular system (<http://www.amc.nl>)

Urinary catecholamines are a useful means of measuring sympathetic activity; but, they have sampling and storage limitations. Urinary metanephrine was found to be a more stable catecholamine metabolite that was less prone to in-vitro interferences and had good sensitivity and specificity to sympathetic activity (Roberts et al., 2010).

Ziegler et al. (1997) found that patients with OSA had increased sympathetic activity and considered it to be the main link in the cause-effect relationship between OSA, hypertension and cardiovascular disease. They found that episodes of hypoxia due to sleep apnoea coincided with increased sympathetic nervous system activity which in turn affected adrenergic receptor functioning, resulting in hypertension. The present study is an attempt to investigate the link between OSA and cardiovascular disease via the SNS pathway.

1.2. Outline of the study

A total of ninety subjects participated in this study over six months. Sixty six subjects were recruited at the “Sleep Laboratory” of Auckland City Hospital where each of them underwent a nocturnal sleep study (polysomnography) under a qualified sleep physiologist. Due to budgetary and resource constraints, the healthy volunteers that participated as control subjects did not undergo polysomnography.

Participants were classified into 3 Groups – without OSA (control), mild OSA and severe OSA. The total number of episodes of AHI per hour of sleep was used to define OSA. An AHI >5/h and <20/h is classified as mild OSA, AHI >20/h is significant OSA.

Each participant contributed urine samples before going to bed and in the morning after the sleep study. An additional sample was collected when CPAP was applied during the duration of the sleep study. The urine samples were tested for uric acid and metanephrine at LabPLUS, Auckland City Hospital. The results of these biochemical tests were compared to the AHI/h and other data obtained from the sleep study.

To the researcher's knowledge, there have not been similar studies conducted in New Zealand that has high prevalence of gout, obesity and OSA. If delta urine uric acid over creatinine can prove to clearly distinguish normal from significant OSA, then with these tests' easy availability and low cost compared to the relatively scarce availability of overnight sleep clinic assessment slots in Auckland, there is potential for these tests to be used for screening of OSA, prioritising the potentially higher yield referrals for confirmatory sleep studies.

Chapter 2

Research Design

2.1. Hypothesis

Direction and magnitude of change (delta) in urine uric acid to creatinine ratio levels before and after sleep are possible markers of overnight tissue hypoxia in obstructive sleep apnoea syndrome.

2.2. Ethics Approval

This study has the approval of the Northern X Regional Ethics Committee (NTX/11/08/069). Approvals were received from the Maori research review committee (MRRC) and Auckland University of Technology ethics committee (AUTEC). The study received institutional approval from the Auckland District Health Board research review committee (ADHB-RRC). Informed consent was obtained from all participants before commencement of the study.

2.3. Study Aim

2.3.1. Primary Aim

To assess the reliability of a positive delta urinary uric acid to creatinine ratio ($\Delta\text{UA:Cr}$) as a marker of overnight tissue hypoxia in a New Zealand population.

2.3.2. Secondary Aim

To assess the possible relationship of urinary metanephrine levels to the sympathetic activity in patients with OSA.

2.4. Screening procedures

Potential volunteers for this study were screened over a fortnight in advance by obtaining clinic lists from the charge sleep physiologist for patients that had scheduled appointments at Auckland City Hospital's (ACH) "Sleep Laboratory".

Each subject that satisfied the recruitment criteria were sent out an invitation along with an information sheet and consent form; one week prior to their sleep study appointment. This gave the participant sufficient time for familiarization with the study design.

2.5. Criteria for recruitment

2.5.1. Inclusion criteria

Participants were aged ≥ 18 and ≤ 80 years and should be able to provide written informed consent. Participants with gout/hyperuricaemia with or without allopurinol treatment were included in this study. Participants on uricosurics e.g. probenecid, benzbromarone, sulfinpyrazone; medications with uricosuric properties e.g. Losartan, Atorvastatin, Fenofibrate, Guaifenesin; medications with antiuricosuric effect e.g. aspirin, thiazide diuretics, pyrazinamide and ethambutol were included in this study but the results were analysed separately as this medication could affect the metabolism or urinary excretion of uric acid.

2.5.2. Exclusion criteria

Participants with severe renal impairment with estimated glomerular filtration rate (eGFR) of less than 30mL/min/1.73m^2 and/or abnormally high creatinine were excluded from the study as high urine creatinine levels would lead to a bias in $\Delta\text{UA:Cr}$ calculations.

2.6. Recruitment of subjects

An invitation to join this study was sent to 124 prospective subjects. Sixty six subjects with suspected OSA symptoms were recruited from the pool of patients referred to ACH, for sleep studies. Their primary symptoms were excessive day time sleepiness and snoring. As per protocol required for diagnosing of OSA (Johns, 1991), each subject was requested to complete a self assessment of their sleepiness using the Epworth sleepiness scale (ESS) questionnaire and the Fatigue assessment score (FAS).

The ESS is not a diagnostic tool in itself, but is the world standard method for making an assessment for day time sleepiness. It is a self-administered questionnaire that asks people to rate their usual chances of dozing off or falling asleep in 8 different situations. The total ESS score can range between 0 and 24. A high score indicates a person's increased level of day time sleepiness (Johns, 1994).

Demographic details of age, ethnicity, gender, body mass index (BMI) and smoking habits were noted on the day the polysomnography (PSG) was performed. Details of the medical history and medication for patients referred to the Sleep Lab were provided by the referring physician.

Twenty four control subjects for this study were selected from a pool of volunteers known to the researcher. They fitted the inclusion and exclusion criteria and had no previous history of day time sleepiness or snoring. Each of them provided details of their medical history and medication. The control subjects did not undergo a PSG but followed the same instructions for sample collection as the other Groups.

2.7. Patient Information Sheets

Each participant was given an information sheet approved by the Northern region ethics committee. The sheet contained detailed information about the study in simple lay terms. It advised the participant about their right to address any concerns about the study and the free services of an independent health and disability advocate under the Health and Disability Commissioner Act (HDCA).

2.8. Patient Consent Forms

As per requirements of the Ethics committee, it was mandatory for each participant to give written informed consent before commencement of the study. The participant thus consented to provide the researcher with information from their medical records, details of age, sex, ethnicity and disease that are specific to this study. The participant also consented to this study, and the researcher analysing the participant's urine sample for urinary uric acid, creatinine and metanephrine.

2.9. Collection of specimens

Collection of specimen for this study required considerable co-ordination with staff and study participants at the sleep lab. Several pictorial instructions (see Figures 3 and 4) to aid staff and participants were displayed for ease of communication.

Urine samples were collected from all participants. One pre-bed urine sample in a 50 ml pot on the night before the sleep study for analysis of urinary uric acid

and creatinine was collected, followed by another collection the next morning, immediately after the sleep study. The morning sample was split into two sub-samples; first in a 50 ml urine pot for uric acid and creatinine measurement. The rest of the sample was put in a 120 ml urine pot that contained acid (1 ml of 6 M HCl), in keeping with LabPLUS procedures, for urinary metanephrine analysis. An additional urine sample in a 50 ml pot was collected before CPAP, for those participants that required its application for the duration of the sleep study.

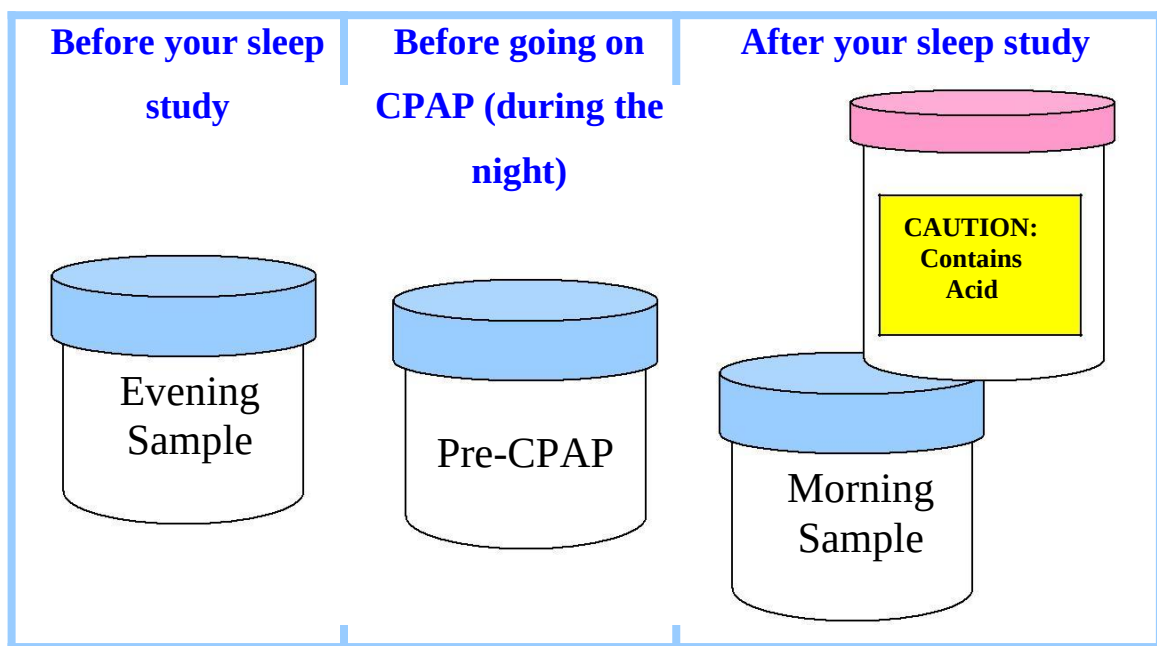
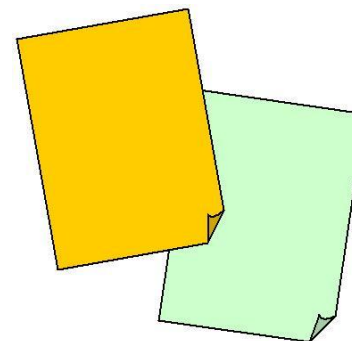


Figure 3: Pictorial instructions displayed at the sleep lab for study patients

Each sample was dispatched via pneumatic Lamson tube to Auckland City Hospital's laboratory, LabPLUS. Urinary uric acid and creatinine levels were analysed within two hours of collection of the specimen while samples for urinary metanephrine levels were stored at -80°C until analysis.



Suitable patients identified
in Admissions book.
Info sheets and consent
forms sent before PSG.



When patients arrive for PSG check study
suitability and collect consent forms. Offer
additional copies where needed.
Note declines in Admissions book.



Urine samples will be sent each
morning to LabPLUS for testing
via Lamson tube.



Collect samples and each
sample in separate Bio bag
with separate form.



Figure 4: Pictorial instructions displayed at the sleep lab for sleep lab staff

2.10. Procedure for withdrawal

Participation in the study was completely voluntary and a participant could withdraw from the study at any stage, without stating a reason.

2.11. Safety parameters

No adverse outcomes were associated with this study. Personnel from the sleep lab followed ADHB-wide procedures and protocol in dealing with emergencies. There were no physical or psychological risks or side-effects to participants or third parties as the study protocol was completely non-invasive and only required collection of urine samples.

2.12. Statistical Analysis

2.12.1. Descriptive data

Minitab statistical software was used for to calculate descriptive data that is expressed as mean, median and $\pm$ Standard deviation (SD). 95% confidence interval (CI) was calculated using the degrees of freedom for the *t*- statistic to describe urinary uric acid and metanephrine data. Patient demographics are presented as mean and standard error for all three Groups using standard statistical tests for comparison. Based on the distribution properties of the data, either parametric (*t*-test or ANOVA) or non-parametric (Mann-Whitney, Kruskal-Wallis or chi-squared) test was used. All tests are two tailed and a *p*-value < 0.05 is considered significant.

To examine correlation between two variables, Pearson single correlation coefficient or Spearman rank correlation was employed where $p < 0.05$ is accepted as significant. Student's paired *t*-test was used to compare Δ UA:Cr data before and after CPAP. Linear regression analysis was used to correlate metanephrine with the arousal index (AI) and Δ UA:Cr with AHI/h.

2.12.2. Power calculation

This study was planned for a continuous response variable from independent control and experimental subjects with 1.0 control(s) per experimental subject. In a previous study (Hasday and Grum, 1987) the response within each subject group was normally distributed with standard deviation 13.7. If the true difference in the experimental and control means is 25, we would need to study 30 experimental subjects and 30 control subjects to be able to reject the null hypothesis that the population means of the experimental and control Groups are equal. The Type I error probability associated with this test of this null hypothesis is 0.05. (DuPont WD, Plummer WD: 'Power and Sample size Calculations for studies involving Linear Regression', Controlled Clinical Trials 1998; 19:589-601).

Thus, in keeping with this, an attempt was made to recruit an equal number of control subjects to the number of participants in each group for ease of statistical analysis. A total of ninety subjects were recruited. The subjects were divided into three Groups as per their AHI/h criteria. Some interesting details about the participant demographics emerged during the course of this study that shall be discussed in the chapter to follow.

Chapter 3

Subject Demographics

3.1. Subject Demographics

New Zealand is a country of diverse ethnicities. Effort was made to include a mixed cohort of all ethnic Groups that commonly receive treatment at ACH. Sixty one adult males and twenty nine adult females between the ages of nineteen and sixty six were recruited. Despite the researcher's efforts, it was not possible to match the ethnic composition of the three Groups. The study is mostly represented by subjects of NZ European ethnicity that only represents participation levels and does not indicate the ethnic distribution of OSA in NZ. 50% of the participants were NZ European, 12% Maori, 12% European, 11% Indian and the rest were of other ethnicities (Figure 5).

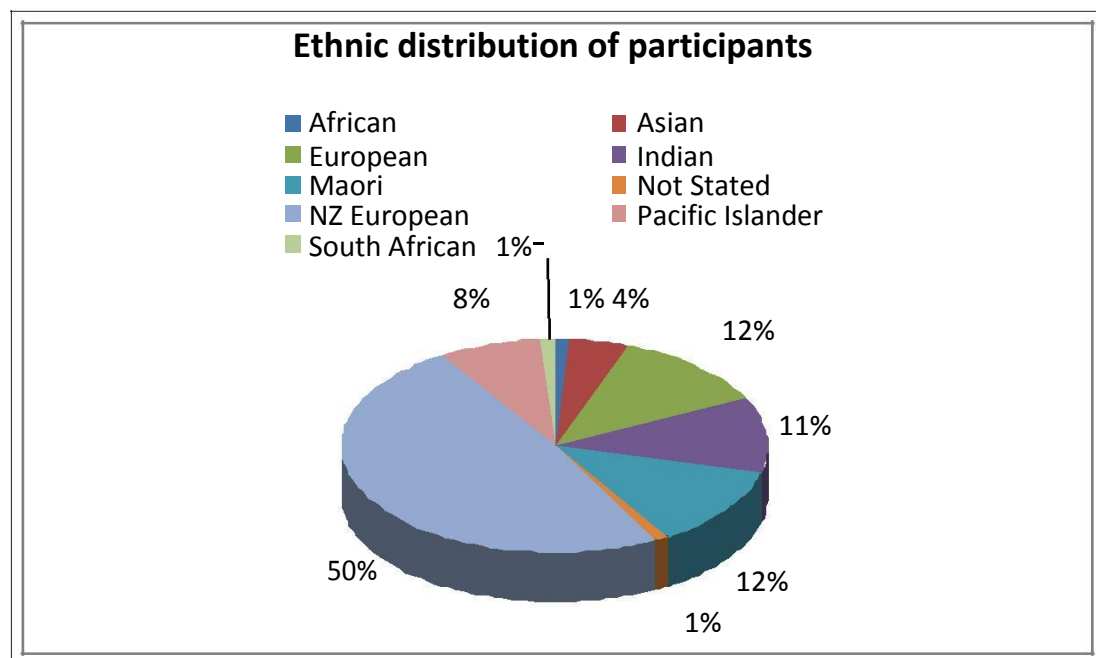


Figure 5: Ethnic distribution of participants

3.2. Anthropometric data

Of the ninety patients recruited, twenty four subjects had no medical history that required medication for diabetes mellitus, gout, hypertension and renal disease assessed by serum creatinine levels and eGFR. These subjects had an average age of 42.5 years and had an average BMI of 26.6 and an average neck circumference of 36.6 cm. This cohort, now referred to as Group 1, was used as 'control subjects' for this study; they did not undergo a PSG.

Sixty six participants that underwent sleep studies were classified into two Groups based on their PSG results, using an AHI/h of 20 as the cut-off value. Group 2 comprising of subjects with an AHI/h of 20 and less, were classified as having mild OSA while subjects in Group 3 were considered to have severe OSA.

Comparison of subject characteristics

Characteristics	Group 1 Control (n=24)	Group 2 Mild OSA (n=33)	Group 3 Severe OSA (n=33)
Anthropometric data *			
Age (years)	42.5±2.57	47.9±2.46	48.2±1.97
Gender			
Male	10	21	30
Female	14	12	3
BMI (Kg/cm)	26.6±0.98	31.0±1.15	39.5±1.84
Neck circumference (cm)	36.6±0.77	38.5±0.57	44.4±0.85

Table 1: *Anthropometric data presented as mean SEM.

Participants of this study did not differ much in age. Control subjects of Group 1 were slightly younger at a mean of 42.5±2.57 while subjects of Group 2 and 3 had means of 47.9±2.46 and 48.2±1.97, respectively. There seemed to be a

higher percentage of males (n=30) diagnosed as having severe OSA in comparison to females (n=3), in Group 3 (Table 1).

Age did not seem to have any correlation to the BMI for Group 1 ($p=0.61$), Group 2 ($p=0.56$) and for Group 3 ($p=0.97$) (Table 1).

Participants of Group 2 were more obese with higher BMI (31.0 ± 1.15 , $p=0.01$) and bigger neck circumference (38.5 ± 0.57 , $p=0.03$) compared to participants of Group 1 that had lower BMI (26.6 ± 0.98) and smaller neck circumference (36.6 ± 0.77). Group 3 were significantly heavier with higher BMI (39.5 ± 1.84 , $p<0.001$) and bigger neck circumference (44.4 ± 0.85 , $p<0.001$) compared to Group 2 (Table 1).

There is a significant relationship between the neck circumference and AHI/h. It is evident from Figure 6 that participants of Group 3 with a greater neck circumference had significantly higher AHI/h compared to participants of Group 2.

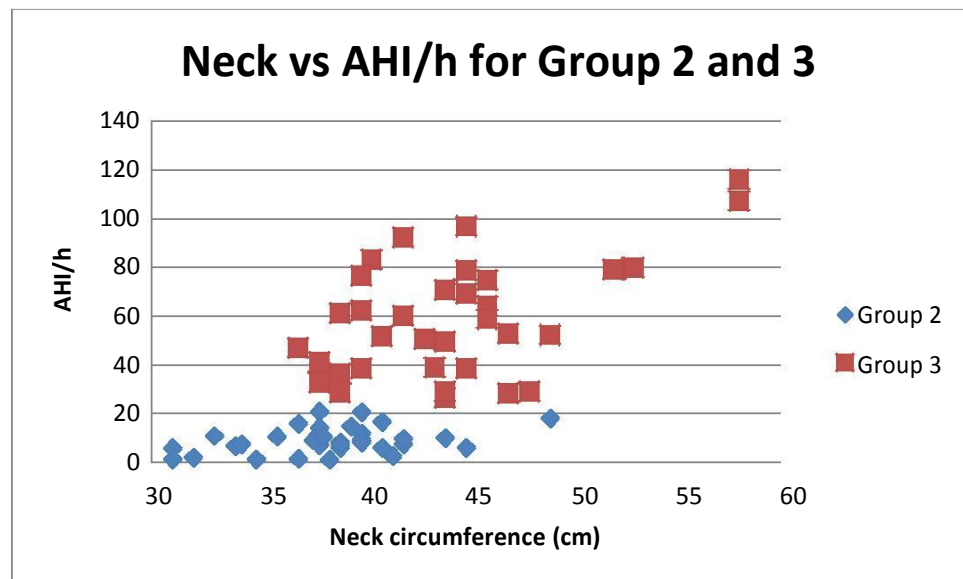


Figure 6: AHI/h vs Neck Circumference for Group 2 & 3

Interestingly, there seemed to be poor correlation between BMI and AHI/h (Figure 7) for participants of Group 2 ($r^2=0.05$, $p=0.12$).

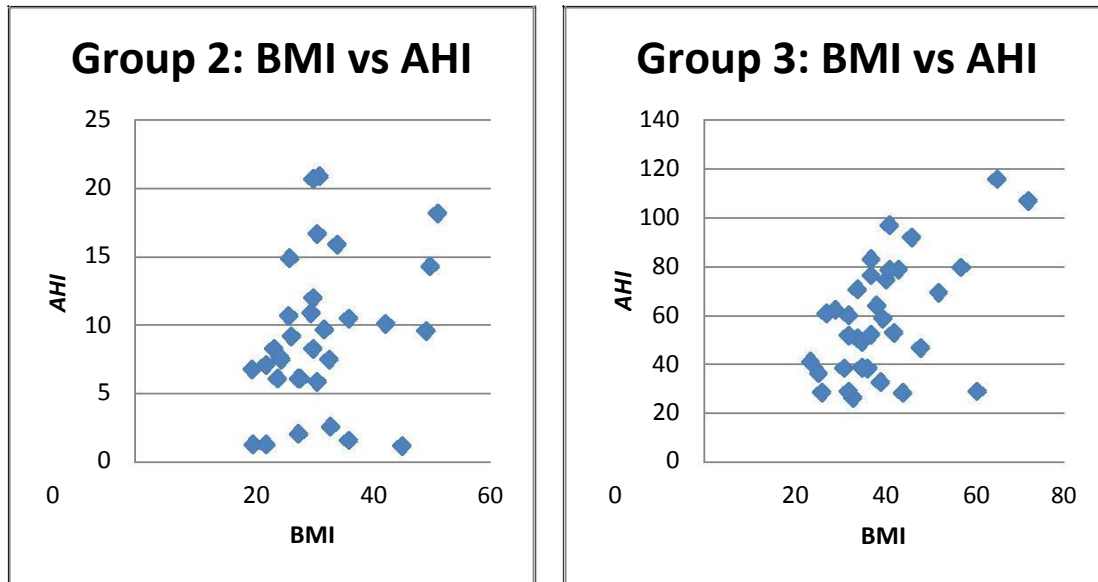


Figure 7: BMI vs AHI/h for Group 2 and 3

The opposite was true for subjects of Group 3 ($r^2=0.29$, $p<0.001$) that showed a significant correlation between BMI and AHI/H.

3.3. Discussion

The ethnic representation of the participants in the present study is not a reflection of the prevalence of OSA in New Zealand. Results of an earlier study (of Paine et al., 2011) on OSA symptoms and risk factors from sleep clinics in New Zealand indicated that observed apnoea and excessive day time sleepiness was significantly higher among Maori men and women compared to non-Maori (all $p<0.001$). However, Maori participation in this study (12%) was not sufficient to substantiate this finding. The same survey reported ethnicity and smoking as a significant predictor of apnoea but Maori ethnicity was a significant predictor only among non-smokers. Smoking may be a good predictor of OSA as half the participants having significant OSA (Table 2) were current smokers.

A high percentage of participants (50%) of this study were of European ethnicity which may be indicative of the ethnic Groups that had greater access to primary healthcare since all participants were sent as referrals by primary healthcare providers. This study may be limited in terms of offering a snapshot of the prevalence of OSA amongst major ethnic groups as it may be representative of only populations of a certain socioeconomic status. Paradoxically, the population that seems to be most at risk of OSA prevalence is under-represented either due to lack of awareness or a lack of access to primary health care.

This study highlighted a rather surprising observation, namely that BMI did not seem to be a reliable predictor of OSA. Obesity has long been documented as a risk factor for developing OSA. However, the present study did not show an overall correlation between BMI and observed apnoea and was found to be significant only in participants of Group3 ($p < 0.001$). However, increasing neck circumference seemed to be a better predictor of OSA than did BMI as there seemed to be a significant ($p < 0.001$) relationship for participants in both Group 2 and 3 (Figure 6). This fact was endorsed by Paine et al. who found neck size to be a risk factor that correlated more closely to OSA than BMI.

The male to female ratio for participants was about 3.5:1 which was consistent with a previous population based study (Huang et.al, 2008) that found a higher percentage of OSA amongst men. The role of various mechanisms to explain gender differences in OSA have been investigated; although no conclusive evidence was available to prove the role of respiratory control of upper airway resistance or the role of progesterone-estrogen/testosterone, Huang et al. believed that females had a more stable upper airway than males. They found that after puberty, boys had a longer upper airway length than did girls, even when normalized for weight. Tsai (2007) showed that males had a greater pharyngeal length and soft palate length that made them more susceptible to OSA. He also found that age distribution for OSA peaked at 55-59 years in both genders.

These findings were reflected in this study where the average age of subjects undergoing PSG for suspected OSA was approximately 49 years.

For several years OSA was considered to be a disease that primarily affected only males. As a result, epidemiological studies that examined the prevalence of OSA included only males. Only recently has OSA been acknowledged to be present in women and it is now the norm for epidemiological studies to have equal representation of both sexes. Although it is well documented that men have a higher incidence of sleep apnoea than women, there is little information available about the differences between men and women upon first clinical presentation. This misconception of greater prevalence of OSA in men than women may perhaps have led to a selection bias for referral from primary healthcare providers, reflected in this study that had a female participation of only 32%. Perhaps these findings are an indication of the high percentage of women with mild or severe OSA that may be under diagnosed.

Gender not only plays an important role in OSA prevalence, it may have a direct influence on uric acid excretion. Garcia et al. (2006) attributed lower uric acid levels in women to estrogens that induce an increase in the clearance of uric acid by the kidneys. Men comparatively maintain a relatively stable uric acid excretion throughout life. Their levels are higher than those of women and, while women experience an increase in uric acid levels after menopause, the prevalence of hyperuricemia is higher in men. While they found this difference in the uric acid levels in the general population, the gender bias did not seem to exist in patients with OSA. Garcia's study reported a high incidence (36%) of hyperuricemia in both sexes that correlated with the number of obstructive respiratory episodes. Similarly, another study carried out in Spain (Corella et al., 1999) that included 1564 healthy working men, found hyperuricemia correlated with the prevalence of cardiovascular risk factors.

Similar results were also reported in a more recent study (Desai et al., 2005) carried out in the general population in which uric acid levels increased in proportion to metabolic risk factors. The prevalence of cardiovascular disease amongst patients diagnosed with OSA is discussed in the next chapter.

Chapter 4

OSA and Syndrome Z

4.1. Metabolic syndrome

Metabolic syndrome is described as a combination of metabolic disturbances and the predisposition of a person to develop type 2 diabetes mellitus and cardiovascular disease. OSA is associated with central obesity, insulin resistance and hypercholesterolemia which are all components of this syndrome (Figure 8). Studies conducted by Coughlin et al. (2007) showed an increasing association of metabolic syndrome with OSA severity. Six weeks of CPAP therapy improved just hypertension but not insulin resistance nor hypercholesterolemia.

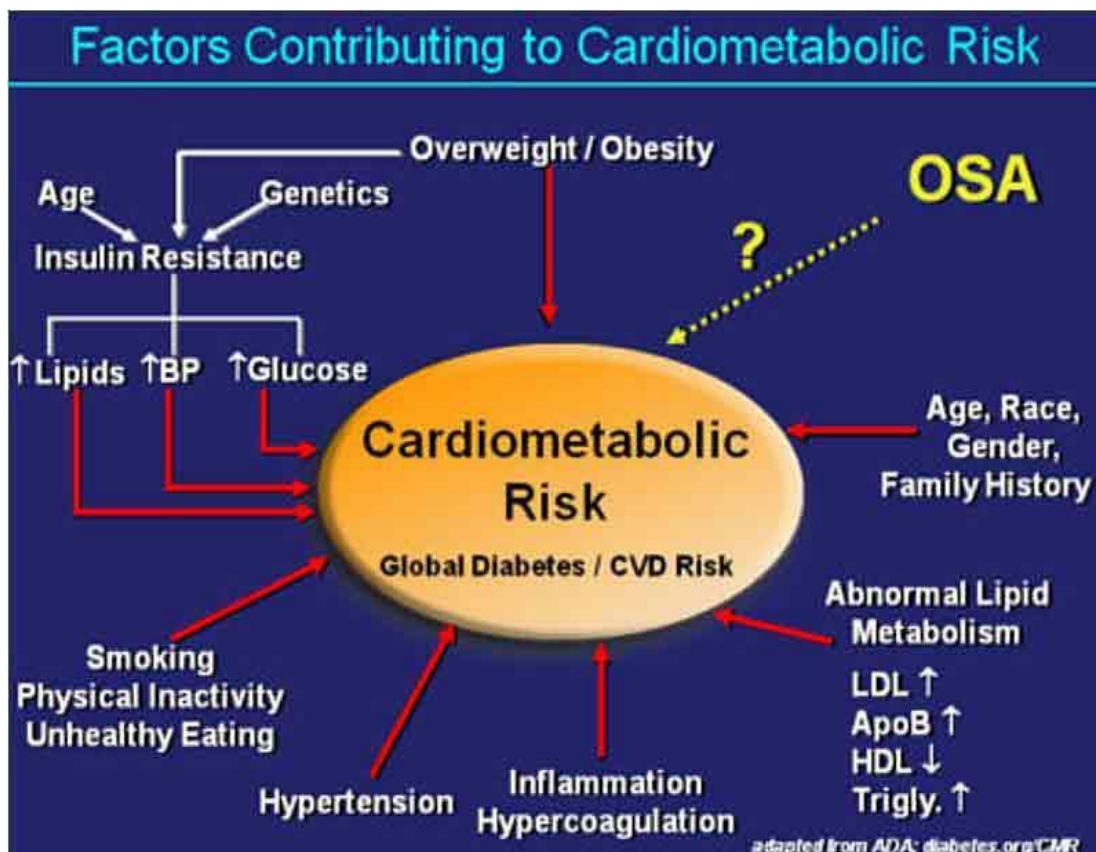


Figure 8: OSA and Cardiovascular risk (adapted from www.diabetes.org/CMR)

Over the years, a lot of data has been presented as evidence to link OSA to cardiovascular disease with higher incidences of coronary events in men than in women. "Syndrome Z", a new term coined to describe the combination of

metabolic syndrome and OSA is increasing worldwide, in part linked to the epidemic of obesity (Wilcox et al., 1998).

4.2. OSA and Cardiovascular risk factors

The association of OSA with cardiovascular disease has been researched with compelling evidence establishing a link between hypertension, coronary artery disease, metabolic syndrome and diabetes mellitus. Some early studies (Schafer et al. 1999; Peker et al. 1999) reported the association of OSA with hypertension, heart disease and stroke but were unable to establish the underlying mechanisms. McCord (2000) theorized that, analogous to ischemia-reperfusion injury, tissue damage resulted from re-oxygenation after hypoxic interludes that led to the production of reactive oxygen species (ROS) during re-oxygenation. ROS are highly reactive molecules that interact with nucleic acids, lipids and proteins and contribute towards the development of cardiovascular disease. People with untreated or undiagnosed OSA having several episodes of hypoxia and re-oxygenation occurring frequently through the night over several decades were thought to be at great risk of developing cardiovascular disease.

Components of cardiovascular risk factors*

Characteristics	Group 1 Control (n=24)	Group 2 Mild OSA (n=33)	Group 3 Severe OSA (n=33)
Smoking	2	46.9	50
Diabetes mellitus	0	28.1	38
Hypertension	1	37.5	50
Cardiac complaints	0	15.6	11.8
Gout	0	3.1	29.4
Hypercholesterolemia	0	40.6	67.7

Table 2: *Data presented as percentage of subjects within their Group receiving medication for diabetes, hypertension, gout, and hypercholesterolemia and are current smokers.

The present study indicates that subjects of Group 3 seemed to have a higher prevalence of hypercholesterolemia, gout and hypertension in comparison to subjects of Group 2 (Table 2). The above data may not definitely identify OSA as the only cause for the prevalence of cardiovascular disease amongst subjects of this study but the following paragraphs do provide compelling evidence to prove a strong association of OSA with cardiovascular disease.

4.3. Smoking

Smoking could well be one of the contributing factors to cardiovascular and pulmonary disease, as reported by Kim et.al (2012), who found many OSA patients to be either current smokers or having a past history of smoking. They

observed that patients that had moderate and severe OSA also had increased thickness and oedema of the uvula mucosa lamina propria caused by their smoking. They also noticed that only smokers had positive staining for calcitonin gene-related peptide, a neuro inflammatory marker for peripheral nerves that was elevated in the uvular mucosa of smokers. This indicated that smoking may either be the cause or worsen OSA through exacerbation of upper airway collapse at the uvula. This is reflected by the findings of this study where almost 50% of subjects in both group 2 and 3 were current smokers.

4.3. Hypertension

A four year follow-up study conducted in 2000 by Peppard et al. found that patients with OSA not only had an increased risk of developing hypertension but the odds of developing hypertension increased linearly with increasing AHI/h. They reported a significant risk of hypertension even at levels of AHI/h that were considered normal (< 5 events/hr).

Aronow (2006) later suggested a direct link between nocturnal intermittent hypoxia and the prevalence of hypertension in persons with OSA due to the body's chemoreceptors having the ability to adapt to long term hypoxia and maintain increased peripheral sympathetic activity and blood pressure long after exposure to hypoxia.

These findings are in keeping with the results of this study where 50% of subjects diagnosed with severe OSA were being prescribed medication for their hypertension compared to 37.5% with mild OSA and only 1% of the control group with hypertension.

A study conducted by Arabi et al. (1997) found that subjects who were free of cardiovascular disease or OSA, developed surges in both sympathetic activity and blood pressure, even during a brief exposure to intermittent nocturnal hypoxia that persisted after subjects recovered from hypoxia. Details of the

relationship between OSA and sympathetic activity shall be discussed in a later chapter.

4.4. Insulin resistance and Diabetes

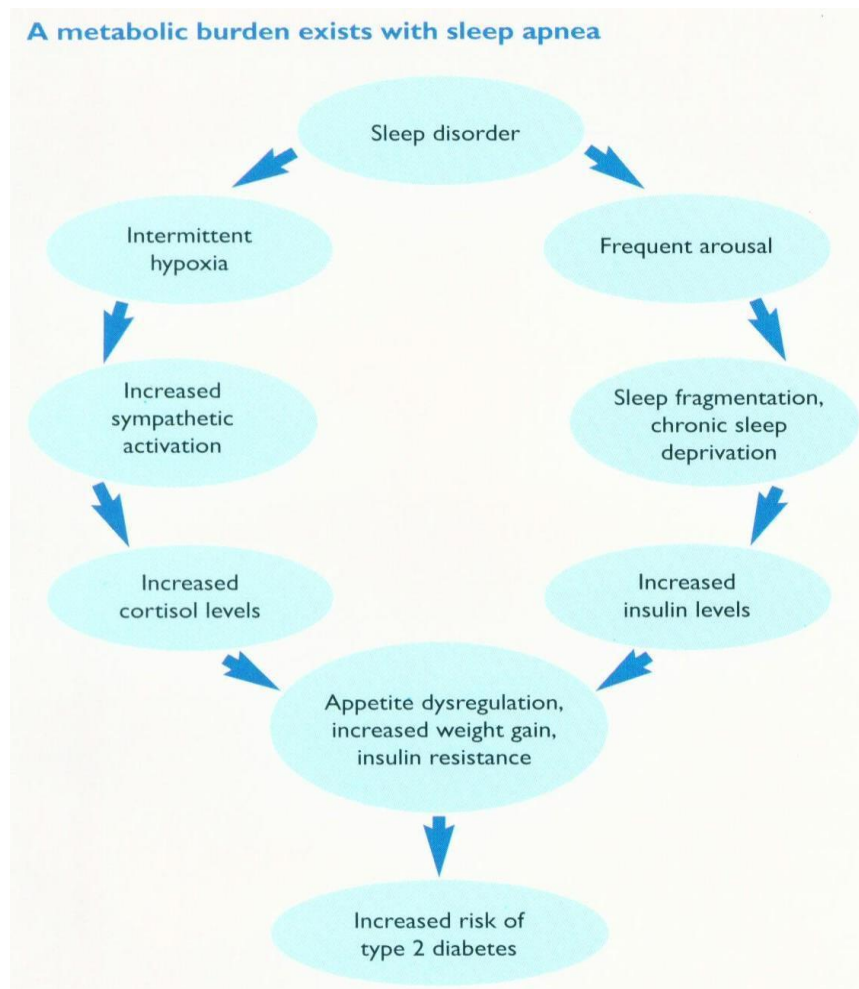


Figure 8: Type II diabetes in OSA patients (<http://allvalleysleepcenter.com>)

38% of subjects enrolled for this study and diagnosed as having severe OSA and 28% of subjects with mild OSA were taking medication for diabetes mellitus. This finding reflects the link of OSA to type 2 diabetes mellitus (Figure 8) that has been reported in several previous papers. A Swedish study (Brooks et al., 1994) reported that obese subjects who snored were seven-fold more likely to develop type 2 diabetes over a ten year follow-up period compared to non-obese non-snorers. They found patients with OSA had higher concentration of blood glucose and plasma insulin levels, independent of obesity. In a similar finding, Strohl et.al (1994)

found a modest correlation between AHI/h and fasting insulin levels. Figure 8 explains the physiology of the development of Type II diabetes in OSA patients. Metabolic dysfunctions caused by sleep fragmentation and sleep deprivation causes insulin levels to rise which in turn leads to appetite deregulation and weight gain culminating in insulin resistance.

4.5. Hypercholesterolemia

The endogenous lipid pathway starts from the intestinal absorption of dietary triglycerides and cholesterol which is bound to locally synthesized chylomicrons. The enzyme lipoprotein lipase (LPL) that is predominantly located in adipose tissue, hydrolysis of the triglyceride content of the chylomicron to form free fatty acids (FFA) and glycerol in the liver. Subsequently, smaller chylomicron particles are synthesised to form very low density lipoproteins (VLDL). The VLDL combines with the cholesterol particle from high density lipoprotein (HDL) and is synthesized to form low-density lipoprotein (LDL) in the liver. (Faiz et al., 2012).

Barceló et al. (2011) showed that people with OSA, having intermittent hypoxia, seem to have increased sympathetic activity that modulates the activity of hormone sensitive lipase (HSL) in the adipose tissue and leads to an influx of FFA in the liver. This subsequently blocks the activity of LPL and decreases LDL clearance. The build-up of LDL cholesterol is said to be the precursor of hypercholesterolemia and atherosclerosis.

Phillips et al. (2011) provided strong evidence to support the beneficial effect of CPAP therapy in reducing triglycerides and LDL, thus proving an association of intermittent hypoxia, characteristic of OSA, with hypercholesterolemia. However, although it is probably not possible to directly identify the cause of the high percentage (67.7%) of subjects in Group 3 taking medication for hypercholesterolemia, the link between OSA and sympathetic activity provides a possible cause of the metabolic derangement.

4.6. Gout

Gout is a common type of arthritis usually associated with or abnormally high uric acid levels in the blood (hyperuricemia). The condition begins with a build-up purines or nitrogen containing compounds that are normally excreted by the kidneys. In conditions where levels of uric acid in blood plasma reach high concentrations, there is a build-up of needle like crystals (monosodium uric acid) in the joints that trigger inflammation and pain, a characteristic of gout.

Huang et al. (2008) found a high occurrence of gout amongst OSA patients. They attributed the increases in purine catabolic products (adenosine and uric acid) and increased pro-inflammatory mediator response to the intermittent hypoxia experienced in OSA. Indeed, 29.4% of subjects in Group 3 of this study (Table 2) who were taking medication for gout were subsequently diagnosed as having severe OSA. Data provided by Huang et al. confirmed earlier theories that associated hypertension, obesity, diabetes, chronic ischaemic heart disease and hypercholesterolemia with OSA.

Thus, all the above findings indicate an epidemiological relationship between OSA and metabolic syndrome. The hypoxia associated with OSA not only promotes syndrome Z but also seems to trigger oxidative stress that shall be discussed in later chapters.

Chapter 5
Polysomnography at the
Sleep Laboratory

5.1. Sleep Studies at Auckland City Hospital

The word polysomnography was derived from the Greek word “somno” meaning sleep and is a graphical recording of many parameters during sleep.

Polysomnography (PSG) or sleep study is an overnight test to evaluate sleep disorders (Figure 9).

Normal sleep consists of cycles of non-rapid eye movement (NREM) and rapid eye movement (REM) that occur every 90 -120 mins, where oxygen saturation remains stable throughout the night with no major respiratory disturbances. However, people with undiagnosed OSA have a transient reduction in oxygen saturation during both REM and NREM sleep (Guilleminault & Dement, 1978). They may display a few symptoms like persistent loud snoring, excessive day time sleepiness, waking with a headache, poor memory/concentration, irritability and depression.

The Auckland District Health Board (ADHB) “Sleep Laboratory” is a fully accredited service that consists of six fully operational PSG beds. It has an additional two beds available for overnight saturation, pressure titration studies and Bi-Level review studies.

During the duration of this study, the “Sleep Laboratory” received an average of six to eight patients per night. Most patients were sent on referrals from other District Health Board (DHBs) and General Practitioners (GPs). The “Sleep Laboratory” is staffed by a team of respiratory specialists, sleep physiologists and nursing staff that assess and diagnose sleep-disordered breathing in adults.

5.2. Preparation for PSG

All participants were sent detailed information about the sleep study before their appointment. They were asked to refrain from consuming caffeinated drinks 24 h before their sleep study.

5.3. Self assessment of sleepiness and fatigue

At the sleep clinic, all patients were required to complete a self-assessment of their day time sleepiness and fatigue by filling in the ESS and FAS questionnaires that assist the sleep physiologist in their diagnosis. Excessive day time sleepiness is often reported in various settings as a symptom of OSA (Young et al., 2002). The severity of sleepiness can be assessed by using the ESS, in which scores range from 0 to 24; a score of above 10 is deemed abnormal. The FAS scores have a range of 0-50; a score of above 24 was deemed as abnormal.

5.4. Polysomnography

PSG was conducted in the privacy of a room where patients were monitored by sleep physiologists via video cameras and microphones that helped make snoring patterns audible. On the night of the sleep study, each participant was given a standardised meal at the “Sleep Laboratory”.

They were required to retire to their individual rooms by 8 pm. Data acquisition started from approximately 9 pm and continued until approximately 6 am the following morning. Diagnosis of OSA relied on established clinical and sleep study criteria as per the protocol at the “Sleep Laboratory”.

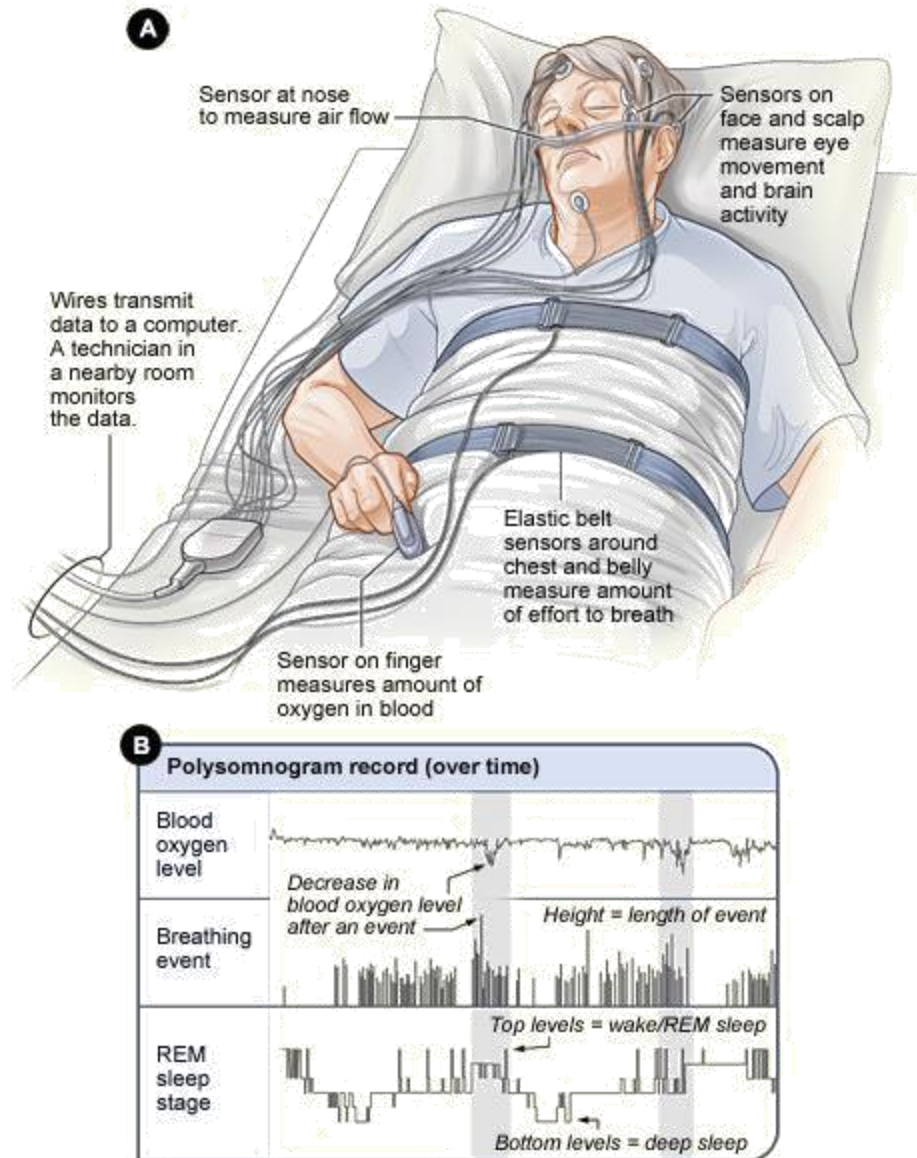


Figure 9: (a) Polysomnography procedure at the sleep lab (b) Polysomnography with encephalogram monitoring (modified from <http://mediconweb.com>)

Participants who underwent polysomnography were constantly monitored with an encephalogram, right and left electro-oculogram, chin and tibialis anterior electromyogram, thoracic and abdominal movements, oronasal airflow, pulse oximetry and electrocardiogram (ECG) with a unipolar precordial lead. Sleep stages and apnoea were scored manually and SaO_2 levels were analysed automatically. Nasal airflow was monitored by a Thermistor (Nihon-Kohden;

Tokyo, Japan), arterial oxygen saturation was measured with a pulse oximeter (Pulsox 7; Minolta; Tokyo, Japan), and thoracoabdominal wall motion was recorded by a respiratory inductance plethysmograph (Respirace; Ambulatory Monitoring; Ardsley, NY). Sleep patterns were monitored from the electro encephalogram (EEG) (C3/A2 and O2/A1), electro-oculogram, and submental electromyogram, with the international 10-20 electrode system being used for EEG.

5.5. PSG parameters

Sleep information included total sleep time, sleep onset, and sleep efficiency, number of awakenings, and number of movement time epochs, REM latency from sleep onset and NREM sleep. Details of average oxygen saturation, lowest oxygen saturation during REM and NREM were recorded.

De-saturation parameters included the minimum SaO_2 , the ratio of oxygen de-saturation time over total sleep time, time with SaO_2 concentrations of more than 90%, less than 60%, 50%, 70%, 80% and 90%, classified as obstructive, central or mixed apnoea/hypopnoea.

Apnoea was defined as cessation of airflow for at least 10 seconds and hypopnoea as a reduction of airflow by at least 50% and lasting for at least 10 seconds of the mean amplitude when the subject is at rest in the supine position, followed by a reduction in SaO_2 of at least 4%. Apnoea/Hypopnoea statistics gave details of obstructive, central and mixed apnoea and hypopnoea episodes in REM and NREM sleep in the supine and non-supine positions.

The AHI/h was calculated as the number of apnoea-hypopnoea events per hour of the total sleeping time.

The AI was calculated as the number of arousals related to disordered breathing events per hour of total sleeping time. Arousal statistics included respiratory,

spontaneous and user-defined arousals as defined by The American Sleep Disorders Association criteria (Guilleminault, 1992).

5.6. Criterion for diagnosis of OSA

The criteria for the diagnosis of OSA at the “Sleep Laboratory” was based on the guidelines sourced from the American Academy of Sleep medicine task force (1999). These guidelines were used to first establish a baseline for normal sleep pattern for each subject which was described as the mean amplitude of stable breathing and oxygenation in the two minutes preceding onset of an event (in individuals who have a stable breathing pattern during sleep) or the mean amplitude of the three largest breaths in the two minutes preceding onset of the event (in individuals without a stable breathing pattern).

Accordingly, a patient was diagnosed with obstructive apnoea if there was over 90% reduction from the baseline in at least either the Thermistor or nasal flow and over 4% oxygen de-saturation or associated arousal over the duration of 10 seconds or more. A diagnosis of obstructive hypopnoea was made if the patient had over 30% reduction from baseline in at least the Thermistor or nasal flow and at least 4% or more oxygen de-saturation or associated arousal over the duration of 10 seconds or more (Meoli et al., 2001). The classification of an arousal was subject to rules and conditions set by guidelines set by The Atlas Task Force (1992) and was diagnosed as an abrupt shift in EEG frequency that may include theta, alpha and/or frequencies greater than 16 Hz but not spindles.

5.7.1. CPAP application

CPAP is the most common treatment for OSA. A CPAP machine (Figure 10) is a pump which delivers air at a positive pressure, through a tube and either a mask on the nose or through a mouthpiece to the throat. The pressure supports the muscles of the soft palate and tongue to keep the throat open and prevent airways from collapsing due to an obstruction, during sleep.

This apparatus is used only during sleep and is known to stop snoring and apnoea, therefore preventing sleep being disrupted and improving the signs and symptoms of OSA.

A short trial of the CPAP machine with the correct size mask was given to all participants prior to bed time. This gave them a chance to experience the feeling of breathing with the machine. Not all participants were required to be given CPAP application.

5.7.2. Criterion for CPAP application

“Sleep Laboratory” staff applied CPAP for patients that had an average AHI/h of over 15/hr four hours after sleep initiation and if the SaO₂ dropped to less than 94%. Alternatively, CPAP was applied for patients with an AHI/h between 5 and 14 with documented symptoms of excessive day time sleepiness, impaired cognition, mood disorders or insomnia, or documented hypertension, ischemic heart disease or history of stroke.



Figure 10: CPAP application (<http://www.snoringcurez.com>)

5.8. PSG Data Analysis

5.8.1. ESS/FAS data

ESS and FAS scores for Group 2 and 3 were analysed and the data indicated that there was no significant difference between Groups 2 and 3 by ESS ($p=0.93$) or by FAS ($p=0.72$), as seen in Table 3.

ESS for subjects in Group 2 was 15 ± 1.33 (range: minimum, 4; maximum, 24) and FAS was 30.1 ± 0.94 (range: minimum, 10; maximum, 39). One participant from this group assessed their ESS score as 24 and FAS as 35 while in actuality, their AHI/h was only 1.2. At the other end of the spectrum, a participant from Group 3 assessed their day time sleepiness score as 7 and FAS as 18 while their AHI/h was calculated at 69.4. This indicated that the ESS and FAS scores did not necessarily reflect the degree of OSA amongst participants of the study.

Polysomnography data in Mild and Severe OSA

Variables	Group 2 (n=33)	Group 3 (n=33)
ESS/24 *	15±1.33	14.8±0.93
FAS/50 *	30.1±0.94	29.5±1.51
AHI/h	9.1±1.02	58.8±4.22 **
Arousal Index	16.2±1.26	45.7±4.04 **
Baseline SaO ₂ , (%)	94.7±0.33	92.4±2.03 **
Lowest SaO ₂ during REM sleep, (%)	86.6±1.29	75.3±1.87 **

Table 3: Data presented as mean ± SEM.

SaO₂ = arterial saturation of oxygen

*p > 0.05 between groups

**p < 0.05 between groups

5.8.2. PSG scoring

The individual analysis of PSG data was done independently by qualified personnel who were blind to any other data collected for this study. Apnoea episodes were selected regularly throughout the night for analysis to avoid any intentional bias. The average measurements were calculated and used as a representative value for each parameter in each subject. To ensure that the

selected apnoeic episodes reflected the overnight data, an average of the minimum SaO₂ associated with the selected apnoeic episodes was calculated.

5.8.2.1. PSG data

Subjects from Group 3 experienced 58.8 ± 4.22 (range: minimum, 26.4; maximum, 115.9) apnoea and hypopnoea events in an hour. Subjects of Group 2 had an AHI/h of 9.1 ± 1.02 (range: minimum, 5.9; maximum, 11.9).

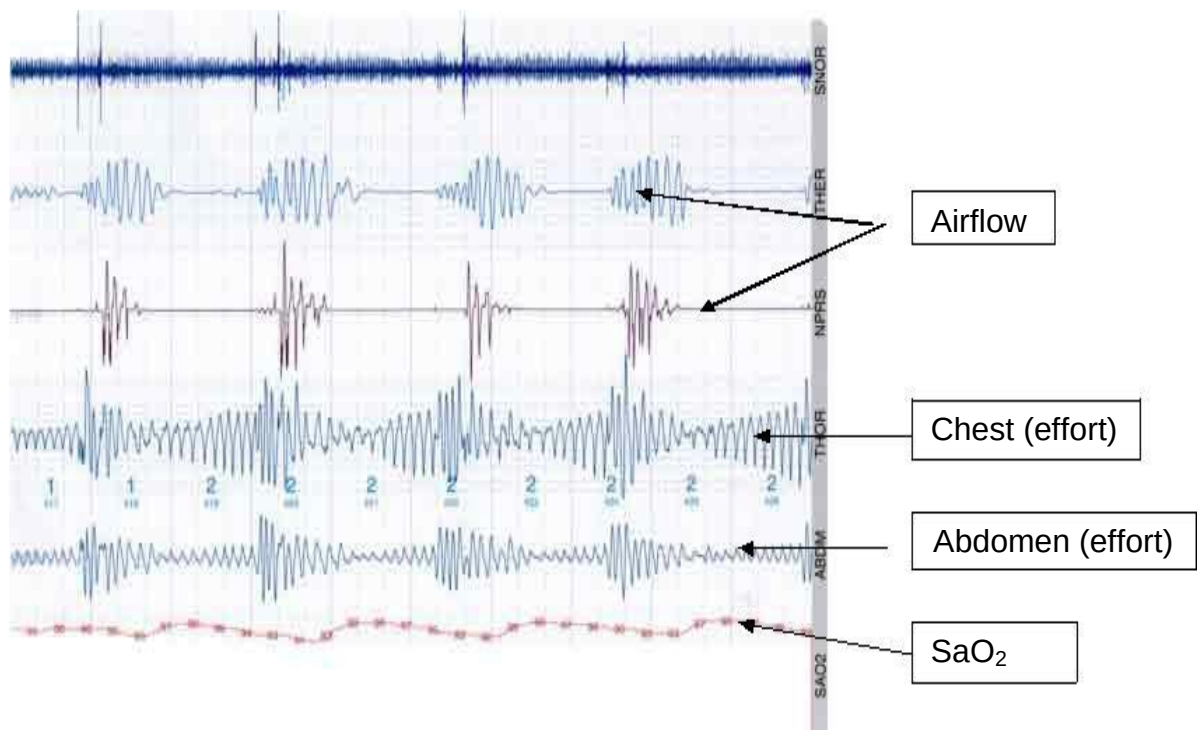


Figure 11: Obstructive Apnoea-absence of airflow with respiratory effort

Data collected for other PSG parameters showed that subjects from Group 3 showed poor sleep quality and experienced more arousals: 45.7 ± 4.04 (range: minimum, 5; maximum, 101.7) which was significantly higher than participants of Group 2 ($p < 0.001$). The baseline SaO₂ for Group 3 (Figure 11) at 92.4 ± 2.03 (range: minimum, 78; maximum, 97.6, $p = 0.006$) was comparatively lower than that of Group 2 at 94.7 ± 0.33 (range: minimum, 93.2; maximum, 97.2). Group 3 showed a considerably higher degree of de-saturation during REM sleep

(75.3 ± 1.87 ; $p < 0.001$) compared to subjects in the mild OSA category (86.6 ± 1.29).

5.9. Discussion

ESS/FAS scores for subjects enrolled in this study indicated that the scores did not always reflect the degree of OSA (Table 3). The scores for subjects in Group 2 were not very different from those in Group 3 although other sleep study parameters indicated that Group 3 subjects had significant OSA and therefore should have had a higher ESS score.

This finding was rather surprising as ESS scores are used worldwide as an indication of day time sleepiness which is a common side effect of OSA. There could be several possible explanations for this discrepancy, one of them being that day time sleepiness could be induced by factors other than OSA; the second possible reason could be that the perception of the degree of sleepiness is very subjective, often clouded by adaptive behaviour (Patel & Schwab, 2007).

The qualitative diagnosis of OSA is based mainly on clinical symptoms like snoring and day time sleepiness followed by a PSG conducted by a qualified sleep physiologist. The diagnosis is linked to the actual number of apnoea and hypopnoea events per hour of sleep, associated to a formal threshold, above which a patient is considered to be suffering from OSA. The degree of the severity of their sleep apnoea is thus quantified. For this study, a range from 5 to 15 events per hour of sleep was deemed to be mild (Group 2) and an AHI/h of over 15 was considered significant OSA (Group 3).

There was a significant difference in the PSG parameters between Groups (Table 3). The actual apnoea events may vary in nature depending on the physiology and other factors (like the age of the participant) that may influence the outcome of the diagnosis. Although available guidelines followed by the “Sleep Laboratory” allowed for a variation in the multi-criteria decision rule for obstructive sleep apnoea, there seemed to be an intrinsic imperfection in the setup of this study design as

some participants that were classified as having OSA also may have had intermittent central apnoea or Cheyne-Stokes respiration.

In central sleep apnoea, the brain's respiratory control centres are imbalanced during sleep. The person stops breathing and as no effort is made to breathe, there is no chest movement and no struggling. During this episode of apnoea, the neurological mechanism is unable to maintain an even respiratory rate and the subsequent breathing after an episode of apnoea; breathing may become faster (hyperpnoea) in an effort to draw more oxygen (Figure 12).

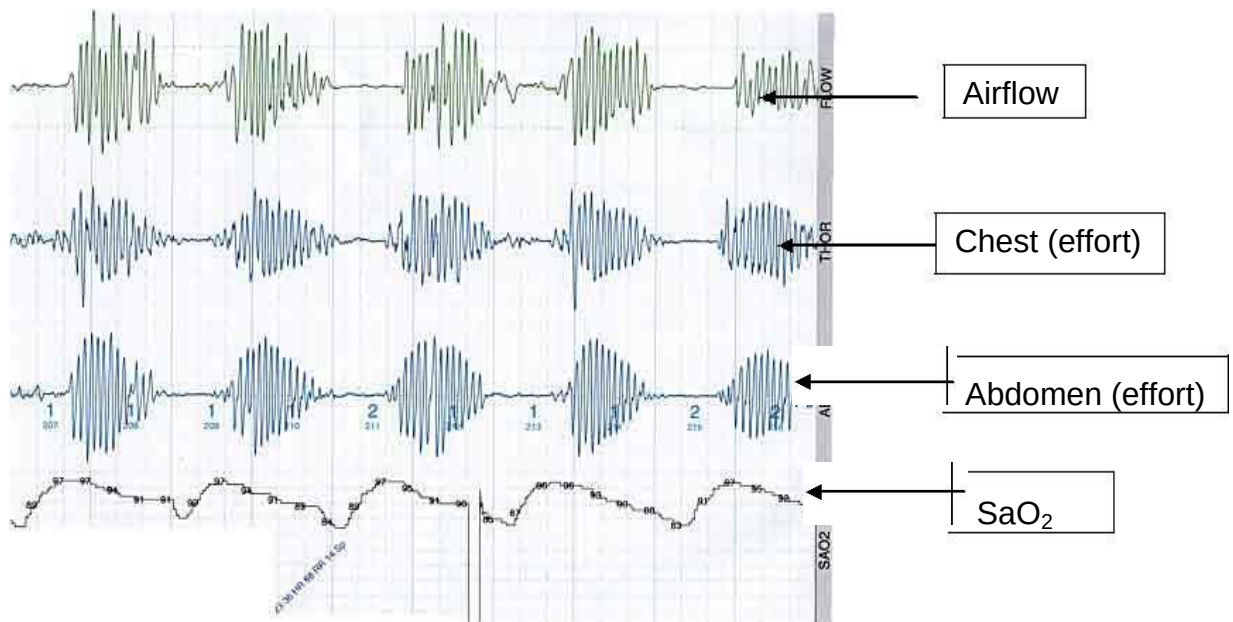


Figure 12: Central Apnoea-absence of airflow with absent respiratory effort

During the transition from episodes of hypoxia (due to an apnoea), to a state of hyperpnoea, the SaO₂% drops and there may consequently be a higher than normal concentration of carbon-dioxide (hypercapnia) which may affect the body's metabolism. The heart rate may increase, along with a compensatory change in the autonomic nervous system; especially in people with long standing OSA. However, the exact mechanism is not yet known (Dernaika et al, 2007).

Due to limitations of resources available for this study, it was not practically possible to exclude participants with mixed apnoea as the diagnosis was made well after the participant had completed their PSG and urine sample collection and laboratory analysis had been completed. Although every effort was made to ensure that only subjects with OSA were included in the study, some participants in Group 3 may have had mixed apnoea.

It is therefore possible that participants with mixed apnoea (OSA and Cheyne-Stokes respiration) may have had slightly biased PSG results which may well have affected not only their uric acid: creatinine but also metanephrine excretion.

Chapter 6

Urine uric acid: creatinine as a Bio-chemical marker of OSA

This study is based on the premise that overnight tissue hypoxia in OSA leads to the formation of uric acid due to increased ATP catabolism. Undoubtedly, there probably are other indices that are more sensitive to tissue hypoxia but testing urinary uric acid is an in-expensive, non invasive method of excluding significant OSA.

6.1. Pre-analytical preparation of urine samples

Before commencement of this study, a pilot test was performed to gauge an optimum method for preventing uric acid precipitation by adding 1:50 2N sodium hydroxide to fresh urine samples. No significant differences were observed in the uric acid results obtained from fresh urine samples, compared to samples that contained sodium hydroxide. Subsequently, fresh urine samples without preservative were collected for uric acid analysis and transported to the laboratory within two hours of collection. Samples were stored at 4 °C when there were delays in analysis.

Each urine sample collected at the “Sleep Laboratory” was accompanied by a request form that had patient details including the participants name, age, hospital number, date and time of collection.

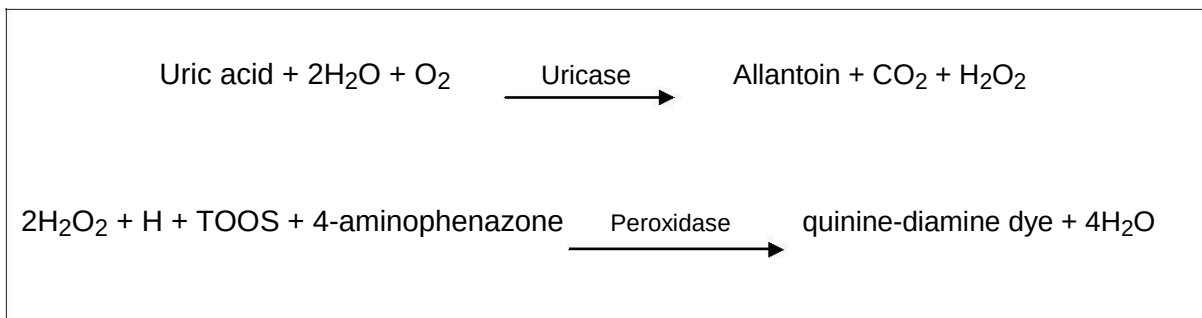
Specimen were analysed at LabPLUS; each sample was given a unique identification number for sample registration and tracking. The pre-analytical process involved centrifugation at 3500 revolutions per minute (RPM) for 5 minutes and the preparation of two aliquots for each urine specimen received. One aliquot was stored at -20 °C for the duration of the study while the other was used for analysis of uric acid and creatinine.

6.2. Method of analysing urinary uric acid:

Uric acid concentration in urine was measured on the Hitachi Modular by an enzymatic colourimetric method using uric acid reagent kits sponsored by Roche

Diagnostics (Cat. No.1875426). Biorad Lyphochek Quantitative Urine Controls, Level 1 (Cat. No. 376) and Level 2 (Cat. No. 377) were used for this study.

The sample was mixed with reagent 1 containing phosphate buffer/ fatty alcohol polyglycol ether and ascorbate oxidase enzyme/TOOS(N-ethyl-N-(2-hydroxy-3-sulphopropyl)-3-methylaniline), then a second reagent (buffer/enzymes/4-aminophenazone) was added. Uricase cleaves uric acid to form allantoin and hydrogen peroxide. The peroxide reacts in the presence of peroxidase, TOOS, and 4-aminophenazone to form a quinine-diamine dye. The intensity of the red colour formed is proportional to the uric acid concentration and is determined photometrically at 546nm.



Equation 1: Uric acid by enzymatic colourimetric method

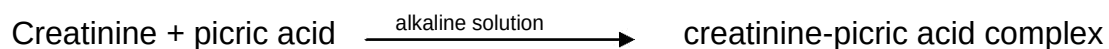
6.2.1. Limitations of this method

This method for urine is linear between 0.1 and 16.4 mmol/L.

Of the drugs tested in-vitro by Roche, α -methyldopa, des-feroxamine and calcium dobesilate (e.g. Dexium) cause interference at therapeutic concentrations (uric acid level artificially low). Uricase reacts specifically with uric acid. Other purine derivatives could inhibit the uric acid reaction. (Ref: Roche Diagnostic's application sheet, 2011-03, 1936131001 V16).

6.3. Method of analysing urinary creatinine

Creatinine in urine was measured on the Hitachi Modular by a kinetic colourimetric Jaffe assay using creatinine reagent kits sponsored by Roche Diagnostics (Cat. No.11875663).



Equation 2: Creatinine by kinetic colourimetric Jaffe method

In alkaline solution, creatinine forms a yellow-orange complex with picrate. The colour intensity is directly proportional to the creatinine concentration and can be measured photometrically. Assays using rate blanking minimise interference by bilirubin.

Due to baseline interference of the Jaffe method by serum proteins, this method was set-up with an intercept of -0.026 mmol/L.

Biorad Lyphochek Quantitative Urine Controls, Level 1 (Cat. No. 376) and Level 2 (Cat. No. 377) were used for this study.

6.3.1. Limitations of this method

This method for urine is linear between 0.4 and 57.5 mmol/L.

The intercept causes a measurement error of $\leq 1\%$ in urine specimens because they do not contain non-specific proteins.

Roche state that antibiotics containing cephalosporin lead to significant false-positive values. Negatively biased results have been reported due to a temporary production of turbidity in the early stages of the reaction. Values in this instance are flagged with a LIMTL and this effect is only seen in freshly drawn, lipaemic samples. Roche also state there is no significant interference from: Acetone up to 8.6 mmol/L, Acetoacetate up to 20 mmol/L and β -hydroxybutyrate up to 25 mmol/L. (Roche Diagnostics application sheet, 2011-03, 1936131001 V16)

6.4. Laboratory results

To investigate the diagnostic utility of urinary uric acid as a possible marker of overnight tissue hypoxia and the relationship of OSA with sympathetic nervous

activity, the laboratory results obtained from processing urine samples was analysed as follows.

6.4.1. Urine uric acid: creatinine

To calculate the overnight increase in urinary uric, the following equation, proposed by Hasday and Grum (1987) was used:

$$\Delta\text{UA:Cr} = 100 \times [(\text{UA:Cr morning} - \text{UA:Cr evening}) / \text{UA:Cr evening} \\ (\text{in mmol/mmol}).$$

Equation 3: Hasday and Grum equation for $\Delta\text{UA:Cr}$

The pattern of $\Delta\text{UA:Cr}$ is reported in Table 4. The analysis of variance demonstrated the excretion of uric acid at night was reduced in normal subjects, leading to a negative ratio for $\Delta\text{UA:Cr}$: -17.9 ± 3.48 (range: minimum, -34.3; maximum, 27.8). Two subjects in the control group had false positive [‡] (see page 65) $\Delta\text{UA:Cr}$ concentration, resulting in a large variation in this group.

[‡] *Note: False negative means that the response was opposite to that expected if the hypothesis was correct, in that OSA elevates uric acid due to nocturnal hypoxia.*

Laboratory results for Groups

Result	Group 1 Control (n=24)	Group 2 Mild OSA (n=33)	Group 3 Severe OSA Before CPAP (n=33)	Group 3 Severe OSA After CPAP (n=23)
$\Delta\text{UA:Cr}$	-17.9±3.48	-24.9±3.73	-16.4±3.64	-33.13±3.25**
AHI/h	<2	9.1±1.02	58.8±4.22	9.81±2.22**

Table 4: Data presented as mean ± SEM.

**p < 0.001vs Group 3, before CPAP

A comparable negative ratio was obtained for subjects classified as having mild OSA in Group 2 (Table 4) for $\Delta\text{UA:Cr}$: -24.9±3.73 (range: minimum, -94.8; maximum, 19.7). 30% (ten) of subjects in this group showed a positive overnight change in the uric acid: creatinine ratio but because 24% (eight) of subjects in this group had false negative [‡](see page 62) results, it led to a large variation in Group 2.

A linear regression analysis showed no correlation between $\Delta\text{UA:Cr}$ and AHI (Figure 14) for subjects in Group 2 (AHI/h=9.1±1.02 versus $\Delta\text{UA:Cr}$ =-24.9±3.73; p=0.010, r^2 =0.013).

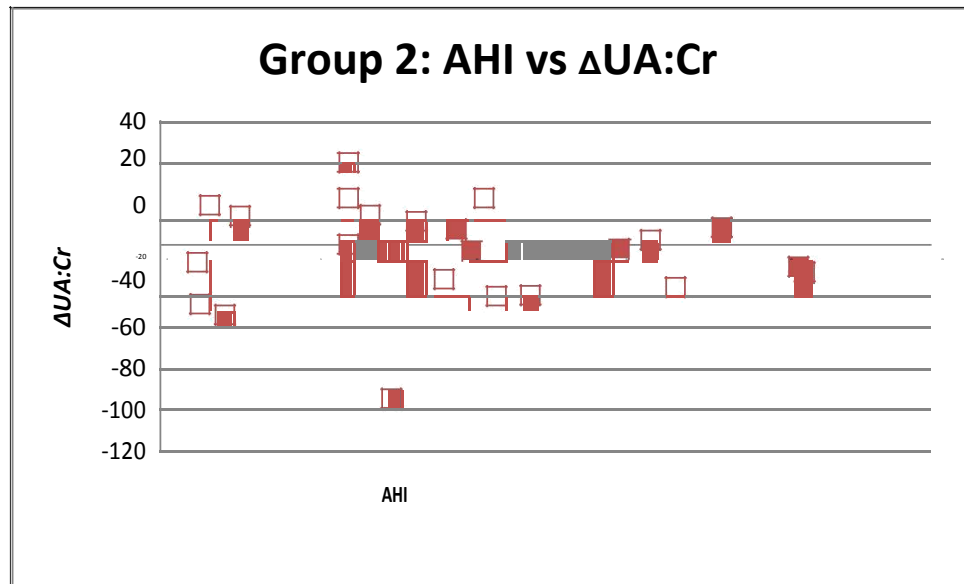


Figure 13: AHI/h vs Δ UA:Cr for Group 2

Data collected from Group 3 reflected the same pattern as seen in the previous two Groups, as a largely negative ratio for Δ UA:Cr: -16.4 ± 3.64 (range: minimum - 56.4; maximum, 27.5). Ten subjects of this group showed a positive Δ UA:Cr, as per expectations. However, due to a high number of false negative [‡] (see page 62) results, the data obtained for this group showed a wide scatter. A linear regression analysis showed no correlation between Δ UA: Cr and AHI/h (Figure 15) for subjects in Group 3 ($r^2=0.015$, $p=0.29$), before CPAP.

There was no significant difference in the Δ UA:Cr (Table 4) between Groups 1, 2 and 3 ($p>0.05$); a largely negative ratio was obtained.

Intervention with CPAP was required for twenty three subjects in Group 3 due to consistently high AHI/h of $>15/\text{hr}$ and low SaO_2 readings at rapid eye movement (REM) sleep (Table 3), during the duration of the PSG. A comparison of parameters showed a significant drop in the Δ UA: Cr ratio after CPAP where subjects who previously had a positive ratio became negative or showed further negative values (Figure 17).

Four subjects that had a baseline positive Δ UA:Cr ratio (range: minimum 0.61; maximum, 27.5), were reduced to negative values (range: minimum, -20.7;

maximum, -53.9). Nineteen subjects that had a negative baseline Δ UA: Cr ratio (range: minimum -13.8; maximum, -37.5) showed a further negative ratio (range: minimum -24.7; maximum, -61.6) after CPAP application. Despite two subjects showing a complete reversal in the Δ UA:Cr ratio, showing a positive ratio after CPAP; there seemed to be a significant reduction in the overall Δ UA:Cr ratio of subjects in Group 3 after CPAP ($p < 0.001$).

The large variation in the individual Δ UA: Cr ratio from patient to patient rendered the comparison of this value in Groups virtually meaningless. The exception being an intra-batch comparison of Group 3 subjects, before and after CPAP (Figure 17).

6.4.2 False negatives results

An analysis of the above data indicates that while false negative[†] results can occur in Group 2 and 3, it is also possible to obtain false positive[‡] results for Group 1 (control), indicating poor specificity of Δ UA:Cr ratio.

Miyamoto et al. (1992) suggested that although elevated concentrations of plasma uric acid represents tissue hypoxia in some organs or tissues, it does not provide any information on potential variations of tissue hypoxia in vital organs such as the brain or heart. It is therefore possible that a number of compensatory and protective mechanisms work in response to arterial hypoxemia before the actual breakdown of ATP occurs. Hence subjects having significant tissue hypoxia, with AHI/H>20 (Group 3), may not necessarily have a positive Δ UA: Cr ratio.

[‡] *Note: False positive means that the response was opposite to that expected, in that elevated Δ UA:Cr due to nocturnal hypoxia was not expected in the control group.*

In a study conducted at the Division of Pulmonary Disease in Novara, Italy, Braghiroli et al. (1993), obtained a very high percentage of false negative results, even in the group of subjects that had the highest concentrations of positive $\Delta\text{UA}:\text{Cr}$ that correlated with a high AHI/H. They reported 40% false negative results in patients with an AHI/h < 30 per hour and a reduced number (20%) of false negative results for patients with an AHI/h threshold = 60. They attributed these findings to inter-individual variability that depends on an individual's cardiac function and circulatory response to hypoxia. This was particularly so in patients with chronic obstructive pulmonary disease (COPD), who develop an adaptation to chronic hypoxia due to slow disease progression.

This is in agreement with earlier theories put forward by McCord (1985) that explained a similar mechanism in an ischemic model. They found patients who had developed tissue hypoxia caused by an alternating pattern of cyclic hypoxemia and reperfusion also had increased production of uric acid and oxygen-derived free radicals induced by an enzymatic conversion requiring only a few seconds in myocardial tissue but up-to 30 min in the liver, spleen, lung and kidney. This delayed response in the enzymatic conversion of uric acid in the kidney could be one of several explanations for the false negative results obtained in this study.

In a 1990 study conducted by McKeon et.al, similar findings as this study were reported. They found high variability of results amongst OSA patients reflected in a substantial number of false negative findings of overnight change in uric acid: creatinine ratio and an absence of any significant correlation between $\Delta\text{UA}:\text{Cr}$ and various parameters of de-saturation. It was implied that the presence of diurnal de-saturation may impair the sensitivity of this test.

Studies conducted by Hasday and Grum as well as Braghiroli et al. had small sample size and did not examine the association of OSA with a positive $\Delta\text{UA}:\text{Cr}$ ratio, specific to gender and ethnicity, as did this study. Although all efforts were

made to include national representation of the general population of NZ adults with OSA, there seemed to be too many variables like age, gender, smoking status, medication, physical activity etc. that were confounding factors that contributed to the variability in the results.

6.5. Δ UA:Cr Before and after CPAP

During the course of their sleep study, twenty three participants from Group 3 had concentrations of SaO_2 that were consistently lower than 88%. CPAP therapy was applied to eliminate or reduce apnoea-hypopnoea and maintain arterial oxyhaemoglobin saturation above 90%.

PSG parameters like the AHI/h and SaO_2 concentrations indicated these subjects to have significant hypoxia, though not all the results for Pre-CPAP Δ UA:Cr were positive; and, as seen in Group 2, there were false negative Δ UA:Cr results in Group 3 before and after CPAP as well. However, despite having considerable over-lap in uric acid: creatinine results like in Group 1 and Group 2, there seemed to be a significant drop in the values, post CPAP.

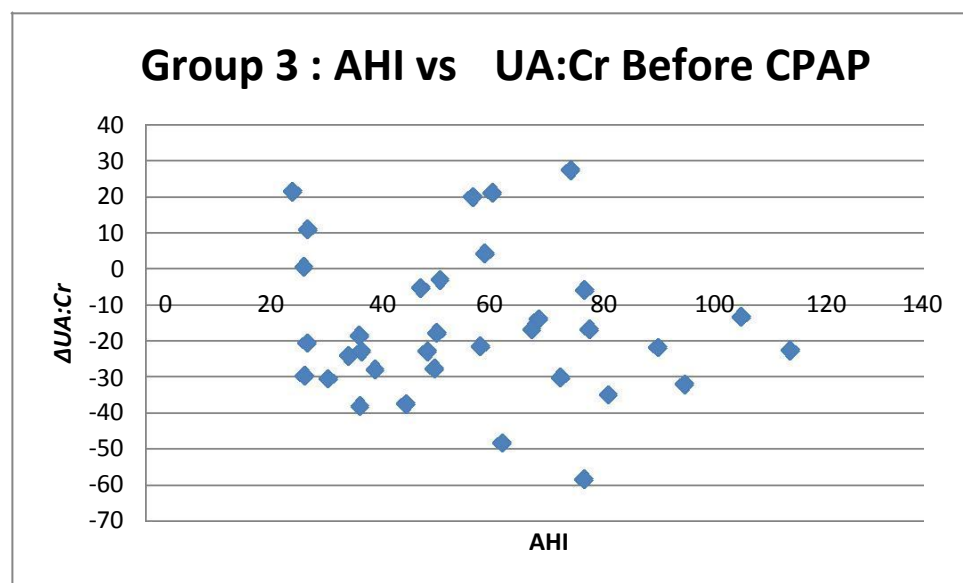


Figure 14: AHI/h vs Δ UA:Cr for Group 3 before CPAP

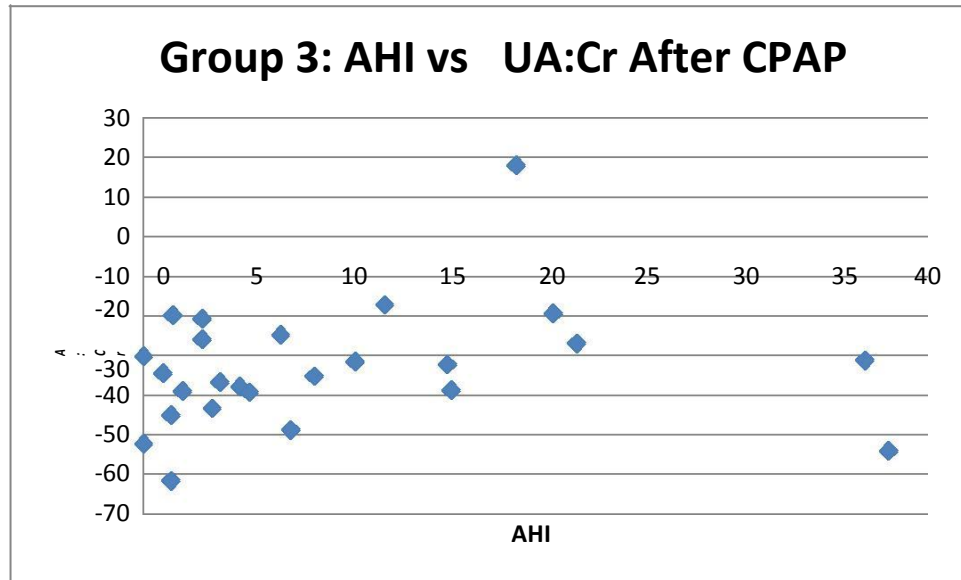


Figure 15: AHI/h vs Δ UA:Cr for Group 3 after CPAP

In twenty one of twenty three subjects (91.3%) of Group 3, that had CPAP⁺⁺ application, Δ UA:Cr was higher before application of CPAP than it was after CPAP. False negative[‡] (see page 62) results were found in only two of the twenty three (8.7%) subjects in Group 3 that had CPAP application, compared to a previous report (McKeon, 1990) that reported 30% false negative results.

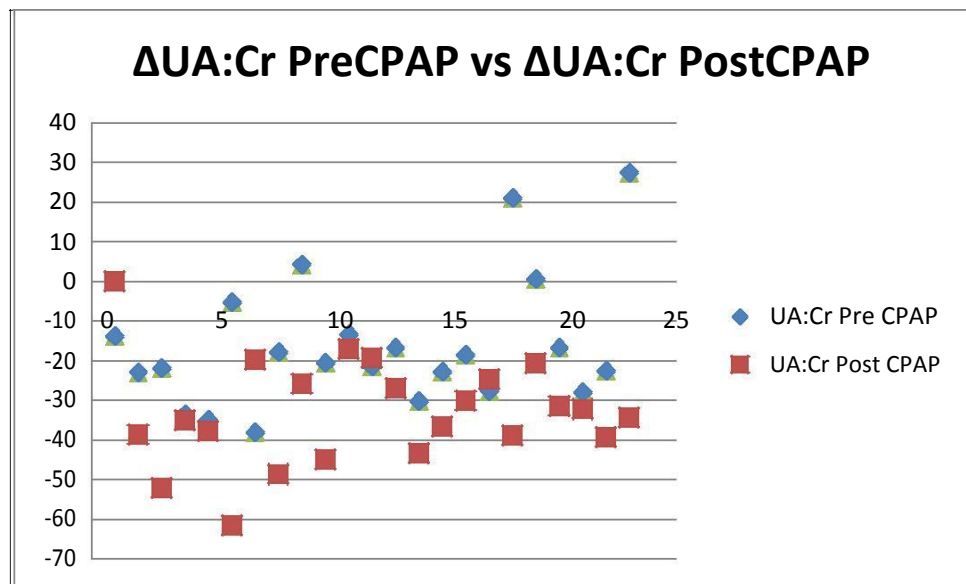


Figure 16: Δ UA:Cr for Group3 before and after CPAP

The mean value of $\Delta\text{UA}:\text{Cr}$ for Group 3 (-16.4 ± 3.64) before CPAP was almost the same as the control group (-17.9 ± 3.48) but showed a significant decrease ($p < 0.001$) having a mean value of -33.13 ± 3.25 , after CPAP^{††}. Four subjects that started with a positive baseline reduced to negative values and nineteen subjects that had a negative baseline showed a further reduction, post CPAP.

These findings may support the suggestion that uric acid excretion has a relationship with nocturnal hypoxemia. However, $\Delta\text{UA}:\text{Cr}$ showed poor correlation with SaO_2 ($r^2 = 0.03$, $p = 0.42$) and with AHI/h ($r^2 = 0.02$, $p = 0.43$), with CPAP application. This is a little puzzling as it seems reasonable to expect to see a strict relationship between this ratio and the indices of nocturnal hypoxemia.

^{††}*Note: No CPAP was applied to participants of Group 1 and Group 2 as they did not satisfy the criterion for CPAP application (see 5.7.2). Therefore, there is no data available to comment on the effects of CPAP application on $\Delta\text{UA}:\text{Cr}$ levels for the control group and participants with mild OSA.*

6.6 Discussion

There may be several reasons to explain these conflicting results, one of which may be related to an individual's adaptation to chronic hypoxia, as seen in participants of Group 3. It is possible that these participants had suffered OSA induced hypoxia for several years before being diagnosed. Braghiroli *et al.* (1993) reported 30% of their study subjects, that had severe de-saturation did not have a positive $\Delta\text{UA}:\text{Cr}$ ratio, demonstrating a poor correlation between partial arterial oxygen (PaO_2) and tissue oxygenation. This may be attributed to the fact that perhaps a spot urine sample being a poor representation of the effective uric acid production. Similarly, Hasday and Grum and McKeon reported 17% of de-saturating subjects having severe OSA, also not showing a positive $\Delta\text{UA}:\text{Cr}$ ratio.

Garcia *et al.* (2006) carried out a cross-sectional retrospective study in a population of 1135 subjects referred to the sleep disordered breathing unit of the

hospital Virgen del Rocío, Spain. They found blood uric acid concentrations positively correlated with the number of obstructive respiratory episodes and oxygen de-saturations during sleep but not independently of various confounding factors such as obesity. They reported that patients having an AHI/h of over 30 had higher serum uric acid concentrations than those with mild or no OSA. However, this difference was not apparent in the analysis of variance in which BMI, cholesterol and triglyceride levels were considered confounding factors. It is therefore highly likely that the same confounding factors that affect the analysis of serum uric acid may well have a role to play in excretion of urinary uric acid. Given the fact that participants of Group 3 were the most obese of all the participants of this study (Table 1) and had the highest incidence of hypercholesterolemia at 67.7% (Table 2), it is possible that $\Delta\text{UA}:\text{Cr}$ is not independent of these factors.

Similar findings were reported by Nagaya *et al.* (1999). No significant correlation was reported between serum uric acid concentrations and arterial oxygen saturation. Controversially, serum uric acid showed a significant negative correlation with the degree of systemic oxygen delivery. They theorized that arterial oxygen decreased sufficiently to enhance tissue hypoxia resulting from reduced cardiac output but insufficiently to have an independent association with serum uric acid concentrations.

The above studies by McCord, Hasday and Grum not only showed that tissue ischemia depleted ATP and activated the purine nucleotide degradation resulting in uric acid over production; it also indicated impairment of uric acid excretion. The degree to which each participant responded to hypoxia would be very individualistic.

These findings suggest that it is possible for diseases associated with metabolic syndrome such as obesity, diabetes, hypertension and hypercholesterolemia to greatly influence the production of uric acid in blood and urine. This complex relationship is acknowledged but not yet fully understood. Therefore, an attempt

to quantify the degree of oxidative metabolic impairment in Group 2 and 3 would probably be just speculation.

It would appear that using $\Delta\text{UA}:\text{Cr}$ as an indicator of estimating overnight tissue hypoxia in OSA is of limited value. However, this test is inexpensive and may be useful in periodic assessment of patients especially in those that have a positive ratio at baseline. $\Delta\text{UA}:\text{Cr}$ could also be used for long term follow up of OSA patients in terms of efficiency of treatment or poor compliance with the CPAP application.

Chapter 7

Urine metanephrine as a marker for sympathetic activity in OSA

Episodes of sleep apnoea are often accompanied by hypoxia, hypercapnia and arousals from sleep which has been known to cause an increase in sympathetic nervous system (SNS) activity. This increase in SNS is often associated with cardiovascular complications, although the mechanism is not yet well understood. It is possible that the SNS in individuals with OSA lack homeostatic mechanism to control SNS activity after episodes of apnoea.

7.1. SNS Activity

Elmasry *et al.* (2002) demonstrated increased SNS activity in patients with OSA and considered it to be the main link between OSA and cardiovascular diseases. They suggested an increased activity of the sympathetic neurons as well as an increased activity of the adrenal gland experienced by patients with OSA was caused by the hypoxia. Normetanephrine is the O-methylated product of norepinephrine resulting from catechol-O-methyltransferase (COMT) activity, primarily in the synaptic cleft of the sympathetic nerve terminals (Forster, 1998). An increased concentration indicates an increase of norepinephrine release and metabolism at nerve terminals. Epinephrine is exclusively formed by the adrenals and almost 97% of metanephrine is derived from that epinephrine. Metanephrine is a metabolite of epinephrine created by action of catechol-O-methyl transferase on epinephrine.

Consequently, measuring metanephrine in urine would be a good reflection of adrenal activity.

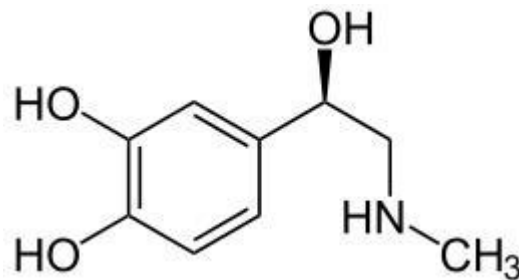


Figure 17 : Epinephrine

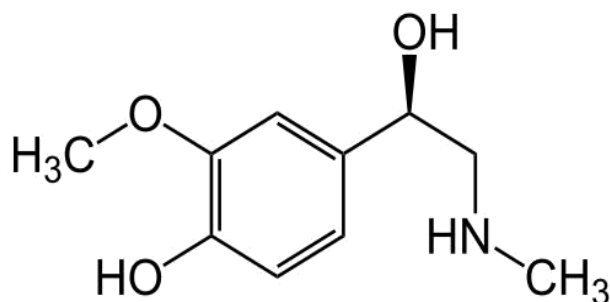


Figure 18: Metanephrine

The aim of this study was to investigate the possible relationship between OSA and sympathetic activity as assessed by measuring urinary metanephrine for the participants of this study. Sixty six out of a total ninety participants were recruited at Auckland City Hospital's "Sleep Laboratory" and underwent an overnight polysomnography.

7.2. Method of analysing urinary metanephrine

The morning sub-sample containing acid were analysed for urine metanephrine metabolites, measured by high performance liquid chromatography (HPLC) method using Recipe® ClinRep® kit sets sponsored by PM Separation.

Metanephrines are three separate analytes: nor-metanephrine, metanephrine and 3-methoxytyramine. They are inactive metabolites of nor-adrenaline, adrenaline and dopamine respectively produced by the action of COMT.

To analyse metanephrine concentrations for this study, urine was first hydrolysed to de-conjugate the metanephrines. Hence this method measures total metanephrines rather than free. Before hydrolysis, urine is first treated with a related internal standard, methoxyhydroxybenzylamine (MHBA) and concentrated acid and then heated close to 100°C for 30 minutes. Subsequently, the hydrolysate is adjusted to pH 6 using an indicator dye and passed through an ion exchange column and washed several times. The metanephrines are eluted into a stabilising solution and analysed by HPLC with electrochemical detection.

The metanephrines are directly oxidised by coulometry and the oxidative current is measured. Urinary Metanephrines for this study were run on HPLC using an Electrochemical Detector, ESA Coulochem III coulometric detector with 5021A conditioning cell and 5011A analytical cell, in a reductive mode since the oxidative mode is more prone to possible interferences. In the Reductive mode, voltage for The Guard was maintained at +460mV, E1cell at +100mV, E2 cell at -450mV, E1 and E2 Range at 200nA.

ClinRep® Analytical Column, 150mm long with an internal diameter of 4.6mm and Phenomenex Security Guard, C18, 4 mm long with an internal diameter of 3.0mm was used for this study. The column temperature was set at 30°C.

The Recipe® ClinRep® complete kit set sponsored by PM Separation containing Internal Standard, urine calibrator, mobile phase, elution reagent, diluting, washing and stabilising solution was used for analysis. The flow rate of the mobile phase was maintained at 1.00mL/minute. The run time was 30 minutes and injection volume was set at 20µL, with a 0 minute delay in injection time.

Commercial controls, Bio-Rad Lyphocheck Quantitative Urine Control Normal and Bio-Rad Lyphocheck Quantitative Urine Control Abnormal were used. Approximate retention time for Normetanephrine was 7.4 ± 2 minutes and 9.7 ± 2 for Metanephrine. (ClinRep® Instruction Manual for the Determination of Metanephrines in Urine by HPLC. Version 6.0 Oct2009 Recipe Chemicals & Instruments GmbH).

A single point calibration and un-weighted linear regression, forced through zero was used. Data was collected and processed on Chromeleon, version 6.80 SP2, Chromatography Management System.

7.2.1. Limitations of this method

The analysis has been tested to be linear up-to 75000 nmol/L at LabPLUS. Acidified specimens for urine metanephrines were collected as per LabPLUS procedure to maintain stability of the analyte over a long storage period.

Interferences that give rise to incorrect metanephrine results may arise due to several reasons, giving an exceptionally tall peak height or a slightly misshapen or widened peak or peaks with a slightly incorrect retention time.

Any drug that effects (nor)adrenergic transmission can cause changes in metanephrine concentrations. Many drugs are designed to expressly do this. The major classes of drugs are listed below. Importantly beta-blockers are widely used and cause increased nor-metanephrine production whereas the other anti-hypertensive drugs (calcium channel blockers, ACE inhibitors, angiotensin receptor blockers, alpha blockers and diuretics) have no effect (Deutschbeiten et al., 2010).

Drug Class	<i>METANEPHRINE</i>	<i>NORMETANEPHRINE</i>
Tricyclic antidepressants (not SSRIs)	-	+++
Phenoxybenzamine	-	+++
Beta-blockers	+	+
MAOIs (monoamine oxidase inhibitors)	+++	+++
Amphetamines & sympathomimetics (e.g pseudoephedrine)	++	++
Alpha-blockers (prazosin, doxazosin), calcium channel blockers (felodipine, verapamil, diltiazem etc), ACEIs (lisinopril etc) ARBs (losartin etc), diuretics (thiazides, frusemide)	-	-

Table 5: Drug interferences with metanephrine and Normetanephrine metabolites

7.3. Urine Metanephrine excretion

Metanephrine and normetanephrine excretion rate was calculated against creatinine. There did not seem to be any significant difference in the metanephrine and normetanephrine excretion between normal, mild and severe OSA subjects (Table 6). There was hardly any inter group variation in this parameter. Normal subjects of Group 1 had a metanephrine concentration of 35.9 ± 3.17 nmol/mmol creatinine (range: minimum 15.8; maximum, 79.3) and a

normetanephrine concentration of 109.5 ± 3.92 nmol/mmol creatinine (range: minimum 36.2; maximum, 332.5). Subjects of Group 2 had metanephrine concentrations of 41.1 ± 3.22 (range: minimum 15.3; maximum, 118.7) and normetanephrine concentration of 118.8 ± 4.52 (range: minimum 45.0; maximum, 190.8). Group 3 had metanephrine concentrations of 41.8 ± 2.74 (range: minimum 14.9; maximum, 63.8) and normetanephrine concentration of 120.3 ± 3.76 (range: minimum 33.9; maximum, 206.8).

Urine metanephrine

Result	<u>Group 1</u> Control (n=24)	<u>Group 2</u> Mild OSA (n=33)	<u>Group 3</u> Severe OSA (n=33)
Metanephrine (nmol/mmol creatinine)	35.9 ± 3.17	41.1 ± 3.22	41.8 ± 2.74
Normetanephrine (nmol/mmol creatinine)	109.5 ± 3.92	118.8 ± 4.52	120.3 ± 3.76

Table 6: Data presented as mean $\pm$ SEM.

A linear regression analysis showed no correlation between urine metanephrine excretion and AI ($r^2=0.031$, $p=0.33$) and normetanephrine excretion and AI ($r^2=0.022$, $p=0.80$) for subjects of Group 2 (Figure 19).

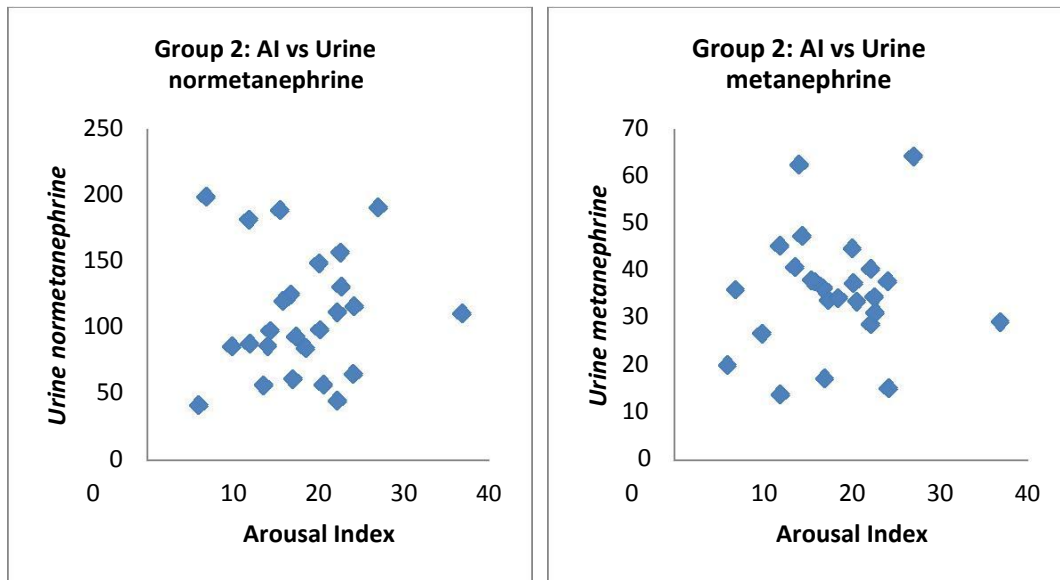


Figure 19: AI vs urine metanephrine/normetanephrine for Group 2

Similar findings were reflected in results for Group 3; no correlation was observed between urine metanephrine excretion and AI ($r^2=0.007$, $p=0.63$) and normetanephrine excretion and AI for Group 3 ($r^2=0.030$, $p=0.88$) as seen in Figure 20.

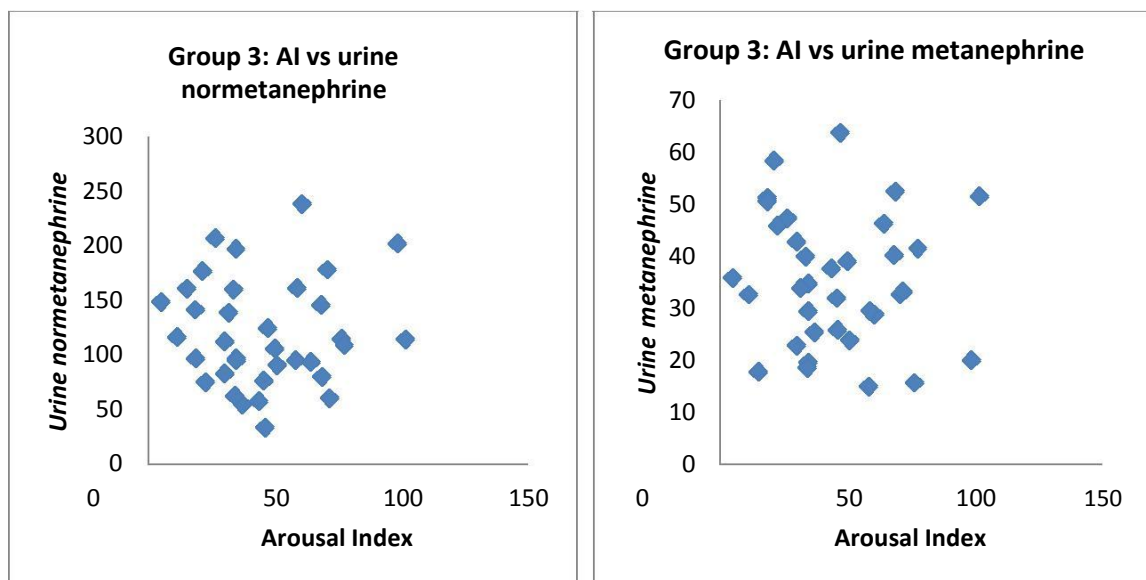


Figure 20: AI vs urine metanephrine/normetanephrine for Group 3

An individual variation of metanephrine and normetanephrine excretion within both Groups prevented any significant association of these metabolites with any other PSG parameters. There were no significant correlations between metanephrine excretion concentrations and SaO₂ concentrations for both Groups (Figure 21) with p=0.55 for Group 2 and p=0.11 for Group 3. As seen in Figure 21, no correlation was observed between normetanephrine excretion concentrations and SaO₂ concentrations for Group 2 (p=0.25) and Group 3 (p=0.73).

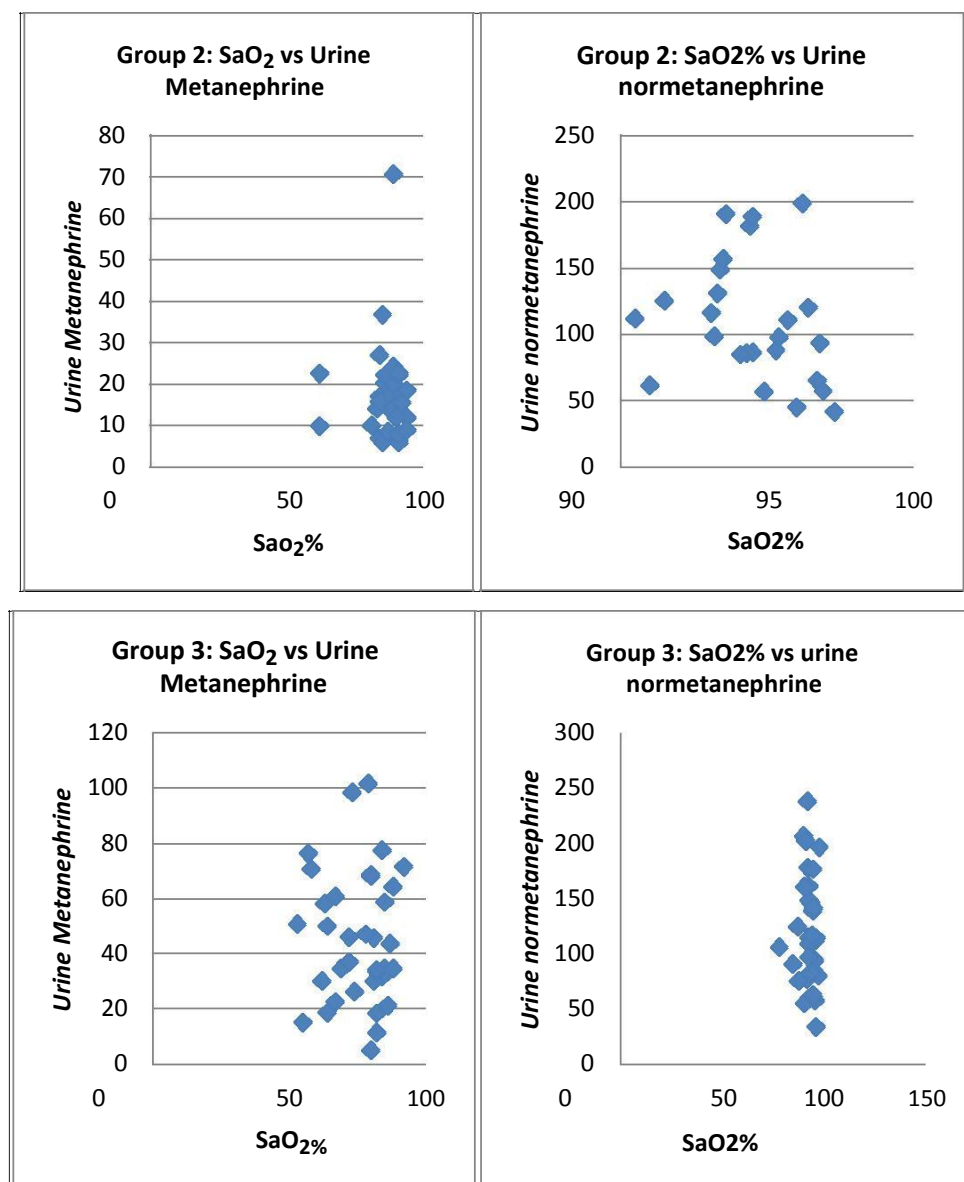


Figure 21: SaO₂ vs urine metanephrine/normetanephrine for Group 2 and 3

7.4. Discussion

The aim of this study was to investigate the possible relationship between OSA and sympathetic activity, as assessed by measuring urinary metaephrine concentrations. However, like in previous studies, small sample size and inadequate correction for age, weight and other factors were found to have confounded SNS activity.

OSA is characterised by the recurrent cessation of respiratory flow during sleep causing sudden arousals that leads to a transient surge in sympathetic activity. Episodes of ensuing hypoxia are followed by repeated arousals in an attempt to restore breathing. A couple of studies (Somers, 1995; Zvillich, 1998) have demonstrated increased SNS activity during episodes of acute apnoea and associated arousal from sleep. They found that patients with severe OSA had resting SNS that was almost twice that of sex- matched controls. Although the above authors had theories that would suggest the resetting of body baro-receptors and chemo-receptors to contribute towards increased nerve activity in patients with OSA, the relationship is not clearly understood and is widely debated.

In an interesting study, Hoy *et al.* (2004) described a series of patients that presented with elevated urinary catecholamines attributed to prolonged sympathetic over-activity due to clinical and biochemical features diagnostic of pheochromocytoma, who were all subsequently diagnosed as OSA. However, on further analysis, it was found that all these patients had elevated noradrenaline that usually reflected neuronal rather than adrenal involvement, which would represent sympathetic nerve activity and synaptic overflow.

Elmasry's (2002) population based study reported increased concentrations of urine normetanephrine in patients with OSA using analysis that controlled for age, weight, blood pressure and drug therapy. Subjects with high normetanephrine concentration had significantly higher prevalence of OSA when compared to those with a low concentration. Amongst all the catecholamines, only urinary

normetanephrine was significantly associated with AHI/h and SaO₂, while no significant elevations were reported in the others. These findings were used as the cornerstone that formulated the outline of this study in an attempt to find the relationship between OSA in an NZ population and increased SNS activity, measured by urinary metanephrine.

Therefore, it was not unreasonable to expect some correlation between AI and normetanephrine excretion in participants of Group 2 and 3, it was however disappointing to find that there was no apparent association for both AI and SaO₂% for participants of both Groups (Figure 21).

In light of the fact that participants of Group 3 had a very high average arousal index (AI) of 45.7 compared to participants of Group 2 that had an average AI of 16.2 (Table 3), it would be expected that Group 3 participants to experience many more episodes of a sudden transient surge in sympathetic activity that would result in higher urinary metanephrine excretion concentrations. However, on analysis, very little difference in the results of the two Groups was found. An analysis of comparison between the degrees of hypoxia (SaO₂) experienced by participants of both Groups to urine metanephrine concentrations also showed no association. A linear regression analysis showed no correlation between urine metanephrine and PSG parameters of AI and SaO₂ for both Groups. There seemed to be little difference in the mean metanephrine excretion between the normal, mild and severe OSA Groups. This finding was probably to be expected as the study was not controlled for medication known to confound the metabolism of metanephrine.

While several reasons could be attributed to these results, it is unclear whether this phenomenon is due to obesity and other co-morbid conditions (Fletcher et al., 1987) as this study was not controlled for age, weight and drug medication; factors that are known to confound metanephrine metabolism.

Another factor for the discrepant results could be the result of the manner in which urine specimen for metanephrine were collected. A limitation of this study

may be that perhaps a spot urine collection is not a representation of true metanephrine excretion that is better seen in a 24 hour collect, normally recommended for this test. However, since this study recruited participants who were on site at the “Sleep Laboratory” only for the duration of the PSG, it was not possible to organise a 24 hour collect.

Despite the association between some catecholamine concentrations (normetanephrine, noradrenaline) and hypoxia, it is not clear whether it was hypoxia alone that accounted for the SNS activity in sleep apnoea. As such, increased catecholamines are also associated with a decrease in sleep efficiency and sleep length (Nishihara et al., 1985). Thus the below average sleep quality often seen in OSA patients may play a role in SNS activity.

It could be debated that the absence of spikes in the metanephrine and normetanephrine concentrations for Group 3 participants may be attributed to the diminished nocturnal SNS activity due to the natural circadian fluctuation in epinephrine, as demonstrated by Cameron et al. (1987) who found epinephrine excretion varied from one individual to another.

Samples for metanephrine were all collected in the morning after participants had undergone nocturnal PSG. Twenty three participants were given CPAP application and although no correlation was seen between $\Delta\text{UA:Cr}$ and AHI/h for both Groups, there was a significant reduction in $\Delta\text{UA:Cr}$ concentrations after CPAP. Similarly, it may be likely that these participants had low metanephrine/normetanephrine concentrations due to CPAP application as reported by Coy *et al.* (1996). However as samples collected before CPAP were not analysed for metanephrine concentrations, it is difficult to ascertain whether the low concentrations of metanephrine are a direct effect of CPAP application itself. Literature on this topic is scanty and no clear relationship between CPAP application duration and catecholamine concentrations is evident (Coy *et al.*).

Chapter 8

Conclusion and

Recommendations

Epidemiological evidence points to the fact that sleep disorders in New Zealand are on the rise. However, OSA is grossly undiagnosed or even misdiagnosed with general practitioners relatively under informed about its clinical features and medical ramifications.

Untreated OSA imposes a significant economic and social cost that includes the cost of treating conditions that are exacerbated by OSA. A conservative estimate of the total annual societal costs of OSA was \$40 million and the incremental net cost of treating OSA was estimated at \$389 per case treated in New Zealand (Paine *et al.*, 2011).

Therefore, along with identifying and addressing issues to reduce OSA risk factors like obesity and smoking, it is essential to develop tools that help in the early diagnosis to mitigate the impact of OSA on the New Zealand population. This study is an attempt to provide a simple and inexpensive biochemical marker for OSA that could be used as a screening tool by primary healthcare providers.

Some very interesting observations came to light during the course of the study. Subject demographics collated for the study predictably demonstrated the under representation of people of Maori ethnicity that perhaps could be a reflection of the lack of awareness or accessibility of the people to primary healthcare. There also seemed to be an under representation of the female gender that may be indicative of the general belief amongst most people to perceive OSA as a syndrome that predominantly effects only males. This fallacy could prove to be detrimental to the health of the female population who may not receive timely treatment compared to their male counterparts.

It was also observed that although obesity has long been associated with OSA, it may not be as reliable a predictor of the disease as neck circumference which seemed to have a significant correlation in all the participants that underwent the PSG.

The predisposition of OSA patients to develop Syndrome Z is indeed alarming. A high percentage of subjects that participated in the study had two or more characteristics that indicated the likelihood of their developing cardiovascular disease.

While collating and analysing the PSG data from the “Sleep Laboratory”, the researcher noticed the dichotomy between the ESS scores of the participants and the actual severity of their disease. The ESS score is often used as a screening mechanism for OSA in the primary health care sector for ADHB referrals to the sleep laboratory. The score is a self-assessment by the patient themselves and it is entirely possible for them to manipulate their responses. Although the ESS score is used only as a guideline, it may not always be a satisfactory screening tool as it lacks both specificity and sensitivity.

It therefore re-enforces the requirement of having an inexpensive and easily accessible tool for OSA screening in the community.

The study indicates that perhaps $\Delta\text{UA:Cr}$ is not an adequate indicator of overnight tissue hypoxia in OSA and that several factors may have contributed to the conflicting results that were obtained. The most significant contributor towards this may be the fact that the study was not controlled for major confounding factors such as obesity and medication that significantly alter the rate of uric acid metabolism. Even so, results obtained in previously controlled studies for the above factors have yet to prove the correlation. The mechanism involved in the excretion of uric acid may follow a complicated pathway that would require a more rigorous research design that could account for all such confounding factors.

Urine metanephrine estimation for this study may not have been very reliable indicator of the true SNS activity of the participant as only spot urine collections were made in the morning after participants had undergone PSG, while a 24 hour collection is the recommended norm. Another factor that may have influenced

catecholamine metabolism was the timing at which samples for metanephrine were collected; one can only speculate what the outcome would have been had the samples for metanephrine were collected before participants underwent PSG. It would have been ideal if samples for metanephrine were analysed before and after CPAP as this would have provided us with a better understanding of whether CPAP application had an effect on SNS activity.

8.1. Advantages of this study

The appropriateness of this model as a screening tool for OSA is debatable with the need for more evaluation. However, it illustrates an approach that could potentially improve referral and promote more effective use of publically funded specialist services.

The usefulness of measuring uric acid excretion in OSA patients before and after CPAP application for subjects of this study did provide some useful information in that that $\Delta\text{UA:Cr}$ could be utilized as a simple and effective biochemical marker to monitor the degree of compliance of a patient's use of the prescribed CPAP apparatus. Hence, if a baseline for a patient's urinary UA: Cr concentration was established before they commenced on CPAP, then the subsequent monitoring of their urinary UA: Cr concentrations after commencement of CPAP therapy would be a good indicator of a patient's improvement in AHI/h even without PSG.

8. 2. Limitations of this study

There are several potential limitations to this study. All the participants of Group 2 and 3 were recruited from the patients referred to the Sleep Laboratory with suspected OSA symptoms. Thus, the study population was biased as participants may have had existing medical conditions and risk factors that may have increased their disposition for hypoxia and increased sympathetic activity in comparison to asymptomatic subjects of Group 1. It is certainly evident from Chapter 4 that participants of Group 3 were more obese and almost all participants of this group had characteristics of developing 'syndrome Z'

PSG was performed for participants of Group 2 and 3. Therefore, comparisons of PSG parameters could only be made between mild and severe OSA Groups. Although this study did include a cohort of volunteers (Group 1) that were asymptomatic for OSA and cardiovascular disease, they did not undergo a PSG due to constraints of budget and hospital resources. It was assumed that these participants would have an AHI/h of < 5 . It is however possible that even if all subjects from Group 1, although asymptomatic, some may have experienced mild apnoea or de-saturations that have gone unreported and may have influenced uric acid and metanephrine concentrations.

The clinical relationship between AHI/h and $\Delta\text{UA}:\text{Cr}$ was difficult to interpret in this study, due to several reasons. The inclusion criteria of patients on allupurinol and medication with uricosuric properties may have caused false negative results. This is especially relevant to participants of Group 3 that had approximately 30% of participants on medication for gout, 67% on medication to reduce hypercholesterolemia and 50% on medication to control hypertension that may have altered the metabolism of uric acid (Wyngaarden & Kelley, 1976). False negative results were obtained for $\Delta\text{UA}:\text{Cr}$ in Group 2 as well, probably for the same reasons.

8. 3. Recommendations

A more stringent criterion for selection of participants for this study would require a larger budget and resources for screening of subjects. As discussed in earlier chapters, patients with central apnoea that were diagnosed post PSG and patients on uricosurics were included in this study. Perhaps the outcome would have been different if that were not the case.

The most common treatment for gout is allopurinol, a pro-drug metabolized to oxypurinol that inhibits xanthine oxidase and forces the excretion of a large proportion of purine catabolite as hypoxanthine rather than uric acid.

Therefore, the urine samples of subjects from Group 3 that were on allopurinol should have had their samples tested for uric acid excretion after oxidation of hypoxanthine.

Chapter 9

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