TITLE:

The interaction between obstructive sleep apnoea and cardiovascular risk factors on cardiovascular disease:

SUMMARY:

Cardiovascular disease (CVD) and obstructive sleep apnoea (OSA) are common disorders that are associated with substantial morbidity and mortality. Large observational studies have consistently found that OSA is an independent risk factor for several CVDs including coronary artery disease, atrial fibrillation, heart failure and stroke, and this has been attributed to physiological derangements during sleep in OSA such as hypoxemia-associated oxidative stress and sympathetic activation. However, the recent Sleep Apnoea cardioVascular Endpoints (SAVE) trial found that continuous positive airways pressure (CPAP) therapy in OSA did not prevent adverse CVD outcomes in those with established CVD. While the SAVE trial has several important limitations, its findings have raised important questions about the nature of OSA-associated CVD risk. These include: (a) whether the usual metric of OSA severity – apnoea hypopnoea index (AHI) – is the most appropriate basis for examining this; (b) whether ***characterisation of OSA according to the magnitude of physiological derangement*** – hypoxaemia, sympathetic activation, proinflammatory status, metabolic imbalance – could ***better identify those at greatest risk of CVD***; and (c) given that known risk factors for CVD (such as obesity, physical inactivity, hypertension, diabetes mellitus and hyperlipidaemia) are highly prevalent in patients with OSA, how might they ***interact with OSA-related physiological derangements*** in determining the ***pathogenesis of CVD***. Better identifying those at greatest risk through examination of these inadequately explored relationships could lead to ***more appropriately targeted interventions for OSA***.

The West Australian Sleep Health Study (WASHS) Prospective Sleep Clinic cohort is a unique resource that provides the opportunity to explore the interaction between OSA and other CVD risk factors in the pathogenesis of CVD. This exceptional cohort of 4,100 consecutive patients who attended the WA Sleep Disorders Research Institute between 2005 and 2010 have been thoroughly phenotyped using questionnaire, anthropometric measurements, in-laboratory polysomnography (PSG) and blood tests. In each patient, detailed information has been obtained on CVD risk factors, CVD and OSA-related physiological derangements during sleep.

We will examine the interaction between OSA-related physiological derangements during sleep and other CVD risk factors on the development of CVD in the WASHS cohort after a ***mean follow- up of 10 years***. The development of CVD will be determined by (a) linking the cohort to statutory WA morbidity and mortality datasets via the WA Health Data Linkage Service (WADLS), (b) a follow-up review with comprehensive health assessments, and (c) detection of sub-clinical CVD in a subset of patients who have no overt CVD. This will provide a clearer understanding of the interaction between OSA and other CVD risk factors on the development of CVD, and help identify which patients with OSA are at greatest risk of developing CVD, enabling risk assessment and intervention to be individualised, consistent with the concept of personalised medicine. Based on our findings, we aim to develop and validate a risk assessment score for CVD that includes OSA. In addition, we will assess if adequate CPAP therapy attenuates the risk of developing CVD in OSA.

AIMS:

To determine

1 Whether the type and severity of OSA-related physiologic derangements during sleep influences the risk of CVD events and subclinical vascular disease by interacting with co-morbid CVD risk factors.
2 Whether the risk of developing CVD events and subclinical vascular disease in OSA can be predicted from a combination of (a) the severity of OSA-related physiologic derangements during sleep, (b) co-existing CVD risk factors and (c) biomarkers (C-reactive protein, fibrinogen, high sensitivity troponin I, B-type naturetic peptide).
3 Whether risk is reduced by treatment with CPAP therapy and, if so, the degree to which this risk reduction relates to how effectively OSA-related physiologic derangements are controlled.

HYPOTHESES:

1. The likelihood of developing CVD and subclinical vascular disease in OSA depends on the interaction of physiologic derangements during sleep with conventional CVD risk factors, and how well both are controlled.
2. The risk of developing CVD and subclinical vascular disease in OSA can be predicted from a combination of (a) the severity of OSA-related physiologic derangements during sleep, (b) co-existing CVD risk factors and (c) risk biomarkers.
3. The effectiveness of CPAP therapy in ameliorating CVD and subclinical vascular disease in patients with OSA depends on how completely the OSA-related physiological derangements during sleep are corrected.

BACKGROUND:

OSA is common chronic disorder characterised by recurrent upper airway obstruction during sleep that may be complete (apnoea) or partial (hypopnoea). The prevalence of OSA of at least moderate severity is estimated at 17% and 9% of middle-aged men and women respectively[1]. OSA events are associated with sleep disruption, hypoxemia and activation of the sympathetic nervous system[2]. OSA is associated with a wide range of adverse consequences including CVD, cognitive impairment, daytime sleepiness, increased risks of motor vehicle and occupational accidents, reduced quality of life and depression. The annual direct and indirect costs of OSA to the Australian economy have been estimated at $21 billion[3].

OSA-related physiologic derangements during sleep. The severity of OSA is usually quantified as the frequency of apnoea's and hypopneas per hour of sleep (AHI) but this may be a sub-optimal metric of CVD risk. Abnormal breathing and arousal in OSA are associated with a range of hemodynamic, autonomic, inflammatory and metabolic effects that are implicated in the development of CVD. The AHI during rapid eye movement (REM) sleep may be more predictive of adverse CVD outcomes than the AHI over the entire night[4]. A consequence of upper airway obstruction during sleep is a reduction in blood oxygen content causing a hypoxemic burden and oxidative stress. *The severity of this oxidative stress may not be reflected in the AHI* because the former is dependent on the duration rather than frequency of upper airway obstruction, and lung oxygen stores. The interaction of this oxidative stress with other CVD risk factors could be more important than the AHI in the development of CVD. In support of this, in the Sleep Health Heart Study, CVD was associated with hypopneas only when accompanied by oxygen desaturation of

>4%[5], and an observational study has found a closer association between longitudinal CV events and hypoxemic burden than with AHI[6]. A second consequence of OSA is *sympathetic activation*[2] induced through a variety of mechanisms including chemoreflexes to hypoxemia and hypercapnia, baroreflexes, upper airway mechanoreceptors, pulmonary afferents, impaired venous return to the heart, alterations in cardiac output and the arousal response. The increase in sympathetic activity increases heart rate and blood pressure (BP) and is thought to be an important mechanism for the development of hypertension and other CV consequences of OSA. Sympathetic activation can be estimated from measurements during an overnight sleep study (such as heart rate variability[7] and non-dipping of BP overnight[8]) or urinary catecholamines[9]. These OSA-related physiologic derangements during sleep may interact with other CVD risk factors to promote CVD.

Relationship between OSA and CVD. CVD is a major cause of morbidity and mortality in Australia. It affects one in six Australians, and was responsible for nearly 30% of all deaths in Australia in 2015. We (CIs Hillman & Hung) were the first to identify OSA as a risk factor for myocardial infarction[10]. Since then, observational studies have consistently shown associations between OSA and hypertension[11], coronary artery disease and heart failure[12], stroke[13] and CV death[14,15]. More recently we (CIs Hillman, Hung, McArdle and AI Cadby) identified OSA as an independent risk factor for incident atrial fibrillation[16]. However not all patients with severe OSA develop CVD, and *little is known about the characteristics of patients with OSA who are most likely to develop CVD*.

Common risk factors for CVD include obesity, physical inactivity, hypertension, diabetes mellitus, hyperlipidaemia, and cigarette smoking. Risk factors for CVD are known to be additive, and this has led to the development of several CVD risk prediction scores based on the number of risk factors present and their severity[17,18]. These scores provide an estimate of personal risk of developing CVD, and allow personalised risk factor modification by changes in life-style or pharmacological therapy. However no CVD risk score has considered the impact of OSA. A recent review has recommended the need to explore new CVD risk predictors (such as OSA) to improve prediction models[19].

A high proportion of patients with OSA have co-existing CVD risk factors including obesity, physical inactivity, hypertension, diabetes mellitus and hyperlipidaemia. Studies examining the relationship between OSA and CVD have attempted to control for these confounding risk factors. However the development of CVD in patients with OSA could be due to an interaction between OSA-related physiologic derangements during sleep with conventional CVD risk factors (e.g. the risk of developing CVD from OSA may be attenuated by increasing age). *The potential interaction between OSA and other CVD risk factors in the development of CVD has not been explored*. Our richly phenotyped cohort provides a unique opportunity to determine how OSA interacts with other CVD risks in the development of CVD.

Effect of CPAP therapy on CVD in OSA. CPAP therapy has been shown to reduce daytime sleepiness[20], hypertension[21] and cardiac dysfunction[22]. Observational studies suggest that CPAP reduces CVD risk[14,15,23]. An important prospective sleep clinic cohort study of 1,651 men found that CPAP was associated with a lower rate of fatal and non-fatal CV events over a 10 year follow-up, compared to those with untreated severe OSA[14]. However randomised controlled trial (RCT) data are limited, and the largest RCT with the longest follow-up (SAVE)[24] found that CPAP treatment did not prevent adverse CVD events. *The results of the SAVE trial should be interpreted with caution* for several reasons. First, the SAVE trial was designed to test whether CPAP would reduce CVD events in a population with established CVD (secondary prevention) so the question of whether CPAP attenuates the development of CVD (primary prevention) remains unanswered. Second, as expected in a sample with established CVD, many participants were elderly (mean age 61 years). This may be an important limitation because the association between OSA and CVD appears weaker in the

elderly[25], possibly due to an OSA survivor effect[26]. Third, as a consequence of emphasis on secondary prevention, participants were not typical of patients who present with OSA symptoms. In particular, participants in the SAVE [and a smaller RCT (RICCASDA)[27]] were not usually sleepy, and severe sleepiness was an exclusion criterion in the SAVE study. This may be another important limitation because sleepiness secondary to OSA has been linked to CVD risk in some studies[28]. In addition, people with severe hypoxaemia were also excluded; a potentially important group to target for CVD risk reduction. Fourth, it is likely that OSA was not adequately controlled because adherence to CPAP treatment was modest (mean 3.3 hours/night), as is common in those without OSA symptoms. Finally, the mean follow up duration of 3.7 years may be too short to demonstrate a treatment effect with sub-optimal CPAP usage in an older population with established CVD in whom OSA may play a smaller role among other contributing CVD risk factors. The findings of the SAVE study underline the importance of better understanding the phenotypes of OSA at risk for CVD. Our proposed 10 year follow up of a large group of carefully phenotyped OSA patients with typical OSA features will provide high quality data to determine sub-groups at greatest risk for the development of CVD. Our data will be crucial to help focus future research in this area and design appropriately targeted intervention studies.

W**ASHS Prospective Sleep Clinic Cohort**. The West Australian Sleep Disorders Research Institute (WASDRI), based at the QEII Medical Centre, has conducted diagnostic sleep studies on ~27,000 patients since 1988. The West Australian Sleep Health Study (WASHS) Prospective Sleep Clinic Cohort, established by CIs Hillman, Hung, Palmer, McArdle, Singh and Mukherjee, is a unique study of >5,000 consecutive consenting patients who attended the WASDRI between 2005 and 2010 for clinical evaluation by a sleep disorders physician and in-laboratory PSG[29]. The participation rate was >98% and OSA was present in 91%. PSGs (n=4,100) were scored manually according to internationally accepted standards and results are stored in an easily accessible database (Profusion neXus Laboratory Management System, Compumedics, Abbotsville, Australia). In addition to the clinical and PSG information, all patients (n=5,187) completed a detailed questionnaire and had anthropometric measurements and spirometry. Most of the cohort (n=3504) also had blood tests for biochemistry and DNA analysis. The detailed questionnaire collected information on *CVD risk factors (physical activity, cigarette smoking, hypertension, hypercholesterolemia and diabetes mellitus*) and *doctor-diagnosed CVD (angina, myocardial infarction, coronary angioplasty or stent, coronary artery bypass graft, heart failure, stroke, and carotid endarterectomy or stent* and other diseases. The questionnaire also collected information on current medications, Epworth sleepiness score, sleep habits, symptoms of sleep disorders, alcohol use, family history of sleep disorders, driving history, educational and employment status, and ethnicity. *Anthropometric measurements* included weight, height, body mass index, neck circumference, upper airway narrowing (Mallampati score, pharyngeal grade), craniofacial measurements (cricomental distance and space) and BP. Biochemical assessments included *fasting glucose and insulin* (to detect insulin resistance and diabetes mellitus), *fasting lipids* (to detect hyperlipidaemia), and *markers of CVD risk (C-reactive protein and fibrinogen)*. Blood samples not used for biochemical or DNA analysis were processed and stored in the WA DNA Bank. Complete data (questionnaire + PSG + blood tests) are available in 2,793 participants. In a subset of patients, additional information was obtained including depression scales (Patient Health Questionnaire-9 and DASS-21) (n=426), additional anthropometric measurements

	Male (N = 1,684)	Female (N = 953)
Characteristics, mean (SD)		
Age, years	49.8 (13.8)	51.5 (13.4)
BMI, kg/m^2	31.9 (6.8)	34.3 (9.0)
Epworth sleepiness score, /24	9.9 (5.5)	10.0 (5.6)
PSG, mean (SD)		
AHI, per hour	41.6 (30.2)	30.5 (27.3)
Nadir SpO_2, %	81.9 (11.0)	84.2 (9.7)

(chest, waist, and hip circumferences, supine abdominal sagittal diameter and height) and full-body dual energy x- ray absorptiometry (DEXA) (GE Lunar Prodigy, Waltham MA) to assess regional fat distribution (n=238). Blood samples from 2,100 participants have been genotyped using a genome wide association scan (GWAS). The WASHS cohort is the largest sample of well characterized OSA cases with DNA samples in the world. Table 1 summarise some baseline characteristics of the WASHS cohort.

About half of the patients in the WASHS cohort subsequently underwent a one month home trial of *CPAP therapy to control OSA under our close supervision*, and about 60% of these patients (~1,200) accepted long-term CPAP therapy. We have sequential clinical assessments on most of these patients, and many remain under our clinical supervision.

Linkage of WASHS to WA Data Linkage System. The WA Data Linkage System (WADLS) was established in 1995 as a facility for linking key datasets (including hospital separations and mortality) for the entire Western Australian population. The WADLS is particularly useful for epidemiological studies because the WA population is relatively stable with a low migration rate. Successful use of the WADLS for investigating heart disease as well as other morbidities has been demonstrated by other authors[30]. The WASHS cohort was fully linked to the core population-based WADLS datasets in 2011 and these links are currently being updated. The Data Linkage will provide CVD classification using international classification of disease (ICD) codes.

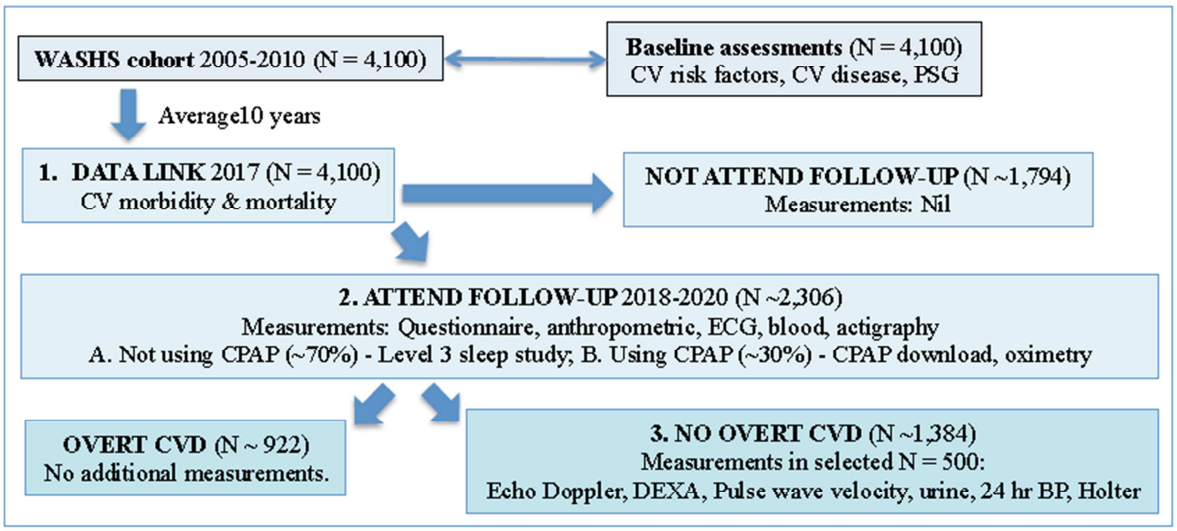
We now seek to conduct a follow-up of the WASHS patients to examine the interaction between the OSA-related physiologic derangements during sleep and other CVD risk factors on the development of CVD over a mean follow-up duration of 10 years. The development of CVD since inclusion in the WASHS study in 2005-2010 will be determined by (a) linking the cohort to databases held by WA Health using the WA Health Data Linkage Unit, (b) a follow-up review with comprehensive health assessments and (c) detection of sub-clinical CVD among those patients who have not developed overt disease using DEXA scan (to measure aortic calcification) and Echo Doppler, and explore pathogenic mechanisms using 24 hour ambulatory BP, pulse wave velocity, and blood risk biomarkers. We will use this data to determine the interaction between OSA phenotypes and CVD risk factors in the development of CVD. Our analyses will give a clearer understanding of which patients with OSA are at risk of developing CVD, i.e. according to OSA and other CVD risk factor phenotypes, enabling risk assessment and intervention to be personalised. We will model the effect of treatment of OSA and other CVD risk factors on CVD risk and determine if there is a dose-response relationship, to determine the OSA phenotypes that are most likely to have a causal role in CVD development. These data will provide vital information to inform subsequent intervention studies directed to OSA sub-groups who are at increased risk of CVD. It will also enable the development and validation of an individualised risk assessment score for CVD incorporating information on OSA. We will develop the model using a random two-thirds sample of the original cohort across the years of enrolment ("derivation cohort") and test its validity in the remaining third of the cohort ("validation cohort")[17]. The developed risk assessment tool will be directly applicable

to typical OSA clinic patients and, although we will perform internal validation, we acknowledge the need for future external validation in other study populations, including general population samples.

RESEARCH PLAN, METHODS AND TECHNIQUES:

Subjects. This project is a 10-year follow-up of 4,100 WASHS subjects in whom we have detailed baseline data on CVD risk factors, CVD, anthropometric measurements and overnight PSG obtained in 2005-2010. The primary outcome of hospital admissions for CVD or CVD mortality will be available on all patients. All other outcomes will be determined by invitation to participate in the follow-up study. We anticipate that about 75% of WASHS subjects will be alive and contactable and, based on prior experience from cohort studies such as the Busselton Health Study, 75% of these subjects will agree to participate. Therefore we anticipate following-up approximately 4,100*0.75*0.75 = 2,306 subjects. An overview of the research plan is presented in Figure 1.

FIGURE 1, STUDY OUTLINE



Baseline measurements:

a) **PSG** (N=4,100). OSA-related physiologic derangements during sleep obtained at baseline PSG will be used to examine the interaction between OSA and other CVD risk factors on the development of CVD. The following derangements will be examined (1) the frequency of obstructive respiratory events (AHI - overall & in REM, respiratory disturbance index), (2) hypoxemic burden (mean and nadir oxygen saturation, total sleep time spent at oxygen saturation <90% (T90), 4% oxygen desaturation index, mean apnoea/hypopnoea duration (3) sleep disruption (cortical arousal index), wake after sleep onset, sleep efficiency, sleep latency, and (4) sympathetic activation (reduced heart rate variability, non-dipping of BP overnight).

b) **CVD risk factors.** CVD risk factors obtained at baseline include (1) demographics (age, sex),

(2) lifestyle (physical activity, cigarette smoking), (3) co-morbidities (obesity, hypertension, diabetes mellitus, hyperlipidaemia) and (4) blood biomarkers (N= 2,793) [insulin resistance (HOMA index), lipid profile, fibrinogen, C-reactive protein]. In addition, from stored plasma obtained at baseline, we will measure additional risk biomarkers for cardiac disease [glycated haemoglobin (HbA1c), hsTn-I and NT-proBNP]. Concentrations of hsTn-I have been associated with increasing severity of OSA (AHI) and hypoxemic burden, suggesting that increasing OSA burden may cause low-grade myocardial injury[38]. We will define co-morbid risk factors using questionnaire, and where appropriate, other measurements (BP) or blood markers. For example diabetes will be defined as disease diagnosed by a doctor, on diabetic medication, fasting blood glucose ≥7.0 mM/L or HbA1c ≥6.5% (48 mM/M).

Follow-up measurements (See also Figure 1):

1. All participants **(N=4,100)**. In all patients, the development of **CVD** will be determined via the WADLS from WA Health datasets for hospital separations for any of the following: (1) coronary artery disease – hospitalization for acute coronary syndrome (unstable angina, AMI, intervention (coronary angioplasty or CABG surgery), (2) cardiac failure, (3) atrial fibrillation (AF) or cardioversion for AF, (4) insertion of permanent pacemaker, (5) sudden cardiac death, (6) ischaemic and haemorrhagic stroke or transient ischaemic attack, and (7) CVD mortality.

2. Measurements in participants who are alive and agree to follow-up **(N~2,306):**

- **Questionnaire** to determine the changes since baseline in (a) CVD risk factors –cigarette smoking, hypertension, hyperlipidaemia, diabetes mellitus, (b) doctor diagnosed CVD not captured in Data Linkage - angina, AMI, AF, coronary angioplasty/stent/CABG, heart failure, stroke, carotid stent/endarterectomy - date of diagnosis and details of where diagnosed (c) OSA treatment history (j.e., CPAP, mandibular advancement splint or surgery, current reported weight), (d) Epworth sleepiness score, (f) sleep behaviour, (g) sleep disorder symptoms and (h) Potential confounding by diet (Australian Diet Quality Tool = DQT[39]), (i) Assessment of healthy user bias - self-efficacy(Generalised Self-efficacy scale[40]), (j) Multi-dimensional Perceived Social Support Scale[41], (k) brief depression scale (PHQ2)[42]. Family history of premature heart disease will be assessed. Newly developed CVD risk factors since baseline will be entered into the model (see analysis).
- **The questionnaire** will be administered by web-based methods (Survey Monkey) or, if the participant is not agreeable or able to use web-based methods, by research interviewer asking the questions over the telephone.
- **CPAP device data download** will be obtained to determine the effectiveness of treatment and objective compliance. CPAP download requires the patient to attend the Sleep service to download the device. We will also measure weight using calibrated scales, this will serve as a check on the accuracy of patient reported weight (see above).
- **Actigraphy:** objective estimate of sleep time when using CPAP: Patients who are using CPAP treatment will be posted a wrist actigraphy device one week prior to the CPAP device download. They will wear this device for a week in addition to their usual CPAP treatment. This device uses accelerometers to sensitively measure movement and will provide an accurate and objective assessment of sleep duration during CPAP treatment.

3. Participants with no overt CV disease at follow-up. Among participants who agree to follow- up, we estimate that about 60% (N~1,384) will not have overt CV disease. A sample of 500 of these subjects stratified by gender, OSA severity (mild, moderate and severe) and CPAP treatment (not using CPAP vs regular CPAP use) will be invited to have further investigations to detect subclinical

CVD. Within this subset we will test the association of CVD risk factor measures, including: abnormal heart rate variability, 24-hour mean BP, abnormal pulse aortic wave velocity, with pre-defined subclinical (quantitative & categorical) CVD endpoints, including: LV longitudinal strain on echo,quantitative coronary calcium score on CT calcium scan and non-invasive imaging of the retinal arteries (OCT).

- **DEXA scans** (Hologic Horizon A, Hologic Australia Pty Ltd) to measure body composition, particularly compartmental fat distribution and visceral fat and to measure aortic calcification as a measure of atherosclerosis.[43]
- **Echo Doppler** (GE Vivid E95) to provide detailed assessment of cardiac dysfunction or structural changes. Left and right ventricular chamber size, wall thickness, mass, systolic and diastolic function, estimation of filling pressures and atrial sizes will be assessed according to established guidelines and imaging protocols[44]. Pulmonary artery systolic pressure will be assessed by spectral Doppler interrogation of the peak tricuspid regurgitation velocity. The echocardiographic pulmonary to left atrial ratio will be determined as a novel assessment of transpulmonary gradient[45]. Echocardiography will be conducted at Sir Charles Gairdner Hospital, where all methods are established, by a highly experienced sonographer.

- **Pulse wave velocity** (SphygmoCor XCEL) to measure arterial compliance and derive central arterial pressures. This will be done by the research assistant at the sleep service.

- **24 hour ambulatory BP** to determine average 24-hour BP and diurnal pattern of BP.

- **CT scan for coronary artery calcium** This will be done at a local private radiology service. This test is widely used on healthy subjects to determine the amount of calcium around their blood vessels and carries an acceptable very low dose of radiation.

- **OCT (Optical Coherence Tomography)-Angiography.** A retinal camera provides >70000 scans/sec with 5.7 μm/pixel resolution and <4s acquisition time. It is a non-invasive imaging technology that enables the visualisation of fine retinal capillary networks in great detail as well as measuring retinal blood flow rate and identify regions of low flow. There is no radiation exposure.

- **In-laboratory PSG.** An overnight sleep study will be undertaken to re-assess OSA severity (N=250) and effectiveness of OSA treatment, in those using CPAP. (N=250)

- **Questionnaire:** Additional questions asked of this sub-group: a) physical activity, b) alcohol consumption, c) medications, and d) selected medical history.

Outcomes.

The primary outcome measure will be the development of incident overt CVD over 10 years defined as a composite of the following: 1) coronary artery disease – hospitalization for unstable angina, AMI, intervention (angioplasty, stent, CABG), (2) cardiac failure, (3) AF or cardioversion for AF, (4) insertion of permanent pacemaker, (5) sudden cardiac death, (6) ischaemic and haemorrhagic stroke or transient ischaemic attack, and (7) CVD mortality. These outcomes will be determined by Data Linkage and, in patients who agree to follow-up, also by self-report.

Secondary outcome measures will be (1) each of the individual components of CVDs listed above and we will also assess the new onset of hypertension. (2) subclinical CVD including the following (a) abnormal cardiac rhythm - abnormal heart rate variability, bradycardia, sinus pauses, (b) reduced arterial compliance, (c) aortic calcification, (d) abnormal Echo Doppler findings, and (e) raised serial CVD biomarkers (e.g., hsTn-I and NT-proBNP) from baseline or at follow-up.

Data analysis

Power calculations

The derivation model (see aim 3) is based on two-thirds of the sample (4,100*2/3 = 2,733). Given that this is a study of moderate to high-risk population, a 20% to 30% absolute 10-year CVD risk is assumed. The power calculations are based on a conservative 20% 10-year CVD risk i.e. new CVD events in 546 participants. A Cox regression of the log hazard ratio on risk variables based on a sample of 2,733 observations achieves a 90% power at the 5% level of significance to detect a regression coefficient equal of 0.0974 (or Hazard Ratio of 1.10)[46]. This calculation takes into consideration the effect of any risk variable of interest being adjusted for other variables in the model, assuming a conservative R-squared of 10%.

A sample size of 500 to assess the association between risk factors and subclinical CVD outcomes will have 90% power and 5% level of significance to detect an increased risk of 7.5% for each standard deviation change in the continuous risk variable[47]. This calculation takes into consideration the effect of any risk variable of interest being adjusted for other variables in the model, assuming a conservative R-squared of 10%.

Aim 1.

1. ***Risk of CVD***
   - **All subjects** (N=4,100). This is the primary analysis using a random 2/3 of the sample (see Aim 2). Predictors: Baseline CVD risk factors (baseline questionnaire, in-lab PSG and blood). Outcomes: Time to develop CVD from Data Linkage (Cox Proportional Hazards Model).
   - **Participants who attend follow-up** (N~2,306). This analysis will allow a more complete understanding of the relationships between conventional CVD risks, risk biomarkers and OSA measures on CVD events. Predictors: Baseline CVD risk factors (from baseline questionnaire, in-lab PSG and blood) + change in risk factors since baseline (from follow-up questionnaire and OSA burden). The potential amelioration of CVD events by CPAP will be estimated by estimating the reduction in OSA burden (CPAP download and actigraphy). The presence of CVD at baseline (20% of sample) will be included in the model to assess whether established CVD influences the relationship between OSA and risk for CVD events. Potential bias from health user effect will be assessed by including Self-efficacy, social support and depression scores in the model. Outcomes: Time to develop CVD (from Data Linkage + follow-up questionnaire) using survival analyses (Cox Proportional Hazards Model). Duration of exposure to CVD risk factors (including those that develop during the 10-year follow-up) will be entered into the model. Interaction terms will be used to examine the modulation of OSA-related risk by other CVD risk factors. We will assess the representativeness of this secondary analysis and adjust for baseline differences in those who agree to follow-up versus all subjects, as applicable.
2. ***Risk of subclinical vascular disease***
   This will be assessed in participants who attend follow-up and have no overt CVD (N=1,384. We will use a stratified sample of N=500. Predictors: Same as a), i) above + 24 hr BP, Holter monitor, urinary catecholamines and pulse wave velocity. Outcomes: Sub-clinical measures of CV disease (aortic calcification, Echo Doppler LV strain) using linear or logistic regression.

Aim 2.

Individualised CVD risk score. We will use a random two-thirds of all subjects (N=2,733) as the derivation cohort for establishing a CVD risk score that incorporates OSA-related physiologic derangements during sleep. We will initially test if OSA is an independent CVD predictor, in those with no CVD at baseline. Where OSA adds significantly to risk prediction, by way of change in C-statistics and net reclassification index (NRI), we will apply our findings to the remaining third of the sample (validation cohort)[17].

Aim 3.

a) ***To assess the effect of CPAP on CVD risk*** (N=2,306). Treatment with CPAP and a treatment "dose" variable will be entered into the model [i.e. efficacy = residual AHI on device download * objective CPAP use (hours/night) * 1/estimated sleep time (hours/night)]. We will undertake a separate propensity score matching analysis to account for non-random application of CPAP treatment (i.e. cases treated with CPAP matched to controls not receiving CPAP therapy, adjusted for differences in baseline CV risk).

b) ***To assess the effect of CPAP on sub-clinical vascular disease risk.*** The stratified sample will include CPAP treatment as a stratification factor (yes: N=250, no: N=250)

SAFETY:

Abnormal investigation results will be referred to the treating physician for further management. Confidentiality of all records will be maintained and data will be kept in a locked filing cabinet and only research staff and authorized members of the Human Research Ethics Committee (HREC) will have access. Electronic data will be protected by codes to which only research staff and authorized members of the hospital HREC will have access.

TIMELINE:

Task	2018	2019	2020	2021
Patient follow up				
Statistical analysis and dissemination of results				

The duration of the study will be 4 years. This project is feasible in the time allowed by the grant and with the proposed resources, staff and equipment. Data collection will take place in years 1-3; we anticipating collecting an average of 3.1 participants per day * 250 working days/year = 775 participants/year. Statistical analysis and dissemination of results will occur in year 4.

FEASIBILITY:

This study is highly feasible because: i) the baseline study data is already available and the linkage of the patients to hospital separations and mortality via the WADLS is fully funded and the anticipated data delivery date is April-May 2017, ii) a proportion of the cohort remains under regular medical review at WASDRI, and iii) the facilities for conducting the follow-up assessments have been identified, and the investigative team has the expertise for conducting and interpreting all measurements.

OUTCOMES AND SIGNIFICANCE:

The SAVE study has raised questions about the validity of the compelling epidemiologic and pathophysiological evidence for OSA as an important risk factor for CVD. As a result, there is uncertainty over the long-term CVD consequences of untreated OSA. A recent systematic review and meta-analysis called for more high-quality studies to evaluate the relationships between OSA and CVD risk and how this risk compares with other modifiable CVD risks, such as hypertension and diabetes[48]. The SAVE study also highlights the importance of identifying the profile of OSA patients who are at increased risk of developing CVD. The WASHS Prospective Sleep Clinic cohort presents a unique opportunity to explore the interaction between OSA and other CVD risk factors on the development of CVD for the following reasons. First, the sample size is large and participants are very well characterized. Second, OSA and physiological derangements during sleep have been quantified by in-laboratory PSG using internationally accepted standards. Third, the follow-up period of 10 years is considerably greater than can be achieved by randomized controlled studies. Fourth, the study provides the opportunity to examine the effects of CPAP treatment for OSA and many of these patients have been closely monitored since enrolment in the study. Fifth, there is stored serum and DNA for additional baseline measurements on the cohort. Sixth, the capacity to link the cohort and physiologic derangements during sleep to statutory morbidity and mortality databases held by WA Health will provide outcome data across the cohort free of biases due to healthy volunteer effects. Finally, as far as we are aware, the WASHS cohort is the largest sample of well-characterized OSA cases with DNA samples in the world, and GWAS results are already available on 60% of these participants, providing an opportunity for genetic studies in the future. The current study is likely to provide invaluable information to clinicians deciding on therapy for individual patients, as well as researchers about mechanisms by which OSA contributes to CVD, and who to target for randomised intervention trials to explore the benefit of OSA therapy on CVD.

B. References

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