

Exploring the link between nocturnal heart rate, sleep apnea and cardiovascular function in African and Caucasian men: the SABPA study

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PREFACE

This study forms part of the SABPA study and the dissertation is submitted in fulfilment of the requirements of the MSc program in Physiology at the North-West University, Potchefstroom Campus. Chapter 1 contains relevant background information and the motivation for this study. Chapter 2 contains a literature overview of all the variables relevant to this study as well as a discussion on obstructive sleep apnea (OSA) and its influence on the cardiovascular function of urbanized South African men. Chapter 3 contains the manuscript “Exploring the link between nocturnal heart rate, sleep apnea and cardiovascular function in African and Caucasian men: the SABPA study”, that will be submitted to the peer-reviewed journal namely: Clinical and Experimental Hypertension. Chapter 4 contains a summary of the main findings and recommendations for future research. Relevant references are provided at the end of each chapter in accordance to the instructions of the journal.

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LIST OF ABBREVIATIONS

ABPM	- Ambulatory Blood Pressure Monitoring
AHI	- Apnea-Hypoapnea Index
BMI	- Body Mass Index
BPM	- Beats per minute
BRS	- Baroreflex sensitivity
BSA	- Body surface area
GGT	- Gamma-glutamyltransferase
COT	- Cotinine
CRP	- C-reactive Protein
CSA	- Central sleep apnea
CVD	- Cardiovascular disease
DBP	- Diastolic blood pressure
HDL	- High-density lipoprotein
HR	- Heart rate
LVH	- Left ventricular hypertrophy
MAP	- Mean arterial pressure
OSA	- Obstructive sleep apnea
PWV	- Pulse wave velocity
RDI	- Respiratory disturbance index
ROS	- Reactive oxygen species
SABPA	- Sympathetic Activity and Ambulatory Blood Pressure in Africans
SBP	- Systolic blood pressure
SDB	- Sleep disordered breathing
TC	- Total cholesterol
TG	- Triglycerides
TPR	- Total peripheral resistance

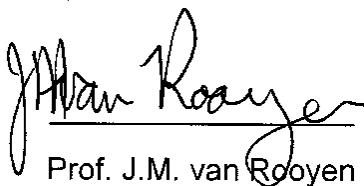
CONTRIBUTION OF AUTHORS

The following list contains the contributions of each researcher that is involved in the study of “The link between nocturnal heart rate, sleep apnea and cardiovascular function of African and Caucasian men: the SABPA study.”

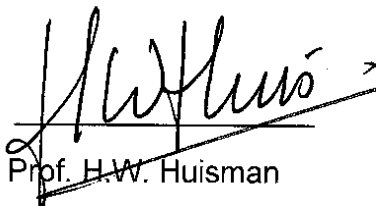
1. Ms Y. van Rooyen (BSc Hons) – Physiologist: Collection of data, literature research, statistical analysis, planning and overall design of the manuscript, interpretation of results and writing of the manuscript.
2. Prof. J.M. van Rooyen (DSc) – Physiologist: Supervisor. Interpretation and supervision of the writing of the manuscript as well as collection of data.
3. Prof H.W. Huisman (PhD) – Physiologist: Co-Supervisor. Collection of data. Interpretation and supervision of the writing of the manuscript.

The following is a statement from the co-authors confirming their roles in this study and giving their permission that the manuscript may form part of this dissertation:

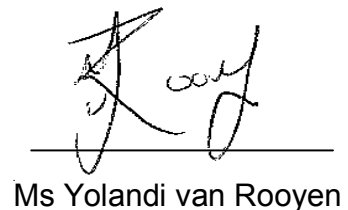
I declare that I have approved the above-mentioned manuscript, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the Master’s degree in Physiology of Yolandi van Rooyen.



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SUMMARY

Title

Exploring the link between nocturnal heart rate, sleep apnea and cardiovascular function in African and Caucasian men: the SABPA study

Motivation: There is a rapid escalation in urbanization amongst South Africans and it is known that urbanized South Africans are subjected to lifestyle factors conducive to an increase in the risk for cardiovascular disease (CVD). Obstructive sleep apnea (OSA) has been described as an independent risk factor for CVD, especially hypertension. OSA has also been associated with insomnia, and plays a contributory role in the co-morbidity of this disorder. The mechanisms employed by OSA, which promote the development of CVD are not fully understood but it has been described that OSA and insomnia both act through an increased sympathetic nervous system activity, which may lead to changes in cardiovascular variability, such as an increase in the nocturnal heart rate and blood pressure. These physiological changes may have adverse cardiovascular outcomes which may be evident in early markers of target organ damage, such as left ventricular hypertrophy. It has recently been shown that ethnicity might contribute to the susceptibility to OSA and that African Americans are more susceptible to OSA when compared to Caucasians. Notwithstanding, studies comparing the prevalence and cardiovascular effects of OSA between African and Caucasian men are limited especially where the effect of the nocturnal heart rate is also taken into consideration. There are also a limited amount of studies investigating the prevalence and co-morbidity of insomnia among African and Caucasian men with OSA.

Objectives: The general aim of this study was to determine whether the risk of sleep apnea and self-reported insomnia are independently associated with nocturnal blood pressure and ECG Cornell product in African and Caucasian men with an elevated nocturnal heart rate. Subsequent objectives included determining

the risk for sleep apnea and the prevalence of self-reported insomnia by respectively implementing the Berlin Questionnaire and the ambulatory diary card. We further aimed to divide the African and Caucasian men separately into two groups based on the nocturnal heart rate and to draw a parallel between the effects of OSA and insomnia on the nocturnal blood pressure and ECG Cornell product within these groups.

Methodology: This study is a subsection of the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study which is a multidisciplinary population comparative study. The study was executed during 2008/2009 on 200 urbanized African and 209 Caucasian school teachers. Our study focused on African and Caucasian men and for the purpose of this study, HIV positive participants were excluded. The final study sample consisted of 88 African men and 101 Caucasian men. The median of the nocturnal heart rate of the 189 men was calculated and the African and Caucasian men were divided into two separate groups, namely those with a nocturnal heart rate ≥ 67 bpm and those with a nocturnal heart rate < 67 bpm.

Ambulatory blood pressure monitoring (ABPM) was performed and the blood pressure measurements took place at 30 minute intervals during the day and 60 minute intervals during the night. Participants completed the ambulatory diary card where they reported on insomnia (hours awake per night). The Berlin Questionnaire for sleep apnea risk was also completed by each participant and anthropometric data was collected by registered biokineticists.

A standard 12-lead ECG was recorded during rest and the ECG left ventricular hypertrophy was determined by using the ECG Cornell product.

Registered nurses collected fasting venous blood samples and the medical history of each participant. The plasma and serum samples were stored at -80°C prior to the analysis of the biochemical markers. Fasting serum samples for total

cholesterol, as well as high-density lipoprotein cholesterol, triglycerides, gamma-glutamyltransferase, cotinine and ultrahigh-sensitivity C-reactive protein were analyzed using two sequential multiple analyzers. Statistical analyses were performed using Statistica version 10.0.

Results: The high risk of sleep apnea based on the Berlin Questionnaire, did not indicate a significant difference between African and Caucasian men but the occurrence of insomnia, based on self-reported hours of wakefulness at night, was significantly higher in African men when compared to Caucasian men. African men showed a more unfavourable cardiovascular profile when compared to Caucasian men with significantly higher values for the nocturnal systolic blood pressure (SBP), nocturnal diastolic blood pressure (DBP) and ECG Cornell product. There was no significant association between the Berlin Questionnaire sleep apnea risk and any cardiovascular variables in any of the groups, but self-reported occurrences of insomnia predicted an increase in both nocturnal systolic blood pressure ($r = 0.469$, $p = 0.001$) and nocturnal diastolic blood pressure ($r = 0.499$, $p \leq 0.001$) in African men with a nocturnal heart rate ≥ 67 bpm. This association was absent in African men with a nocturnal heart rate < 67 bpm and in the case of both Caucasian groups.

Conclusions: The risk for sleep apnea between African and Caucasian men based on the Berlin Questionnaire does not differ and further validation of the Berlin Questionnaire, especially in the African population might be necessary to determine the sensitivity and specificity when predicting sleep apnea in this population group. Self-reported insomnia is associated with an increase in nocturnal blood pressure in African men with an increased nocturnal heart rate. The contributory physiological role of insomnia and the accompanying cardiovascular effects, especially the augmentation in nocturnal blood pressure should be considered when investigating OSA.

OPSOMMING

Titel

Verkenning van die skakel tussen nagtelike harttempo, slaap apnee en kardiovaskulêre funksie in Afrikane en Kaukasiër mans: die SABPA studie

Motivering: Daar is 'n geweldige toename in verstedeliking van Suid-Afrikaners en dit is bekend dat verwesterde Suid-Afrikaners blootgestel word aan faktore wat hul risiko vir die ontwikkeling van kardiovaskulêre siektes kan verhoog. Obstruktiewe slaap apnee (OSA) word beskryf as 'n onafhanklike risiko faktor vir kardiovaskulêre siektes, veral hipertensie. OSA word ook geassosieër met slaaploosheid, wat 'n bydraende faktor mag wees tot die kardiovaskulêre effekte van OSA. Die meganismes waardeur OSA tot kardiovaskulêre siektes lei, kan huidiglik nie ten volle verklaar word nie, maar volgens navorsing lei beide OSA en slaaploosheid, weens die meganisme van verhoogde simpatiese aktiwiteit, tot veranderinge in kardiovaskulêre variabiliteit, met insluiting van 'n verhoogde nagtelike harttempo en bloeddruk. Hierdie fisiologiese veranderinge mag tot 'n aantal kardiovaskulêre uitkomstes lei, wat waarneembaar sou wees in eind-orgaan skade merkers soos linkerventrikulêre hipertrofie. Daar is onlangs bevind dat etnisiteit 'n risiko faktor vir OSA is, en dat die voorkoms van OSA hoër is onder Afro-Amerikane as onder Kaukasiërs. Daar is egter 'n beperkte hoeveelheid studies betreffende die voorkoms en kardiovaskulêre effekte van OSA in Afrikane vergeleke by die Kaukasiërs in Suid-Afrika, in besonder wanneer die nagtelike harttempo ook in ag geneem word. Daar is ook 'n beperkte aantal studies wat die voorkoms en samewerkende fisiologiese invloed van slaaploosheid onder Afrikane en Kaukasiërs met OSA bestudeer.

Objektiewe: Die hoofdoel van die studie is om te bepaal of die risiko vir slaap apnee en self-gerapporteerde slaaploosheid onafhanklik geassosieër is met nagtelike bloeddruk en die EKG Cornell produk in Afrikane en Kaukasiër mans

met 'n verhoogde nagtelike harttempo. Verdere doelwitte sluit in die bepaling van die risiko vir slaap apnee en die voorkoms van self-gerapporteerde slapeloosheid in Afrikane en Kaukasiër mans deur onderskeidelike implementering van die Berlyn vraelys en die ambulatoriese dagboek kaart. Verder is daar beoog om die Afrikane en Kaukasiër mans afsonderlik te groepeer volgens nagtelike harttempo en om sodoende die effekte van OSA en slaaploosheid op die nagtelike bloeddruk en die EKG Cornell produk binne hierdie groepe te bestudeer.

Metodologie: Hierdie studie is deel van die SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) studie, wat 'n teiken populasie, vergelykende studie is. Die studie is uitgevoer in 2008/2009 op 200 verwesterde Afrikane en 209 Kaukasiër onderwysers. Hierdie spesifieke studie het gefokus op Afrikane en Kaukasiër mans met uitsluiting van MIV positiewe deelnemers. Die finale studiegroep het bestaan uit 88 Afrikane- en 101 Kaukasiër mans. Die mediaan van die nagtelike harttempo is bepaal en daarvolgens is die Afrikane- en Kaukasiër mans afsonderlik ingedeel volgens deelnemers met 'n nagtelike harttempo ≥ 67 slae per minuut en deelnemers met 'n nagtelike harttempo < 67 slae per minuut.

Ambulatoriese bloeddruk monitering (ABPM) is uitgevoer. Die meting van bloeddruk het plaasgevind in 30 minuut intervalle gedurende die dag en 60 minuut intervalle gedurende die nag. Deelnemers het die ambulatoriese dagboek kaart voltooi waarop hul gerapporteer het oor slaaploosheid wat hul ondervind het (aantal ure per nag waartydens die deelnemer nie kon slaap nie). Die Berlyn vraelys vir slaap apnee risiko is ook deur elke deelnemer voltooi. Antropometriese data is versamel deur geregistreerde biokinetici.

'n Standaard 12-afleiding EKG is geneem gedurende rus en die EKG linkerventrikulêre hipertrofie is bepaal deur middel van die Cornell produk.

'n Vastende veneuse bloedmonster sowel as die mediese geskiedenis van elke deelnemer, is deur 'n geregistreerde verpleegster versamel. Beide die plasma

en serum monsters is teen 'n temperatuur van -80°C gestoor voordat die biochemiese merkers geanaliseer is. Vastende serum monsters van die totale cholesterol, hoë-digtheid lipoproteïen cholesterol, trigliseriedes, gamma-glutamyltransferase, kortisol en ultrahoë-sensitiewe C-reaktiewe proteïen is geanaliseer deur middel van twee veelvuldige analiseerders. Statistiese analyses is uitgevoer deur gebruik te maak van Statistica, weergawe 10.

Resultate: Die hoë risiko vir slaap apnee, soos bepaal met behulp van die Berlyn vraelys, het nie betekenisvol verskil vergelykende die Afrikane en Kaukasiër mans nie, alhoewel die voorkoms van die self-gerapporteerde slaaploosheid wel betekenisvol hoër was in die Afrikane mans. Afrikane mans het 'n meer ongunstige kardiovaskulêre profiel in vergelyking met die Kaukasiër mans, aangesien die Afrikane mans betekenisvolle hoër nagtelike bloeddruk en EKG Cornell produk waardes getoon het. Daar was geen betekenisvolle assosiasie tussen die Berlyn vraelys risiko vir slaap apnee en die kardiovaskulêre veranderlikes in enige van die groepe nie maar self-gerapporteerde slaaploosheid het 'n verhoging in die nagtelike sistoliese bloeddruk voorspel ($r = 0.469$, $p = 0.001$) sowel as die nagtelike diastoliese bloeddruk ($r = 0.499$, $p \leq 0.001$) in die Afrikane mans met 'n hoër nagtelike harttempo (≥ 67 slae per minuut). Hierdie verhouding is afwesig in die Afrikane mans met 'n laer nagtelike harttempo (< 67 slae per minuut) asook in beide die Kaukasiër mans groepe.

Gevolgtrekkings: Volgens die Berlyn vraelys, verskil die risiko vir slaap apnee tussen die Afrikane en Kaukasiër mans nie betekenisvol nie, wat wys op die moontlikheid dat verdere studies, gemik op die validering van hierdie vraelys, in besonder met betrekking tot die Afrika populasie, toegepas moet word om die sensitiwiteit en spesifisiteit vir die voorspelling van slaap apnee in hierdie populasie groep te bevestig. Self-gerapporteerde slaaploosheid is geassosieer met 'n verhoging in die nagtelike bloeddruk in Afrikane mans het 'n hoër nagtelike harttempo. Dit is moontlik dat die bydraende fisiologiese rol van slaaploosheid

oorweeg moet word in die toekoms wanneer die effekte van OSA bestudeer word.



CHAPTER 1

Introduction and motivation.

1.1 Introduction

The primary causes of morbidity and mortality in Sub-Saharan Africa have previously been attributed to infectious diseases and malnutrition(1), however there is convincing evidence that many communities in Sub-Saharan Africa are in transition from a rural to urbanized lifestyle with epidemiological consequences such as the emergence of more contributing factors that may facilitate the development of cardiovascular disease (CVD) (2). In South Africa there is a rapid increase in the number of individuals who are urbanized and this trend is especially evident in the African population. It has now become known that there is an escalating incidence of cardiovascular disease (CVD) among urban Africans, especially in South Africa (2-4). The increase in the emergence of CVD in urbanized individuals is not limited to the African population and it has been estimated that premature deaths among African, as well as Caucasian South Africans of a working age (35-64 years) due to CVD, are expected to escalate to 41% between 2000 and 2030 (5). This increase in CVD related morbidity and mortality might be caused by changes in lifestyle associated with urbanisation, in particular dietary changes, increases in weight and obesity, decreased physical activity, high levels of stress and increasing alcohol and tobacco consumption (6).

According to the Heart and Stroke foundation, CVD comprises any disease of the heart and blood vessels. Obstructive sleep apnea (OSA) is an independent and potentially reversible risk factor for CVD (7) and there is growing evidence that OSA might contribute to both the initiation and progression of diseases such as hypertension, heart failure, cardiac ischemia and stroke (7). It has also been found that patients with sleep apnea may have increased mortality rates (8,9).

OSA is a syndrome symptomised by a number of respiratory cessations during sleep, which causes abrupt arousals (10) and insomnia (11) and OSA has been described as a risk factor for insomnia (12). The mechanisms through which OSA leads to the development of CVD are still not fully understood (10). A multifactor process involving a complex range of mechanisms is suspected to be the most likely cause (10). One of the

mechanisms in this causative process is the over-activity of the sympathetic nervous system (10). The high level of sympathetic activity in OSA is associated with alterations in cardiovascular variability and heart rate is markedly increased in patients with OSA (13). An increase in the sympathetic nervous system activity has also been found to be present in those with insomnia associated with OSA (14). This has been suggested by the elevation of certain autonomic indicators present in those suffering from insomnia that is associated with OSA, which includes an increased heart rate and an enhanced adrenalin secretion (15).

Sympathetic neural tone is synergistically increased by the hypoxia and hypercapnia that is associated with OSA (16,17). The increased sympathetic neural tone causes vasoconstriction and when breathing is resumed, the cardiac output is delivered into the constricted peripheral vasculature resulting in marked increases in arterial pressure (10). It has been demonstrated that the sympathetic over-activity that occurs in OSA may increase the future risk of hypertension and the associated hypertensive end-organ damage such as left ventricular hypertrophy (LVH) (18,19). Various reports indicate that left ventricular mass is significantly higher in patients with OSA (20) and this signifies the importance of early diagnosis of the disease in order to slow the progression of end-organ damage.

1.2 Motivation

Unless preventative measures are taken to stem the trend, the burden of disease that is attributable to non-communicable diseases such as CVD, is predicted to show a substantial increase in South Africa over the next decades (21). A considerable amount of evidence exists in support of the independent association between OSA and CVD, with this association evidently being strong for arterial hypertension (7). The risk factors for OSA have previously been described as obesity, male sex and increasing age (22). However, ethnicity has recently been identified as an accompanying risk factor (22). It has been demonstrated that apnea is more frequent in African Americans than in Caucasians (23). A relationship between OSA and insomnia has also been established (12) and it has been found that a limited period of sleep is associated with an increased risk for CVD in population-based studies (24-26). It is possible that insomnia may predispose to factors that could increase the mortality risk (27). These findings support the validity of the co-morbidity of insomnia when investigating the cardiovascular effects of OSA. The increase in nocturnal heart rate in people with an enhanced sympathetic activity that is present in both OSA and insomnia has been confirmed by several studies (13-15,28) and the possibility exists that the cardiovascular effects of OSA and insomnia differ in those with an increased nocturnal heart rate when compared to those with a normal nocturnal heart rate.

Studies comparing the association of OSA, insomnia and the cardiovascular outcomes in African and Caucasian South African men, taking into account the nocturnal heart rate, are limited. The quantification of the extent of non-communicable diseases in South Africa and the identification of possible risk factors may prove to be essential for effective treatment and action. An investigation as to the relationship of OSA and the insomnia that is associated with OSA, and cardiovascular disease might therefore be of importance in the understanding of CVD in South Africa and might contribute to the improvement of the health status of South Africans by informing people of the early signs and symptoms of CVD, thus enabling these individuals to seek assistance early in

diagnosing their conditions. Early treatment could prevent serious long-term complications.

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CHAPTER 2

Literature study, Aims and Objectives,
Hypotheses.

1. The burden of cardiovascular disease in South Africa

Cardiovascular disease (CVD) is a major health concern that has reached near epidemic proportions in Africa (1). The *World Health Report 2002*, stated that CVD contributed to 9.2% of total deaths in large regions of Africa (1) and that the mortality due to CVD is higher in developing countries than in developed ones (2). Even in low to middle income countries such as South Africa, CVD is responsible for up to 10% of healthy life years lost, being second only to HIV/AIDS (1).

South Africans are undergoing a health transition, characterised by an increase in the prevalence of infectious as well as non-communicable diseases (3). During the course of the past 15 years, the prevalence of non-communicable diseases, such as CVD, has shown a marked increase, driven by a foregoing increase in the relevant risk factors in urban as well as rural environments (4). Several changes in lifestyle, such as dietary changes, obesity, a low level of physical activity, high levels of stress and an increase in tobacco and alcohol consumption, contribute to the emergence of the well-known risk-factors for CVD (5-7).

2. Sleep apnea as a risk factor for cardiovascular disease.

In normal subjects, several important physiological changes in respiratory and cardiovascular functions occur during sleep (8). These changes include an augmentation in cardiac vagal modulation and a decrease in sympathetic stimulation to cardiac as well as vascular targets resulting in a reduction in blood pressure and mean heart rate (9). Sleep-related breathing disorders, such as sleep apnea, is an increasingly commonplace problem affecting sleep (10).

Sleep apnea syndromes are characterised by a large number of respiratory cessations during sleep and these lead to partial arousals and interfere with the normal physiological cyclic shift between the various stages of sleep (8). Sleep apnea episodes are classified as being central (CSA), obstructive (OSA) or mixed (8). CSA is caused by a dysfunction of neural centres that regulate respiration whereas OSA occurs due to upper airway obstruction (8), which, in turn, is triggered by complete or partial collapse of the pharynx (11).

OSA is an increasingly frequent disorder (12) and it has been estimated to affect 24 % and 9 % of middle-aged men and women, respectively (12). OSA is an independent and potentially reversible risk factor for CVD (13) and evidence supports the contributory role of OSA in both the initiation and progression of cardiovascular diseases such as hypertension, heart failure, cardiac ischemia and stroke (13). Marin et al., (14) has shown that severe OSA in men significantly increases the composite risk of fatal cardiovascular events. According to some uncontrolled studies, untreated OSA is significantly associated with increased rates of cardiovascular events after a relatively short follow-up (15,16). Other longitudinal studies have also confirmed increased cardiovascular morbidity in OSA patients (17,18).

3. Diagnosis of OSA

The successful recognition of OSA by community physicians world-wide, is negligible. According to the outcomes of the Wisconsin sleep cohort study (19), only 7 per cent of women and 12 per cent of men with moderate to severe OSA could be identified by the community physicians. Studies regarding the improved success rate of OSA recognition by physicians concluded that specialist intervention with diagnostic equipment (20) or alternatively, training of physicians taking sleep history, (21) would promote reliable recognition of OSA. However, both these approaches would require significant professional and technical resources.

OSA diagnosis is most commonly made by sleep laboratory polysomnographic examinations which involve the monitoring of a number of variables such as nasal air flow, snoring sounds, thoracic movements and blood oxygen saturation in a controlled environment conducive to an entire night of sleep and polysomnography is known as the golden standard for the diagnosis of sleep apnea (10). The access of this examination is limited due to the prohibitive cost of the test, technical demand and long waiting lists (22).

3.1 Validation of the Berlin Questionnaire to identify patients at risk for OSA.

The Berlin Questionnaire, which is a collection of questions regarding snoring behaviour, daytime fatigue and the presence of obesity and hypertension, was one of the outcomes of the Conference on Sleep in Primary Care (23). This conference involved 120 pulmonary and primary care physicians from the USA and Germany. During this conference, questions were carefully selected from the literature with the aim to determine and raise factors and behaviours that predicted the presence of sleep disordered breathing (SDB) on a consistent basis (24-26). The overall focus of the questionnaire was aimed at a limited set of known risk factors for sleep apnea (23).

A study to determine the success of the Berlin Questionnaire as a tool for identifying patients at risk of obstructive sleep apnea, was conducted by Netzer et al.,(23) and the report of the study is the first to use a survey, the Berlin Questionnaire, to screen for sleep apnea in a primary care population. During this study, one thousand questionnaires in batches of 200 per study site were distributed to individual physicians at five sites in Cleveland, Ohio. The study sites were geographically and socio-economically diverse. Questionnaires were handed out to consecutive patients visiting the physician for various reasons or ailments and patients were advised to return the questionnaire within one month. Portable monitoring of respiratory disturbances during sleep was performed on 100 patients, 69 in the high risk group and 31 in the low risk group. Monitoring was performed with the Eden Tec recorder, which measures variables such as nasal and oral airflow, chest wall movement, oxygen saturation and pulse rate. Measurements from a full disclosure printout were manually scored for a respiratory disturbance index. Questions regarding the symptoms in the questionnaire demonstrated internal consistency, with Cronbach α coefficients of 0.86 to 0.92. Risk grouping by the questionnaire was also useful in the prediction of the respiratory disturbance index (RDI). The questionnaire predicted the RDI with a sensitivity of 0.86 and a specificity of 0.77, a positive predictive value of 0.89 and a likelihood ratio of 3.79. Netzer et al., concluded that the Berlin Questionnaire is an accurate tool for identifying patients who are likely to suffer from sleep apnea (23).

In another study, conducted by Chung et al.,(27), the result of the Berlin Questionnaire was evaluated against the apnea-hypoapnea index from in-laboratory polysomnography. The study validated the use of the Berlin Questionnaire as a screening tool for OSA patients and the questionnaire displayed a high level of sensitivity, ranging from 65.6% to 87.2% for different apnea-hypoapnea cut-offs.

The Berlin Questionnaire has also been used for the preoperative identification of sleep apnea risk in surgical patients (28). During this study, the Berlin Questionnaire correctly identified all patients previously diagnosed with sleep apnea as being at high risk (28). In a study conducted in 2004 to determine the association of atrial fibrillation with OSA, OSA was diagnosed with the Berlin Questionnaire (29). The accuracy of the questionnaire compared with polysomnography was also assessed in a sample of the study population and the questionnaire performed with 0.86 sensitivity, 0.89 specificity and 0.97 positive predictive value (29).

Some limitations of the Berlin Questionnaire have also been reported. Netzer et al., (23) reported that the prevalence of patients at high risk for sleep apnea, based on the Berlin Questionnaire, in this study exceeded the estimates from community-based surveys. The predictive value of the Berlin Questionnaire varies among different patient populations and it has been demonstrated that the sensitivity of the Berlin Questionnaire is lower with higher cut-off points of the apnea-hypoapnea index (23) allowing for over-diagnosis of mild to moderate sleep apnea by the questionnaire (28). When a positive result is found with the Berlin Questionnaire, it is advisable to consider further direct clinical examinations regarding sleep apnea. A further possible limitation of the Berlin Questionnaire is its use in hypertensive population samples. In a recent study to explore the prevalence of OSA in a group of hypertensive patients and to evaluate the sensitivity and specificity of the Berlin Questionnaire to detect OSA as compared to polysomnography in this group, it was found that the Berlin Questionnaire is not a dependable screening tool for the detection of OSA in hypertensive patients (30). Lisi et al., (30) attributes this finding to insufficient questions regarding hypertension and suggests that a specific version of the Berlin Questionnaire or scoring criteria applicable to hypertensive subjects are needed for a reliable screening of OSA in this population.

4. The relationship between OSA and insomnia.

The presence of OSA may be suggested by symptoms such as habitual and intermittent snoring, witnessed apneas and abrupt arousals during sleep (8). These multiple electroencephalographic arousals occur due to the repeated breathing disturbances associated with OSA and result in sleep fragmentation and insomnia (31). OSA has been described as a risk factor for insomnia (32). However, studies regarding insomnia in patients with OSA as well as the co-occurrence and possible interactions of these conditions have been limited, despite the increasing prevalence and physiological consequences. A relationship between insomnia and OSA has previously been successfully described in patients seeking treatment in primary care as well as sleep clinic settings (32). Studies have reported that insomnia symptoms are present in 40-50% of patients suffering from sleep-disordered breathing (32,33), emphasizing the importance of applying insomnia research in cases of sleep disorders such as OSA and taking into account the possible physiological effects of insomnia.

5. Epidemiology of OSA.

The prevalence of OSA might vary according to various settings and is likely to be influenced by the characteristics of the study population (8). It is estimated that approximately 20% of a general population display obstructive apneas (34,35) with the peak of prevalence being in middle-aged subjects, followed by a decline after the age of 65 (36). The prevalence of OSA might increase in postmenopausal women, especially in women who do not make use of hormone replacement therapy, the prevalence, notwithstanding, remains lower than in men of the same age (37-40). Male gender is a well-known risk factor for OSA and it has been found that men are twice as likely to develop OSA as are women (41). The principal epidemiological factor associated with OSA is increased body mass (8) and the increased prevalence of OSA is parallel to the progressive increase in obesity (36,42).

6. Racial differences in the epidemiology of OSA and insomnia exist.

The well-known risk factors for OSA have previously been obesity, male gender and increasing age (43). Some recently discovered risk factors, including menopausal status and ethnic origin, have been identified (43). Several studies have suggested that ethnicity might prove to be an important risk factor in OSA (44,45). Previous studies have demonstrated that apnea is more frequent in African Americans than in Caucasians, independent of confounding variables (45). The anatomical and physiological mechanisms underlying this predisposition are unclear; however differences in the soft tissue and bony structure of the upper airways are the most likely explanations (43).

Limited epidemiological research exists on ethnic differences with regards to insomnia and studies that have addressed this topic have brought about controversial results. However, in the CARDIA study, which was conducted by Lauderdale et al., (46), it was found that Africans have a lower mean sleep duration when compared to Caucasians, this notwithstanding adjustments made for socioeconomic and demographic factors (47).

7. Physiological effects of OSA.

During normal non-rapid eye movement sleep, a decrease in sympathetic nerve activity, blood pressure, metabolic rate and heart rate occurs (48,49). OSA disrupts these cardiovascular changes by triggering acute changes in autonomic, hemodynamic, chemical, inflammatory and metabolic effects. This leads to chronic physiological changes which could potentially exacerbate CVD (Fig 2.1) (50).

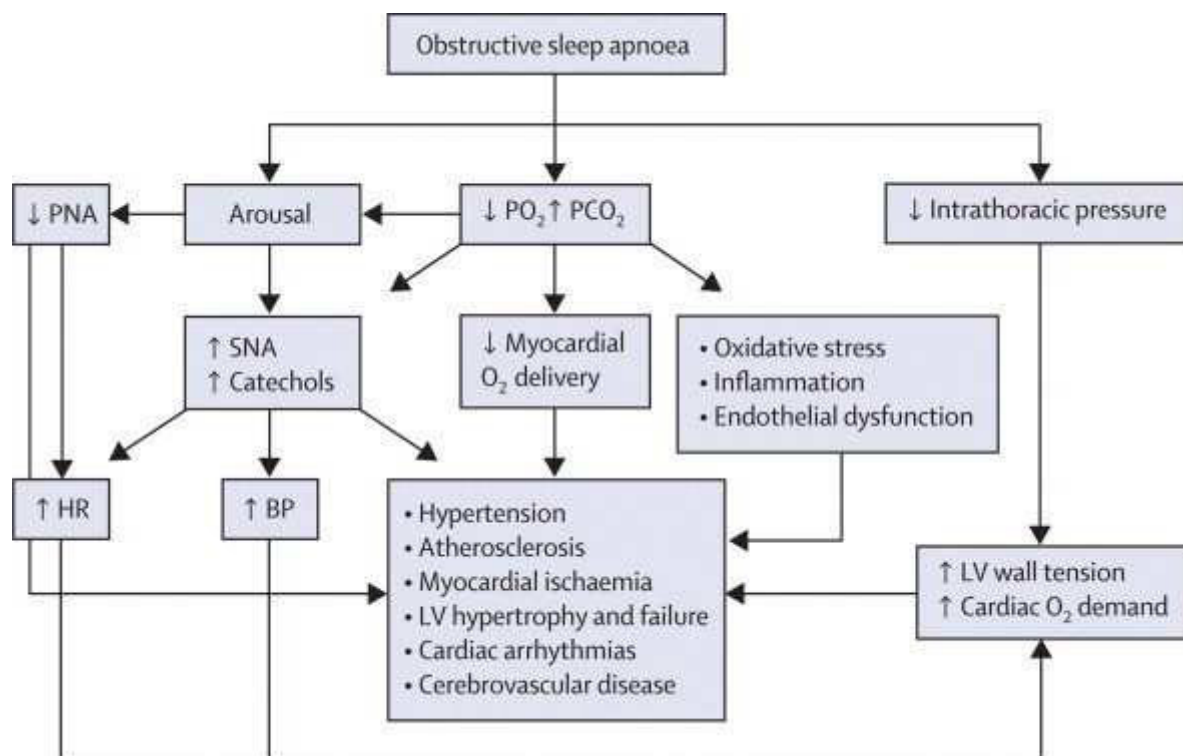


Figure 2.1: Pathophysiological effects of obstructive sleep apnea on the cardiovascular system. PNA= parasympathetic nervous system activity, PO₂= partial pressure of oxygen, PCO₂= partial pressure of carbon dioxide, SNA= sympathetic nervous system activity, HR= heart rate, BP= blood pressure, LV= left ventricular. From Bradley et al., 2009.

7.1 Mechanical and Hemodynamic effects.

The occlusion of the pharynx during sleep apnea leads to great inspiratory efforts which cause abrupt reductions in intrathoracic pressure (11). These reductions in intrathoracic pressure lead to an augmented left-ventricular transmural pressure, and consequently to an increase in the ventricular after load (51). An enhanced venous return results in right ventricular distension, displacing the interventricular septum to the left, which lowers filling of the left ventricle (52). Diminished left ventricular preload and increased left ventricular after load act simultaneously to reduce stroke volume and eventually the cardiac output (53).

7.2 Neurohumoral effects

7.2.1 Neurohumoral effects of insomnia

It has been suggested that insomnia associated with OSA represents a state of physiological hyperarousal (54,55) and Monroe (56) was one of the first to report the elevation of certain autonomic indicators among those who suffer from insomnia and since then other researchers have found an association of insomnia with an increased heart rate and an augmented adrenalin secretion (57). Increased heart rate and adrenalin secretion are indicators of an increased sympathetic nervous system activity and in a study conducted by Bonnet et al.,(58) to investigate whether patients suffering from insomnia displayed an increased heart rate, the result was positive and it was suggested that the increased sympathetic activity associated with insomnia could increase the risk for cardiovascular diseases that are related to an increased sympathetic activity, such as coronary artery disease.

7.2.2 Neurohumoral effects of OSA

Increased sympathetic nervous system activity is also one of the key features of OSA (11). Under normal conditions, sympathetic discharge is suppressed by a reflex arising from pulmonary stretch receptors, however, this reflex ceases to occur during apnea (11), resulting in augmented sympathetic activity. Sympathetic activity is further augmented by hypoxia and hypercapnia through stimulation of peripheral and

central chemoreceptors (59). It has previously been demonstrated that patients suffering from OSA display increased pressor responses to hypoxia by an increased peripheral chemoreceptor sensitivity (60,61). The stimulation of chemoreceptors has an enhanced vasoconstriction effect which will augment the peripheral resistance while increased cardiac sympathetic stimulation increases the heart rate and reduces heart rate variability (62). When breathing is resumed after an apnea/hypoapnea episode, the venous return is increased and this results in a higher cardiac output that is delivered into the constricted peripheral vasculature, increasing the arterial pressure (63).

There are a number of neural and humoral mechanisms that might serve to maintain the augmented sympathetic activity and arterial pressure in OSA (63). Evidence regarding the pathogenic role of OSA in cardiovascular complications through changes in the autonomic nervous system control has been obtained by studying the baroreflex control of the heart (63).

7.3 Chronic physiological effects of OSA on the baroreflex sensitivity.

Baroreflex sensitivity (BRS) evaluation is a widely used tool in the assessment of autonomic control of the cardiovascular system (64). In a healthy individual, the activation of the baroreceptors in the carotid sinus and aortic arch by an increase in blood pressure, reflexively inhibits sympathetic outflow, increases cardiac vagal outflow and reduces heart rate (65). It is thus evident that a decrease in baroreceptor sensitivity could contribute to enhanced sympathetic activation and a decrease in parasympathetic nerve activity (66). In patients suffering from OSA, the repetitive nocturnal surge in arterial pressure might down regulate baroreceptors and decrease the baroreceptorsensitivity (65). It has been determined that OSA patients display a characteristic reduction in baroreceptor sensitivity, with this characteristic being present during wakefulness and periods of sleep (66). Tkacova and colleagues demonstrated that acute elimination of OSA by treatment with continuous positive airway pressure (CPAP) administration resulted in an immediate increase in baroreflex sensitivity (67). This suggests the possibility that OSA increases the set point of the baroreflex which will therefore lower the sensitivity (65).

7.4 OSA and altered cardiovascular variability

The high level of sympathetic activity present in OSA, due to baroreflex (66,68) and chemoreflex (68,69) dysfunction, is associated with certain alterations in cardiovascular variability (Fig 2.2) which is not restricted to sleep and persists into wakefulness as well (63). Both blood pressure variability and heart rate **are** markedly increased in OSA (Fig 2.2)

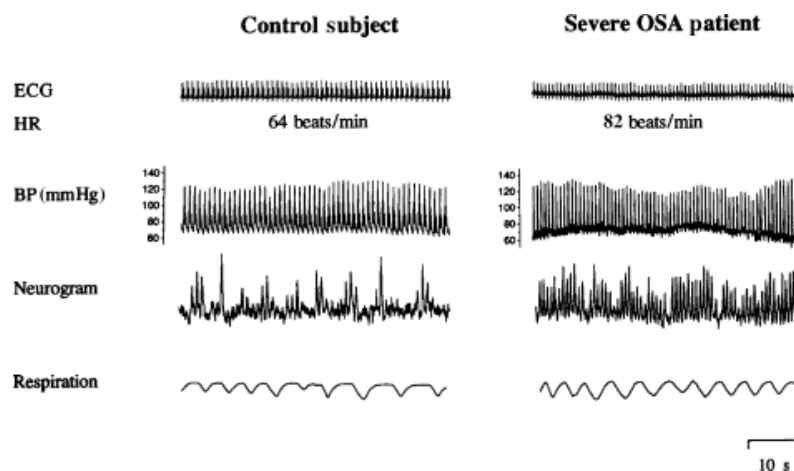


Figure 2.2.Electrocardiogram (ECG), bloodpressure, sympatheticneurograms, andrespiration in acontrolsubject (left) and in apatientwithsevereobstructive sleep apnoea (OSA; right) showing faster heart rate, increased blood pressure variability and markedly elevated muscle sympathetic nerve activity in the patient with OSA – from Narkiewicz et al.,(1998)

7.5 OSA and nocturnal heart rate

The value of heart rate evaluation as a prognostic tool has previously been underestimated (70), and it has recently become evident that an association between heart rate and cardiovascular deaths exists (71,72). It has been concluded that an increased heart rate and the sympathetic outflow imbalance that it reflects, has pathophysiological consequences, beyond the association with hypertension (70). In a study performed to determine whether non-dipping of the nocturnal heart rate determines future CVD, it was found that the risk for future CVD was 2.4 times higher in those who did not display the normal decline in nocturnal heart rate (73).

The degree to which abnormal cardiovascular variability occurs is linked to the severity of OSA (62). These cardiovascular abnormalities in blood pressure and heart rate variability are also present in normotensive OSA patients, who otherwise appear to be in good health and are free of CVD (63). The abnormalities in cardiovascular variability might increase the risk of these individuals for future hypertension and hypertensive end-organ damage (74). It may therefore be possible that abnormal cardiovascular variability will precede, and possibly even predispose to the development of CVD in patients suffering from OSA (63).

8. The clinical relevance of OSA: Association with hypertension and cardiovascular end-organ damage.

The possible consequences of OSA have previously been focused on the possibility that the intermittent hypoxia associated with OSA, might lead to pulmonary hypertension (8). However, it has now become clear that OSA mainly affects the systemic circulation (8). Various case-control studies have reported that the association between hypertension and OSA is independent of confounders, including that of obesity. OSA and hypertension are likely to exist synergistically in an individual and it has been said that 50% of OSA patients are hypertensive and that 30% of obese hypertensive patients are prone to OSA (75). OSA is also very common in patients suffering from resistant hypertension, with the prevalence being 96% in men and 65% in women. The European Hypertension Guidelines have emphasized that OSA plays an important role as a determinant of hypertension (76).

When the risk of hypertension as a result of OSA is expressed as an odds ratio, the data from various studies range from 1.3 to as high as 9.7, even after adjusting for confounders (77,78). Assessment of blood pressure in OSA patients by means of office readings alone could lead to the under diagnosis of hypertension and Baguet et al.,(79) have previously indicated that ambulatory blood pressure monitoring (ABPM) might be important in diagnosing hypertension in OSA patients. In the study conducted by Baguet et al.,(79) it was shown that 58% of OSA patients had elevated daytime ambulatory blood pressure and 76% were suffering from nocturnal hypertension (Fig 2.3)

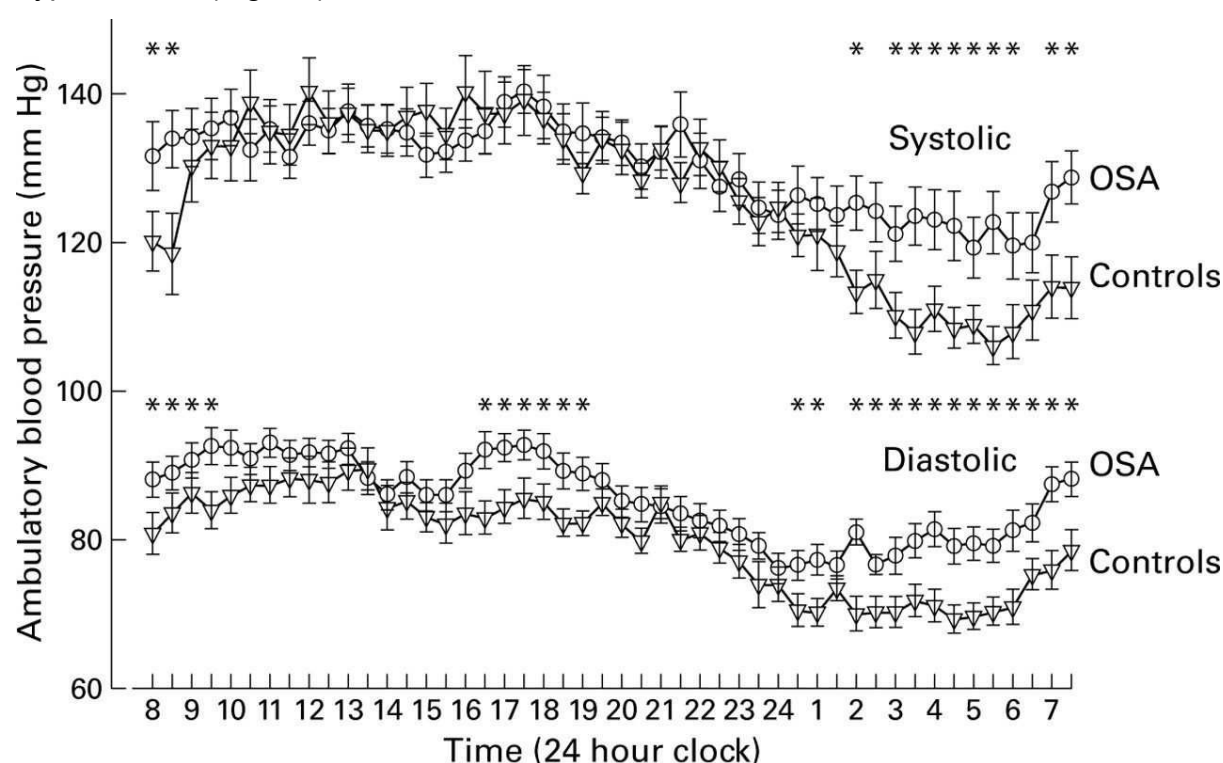


Figure 2.3: Ambulatory blood pressure profiles in group of patients with OSA (n=45) and in a group of controls (n=45) matched for age ($\pm 10\%$), body mass index (BMI; $\pm 5\%$), alcohol intake (> 10 or < 10 units per week), smoking (never vs ex/current), treated hypertension, ischemic heart disease and diabetes. Data are separately shown for systolic and diastolic blood pressure. Note clear differences at night with reduced nocturnal fall in systolic and diastolic blood pressure in patients with OSA, accompanied by increased diastolic blood pressure during the morning and afternoon. Asterisks indicate times at which differences in hourly blood pressure reached a significant level of $p < 0.05$. From Davies et al., 2000.

OSA patients frequently display a reduced fall in nocturnal blood pressure, also known as non-dipping (80) which may lead to the exacerbation of hypertension in OSA patients as well as to several forms of hypertensive target end-organ damage, including left ventricular hypertrophy (LVH) (81).

8.1 The association of OSA with left ventricular hypertrophy (LVH)

LVH is one of the leading causes of morbidity and mortality (82). An increase in the incidence of clinical events due to CVD can be predicted by an increase in left ventricular mass (83,84). There are several unfavorable effects that OSA may impose on the heart. Firstly, due to significant inspiratory efforts, large negative intra thoracic pressures are generated that increase the transmural pressure across the myocardium, occasioning an augmentation in the ventricular after load (82). Secondly, there is a reduction in the oxygen supply to the myocardium as a result of the hypoxia associated with OSA, which will promote the onset of angina or arrhythmias (82). Lastly, the enhanced sympathetic activity which is so characteristic of patients with OSA, leads to an accompanying elevation of catecholamines, both in the plasma and urine (82). The detrimental effects of the repetitive episodes of an augmented ventricular after load on the heart is not necessarily limited to sleep and could persist into daytime, supporting the notion that OSA may contribute to LVH (85-87). In a study conducted by Cloward and colleagues, it was found that LVH was present in high frequency in subjects with severe OSA, with 88% of OSA subjects displaying LVH (82). The LVH also regressed after 6 months of continuous positive airway pressure (CPAP) treatment (82). Other investigators have also characterized cardiac structure and function in OSA (88,89). Hedner et al., compared 61 men with OSA to 61 control subjects and reported that left ventricular mass was significantly higher among the OSA patients (85). Left ventricular mass also varies with ethnicity. In a study conducted by Drazner et al.,(90) it was found that ethnic differences between African American and Caucasian individuals persisted in multivariate models even after adjustment for body composition, systolic blood pressure (SBP), age, gender and socio-economic status and they concluded that African Americans had a significantly higher ventricular mass when compared to Caucasians.

Aim:

The general aim of this study was therefore to determine whether the risk of sleep apnea and self-reported insomnia are independently associated with nocturnal blood pressure and ECG Cornell product in African and Caucasian men with an elevated nocturnal heart rate.

The detailed objectives were:

- To determine and compare the risk of sleep apnea in African and Caucasian men by implementation of the Berlin Questionnaire.
- To determine and compare the incidence of self-reported insomnia among African and Caucasian men.
- To determine whether African and/or Caucasian men display an increased nocturnal heart rate when divided into groups based on the median of the nocturnal heart rate.
- To compare the association of the Berlin sleep apnea risk and self-reported insomnia with hypertension and a marker of end-organ damage, namely the ECG Cornell Product in African and Caucasian men with a nocturnal heart rate ≥ 67 bpm and < 67 bpm.

Hypotheses

Based on literature overview, the following hypotheses were made:

- African men display a higher risk of sleep apnea when compared to Caucasian men, based on the Berlin questionnaire.
- African men display a higher incidence of self-reported insomnia when compared to Caucasian men.
- African men have a higher nocturnal heart rate when compared to Caucasian men.
- The Berlin sleep apnea risk and self-reported insomnia is positively associated with blood pressure and an increased ECG Cornell Product, this association being stronger in men with an increased nocturnal heart rate.

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CHAPTER 3

Manuscript

Instructions for authors: Clinical and Experimental Hypertension

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Abstract: Should not exceed 200 words. Abbreviations, diagrams and references to the text should be avoided.

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Chapter in Book: Kaplan NM, Ed. Primary hypertension: natural history and evaluation. In: Kaplan's Clinical Hypertension, 8th ed. Philadelphia: Lippincott Williams & Wilkins, 2002,163.

Conference proceeding: Kario K, Rapoport D. Sleep-disordered breathing as a determinant of non-dipping status of nocturnal blood pressure independent of age and body mass index. The 73rd Scientific Sessions, American Heart Association, Atlanta, GA, November 12-15, 2000.

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Exploring the link between nocturnal heart rate, sleep apnea and cardiovascular function in African and Caucasian men: the SABPA study

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Running head title: The link between sleep apnea and cardiovascular function.

ABSTRACT

Aim: Obstructive sleep apnea is regarded as a risk factor for cardiovascular disease such as hypertension and is associated with an increase in sympathetic nervous system activity. We aimed to determine whether the risk of sleep apnea and self-reported insomnia are independently associated with nocturnal blood pressure and ECG Cornell product in African and Caucasian men with an elevated nocturnal heart rate (n=189). *Methods:* The sleep apnea risk and insomnia was established by the Berlin Questionnaire and ambulatory diary card, respectively. Nocturnal blood pressure and heart rate data was obtained from ambulatory blood pressure monitoring. African and Caucasian men were subdivided into two groups based on the nocturnal heart rate. A standard 12-lead resting ECG was recorded. ECG left ventricular mass was determined by the Cornell product. *Results:* The risk for sleep apnea did not differ significantly between African and Caucasian men but the prevalence of self-reported insomnia was significantly higher in African men. There was no significant association between the Berlin sleep apnea risk and the cardiovascular variables but self-reported insomnia was associated with an increased nocturnal systolic blood pressure ($r = 0.469$, $p = 0.001$) and nocturnal diastolic blood pressure ($r = 0.499$, $p \leq 0.001$) in African men with a higher nocturnal heart rate. *Conclusions:* African and Caucasian men have the same risk for sleep apnea, based on the Berlin Questionnaire. Self-reported insomnia is associated with an increase in nocturnal blood pressure in African men with an elevated nocturnal heart rate.

Word count: 228

Keywords: Obstructive sleep apnea, insomnia, Berlin Questionnaire, nocturnal heart rate, nocturnal blood pressure, ECG Cornell product.

INTRODUCTION

Cardiovascular disease (CVD) is the dominant originator of mortality in people from developing regions worldwide (1). A number of socio-economic factors in Sub-Saharan Africa are currently facilitating a shift of previously isolated rural groups into increasingly urban areas and large parts of Africa are undergoing an epidemiological transition (1). Epidemiological transition is associated with an upsurge of factors giving rise to CVD and convincing evidence exists that CVD has become a challenging public health issue in Sub-Saharan Africa (2). According to estimation, premature deaths of working aged (35-64 years) South Africans, due to CVD, are expected to increase by 41% between 2000 and 2030 (3).

Obstructive sleep apnea (OSA) is a disorder in which repetitive apneas due to loss of pharyngeal dilator muscle tone during sleep, expose the cardiovascular system to detrimental effects such as cycles of hypoxia, augmented negative intra-thoracic pressure and frequent arousals (4). These frequent arousals that occur due to multiple breathing disturbances during sleep, lead to sleep fragmentation and insomnia (5) and a positive relationship between OSA and insomnia has been established (6).

There is an independent association between OSA and CVD and this association is especially strong for hypertension (7). OSA has also been identified as an independent risk factor for myocardial and cerebral infarction (8). Short periods of disturbed sleep, which may be caused by insomnia, has recently been found to also be associated with an increased risk for CVD (9-11) and could possibly predispose to factors that increase the mortality risk.

OSA as well as the associated insomnia has been found to be related to abnormalities in cardiovascular variability such as enhanced adrenalin secretion, (12) an increase in diurnal as well as in nocturnal heart rate (13) and a reduction in heart rate variability (14). Nocturnal non-dipping of the heart rate has been shown to increase the future risk of CVD (15).

These changes in cardiovascular variability that occur in OSA as well as in insomnia originate in an enhanced sympathetic activity that has been found in both these conditions (13,16). The enhancement of sympathetic activity in OSA may occur due to chemo reflex-mediated hypoxic stimulation, resulting in an increase in the peripheral vascular resistance as well as in increases in nocturnal blood pressure (16). OSA might be a contributing factor in the nocturnal non-dipping of blood pressure that is associated with hypertension and may lead to hypertensive end-organ damage such as left ventricular hypertrophy (17,18).

Ethnicity has recently been identified as an accompanying risk factor for OSA, along with the previous known risk factors which include male gender, obesity and increasing age (19). It has been established that sleep apnea is more frequent in African Americans than in Caucasians (20), however studies comparing the frequency of OSA between African and Caucasian men in South Africa, as well as the accompanying cardiovascular effects of this syndrome have been limited. There have also been a limited amount of studies investigating the cardiovascular effects of OSA in African and Caucasian men with an increased nocturnal heart rate compared to those with a normal nocturnal heart rate. Therefore, we aimed to explore the link between nocturnal heart rate, sleep apnea and cardiovascular function in an urban group of African and Caucasian men.

METHODS

Participants and study design

This study forms part of the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) study which commenced in February, 2008 and was implemented on urbanized Africans for 10 weeks (Phase I). Seasonal effects were minimized by the repetition of the same protocol on urban Caucasian South Africans in February 2009 (Phase II). A total of 200 apparently healthy African and 209 Caucasian school teachers from the Dr. Kenneth Kaunda Education District of the North-West Province of South Africa were included as participants. These participants were of the same socio-economic status, aged between 25 and 65 years which ensured a homogenous study sample. Criteria according to which participants were excluded were an elevated ear temperature, pregnancy, lactation, blood donation and the use of psychotropic substances. For the purpose of this study, female participants as well as HIV positive participants were excluded and the final study sample consisted of 88 African men and 101 Caucasian men. The median of the nocturnal heart rate of the 189 men was calculated and the African and Caucasian men were divided into two separate groups, namely those with a nocturnal heart rate ≥ 67 bpm and those with a nocturnal heart rate < 67 bpm.

Permission for participation was granted by the North-West Department of Education and the South African Democratic Teachers Union. The study was approved by the Research Ethics Committee of the North-West University. All participants were informed of the procedures regarding the study, prior to their recruitment. The participants who wished to participate were asked to sign the study informed consent form, which is in compliance to the World Medical Association Declaration of Helsinki.

Organizational procedures

An Ambulatory Blood Pressure Monitoring (ABPM) apparatus (Meditech CE120® Cardiotens, Budapest, Hungary) was fitted to the non-dominant arm of four participants each morning between 0700 hours and 0800 hours from Monday to Thursday. The apparatus measured the blood pressure at 30 minute intervals during the day and 60 minute intervals during the night. Each participant's physical activity was assessed by fitting the Actical accelerometer (Mini-Mitter Co., Inc, Montréal, Québec, Canada) to each participant during a normal working day. Participants were transported to the Metabolic Unit Research Facility of the North-West University at 1630 hours and after having been familiarized with the protocol, received HIV pre-counselling and completed the Berlin Sleep Apnea Risk questionnaire. A high risk or low risk for sleep apnea was based on the response in three different categories of the Berlin Questionnaire. In category 1, a high risk was defined as persistent symptoms (in excess of 3 to 4 times a week) in two or more questions regarding snoring. A high risk in category 2 was defined as persistent (in excess of 3 to 4 times a week) daytime sleepiness, drowsiness while driving, or both. A high risk in category 3 was defined as a positive history of high blood pressure or a body mass index exceeding 30 kg/m². An overall high risk for sleep apnea was defined as a high risk result for at least two of the categories. The participants also completed an ambulatory diary card during the time they had to wear the Cardiotens®, where they reported on insomnia (hours awake per night). The participants received a standardized dinner at 2030 hours and abstained from consuming alcohol and caffeine. Participants were not allowed to smoke after this time and were encouraged to be in bed by approximately 2200 hours. The participants were woken at 0545 hours the next morning and the Cardiotens® apparatus was removed after the last recording was made at 0600 hours. A urine sample was taken and the anthropometric data was collected.

Anthropometric measurements

Anthropometric data was obtained by registered biokineticists. A stadiometer (Invicta Stadiometer, IP 1465, London, U.K.) was used for the assessment of each participant's stature. The mass of each participant was determined by use of a Precision Health Scale (A & D Company, Japan). The body mass index (BMI) was calculated afterwards according to the height and weight of each participant.

Cardiovascular measurements

After the anthropometric data was collected, continuous blood pressure monitoring was performed by use of the Finometer (Finapres Medical Systems, Amsterdam, the Netherlands) (21,22). A 5 minute blood pressure recording of each participant, under resting conditions, was performed. The finger pressure was calibrated with the brachial pressure (return-to-flow systolic calibration) after the first two minutes. The baroreflex sensitivity (BRS) was calculated by use of the cross-correlation baroreflex sensitivity method (23). A standard 12-lead ECG (PC 1200, v5.030, Norav Medical, Yokneam, Israel) was recorded during rest and the ECG left ventricular hypertrophy was determined by using the Cornell product (24,25). The ABPM data as well as the ECG data was imported to a database by using CardioVisions 1.15 Personal Edition (Meditech, Budapest, Hungary). In instances where 75% of the recordings of a participant were not successful, the ABPM recordings were repeated the following day.

Biochemical samples

A fasting venous blood sample was collected by a registered nurse from the ante brachial vein branches with a winged infusion set. The plasma and serum samples from fasting blood were stored at - 80°C prior to the analysis of the biochemical markers. Serum samples for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), sodium fluoride glucose (NaF), high sensitivity C-reactive protein (hs-CRP), and γ -glutamyltransferase (GGT) were analysed by two sequential multiple analyzers (Konelab 20i, Thermo Scientific, Vantaa, Finland, Unicel DXC 800- Beckman and Coulter®, Germany). Serum cotinine levels were determined as a marker of tobacco use with a homogenous immunoassay (Integra 400, Roche, Basel, Switzerland).

Statistical analysis

Statistical analyses were performed using Statistica version 10.0 (Statsoft, Inc., Tulsa, OK, USA, 2009). Variables that did not display a normal distribution were logarithmically transformed and expressed as the geometric mean and the 5th and 95th percentile intervals. Independent *t*-tests and Chi-square tests were used to compare means and proportions, respectively. A median split of the nocturnal heart rate was performed to divide the men into four groups: African men with a nocturnal heart rate ≥ 67 bpm ($n=51$), Caucasian men with a nocturnal heart rate ≥ 67 bpm ($n=31$), African men with a nocturnal heart rate ≥ 67 bpm ($n=37$) and Caucasian men with a nocturnal heart rate < 67 bpm ($n=70$). Partial correlations between the variables of the African and Caucasian men were performed with adjustment for confounders, including age, body surface area, physical activity, cotinine and GGT. Forward stepwise regression analysis was performed, with the ECG Cornell product, nocturnal SBP and nocturnal DBP as the dependent variables to investigate the adjusted associations between these three variables,

the Berlin sleep apnea risk, self-reported insomnia and other cardiovascular variables. A p-value was regarded as significant when $p \leq 0.05$.

RESULTS

Characteristics of the study population

As presented in table 3.1, the age of the African and Caucasian men were similar. With regards to the anthropometric measurements, the body mass index (BMI) of the African and Caucasian men did not differ significantly but Caucasian men displayed a significantly higher neck circumference. African men showed a more unfavorable cardiovascular profile compared to Caucasian men, with significantly higher values for 24h SBP (137 ± 16.6 vs. 128 ± 10.4 mmHg; $p \leq 0.001$), 24h DBP (88 ± 11.2 vs. 80.74 mmHg; $p \leq 0.001$) and the 24h HR (79 ± 11.4 vs. 72 ± 11.1 bpm; $p \leq 0.001$). The nocturnal blood pressure and heart rate values of the African and Caucasian men were also significantly different, with African men having a significantly higher nocturnal SBP (129 ± 18.7 vs. 117 ± 11.6 mmHg; $p \leq 0.001$), nocturnal DBP (79 ± 12.8 vs. 69 ± 8.3 mmHg; $p \leq 0.001$) and nocturnal HR (71 ± 11.3 vs. 63 ± 11.0 bpm; $p \leq 0.001$). The ECG Cornell product was also significantly higher in the African men. The BRS (9.35 ± 7.36 vs. 10.4 ± 6.77 ms/mmHg; $p = 0.336$) of the African and Caucasian men were similar. The high risk of sleep apnea based on the Berlin Questionnaire, did not show a significant difference between African and Caucasian men but the occurrence of insomnia, based on self-reported hours a night that one is awake, was significantly higher in African men when compared to Caucasian men (0.91 ± 1.20 vs. 0.30 ± 0.53 h; $p \leq 0.001$).

Adjusted analysis

The partial correlations between the dependent variables, nocturnal blood pressure and the ECG Cornell product, and the independent variables were performed separately in African and Caucasian men with a nocturnal heart rate ≥ 67 bpm (Table 3.2a) and a nocturnal heart rate < 67 bpm (Table 3.2b) adjusting for age, body surface area, physical activity, alcohol consumption and tobacco use as confounders. In table 3.2a there was a significant positive association between self-reported insomnia and nocturnal SBP ($r = 0.469$, $p = 0.001$) as well as nocturnal DBP ($r = 0.499$, $p \leq 0.001$) in African men. This association was not present in Caucasian men. There was no significant association between the Berlin Questionnaire and any of the dependent variables in Africans or Caucasians with a nocturnal heart rate < 67 bpm. However, a negative association ($r = -0.266$, $p = 0.040$) between the Berlin Questionnaire and the nocturnal DBP was present in Caucasian men with a nocturnal heart rate ≥ 67 bpm (Table 3.2b).

Forward stepwise regression analysis was performed separately for each of the dependent variables, ECG Cornell product (Table 3.3a), nocturnal SBP (Table 3.2b) and the nocturnal DBP (Table 3.3c), adjusting for confounders. There was no relationship between the ECG Cornell product, self-reported insomnia and the Berlin Questionnaire sleep apnea risk in African or Caucasian men (Table 3.3a). Self-reported insomnia predicted a higher nocturnal SBP in African men with a nocturnal heart rate ≥ 67 bpm ($\beta = 0.452$, $p = 0.003$) and this relationship was not displayed in Caucasian men (Table 3.3b). There was also no relationship between the nocturnal SBP or the nocturnal DBP and the Berlin Questionnaire sleep apnea risk in African or Caucasian men. Self-reported insomnia also predicted an increase in the nocturnal DBP ($\beta = 0.517$, $p = 0.001$) in African men with a nocturnal heart rate ≥ 67 bpm and this relationship was also not present in Caucasian men (Table 3.3c).

Table 3.1: Characteristics of the study population.

	African men	Caucasian men	p-value
N	88	101	
Age (years)	43 ± 8.24	45 ± 11.1	0.187
<i>Anthropometric measurements</i>			
Body mass index (kg/m ²)*	27.0 (20.2;37.7)	28.6 (22.0;41.1)	0.070
Neck circumference (cm)*	37.6 (33.2;42.7)	40.8 (36.1;46.4)	≤ 0.001
Body surface area (m ²)	1.94 ± 0.23	2.18 ± 0.21	≤ 0.001
<i>Biochemical markers</i>			
TC:HDL (mmol/L)	5.05 ± 2.57	5.88 ± 1.49	0.006
Triglycerides (mmol/L)*	1.44 (0.64;4.96)	1.30 (0.58;3.01)	0.113
NaF glucose (mmol/L)	6.07 ± 2.13	5.98 ± 0.90	0.684
C-Reactive protein (mg/L)*	2.75 (0.27;16.1)	1.80 (0.99;8.00)	0.001
ROS (mg/L)*	80.5 (50.9;114.0)	75.2 (55.2;106)	0.017
<i>Cardiovascular measurements</i>			
BRS (ms/mm Hg)	9.35 ± 7.36	10.4 ± 6.77	0.336
24 h SBP (mmHg)	137 ± 16.6	128 ± 10.4	≤ 0.001
24 h DBP (mmHg)	88 ± 11.2	80 ± 7.4	≤ 0.001
24 h HR (bpm)	79 ± 11.4	72 ± 11.1	≤ 0.001
Nocturnal SBP (mmHg)	129 ± 18.7	117 ± 11.6	≤ 0.001
Nocturnal DBP (mmHg)	79 ± 12.8	69 ± 8.3	≤ 0.001
Nocturnal HR (bpm)	71 ± 11.3	63 ± 11.0	≤ 0.001
Non-dippers, n %	30.3	34.7	0.038
ECG Cornell product (mV)	82.1 ± 47.9	64.1 ± 27.8	0.002
<i>Sleep apnea and insomnia variables</i>			
Berlin Questionnaire high risk for sleep apnea, n %	25.1	37.9	0.714
Self-reported insomnia (hours awake per night)	0.91 ± 1.20	0.30 ± 0.53	≤ 0.001
<i>Lifestyle factors</i>			
Cotinine (ng/ml)*	0.046 (0.01;151)	0.008 (0.01;243)	0.082
GGT (U/L)*	62.3 (23.6;280)	27.3 (11.0;90.0)	≤ 0.001
Physical activity (kcal)*	2585 (1725;4021)	3478 (2547;4679)	≤ 0.001

With TC as total cholesterol, HDL-c as high-density lipoprotein cholesterol, ROS as reactive oxygen species, BRS as baroreflex sensitivity, SBP as systolic blood pressure, DBP as diastolic blood pressure, HR as heart rate, ECG as electro cardiogram, GGT as serum gamma glutamyltransferase. p-value as the statistical test of difference in effects between Africans and Caucasians with $p \leq 0.05$ regarded as significant.* Log transformed values expressed as geometric mean ± CI (5%-95%)

Table 3.2a: Partial correlations of the independent sleep variables with the dependent variables, Nocturnal BP and the ECG Cornell product (mV), in men with a nocturnal heart rate ≥ 67 bpm.

African men (n=51)				Caucasian men (n=31)		
	Nocturnal SBP (mmHg)	Nocturnal DBP (mmHg)	ECG Cornell product (mV)	Nocturnal SBP (mmHg)	Nocturnal DBP (mmHg)	ECG Cornell product (mV)
Nocturnal SBP (mmHg)			$r = 0.333$ $p = \mathbf{0.031}$			$r = -0.181$ $p = 0.386$
Nocturnal DBP (mmHg)			$r = 0.210$ $p = 0.182$			$r = -0.025$ $p = 0.908$
BRS (ms/mmHg)	$r = 0.154$ $p = 0.330$	$r = 0.201$ $p = 0.203$	$r = 0.120$ $p = 0.449$	$r = 0.289$ $p = 0.162$	$r = 0.160$ $p = 0.445$	$r = -0.175$ $p = 0.402$
Nocturnal HR (bpm)	$r = 0.248$ $p = 0.097$	$r = 0.126$ $p = 0.405$	$r = -0.006$ $p = 0.969$	$r = -0.228$ $p = 0.262$	$r = -0.341$ $p = 0.089$	$r = -0.217$ $p = 0.298$
Berlin Questionnaire Sleep Apnea Risk	$r = -0.079$ $p = 0.656$	$r = -0.152$ $p = 0.390$	$r = -0.321$ $p = 0.073$	$r = 0.058$ $p = 0.778$	$r = -0.082$ $p = 0.690$	$r = 0.082$ $p = 0.696$
Self-reported insomnia (hours awake per night)	$r = 0.469$ $p = \mathbf{0.001}$	$r = 0.499$ $p \leq \mathbf{0.001}$	$r = 0.105$ $p = 0.507$	$r = -0.125$ $p = 0.542$	$r = 0.157$ $p = 0.445$	$r = 0.102$ $p = 0.626$

Where BP= blood pressure, BPM = beats per minute, ECG= electrocardiogram, SBP = systolic blood pressure, DBP = diastolic blood pressure, BRS = baroreflex sensitivity, HR = heart rate. P-values ≤ 0.05 regarded as significant, indicated by bold text.

Adjusted for age, body surface area, physical activity, cotinine and GGT

Table 3.2b: Partial correlations of the independent sleep variables with the dependent variables, Nocturnal blood pressure and the ECG Cornell product (mV), in men with a nocturnal heart rate < 67 bpm.

African men (n=37)			Caucasian men (n=70)			
	Nocturnal SBP (mmHg)	Nocturnal DBP (mmHg)	ECG Cornell product (mV)	Nocturnal SBP (mmHg)	Nocturnal DBP (mmHg)	ECG Cornell product (mV)
Nocturnal SBP (mmHg)			$r = 0.557$ $p = \mathbf{0.005}$			$r = 0.023$ $p = 0.864$
Nocturnal DBP (mmHg)			$r = 0.505$ $p = \mathbf{0.012}$			$r = 0.087$ $p = 0.511$
Nocturnal HR (bpm)	$r = 0.094$ $p = 0.663$	$r = 0.013$ $p = 0.953$	$r = 0.108$ $p = 0.617$	$r = -0.013$ $p = 0.919$	$r = 0.071$ $p = 0.589$	$r = -0.129$ $p = 0.326$
BRS (ms/mmHg)	$r = -0.138$ $p = 0.530$	$r = -0.154$ $p = 0.482$	$r = -0.175$ $p = 0.424$	$r = -0.068$ $p = 0.612$	$r = 0.010$ $p = 0.938$	$r = 0.395$ $p = \mathbf{0.002}$
Berlin Questionnaire Sleep Apnea Risk	$r = 0.332$ $p = 0.153$	$r = 0.315$ $p = 0.176$	$r = -0.111$ $p = 0.640$	$r = -0.174$ $p = 0.184$	$r = -0.266$ $p = \mathbf{0.040}$	$r = -0.158$ $p = 0.227$
Self-reported insomnia (hours awake per night)	$r = -0.063$ $p = 0.771$	$r = -0.118$ $p = 0.582$	$r = 0.156$ $p = 0.466$	$r = -0.136$ $p = 0.299$	$r = -0.051$ $p = 0.702$	$r = 0.235$ $p = 0.071$

Where BP= blood pressure, BPM= beats per minute, ECG= electrocardiogram, SBP = systolic blood pressure, DBP = diastolic blood pressure, BRS = baroreflex sensitivity, HR = heart rate. P-value ≤ 0.05 regarded as significant, indicated by bold text.

Adjusted for age, body surface area, physical activity, cotinine and GGT.

Table 3.3a: Forward stepwise regression analysis with the ECG Cornell product (mV) as the dependent variable

	African men		Caucasian men	
	Nocturnal HR \geq 67 bpm	Nocturnal HR < 67 bpm	Nocturnal HR \geq 67 bpm	Nocturnal HR < 67 bpm
	ECG Cornell Product (mV)	ECG Cornell Product (mV)	ECG Cornell Product (mV)	ECG Cornell Product (mV)
Independent variables	$R^2 = 0.281$	$R^2 = 0.368$	$R^2 = 0.041$	$R^2 = 0.327$
Age (years)				
BSA (m ²)				$\beta = -0.317$ $p = 0.005$
Nocturnal SBP (mmHg)	$\beta = 0.249$ $p = 0.103$	$\beta = 0.591$ $p = 0.012$		
BRS (ms/mmHg)				$\beta = 0.361$ $p = 0.001$
Berlin Questionnaire Sleep Apnea Risk	$\beta = -0.301$ $p = 0.053$	$\beta = -0.299$ $p = 0.093$		
Self-reported insomnia (hours awake per night)				$\beta = 0.156$ $p = 0.158$
GGT (U/L)	$\beta = -0.155$ $p = 0.320$		$\beta = 0.268$ $p = 0.160$	
Cotinine (ng/ml)				$\beta = -0.181$ $p = 0.095$
Physical activity (kcal)		$\beta = -0.338$ $p = 0.085$	$\beta = 0.250$ $p = 0.190$	

Independent variables included in model: Age, BSA, Nocturnal SBP, BRS, Berlin Questionnaire Sleep Apnea Risk, Self-reported insomnia, GGT, Cotinine and physical activity. With BSA as body surface area, SBP as systolic blood pressure, DBP as diastolic blood pressure, BRS as baroreflex sensitivity, HR as heart rate and GGT as gamma glutamyltransferase. P-value ≤ 0.05 regarded as significant, indicated by bold text.

Table 3.3b: Forward stepwise regression analysis with the Nocturnal SBP (mmHg) as the dependent variable

Independent variables	African men		Caucasian men	
	Nocturnal HR \geq 67 bpm	Nocturnal HR < 67 bpm	Nocturnal HR \geq 67 bpm	Nocturnal HR < 67 bpm
	Nocturnal SBP $R^2=0.252$	Nocturnal SBP $R^2=0.403$	Nocturnal SBP $R^2=0.339$	Nocturnal SBP $R^2=0.207$
Age (years)				$\beta = -0.178$ $p = 0.123$
BMI (kg/m ²)	$\beta = 0.240$ $p = 0.098$		$\beta = 0.436$ $p = 0.007$	
BRS (ms/mmHg)		$\beta = -0.148$ $p = 0.373$	$\beta = 0.349$ $p = 0.048$	
Berlin Questionnaire Sleep Apnea Risk		$\beta = 0.214$ $p = 0.210$		$\beta = -0.149$ $p = 0.212$
Self-reported insomnia (hours awake per night)	$\beta = 0.452$ $p = 0.003$		$\beta = -0.336$ $p = 0.057$	$\beta = -0.138$ $p = 0.213$
GGT (U/L)		$\beta = 0.252$ $p = 0.151$		$\beta = 0.171$ $p = 0.154$
Cotinine (ng/ml)			$\beta = -0.174$ $p = 0.277$	$\beta = 0.299$ $p = 0.009$
Physical activity (kcal)		$\beta = 0.380$ $p = 0.024$		

Independent variables included in model: Age, BSA, BRS, Berlin Questionnaire Sleep Apnea Risk, Self-reported insomnia, GGT, Cotinine and physical activity. With BMI as body mass index, SBP as systolic blood pressure, BRS as baroreflex sensitivity, HR as heart rate and GGT as gamma glutamyltransferase. P-value ≤ 0.05 regarded as significant, indicated by bold text.

Table 3.3c: Forward stepwise regression analysis with the Nocturnal DBP (mmHg) as the dependent variable

	African men		Caucasian men	
	Nocturnal HR \geq 67 bpm	Nocturnal HR < 67 bpm	Nocturnal HR \geq 67 bpm	Nocturnal HR < 67 bpm
	Nocturnal DBP $R^2=0.278$	Nocturnal DBP $R^2=0.356$	Nocturnal DBP $R^2=0.288$	Nocturnal DBP $R^2=0.186$
Independent variables				
Age (years)		$\beta = 0.217$ $p = 0.197$	$\beta = 0.316$ $p = 0.060$	
BMI (kg/m ²)			$\beta = 0.502$ $p = 0.004$	
Berlin Questionnaire Sleep Apnea Risk	$\beta = -0.212$ $p = 0.131$	$\beta = 0.275$ $p = 0.112$		$\beta = -0.253$ $p = 0.058$
Self-reported insomnia (hours awake per night)	$\beta = 0.517$ $p = 0.001$			
GGT (U/L)		$\beta = 0.341$ $p = 0.059$		$\beta = 0.194$ $p = 0.105$
Cotinine (ng/ml)			$\beta = -0.200$ $p = 0.216$	$\beta = 0.211$ $p = 0.063$
Physical activity (kcal)		$\beta = 0.406$ $p = 0.020$		

Independent variables included in model: Age, BSA, BRS, Berlin Questionnaire Sleep Apnea Risk, Self-reported insomnia, GGT, Cotinine and physical activity. With BMI as body mass index, DBP as diastolic blood pressure, HR as heart rate and GGT as gamma- glutamyltransferase. P-value ≤ 0.05 regarded as significant, indicated by bold text.

DISCUSSION:

The aim of this study was to determine whether the risk of sleep apnea and self-reported insomnia are independently associated with nocturnal blood pressure and ECG Cornell product in African and Caucasian men with an elevated nocturnal heart rate.

The main finding of this study is that the incidence of self-reported insomnia was significantly higher in African men, when compared to Caucasian men. It has been established that insomnia symptoms are common in those suffering from OSA (5), and this result confirms previous findings from the CARDIA study (26), which indicated that African Americans had a lower average sleep duration when compared to Caucasians.

A further key finding of this study was that the self-reported insomnia was significantly related to both the nocturnal SBP and DBP in African men with a nocturnal heart rate ≥ 67 bpm. This relationship was absent in the Caucasian men with the same nocturnal heart rate. This relationship was also not evident in the African men with a nocturnal heart rate that was lower than 67 bpm. In the multiple regression analysis with the nocturnal SBP and nocturnal DBP as dependent variables, self-reported insomnia predicted an increase in both the nocturnal SBP and DBP.

A possible mechanism that could contribute to this finding is that of altered autonomic function caused by an increase in the activity of the sympathetic nervous system, which is associated with increases in both diurnal and nocturnal heart rate. Insomnia is a condition that has previously been linked to a state of physiological hyperactivity and an increase in sympathetic activity in this condition has been confirmed by an increase in heart rate and adrenalin secretion. Malan et al. (27) reported an enhanced sympathetic nervous system activity of Africans during urbanization which may be a possible explanation as to why the occurrence of insomnia is higher in the African men and why the relationship of the insomnia with the nocturnal blood pressure is only present when the nocturnal heart rate is increased. The positive relationship between insomnia and

the nocturnal blood pressure may possibly be enhanced by the increased sympathetic activity associated with insomnia, increasing the blood pressure by the vasoconstrictor effects of the catecholamines (4). This result may further support the findings of Van Rooyen et al (28), which indicated that African men exhibit exaggerated peripheral resistance responses, which especially occur during high stress situations such as urbanization.

Contradictory to previous studies which found that African Americans are at a greater risk for sleep apnea (29,30), there was no significant difference between the high risk of sleep apnea that was based on the Berlin Questionnaire, between the African and Caucasian men in this study. This finding may in part be due to the fact that the risk for sleep apnea in this study was only determined by a questionnaire and sleep laboratory polysomnography remains the golden standard for the diagnosis of sleep apnea (31). Although polysomnography is needed for the definitive diagnosis of OSA, the Berlin Questionnaire still serves as an important diagnostic tool and has been validated to identify patients at risk for OSA (32). Due to the high cost and limited availability of polysomnography, the screening of a large undiagnosed population may prove to be more cost effective.

Furthermore, another prominent result of this study showed that the Berlin Questionnaire risk for sleep apnea, did not display any association with either the nocturnal blood pressure or the ECG Cornell product. This result is in contradiction to previous findings where it has been stated that OSA and hypertension often co-exist in an individual (33) and it could be expected that a risk for sleep apnea may possibly predict an increase in blood pressure. However, in the multiple regression analyses that included the nocturnal blood pressure, the Berlin Questionnaire sleep apnea risk did not enter the final model in any of the groups.

The lack of association between the Berlin sleep apnea risk and the cardiovascular variables may possibly be due to insufficient validation of the questionnaire in African populations. Validation of the questionnaire has been performed in western Caucasian

populations and further validation may need to be done in large samples of the African population to determine the sensitivity and specificity of the questionnaire to predict sleep apnea in this population. It has recently been shown that the use of the Berlin Questionnaire for the detection of OSA in hypertensive patients may not be a dependable diagnostic tool, due to a lack of sufficient questions regarding hypertension. Lisi et al., suggest that a specific version of the Berlin Questionnaire or a scoring criterion that is applicable to hypertensive subjects may be needed for a reliable screening of OSA in a hypertensive study sample (34).

African populations have been described to have an increased left ventricular mass (35) and it has been found that ECG left ventricular hypertrophy contributed to a risk of cardiovascular mortality that was more pronounced in Africans than in Caucasians (36). These findings were supported by this study and the ECG Cornell product of the African men was significantly higher, compared to the Caucasian men. However, although the African men displayed a significant higher ECG Cornell product compared to Caucasian men, the positive association of OSA and left ventricular hypertrophy that was established by previous studies (37-40), was not present in this study.

Our findings may be of clinical importance in the treatment of hypertension, especially in the African population. Our results indicate that there is an ethnic-specific difference in the prevalence of insomnia in this study population and that the relationship between insomnia and nocturnal blood pressure is evidently strong in African men with an increased nocturnal heart rate. The early detection and treatment of insomnia could possibly play a role in the improvement of the cardiovascular health of African men by reducing the nocturnal blood pressure, facilitating a nocturnal dipping pattern in the blood pressure of African men, which could possibly reduce their risk of hypertension and related target end-organ damage such as left ventricular hypertrophy.

The strengths of our study included a highly controlled study environment, a wide range of cardiovascular measurements and the ability to compare the data of African men with Caucasian men to investigate any ethnic specific differences. However, our study does

pose potential limitations as well. This population comparative study prohibits the extrapolation of our data to a general population and could also not infer causality. Sleep apnea was not determined by polysomnography but by the validated Berlin Questionnaire. The determination of insomnia in this study was based on self-report and may pose possible reporting biases. Left ventricular mass was not determined by echocardiography, but by use of a validated electrocardiographic method, namely the Cornell product.

CONCLUSION

In conclusion, the data from this study indicates that self-reported insomnia was significantly higher among African men and was strongly associated with an increase in nocturnal blood pressure in the African men with an elevated nocturnal heart rate. The risk for sleep apnea as established by the Berlin Questionnaire does not differ significantly between African and Caucasian men. There is no significant relationship between the Berlin Questionnaire risk for sleep apnea, and the nocturnal blood pressure or ECG Cornell product in African or Caucasian men.

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CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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CHAPTER 4

Summary of main findings, limitations and
recommendations for future studies.

4.1 Summary of main findings

The general aim of this study was to determine whether the risk of sleep apnea and self-reported insomnia are independently associated with nocturnal blood pressure and ECG Cornell product in African and Caucasian men with an elevated nocturnal heart rate.

Upon completion of this study, it is now possible to review the hypotheses that were made and to determine whether the hypotheses were supported by the findings of this study.

Hypothesis 1: *African men display a higher risk of sleep apnea when compared to Caucasian men, based on the Berlin Questionnaire.*

No significant difference was encountered between the high risk for sleep apnea that was based on the Berlin Questionnaire, between the African and Caucasian men of this study population, therefore hypothesis 1 is rejected. There are a considerable number of epidemiological studies regarding the prevalence of OSA that have been done in various ethnic groups. In a study that was performed to determine ethnic differences in sleep-disordered breathing in African Americans and Caucasians it was found that sleep apnea is more frequent in African Americans (1). The result from our study is in contradiction to the above mentioned and studies regarding the prevalence of OSA in Africans have been limited. Our findings may be attributable to the lack of validation of the Berlin Questionnaire in African populations. The Berlin Questionnaire has been validated as a diagnostic tool in Western populations (2,3) but little evidence with regards to the specificity and sensitivity of the questionnaire to determine sleep apnea risk in African populations, exist. The screening of participants for the risk of sleep apnea with the Berlin Questionnaire and comparing this data to polysomnographic data of the same study sample can provide insight as to the sensitivity and specificity of the Berlin Questionnaire.

Hypothesis 2: *African men display a higher incidence of self-reported insomnia when compared to Caucasian men.*

African men displayed a significantly higher prevalence of self-reported insomnia, compared to Caucasian men. Limited research exists to support ethnic differences with regards to insomnia and results from studies that have addressed this have rendered controversial results. Our data can, however, be supported by findings from the CARDIA study that demonstrated that African-Americans have a lower sleep duration when compared to Caucasians (4). We can therefore accept this hypothesis based on the findings from this study.

Hypothesis 3: *African men have a higher nocturnal heart rate when compared to Caucasian men.*

The nocturnal heart rate of the African men was significantly higher compared to the Caucasian men in this study population and our hypothesis is accepted. Previous findings, including those from the SABPA study, have shown that Africans exhibit an increased sympathetic nervous system activity during situations of high stress, such as urbanization (5,6). It has been demonstrated that an increase in the activity of the sympathetic nervous system may be suggested by an increase in heart rate, especially during sleep (7). The data from our study might suggest that African men display an increase in sympathetic nervous system activity, which can be seen in changes in cardiovascular variability, such as the nocturnal heart rate (8). Non-dipping of the nocturnal heart rate has been associated with a 2.4 times greater risk for future CVD (9) and early detection may prove valuable in the improvement of the cardiovascular health of the African population, however, the result from our study needs to be confirmed by other studies in African populations.

Hypothesis 4: The Berlin sleep apnea risk and self-reported insomnia is positively associated with blood pressure and an increased ECG Cornell Product, this association being stronger in men with an increased nocturnal heart rate.

After dividing the African men and the Caucasian men separately into those with a nocturnal heart rate ≥ 67 bpm and those with a nocturnal heart rate < 67 bpm by a median split, a highly significant positive relationship was found between self-reported insomnia and both the nocturnal SBP and DBP in the African men with an increased nocturnal heart rate. Our result is in accordance with studies that have found that insomnia is associated with an increased risk for hypertension (10). This relationship was not present in the African men with a normal nocturnal heart rate or the Caucasian men. Self-reported insomnia predicted an increase in the nocturnal SBP and DBP in African men with an increased nocturnal heart rate after adjusting for confounders, however there was no association between insomnia and the ECG Cornell Product. It is evident that the co-morbidity of insomnia should also be taken into account when studying the cardiovascular effects of OSA, especially hypertension.

No relationship was found between the Berlin Questionnaire sleep apnea risk and any of the cardiovascular variables. Our result is in contradiction to previous studies that have demonstrated a positive association between OSA and hypertension, as well as left ventricular hypertrophy. The lack of association may be explained by a lack of validation of the questionnaire in our study sample, as previously discussed. Our hypothesis can only be partly accepted, due to the lack of association between the Berlin Questionnaire and the independent variables.

4.2 Strengths and limitations and recommendations for future studies.

Strengths and limitations

The strengths of our study are based on a highly controlled study environment. All participants spent the night at the Metabolic Unit of the North-West University, Potchefstroom Campus. The participants underwent an extensive range of cardiovascular measurements that were conducted by trained professionals. The participants were of the same socio-economic status, aged between 25 and 65 years, which ensured a homogenous study sample. The collection of data from African and Caucasian participants made it possible to compare the data to determine any ethnic-specific differences. However, our study does pose potential limitations. Self-reported insomnia was determined by means of the diary card that is provided for ambulatory blood pressure measurement. The hourly blood pressure measurements may therefore have an influence on the amount of time that a participant is awake. The cross-sectional design of our study prohibits the extrapolation of our data to a general population and it is recommended that a longitudinal study with large population samples should be performed, making it possible to determine causality. Our study consisted of a group of urban school teachers and collection of the same data from a rural population sample may provide insight as to the effect of urbanization on sleeping patterns and the association with cardiovascular function. OSA has been associated with various other forms of target organ damage, such as endothelial dysfunction (11) and atherosclerosis (12) and investigating the association of OSA with these markers in the African population may provide further valuable information on the physiological effects of OSA.

Confounding

Although provision was made for the adjustment for several confounding factors, which included age, body composition, physical activity, tobacco use and alcohol consumption, there still remains other factors that may have induced a confounding effect on the data of our study. The use of the Berlin Questionnaire and self-reported insomnia may have had reporting biases and to exclude the effect of bias, a direct method such as a sleep laboratory study can be performed. Upon dividing the African and Caucasian men into subgroups based on the nocturnal heart rate, the groups were small, although they were a highly specific study sample. Participants that were under the treatment of anti-hypertensive medication were not excluded. The confounding effect of hypertension with regards to the Berlin Questionnaire as a screening tool (13) should be considered and in future studies it may prove to be beneficial to exclude hypertensive participants from the study sample.

Recommendations for future studies

- The determination of insomnia by means of a more direct method such as sleep laboratory studies.
- The determination of OSA by utilizing the gold standard of polysomnography.
- The validation of the Berlin Questionnaire in African populations as well as hypertensive population groups.
- Comparing data from an urban population group with a rural population group to determine whether socio-economic factors contribute to the prevalence of OSA and insomnia and the effect of these conditions on the cardiovascular system.

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APPENDIX

Participant information and informed consent form
of the SABPA study, General Health
Questionnaire of the SABPA study, Ambulatory
diary card of the SABPA study, Berlin
Questionnaire.

Appendix A

**NORTH-WEST UNIVERSITY
POTCHEFSTROOM CAMPUS
SCHOOL FOR PHYSIOLOGY, NUTRITION AND CONSUMER SCIENCES
PARTICIPANT INFORMATION AND CONSENT FORM**

PART 1

PRINCIPAL RESEARCHER: Dr Leoné Malan, Subject Group Physiology

PROJECT LEADER: Dr. Leoné Malan, Subject Group Physiology

Associate Researcher(s): The postdoctoral fellow involved in this trial is Dr. Szabolcs Péter. Other persons assisting in the study are Dr. Hugo W. Huisman, Prof. Johannes M. van Rooyen, Prof. Nico T. Malan, Dr R Schutte, Mrs. Carla M.T. Fourie, Mrs. Tina Scholtz (Cardiovascular research group, Physiology), Prof. Salomé Kruger & Dr. Ramoteme Mamabolo, (Physical activity), Proff. Hans de Ridder (Anthropometry), Marié Wissing (Psychology), Linda Brand & Brian Harvey (Pharmacology), Kobus Mentz (Education), Francois van der Westhuizen (Biochemistry), Hester Klopper (Nursing), Nancy Frasure-Smith & Francois Lespérance (Psychology, Canada), Alaa Alkerwi (Epidemiology, Luxembourg), Yackoob Seedat (ECG, Kwazulu Natal), Paul Rheeder (Sonar, Pretoria University), Drs. Johan Potgieter & Michael Temane & Mr Thumi Khumalo (Psychology), Mrs Gedina de Wet (Nursing).

This Participant Information and Consent Form is **7** pages long. Please make sure you have all the pages.

Your Consent

You are invited to take part voluntarily in this research project.

This participant information document contains detailed information about the research project which has been explained to you verbally. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part.

Please read this *Participant Information Form* carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information and Consent Form to keep as a record.

What is the study about?

The aim of this project is to have an impact on the eventual prevention and treatment of lifestyle diseases in Africans from South Africa. New knowledge regarding the relationship between higher nervous system activity implicating cardiovascular, metabolic and psychological well-being will improve understanding and change strategies at the roots of treatment and prevention of lifestyle diseases.

Our research has shown that lifestyle diseases in urbanised Africans present higher obesity levels, high blood pressure or hypertension prevalence rates and the experiencing of more stress. This pattern is enhanced during psychosocial stress/urbanisation in participants with a specific coping style. Hence the planned SABPA project, which is the first study in South Africa where coping and direct markers of nervous system activity in Africans will be measured.

Purpose of study

The purpose of this study is to investigate biological markers associated with higher sympathetic nervous system activity in urbanised teachers with a specific coping style.

To investigate the relationship between blood pressure, inflammation, obesity, stress and coping in more detail we are going to perform this study in 400 men and women from the North-West province, aged 25-60 years. A comprehensive assessment of the cardiovascular and nervous systems by means of non-invasive painless techniques will be performed and a blood sample will be taken by an experienced research doctor and nurse to determine your blood sugar, cardiovascular, inflammation and stress hormone levels amongst other health markers.

Procedures

All measurements are performed in the Metabolic Unit (lipid clinic) of the University. A researcher has explained the entire procedure in detail and while you are reading this information document you have time to ask questions and to have clarified matters. If you are fine with the explained procedure you are requested to sign a *consent form (at the end of this document). Remember all personal data will be handled with care and remain confidential.

**By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for the purposes noted above. Your blood will also be tested for preliminary results on HIV status, since your HIV status may directly influence the main purposes of this study. If you would like to know what your HIV-status is, we will provide it. If tested positive we will refer you to your doctor and he/she will perform the necessary tests which will allow you to apply for chronic medication benefit. Also, the blood cells from your donated blood sample will be used to investigate the molecular genetics of higher nervous system activity and type 2 diabetes in order to enable pre-symptomatic diagnosis of hypertension and diabetes in the long term.*

Why was I chosen? Teachers are exposed to changing curricula and disciplinary problems whilst living in an urbanised environment adding to higher stress experiencing and nervous system activity.

How was I chosen?

Inclusion criteria:

Phase I: 200 black Africans aged 25-60 years (male=100, female = 100)

Phase II: 200 white Africans (n = male, 100 = female) aged 25-60 years.

Exclusion criteria: *pregnancy, lactation, any acute/chronic medication (e.g. high blood pressure, TB/tuberculosis, high sugar/diabetes, arthritis, anti-clotting/stroke factors, epilepsy/mental diseases or being treated for it as well being addicted to the medicine). You can not be included if you have been vaccinated in the previous 3 months and if you are a regular blood donor.*

What will be expected of me?

You, as participant will be screened once by a registered nurse to be eligible complying to the inclusion criteria. The following procedures will be followed:

- Recruitment, screening and informed sessions with all participants will be done two months prior to the study (October - November 2007, Phase I, and November, 2008, Phase II) and informed consent forms will be signed.
- After selection of all participants, the details of the project will be discussed with you in English or your home language, i.e. what the exact objectives of the study are, what procedures will be taken and what will be expected from each of you (e.g. overnight stay, resting blood pressure procedures and fasting urine and blood samples are required, importance of complying with the correct sampling methods, incentives). You will be given the opportunity to ask questions.
- Data collection for each participant will involve two days (15min in the morning and 2½ hours in the evening) on Day I; and 2 hours on Day II):

DAY I

- On day I at 07:00, the blood pressure apparatus, which will measure your blood pressure and heart function as well as a physical activity meter will be applied to your arm and waist at your school and you can then resume your normal daily activities. In the afternoon you must complete the Neethling Brain Instrument questionnaire which measures thought processes of the brain.
- At the end of Day I (± 16:30) you will be transported from your schools to overnight in the Metabolic Unit Research Facility of the North-West University. This unit is a research unit for human studies and equipped with 10 well furnished bedrooms, a kitchen, two bathrooms and a television room. Each of you will be subjected to the following procedures:
 - At the end of Day I between ± 17:15 and 18:00 you will be welcomed and each of you will receive your own private bedroom.

- The procedures, which will be done, will be explained again and each of you will then complete a general socio-demographic health questionnaire. Afterwards you will receive dinner.
- After dinner, psychological questionnaires will be completed under supervision of registered education specialists and psychologists. Completion of questionnaires will take approximately 40 min, including a break of 20 minutes with coffee/tea and biscuits. This will be your last meal for Day I as you must be fasting on Day II for obtaining good results.
- Thereafter, you can relax and watch television or socialise with your c-participants. It will be wise to go to bed not later than 22:00 as the blood pressure apparatus will take measurements every hour during the night and it can be tiring.

DAY II

- At 06:45 on Day II the AMBP will be removed and an urine sample collected. Once this has been done you will be directed to the anthropometric station where your weight, height and body circumferences will be measured.
- The next station involves the blood pressure measurement station. Whilst in a sitting position your blood pressure will be taken in duplicate with the sphygmomanometer (the same as used at clinics) with a resting period of 5 minutes in between. Our registered research doctor/nurse will take a fasting saliva sample as well as a blood sample of 45ml from a vein in your dominant arm. The infusion set will be left in your arm to lessen the effect of inserting a needle again for blood sampling after exposure to the two stressors. A small amount of diluted heparin will be left in the infusion set in your arm to prevent clotting.

Next the cardiovascular measurements will follow consisting of three separate procedures:

- The 1st measurement involves an ECG apparatus, which measures heart function, with 12 leads, which will be placed into position on your rib cage/front part of the body.
- The 2nd measurements are non-invasive and will be done by means of the Finometer device which also involves the assessment of heart functioning such as pulse (beats per minute), stroke volume (blood volume ejected by the heart per beat), cardiac output (blood volume ejected by the heart per minute), total peripheral resistance (resistance against the blood flow created by small arteries), central resistance (resistance against which the heart has to work while ejecting the blood into the aorta) as well as the elasticity of your large arteries (compliance). For this procedure a blood pressure cuff will be placed around your left arm and middle finger which will be inflated and stepwise deflated. You will not have more discomfort than during a common blood pressure measurement. This will take about 5 minutes.
- The stressor application procedure follows: You will now be exposed to a stressor for 1 minute whilst your blood pressure and ECG will still be taken. After exposure a saliva and blood sample (45ml) will be taken. After 10 minutes another saliva sample will be taken. Then the stressor application procedure will be repeated with the second stressor.
- At another station your 3rd measurement includes the assessment of pulse wave velocity, i.e. how fast your blood travels through your arteries. This measurement gives us an indication about how stiff your vessel walls are. The stiffer your vessel

wall is the faster the blood travels from one point of your body to another. These painless measurements will require two technicians using blunt probes (tonometer) putting light pressure on the neck and on the foot to measure the velocity of the pulse waves. This takes only a few minutes. An ultrasound device will be taken of your arteries in the neck with a blunt probe to indicate the intrinsic thickness of your arteries which contributes to high blood pressure.

The two stressors you will be exposed to for one minute include:

1. The *Colour-Word-Conflict Chart (applied for 1 minute)* is written in various colours. You must say or select the ink colour rather than the name of the colour spelled out by the word. A sliding scale with monetary incentives (maximum of R55.00) will be given if you can complete reading the chart.
 2. The *Cold Pressor Test (Foot) (applied for 1 minute)*: Immersion of your foot up to the wrist in ice water (4 degrees Celcius). As the cold can make you hold your breath you must quietly count to yourself during cold exposure to breath more rhythmic.
- You have reached the end of the sampling phase.
 - **Thank you for your participation! You now will have the opportunity to shower and a take away breakfast will be given.**
 - Immediate feedback on your HIV/AIDS status, obesity, blood pressure and blood glucose/sugar values will be given. *HIV/AIDS post-test counselling will be arranged if you are tested positive.*
 - You are now transported back to your school and after one week you will receive your Neethling Brain Instrument and 24-hour blood pressure reports.

Possible Risks

The measurements performed in our study will include only non-invasive techniques that are not expected to reveal any risks but might cause little discomfort. The taking of blood samples is an invasive procedure with a minimal risk of bleeding. Thus the procedure may cause only a few seconds of light discomfort. All tests will be performed by experienced research nurses of our department. There may be additional unforeseen or unknown risks.

Precautions to protect the participant

The Metabolic Unit facility of the NWU is fully equipped, and in case of an emergency which could not be handled by the registered nurse, the supervising medical doctor Emile Kotzé will be contacted. Dr. Kotzé was notified before the study commenced that this study will be taking place, and that there is a slight possibility that he may be contacted. Supporting medical treatment care facilities will be at hand anytime if needed.

Other Treatments Whilst on Study

It is important to tell the research staff about any treatments or medications you may be taking, including non-prescription medications, vitamins or herbal remedies during your participation in the study.

Incentives

1. All teachers will receive feedback on their health profile and if necessary references will be given to physicians/clinics/hospitals.
2. Printout feedback on 24 hour blood pressure monitoring report (normally costing R637.60), sonar of the artery (R1200.00), resting ECG (R600.00) and other variables (R500.00). Your benefit of participation is a comprehensive assessment of the cardiovascular and metabolic condition including investigation of blood pressure, inflammatory status and psychological well-being. These examinations will help us to assess the degree of vascular impairment of the arteries and to predict your risk of possible cardiovascular events such as heart attacks and stroke. The results may assist your doctor in decision making for further treatment or for instituting preventive measures. Our study will also contribute to the identification of possible factors leading to high blood pressure. As 24 hour ambulatory blood pressure monitoring is required for the diagnosis of hypertension, medical aids insist on this method of diagnosis to qualify for chronic medication. Additional testing could also reveal illnesses of a chronic nature and would serve as a motivation to qualify for chronic medication, such as metabolic syndrome, anti-inflammatory and cholesterol-lowering drugs.
3. Monetary incentive on completion of the colour word conflict chart (\pm R55.00).
4. Dinner and breakfast (\pm R24.00).
5. Neethling Brain Instrument profiles done by registered user of the Whole Brain (normally costing \pm R350.00).
6. Coping skills workshop will be arranged on request.

Privacy, Confidentiality and Disclosure of Information

By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for purposes noted above. Your blood samples will be discarded immediately after analysis. All information provided by you and the results of tests will be treated in the strictest confidence, and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law. The results of your medical tests will be labelled only with a code number, and will be stored separately from any identifying information. When the results are analysed we will be looking for differences between groups of people, not at the results of individuals. No information that could identify any person taking part in the study will be revealed when the results are reported.

Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the North-West University.

Before you make your decision, a member of the research team will be available so that you can ask any questions you have about the research project. You can ask for any information

you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team before you withdraw.

Ethical Guidelines

This project will be carried out according to Ethical Guidelines of the Helsinki declaration from 2000, with additional notes in 2002. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of ***North-West University Potchefstroom***.

Further Information or Any Problems

If you require further information or if you have any problems concerning this project, you can contact the principal researcher or *the other* researchers responsible for this project.

Dr Leoné Malan (018-299 2438)

Signature:

Sr. Chrissie Lessing (018-299 2480)

PART 2

Project Leader: Dr Leoné Malan

To the subject signing the consent as in part 3 of this document

You are invited to participate in a research project as described in paragraph 2 of Part 1 of this document. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project: Participation in this project is voluntary.

1. **It is possible that you personally will not derive any benefit from participation in this project, although the knowledge obtained from the results may be beneficial to other people.**
2. **You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.**
3. **The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.**
4. **We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.**

PART 3

Consent

Title of the project:

"THE SABPA STUDY (SYMPATHETIC ACTIVITY AND AMBULATORY BLOOD PRESSURE IN AFRICANS)".

I, the undersigned (full names)
read/listened to the information on the project in PART 1 and PART 2 of this document and I
declare that I understand the information. I had the opportunity to discuss aspects of the project
with the project leader and I declare that I participate in the project as a volunteer. I hereby give
my consent to be a subject in this project.

(Signature of the subject)

Signed at on2008/2009

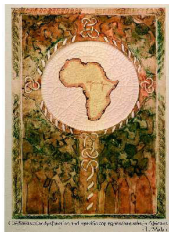
Witnesses

1.

2.

Signed at on2008/2009

Appendix B



SABPA Project

General Health and Sociodemographic Questionnaire

2008

PARTICIPANT NUMBER

Gender ☐

White Black Indian Coloured

RACE

Date of BIRTH

HOUSE N: P.BOX N:

STREET:

Post Code:TOWN.....

MOBILE phone number.....

P_DUR Number of years staying in Potchefstroom.

Question 1:

Marital status.

- MS_SI ☐ Unmarried
MS_SIP ☐ Unmarried, living with partner
MS_MA ☐ Married, living with "legal" wife/husband
MS_MAP ☐ Married, partner other than "legal" husband/wife
MS_DI ☐ Divorced, not living with new partner
MS_DIP ☐ Divorced, living with new partner
MS_WW ☐ Widow or widower, not living with new partner
MS_WWP ☐ Widow or widower, living with new partner

Question 2: Education

Still attending school?

SC_NOW ☐ Now ?

SC_LOC School or institution

EDU DI ☐ Completed DIPLOMA

EDU DE ☐ Completed DEGREE

Question 3: Past occupation.

P_HINS ☐ Long-lasting health problems

P_DUR ☐☐☐ Number of years

P_P_LOC ☐☐☐☐☐☐ Address

Question 4:

SALARY ☐ Employee receiving salary

S_FULL ☐ Full-time basis

S_PART ☐ Part-time basis

S_SUBE ☐☐☐ Persons subordinated to you

Question 5:

EDU DI ☐ DIPLOMA

EDU DE ☐ DEGREE

EDU WW ☐ Hours of work per week

Question 6: (Family members alive)

FH_F ☐ Father

FH_GFf ☐ Grandfather (father's side)

FH_GMf ☐ Grandmother (father's side)

FH_M ☐ Mother

FH_GFm ☐ Grandfather (mother's side)

FH_GMm ☐ Grandmother (mother's side)

FH_Ch ☐ Children

FH_GCh ☐ Grandchildren

FH_BSf ☐ Brothers or sisters of your father

FH_BSm ☐ Brothers or sisters of your mother

FH_BS ☐ Own brothers or sisters

Question 7:

C_DIS	<input type="checkbox"/> Disease affecting your heart or blood vessels	
	Disease	Starting date
C_COD1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_BMY1 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
C_EMY1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_NAGP1*
	Disease	Starting date
C_COD2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_BMY2 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
C_EMY2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_NAGP2*
	Disease	Starting date
C_COD3	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_BMY3 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
C_EMY3	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_NAGP3*
	Disease	Starting date
C_COD4	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_BMY4 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
C_EMY4	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	C_NAGP4*

Question 8:

K_DIS	<input type="checkbox"/> Disease affecting your kidneys or urinary tract	
	Disease	Starting date
K_COD1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_BMY1 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
K_EMY1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_NAGP1*
	Disease	Starting date
K_COD2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_BMY2 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
K_EMY2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_NAGP2.....*
	Disease	Starting date
K_COD3	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_BMY3 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
K_EMY3	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_NAGP3.....*
	Disease	Starting date
K_COD4	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_BMY4 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
K_EMY4	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	K_NAGP4*

Question 9:

L_DIS	<input type="checkbox"/> Kidney stones or stones in you urinary tract
L_REPC	<input type="checkbox"/> Repeated pain attacks
L_EVAC	<input type="checkbox"/> Passed a stone with urine
L_OPER	<input type="checkbox"/> Surgical treatment
L_NOW	<input type="checkbox"/> Still suffering from kidney stones or stones in urinary tract

Question 10:

DIABET ☐ Diabetes
D_DIET ☐ Diet and avoiding sweet foodstuffs
D_ORAL ☐ Pills
D_INS ☐ Insulin

Question 11:

HYPERT ☐ Hypertension
HY_MY ☐☐☐☐ When ?
HY_Th ☐ Treatment

Question 12:

DISEAS	<input type="checkbox"/> Currently in good health	
	Disease	Starting date
DS_CD1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_BMY1 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	Date of cure	Treating physician
DS_EMY1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_NAGP1.....*
	Disease	Starting date
DS_CD2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_BMY2 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	Date of cure	Treating physician
DS_EMY2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_NAGP2*
	Disease	Starting date
DS_CD3	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_BMY3 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	Date of cure	Treating physician
DS_EMY3	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_NAGP3*
	Disease	Starting date
DS_CD4	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_BMY4 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
	Date of cure	Treating physician
DS_EMY4	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	DS_NAGP4*

	Disease	Starting date
DS_CD5	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DS_BMY5 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
DS_EMY5	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DS_NAGP5.....*

	Disease	Starting date
DS_CD6	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DS_BMY6 <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	Date of cure	Treating physician
DS_EMY6	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DS_NAGP6.....*

Question 13:

D_HYPT	<input type="checkbox"/> Drugs to lower blood pressure	
DH_NOW	<input type="checkbox"/> Now ?	
	Name of drug	Tablets/day/dosage
DH_CD1	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DH_DO1 <input type="text"/> <input type="text"/> <input type="text"/>
	Name of drug	Tablets/day/dosage
DH_CD2	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DH_DO2 <input type="text"/> <input type="text"/> <input type="text"/>
	Name of drug	Tablets/day/dosage
DH_CD3	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DH_DO3 <input type="text"/> <input type="text"/> <input type="text"/>
	Name of drug	Tablets/day/dosage
DH_CD4	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	DH_DO4 <input type="text"/> <input type="text"/> <input type="text"/>

Question 14:D_DIUR ☐ DiureticsDD_NOW ☐ Now ?

Name of drug

Tablets/day/dosage

DD_CD1 DD_DO1

Name of drug

Tablets/day/dosage

DD_CD2 DD_DO2

Name of drug

Tablets/day/dosage

DD_CD3 DD_DO3

Name of drug

Tablets/day/dosage

DD_CD4 DD_DO4

Question 15:D_ANAL ☐ Taking pain-killersDA_YE How many years ?DA_SAL ☐ Salicylic acid (Disprin)DA_PAR ☐ Paracetamol (Grand-Pa)DA_OTH ☐ Analgesic drugs for arthritis (Brufen)

Name of drug

Units/week

DA_CD1 DA_DO1

Name of drug

Units/week

DA_CD2 DA_DO2

Name of drug

Units/week

DA_CD3 DA_DO3

Name of drug

Units/week

DA_CD4 DA_DO4

Question 16:

DR_2WK	<input type="checkbox"/> Medication during last 2 weeks		
	Name of medicine		Units/day
DR_CD1	<input type="text"/>	DR_DO1	<input type="text"/>
	Name of medicine		Units/day
DR_CD2	<input type="text"/>	DR_DO2	<input type="text"/>
	Name of medicine		Units/day
DR_CD3	<input type="text"/>	DR_DO3	<input type="text"/>
	Name of medicine		Units/day
DR_CD4	<input type="text"/>	DR_DO4	<input type="text"/>
	Name of medicine		Units/day
DR_CD5	<input type="text"/>	DR_DO5	<input type="text"/>
	Name of medicine		Units/day
DR_CD6	<input type="text"/>	DR_DO6	<input type="text"/>

Question 17:

T_NOW	<input type="checkbox"/> Currently smoking
T_CTf	<input type="text"/> Cigarettes with filter per day
T_CT	<input type="text"/> Cigarettes without filter per day
T_CTgt	<input type="text"/> Grams of tobacco per day
T_Plgt	<input type="text"/> Grams of tobacco per week for pipe
T_SCgr	<input type="text"/> Small cigars per week
T_Cgar	<input type="text"/> Cigars per week
T_AGE	<input type="text"/> When started smoking ? (age)
T_INHA	<input type="checkbox"/> Inhalation

Question 18:

T_P_PAST	<input type="checkbox"/> Smoking in the past
T_P_1CDY	<input type="checkbox"/> At least one cigarette per day during one year
T_P_AGE	<input type="text"/> Age at which participant quit smoking

T_P_CTf	<input type="checkbox"/> <input type="checkbox"/>	Cigarettes with filter per day
T_P_CT	<input type="checkbox"/> <input type="checkbox"/>	Cigarettes without filter per day
T_P_CTgt	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Grams of tobacco per day
T_P_Plgt	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Grams of tobacco per week for pipe
T_P_SCgr	<input type="checkbox"/> <input type="checkbox"/>	Small cigars per week
T_P_Cgar	<input type="checkbox"/> <input type="checkbox"/>	Cigars per week
T_P_WHY	<input type="checkbox"/> <input type="checkbox"/>	Reason to stop smoking

Question 19:

E_NOW	<input type="checkbox"/>	Current consumption alcoholic beverages
E_BEER	<input type="checkbox"/> <input type="checkbox"/>	Glasses of beer per day
E_TBEER	<input type="checkbox"/> <input type="checkbox"/>	Glasses of traditional beer per day
E_WINE	<input type="checkbox"/> <input type="checkbox"/>	Bottles of wine per week
E_THLOKd	<input type="checkbox"/> <input type="checkbox"/>	Boxes of Thlokwe per day
E_THLOKw	<input type="checkbox"/> <input type="checkbox"/>	Boxes of Thlokwe per week
E_SPIRITt	<input type="checkbox"/> <input type="checkbox"/>	Tot Spirits per day
E_SPIRITb	<input type="checkbox"/> <input type="checkbox"/>	Bottle of Spirits per week
E_LIQR	<input type="checkbox"/> <input type="checkbox"/>	Bottle of Liquor per week
E_AGE	<input type="checkbox"/> <input type="checkbox"/>	When started drinking alcohol regularly ? (age)

Question 20:

E_P_PAST	<input type="checkbox"/>	Consumption of alcoholic beverages in the past
E_P_AGE	<input type="checkbox"/> <input type="checkbox"/>	When stopped ? (age)
E_BEER	<input type="checkbox"/> <input type="checkbox"/>	Glasses of beer per day
E_TBEER	<input type="checkbox"/> <input type="checkbox"/>	Glasses of traditional beer per day
E_WINE	<input type="checkbox"/> <input type="checkbox"/>	Bottles of wine per week
E_THLOKd	<input type="checkbox"/> <input type="checkbox"/>	Boxes of Thlokwe per day
E_THLOKw	<input type="checkbox"/> <input type="checkbox"/>	Boxes of Thlokwe per week
E_SPIRITt	<input type="checkbox"/> <input type="checkbox"/>	Tot Spirits per day
E_SPIRITb	<input type="checkbox"/> <input type="checkbox"/>	Bottle of Spirits per week
E_LIQR	<input type="checkbox"/> <input type="checkbox"/>	Bottle of Liquor per week
E_P_WHY	<input type="checkbox"/> <input type="checkbox"/>	Why stopped consuming alcoholic beverages ?

Question 21:

- C_NOW ☐ Now consumption of caffeine-containing beverages
- C_REG ☐☐ Cups of coffee
- C_COKE ☐☐ Glasses of Coca-cola
- C_OTH ☐☐ Other
- C_TEA ☐ Tea
- C_DECAF ☐ Decaffeinated coffee
- C_DECA_N ☐☐ Number of cups of decaffeinated coffee per day
-

Question 22:

- M-NOW ☐ Periods
-

Question 23:

- DCCET ☐ Ever taken "the pill" ?
- DC_NOW ☐ "The pill" now ?
- DC_COD ☐☐☐☐☐☐ Name of "the pill"
- DC_YE ☐☐☐☐ How long ? (years, months)
-

Question 24:

- PR_PST ☐ Pregnant before
- PR_N ☐☐ Number of pregnancies
- PR_ABO ☐ Number of miscarriages
- PR_LIB ☐ Children born alive
- PR_STB ☐ Children stillborn
-

Question 25:

- M_NOW ☐ Still periods
- M_IRYE ☐☐☐☐ Since when irregular periods ?
- M_DISYE ☐☐☐☐ Since when periods completely disappeared ?
- M_P_SPON ☐ Spontaneous disappearance

M_P_HYST ☐ Removal of only womb
M_HYSTYE Date (month/year)
M_P_OVRR ☐ Removal of only right ovary
M_OVRRYE Date (month/year)
M_P_OVRL ☐ Removal of only left ovary
M_P_OVR2 ☐ Removal of both ovaries
M_OVR2YE Date (month/year)
M_P_ORHR ☐ Removal of right ovary together with womb
M_ORHRYE Date (month/year)
M_P_OLHR ☐ Removal of left ovary together with womb
M_OLHRYE Date (month/year)
M_P_HRT ☐ Removal of both ovaries and womb
M_HRTstart Date (month/year)
MS_COD1 Underlying disease 1
MS_COD2 Underlying disease 2
MS_COD3 Underlying disease 3
M_P_DRUG ☐ Periods suppressed by taking "the pill"
MD_P_COD Name of "the pill"
MD_P_MN Number of months

Question 26:

E_EXCS ☐ Results sent only to yourself
R-EXGP ☐ Results sent only to your family doctor
E_S_GP ☐ Results sent to yourself and your family doctor

Question 27:

C-GP ☐ Consent to contact the subject's physician(s)

Appendix C

Subject Group Physiology

AMBP card 2008/2009

SURNAME -----

NAME -----

CONTACT NAME AND NUMBER -----

DBIRTH Day□□ Month□□ Year□□□□ date of birth

CPNBR □□□□□□□□ identification number

DATABP_S □□□□□□□□ date of ABP recording

Please indicate if you experience/do any of the following during the 24 hour recording:

C_DIZ ☐ **dizziness** **1 = yes, 2 = no**

TDIZ_S1 □□□□ time of start of dizziness 1

TDIZ_E1 □□□□ time of end of dizziness 1

TDIZ_S2 □□□□ time of start of dizziness 2

TDIZ-E2 □□□□ time of end of dizziness 2

C_FAT ☐ **fatigue** **1 = yes, 2 = no**

TFAT_S1 □□□□ time of start of fatigue 1

TFAT_E1 □□□□ time of end of fatigue 1

TFAT_S2 □□□□ time of start of fatigue 2

TFAT_E2 □□□□ time of end of fatigue 2

C_VIS ☐ **visual problems** **1 = yes, 2 = no**

TVIS_S1 □□□□ time of start of visual problems 1

TVIS_E1 □□□□ time of end of visual problems 1

TVIS_S2 □□□□ time of start of visual problems 2

TVIS_E2 □□□□ time of end of visual problems 2

C_HEAD ☐ **headache** **1 = yes, 2 = no**

THEAD_S1 □□□□ time of start of headache 1

THEAD_E1 □□□□ time of end of headache 1

THEAD_S2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of headache 2	
THEAD_E2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of headache 2	
C_HOT	<input type="checkbox"/>	hot flushes	1 = yes, 2 = no
THOT_S1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of flushes 1	
THOT_E1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of flushes 1	
THOT_S2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of flushes 2	
THOT_E2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of flushes 2	
C_VOM	<input type="checkbox"/>	nausea and/or vomiting	1 = yes, 2 = no
TVOM_S1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of nausea 1	
TVOM_E1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of nausea 1	
TVOM_S2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of nausea 2	
TVOM_E2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of nausea 2	
C_PALP	<input type="checkbox"/>	palpitations/fast heart beat	1 = yes, 2 = no
TPALP_S1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of palpitations 1	
TPALP_E1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of palpitations 1	
TPALP_S2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of palpitations 2	
TPALP_E2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of palpitations 2	
C_SYNC	<input type="checkbox"/>	syncope/faint feeling	1 = yes, 2 = no
TSYNC_S1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of syncope 1	
TSYNC_E1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of syncope 1	
TSYNC_S2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of syncope 2	
TSYNC_E2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of syncope 2	
C_OTHICD	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	ICD code for other symptoms if present	
TOTH_S1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of other 1	
TOTH_E1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of other 1	
TOTH_S2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of other 2	
TOTH_E2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of other 2	
A_LPH	<input type="checkbox"/>	light physical activity	1 = yes, 2 = no
TLPH_S1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of start of LPH 1	
TLPH_E1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	time of end of LPH 1	

TLPH_S2 ☐☐☐☐ time of start of LPH 2
 TLPH_E2 ☐☐☐☐ time of end of LPH 2
 TLPH_S3 ☐☐☐☐ time of start of LPH 3
 TLPH_E3 ☐☐☐☐ time of end of LPH 3
A_PH ☐ **physical effort** **1 = yes, 2 : no**
 TPH_S1 ☐☐☐☐ time of start of physical effort 1
 TPH_E1 ☐☐☐☐ time of end of physical effort 1
 TPH_S2 ☐☐☐☐ time of start of physical effort 2
 TPH_E2 ☐☐☐☐ time of end of physical effort 2
 TPH_S3 ☐☐☐☐ time of start of physical effort 3
 TPH_E3 ☐☐☐☐ time of end of physical effort 3
S-SST ☐ **slightly stressed** **1 = yes, 2 = no**
 TSST_S1 ☐☐☐☐ time of start of slightly stressed 1
 TSST-E1 ☐☐☐☐ time of end of slightly stressed 1
 TSST_S2 ☐☐☐☐ time of start of slightly stressed 2
 TSST-E2 ☐☐☐☐ time of end of slightly stressed 2
 TSST_S3 ☐☐☐☐ time of start of slightly stressed 3
 TSST-E3 ☐☐☐☐ time of end of slightly stressed 3
S_ST ☐ **stress** **1 = yes, 2 = no**
 TST_S1 ☐☐☐☐ time of start of stress 1
 TST_E1 ☐☐☐☐ time of end of stress 1
 TST_S2 ☐☐☐☐ time of start of stress 2
 TST_E2 ☐☐☐☐ time of end of stress 2
 TST_S3 ☐☐☐☐ time of start of stress 3
 TST_E3 ☐☐☐☐ time of end of stress 3
TSLEEP ☐☐☐☐ **time of sleep**
TGUP ☐☐☐☐ **time of getting-up**
THSLEEP ☐☐☐☐ **hours of sleep per night**
TASLEEP ☐☐☐☐ **hours awake/can't sleep per night**
TMEAL ☐☐☐☐ **time of main meal**

DR_CD1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> drug 1 (coded as in the standard questionnaire)
DR_DO1	<input type="checkbox"/> <input type="checkbox"/> amount of tablets taken
TIMDR1	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> time at which the medication was taken
DR_CD2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> drug 2 (coded as in the standard questionnaire)
DR_DO2	<input type="checkbox"/> <input type="checkbox"/> amount of tablets taken
TIMDR2	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> time at which the medication was taken
DR_CD3	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> drug 3 (coded as in the standard questionnaire)
DR_DO3	<input type="checkbox"/> <input type="checkbox"/> amount of tablets taken
TIMDR3	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> time at which the medication was taken
DR_CD4	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> drug 4 (coded as in the standard questionnaire)
DR_DO4	<input type="checkbox"/> <input type="checkbox"/> amount of tablets taken
TIMDR4	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> time at which the medication was taken
DR_CD5	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> drug 5 (coded as in the standard questionnaire)
DR_DO5	<input type="checkbox"/> <input type="checkbox"/> amount of tablets taken
TIMDR5	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> time at which the medication was taken
DR_CD6	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> drug 6 (coded as in the standard questionnaire)
DR_DO6	<input type="checkbox"/> <input type="checkbox"/> amount of tablets taken
TIMDR6	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> time at which the medication was taken.

Appendix D

Berlin Questionnaire (for sleep apnea)

Scoring Berlin questionnaire

Adapted from: Table 2 from Netzer, et al., 1999. (Netzer NC, Stoohs RA, Netzer CM, Clark K, Strohl KP. Using the Berlin Questionnaire to identify patients at risk for the sleep apnea syndrome. Ann Intern Med. 1999 Oct 5;131(7):485-91).

The questionnaire consists of 3 categories related to the risk of having sleep apnea. Patients can be classified into High Risk or Low Risk based on their responses to the individual items and their overall scores in the symptom categories.

Categories and scoring:

Category 1: items 1, 2, 3, 4, 5.

Item 1: if 'Yes', assign **1 point**

Item 2: if 'c' or 'd' is the response, assign **1 point**

Item 3: if 'a' or 'b' is the response, assign **1 point**

Item 4: if 'a' is the response, assign **1 point**

Item 5: if 'a' or 'b' is the response, assign **2 points**

Add points. Category 1 is positive if the total score is 2 or more points

Category 2: items 6, 7, 8 (item 9 should be noted separately).

Item 6: if 'a' or 'b' is the response, assign **1 point**

Item 7: if 'a' or 'b' is the response, assign **1 point**

Item 8: if 'a' is the response, assign **1 point**

Add points. Category 2 is positive if the total score is 2 or more points

Category 3 is positive if the answer to item 10 is 'Yes' OR if the BMI of the patient is greater than 30kg/m².

(BMI must be calculated. BMI is defined as weight (kg) divided by height (m) squared, i.e., kg/m²).

High Risk: if there are 2 or more Categories where the score is positive

Low Risk: if there is only 1 or no Categories where the score is positive

Additional question: item 9 should be noted separately.

BERLIN QUESTIONNAIRE

Height (m) _____ Weight (kg) _____ Age _____ Male / Female
Please choose the correct response to each question.

CATEGORY 1

1. Do you snore?

- ☐ a. Yes
- ☐ b. No
- ☐ c. Don't know

If you snore:

2. Your snoring is:

- ☐ a. Slightly louder than breathing
- ☐ b. As loud as talking
- ☐ c. Louder than talking
- ☐ d. Very loud – can be heard in adjacent Rooms

3. How often do you snore

- ☐ a. Nearly every day
- ☐ b. 3-4 times a week
- ☐ c. 1-2 times a week
- ☐ d. 1-2 times a month
- ☐ e. Never or nearly never

4. Has your snoring ever bothered other people?

- ☐ a. Yes
- ☐ b. No
- ☐ c. Don't Know

5. Has anyone noticed that you quit breathing during your sleep?

- ☐ a. Nearly every day
- ☐ b. 3-4 times a week
- ☐ c. 1-2 times a week
- ☐ d. 1-2 times a month
- ☐ e. Never or nearly never

CATEGORY 2

6. How often do you feel tired or fatigued after your sleep?

- ☐ a. Nearly every day
- ☐ b. 3-4 times a week
- ☐ c. 1-2 times a week
- ☐ d. 1-2 times a month
- ☐ e. Never or nearly never

7. During your waking time, do you feel tired, fatigued or not up to par?

- ☐ a. Nearly every day
- ☐ b. 3-4 times a week
- ☐ c. 1-2 times a week
- ☐ d. 1-2 times a month
- ☐ e. Never or nearly never

8. Have you ever nodded off or fallen asleep while driving a vehicle?

- ☐ a. Yes
- ☐ b. No

If yes:

9. How often does this occur?

- ☐ a. Nearly every day
- ☐ b. 3-4 times a week
- ☐ c. 1-2 times a week
- ☐ d. 1-2 times a month
- ☐ e. Never or nearly never

CATEGORY 3

10. Do you have high blood pressure?

- ☐ Yes
- ☐ No
- ☐ Don't know