

**Morning blood pressure surge and its
association with arterial function and subclinical
target organ damage in young South Africans:
The African-PREDICT study**

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degree *Doctor of Philosophy* in *Science (Physiology)* at the
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PREFACE

This thesis is presented in the article format, consisting of peer-reviewed published or submitted articles. This format is approved, supported and defined by the North-West University guidelines for post-graduate Ph.D. level studies. This thesis comprises of six chapters and forms part of the *Doctor of Philosophy in Science (Physiology)* program. Chapter 1 contains the general introduction as well as the literature overview and motivation to elucidate the purpose of the study. Chapter 2 contains the methodology of the study. Chapter 3, 4 and 5 are the research articles that were submitted for publication to peer reviewed journals, namely, *Journal of Human Hypertension* (published), *Journal of Hypertension* (under review) and *Heart, Lung and Circulation* (published), respectively. The final chapter (Chapter 6) summarises the main findings of the study, and includes a reflection on the hypotheses and provides recommendations. All references at the end of Chapter 1, 2, and 6 are indicated according to the *Vancouver* referencing style. The relevant references provided at the end of Chapter 3, 4 and 5 are according to the instructions for authors of the specific journal in which the articles were published or submitted for publication.

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CONTRIBUTION OF AUTHORS

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Prof. W Smith: Supervised writing of the second manuscript (Chapter 4).

The following statement from the co-authors confirms their individual involvement in this study and give their permission that the relevant research articles may form part of this thesis:

Hereby, I declare that I approved the abovementioned thesis and that my role in this study (as stated above) is representative of my contribution towards the manuscripts and supervised postgraduate study. I also give my consent that the manuscripts may be published as part of the *Doctor of Philosophy in Science (Physiology)* thesis of Gontse Gratitude Mokwatsi.



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SUMMARY

Motivation

The accurate measurement of blood pressure remains the most essential technique for the diagnosis and management of hypertension. In addition, there is increasing evidence that the measurement of clinic blood pressure may yield incorrect estimates of a patient's true blood pressure status. This is due to blood pressure fluctuations throughout the day-night period, with reported higher blood pressure during the day and lower at night, reflecting the physiological dipping pattern of night-time blood pressure. Furthermore, studies indicated the occurrence of cardiovascular events such as stroke, myocardial infarction and sudden cardiac death more frequently during the morning hours after waking up, due to increased blood pressure.

The morning blood pressure surge (MBPS) is a normal physiological response to changes in the activity of the sympathetic nervous system associated with the process of awakening and other factors (including age, ethnicity, sex, hypertensive status, health behaviours, or dipping status). A MBPS up to 37 mmHg is considered physiological, while an exaggerated MBPS above 37 mmHg is pathological, and associated with cardiovascular events, mainly in hypertensive and elderly individuals. A majority of previous studies focusing on exaggerated MBPS were conducted in elderly and hypertensive individuals, but there is scant knowledge on ethnic differences, prevalence and effects of an exaggerated MBPS on subclinical hypertension-mediated organ damage in young healthy individuals. This study therefore investigated MBPS in young black and white normotensive adults, and the relationships between the MBPS, measures of arterial function and autonomic function in healthy black and white South African men and women, aged between 20 and 30 years.

Aim

The overarching aim of this Ph.D. thesis was to obtain a better understanding regarding the MBPS and how it relates to arterial function and subclinical hypertension-mediated organ damage in a healthy bi-ethnic South African population, aged between 20 and 30 years.

Methodology

This thesis used data (presented in Chapter 3, 4 and 5) collected from the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT). We included the first consecutive 845 black and white men and women in total. Groups were stratified by gender and ethnicity, and dipping status as specified by statistical interaction terms. Cardiovascular measurements included ambulatory blood pressure measurements, MBPS (sleep-trough and dynamic morning surge) and pulse wave velocity. Using fundoscopy, we also included measurements of the microvasculature, namely retinal vessel

calibres. As part of ambulatory blood pressure and electrocardiogram monitoring, we measured 24-hour heart rate variability. Baroreceptor sensitivity was determined using the validated cross-correlation baroreflex sensitivity (xBRS) method. Using accelerometry, we were able to measure total energy expenditure and activity energy expenditure.

Results and conclusions of each manuscript

The first manuscript (Chapter 3) aimed to determine interactions of sex and black and white ethnicity across increasing quartiles of MBPS. This manuscript also aimed to explore whether differences in cardiovascular-related measures and specific health behaviours exist across increasing MBPS quartiles. We also aimed to determine if an exaggerated MBPS already occurs in young black and white adults. We found an interaction of ethnicity on the relationship between sleep-trough surge with cardiovascular markers and health behaviours ($p<0.01$). Interaction terms of ethnicity ($p<0.001$) and sex ($p=0.016$) on the relationship between dynamic surge with cardiovascular markers and health behaviours were observed. In white men, both sleep-trough and dynamic morning surge were higher than the black group (all $p<0.01$). A higher proportion of white individuals had an exaggerated sleep-trough (6.16% vs 3.42%; $p=0.21$) and dynamic morning (15.8% vs 14.6%; $p=0.63$) surge when compared to the black population. On the other hand, more black participants were non-dippers than whites (44% vs 34%; $p=0.004$). MBPS related independently and positively with night-time percentage dipping in all ethnic groups (all $p<0.01$). No consistent independent associations of health behaviours with MBPS were obtained. However, we confirmed ethnic differences in MBPS in young adults, with a higher, but normal MBPS in white men, however, the non-dipping night-time pattern in young black adults may serve as a potential risk factor for future cardiovascular disease.

The aim of the second manuscript (Chapter 4) was to explore whether the MBPS is associated with measures of the microvascular structure and macrovascular function in dippers and non-dippers. Dippers had a higher, but normal sleep-trough surge ($p<0.001$). Measures of microvascular structure (central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE)) and macrovascular function (pulse wave velocity) were comparable between dippers and non-dippers. Partial correlations (adjusted for age, sex and ethnicity) indicated a positive association between central retinal artery equivalent and sleep-trough surge ($r=0.20$; $p=0.021$) as well as a negative association between central retinal artery equivalent and night-time diastolic blood pressure ($r=-0.24$; $p=0.004$) in dippers. Multiple regression analysis confirmed a positive relationship of CRAE with sleep-trough surge (adjusted $R^2=0.33$; $\beta=0.16$; $p=0.040$) in dippers only. These associations were absent in non-dippers. Our results suggest that a normal MBPS, evident in dippers, may play an important role to preserve retinal artery diameter.

To better understand the involvement of autonomic function on MBPS in dippers and non-dippers, we also investigated associations of the MBPS with heart rate variability and baroreceptor sensitivity in the African-PREDICT population of young healthy adults, including dippers and non-dippers (Chapter 5). The majority of non-dippers in this population were black individuals (70.4%), with apparent lower sleep-trough and dynamic morning surge (all $p < 0.001$) compared to dippers. Baroreceptor sensitivity was higher in non-dippers ($p = 0.021$), while heart rate variability was comparable between dippers and non-dippers. There was an inverse association of both sleep-trough ($\beta = -0.25$; $p = 0.039$) and dynamic morning surge ($\beta = -0.14$; $p = 0.047$) with 24-hour heart rate variability (total power) in non-dippers only. This result suggests increased autonomic function involvement in the observed lower MBPS of non-dippers.

General conclusion

In this study, ethnic differences in MBPS is evident in young and healthy adults, with a higher proportion of white individuals presented with an exaggerated MBPS. When reviewing factors that relate to MBPS, no associations were found with any health behaviours such as obesity or salt intake, however the positive association of MBPS with night-time blood pressure indicates the important role of dipping status when interpreting MBPS. The positive association between MBPS and the central retinal artery equivalent observed in dippers suggests the important role of normal morning surge (dependent on night-time dipping) in preserving retinal arteriolar diameter of dippers. Apart from the association with the microvasculature, MBPS also related inversely with increased autonomic function in non-dippers. Although non-dippers had a lower MBPS, their blunted night-time blood pressure dipping pattern (in response to increased autonomic function) may increase their risk for future cardiovascular disease and adverse outcomes.

Keywords: ambulatory blood pressure, autonomic nervous activity, dipper, macrovascular, microvascular, morning blood pressure surge, night-time blood pressure, non-dipper

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LIST OF ABBREVIATIONS

AEE:	Activity energy expenditure
African-PREDICT:	African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension
BMI:	Body mass index
bpm:	Beats per minute
BRS:	Baroreceptor sensitivity
cm:	Centimetres
CRAE:	Central retinal artery equivalent
CRP:	C-reactive protein
CRVE:	Central retinal vein equivalent
CVD:	Cardiovascular disease
ECG:	Electrocardiogram
ECM:	Extracellular matrix
DBP:	Diastolic blood pressure
DVA:	Dynamic Retinal Vessel Analyzer
GGT:	Gamma(γ)-glutamyl transpeptidase
GFR:	Glomerular filtration rate
HDL:	High-density lipoprotein
HIV:	Human immunodeficiency virus
HRV:	Heart rate variability
LDL:	Low-density lipoprotein
kCal:	Kilocalorie
kCal/kg/day:	Kilocalorie per kilogram per day
Kg:	Kilogram
kg/m²:	Kilograms per meter squared
L:	Litre
m²:	Square meter
MBPS:	Morning blood pressure surge
m/s:	Meters per second
mg/g:	Milligrams per gram
mg/L:	Milligrams per litre
mL:	Milliliter
mm:	Millimetre

mmHg:	Millimetres mercury
mmol/day:	Millimole per day
mmol/L:	Millimole per litre
MU:	Measuring units
N:	Number of
ng/ml:	Nanogram per milliliter
p:	Probability value
PWV:	Pulse wave velocity
r:	Regression coefficient
RAAS:	Renin-angiotensin-aldosterone system
ROS:	Reactive oxygen species
SBP:	Systolic blood pressure
SE:	Standard error
SES:	Socio-economic status
SD:	Standard deviation
TEE:	Total energy expenditure
vs:	Versus

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CHAPTER 1

Introduction and Literature overview

1.1 General introduction

Cardiovascular diseases remain a major health concern globally, and also in sub-Saharan Africa (1-3). Cardiovascular diseases such as coronary artery disease, heart failure, stroke and peripheral arterial disease, are mainly attributed by risk factors associated with hypertension (4-6). Factors associated with hypertension include obesity and health behaviours such as unhealthy diets, sedentary lifestyle, excessive alcohol consumption and smoking (4-8). Hypertension was reported as the leading cause of global deaths in 2015, and 1.13 billion individuals were reported to suffer from hypertension in 2015, while the numbers are increasing (9-11). In South Africa, the prevalence of hypertension is also high, especially in the black adult population (12).

Clinic blood pressure measurements were reported to possibly yield incorrect estimates of patients' true blood pressure (13). Over the past decade the value of not only obtaining a single blood pressure measurement, but rather a 24-hour blood pressure recording became clear (2, 14-17). This is due to blood pressure fluctuations throughout the day-night period, with reported higher blood pressures recorded during the day (06:00 – 22:00), and lower during night (18, 19). Furthermore, studies have indicated the occurrence of cardiovascular events such as stroke, myocardial infarction and sudden cardiac death (in association with increased blood pressure) being more frequent during the morning hours after waking up (14, 18, 20-23).

Increasing blood pressure during the morning is a normal physiological phenomenon termed the morning blood pressure surge (MBPS), and the degree of the MBPS was proven to be exaggerated in hypertensive patients (4, 24). MBPS occurs as a consequence of changes in the activity of the sympathetic nervous system associated with the process of awakening, and other factors such as elevated activity of the renin-angiotensin-aldosterone-system, hypertension, endothelial dysfunction, oxidative stress and inflammation (4, 23, 25). Increased sympathetic nervous system activity induces a significant rise in cortisol, epinephrine and norepinephrine (25-28). Elevated levels of cortisol, epinephrine and norepinephrine result in an increase in blood pressure through vasoconstriction of systemic arteries (27, 29). An exaggerated MBPS received research attention due to its role in the development of cardiovascular diseases, events and mortality (30, 31). The majority of previous studies focusing on exaggerated MBPS, and its effects, were conducted in the elderly and hypertensive individuals (4, 23, 24, 32-35).

A study found that an exaggerated MBPS was associated with vascular damage throughout the circulation including the myocardium, large arteries and other organs (2).

1.2 Morning blood pressure surge

The morning blood pressure surge (MBPS) is a normal physiological phenomenon that is characterized by increased blood pressure during the morning hours (4, 24). The magnitude of the MBPS is influenced by various factors including ethnicity, age, sex (18, 36-38), cardiovascular factors (hypertensive status, dipping status and impaired sympathetic nerve activation) (4, 16, 39-43), biochemical factors (inflammation, oxidative stress, decreased bioavailability of nitric oxide and increased activity of the renin-angiotensin-aldosterone system) (4, 16, 43) and lifestyle factors (tobacco use, physical activity and habitual alcohol consumption) (44-48).

1.2.1 Definitions of physiological morning blood pressure surge

There is no consensus on the definition of the MBPS, but studies describe distinct ways of quantifying MBPS either based on waking time (Figure 1) or by using the 24-hour clock-based MBPS if waking time is not available (4, 39, 49, 50).

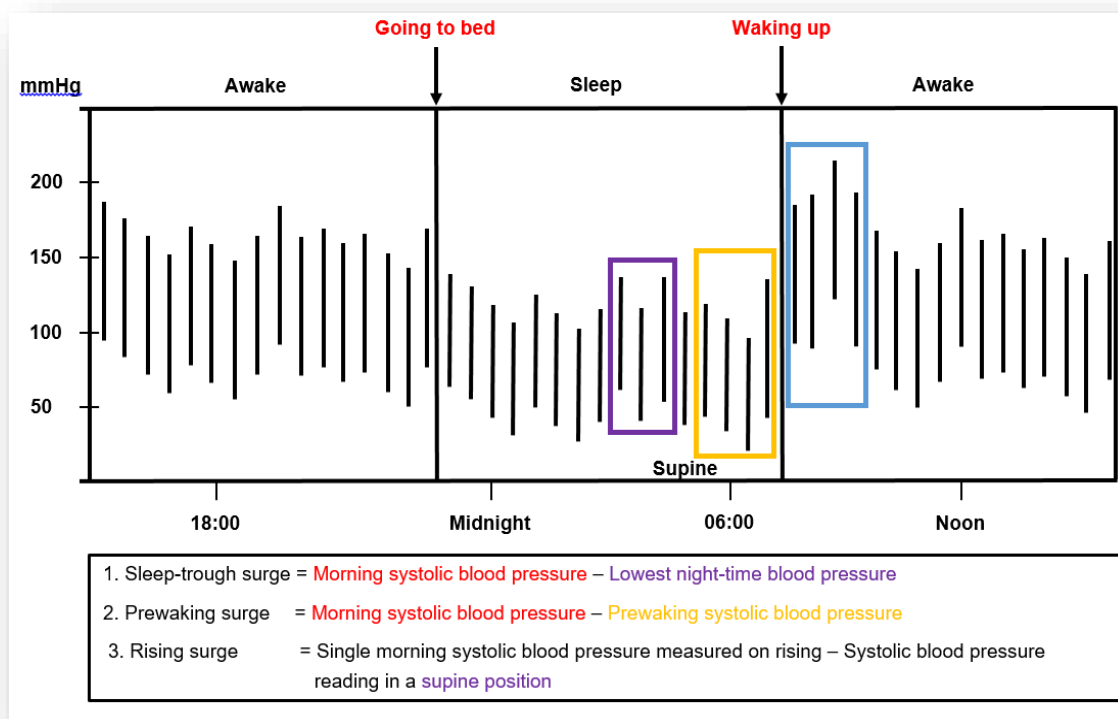


Figure 1. Definition of morning blood pressure surge based on waking time (4)

Different definitions of MBPS based on waking times have been described as follows:

(1) **Sleep-trough surge** is defined as the morning systolic blood pressure (2-hour average of four 30-minute systolic blood pressure readings just after waking) minus the lowest nocturnal systolic blood pressure (average blood pressure of 3 readings centred on the lowest night-time reading). Sleep-trough surge is considered as one of the dynamic diurnal surges during the specific period of time (from sleep to early morning) when the cardiovascular risk is exaggerated (4). It is thus important to exclude the effects of circadian rhythm in blood pressure when establishing the clinical implications of sleep-trough surge (4);

(2) **Prewaking surge** is calculated as morning systolic blood pressure minus the prewaking systolic blood pressure (2-hours average of four systolic readings just before waking). Prewaking surge is considered to largely reflect the day-night difference (4);

(3) **Rising surge** is defined as a single morning systolic blood pressure measured on rising, minus the systolic blood pressure reading in a supine position, 30 minutes before waking (4, 39, 49).

All of the above-mentioned morning blood pressure surge definitions are associated with cardiovascular events (4). A previous study reported that a MBPS up to 135/85 mmHg is considered physiological, while an exaggerated MBPS above 135/85 mmHg is considered pathological, triggering cardiovascular events, mainly in hypertensive individuals (2).

Several studies have proposed sleep-trough surge as the preferred method for quantifying MBPS (4, 35) since rising surge may detect the morning risk just after rising, but it may underscore the blood pressure surge subsequently augmented by physical activity in the morning (4). Another reason is that sleep-trough surge remains significantly associated with cardiovascular risk after controlling for dipping status of nocturnal blood pressure or the mean nocturnal blood pressure level compared to the other MBPS quantifications (4). A study conducted by Kario *et al.* reported that prewaking surge was not significantly associated with stroke, and that sleep-trough gives a more clinically relevant definition of the MBPS (35).

As mentioned above, there are other definitions of MBPS for quantifying MBPS if time of awakening is not available (50). These definitions are as follows:

(1) **Average morning surge** is defined as average morning systolic blood pressure (between 07:00 and 09:00) minus average night-time systolic blood pressure (between 01:00 and 06:00);

(2) **Dynamic morning surge** is defined as the moving peak morning systolic blood pressure (highest 1 hour moving average of consecutive systolic blood pressures between 06:00 and 10:00) minus moving lowest night-time systolic blood pressure (lowest 1 hour moving average of consecutive systolic blood pressures between 01:00 and 06:00);

(3) **Maximum dynamic morning surge** is defined as maximum morning systolic blood pressure (maximum morning systolic blood pressure between 06:00 and 10:00) minus minimum night-time systolic blood pressure (minimum night-time systolic blood pressure [one systolic blood pressure] during 01:00 and 06:00) and;

(4) **Perimorning surge** is defined as the maximum morning systolic blood pressure (maximum morning systolic blood pressure [one systolic blood pressure] between 06:00 and 10:00) minus minimum morning systolic blood pressure (minimum morning systolic blood pressure [one systolic blood pressure] between 06:00 and 10:00) (50-53).

To the best of our knowledge, information regarding the preferred method for quantifying 24-hour clock-based MBPS is limited. For this current study, we chose dynamic morning surge as an ideal 24-hour clock-based MBPS, because its principles are similar to those of sleep-trough surge, which is considered as the preferred method for quantifying MBPS (4, 35).

1.3 Factors contributing towards exaggerated morning blood pressure surge

1.3.1 Ethnicity, sex and age

Previous studies have indicated that the MBPS is lower (dependent on night-time blood pressure) in black individuals compared to white individuals aged between 40 and 50 years, whether normotensive or hypertensive (18, 37, 38). However, these studies had several limitations such as a lack of information regarding potential demographic confounders, and only included a small number of black participants (N=71) compared to white counterparts (N=1172) (18, 38). These limitations make it difficult to identify mechanisms related to the lower MBPS in blacks compared to whites (37). It was previously reported that the ethnic difference in the MBPS may be as a result of differences in plasma renin activity in these ethnic groups (37). This study indicated that the increased MBPS in whites is caused by higher plasma renin activity evident in whites compared to blacks, after consumption of a controlled diet (with similar salt intake) for a three-week period (37). These results were also supported by a study conducted by Hurwitz *et al.* which reported that plasma renin activity is higher during the latter part of sleep and pre-waking period, possibly leading to exaggerated MBPS (54).

The MBPS was further proven to be higher in hypertensive men compared to hypertensive women aged between 40 and 80 years (36), with the authors arguing that the difference may be caused by genetic factors including XY sex chromosome and some hormonal genes (36, 55, 56). There is limited evidence on the potential role of sex when investigating MBPS in young and healthy individuals.

Aging is a dominant determinant of functional and structural changes of the arterial wall (57-59) and contributes to these changes through mechanisms, such as medial hypertrophy and increased accumulation of collagen and elastin breakdown of the extracellular matrix (57, 59-63). These structural changes increase arterial wall thickness and decreased distensibility and compliance of arteries, resulting in increased arterial stiffness as measured with pulse wave velocity (59, 64, 65). Apart from distensibility, the intima-media thickness of arteries increases by two-to-threefold from the age of 20 years and is associated with luminal dilation and increased vascular wall stiffness (61, 65). It has also been reported that elastin degrades with age (61, 66). This degradation leads to increased collagen turn-over and extracellular matrix remodeling which contributes to the stiffness of arteries (57, 61, 62). Kario *et al.* reported in a research study that arterial stiffness in large arteries acts as one of the important leading causes of exaggerated MBPS through a decrease in baroreceptor sensitivity (4). Furthermore, the activity of the sympathetic nervous system was reported to be elevated in older individuals, especially women (67-69). However, little is known about the effects of sex on age-related changes in sympathetic nervous system activity or in younger healthy individuals (67). Increased sympathetic activity in adults may be caused by factors such as increases in total and abdominal adiposity and circulating adipose-derived signals such as leptin, and increased subcortical suprabulbar brain noradrenergic activity, as correlated from measurements of norepinephrine turnover from the cerebrovascular circulation (68, 69).

1.3.2 Cardiovascular factors

1.3.2.1 Sympathetic nervous system activity

The sympathetic nervous system is one of the two main divisions of the autonomic nervous system, also known to stimulate the body's fight-or-flight response (70). The stimulation of sympathetic nervous system also plays an important role in blood pressure regulation by increasing both cardiac output by the heart and blood vessel resistance to blood flow, leading to acute increases in arterial pressure (70). The parasympathetic nervous system, which is the other main division of the autonomic nervous system is responsible for decreasing the arterial pressure by increasing vasodilation of veins and arterioles, and by also decreasing heart rate and strength of heart contraction (71). Sympathetic nervous system activity increases steeply in the morning when individuals wake up and resume with daily activities (possibly leading to exaggerated MBPS) and decreases at night when sleeping (19). Alpha adrenergic activity of the sympathetic nervous system has vasoconstrictor effects and increases vascular tone and remodeling in resistance arteries, which may lead to an exaggerated MBPS in older hypertensive individuals (2, 4, 24, 30). Black individuals were reported to have a higher density of alpha-adrenergic receptors responsible for higher peripheral vasoconstriction and BP reactivity (72). The increased activity of the sympathetic nervous system at night, as observed in black individuals, may explain the lower MBPS evident in blacks (73). Since MBPS is quantified by the difference between morning blood pressure and night-time blood pressure, the lower MBPS observed in blacks is not surprising. A surge quantified by a difference of high nocturnal blood pressure observed in blacks (74) (possibly due to increased activity of sympathetic nervous system) from an increased morning blood pressure associated with the process of waking up, may result to the low MBPS as observed in blacks. Various indices of the autonomic neural activity such as heart rate variability, baroreceptor sensitivity and dipping status have been associated with the sympathetic nervous system (75-77).

1.3.2.2 Heart rate variability

Heart rate variability (HRV) reflects beat-to-beat changes in R-R intervals, which are related to the on-going interplay between the two divisions of the autonomic nervous system (78). HRV is considered as a measure of the autonomic neural activity and is well known to associate with increased sympathetic nervous system activity (79, 80). Changes in the heart rate may occur in response to autonomic neural activity imbalance, with a shift towards increased sympathetic activity due to mental or physical stress (78). It was found that HRV decreases with an increase in sympathetic nervous system activity (79, 80).

Various quantification methods, including time and frequency domain analysis (to assess spontaneous oscillations resulting from sinus node depolarization obtained from ~3.5 h of valid analysable ambulatory ECG data) as well as geometric domain analysis (an index of the pulse variability based on a triangular interpolation method in the given time interval where cardiovascular risk 0-15 is high; 15-20 is mid; >20 is low) are used to quantify HRV, to give an indication of the influence of either sympathetic or parasympathetic activity on HRV (81, 82). For the purpose of this Ph.D. study, focus will be placed on frequency domain analysis only. Frequency domain analysis involves low frequency HRV (a major index of sympathetic cardiac tone but also has a parasympathetic component), high frequency HRV (a major indicator of parasympathetic activity), low frequency-to-high frequency ratio (reflector of sympatho-vagal autonomic balance) and HRV total power (global determinant of overall autonomic modulation) (81). To the best of our knowledge, there is limited information regarding the associations between HRV and MBPS.

1.3.2.3 Baroreceptor sensitivity

Baroreceptor sensitivity is a physiological measure used to assess the control of the baroreflex within the cardiovascular system (42, 75, 83). The baroreflex is essential for acute and chronic control of blood pressure, as it buffers acute changes in blood pressure (42). The baroreflex provides a rapid negative feedback loop, in which an elevated blood pressure reflexively causes the heart rate, and subsequently, blood pressure to decrease (42). Baroreceptor sensitivity can be defined by measures of the reflex-mediated change in the R-R interval produced by a change in blood pressure through stretch-sensitive receptors called baroreceptors, which are located on the arterial wall (localized mainly in the carotid sinus of the carotid arteries and in the walls of the aortic arch) (71, 84). Arterial baroreceptors are innervated and remain under the reflex neural control (84). An increase in systolic blood pressure causes the arterial baroreceptors to stretch, and receptors respond by increasing their rate at which action potentials are generated (84). As a result, compensatory responses are initiated to decrease systolic blood pressure (75, 84). This is achieved through increased parasympathetic outflow and a decrease in the sympathetic outflow to the heart and blood vessels, and the resultant effect is a decrease in heart rate, cardiac contractility, stroke volume, peripheral vascular resistance and venous return (71, 75, 84). Previous studies reported an association between impaired baroreceptor sensitivity and exaggerated MBPS (4, 42, 85). Baroreceptor sensitivity reduces in the morning hours, impacting blood pressure variability control and possibly leading to exaggerated MBPS (4, 85). Impaired baroreceptor

sensitivity may be caused by various factors such as the activation of the sympathetic nervous system and an increase in large artery stiffness (4, 42).

1.3.2.4 Dipping status

Blood pressure decreases at night and this is known as nocturnal blood pressure dipping (18, 19, 53). Blood pressure declines more than 10% in most individuals who are classified as dippers (Figure 2), whereas the nocturnal systolic blood pressure of other individuals declines by <10%, classified as non-dippers (Figure 2) (32, 86). In addition, there are individuals classified as riser, whose nocturnal blood pressure declines by <0% (53). The non-dipping patterns were reported to be associated with poor sleep quality, high body mass index, and suppressed decline in activity of sympathetic nervous system during sleep hours (77). Non-dipping is reportedly common in the black population, increasing the risk for cardiovascular disease (32, 77, 87, 88). In addition, there are individuals classified as extreme dippers ($\geq 20\%$ nocturnal decline in SBP) (Figure 2), whose blood pressure can decrease to as low as 96/62 mmHg (32, 53). Extreme nocturnal dipping is associated with increased risk of stroke events compared to normal dippers (53) and can predict cardiovascular morbidity and mortality in elderly individuals (32, 89-91).

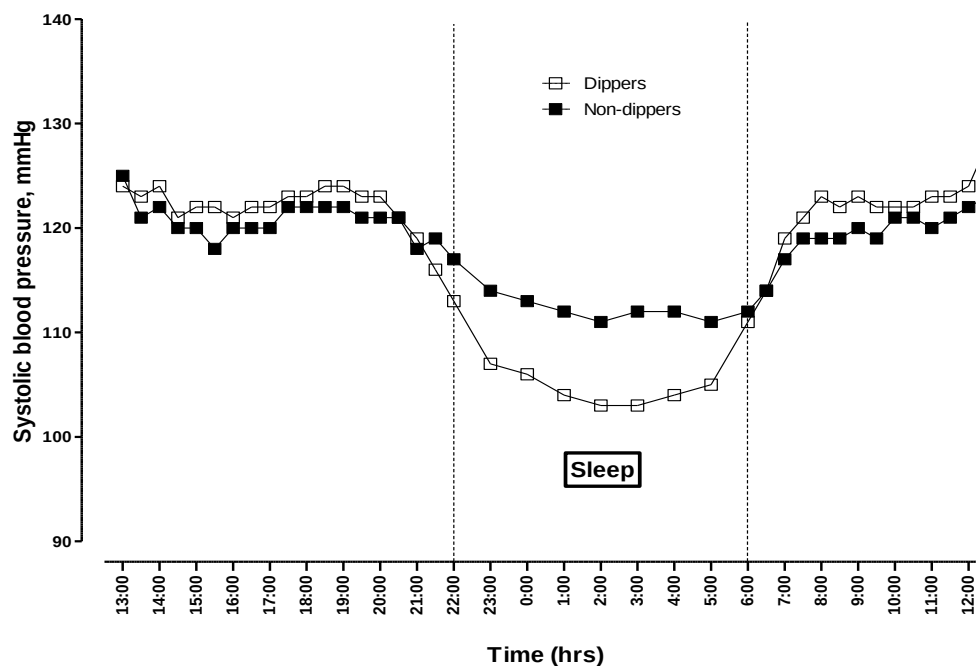


Figure 2. Patterns of night-time systolic blood pressure dipping

Previous studies indicated the effects of nocturnal dipping on MBPS, where non-dippers were reported to show a lower MBPS compared to dippers (32, 33, 86, 92). Evident lower MBPS in non-dippers may be influenced by the suppressed night-time blood pressure dipping of non-dippers, considering that MBPS is quantified by the difference between morning and night-time blood pressure. Authors proposed that increased activity of the sympathetic nervous system may lead to blunted nocturnal dipping in non-dippers (93). Impaired dipping of blood pressure at night was identified as a risk factor for cardiovascular (32, 33). However, mechanisms by which blunted nocturnal dipping leads to elevated (in dippers) or lower (in non-dippers) MBPS have not been fully elucidated.

1.3.2.5 Hypertensive status

Hypertension may cause exaggerated MBPS due to increased alpha adrenergic activity of the sympathetic nervous system in the morning hours (94-98). Hypertension may increase the alpha-adrenergic receptor sensitivity through pressure induced endothelial dysfunction with relative enhancement of alpha-2 receptor mediated vasoconstriction (94-98). As a result of the increased receptor sensitivity, alpha adrenergic activity increases vascular tone in small resistance arteries, leading to exaggerated MBPS in hypertensive individuals (2, 4, 24, 30, 94).

1.3.3 Biochemical factors

1.3.3.1 Inflammation

Previous studies reported that vascular inflammation is associated with exaggerated MBPS in hypertensive patients (4, 41). A study conducted by Savoia *et al.* indicated that the occurrence of vascular inflammation in hypertensive individuals may be caused by mechanical, structural or functional alterations (due to increased blood pressure) that lead to vascular remodeling and up-regulation of inflammatory mediators and cells (99). In addition, levels of inflammatory markers such as C-reactive protein, interleukin-6 and tumor necrosis factor alpha are higher in hypertensive patients due to vascular remodeling (41, 99). Elevated wall stress can promote the production of proteoglycans that bind and retain lipoproteins. This escalates into oxidative alterations and initiates an inflammatory response, which in turn cascades into lesion formation in arterial smooth muscle cells (100). To the best of our knowledge, mechanisms by which inflammation leads to elevated MBPS are not yet clear.

1.3.3.2 Oxidative stress

Reactive oxygen species (ROS) such as oxygen ions, free radicals (superoxide and hydroxyl radicals) and peroxides (hydrogen peroxide) are products of the normal oxygen consuming metabolic process in the body (101). Reactive oxygen species are highly reactive molecules with important cell signalling roles when maintained at proper cellular concentrations (101). Reactive oxygen species can modify other oxygen species, deoxyribonucleic acid (DNA), proteins, or lipids (101). Oxidative stress is defined as “an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage” (102). Factors such as increased activity of the renin-angiotensin-aldosterone system and lifestyle choices such as smoking and excessive alcohol consumption, may lead to oxidative stress (2, 103). A biological marker for excessive alcohol use, serum γ -glutamyl transpeptidase (GGT), which is also considered as a marker of oxidative stress (104, 105), was positively associated with the MBPS in hypertensive patients (106). Production of ROS is elevated in hypertensive individuals with an exaggerated MBPS (2, 107). In addition, elevated blood pressure activates mononuclear cells and polymorphonuclear leukocytes which release ROS, leading to oxidative stress (107). As a result, increased ROS can further increase exaggerated MBPS in hypertensive patients through various mechanisms including altered endothelial function, sympathetic over-activity and inflammation (2, 19, 103, 108).

1.3.3.3 Decreased bioavailability of nitric oxide

A study conducted by Otto *et al.* proposed that endothelial dysfunction is evident in the morning, also in healthy individuals (4, 109). Endothelial function in healthy subjects was measured with ultrasound measures of flow-mediated endothelium-dependent vasodilation and endothelium-independent vasodilation of the brachial artery (109). Flow-mediated endothelium-dependent vasodilation was blunted in the early morning hours, indicating endothelial dysfunction (109). Although no specific mechanisms responsible for endothelial dysfunction in the morning were identified, factors such as increased activation of the sympathetic nervous system and adrenergic receptor sensitivity may contribute to endothelial dysfunction in the early hours of the morning, in healthy subjects (109). Endothelial dysfunction may also contribute to an exaggerated MBPS due to this reduced capacity for vasodilation, leading to increased blood pressure during the morning hours (109).

1.3.3.4 Renin-angiotensin-aldosterone-system activity

As previously mentioned, studies examining the MBPS in hypertensive adults reported that elevated MBPS may be due to neuro-humoral factors of which one is increased activation of the renin-angiotensin-aldosterone system (RAAS) in the morning (2, 18, 20, 26, 40, 110). The activity of RAAS, which is one of the main blood pressure regulatory mechanisms (111), and aldosterone levels were shown to increase before awakening and further increased after awakening in normotensive individuals (4, 112). The RAAS also plays a major role in cellular growth and cardiovascular remodeling (113-115). Its components have been linked to hypertension and hypertension-mediated organ damage leading to cardiovascular morbidity and mortality (116). In the classical RAAS, the substrate angiotensinogen is degraded by renin into angiotensin I, which is then converted into angiotensin II by angiotensin converting enzyme. Angiotensin II is the main effector molecule of the system that stimulates signal pathways in the heart, vasculature and kidneys that result in physiological and pathophysiological effects attributable to the RAAS (117-119).

Elevated RAAS activation leads to a higher blood pressure through various mechanisms, including increased vascular tone through direct vasoconstrictor, pro-inflammatory, pro-fibrotic, hypertrophic effects of angiotensin II, as well as increased production of ROS (2, 99, 120). Low activity of the RAAS system is a well-known phenomenon in the black population (121, 122). As a result, the role of RAAS in the maintenance of blood pressure and development of hypertension in the black populations that are commonly affected by low-renin hypertension, is questionable. Due to this reason, antihypertensive medications such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs) are not as effective in black populations as in white populations (122).

1.3.4 Lifestyle and health behaviours

1.3.4.1 Tobacco use

Nicotine is a compound of tobacco that is associated with the physiological mechanisms of blood pressure regulation, which explains the influences of tobacco on acute increases in blood pressure (47, 123). To the best of our knowledge, no study has reported the effects of nicotine on the MBPS; however, the following mechanisms may highlight the effects of nicotine on MBPS. Smoking may lead to a decrease in nitric oxide resulting in vasoconstriction, leading to an increase in blood pressure (124). Furthermore, nicotine activates the sympathetic nervous system which is the primary mechanism responsible for exaggerated MBPS (125).

Cigarette smoke also contains chemically active oxidizing agents (126, 127) that increase ROS production, eventually leading to the occurrence of oxidative stress, and consequently tissue damage (124, 127-129). Furthermore, cigarette smoke also activates monocytes, platelets, leukocytes, and endothelial cells which increase the burden of ROS, contributing to inflammatory responses, which were previously described as factors that lead to exaggerated MBPS (2, 4, 41, 99, 120, 126).

1.3.4.2 Physical activity

An increase in the MBPS has been associated with the onset of physical activity immediately after waking in the morning, independent of age, smoking status, body mass index and time of waking (48, 130). The onset of physical activity after waking is associated with a direct sympathetic neural input to the heart and vasculature, leading to increased blood pressure in the morning (48, 131). Furthermore, for a given level of physical activity, hypertensive patients have a higher MBPS than normotensive individuals (48). Although intense physical activity (when carried out immediately after waking up in the morning) has a negative effect on MBPS, it is important to highlight reports from other studies indicating that regular physical activity reduces blood pressure in hypertensive individuals and may also prevent the development of hypertension (132-134).

1.3.4.3 Habitual alcohol consumption

Blood pressure levels were reported to decrease soon after consumption of alcohol at night-time, and, only increase in the early hours of the morning (approximately 10 hours later), leading to exaggerated MBPS (46). However, mechanisms by which alcohol consumption leads to elevated MBPS are unclear. Possible mechanisms that were proposed in previous studies include stimulation of sympathetic activity and the RAAS and endothelial dysfunction (46, 47). A marker of excessive alcohol consumption, serum GGT, was previously associated with elevated MBPS (106). The GGT may contribute to an exaggerated MBPS due to its pro-oxidative effect, as it is involved in the degradation of the antioxidant glutathione and has an indirect pro-oxidative effect by causing low-density lipoprotein cholesterol oxidation in the presence of iron (106).

1.4 Effects of morning blood pressure surge on the vasculature and subclinical hypertension-mediated organ damage

Apart from cardiovascular mortality (135), an exaggerated MBPS has been associated with hypertension-mediated organ damage such as left ventricular hypertrophy and kidney damage, as well as retinal artery narrowing, venular widening, arterial stiffness and carotid intima-media thickness as shown in Figure 3 (4, 20, 24, 136). The age of the population included in the previously mentioned studies ranged between 45 and 72 years (4, 20). It is therefore important to investigate the effects of an elevated MBPS on subclinical hypertension-mediated organ damage in young individuals to potentially identify new mechanisms to delay the occurrence of hypertension-mediated organ damage.

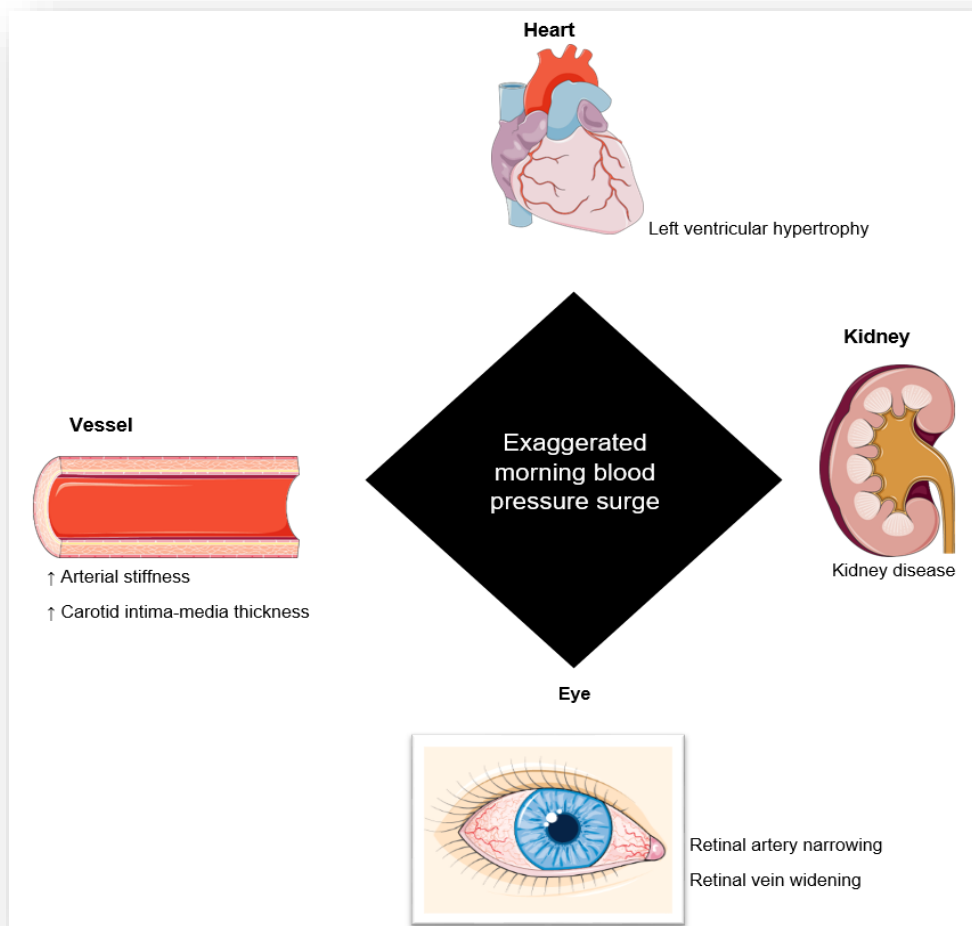


Figure 3. Morning blood pressure surge and its relation with hypertension-mediated organ damage

1.4.1 The arterial wall

The arterial wall contains the extracellular matrix (ECM) which provides a structural framework essential in the functional properties of arteries (61, 137, 138). Figure 4 shows the vascular wall containing three layers embedded in the ECM, namely the tunica intima (inner layer), tunica media (middle layer) and tunica adventitia (outer layer) in the ECM (61, 137, 139). Each layer plays an essential role in the vascular system. The tunica intima layer comprises of internal elastic lamina, fibro-collagenous tissue and a single layer of endothelial cells (61). The medial layer consists of vascular smooth muscle cells important for depositing ECM proteins. Two of these important proteins, namely elastin and collagens, are essential in giving arteries their elastic properties (61, 137, 139-142).

The adventitia contains fibroblasts and consists of external elastic lamina embedded between two fibro-collagenous layers (61, 137). Elastin is the most abundant protein and is important for the elasticity of arteries and regulation of arterial compliance (61, 137, 140-142). Collagens are essential for structural support to local cells and prevention of rupture of vessel walls in response to volume changes (61, 137, 140-142).

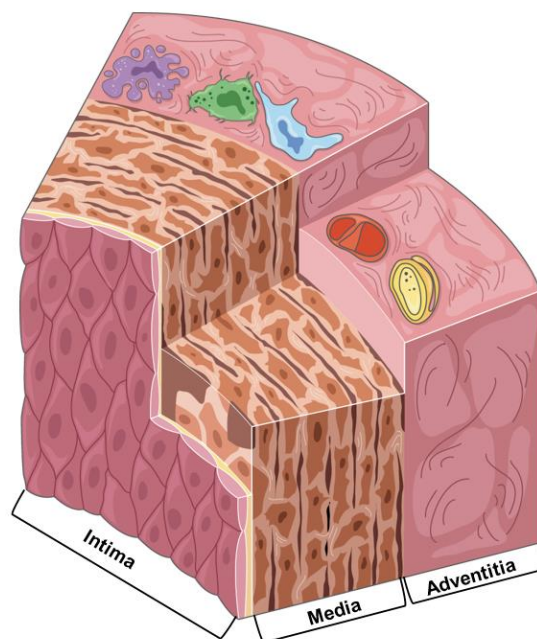


Figure 4. Cross-sectional view of the arterial wall

Previous studies reported the importance of the microvasculature because microvascular changes are considered to precede macrovascular pathologies (143, 144). Exaggerated MBPS was shown to cause direct damage to small blood vessels through increased

mechanical pressure and shear stress on the vessel wall, leading to vascular remodeling (4, 24, 34). Vascular remodeling is characterized by increased collagen and enhanced elastin breakdown, and the proliferation of vascular smooth muscle cells (57, 61, 62, 99). Furthermore, the presence of structural alterations in the microvessels may play an important role in the development of cerebral ischemic attacks, heart failure, ischemic heart disease and renal failure (34).

1.4.2 The retinal microvasculature

The retinal microvasculature has gained increasing scientific interest as it bears a close resemblance with the coronary and cerebral vasculature (145) and can be easily studied using non-invasive devices such as a fundoscope as implemented in the Dynamic Retinal Vessel Analyzer (DVA) (146, 147). The DVA makes it possible to summarize equivalents for the calibres of the central retinal artery (CRAE) and vein (CRVE) (146-148). Retinal microvascular diameter changes in response to blood pressure fluctuations (149). This is a physiological phenomenon termed autoregulation, which is essential for protecting the capillary network against long-term high blood pressures (149). However, hypertension is associated with adverse structural changes of the microvasculature, including retinal arteriolar narrowing (presented with a reduced CRAE) and venular widening (presented with a wide CRVE) (150). Retinal arteriolar narrowing, which may reflect greater peripheral resistance, is related to the prediction of hypertension, coronary heart disease and arterial stiffness, whereas venular widening is associated to atherosclerosis and incident stroke (151-154). It is noteworthy to mention that the relationships between the MBPS and retinal vessel calibres have, to the best of our knowledge not yet been studied.

1.4.3 Kidney function

Studies reported that elevated MBPS may induce renal pathophysiology in non-diabetic individuals and those with diabetes and hypertension, leading to renal damage (155-157). Markers such as estimated glomerular filtration rate and urinary albumin-to-creatinine ratio are used to estimate renal function (158). Glomerular filtration rate (GFR) is defined as the amount of filtrate produced in the kidneys each minute (159). A cut-off value for glomerular filtration rate that is 60 milliliter (mL) per minute or above is associated with normal kidney function, whereas a value below 60 mL per minute is associated with kidney disease, whereas GFR <15 mL per minute is associated with kidney failure (Figure 5) (160).

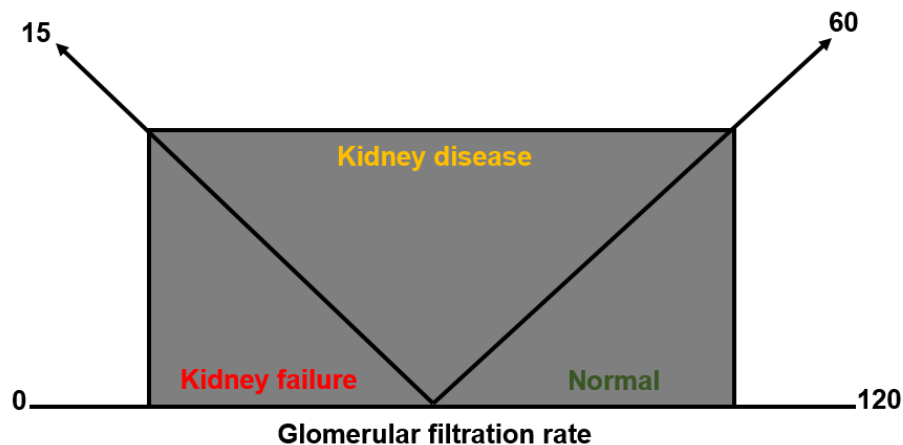


Figure 5. Cut-off values of glomerular filtration rate (160)

Urinary albumin excretion is used as an early sign of increased risk for developing kidney disease (161, 162). A urinary albumin-to-creatinine ratio that is 30 mg/g or higher is associated with chronic kidney disease (162). Significant associations between albumin-to-creatinine ratio and ambulatory blood pressures were reported in non-diabetic South African men with normal kidney function (163), but to the best of our knowledge, information regarding the mechanisms that explain kidney damage due to exaggerated MBPS is scant (157). However, a study conducted by Turak *et al.* reported that elevated MBPS may cause structural changes in the small resistance arteries of the kidneys, leading to kidney damage and chronic kidney disease (157).

1.4.4 Large artery stiffness

Arterial stiffness is independently associated with both hypertension-mediated organ damage (heart and kidneys) and an increased risk for both cardiovascular morbidity and mortality (164). The contribution of an exaggerated MBPS towards the development of arterial stiffness was previously investigated in hypertensive patients with an average age of 73 years (4, 14). These investigations found a positive relationship between exaggerated MBPS and arterial stiffness indices through mechanisms such as vascular remodeling (4, 24, 165).

Large elastic arteries are important for effective cardiac function by serving as elastic reservoirs to ensure adequate blood flow to tissues and organs according to their metabolic requirements (137, 166). It also enables the arterial tree to undergo volume changes with minor changes in arterial pressure (137, 166). Elastic arteries store a portion of blood volume

from the left ventricle during systole and discharge it during diastole (137). This helps to reduce the load on the heart and it also minimizes systolic volume while maximizing diastolic volume in the arterioles (137). Arteries also react to changes in physical conditions such as exaggerated MBPS, adapting to the new surroundings through vascular ECM remodeling (138, 167). Smaller ECM proteins and components such as laminins, fibrilin, fibulins, integrins and matrix metalloproteinase are also involved in ECM alterations, and partly linked to arterial stiffness (61, 137, 168, 169). Blood pressure determines arterial wall stretch and shear stress (166). Elevated blood pressure causes tension on the arterial wall, initiating the response of smaller arteries to the force through vascular smooth muscle cell hypertrophy and ECM remodeling as shown in Figure 6. This allows arteries to withstand the increased pressure load (138, 166). Remodeling results in arterial stiffness and decreased arterial compliance and distensibility, reducing arterial elastic properties (138, 170). Alterations in parameters, such as pulse wave velocity and the carotid intima-media thickness, are associated with the occurrence of arterial stiffness (61, 171, 172). Pulse wave velocity is defined as the speed at which the forward pressure wave generated by the heart is transmitted from the aorta and reflected from the peripheral sites back to the heart (173, 174). The proposed threshold for pulse wave velocity in adults is 10 m/s and the higher the pulse wave velocity, the stiffer the arteries (175-177).

Furthermore, three studies reported positive associations between carotid-femoral pulse wave velocity and MBPS in type 2 diabetic patients as well as both untreated and treated hypertensive individuals (165, 167, 178). Carotid intima-media thickness is a measurement of the thickness of tunica intima and tunica media (61). Elevated MBPS was previously associated with increased intima-media thickness in patients with pre-hypertension and cardiac syndrome-X (179, 180).

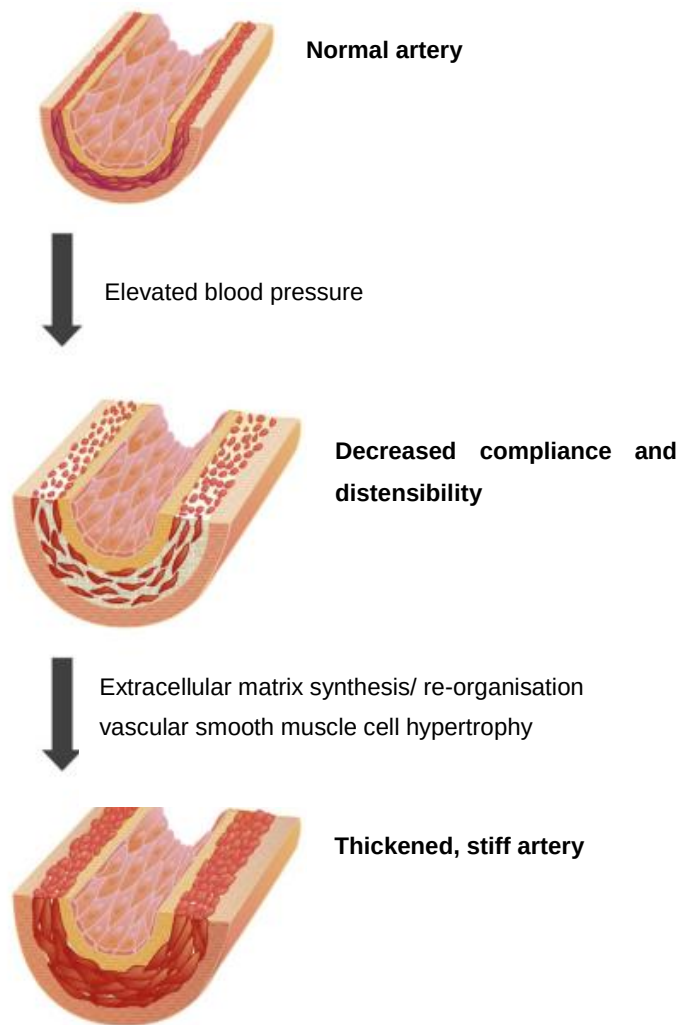


Figure 6. Schematic presentation of arterial remodeling (138)

1.4.5 Cardiac function

Exaggerated MBPS was shown to associate positively with indices of hypertensive heart disease, such as left ventricular hypertrophy and left ventricular mass index; and inversely with left ventricular diastolic function in hypertensive patients (4, 20, 40, 181). Increased arterial stiffness, possibly due to elevated MBPS, may lead to cardiac hypertrophy through impaired baroreceptor sensitivity (24) and increased pulse wave velocity (182, 183). Increased pulse wave velocity may lead to an early return of reflected waves from the peripheral vasculature to the aorta (183). In turn, the aortic and left ventricular pressures increase during systole whereas the mean diastolic pressure decreases. Synchronization between the ejected and reflected wave is now disturbed, which reaches the aorta during systole in older individuals, increasing left ventricular afterload and leading to hypertrophy of the left ventricle (184).

1.5 Importance of exaggerated morning blood pressure surge in cardiovascular outcomes

Elevated MBPS, has independent predictive value for cardiovascular mortality and events (that occur during the morning hours after waking up) such as stroke, myocardial infarction and sudden cardiac death independent of blood pressure dipping status, age and 24-hour ambulatory blood pressure (14, 18, 20-23, 43, 185). Longitudinal studies found that MBPS predicts cardiovascular outcome in elderly, normotensive and hypertensive individuals (4, 23, 32, 35, 43). A previous study conducted in 8 different populations from different countries reported that a sleep-trough MBPS that is equal or greater than 37 mmHg can be used as a cut-off value for prediction of increased cardiovascular risk and is associated with 30% increase in cardiovascular morbidities and mortality (22, 43). Thus, MBPS may be presented as an important biomarker of cardiovascular risk (22). Given the predictive value of elevated MBPS, identifying ways to reduce exaggerated surge of blood pressure in the morning hours to avert cardiovascular events, is essential. A better physiological understanding on exaggerated MBPS in the youth may aid in better future interventions to prevent or delay cardiovascular events.

1.6 Problem statement and motivation

Previous studies conducted in other countries reported that the MBPS is lower in older normotensive and hypertensive black individuals compared to their white counterparts, aged between 40 and 50 years (18, 37, 38). The lower prevalence of exaggerated MBPS in black populations is unexpected, since hypertension has a high prevalence in the black population and is also known to develop at a young age (186-189). Furthermore, in South Africans, it was reported that increased sympathetic nervous system activity, as shown by cardiovascular reactivity to stress, may be an important factor that increases blood pressure in the black population through increased total peripheral resistance and heart rate (190). As previous studies investigating the MBPS in black populations were conducted in black populations not residing in Africa (18, 37, 38), it is important to know if young black South Africans also present with lower MBPS when compared with their white counterparts. There is limited knowledge of the MBPS profile in young healthy individuals. This study will specifically include young black and white adults in an attempt to provide a better physiological and pathophysiological understanding of MBPS profile, and its association with the vasculature and specific markers of subclinical hypertension-mediated organ damage in a young black population, prior to disease development. This is particularly to investigate in black populations, prone to the development of hypertension (103, 187, 191-193). Knowledge gained during this study may

also give a better understanding of the early processes of cardiovascular disease development. To the best of our knowledge, no study has been conducted to investigate the MBPS in young black and white normotensive adults, and also the relationships between the MBPS, markers of arterial function and subclinical hypertension-mediated organ damage in healthy South African men and women, aged between 20 and 30 years.

1.8 Aims and objectives

This study aimed to obtain a better understanding regarding the MBPS and how it relates to arterial function and subclinical hypertension-mediated organ damage in a healthy bi-ethnic South African population aged between 20 and 30 years.

The objectives were:

- To determine the interactions of sex and black and white ethnicity across increasing quartiles of MBPS. Also, to explore whether differences in cardiovascular markers and specific health behaviours exist across increasing MBPS quartiles.
- To determine if an exaggerated MBPS already occurs in young black and white adults.
- To determine whether the MBPS is associated with measurements of the microvasculature (retinal calibres - CRAE and CRVE) and macrovasculature (pulse wave velocity); and
- To establish whether the MBPS is related to measures of sympathetic nervous system activity (low frequency HRV and baroreceptor sensitivity).

1.9 Hypotheses

- There will be an interaction of sex and ethnicity, and both cardiovascular and health behaviour markers will be augmented in higher MBPS quartiles.
- A larger proportion of white adults will present an exaggerated MBPS than black adults.
- MBPS will relate positively to CRVE and pulse wave velocity, and negatively with CRAE; and
- The MBPS will relate inversely with low frequency HRV and baroreceptor sensitivity.

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CHAPTER 2

Methods

2.1 Study design

The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) is an on-going longitudinal study, which included 1202 healthy black and white individuals aged between 20 and 30 years at baseline and is currently in the first phase of a 5-year follow-up and will continue over a follow-up period of 10 years. The aim of the African-PREDICT study is to understand the early pathophysiology accompanying cardiovascular disease development, and to identify novel early markers or predictors for the development of cardiovascular disease by following young, healthy adults over time. This Ph.D. study included cross-sectional data of the first consecutive 845 participants with completed data. Self-reported black or white participants (men and women) who are apparently healthy and normotensive or pre-hypertensive were included in the study.

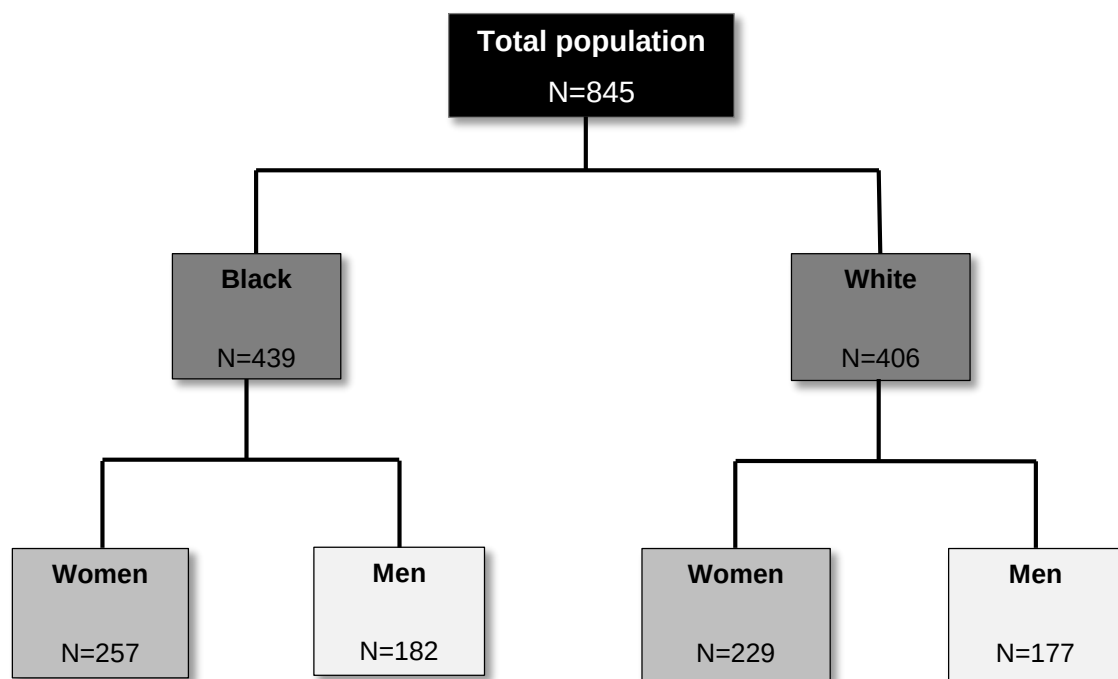


Figure 1. An illustration of the African-PREDICT sub-sample used in this Ph.D. study

2.2 Materials and methods

2.2.1 Organizational procedures

The African-PREDICT study is conducted at the Hypertension Research and Training Clinic on the Potchefstroom campus of the North-West University and is registered on ClinicalTrials.gov (NCT03292094). This study complied with all applicable requirements set out in the Declaration of Helsinki and also obtained approval from the Health Research Ethics Committee of the North-West University (NWU-00001-12-A1). This present Ph.D. study received additional ethical approval from the Health Research Ethics Committee of the North-West University (NWU-00008-17-A1) to investigate the MBPS (Appendix A). All participants provided written informed consent.

2.2.2 Recruitment and Screening

Participants were recruited through different strategies such as advertisements in newspapers and on notice boards, health screening in public places and also by direct recruitment at workplaces. Trained recruiters explained the details of the study and all procedures involved to each prospective participant. Participants had the opportunity to listen and ask questions prior to deciding if they wish to participate. Those who agreed to participate in the study were requested to complete and sign an informed consent form. Prospective participants were ensured voluntary participation and they were able to withdraw from participating in the study at any time point without any harm to them. Voluntary participation was explained to participants by a research nurse acting as an independent mediator between the Principal Investigator and the participants. After completion and signing of informed consent forms, basic testing for the screening procedure followed in a private room to ensure the privacy of participants. Tests involved in the screening procedure of the study were clearly explained to participants and included: clinic brachial blood pressure assessments, a urine sample dipstick test (to test for urine pH and presence of protein, glucose, ketone, blood, nitrite and leukocyte), body composition (height, weight as well as neck, waist and hip circumference), finger prick test for glucose and total cholesterol levels and human immunodeficiency virus (HIV) by a trained counsellor with pre- and post-counselling. The HIV status of the participants was determined with the ABON HIV 1/2/O Tri-Line Rapid Test (ABON Biopharm, Hangzhou, China) using whole blood. If positive, the HIV test was repeated with a First Response (PMC Medical, Nani Daman, India) rapid HIV test. Test results were communicated to individuals by trained counsellors in a private room and those who tested positive for HIV were referred to their physician, local clinic or hospital for to measure their CD4 count. Participants with a clinic

systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg, who were infected with HIV, previously diagnosed with a chronic disease (such as diabetes or hypertension), current pregnancy, or breastfeeding were excluded.

2.2.3 Inclusion in the African-PREDICT study

After screening, eligible participants were invited to participate in the research phase of the study. Those who agreed to participate were provided with a detailed explanation and another informed consent form and were given an opportunity to review the detailed procedures of the study and then sign the informed consent prior to any further participation. Furthermore, participants were requested to complete the consent form before commencing with the research study measurements, with the research nurse acting as an independent mediator. Each participant had an appointment scheduled and transport was provided (to and from the Hypertension Research and Training Clinic at the North-West University Potchefstroom Campus) if required. Participants were requested to fast on the research study day and were provided with a meal during the day after completing fasting-required procedures.

2.2.4 Questionnaires

Participants completed a standard general health and demographic questionnaire to obtain basic details regarding their medical history, lifestyle, socio-economic status and traditional risk factors (including sex, age, smoking and alcohol consumption). Participants' socio-economic status score was determined using a point system that was adapted from the Kuppuswamy's socio-economic status scale, based on their skills level, education and household income (1). The Berlin questionnaire was used to assess sleep apnea risk (1). Sleep apnea was suggested to induce sympathetic nervous system activity and increasing night-time blood pressure, thereby leading to exaggerated MBPS (2).

2.2.5 Body composition and accelerometry

Anthropometric measurements included body height (SECA stadiometer, SECA) and weight (SECA electronic scales, SECA, Birmingham, UK). Waist circumference was measured in triplicate, using a validated non-flexible tape measure (Holtain, Crymych, UK) (3). Body mass index is used as a classification system for obesity (4) and was calculated from body weight divided by height (m) in squared meters. Exaggerated MBPS was reportedly associated with a high body mass index (possibly due to physical inactivity) in patients with essential hypertension (5). All anthropometric measurements were performed according to the guidelines and procedures as described by the International Society for the Advancement of

Kinanthropometry. Anthropometric measurements were done in private rooms by trained researchers, with participants in minimal clothing to ensure accurate results.

Body fat percentage and lean mass were obtained through bio-electrical impedance assessment (Biostat 1500, Biostat Ltd, Isle of Man, UK).

Each participant was also fitted with a combined heart rate and accelerometry device (ActiHeart, CamNtech Ltd., Cambridge, UK) which was worn on the chest to record physical activity, total energy expenditure and activity energy expenditure continuously for 7 consecutive days. Immediate onset of physical activity after waking up was associated with exaggerated MBPS (6). Physical activity has well-known positive effects on cardiovascular disease risk and all-cause mortality (7). In addition, regular physical activity was previously associated with reducing blood pressure and preventing the development of hypertension (8-10).

2.2.6 Cardiovascular measurements

2.2.6.1 Clinic brachial blood pressure measurements

Clinic brachial blood pressure and heart rate were measured using the Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA) with an appropriately sized GE Critikon latex-free Dura-Cuffs. As indicated by the European Society of Hypertension guidelines, blood pressure was measured in duplicate on both arms with participants in a sitting position, after a resting period of 10 minutes (11).

2.2.6.2 Ambulatory blood pressure over 24-hours

Participants were fitted with a validated 24-hour ambulatory blood pressure and ECG apparatus (Card(X)plore®, CE0120, Meditech, Budapest, Hungary) (12) and an appropriately sized cuff was fitted to the participant's non-dominant arm to measure ambulatory blood pressure. Ambulatory blood pressure was used to determine morning blood pressure surge (MBPS), dipping status and heart rate variability (HRV). The apparatus was programmed, according to guidelines (13), to take blood pressure recordings every 30 minutes during the day (6am to 10pm) and every hour during the night (10pm to 6am). Over the 24-hour period that ambulatory devices were worn by participants, 89% successful mean inflation rate was achieved for this Ph.D. study sample. Masked hypertension was classified as normal clinic blood pressure (<140 and <90 mmHg) but elevated 24-hour systolic and/or diastolic blood pressure $\geq 130/80$ mmHg; daytime systolic and/or diastolic blood pressure $\geq 135/85$ mmHg;

and/or night-time systolic and/or diastolic blood pressure $\geq 120/70$ mmHg (11). Several subtypes of masked hypertension exist (14). Morning hypertension was identified as one of the most common forms of masked hypertension and may influence the magnitude of MBPS (14).

Night-time blood pressure dipping was determined as a categorical variable determined from the CardioVisions software (classified as non-dipper when the diurnal index was $<10\%$ and dippers when diurnal index was $>10\%$) or as a continuous variable (percentage dipping) by calculating the percentage SBP dipping $[1 - (\text{SBP}_{\text{sleeping}} / \text{SBP}_{\text{awake}}) \times 100\%]$ (15-17).

2.2.6.3 Morning blood pressure surge

Morning blood pressure surge was calculated using two methods; (1) based on wake up and sleep times of participants and (2) based on a method known as 24-hour clock-based MBPS, which does not require the wake up and sleep times of participants. Three measures of MBPS based on the sleep and wake up times of participants namely, sleep-trough surge, prewaking surge and rising were quantified, using the following formulas:

Sleep-trough surge: morning systolic blood pressure (2-hour average of four 30-minute systolic blood pressure readings just after waking) minus the lowest nocturnal systolic blood pressure (average blood pressure of 3 readings centered on the lowest nighttime reading).

Prewaking surge: morning systolic blood pressure minus the prewaking systolic blood pressure (2-hours average of four systolic readings just before waking).

Rising surge: single morning systolic blood pressure measured on rising minus the systolic blood pressure reading in a supine position, 30 minutes before waking.

The wake up and sleep times of participants were obtained from heart rate data of participants that were acquired from the ActiHeart software (ActiHeart, CamNtech Ltd., Cambridge, UK). The wake up and sleep times of participants whose data was missing on the ActiHeart software were obtained from the ABPM diary card where participants recorded their wake up and sleep times.

The 24-hour clock-based MBPS was calculated for all participants. The additional types of MBPS are as follows:

Average morning surge: average morning systolic blood pressure (2-hr average of morning systolic blood pressures between 7am and 9am) minus average night-time systolic blood pressure (average of nighttime systolic blood pressures between 1am and 6am).

Dynamic morning surge: the moving peak morning systolic blood pressure (highest 1-hr moving average of two consecutive systolic blood pressures between 6am and 10am) minus moving lowest night-time systolic blood pressure (lowest 1-hr moving average of consecutive systolic blood pressures between 1am and 6am).

Maximum dynamic morning surge: maximum morning systolic blood pressure (maximum morning systolic blood pressure between 6am and 10am) minus minimum night-time systolic blood pressure (minimum night-time systolic blood pressure during 1am and 6am).

Perimorning surge: the maximum morning systolic blood pressure (maximum morning systolic blood pressure between 6am and 10am) minus minimum morning systolic blood pressure (minimum morning systolic blood pressure between 6am and 10am).

As mentioned in Chapter 1 of this thesis, sleep-trough surge is considered as the preferred method for quantifying MBPS, and for the purposes of this study, we chose dynamic morning surge as the ideal method for quantifying the 24-hour clock-based MBPS due to its quantification similarities to those of sleep-trough surge.

2.2.6.4 Heart rate variability

The 24-hour HRV was measured automatically by the Cardio Visions 1.15.2 Personal Edition (Meditech, Budapest, Hungary) software, and analyses was taken from time, frequency and geometric domains. The time domain analysis included the Standard Deviation of Normal to Normal Interval, which represents the overall HRV activity in the time domain (18). The frequency domain analysis, determined by the fast Fourier transformation involve high frequency (a major indicator of parasympathetic activity) and low frequency (a major index of sympathetic cardiac tone but also has a parasympathetic component) (18). Low frequency-to-high frequency ratio (reflector of sympatho-vagal autonomic balance) and HRV total power (global determinant of overall autonomic modulation) were also determined using the frequency domain (18). Geometric domain represents the entire HRV, using the HRV triangular index and is determined by the total number of NN intervals divided by the number of NN intervals in the modal bin of the NN interval histogram (19).

2.2.6.5 Continuous arterial blood pressure assessments for calculation of baroreceptor sensitivity

Continuous arterial blood pressure measurements were acquired with a validated Finometer device (Finapres Medical Systems, Amsterdam, the Netherlands) (20). The Finometer device was connected with a finger cuff placed on the middle phalanx of the middle left finger and a cuff was also placed on the left upper arm. Participants were requested to lie in the Fowler's position with their arm at heart level. A two-minute calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure. Continuous measurement of resting cardiovascular variables was performed for a 5-minute period. The cardiovascular data collected with the Finometer was processed using the Beatscope v1.1 software (Finapres Medical Systems, Amsterdam, the Netherlands) to provide baroreceptor sensitivity. Baroreceptor sensitivity was calculated using the validated cross-correlation baroreflex sensitivity (xBRS) method (21). This method was proven to yield lower within-patient variance than other methods and was recommended to be used in clinical and experimental settings (21). This method quantifies the correlation between beat-to-beat systolic blood pressure and R-R interval, resampled at 1 Hz, over 10-sec sliding windows. This is a timespan sufficient to accommodate a 10-sec variability in rhythm, or several cycles at ventilatory frequencies (21).

2.2.6.6 Central pulse pressure and pulse wave velocity

Central systolic blood pressure and the “golden standard” measurement of arterial stiffness called carotid-to-femoral pulse wave velocity (along the descending thoracic-abdominal aorta – Figure 2) were respectively, determined in duplicate with the validated SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) (22). Participants were requested to be in a supine position and relaxed state for approximately 5 minutes before commencement of the measurement. The device was firstly used to estimate central systolic blood pressure by performing pulse wave analysis, using a general transfer function, with a brachial cuff placed on the right upper-arm. Afterwards, the participants' right carotid artery pulse wave was recorded by a tonometer while the femoral pulse was measured with a femoral cuff placed around the upper thigh of the participant. The distance between the carotid pulse point and upper femoral cuff were measured, and 80% of these distances were used as the pulse wave travelled distance (23).

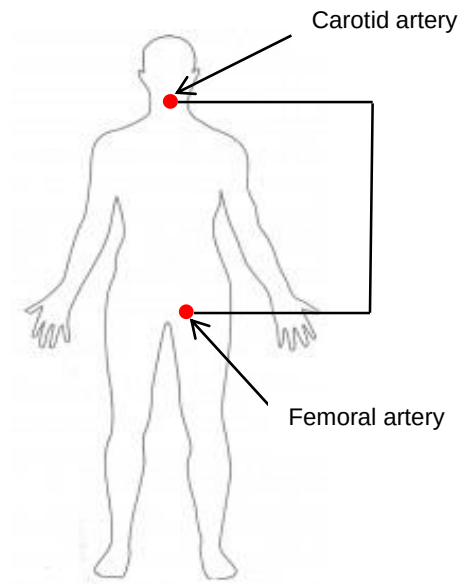


Figure 2. Points of measuring carotid to femoral pulse wave velocity

2.2.6.7 Retinal vessel calibres

Retinal photography was performed using the Dynamic Retinal Vessel Analyzer (Imedos, Jena, Germany) fitted with a Zeiss Fundus camera FF-450plus, to assess non-invasive measurements of retinal vessel calibres during resting conditions (static) (24-26). Static retinal measurements provide information on retinal vessel calibres such as central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE). Food and fluid (such as caffeine) intake as well as smoking and exercise were refrained 1 hour prior to the measurement due to their retinal arteriolar narrowing and/or venular widening effects thereof (27-29). The retinal vessel measurements were conducted in a half-lit temperature-controlled room. A drop of Tropicamide (1% Alcon) was administered in the right (used the majority of the time) eye to induce mydriatic conditions, fifteen to thirty minutes prior to the measurement. The left eye was used only if the right eye was not suitable.

Colour and monochrome retinal images were captured using the Visualis 2.81 software with the camera positioned at a 50° angle. The monochrome image underwent vessel analysis using VesselMap2 software to determine retinal vessel calibres. When the monochrome image was not of a suitable quality, the colour image was used. All vessel trunks located between 0.5 and 1.0 optic disc diameters (Figure 3) from the outer margin of the optic disc were marked as either arteriole or venule.

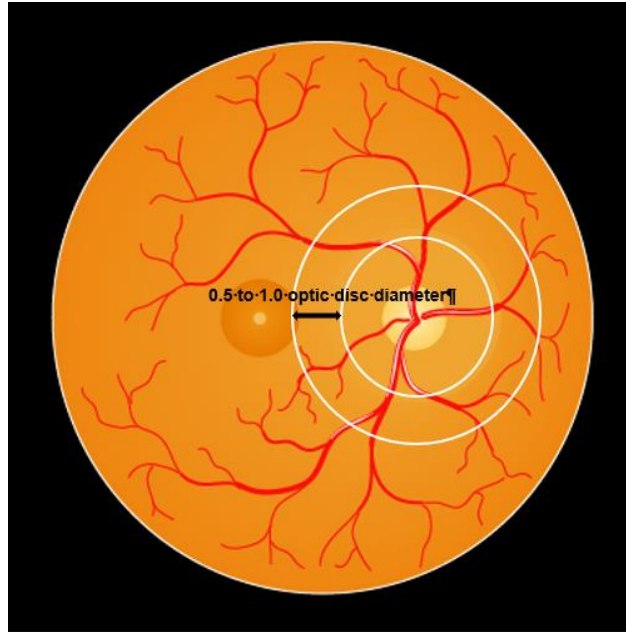


Figure 3. Vessel segments located within 0.5 – 1.0 optic disc diameters

The retinal calibres were measured in Measuring Units, where 1 Measuring Unit is equivalent to $1\mu\text{mol/l}$ in the normal Gullstrand eye. The six largest arteries (red) and veins (blue) (Figure 4) in the measuring area were used for the calculation of CRAE and CRVE using revised formulas (30). Both CRAE and CRVE were measured in measuring units (MU), where 1MU is equivalent to $1\mu\text{M}$ if the dimensions of the eye are similar to the normal Gullstrand eye.

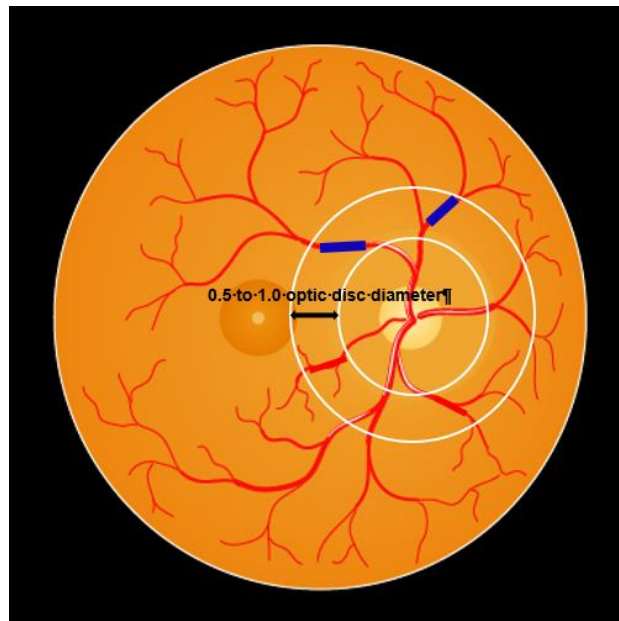


Figure 4. Measurement of retinal arteries and veins

2.2.7 Biological sampling and biochemical analyses

All participants were required to fast overnight for a period of eight to ten hours. In the early morning, mid-stream spot urine and blood samples were collected. A registered research nurse used a sterile needle to draw blood from the ante-brachial vein. Trained staff members also explained the collection protocol of the 24-hour urine sample according to World Health Organization standards (31). The 24-hour urine samples were collected to determine daily salt intake. Furthermore, participants were allowed to collect the sample on a day that was convenient for them, which was noted. The “first-pass urine” was discarded, and all urine passed thereafter was collected in the container provided, including the first urine of the following morning. Participants recorded the start and finish times of the collection period. Incomplete urine collections were defined as a volume <300 mL per 24-hours and/or a 24-hour creatinine excretion of <4 mmol or >25 mmol in women, and <6 mmol or >30 mmol in men (32).

Whole blood, serum and plasma samples were prepared according to standard procedures and stored at -80°C until they were analysed. The following biomarkers were analysed and used for this Ph.D. sub-study: γ -glutamyl transpeptidase, high sensitivity C-reactive protein, cortisol, cotinine and daily salt intake (sodium chloride). All biochemical variables were tested for intra- and inter-assay variability to ensure the specificity, sensitivity and reliability. Intra-assay variability for all biochemical variables should be <10%, however the inter-assay variability for all biochemical variables should be <20%.

> The γ -glutamyl transpeptidase is considered a biomarker of increased alcohol consumption (33) and was previously suggested to contribute to exaggerated MBPS due to its pro-oxidative effect (33). γ -glutamyl transpeptidase was determined in serum using the Cobas Integra 400plus (Roche, Basel, Switzerland). Intra- and inter-assay variability for γ -glutamyl transpeptidase were 1.80% and 1.80%, respectively.

> High sensitivity C-reactive protein is a robust clinical biomarker for systemic inflammation, which was previously associated with exaggerated MBPS in hypertensive individuals (34, 35). C-reactive protein was determined in serum using the Cobas Integra 400plus (Roche, Basel, Switzerland). Intra- and inter-assay variability for C-reactive protein were 1.30% and 3.50%, respectively.

> Cortisol is a glucocorticoid (36) which is considered as a potential mechanism (in combination with increased autonomic function) that increases blood pressure during the process of waking up (37). Cortisol was quantified using the Roche e411 apparatus (Basel, Switzerland).

> Aldosterone is a steroid hormone which forms part of the renin-angiotensin-aldosterone-system that is regarded as one of the main blood pressure regulatory mechanisms (38). Aldosterone levels were shown to increase before awakening and further increased after waking up (39). Aldosterone levels were determined with the Radio Immuno Assay Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic). Intra- and inter-assay variability for aldosterone were 2.13% and 2.42%, respectively.

> Cotinine is a nicotine metabolite, and considered as a biomarker of both passive exposure and active smoking (40). Smoking is one of the major risk factors for both the development and progression of cardiovascular disease and vascular disease (41), and is associated with increased inflammatory markers which were previously described as factors that lead to exaggerated MBPS in hypertensive patients (34, 35, 42). Cotinine was quantified using the chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Intra- and inter-assay variability for cotinine were 10.7% and 5.50%, respectively.

> Salt sensitivity was previously linked with the development of hypertension, especially in black individuals (12). Daily salt intake (sodium chloride) was determined by multiplying the mmol/day sodium by 58.9 (combined molecular weight of sodium and chloride) (43).

To describe the general characteristics of our study population we included basic information:

> Total cholesterol (intra- and inter-assay variability were 0.51% and 1.90%, respectively), low-density lipoprotein cholesterol (intra- and inter-assay variability were 1.5% and 1.9%, respectively), high-density lipoprotein cholesterol (intra- and inter-assay variability were 1.13% and 1.00%, respectively), urinary sodium (intra- and inter-assay variability were 0.29% and 0.89%, respectively) and triglycerides (intra- and inter-assay variability were 1.6% and 1.9%, respectively) were determined in serum and fasting glucose (intra- and inter-assay variability were 1.8% and 2.1%, respectively) in fluoride plasma (Cobas Integra 400 plus Roche, Basel, Switzerland).

2.2.8 Statistical analyses

The G*power v3.1.9.2 software was used to compute the power for this study and a probability value of ≤ 0.05 to determine significance. A population of 800 was indicated as sufficient for 80% power to accurately assume probability for the posed research question. Statistical analyses were performed using the IBM® SPSS® Statistics, Version 25 software (IBM Corporation, Armonk, New York). All variables used in the statistical analyses were tested for normality by visual inspection of histograms and also reviewing the coefficients of skewness and kurtosis. In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable. Comparison of mean values between groups was performed using independent T-tests and analysis of variance, and also performed Chi-square tests to compare proportions. Pearson and partial correlations were performed to determine the potential link between MBPS and relevant variables. Multiple regression analysis was performed to determine independent relations between MBPS and relevant cardiovascular, health behaviour and biochemical variables. Logistic regression analysis was performed to compare the likelihood of various independent variables to increase MBPS in individuals with a lower and higher surge. The detailed statistical methodology used for Chapters 3, 4 and 5 are described within each chapter, as appropriate.

2.2.9 Contributions of Ph.D. student

Initially, I was involved in the Arterial Stiffness in Offspring Study (ASOS) where I was responsible for measuring continuous arterial blood pressure using the Finometer device (Finapres Medical Systems, Amsterdam, the Netherlands). Furthermore, I assessed skin advanced glycation end-products with the DiagnOptics AGE Reader, Standard Unit & Software v2.4.0.1 (DiagnOptics Technologies, Groningen, The Netherlands) and pulse wave velocity using the Complior SP Acquisition System (Artech Medical, Pantin, France). I assisted in the laboratory with aliquoting and storing urine samples, and also performed data capturing on an electronic database called REDCap.

I was involved in the initial screening phase of the African-PREDICT study where I was responsible for clinic brachial blood pressure measurements, urine sample dipstick tests as well as performing finger prick tests for glucose and cholesterol levels and determining blood groups. In the African-PREDICT study, these data were used to determine whether participants were eligible to take part in the research study. I was also involved in laboratory work, where I was responsible for aliquoting urine samples as well as centrifuging, separating blood samples into individual cryovials and storing them in bio-freezers. Furthermore, I was

responsible for doing continuous arterial blood pressure measurements using the Finometer device (Finapres Medical Systems, Amsterdam, the Netherlands). In the African-PREDICT study I also performed a thorough analysis of all 24-hour ambulatory blood pressure data, including diary card information (sleep-time and wake up times) in the dataset, analysing the data per hour and per 30 minutes, quantifying MBPS, and linking information from accelerometry data to the ambulatory blood pressure information.

I am currently involved in the Exercise, Arterial Modulation and Nutrition in Youth South Africa (ExAMIN Youth SA) study in which I am responsible for assessment of static retinal vessel diameters using a Static Retinal Vessel Analyzer (SVA-T, Imedos Systems UG, Jena, Germany). In addition, I am also responsible for collection of saliva samples.

2.3 References

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CHAPTER 3

Manuscript 1



Morning blood pressure surge in young black and white adults: The African-PREDICT Study

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INSTRUCTIONS FOR AUTHORS

- Original articles: Include an extra table to be named 'Summary Table', with two parts: firstly, the heading 'What is known about topic', and then secondly: 'What this study adds'. This should be two or three bullet points for each, with one or two short sentences for each bullet point.
- Title Page information must include: Title of the paper, Running title and Full names of all the authors and their affiliations, as well as the e-mail address of the corresponding author.
- Articles must be prepared with an unstructured abstract (maximum 250 words) designed to summarise the essential features of the paper in a logical and concise sequence.
- The manuscript must be structured as follows: Introduction, Material and methods, Results, Discussion, Acknowledgements, Conflict of Interest, Funding and References.
- References: only papers directly related to the article should be cited. Exhaustive lists should be avoided. References should follow the Vancouver format. In the text they should appear as numbers starting at one and at the end of the paper they should be listed (double-spaced) in numerical order corresponding to the order of citation in the text. All authors should be listed for papers with up to six authors; for papers with more than six authors, the first six only should be listed, followed by et al. Abbreviations for titles of medical periodicals should conform to those used in the latest edition of Index Medicus.

Abstract

An exaggerated morning blood pressure surge (MBPS) has independent predictive value for cardiovascular mortality and is suggested to be prevalent in elderly hypertensive patients; men and white populations. To better understand the MBPS profile in a young and normotensive population, we evaluated the MBPS in young adults and explored associations with demographic, cardiovascular and health behaviour measurements. We included 845 black (n = 439) and white (n = 406) men and women aged between 20 and 30 years. We calculated the sleep-trough and dynamic morning surge, and compared demographic data, health behaviours and ambulatory blood pressure according to MBPS quartiles. In the total group, higher waist circumference, socio-economic score, lean mass, ambulatory blood pressure (24-hour, daytime blood pressure) and increased night-time dipping (all $p < 0.05$) were found in the highest sleep-trough and dynamic morning surge quartiles. In the total white group, particularly men, both sleep-trough and dynamic morning surge were higher than the black group (all $p < 0.013$). More black participants were non-dippers than whites (44% vs 34%; $p = 0.004$). In multivariable adjusted regression in the total group, we found no consistent associations of MBPS with demographic and health behaviour measurements. MBPS related independently and positively with night-time percentage dipping in all ethnic groups (all $p < 0.01$). Ethnic differences in MBPS is evident in young adults, with a higher, but normal MBPS in white men. A non-dipping night-time pattern in young black adults (with reduced MBPS) and a higher MBPS (observed in dippers) may serve as potential risk factors for cardiovascular disease.

Keywords: dynamic morning surge, ethnicity, morning blood pressure surge, night-time dipping, sleep-trough surge

Introduction

An increase in morning blood pressure is a normal physiological phenomenon termed the morning blood pressure surge (MBPS) [1, 2], but MBPS was reported to be exaggerated in hypertensive patients [2, 3]. Elevated MBPS has independent predictive value for cardiovascular mortality and events such as stroke, myocardial infarction and sudden cardiac death [4-8]. MBPS occurs as a consequence of changes in the activity of the sympathetic nervous system associated with the process of awakening [8, 9]. Increased sympathetic nervous system activity induces a significant rise in both epinephrine and norepinephrine which results in an increase in blood pressure through vasoconstriction of systemic arteries [9-12]. The magnitude of the MBPS is further influenced by health behaviours including tobacco use, alcohol consumption and physical activity as well as night-time blood pressure dipping [13-17].

Distinct ways of quantifying MBPS exist and are either established on sleep and waking times or a 24-hour clock-based calculation [2, 18-20]. The quantification method most often used to determine MBPS based on waking times include the sleep-trough surge, which is defined as average morning systolic blood pressure minus moving lowest night-time systolic blood pressure [20]. Sleep-trough surge is considered the preferred method [2, 21] since it remains significantly associated with cardiovascular risk after controlling for dipping status [2]. Less is known regarding the preferred method for quantifying 24-hour clock-based MBPS. One such measure is the dynamic morning surge which is defined as average morning systolic blood pressure minus average night-time systolic blood pressure [20].

Previous studies focusing on exaggerated MBPS were mainly conducted in elderly and hypertensive populations [2, 3, 8, 21-24] with scant knowledge of exaggerated MBPS occurrence in young healthy individuals. High prevalence of hypertension is evident in black adults, however, the sleep-trough surge was found to be greater in white compared to black populations [1, 4, 25]. The MBPS was further found to be higher in older men compared to women (aged between 40 and 80 years) [26]. The aim of this study was to evaluate MBPS and its potential determinants (demographic, cardiovascular and health behaviour measures) in young (20 to 30 years) black and white men and women.

Materials and methods

Study design, population demographics and basic procedures

This study is embedded in the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT). The aim of the African-PREDICT study is to understand the early pathophysiology accompanying cardiovascular disease (CVD) development and to identify novel early markers or predictors for the development of CVD by following young, healthy adults over a period of 10–20 years. The African-PREDICT study is currently conducted at the Hypertension Research and Training Clinic on the Potchefstroom campus of the North-West University and is registered on ClinicalTrials.gov (NCT03292094). This study complied with all applicable requirements of the Declaration of Helsinki and also obtained approval from the Health Research Ethics Committee of the North-West University, South Africa. Participants were recruited from the Potchefstroom and surrounding areas by field workers through their workplace or advertisement by means of local newspapers or radio stations. Young (20–30 years of age), apparently healthy black and white men and women were included in the study after an initial screening day. Participants with known disease or risk factors that may influence cardiovascular health, such as individuals with mean BP out of 4 measurements ≥ 140 mm Hg and/or ≥ 90 mm Hg, who were human immunodeficiency virus infected or previously diagnosed with a chronic disease were excluded. Furthermore, pregnant or breastfeeding women were also excluded due to known effects of hormones on cardiovascular health. All procedures were explained to the participants before measurements commenced and informed consent was obtained from all participants. This study included cross-sectional baseline data from the first 845 participants of the African-PREDICT study, with complete ambulatory blood pressure recordings.

Body composition measurements

Anthropometric measurements including body height (SECA stadiometer, SECA), weight (SECA electronic scales, SECA, Birmingham, UK) and waist circumference (validated non-flexible tape measure (Holtain, Crymych, UK)) were measured in triplicate. Body mass index (BMI) was calculated. Body fat percentage and lean mass were obtained through bio-electrical impedance assessment (Biostat 1500, Biostat Ltd, Isle of Man, UK).

Questionnaire data

A general health and demographic questionnaire was completed by each participant to obtain basic details regarding medical history, lifestyle, socio-economic status and traditional risk factors (sex, age, smoking and alcohol consumption). Socio-economic status was calculated using a point system that was adapted from the Kuppuswamy's Socioeconomic Status Scale [27] for a South African environment. The adapted version scored participants in three categories: skill level, education and household income. Skill level was classified according to the South African Standard Classification of Occupation. The Berlin questionnaire was used to assess sleep apnea risk of participants.

Physical activity

Each participant was fitted with a combined heart rate and accelerometer device (ActiHeart, CamNtech Ltd., Cambridge, UK) which was worn on the chest and recorded physical activity, total energy expenditure and activity energy expenditure for a maximum of 7 consecutive days. Total energy expenditure and activity energy expenditure were standardised for weight.

Cardiovascular measurements

Clinic blood pressure was measured with a Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA) in duplicate on both arms with participants in a sitting position, after a resting period of 10 minutes. Participants were also fitted with a 24-hour ambulatory blood pressure monitor (ABPM) and ECG apparatus (CardioXplore®, CE0120, Meditech, Budapest, Hungary). The apparatus measured daytime blood pressure recordings every 30 minutes (6am to 10pm) and night time blood pressure every hour (10pm to 6am). Over the 24-hour period that ambulatory devices were worn by participants, we achieved 89% successful mean inflation rate. Masked hypertension was defined as a normal clinic blood pressure (<140 and <90 mmHg), but elevated 24-hour systolic and/or diastolic blood pressure $\geq 130/80$ mmHg; daytime systolic and/or diastolic blood pressure $\geq 135/85$ mmHg; and/or night-time systolic and/or diastolic blood pressure $\geq 120/70$ mmHg [28]. Morning blood pressure surge was quantified from ABPM blood pressure measurements as previously described [29, 30]. Sleep-trough and dynamic morning surge were determined from valid 24-hour systolic blood pressure measurements. Sleep-trough surge was determined as the morning systolic blood pressure (2-hour average of four 30-minute systolic blood pressure readings just after waking) minus the lowest nocturnal systolic blood pressure (average blood pressure of 3 readings centred on the lowest night-time reading) [2]. Dynamic surge was

determined as the moving peak morning systolic blood pressure (highest 1 hour moving average of consecutive systolic blood pressures between 6am and 10am) minus moving lowest night-time systolic blood pressure (lowest 1 hour moving average of consecutive systolic blood pressures between 1am and 6am) [31, 32].

Biochemical sampling and analyses

Blood and 24-hour urine collection

Participants were required to fast overnight for a period of eight to ten hours. Blood samples were obtained with a sterile winged infusion set and syringes from the antebrachial vein. Trained staff members explained the collection protocol of the 24-hour urine sample according to World Health Organization standards [33]. Furthermore, they were allowed to collect the sample on a day that was convenient for them, which was noted. The “first-pass urine” was discarded, and all urine passed thereafter was collected in the container provided, including the first urine of the following morning. Participants recorded the start and finish times of the collection period. All samples were prepared according to standard procedures and stored at -80°C for future biochemical analyses. Serum γ -glutamyl transpeptidase was quantified with an enzymatic colorimetric method, whereas urinary sodium (mmol/day) was quantified with ion-selective electrode potentiometry and high sensitivity C-reactive protein was determined in serum and fasting glucose in fluoride plasma (Cobas Integra 400 plus Roche, Basel, Switzerland). We calculated daily salt intake (sodium chloride) in g/day by multiplying the mmol/day sodium by 58.9 (combined molecular weight of sodium and chloride). Cotinine was quantified with a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany).

Statistical analyses

We used IBM® SPSS® Statistics, Version 24 software (IBM Corporation, Armonk, New York) for statistical analyses. The difference in the sample size for sleep-trough ($n=650$) and dynamic morning surge ($n=845$) is a consequence of missing data regarding waking and sleep times recorded by participants, which are important for quantifying sleep-trough surge. Sample size estimation was performed for this study, and a population of 600 was indicated as sufficient for 80% power to accurately assume probability for the posed research question. All variables were tested for normality by visual inspection and skewness and kurtosis coefficients. In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable (cotinine, γ -glutamyl transpeptidase and estimated salt intake). Interactions of sex and ethnicity were tested for the relationship between MBPS and each cardiovascular (ambulatory blood pressures) as well as health behaviour related markers

(physical activity, urinary sodium, smoking status, alcohol consumption and socio-economic status), respectively. Analyses of variance were performed to compare characteristics of the study population between quartiles of MBPS in the total group. Multiple regression analyses were performed for the total group, dippers, non-dippers and black and white men and women, with sleep-trough and dynamic morning surge as dependent variables. Independent variables (age, ethnicity, sex, waist circumference, socio-economic status score, 24-hour systolic blood pressure, activity energy expenditure, cotinine, γ -glutamyl transpeptidase, C-reactive protein, sleep apnea risk and night-time dipping) added in the regression models were those that significantly associated with sleep-trough and dynamic morning surge in bivariate regression analyses. Logistic regression analysis was also performed to compare the likelihood of various independent variables (sex, ethnicity, age, waist circumference, socio-economic status score, 24-hour systolic blood pressure, activity energy expenditure, cotinine, γ -glutamyl transpeptidase, C-reactive protein, sleep apnea risk and night-time dipping) to increase MBPS in individuals with a lower (quartile 1-3) and higher surge (quartile 4).

Results

Interaction terms indicated an interaction of ethnicity on the relationship between sleep-trough surge with cardiovascular markers and health behaviours ($p < 0.01$). Interaction terms of ethnicity ($p < 0.001$) and sex ($p = 0.016$) were observed on the relationship between dynamic surge with cardiovascular markers and health behaviours. The general characteristics of the study population are provided in Table 1, Table 2 and Supplementary Table 1. In Table 1, there were 36% black individuals in the highest sleep-trough quartile compared to 51% in quartile 1. Body composition was comparable between quartiles of sleep-trough surge, except for individuals within the highest MBPS (fourth quartile) who presented with increased measures of obesity including waist circumference ($p = 0.004$) and overall body size reflected by lean muscle mass ($p = 0.001$). The same trend was observed for socio-economic score ($p = 0.011$), 24-hour systolic ($p < 0.001$) and diastolic blood pressure ($p = 0.002$), daytime systolic ($p < 0.001$) and diastolic blood pressure ($p < 0.001$), masked hypertension ($p = 0.006$), percentage dipping ($p < 0.001$) and γ -glutamyl transpeptidase ($p = 0.045$). Lower night-time systolic blood pressure and diastolic blood pressure (all $p < 0.001$) were indicated with increasing sleep-trough surge quartiles.

When comparing the quartiles in dynamic morning surge in Table 2, findings for ethnicity, waist circumference, lean mass, socio-economic score, 24-hour systolic and diastolic blood pressure, daytime systolic and diastolic blood pressure, night-time systolic and diastolic blood pressure and percentage dipping were replicated as observed for sleep-trough surge. In addition, more men (51%) were in quartile four compared to quartile one (38%); as were individuals presented with an increased waist-to-hip ratio ($p=0.002$); and reported to consume more alcohol ($p=0.005$).

Table 1. Characteristics of the total study population by quartiles of sleep-trough morning blood pressure surge (N=650)

	Q1 (n = 156)	Q2 (n = 161)	Q3 (n = 160)	Q4 (n = 173)	P
MBPS range, mmHg	<10	≥10 to <17	≥17 to <25	≥25	
Age, years	24.6 ± 3.00	24.9 ± 3.04	24.9 ± 3.00	25.2 ± 2.96	0.39
Ethnicity (black), n (%)	80 (51)	82 (51)	83 (52)	62 (36)	0.008
Sex (men), n (%)	79 (51)	61 (38)	53 (33)	82 (47)	<0.001
Body composition					
Body mass index, kg/m ²	24.6 ± 5.24	25.3 ± 5.96	24.6 ± 4.94	25.9 ± 5.41	0.078
Waist circumference, cm	79.1 ± 11.7	79.9 ± 13.4	77.8 ± 12.0	82.7 ± 13.1	0.004
Waist-to-hip ratio	0.78 ± 0.08	0.77 ± 0.07	0.77 ± 0.08	0.79 ± 0.08	0.11
Body fat percentage, %	24.2 ± 9.94	30.4 ± 38.5	27.2 ± 9.65	26.4 ± 10.2	0.091
Lean mass, kg	53.4 ± 11.5	50.7 ± 11.9	49.3 ± 9.71	54.5 ± 13.3	0.001
Obese, n (%)	24 (15)	27 (17)	26 (16)	35 (20)	0.89
Socio-economic status score	20.3 ± 5.99	21.0 ± 6.27	21.7 ± 5.89	22.5 ± 6.60	0.011
Ambulatory blood pressures					
24-hour SBP, mmHg	117 ± 8.68	115 ± 9.18	114 ± 8.24	119 ± 8.88	<0.001
24-hour DBP, mmHg	69.3 ± 6.26	68.6 ± 5.63	67.7 ± 5.34	70.0 ± 5.42	0.002
24-hour heart rate, bpm	73.7 ± 10.2	75.7 ± 11.2	74.8 ± 8.98	75.0 ± 10.5	0.43
Daytime SBP, mmHg	120 ± 8.85	120 ± 9.45	119 ± 8.62	126 ± 9.22	<0.001
Daytime DBP, mmHg	73.1 ± 6.68	73.2 ± 5.96	72.7 ± 5.91	75.9 ± 6.16	<0.001
Daytime heart rate, bpm	77.5 ± 10.9	80.4 ± 11.6	79.5 ± 9.61	79.3 ± 12.6	0.17
Night-time SBP, mmHg	111 ± 9.70	107 ± 9.88	104 ± 8.98	106 ± 9.51	<0.001
Night-time DBP, mmHg	61.7 ± 7.42	59.6 ± 6.47	58.0 ± 6.00	58.2 ± 5.75	<0.001
Night-time heart rate, bpm	66.2 ± 10.7	67.7 ± 11.5	66.0 ± 9.45	65.6 ± 10.4	0.33
Masked hypertension, n (%)	27 (17)	16 (10)	11 (7.0)	30 (17)	0.006
Night-time dipping, %	7.59 ± 4.59	9.95 ± 5.02	11.3 ± 5.72	14.8 ± 5.37	<0.001
Biochemical variables					
24-hour urinary sodium, mmol/L	113 ± 51.5	122 ± 58.5	112 ± 49.2	123 ± 51.8	0.20
Cotinine, ng/mL	51.9 (30.3 ; 73.5)	65.0 (43.0 ; 87.0)	44.8 (27.2 ; 62.4)	70.6 (48.3 ; 92.9)	0.11
γ-glutamyl transpeptidase, U/L	26.7 (23.7 ; 29.7)	22.5 (19.9 ; 25.1)	25.3 (21.4 ; 29.2)	27.1 (24.0 ; 30.2)	0.045
C-reactive protein, mg/L	2.40 ± 3.50	2.56 ± 3.99	2.30 ± 4.12	3.27 ± 5.71	0.23
Health behaviour					
Physical activity, kCal	1.38 ± 0.14	1.40 ± 0.15	1.49 ± 0.78	1.40 ± 0.13	0.15
Activity Energy Expenditure, kCal/kg/day	15.7 ± 7.60	16.1 ± 8.16	17.3 ± 7.16	17.1 ± 7.53	0.30
Total Energy Expenditure, kCal/kg/day	91.8 ± 13.7	90.6 ± 14.6	95.1 ± 52.6	92.3 ± 14.4	0.62
Estimated salt intake, g/day	9.49 (8.16 ; 10.8)	9.43 (8.19 ; 10.7)	9.19 (8.11 ; 10.3)	9.12 (8.20 ; 10.0)	0.96
Smoking, n (%)	29 (19)	37 (23)	28 (18)	46 (27)	0.15
Alcohol consumption, n (%)	94 (60)	86 (54)	85 (53)	103 (60)	0.39

Abbreviations: DBP – diastolic blood pressure; MBPS – Morning blood pressure surge; SBP – systolic blood pressure.

Table 2. Characteristics of the total study population by quartiles of dynamic morning blood pressure surge (N=845)

	Q1 (n = 202)	Q2 (n = 207)	Q3 (n = 207)	Q4 (n = 229)	P
MBPS range, mmHg	<17	≥17 to <24	≥24 to <32	≥32	
Age, years	24.4 ± 3.01	24.7 ± 3.15	24.9 ± 3.29	25.0 ± 2.87	0.15
Ethnicity (black), n (%)	134 (66)	112 (54)	92 (44)	102 (45)	<0.001
Sex (men), n (%)	77 (38)	85 (41)	80 (39)	117 (51)	0.001
Body composition					
Body mass index, kg/m ²	25.0 ± 5.80	25.0 ± 5.92	25.4 ± 5.32	25.5 ± 5.23	0.78
Waist circumference, cm	78.5 ± 12.3	78.9 ± 13.4	80.7 ± 12.5	81.9 ± 12.3	0.021
Waist-to-hip ratio	0.77 ± 0.07	0.77 ± 0.08	0.78 ± 0.07	0.79 ± 0.08	0.002
Body fat percentage, %	26.2 ± 10.6	28.0 ± 34.2	27.7 ± 10.1	24.7 ± 9.97	0.30
Lean mass, kg	50.0 ± 11.7	51.7 ± 11.6	51.7 ± 11.1	54.8 ± 13.1	0.001
Obese, n (%)	34 (17)	35 (17)	43 (21)	42 (18)	0.90
Socio-economic status score	19.3 ± 5.92	20.3 ± 6.42	21.5 ± 6.41	22.2 ± 6.32	<0.001
Ambulatory blood pressures					
24-hour SBP, mmHg	115 ± 9.56	115 ± 9.47	116 ± 8.88	119 ± 8.48	<0.001
24-hour DBP, mmHg	67.9 ± 6.05	67.9 ± 5.35	69.0 ± 5.53	69.9 ± 5.63	<0.001
24-hour heart rate, bpm	74.6 ± 10.8	74.2 ± 10.8	75.2 ± 9.43	75.1 ± 10.5	0.77
Daytime SBP, mmHg	118 ± 9.63	120 ± 9.49	121 ± 8.93	125 ± 8.89	<0.001
Daytime DBP, mmHg	71.3 ± 6.20	72.3 ± 5.83	74.0 ± 5.93	75.9 ± 6.26	<0.001
Daytime heart rate, bpm	78.6 ± 11.4	78.3 ± 11.1	79.8 ± 10.0	79.7 ± 12.1	0.42
Night-time SBP, mmHg	110 ± 10.7	108 ± 10.6	106 ± 9.86	106 ± 9.17	0.002
Night-time DBP, mmHg	61.5 ± 7.38	59.1 ± 6.51	59.3 ± 5.91	58.1 ± 6.29	<0.001
Night-time heart rate, bpm	67.3 ± 11.8	66.9 ± 10.9	66.5 ± 10.2	65.7 ± 10.6	0.47
Masked hypertension, n (%)	31 (15)	29 (14)	20 (10)	38 (17)	0.22
Night-time dipping, %	6.96 ± 4.11	9.38 ± 5.15	11.5 ± 5.29	14.3 ± 5.77	<0.001
Biochemical variables					
24-hour urinary sodium, mmol/L	124 ± 53.0	119 ± 60.1	115 ± 50.1	126 ± 56.3	0.24
Cotinine, ng/mL	48.0 (29.8 ; 66.2)	63.3 (43.8 ; 82.8)	56.0 (39.4 ; 72.6)	71.4 (51.9 ; 90.5)	0.34
γ-glutamyl transpeptidase, U/L	26.2 (23.1 ; 29.3)	25.3 (22.8 ; 27.9)	25.3 (21.7 ; 28.9)	27.4 (24.8 ; 30.0)	0.14
C-reactive protein, mg/L	2.88 ± 5.39	2.23 ± 3.18	2.70 ± 4.17	2.89 ± 5.48	0.49
Health behaviour					
Physical activity, kCal	1.41 ± 0.15	1.46 ± 0.74	1.40 ± 0.14	1.41 ± 0.13	0.57
Activity Energy Expenditure, kCal/kg/day	17.2 ± 8.44	15.9 ± 8.13	16.5 ± 7.71	17.6 ± 6.91	0.25
Total Energy Expenditure, kCal/kg/day	91.4 ± 16.1	93.6 ± 49.2	90.8 ± 14.5	93.2 ± 13.6	0.77
Estimated salt intake, g/day	9.37 (8.39 ; 10.4)	9.79 (8.54 ; 11.0)	9.14 (8.30 ; 9.98)	9.63 (8.57 ; 10.7)	0.84
Smoking, n (%)	32 (16)	49 (24)	49 (24)	58 (25)	0.085
Alcohol consumption, n (%)	107 (53)	130 (63)	101 (49)	141 (62)	0.005

Abbreviations: DBP – diastolic blood pressure; MBPS – Morning blood pressure surge; SBP – systolic blood pressure.

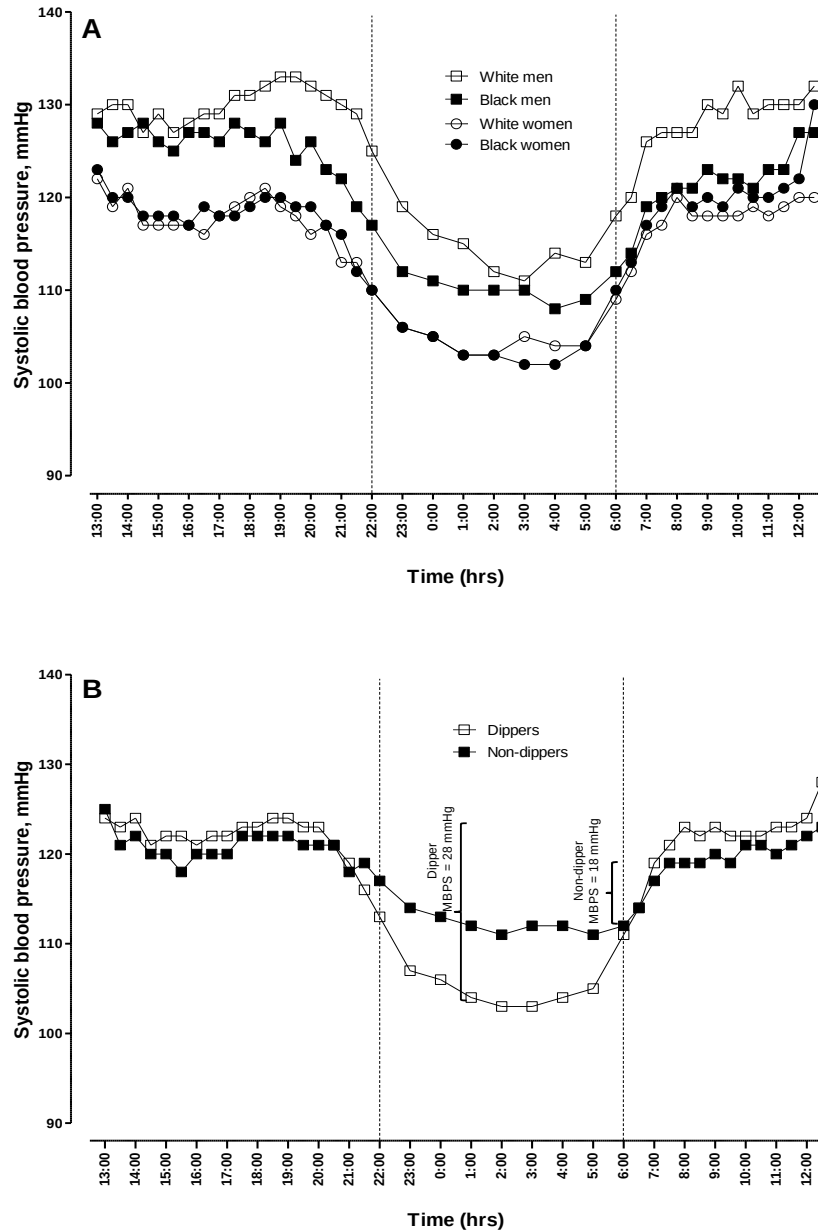


Figure 1. Twenty-four hour systolic blood pressure profiles of black and white men and women (A) as well as dippers and non-dippers (B)

Twenty-four hour systolic blood pressure recordings indicated that morning blood pressure was highest in white men, followed by black men compared to their female counterparts ($p < 0.001$) (Figure 1(A)). The sleep-trough surge of dippers was 28 mmHg compared to 18 mmHg of non-dippers ($p < 0.001$) (Figure 1(B)). More black participants were non-dippers (44% vs 34%; $p = 0.004$) and they also indicated a lower night-time percentage dipping (10.2 ± 5.57) vs (11.0 ± 6.07) compared to their white counterparts ($p = 0.048$). Sleep-trough ($p = 0.59$) and dynamic surge ($p = 0.32$) of normotensive and masked hypertensive participants were comparable (Supplementary Figure 1).

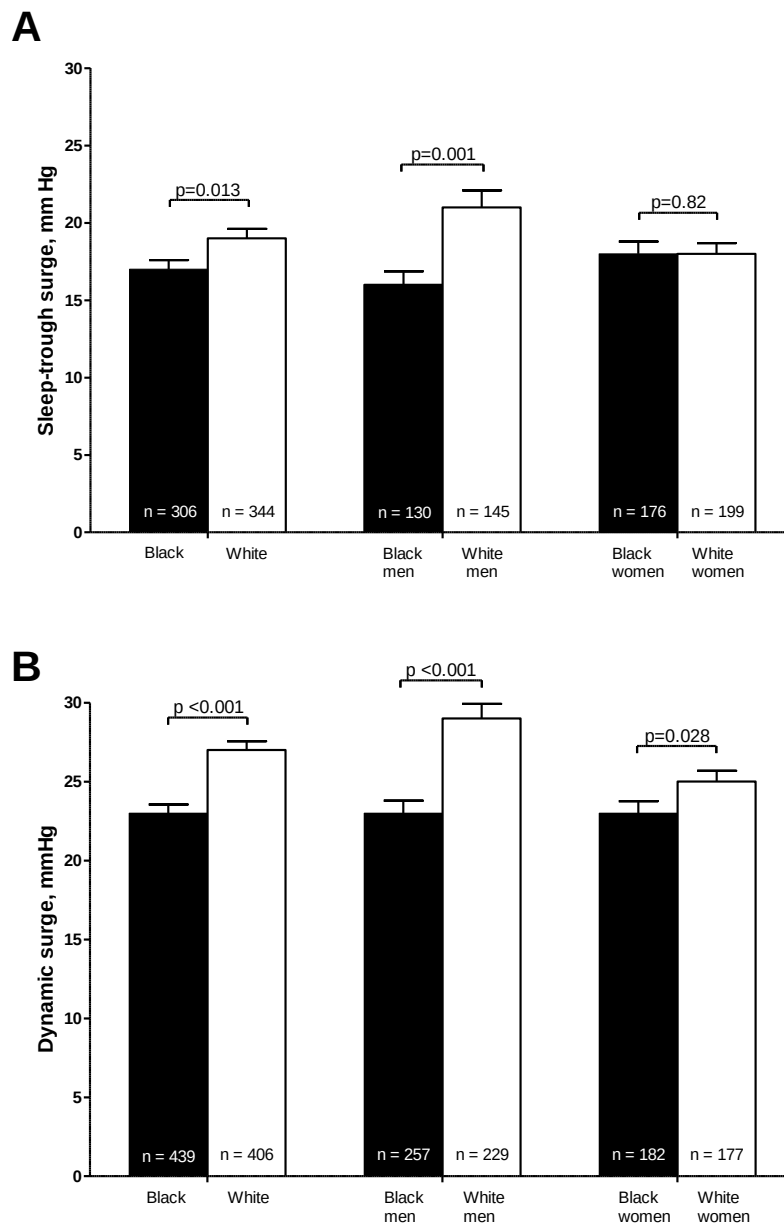


Figure 2. Sleep-trough (A) and dynamic morning surge (B) for black and white men and women

In Figure 2(A), sleep-trough surge was lower in the total black group ($p=0.013$) and black men ($p=0.001$) compared to their white counterparts, with no difference observed in women ($p=0.82$). Similar findings for the total black group and black men were observed when comparing dynamic surge in Figure 2(B). In addition, lower dynamic surge was observed in black women compared to their white counterparts ($p=0.028$). As expected, upon adjustment for night-time dipping (Supplementary Figure 2), the differences in sleep-trough surge between the total black and white groups ($p=0.080$) and dynamic morning surge between black and white women ($p=0.19$) became non-significant. However, the significant differences

in sleep-trough ($p=0.010$) and dynamic surge ($p<0.001$) between black and white men remained after adjustment for dipping status.

Multiple regression analyses were performed to determine the independent associations of sleep-trough surge (Table 3) and dynamic morning surge (Table 4) (as continuous variables) with demographic, blood pressure and health behaviour measures. In the total group, the regression analysis confirmed positive relationships of sleep-trough surge with 24-hour systolic blood pressure ($\beta=0.14$; $p=0.012$), activity energy expenditure ($\beta=0.10$; $p=0.034$) and cotinine ($\beta=0.09$; $p=0.041$), in a model when percentage dipping was not included as an independent variable. When percentage dipping was added to the model, positive relationships between sleep-trough surge and 24-hour systolic blood pressure ($\beta=0.18$; $p<0.001$) and percentage dipping ($\beta=0.46$; $p<0.001$) were obtained in the total group. In dippers, sleep-trough surge indicated additional independent associations with white ethnicity ($\beta=0.13$; $p=0.044$) and 24-hour systolic blood pressure ($\beta=0.29$; $p<0.001$), whereas in non-dippers, associations with women ($\beta= -0.22$; $p=0.020$) were indicated. We found positive relationships of sleep-trough surge with percentage dipping in black men and women as well as in white men and women (all $p<0.001$); and 24-hour systolic blood pressure only in white men ($\beta=0.22$; $p=0.013$) and women ($\beta=0.23$; $p=0.013$).

In Table 4, the regression analyses indicated positive associations between dynamic morning surge with socio-economic score ($\beta=0.15$; $p=0.004$) and 24-hour systolic blood pressure ($\beta=0.14$; $p=0.011$) in the total group when percentage dipping was not included in the model. Findings for 24-hour systolic blood pressure and percentage dipping for the total group in a model where percentage dipping was added were replicated as observed for regression analysis of sleep-trough surge. In dippers ($\beta=0.23$; $p=0.001$) and non-dippers ($\beta=0.22$; $p=0.013$), we found positive relationships between dynamic morning surge and 24-hour systolic blood pressure. When viewing the four groups according to ethnicity and sex, positive relationships between dynamic morning surge and percentage dipping in all groups (all $p<0.001$) were found. Furthermore, positive relationships of dynamic morning surge with socio-economic score ($\beta=0.16$; $p=0.040$) were obtained in white women; 24-hour systolic blood pressure in white ($\beta=0.23$; $p=0.007$) and black women ($\beta=0.27$; $p=0.001$); and with C-reactive protein ($\beta=0.18$; $p=0.008$) in white women only.

We also performed logistic regression analysis (Supplementary Table 2), comparing the likelihood of various independent variables to increase sleep-trough and dynamic morning surge in individuals with a lower (quartile 1-3) and higher surge (quartile 4). The odds ratios indicate that age, 24-hour systolic blood pressure and percentage dipping are respectively, 1.15, 1.06 and 1.35 times more likely to increase sleep-trough surge in individuals with the

highest surge quartile when compared to the group with a lower sleep-trough surge (quartile 1-3). For dynamic morning surge, results for 24-hour systolic blood pressure (1.08) and percentage dipping (1.40) were replicated as observed for sleep-trough surge. In addition, socio-economic score and sleep-apnea risk are respectively, 1.08 and 6.60 times more likely to increase dynamic morning surge of individuals with the highest dynamic surge (quartile 4).

Table 3. Multiple regression analysis with sleep-trough surge as dependent variable, according to dipping status, ethnicity and sex

	Total group – without percentage dipping	Total group – with percentage dipping	Dippers	Non-dippers
n	651	651	377	216
R ²	0.05	0.26	0.09	0.06
Adjusted R ²	0.03	0.24	0.06	0.01
	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)
Sex, women/men	–0.06 (–3.63 ; 0.95)	–0.05 (–3.23 ; 0.82)	–0.04 (–3.61 ; 1.93)	–0.22 (–6.55 ; –0.57)*
Ethnicity, black/white	0.06 (–0.98 ; 3.49)	0.05 (–0.83 ; 3.13)	0.13 (0.11 ; 5.31)*	–0.05 (–3.65 ; 2.02)
Age, years	0.02 (–0.27 ; 0.41)	0.03 (–0.20 ; 0.40)	0.03 (–0.30 ; 0.53)	–0.03 (–0.51 ; 0.34)
Waist circumference, cm	–0.01 (–0.15 ; 0.05)	–0.05 (–0.15 ; 0.03)	–0.20 (–0.29 ; –0.04)	–0.07 (–0.16 ; 0.08)
Socio-economic score	0.08 (–0.05 ; 0.32)	0.02 (–0.14 ; 0.19)	0.01 (–0.22 ; 0.25)	0.13 (–0.05 ; 0.39)
24-hour SBP, mmHg	0.14 (0.03 ; 0.30)*	0.18 (0.09 ; 0.33)†	0.29 (0.17 ; 0.49)†	0.19 (–0.01 ; 0.33)
AEE, kCal/kg/day	0.10 (0.27 ; 7.01)*	0.08 (0.07 ; 5.90)	0.10 (–0.55 ; 7.47)	0.03 (–3.47 ; 5.24)
Cotinine, ng/mL	0.09 (0.04 ; 2.05)*	0.04 (–0.44 ; 1.34)	–0.03 (–1.44 ; 0.87)	0.17 (0.10 ; 3.00)
γ-glutamyl transpeptidase, U/l	0.02 (–3.15 ; 5.20)	0.03 (–2.32 ; 5.07)	0.09 (–1.73 ; 8.66)	0.02 (–4.63 ; 5.87)
C-reactive protein, mg/L	0.02 (–0.16 ; 0.25)	0.02 (–0.13 ; 0.24)	0.01 (–0.20 ; 0.29)	0.06 (–0.17 ; 0.35)
Sleep apnea risk	0.07 (–0.77 ; 6.45)	0.06 (–0.66 ; 5.73)	0.12 (0.07 ; 8.69)	–0.06 (–6.43 ; 3.04)
Night-time dipping, %		0.46 (0.73 ; 1.01)†		

	Black women	Black men	White women	White men
n	177	130	199	145
R ²	0.30	0.26	0.24	0.37
Adjusted R ²	0.25	0.19	0.19	0.31
	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)
Age, years	–0.03 (–0.62 ; 0.43)	0.01 (–0.63 ; 0.67)	0.11 (–0.18 ; 0.91)	0.02 (–0.77 ; 0.99)
Waist circumference, cm	–0.06 (–0.22 ; 0.16)	0.07 (–0.20 ; 0.34)	–0.23 (–0.34 ; –0.02)	–0.12 (–0.35 ; 0.08)
Socio-economic score	0.01 (–0.27 ; 0.31)	–0.03 (–0.41 ; 0.32)	0.14 (–0.06 ; 0.54)	–0.06 (–0.61 ; 0.33)
24-hour SBP, mmHg	0.15 (–0.02 ; 0.43)	0.05 (–0.19 ; 0.31)	0.23 (0.06 ; 0.46)*	0.22 (0.08 ; 0.71)*
AEE, kCal/kg/day	0.03 (–3.82 ; 5.74)	0.16 (–1.31 ; 14.9)	0.14 (–0.51 ; 11.9)	0.09 (–3.24 ; 10.8)
Cotinine, ng/mL	0.11 (–0.55 ; 3.41)	0.03 (–1.39 ; 1.93)	0.10 (–0.52 ; 2.99)	–0.03 (–2.55 ; 1.73)
γ-glutamyl transpeptidase, U/l	0.07 (–3.86 ; 9.64)	0.03 (–6.45 ; 9.09)	–0.07 (–9.45 ; 3.88)	0.06 (–6.95 ; 13.4)
C-reactive protein, mg/L	0.05 (–0.21 ; 0.38)	–0.05 (–0.83 ; 0.51)	0.07 (–0.15 ; 0.43)	0.04 (–0.41 ; 0.67)
Sleep apnea risk	0.03 (–4.93 ; 7.80)	–0.13 (–18.2 ; 4.28)	0.14 (–0.53 ; 0.79)	0.08 (–3.11 ; 8.79)
Night-time dipping, %	0.50 (0.69 ; 1.24)†	0.45 (0.46 ; 1.11)†	0.34 (0.32 ; 0.79)†	0.54 (0.81 ; 1.48)†

Abbreviations: AEE – activity energy expenditure; CI – confidence interval; SBP – systolic blood pressure.* indicates p<0.05; † indicates p<0.01; ‡ indicates p<0.001.

Table 4. Multiple regression analysis with dynamic morning surge as dependent variable, according to dipping status, ethnicity and sex

	Total group – without percentage dipping	Total group – with percentage dipping	Dippers	Non-dippers
n	846	846	467	310
R²	0.07	0.28	0.10	0.06
Adjusted R²	0.05	0.27	0.08	0.01
	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)
Sex, women/men	0.03 (–1.52 ; 3.04)	0.04 (–1.10 ; 2.92)	0.07 (–1.09 ; 4.17)	–0.09 (–5.02 ; 1.49)
Ethnicity, black/white	0.07 (–0.71 ; 3.75)	0.06 (–0.56 ; 3.37)	0.09 (–0.76 ; 4.39)	0.02 (–2.80 ; 3.39)
Age, years	–0.002 (–0.34 ; 0.33)	0.01 (–0.27 ; 0.32)	–0.01 (–0.41 ; 0.38)	0.05 (–0.42 ; 0.50)
Waist circumference, cm	–0.01 (–0.13 ; 0.07)	–0.05 (–0.13 ; 0.04)	–0.10 (–0.20 ; 0.04)	–0.07 (0.17 ; 0.09)
Socio-economic score	0.15 (0.09 ; 0.45) [†]	0.08 (–0.01 ; 0.31)	0.12 (–0.03 ; 0.41)	0.12 (–0.05 ; 0.43)
24-hour SBP, mmHg	0.14 (0.04 ; 0.30) [†]	0.17 (0.10 ; 0.34) [‡]	0.23 (0.11 ; 0.41) [‡]	0.22 (0.05 ; 0.42) [*]
AEE, kCal/kg/day	0.07 (–0.55 ; 6.17)	0.05 (–0.93 ; 4.98)	0.06 (–1.67 ; 5.93)	0.03 (–3.81 ; 5.69)
Cotinine, ng/mL	0.07 (–0.19 ; 1.81)	0.01 (–0.72 ; 1.05)	–0.02 (–1.33 ; 0.86)	0.09 (–5.47 ; 2.57)
γ -glutamyl transpeptidase, U/l	–0.04 (–5.75 ; 2.57)	–0.03 (–4.86 ; 2.46)	–0.01 (–5.39 ; 4.46)	–0.01 (–5.52 ; 5.92)
C-reactive protein, mg/L	0.03 (–0.13 ; 0.29)	0.04 (–0.09 ; 0.27)	0.05 (–0.12 ; 0.35)	–0.01 (–0.30 ; 0.25)
Sleep apnea risk	0.02 (–2.69 ; 4.51)	0.01 (–2.58 ; 3.75)	0.07 (–1.69 ; 6.49)	–0.06 (–7.28 ; 3.06)
Night-time dipping, %		0.47 (0.79 ; 1.07) [‡]		

	Black women	Black men	White women	White men
n	258	182	229	117
R²	0.25	0.31	0.34	0.33
Adjusted R²	0.21	0.25	0.30	0.28
	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)	Std β (95% CI)
Age, years	–0.01 (0.62 ; 0.52)	0.08 (–0.36 ; 0.90)	–0.08 (–0.82 ; 0.24)	0.08 (–0.45 ; 1.12)
Waist circumference, cm	–0.13 (–0.31 ; 0.05)	0.16 (–0.07 ; 0.46)	–0.18 (–0.31 ; 0.02)	–0.04 (–0.23 ; 0.16)
Socio-economic score	0.08 (–0.14 ; 0.50)	–0.12 (–0.58 ; 0.13)	0.16 (0.01 ; 0.59) [*]	0.05 (–0.31 ; 0.52)
24-hour SBP, mmHg	0.27 (0.19 ; 0.68) [‡]	0.04 (–0.20 ; 0.29)	0.23 (0.07 ; 0.47) [†]	0.11 (–0.09 ; 0.47)
AEE, kCal/kg/day	0.05 (–3.06 ; 7.37)	–0.09 (–12.3 ; 3.60)	0.02 (–5.18 ; 6.84)	0.15 (–0.34 ; 12.2)
Cotinine, ng/mL	0.06 (–1.36 ; 2.95)	0.01 (–1.58 ; 1.68)	0.05 (–1.02 ; 2.38)	0.01 (–1.82 ; 2.01)
γ -glutamyl transpeptidase, U/l	–0.04 (–9.56 ; 5.17)	–0.01 (–7.95 ; 7.29)	–0.06 (–9.01 ; 3.89)	–0.001 (–9.14 ; 8.99)
C-reactive protein, mg/L	0.02 (–0.28 ; 0.36)	–0.01 (–0.64 ; 0.67)	0.18 (0.10 ; 0.66) [*]	–0.09 (–0.75 ; 0.22)
Sleep apnea risk	0.07 (–3.60 ; 10.3)	–0.07 (–14.9 ; 7.15)	0.09 (–2.05 ; 9.39)	–0.05 (–6.85 ; 3.79)
Night-time dipping, %	0.41 (0.62 ; 1.20) [‡]	0.55 (0.73 ; 1.37) [‡]	0.50 (0.65 ; 1.10) [‡]	0.52 (0.73 ; 1.32) [‡]

Abbreviations: AEE – activity energy expenditure; CI – confidence interval; SBP – systolic blood pressure. * indicates $p < 0.05$; [†] indicates $p < 0.01$; [‡] indicates $p < 0.001$

Discussion

Previous studies indicated an exaggerated MBPS in elderly hypertensive individuals, men and white populations [1, 4, 16, 25, 26, 34-37]. Our study included a young population aged 20-30 years, which is the first to demonstrate a greater surge in young white than black adults, independent of whether we used sleep-trough or dynamic morning surge. However, this was independent of night-time dipping. We therefore support the notion of higher MBPS in white populations as previously reported [1, 4, 25].

In our study population, 40 participants had an exaggerated sleep-trough surge whereas 128 had an exaggerated dynamic morning surge, defined by a surge greater than 37 mmHg [2]. Blood pressure decreases at night and this physiological phenomenon is known as nocturnal dipping. Individuals whose night-time blood pressure declines more than 10% are classified as dippers, [17, 22] whereas the night-time blood pressure of non-dippers declines by <10% [17, 22]. Non-dipping patterns are common in black populations, increasing the risk for cardiovascular disease [22, 38-40]. A previous study indicated that the sleep-trough surge is likely to be associated with night-time dipping [2]. This is not surprising and also evident in our study as sleep-trough and dynamic surge of dippers were greater compared to non-dippers. To support this, significant differences were lost for sleep-trough surge between black and white groups, and dynamic morning surge between black and white women after adjusting for dipping. Furthermore, a higher percentage of dipping was found in higher quartiles of sleep-trough and dynamic morning surge. These results highlight the importance of non-dipping status when interpreting results of the MBPS. It is also important to mention that the significant differences in sleep-trough and dynamic surge between black and white men remained after adjustment for dipping status. These results may perhaps be influenced by increased plasma renin activity (evident in white individuals) which was previously proven to lead to exaggerated MBPS [1].

Due to the exclusion criteria of this study, individuals with elevated clinic blood pressure were excluded. Besides all clinic blood pressure values being in the normal range, white men in our study had significantly higher daytime and night-time systolic blood pressure compared to black men, and they also had a greater morning surge. Our young study population reflects no cardiovascular disease and a normal MBPS (Figure 2). Differences in night-time dipping between the black and white groups in our study is supported by previous reports suggesting heightened α -adrenergic receptor sensitivity in blacks [39]. Mechanisms indicating the cause of non-dipping patterns due to elevated sensitivity of the α -adrenergic receptors have not been fully elucidated. However, a higher density of α -adrenergic receptors contributes to higher peripheral vasoconstriction and blood pressure reactivity, and may lead to impaired decline in

night-time blood pressure [40]. It is important to highlight the controversy about MBPS as a risk factor. Several articles proposed that a lower MBPS (observed in non-dippers) is associated with a higher risk of morbidity and mortality, whereas others have reported a higher MBPS (observed in dippers) predicts morbidity and mortality [41, 42]. Importantly, the predictive value of MBPS is needed from longitudinal studies [43]. It is well known that a non-dipping pattern is a predictor of cardiovascular morbidity and mortality [22, 23]. Therefore, despite black men having a lower MBPS, the combination of non-dipping status with low MBPS may indicate a risk profile, notwithstanding the low MBPS [5].

Previous studies reported associations between MBPS and health behaviours [13, 44, 45]. Despite using sensitive and objective measures of health behaviours in our study, we found no consistent independent multivariable associations of health behaviours with MBPS. However, we found associations with activity energy expenditure and cotinine in sleep-trough in the total group. These markers do not seem to play an important role in elevated MBPS in young individuals, but may contribute towards an exaggerated MBPS in the future as cardiovascular disease initiates and progresses in our study population.

We found a positive association between sleep-trough surge and ethnicity in dippers. This positive relationship may be influenced by white ethnicity, with previous studies indicating a lower MBPS in black compared to white individuals [1, 4, 25]. Lower MBPS observed in blacks may be due to non-dipping patterns that are common in black population [22, 38-40]. As previously indicated, higher MBPS evident in white individuals may be as a result of increased plasma renin activity, which was previously proven to lead to exaggerated MBPS [1]. Furthermore, we found a negative association of sleep-trough surge with sex in black non-dipping women. This observation is not surprising, because a higher surge was reported in men compared to women (aged between 40 and 80 years), mainly due to the influence of sex-related factors including sex chromosomes and hormones [26, 46, 47]. However, there is limited information on the potential role of sex when investigating MBPS in young and healthy individuals.

In our study population, a larger proportion of white participants was from a high socio-economic status when compared to black participants. This observation could explain why a positive relation of dynamic surge and socio-economic score was obtained in white women. A westernized lifestyle, associated with higher socio-economic status is likely to involve factors such as cigarette smoking (or similar products) and unhealthy dietary choices such as excess intake of saturated fats and alcohol [48] which were previously associated with a higher MBPS [13-15, 45, 49]. In addition, smoking was previously associated with an increase in inflammatory markers such as C-reactive protein [50]. This may support the positive

relationship between dynamic surge and C-reactive protein in white women. However, to the best of our knowledge, mechanisms by which inflammation leads to elevated MBPS have not been fully elucidated.

A secondary objective of this study was to compare results obtained for sleep-trough and dynamic morning surge. Overall, results between the two MBPS quantification methods seem interchangeable, although there were slight differences in the results we obtained for multiple regression analysis. To the best of our knowledge, there is scant knowledge on mechanisms that may lead to these differences. However, it is important to highlight that both sleep-trough (established on sleep and waking times) and dynamic morning surge (24-hour clock-based calculation) were based on different calculations. Despite the fact that sleep-trough surge is considered to be the preferred method as it remains significantly associated with cardiovascular risk after controlling for dipping status [2], it seems as if the dynamic morning surge may be more sensitive in establishing sex differences as indicated by the interaction terms and results presented in Figure 2. However, interpretation and comparison of results for sleep-trough and dynamic surge should be done with caution since there is scant information regarding the predictive value of dynamic morning surge.

This study has to be interpreted within the context of its limitations and strengths. Due to the cross-sectional study design, causality cannot be inferred. Since we did not include data on the renin-angiotensin system, we could not investigate the low renin phenotype (typically observed in black populations [51]) or high renin, and the association thereof with MBPS. Sympathetic activity was also not available for analysis. Overall, this study was conducted in highly controlled conditions in a well-equipped research facility. This was the first study to investigate ethnic differences in sleep-trough and dynamic morning surge among a young bi-ethnic population. With studies indicating non-dipping status as a predictor of future cardiovascular complications, it is important for future longitudinal studies to compare the predictive value of lower sleep-trough vs dynamic morning surge by taking nocturnal non-dipping blood pressure into account regarding cardiovascular outcome.

In conclusion, we found a higher, but normal MBPS in young normotensive white men (independent of night-time dipping) and higher ambulatory blood pressure compared to their black counterparts. Despite black individuals having a lower MBPS, non-dipping night-time patterns presented in them may serve as an important risk factor and potential predictor of future cardiovascular disease. Since MBPS is influenced by dipping patterns, the potential of a non-dipping nocturnal pattern in blacks (with reduced MBPS) and a higher MBPS (observed in dippers and extreme dippers) as potential predictors of cardiovascular disease should be investigated in future longitudinal studies.

Summary Table

What is known about topic
• Ethnic differences of MBPS have been mainly proven in hypertensive and elderly populations. • Non-dipping patterns observed in healthy black adults may serve as a potential risk factor for cardiovascular disease. • The magnitude of the MBPS is influenced by health behaviours including tobacco use, alcohol consumption and physical activity as well as dipping status.
What this study adds
• Young and healthy white men had a higher, but normal MBPS. • Young black adults had a lower MBPS partially due to non-dipping patterns.

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Conflicts of interest

There are no conflicts of interest.

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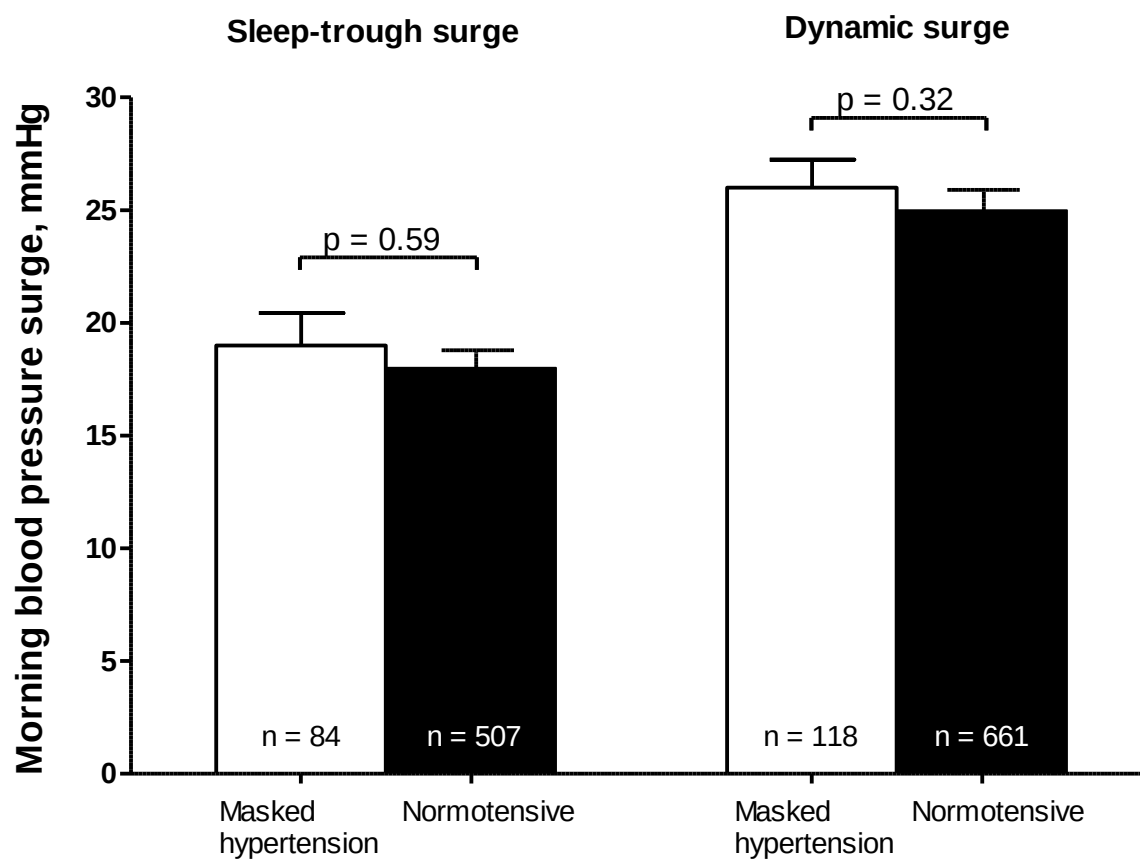
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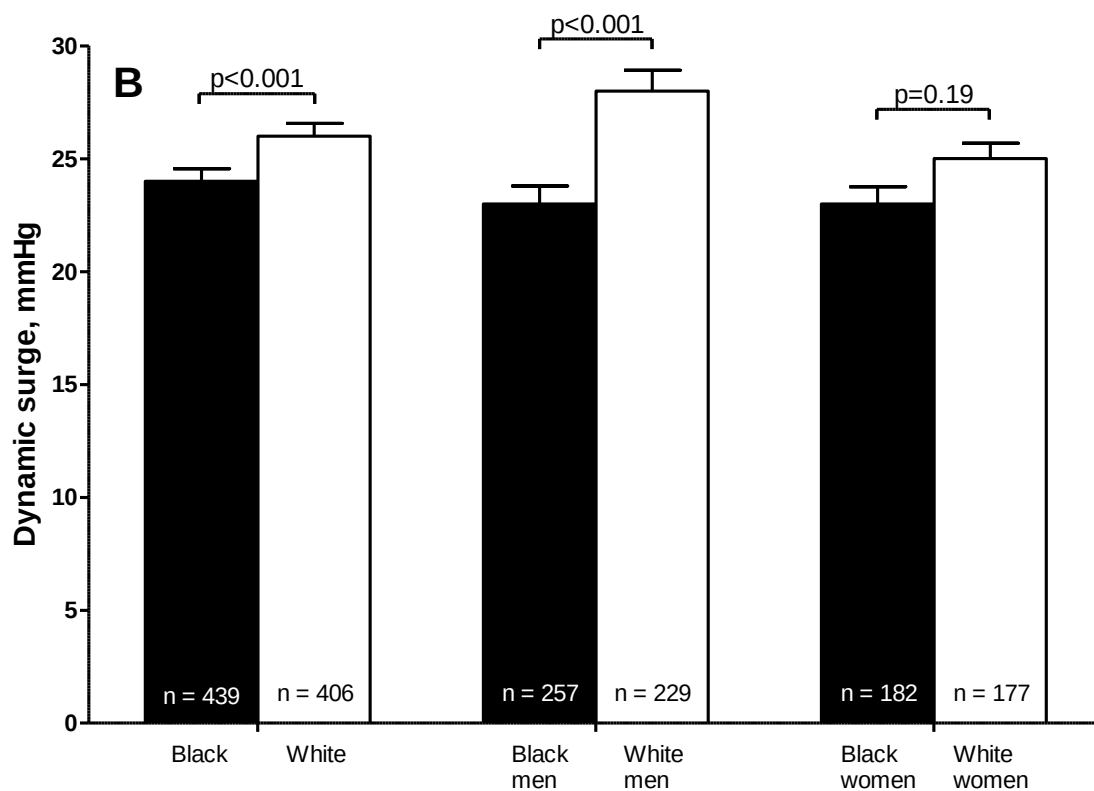
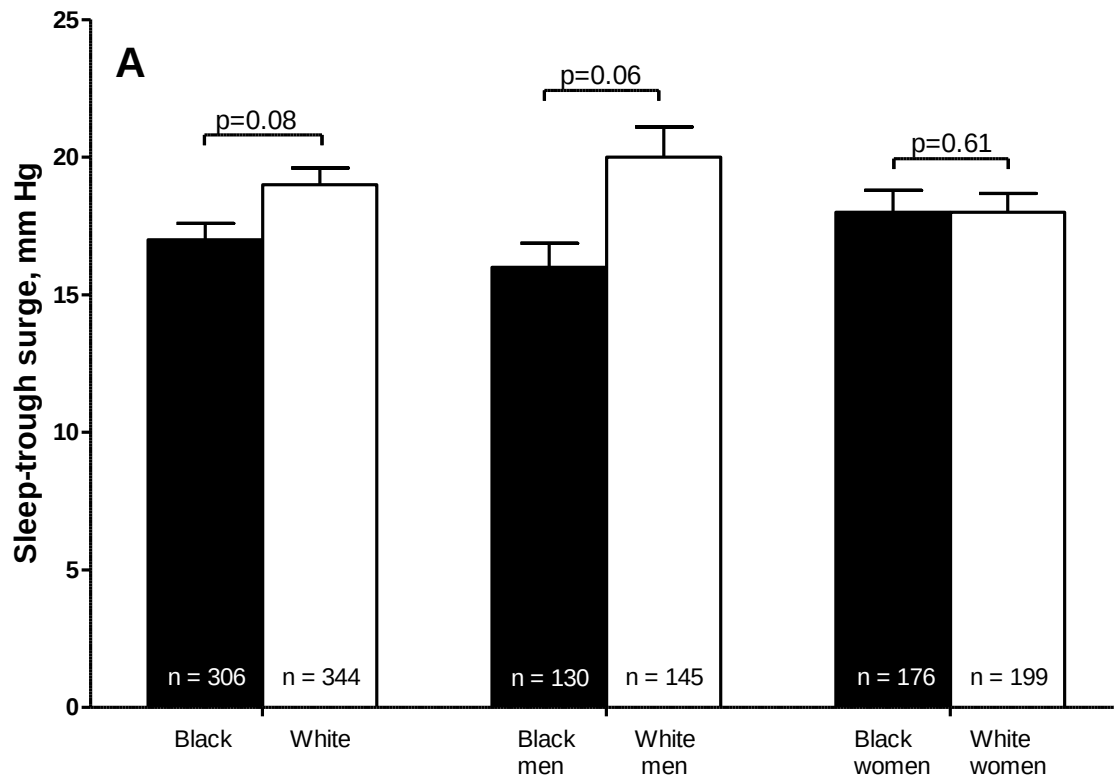
Supplementary Table 1. Characteristics of the total study population (N=845)

	Men			Women		
	Black n = 182	White n = 177	P	Black n = 257	White n = 229	P
Age, years	24.3 ± 3.08	25.3 ± 2.93	0.001	24.6 ± 3.29	25.0 ± 2.90	0.10
Body composition						
Body mass index, kg/m ²	22.2 ± 4.07	27.2 ± 5.12	<0.001	26.6 ± 5.91	24.6 ± 5.48	<0.001
Waist circumference, cm	75.9 ± 8.82	90.5 ± 12.3	<0.001	78.7 ± 12.1	76.8 ± 12.3	0.08
Waist-to-hip ratio	0.81 ± 0.06	0.86 ± 0.06	<0.001	0.74 ± 0.07	0.74 ± 0.06	0.49
Body fat percentage, %	16.2 ± 5.79	19.1 ± 7.06	<0.001	35.7 ± 28.7	29.4 ± 8.52	0.002
Lean mass, kg	53.8 ± 8.54	69.6 ± 8.50	<0.001	43.4 ± 6.08	47.8 ± 6.57	<0.001
Obese, n (%)	11 (6)	43 (24)	<0.001	69 (27)	33 (14)	<0.001
Socio-economic status score	17.3 ± 5.74	24.3 ± 5.76	<0.001	18.4 ± 5.55	23.8 ± 5.40	<0.001
Morning blood pressure surge						
Sleep-trough surge, mmHg	15.9 ± 9.90	20.6 ± 13.2	0.001	17.9 ± 10.6	18.2 ± 9.66	0.79
Dynamic morning surge, mmHg	23.4 ± 10.7	28.5 ± 12.3	<0.001	22.9 ± 12.2	25.2 ± 10.3	0.028
Ambulatory blood pressures						
24-hour SBP, mmHg	119 ± 8.03	124 ± 7.37	<0.001	112 ± 7.75	113 ± 8.63	0.41
24-hour DBP, mmHg	69.4 ± 5.80	70.5 ± 5.79	0.06	67.9 ± 5.34	67.9 ± 5.65	0.93
24-hour heart rate, bpm	50.1 ± 6.59	53.4 ± 6.86	<0.001	44.4 ± 5.51	45.1 ± 5.86	0.19
Daytime SBP, mmHg	124 ± 8.30	129 ± 7.93	<0.001	117 ± 8.32	118 ± 9.12	0.11
Daytime DBP, mmHg	74.3 ± 6.33	75.1 ± 6.42	0.23	72.1 ± 6.44	72.9 ± 6.18	0.21
Daytime heart rate, bpm	49.7 ± 6.71	53.5 ± 7.29	<0.001	44.5 ± 5.93	45.1 ± 6.04	0.31
Night-time SBP, mmHg	111 ± 9.54	114 ± 9.26	<0.001	104 ± 8.64	103 ± 9.40	0.43
Night-time DBP, mmHg	59.4 ± 6.89	61.1 ± 7.43	<0.029	59.5 ± 6.00	58.2 ± 6.30	0.022
Night-time heart rate, bpm	51.0 ± 7.70	53.3 ± 7.91	0.008	44.6 ± 6.32	45.3 ± 6.67	0.28
Masked hypertension, n (%)	26 (14)	61 (35)	<0.001	17 (6)	17 (7)	0.61
Percentage dipping, %	10.3 ± 5.64	10.6 ± 6.26	0.64	10.2 ± 5.53	11.4 ± 5.91	0.024
Biochemical analyses						
24-hour urinary sodium, mmol/L	131 ± 53.2	114 ± 46.7	0.004	122 ± 60.8	118 ± 56.8	0.50
Cotinine, ng/mL	114 (88.3 ; 140)	74.5 (54.3 ; 94.7)	0.054	31.1 (18.6 ; 43.6)	35.7 (21.1 ; 50.2)	0.86
γ-glutamyl transpeptidase, U/L	33.2 (29.1 ; 37.3)	30.7 (27.1 ; 34.3)	0.38	26.4 (24.2 ; 28.6)	16.8 (15.2 ; 18.4)	<0.001
C-reactive protein, mg/L	1.57 ± 3.28	2.04 ± 4.05	0.27	3.83 ± 5.74	2.76 ± 4.96	0.034
Health behaviour						
Physical activity, kCal	1.36 ± 0.16	1.33 ± 0.10	0.014	1.48 ± 0.16	1.48 ± 0.67	0.96
Activity Energy Expenditure, kCal/kg/day	365 (337 ; 393)	381 (350 ; 412)	0.98	485 (452 ; 518)	430 (398 ; 462)	0.18
Total Energy Expenditure, kCal/kg/day	2230 ± 331	2612 ± 438	<0.001	2176 ± 404	2223 ± 920	0.51
Estimated salt intake, g/day	10.7 (9.33 ; 12.1)	9.47 (8.67 ; 10.3)	0.52	9.14 (8.26 ; 10.0)	9.14 (8.06 ; 10.2)	0.54
Smoking, n (%)	78 (43)	50 (28)	0.005	25 (10)	37 (16)	0.032
Alcohol consumption, n (%)	111 (61)	116 (66)	0.38	134 (52)	129 (56)	0.43

Abbreviations: DBP – diastolic blood pressure; SBP – systolic blood pressure



Supplementary Figure 1. Morning blood pressure surge of normotensive and masked hypertensive participants



Supplementary Figure 2. Sleep-trough (A) and dynamic morning surge (B) for black and white men and women after adjusting for night-time dipping

Supplementary Table 2. Odds ratios of quartile 1-3 versus quartile 4 of sleep-trough and dynamic morning surge

Sleep-trough surge		
Odds Ratio		
	Q1-3 versus Q4	95% CI
Sex, women/men	0.79	0.34 ; 1.81
Ethnicity, black/white	1.17	0.52 ; 2.63
Age, years	1.15	1.01 ; 1.31*
Waist circumference, cm	0.98	0.95 ; 1.02
Socio-economic score	1.02	0.95 ; 1.10
24-hour SBP, mmHg	1.06	1.01 ; 1.12*
AEE, kCal/kg/day	2.06	0.39 ; 10.8
Cotinine, ng/mL	1.00	1.00 ; 1.01
γ-glutamyl transpeptidase, U/l	0.43	0.09 ; 2.11
C-reactive protein, mg/L	1.06	0.98 ; 1.15
Sleep apnea risk	2.63	0.75 ; 9.23
Night-time dipping, %	1.35	1.25 ; 1.45†
Dynamic morning surge		
Odds Ratio		
	Q1-3 versus Q4	95% CI
Sex, women/men	0.55	0.25 ; 1.22
Ethnicity, black/white	1.33	0.62 ; 2.88
Age, years	1.04	0.91 ; 1.19
Waist circumference, cm	0.96	0.93 ; 1.00
Socio-economic score	1.08	1.00 ; 1.15*
24-hour SBP, mmHg	1.08	1.03 ; 1.14*
AEE, kCal/kg/day	1.04	0.27 ; 4.01
Cotinine, ng/mL	1.00	1.00 ; 1.01
γ-glutamyl transpeptidase, U/l	0.86	0.19 ; 3.93
C-reactive protein, mg/L	1.01	0.95 ; 1.08
Sleep apnea risk	6.60	1.60 ; 27.2†
Night-time dipping, %	1.40	1.29 ; 1.51‡

Abbreviations: AEE – activity energy expenditure; SBP – systolic blood pressure; Q – quartile.

* indicates $p < 0.05$; † indicates $p < 0.01$; ‡ indicates $p < 0.001$

CHAPTER 4

Manuscript 2

Morning blood pressure surge and the vasculature: A comparison between young dippers and non-dippers from the African-PREDICT Study

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Short title: Morning surge and the vasculature

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Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.

Conflicts of interest

The authors report no conflict of interest.

This manuscript is under review at the *Journal of Hypertension*.

AUTHORS INSTRUCTIONS

- The title page should carry: title of the paper, a brief short title, all authors' names, the affiliations of all the authors, the sources of any support, a statement on potential conflicts of interest and the name and address of the author responsible for correspondence concerning the manuscript.
- A structured abstract of no more than 250 words. The abstract should state the Objective(s) of the study or investigation, basic Methods (selection of study subjects or laboratory animals; observational and analytical methods), main Results (giving specific data and their statistical significance, if possible), and the principal Conclusions.
- The manuscript must be structured as follows: Introduction, Methods, Results, Discussion, Acknowledgements and References.
- References should be numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numerals, which should be given in brackets, e.g. [17]. References should include the names of all authors and any Study Group named in the primary author list when six or fewer; when seven or more, list only the first six names and add et al. Members of the Study Group should not be listed. References should also include full title and source information. Journal names should be abbreviated as MEDLINE.

Abstract

Objectives: Adverse changes in large arteries and retinal microvascular calibres are predictors for hypertension and stroke. These adverse changes were reported in response to the suppressed night-time dipping as well as in hypertensive individuals with exaggerated morning surge. However, it is unknown whether young healthy individuals with normal morning surge, but with non-dipping night-time blood pressure profile, will already present with early onset of micro and macrovascular changes. We investigated the relationship of morning surge with retinal vessel calibres and large artery stiffness in healthy young dippers and non-dippers.

Methods: We included black and white participants (N=323), aged 20-30 years, determined sleep-trough surge. We determined central retinal artery and vein equivalents from fundus images, as well as carotid-femoral pulse wave velocity.

Results: Dippers had a greater morning surge (within normal range) ($p<0.001$) and a greater proportion of individuals with exaggerated morning surge ($p<0.001$). In dippers only, we found a positive relationship between central retinal artery equivalent and morning surge ($\beta=0.16$; $p=0.040$). In sensitivity analysis, the association between central retinal artery equivalent and morning surge in dippers lost significance ($\beta=0.13$; $p=0.09$) when 24-hour diastolic blood pressure was replaced with night-time diastolic blood pressure. However, an independent negative association between central retinal artery equivalent and night-time diastolic blood pressure ($\beta=-0.30$; $p<0.001$) emerged.

Conclusion: Normal morning surge of dippers was positively associated with central retinal artery equivalent (dependent on night-time dipping). Normal morning surge (due to normal night-time dipping) may therefore be involved in preserving retinal arteriolar diameter of dippers, which may reflect a lower vascular resistance.

Keywords: arterial stiffness, morning surge, night-time blood, retinal vessel calibres

Introduction

The accurate measurement of blood pressure remains the most essential practice for the diagnosis of hypertension and prediction of cardiovascular disease risk [1]. Blood pressure data reflecting day-night variation obtained with ambulatory monitoring, was shown to be superior to standard office measurements in predicting cardiovascular morbidity and mortality [1].

Higher blood pressure upon awakening in the morning (in response to increased sympathetic nervous system activity) is a normal physiological phenomenon termed the morning blood pressure surge [2]. There is, however, a marked difference in the magnitude of the morning surge between individuals who experience a normal lowering in night-time blood pressure ($>10\%$) – known as dippers – when compared to those with a blunted lowering in night-time pressure ($<10\%$), namely non-dippers [3]. This is largely explained by increased sympathetic nervous system activity in non-dippers [4]. We previously reported lower morning surge in young and healthy non-dippers compared to their dipping counterparts (with normal morning surge) [5]. In older non-dippers, higher night-time blood pressure was reported to pose an increased risk for cardiovascular disease [2].

Exaggerated morning surge (greater than 37 mmHg) was previously reported in elderly (aged between 49-72 years) and hypertensive individuals [2], and is considered pathological since it is associated with an increased risk for cardiovascular events, independent of clinic or 24-hour blood pressure [6]. Apart from cardiovascular events [6], exaggerated morning surge is also associated with target organ damage including vascular remodeling of small vessels and larger arteries [2].

Research interest in the retinal microvasculature has increased due to its anatomical and physiological similarities with the coronary and cerebral circulation, hence offering non-invasive access to study the microcirculation [7, 8]. Several studies reported that retinal arteriolar narrowing is strongly related to arterial stiffness, coronary heart disease and the prediction of hypertension, whereas venular widening relates more to atherosclerosis (such as inflammation and adverse lipid profiles) and incident stroke [7-10]. While some studies could not identify retinal vessel calibre changes in dippers vs non-dippers [11, 12], a South African study found venular widening in black non-dippers [7].

Increased pulse wave velocity, as a measure of large artery stiffness, is associated with arterial structural changes such as increased accumulation of collagen and enhanced elastin breakdown [13]. Arterial stiffness is known to be increased in non-dippers [14] and predicts

the risk for cardiovascular events such myocardial infarction and stroke as well as cardiovascular mortality [15, 16].

To better understand the involvement of morning surge in the early onset of micro- and macrovascular changes we investigated the relationships between morning surge and retinal vessel calibres as well as large artery stiffness in healthy young adults (20-30 years). We previously indicated that dipping status is a strong predictor for the magnitude of the morning surge in young adults [17], therefore, we will explore these relationships in groups stratified by dipping status.

Methods

Study design, population demographics and basic procedures

This study is embedded in the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT) in which data was collected from young, healthy adults aged between 20 and 30 years. The African-PREDICT study complies with all applicable requirements of the Declaration of Helsinki and obtained approval from the Health Research Ethics Committee of the North-West University (NWU-00001-12-A1) and is registered on ClinicalTrials.gov (NCT03292094). Written informed consent was obtained from all participants.

Participants were recruited on a voluntary basis from Potchefstroom and surrounding areas by field workers through their workplace or advertisement by means of local newspapers or radio stations. Recruited participants firstly underwent screening procedures to determine eligibility for participation in the African-PREDICT study. Participants with a clinic blood pressure of ≥ 140 mmHg and/or ≥ 90 mmHg, those who were infected with human immunodeficiency virus, previously diagnosed with a chronic disease, pregnant, or breastfeeding were excluded. All measurements took place in the morning under highly controlled conditions in a well-equipped research facility. This sub-study includes cross-sectional baseline data from the first consecutive 323 participants with complete data for morning surge, dipping status, micro- and macrovascular measurements.

Cardiovascular measurements

Participants were fitted with a 24-hour ambulatory blood pressure and ECG apparatus (Card(X)plore®, CE0120, Meditech, Budapest, Hungary). The apparatus recorded blood pressure every 30 minutes during the day (6am to 10pm) and every hour during the night (10pm to 6am). Over the 24-hour period that ambulatory devices were worn by participants,

we achieved 91% successful mean inflation rate. Masked hypertension was defined as a normal clinic blood pressure (<140 and <90 mmHg), but elevated 24-hour systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) $\geq 130/80$ mmHg; daytime SBP and/or DBP $\geq 135/85$ mmHg; and/or night-time SBP and/or DBP $\geq 120/70$ mmHg [18].

Sleep-trough surge was determined as the morning SBP (2-hour average of four 30-minute blood pressure readings just after waking) minus the lowest night-time SBP (average blood pressure of 3 readings centred on the lowest night-time reading) [2]. Dynamic surge was determined as the moving peak morning systolic blood pressure (highest 1 hour moving average of consecutive systolic blood pressures between 6am and 10am) minus moving lowest night-times systolic blood pressure (lowest 1 hour moving average of consecutive systolic blood pressures between 1am and 6am) [19]. Night-time blood pressure dipping was determined as a categorical variable determined from the CardioVisions software (classified as non-dipper when the diurnal index was <10% and dippers when diurnal index was >10%) or as a continuous variable (percentage dipping) by calculating the percentage SBP dipping $[1 - (SBP_{\text{sleeping}} / SBP_{\text{awake}}) \times 100\%]$ [20].

Retinal photography was performed using the Dynamic Retinal Vessel Analyzer (Imedos, Jena, Germany) fitted with a Zeiss Fundus camera FF-450plus [7]. Food and fluid intake as well as smoking and exercise were refrained from 1 hour prior to the measurement. A drop of Tropicamide (1% Alcon) was administered in the right eye of participants to induce mydriatic conditions, fifteen to thirty minutes prior to the measurement. Colour and monochrome retinal images were captured using the Visualis 2.81 software with camera positioned at a 50° angle. The monochrome image underwent vessel analysis using VesselMap2 software to determine retinal vessel calibres. When the monochrome image was not of a suitable quality, we used the colour image. All vessel trunks located between 0.5 and 1.0 optic disc diameters from the outer margin of the optic disc were marked as either arteriole or venule. The central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) were quantified using the revised formulas [21]. Only the six largest arteriole and six largest venular segments were included for quantification. The retinal calibres were measured in measuring units, where 1 measuring unit is equivalent to 1 μ mol/l in the normal Gullstrand eye.

Both central SBP and pulse wave velocity (PWV) (along the descending thoracic-abdominal aorta) were determined in duplicate using the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). Participants were requested to be in a supine position and relaxed state for approximately 5 minutes before commencement of the measurement. The device was firstly used to estimate central SBP by performing pulse wave analysis, using a general transfer function, with a brachial cuff placed on the right upper-arm. Afterwards, the

participant's right carotid artery pulse was measured by a tonometer while the femoral pulse was measured with a femoral cuff placed around the thigh of the participant. Distances between the carotid pulse point and upper femoral cuff were measured, and 80% of these distances were used as the pulse wave travelled distance [22].

Biological Sampling and Biochemical analyses

Participants were required to fast overnight for a period of eight to ten hours. Blood samples were collected from the antebrachial vein with a sterile winged infusion set and syringes. Samples were prepared according to standard procedures and stored at -80°C for future biochemical analyses. The γ -glutamyl transpeptidase, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and high sensitivity C-reactive protein were determined in serum and fasting glucose in sodium fluoride plasma (Cobas Integra 400 plus Roche, Basel, Switzerland). Cotinine was quantified using the chemiluminescence method on the Immulite (Siemens, Erlangen, Germany) and cortisol was quantified using the Roche e411 apparatus (Basel, Switzerland).

Body Composition

Anthropometric measurements including body height (SECA stadiometer, SECA), weight (SECA electronic scales, SECA, Birmingham, UK) and waist circumference using a validated non-flexible tape measure (Holtain, Crymych, UK), were measured in triplicate [23]. Body mass index was calculated. Bio-electrical impedance assessment (Biostat 1500, Biostat Ltd, Isle of Man, UK) was used to obtain lean mass and body fat percentage.

Questionnaires

Basic details of participants regarding traditional risk factors (including sex, age, smoking and alcohol consumption), lifestyle and socio-economic status were reported by participants through a general health and demographic questionnaire. The socio-economic status score of participants was obtained using a point system that was adapted from the Kuppuswamy's socio-economic status scale, based on their skills level, education and household income [24]. Sleep apnea risk was assessed using the Berlin questionnaire [25].

Statistical analyses

All statistical analyses were performed with IBM® SPSS® Statistics, Version 25 software (IBM Corporation, Armonk, New York). All variables were tested for normality by QQ-plots and the skewness and kurtosis coefficients. In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable (glucose, total cholesterol, low-

density lipoprotein cholesterol, cotinine, triglycerides, C-reactive protein, cortisol, γ -glutamyl transpeptidase). Independent T-tests, analysis of covariance (ANCOVA) and Chi-square tests were used to compare characteristics of dippers and non-dippers. Pearson and partial correlations (adjusted for age, sex and ethnicity) were performed to determine whether relationships exist between morning surge and measures of microvascular structure and macrovascular function. Partial correlations for PWV were additionally adjusted for mean arterial pressure. Independent associations of microvascular structure measures (CRAE and CRVE) and macrovascular function (pulse wave velocity) with morning surge were determined by performing multiple regression analyses. Several variables were considered for entry into the regression models by using bivariate regression analyses. Covariates considered when exploring associations between CRAE or CRVE with morning surge included age, sex, ethnicity, socio-economic status score, sleep apnea risk, 24-hour DBP, low-density lipoprotein cholesterol, glucose, C-reactive protein and cortisol. The same covariates were included for the association between PWV and morning surge, except that 24-hour DBP was replaced with mean arterial pressure. When CRAE or CRVE was selected as the dependent variable, CRVE or CRAE was, respectively, included as covariates [26]. In sensitivity analyses, we excluded individuals with exaggerated morning surge (N=13) and those with masked hypertension (N=36), separately. Additionally, 24-hour DBP was substituted with night-time DBP and 24-hour SBP separately.

Results

The general characteristics of dippers and non-dippers are presented in Table 1, where 35% of our study sample consisted of non-dippers. Dippers were slightly older ($p=0.008$) than non-dippers and had a greater socio-economic status score ($p=0.028$). Dippers also presented with a greater morning surge than non-dippers, albeit in the normal range (20.3 ± 9.13 versus 12.3 ± 8.31 ; $p<0.001$) and a higher proportion of individuals with exaggerated morning surge (5.21% versus 1.79%; $p<0.001$). Body composition was comparable between dippers and non-dippers. As expected, night-time ambulatory blood pressures (all $p<0.001$) were higher in non-dippers (Figure 1), whereas daytime SBP and DBP (all $p<0.05$), and night-time percentage dipping ($p<0.001$) were higher in dippers. Microvascular calibres (CRAE, and CRVE), macrovascular function (pulse wave velocity) and all biochemical markers were similar between dippers and non-dippers.

Table 1. Characteristics of the total study population (N=323)

	Dippers N = 211	Non-dippers N = 112	P
Age, years	25.3 ± 3.02	24.4 ± 2.94	0.008
Ethnicity (black), n (%)	86 (40.8)	55 (49.1)	0.28
Sex (men), n (%)	80 (37.9)	53 (47.3)	0.24
Socio-economic status score	22.8 ± 6.33	21.2 ± 6.45	0.028
Body composition			
Body mass index, kg/m ²	25.4 ± 5.56	24.9 ± 5.00	0.38
Waist circumference, cm	80.8 ± 13.4	79.1 ± 11.7	0.27
Waist-to-hip ratio	0.78 ± 0.08	0.77 ± 0.07	0.38
Body fat percentage, %	27.8 ± 9.61	25.7 ± 9.56	0.064
Lean mass, kg	52.0 ± 11.5	52.9 ± 12.5	0.52
Cardiovascular profile			
Sleep-trough surge, mmHg	20.3 ± 9.13	12.3 ± 8.31	<0.001
Dynamic morning surge, mmHg	28.4 ± 10.1	18.1 ± 9.74	<0.001
Exaggerated sleep-trough surge, n (%)	11 (5.21)	2 (1.79)	<0.001
24-hour systolic blood pressure, mmHg	116 ± 9.32	117 ± 9.18	0.39
24-hour diastolic blood pressure, mmHg	68.2 ± 5.42	69.2 ± 5.46	0.14
24-hour heart rate, bpm	74.9 ± 9.20	73.5 ± 10.3	0.20
Daytime systolic blood pressure, mmHg	122 ± 8.59	119 ± 9.59	0.012
Daytime diastolic blood pressure, mmHg	74.7 ± 5.78	71.6 ± 5.89	<0.001
Daytime heart rate, bpm	79.7 ± 9.64	77.5 ± 11.0	0.062
Night-time systolic blood pressure, mmHg	104 ± 8.25	112 ± 9.37	<0.001
Night-time diastolic blood pressure, mmHg	58.3 ± 5.84	61.4 ± 6.99	<0.001
Night-time heart rate, bpm	65.9 ± 9.94	66.1 ± 9.94	0.85
Percentage dipping	14.5 ± 3.23	5.89 ± 4.02	<0.001
Clinic central systolic blood pressure, mmHg	108 ± 9.77	108 ± 10.1	0.78
Clinic central pulse pressure, mmHg	34.0 ± 6.01	34.2 ± 5.66	0.77
Central retinal artery equivalent, MU	161 ± 11.8	160 ± 13.7	0.42
Central retinal vein equivalent, MU	250 ± 16.8	249 ± 16.6	0.63
Pulse wave velocity, m/s ^a	6.29 ± 0.89	6.31 ± 0.77	0.25
Biochemical analyses			
Glucose, mmol/L	4.74 (4.62; 4.86)	4.68 (4.52; 4.84)	0.99
Total cholesterol, mmol/L	4.56 (4.39; 4.73)	4.33 (4.13; 4.53)	0.058
Low density lipoprotein cholesterol, mmol/L	2.96 (2.82; 3.10)	2.75 (2.57; 2.93)	0.053
High density lipoprotein cholesterol, mmol/L	1.38 ± 0.41	1.34 ± 0.36	0.41
Triglycerides, mmol/L	1.03 (0.93; 1.13)	0.93 (0.84; 1.05)	0.20
γ-glutamyl transpeptidase, U/L	25.5 (22.2; 28.8)	25.0 (21.5; 28.5)	0.69
Cotinine, ng/ml	54.9 (38.7; 71.1)	40.0 (20.9; 59.1)	0.10
C-reactive protein, mg/L	2.28 (1.62; 2.89)	1.89 (1.40; 2.38)	0.075
Cortisol, nmol/L	460 (426; 494)	477 (428; 526)	0.43
Health behaviours			
Self-reported smoking, n/total (%)	48/210 (22.9)	16/112 (14.3)	0.18
Self-reported alcohol consumption, n/total (%)	122/209 (58.4)	57/110 (51.8)	0.23
Sleep apnea risk, n (%)	16 (7.58)	5 (4.46)	0.40
Masked hypertension, n (%)	14/187 (7.49)	22/85 (25.9)	0.001

^aAdjusted for mean arterial pressure.

Values are expressed as arithmetic mean±SD, geometric mean (5th and 95th percentiles) or % of n.

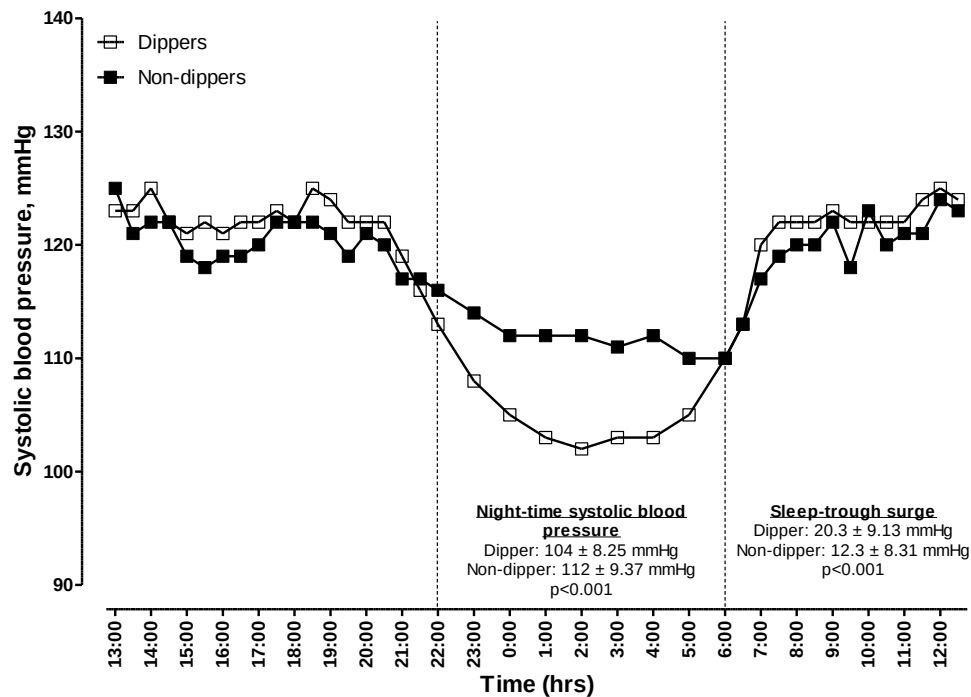


Figure 1. Twenty-four hour systolic blood pressure profiles of dippers and non-dippers

We performed Pearson correlations (Figure 2 and Supplementary Figure 1) between measures of microvascular calibres, macrovascular function (CRAE, CRVE and PWV) and night-time SBP with morning surge in dippers and non-dippers. In dippers only, CRAE correlated positively with morning surge ($r=0.20$; $p=0.021$; non-dippers $r=0.01$; $p=0.96$), and this was confirmed after adjustments were applied for age, sex and ethnicity ($r=0.20$; $p=0.017$; Supplementary Table 1) in partial correlations. No associations were evident between PWV and morning surge.

Multivariable-adjusted regression analyses (Table 2) confirmed a positive relationship of CRAE with morning surge in dippers (adjusted $R^2=0.33$; $\beta=0.16$; $p=0.040$), independent of the listed covariates. In addition, we found a negative association between CRAE and 24-hour DBP in dippers (adjusted $R^2=0.33$; $\beta=-0.34$; $p<0.001$) and non-dippers (adjusted $R^2=0.32$; $\beta=-0.26$; $p=0.021$). In non-dippers a positive association between CRAE and white ethnicity was found (adjusted $R^2=0.32$; $\beta=0.33$; $p=0.012$).

After excluding 4% ($N=13$) of participants with exaggerated morning surge and 13% with masked hypertension ($N=36$), we confirmed our main findings in dippers, namely a positive association between CRAE and morning surge (adjusted $R^2=0.33$; $\beta=0.32$; $p<0.001$ and adjusted $R^2=0.36$; $\beta=0.39$; $p<0.001$, respectively).

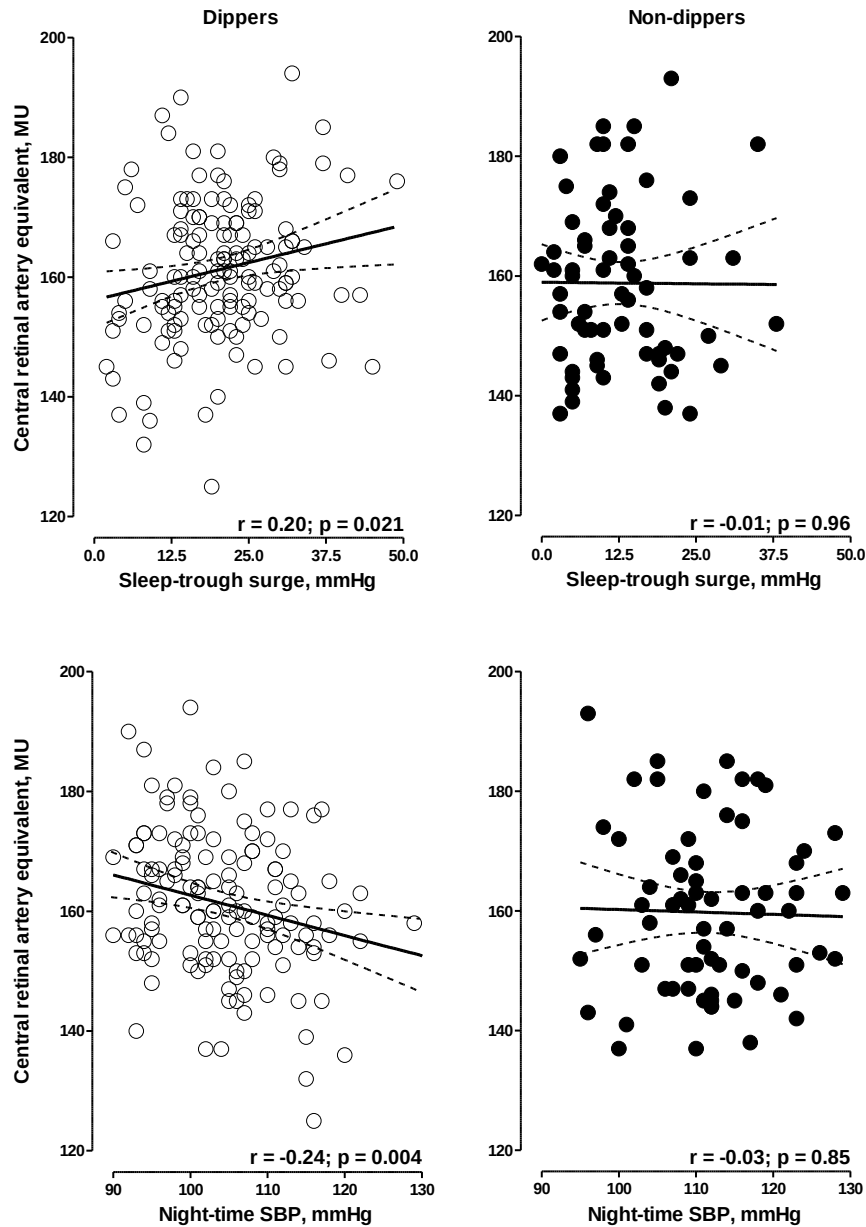


Figure 2. Scatterplots depicting the relationship between central retinal artery equivalent, sleep-trough surge and night-time systolic blood pressure in dippers and non-dippers

Considering the reported association of morning surge with night-time blood pressure [27], we replaced 24-hour DBP with night-time DBP in sensitivity analysis. In dippers, the association between CRAE and morning surge lost significance (adjusted $R^2=0.31$; $\beta=0.13$; $p=0.09$), but an independent negative association between CRAE and night-time DBP (adjusted $R^2=0.31$ $\beta=-0.30$; $p<0.001$) was shown. We also replaced 24-hour DBP with 24-hour SBP, and a negative association between CRAE and 24-hour SBP was confirmed in dippers only (adjusted $R^2=0.30$ $\beta=-0.18$; $p=0.020$).

Table 2. Multiple regression analysis of central retinal artery equivalent and sleep-trough surge in dippers and non-dippers

	Central retinal artery equivalent, MU	
	Dippers N = 211	Non-dippers N = 112
R ²	0.39	0.45
Adjusted R ²	0.33	0.32
	Std β ($\pm$ 95% CI)	Std β ($\pm$ 95% CI)
Sleep-trough surge, mmHg	0.16 (0.01 ; 0.39)*	0.04 (−0.35 ; 0.37)
Age, years	−0.09 (−1.06 ; 0.33)	−0.05 (−1.02 ; 1.52)
Sex, women/men	−0.01 (−4.68 ; 4.15)	0.18 (−1.38 ; 11.1)
Ethnicity, black/white	0.12 (−1.34 ; 8.09)	0.33 (2.10 ; 16.4)*
Socio-economic status score	−0.01 (−0.44 ; 0.40)	−0.11 (−0.89 ; 0.41)
Sleep apnea risk	0.04 (−5.78 ; 9.52)	−0.09 (−17.3 ; 7.10)
24-hour diastolic blood pressure, mmHg	−0.34 (−1.11 ; −0.39)†	−0.26 (−1.28 ; −0.11)*
LDL cholesterol, mmol/L	0.02 (−12.6 ; 16.2)	−0.05 (−27.6 ; 19.1)
Glucose, mmol/L	−0.04 (−5.00 ; 8.69)	0.20 (−3.04 ; 29.5)
C-reactive protein, mg/L	−0.15 (−6.03 ; 0.19)	−0.004 (−5.84 ; 6.05)
Cortisol, nmol/L	−0.01 (−6.11 ; 5.21)	−0.06 (−16.9 ; 9.33)
Central retinal vein equivalent, MU	0.38 (0.17 ; 0.38)‡	0.51 (0.22 ; 0.58)‡

Abbreviations: LDL – Low density lipoproteins. * indicates $p < 0.05$; † indicates $p < 0.01$; ‡ indicates $p < 0.001$

Discussion

We found a positive association between a measure of central retinal artery diameter and morning surge, but only in young adults with a normal night-time blood pressure dipping profile. This association was dependent on night-time DBP. Our results suggest that a physiological morning surge in healthy dippers may play an important role in maintaining an adequate retinal artery diameter as opposed to individuals with an exaggerated morning surge which is considered to increased cardiovascular risk [9]. Our observed results may perhaps also reflect what is happening to the total peripheral resistance of the vasculature [9]. This is important in light of consistent reports that retinal arteriolar narrowing is associated with hypertension and adverse cardiovascular outcomes [8, 9]. It is noteworthy that this association was absent in young non-dippers. We found no associations of morning surge with the central retinal vein calibre or large artery stiffness in any group. In addition, we found no significant association between central retinal artery diameter and dynamic in dippers. Considering there

is limited research conducted on dynamic morning surge, it is difficult to speculate which factors may lead to the lack of association, as observed with sleep-trough surge.

Although retinal arteriolar diameter (CRAE) was comparable between dippers and non-dippers in our study, only in dippers with a lower night-time blood pressure, we found CRAE to be positively associated with morning surge, but independently of 24-hour DBP. Importantly, the positive association between CRAE and morning surge strengthened when individuals with exaggerated morning surge and masked hypertension were removed from the study population. Although this association was independent of 24-hour blood pressure, it is the blood pressure variation or pattern that seems essential in understanding this finding. This is confirmed by our sensitivity analysis indicating that the association between CRAE and morning surge was dependent on night-time blood pressure. This is supported by our previous findings in this population indicating morning surge to be positively related to night-time blood pressure dipping [17]. These results suggest that the normal night-time blood pressure of dippers is important in maintaining an adequate microvascular arteriolar diameter.

To the best of our knowledge, this is the first study to report a negative association between retinal arteriolar diameter and night-time blood pressure in young healthy dippers – but not in non-dippers. In a study including healthy individuals with an average age of 27 years, changes in retinal artery diameter were suggested to change in response to blood pressure fluctuations [28]. This physiological phenomenon termed autoregulation, is important for protecting the capillary network against long-term high blood pressure [28]. This observation was further confirmed by the negative association we obtained between CRAE and 24-hour DBP in dippers and non-dippers, demonstrating a wider retinal artery diameter in response to lower blood pressure, and vice versa. Preserved CRAE of dippers may be achieved by their sustained normal night-time blood pressure, maintaining adequate capillary pressure and perfusion to retinal tissues and retinal artery diameter [28].

Apart from the effects of exaggerated morning surge on microvascular remodeling, it is also important to highlight that increased resistance of small arteries due to remodeling may contribute to increased morning surge [2]. Elevated activation of neurohumoral factors such as the sympathetic nervous system and renin angiotensin system in response to the process of awakening, further increase vascular tone in small resistant arteries, contributing to an exaggerated morning surge [2].

Suppressed night-time dipping is associated with increased risk for cardiovascular disease [2, 27] and thus suggest increased risk for early onset of hypertension in the young non-dippers of our study [29]. Non-dippers did not present a negative association between retinal arteriolar

diameter and night-time blood pressure, as we found in dippers. Potentially autoregulation of non-dippers may already be compromised due to their higher night-time blood pressure, possibly leading to the absence of association between central retinal artery diameter and morning surge in non-dippers [28].

Although arterial stiffness is reportedly increased in non-dippers (14), PWV was comparable between young dippers and non-dippers in our study (6.29 ± 0.89 versus 6.31 ± 0.77 m/s) and well below the cut-off of 10 m/s [22]. Alterations in microvascular structure is anticipated prior to those in the macrovasculature in the process of hypertension development [30], since microvessel diameter changes in response to sustained increased blood pressure [28, 30]. Prolonged elevated blood pressure increases the thickness of microvessel walls impacting the wall-to-lumen ratio and eventually leads to abnormalities in vessel function, inducing multiple clinical manifestations such as hypertension [28, 30].

This study has to be interpreted within the context of its limitations and strengths. Due to the cross-sectional study design, causality cannot be inferred. This study population was recruited from a specific region and our findings cannot be generalised to the entire South African population. In the future, longitudinal African-PREDICT study data will be used to investigate whether higher night-time blood pressures of healthy non-dippers contribute to retinal arteriolar narrowing over time.

In conclusion, the morning surge associated positively with retinal artery calibre in young adults with normal night-time blood pressure dipping, but not in non-dippers. This finding suggests that a normal morning surge in those with normal night-time blood pressure dipping may play an important role in preserving adequate microvascular diameter, and perhaps total peripheral resistance. This association was absent in non-dippers possibly due to their higher night-time blood pressure.

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Supplementary Table 1. Partial correlations of measures of microvascular structure and macrovascular function with sleep-trough surge in dippers and non-dippers

	Sleep-trough surge, mmHg	
	Dippers	Non-dippers
	N = 211	N = 112
Central retinal artery equivalent, MU	r = 0.20; p = 0.017	r = -0.07; p = 0.57
Central retinal vein equivalent, MU	r = 0.07; p = 0.44	r = -0.06; p = 0.59
Pulse wave velocity, m/s ^a	r = 0.06; p = 0.57	r = 0.02; p = 0.75

Adjustments applied for age, sex and ethnicity. ^a – additionally adjusted for mean arterial pressure.

Supplementary Table 2. Multiple regression analysis of central retinal artery equivalent and dynamic morning surge in dippers and non-dippers

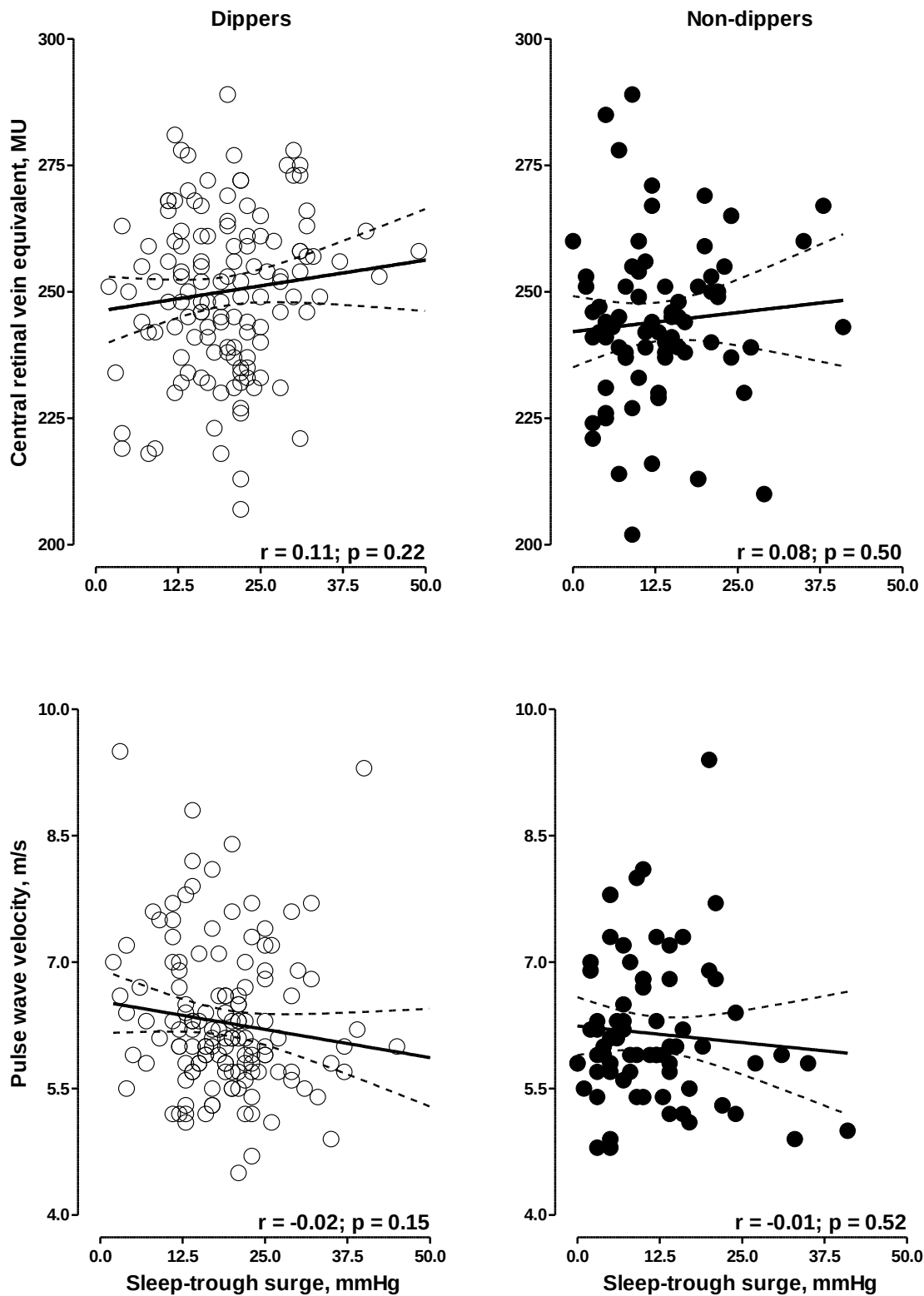
	Central retinal artery equivalent, MU	
	Dippers N = 317	Non-dippers N = 230
R ²	0.37	0.33
Adjusted R ²	0.34	0.27
	Std β ($\pm 95\%$ CI)	Std β ($\pm 95\%$ CI)
Dynamic morning surge, mmHg	0.06 (−0.06 ; 0.19)	−0.01 (−0.20 ; 0.18)
Age, years	0.04 (−0.31 ; 0.63)	−0.01 (−0.77 ; 0.73)
Sex, women/men	0.01 (−2.73 ; 3.05)	0.10 (−1.50 ; 6.24)
Ethnicity, black/white	0.14 (−0.04 ; 6.82)	0.23 (1.26 ; 10.3)*
Socio-economic status score	−0.02 (−0.28 ; 0.27)	0.09 (−0.22 ; 0.57)
Sleep apnea risk	0.03 (−4.07 ; 6.68)	−0.11 (−11.8 ; 1.84)
24-hour diastolic blood pressure, mmHg	−0.32 (−0.84 ; 0.38)†	−0.27 (−0.94 ; −0.26)†
LDL cholesterol, mmol/L	0.04 (−6.60 ; 13.3)	0.02 (−11.2 ; 14.7)
Glucose, mmol/L	0.01 (−5.69 ; 7.12)	−0.07 (−10.5 ; 3.57)
C-reactive protein, mg/L	−0.14 (−5.10 ; −0.25)*	0.09 (−1.50 ; 5.15)
Cortisol, nmol/L	−0.02 (−5.81 ; 4.31)	0.06 (−5.45 ; 11.6)
Central retinal vein equivalent, MU	0.46 (0.24 ; 0.40)‡	0.46 (0.22 ; 0.43)‡

Abbreviations: LDL – Low density lipoproteins. * indicates $p < 0.05$; † indicates $p < 0.01$; ‡ indicates $p < 0.001$

Supplementary Table 3. Multiple regression analysis of central retinal vein equivalent, pulse wave velocity and sleep-trough surge in dippers and non-dippers

	Central retinal artery equivalent, MU	
	Dippers	Non-dippers
	N = 211	N = 112
R ²	0.27	0.37
Adjusted R ²	0.19	0.21
	Std β ($\pm 95\%$ CI)	Std β ($\pm 95\%$ CI)
Sleep-trough surge, mmHg	0.06 (−0.06 ; 0.19)	−0.01 (−0.20 ; 0.18)
Age, years	0.04 (−0.31 ; 0.63)	−0.01 (−0.77 ; 0.73)
Sex, women/men	0.01 (−2.73 ; 3.05)	0.10 (−1.50 ; 6.24)
Ethnicity, black/white	0.14 (−0.04 ; 6.82)	0.23 (1.26 ; 10.3)*
Socio-economic status score	−0.02 (−0.28 ; 0.27)	0.09 (−0.22 ; 0.57)
Sleep apnea risk	0.03 (−4.07 ; 6.68)	−0.11 (−11.8 ; 1.84)
24-hour diastolic blood pressure, mmHg	−0.32 (−0.84 ; 0.38)‡	−0.27 (−0.94 ; −0.26)†
LDL cholesterol, mmol/L	0.04 (−6.60 ; 13.3)	0.02 (−11.2 ; 14.7)
Glucose, mmol/L	0.01 (−5.69 ; 7.12)	−0.07 (−10.5 ; 3.57)
C-reactive protein, mg/L	−0.14 (−5.10 ; −0.25)*	0.09 (−1.50 ; 5.15)
Cortisol, nmol/L	−0.02 (−5.81 ; 4.31)	0.06 (−5.45 ; 11.6)
Central retinal artery equivalent, MU	0.46 (0.24 ; 0.40)‡	0.46 (0.22 ; 0.43)‡
	Pulse wave velocity, m/s	
	Dippers	Non-dippers
	N = 317	N = 230
R ²	0.28	0.33
Adjusted R ²	0.21	0.27
	Std β ($\pm 95\%$ CI)	Std β ($\pm 95\%$ CI)
Sleep-trough surge, mmHg	0.06 (−0.06 ; 0.19)	−0.01 (−0.20 ; 0.18)
Age, years	0.04 (−0.31 ; 0.63)	−0.01 (−0.77 ; 0.73)
Sex, women/men	0.01 (−2.73 ; 3.05)	0.10 (−1.50 ; 6.24)
Ethnicity, black/white	0.14 (−0.04 ; 6.82)	0.23 (1.26 ; 10.3)*
Socio-economic status score	−0.02 (−0.28 ; 0.27)	0.09 (−0.22 ; 0.57)
Sleep apnea risk	0.03 (−4.07 ; 6.68)	−0.11 (−11.8 ; 1.84)
24-hour diastolic blood pressure, mmHg	−0.32 (−0.84 ; 0.38)‡	−0.27 (−0.94 ; −0.26)†
LDL cholesterol, mmol/L	0.04 (−6.60 ; 13.3)	0.02 (−11.2 ; 14.7)
Glucose, mmol/L	0.01 (−5.69 ; 7.12)	−0.07 (−10.5 ; 3.57)
C-reactive protein, mg/L	−0.14 (−5.10 ; −0.25)*	0.09 (−1.50 ; 5.15)
Cortisol, nmol/L	−0.02 (−5.81 ; 4.31)	0.06 (−5.45 ; 11.6)
Mean arterial pressure, mmHg	0.59 (0.03 ; 0.14)†	0.88 (0.05 ; 0.17)†

Abbreviations: LDL – Low density lipoproteins. * indicates $p < 0.05$; † indicates $p < 0.01$; ‡ indicates $p < 0.001$



Supplementary Figure 1. Scatterplots depicting the relationship between measures of microvascular structure and macrovascular function with sleep-trough surge in dippers and non-dippers

CHAPTER 5

Manuscript 3

Morning Blood Pressure Surge Relates to Autonomic Neural Activity in Young Non-Dipping Adults: The African-PREDICT Study

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AUTHORS INSTRUCTIONS

- Essential title page information must include: Full title of the manuscript, author names and affiliations and corresponding author.
- A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. References should be avoided.
- Immediately after the abstract, provide at least 2 keywords associated with their paper using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible.
- The manuscript must be structured as follows: Introduction, Material and methods, Results, Discussion and Conclusions.
- Consecutive numbers in square brackets should be used to indicate references in the text, e.g., [1, 2], as part of the text and not raised above it. The full reference should be cited in a numbered list essentially according to the Vancouver Uniform Requirements (see 5th ed., Ann Intern Med 1997;126(1):36-47). Journal References should contain the names of the first 6 authors (surnames followed by initials), followed by "et al." Title of communication in lower case lettering, Title of Journal [abbreviated according to International Serials Data System-List of Serial title Word Abbreviations, 1985 (ISDS-ISO International Centre, 20 rue Bachaumont, 75002 Paris, France)], year of publication; volume number: first and last page number. For communications which have been accepted for publication, but not yet printed, the reference must contain the journal name and year.

Abstract

Background: It is well established that an exaggerated morning blood pressure surge (MBPS) is associated with an increased risk for cardiovascular disease development in hypertensive individuals. However, in non-dipping individuals, a lower surge was reportedly associated with increased cardiovascular risk. Sympathetic nervous system activity is involved in 24-hour blood pressure fluctuations, including night-time dipping and the MBPS. To better understand this interaction, we investigated associations of MBPS with heart rate variability and baroreceptor sensitivity in young healthy dippers and non-dippers.

Methods: We included black and white men and women (N=827), aged 20-30 years and determined the MBPS using two formulas: the sleep-trough and dynamic morning surge. For autonomic function we determined baroreceptor sensitivity and heart rate variability.

Results: The majority of non-dippers in this population were black (70.4%), presenting lower sleep-trough and dynamic morning surge (all $p < 0.001$). Heart rate variability was comparable between dippers and non-dippers, whereas baroreceptor sensitivity was higher in non-dippers ($p = 0.021$). Despite a suppressed MBPS profile in non-dippers, we found both sleep-trough ($\beta = -0.25$; $p = 0.039$) and dynamic morning surge ($\beta = -0.14$; $p = 0.047$) to be inversely and independently associated with 24-hour heart rate variability (total power). These results were absent in dippers.

Conclusions: In conclusion, we found a higher night-time blood pressure coupled with lower MBPS in young healthy non-dippers. Furthermore, this lower MBPS was independently and negatively associated with autonomic neural activity, suggesting increased autonomic function involvement in MBPS suppression of non-dippers. The predictive value of suppressed nocturnal dipping pattern should be investigated while taking autonomic neural activity into account.

Keywords: Autonomic neural activity • Dippers • Morning blood pressure surge • Non-dippers

Introduction

The value of not only obtaining a single blood pressure measurement, but a 24-hour blood pressure recording became clear over the past decade [1-4]. This is due to blood pressure fluctuations throughout the day-night period, with higher blood pressure during the day and lower at night [5, 6], reflecting the physiological dipping pattern of nocturnal blood pressure. Morning blood pressure surge (MBPS) is also a normal physiological response to increased sympathetic nervous system activity characterised by increased blood pressure in the morning hours at awakening [7-10]. In hypertensive patients, MBPS is exaggerated and has predictive value for cardiovascular events and mortality [2, 5, 7-9, 11-13].

The sympathetic nervous system plays an important role in blood pressure regulation, including the MBPS. Its regulatory role includes increasing both blood propulsion by the heart and blood vessel resistance to blood flow, leading to acute increases in arterial pressure [14]. The magnitude of the MBPS is further influenced by various factors including alcohol use, smoking and physical activity [13, 14]. Importantly, MBPS is mainly influenced by blunted nocturnal blood pressure dipping which is common in black populations, increasing the risk for cardiovascular disease [15-21]. In non-dippers, increased activity of the sympathetic nervous system is associated with insufficient dipping of nocturnal blood pressure [15]. In fact, increased sympathetic nervous system activity may lead to blunted nocturnal dipping as reported in other studies [16, 17, 22], with impaired nocturnal blood pressure dipping identified as a risk factor for cardiovascular events [18, 23].

To better understand the involvement of autonomic function on the MBPS, we investigated associations of the MBPS with heart rate variability and baroreceptor sensitivity in a large group of young healthy adults, including dippers and non-dippers.

Materials and methods

Study design, population demographics and basic procedures

This study is embedded in the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT). The aim of the African-PREDICT study is to understand the early pathophysiology accompanying cardiovascular disease (CVD) development and to identify novel early markers or predictors for the development of CVD by following young, healthy adults over a period of 10 years. The African-PREDICT study is conducted at the Hypertension Research and Training Clinic on the Potchefstroom campus of the North-West University and is registered on ClinicalTrials.gov (NCT03292094). This study complies with all applicable requirements of the Declaration of Helsinki and also obtained approval from the Health Research Ethics Committee of the North-West University, South Africa. Black and white men and women were included in the study after an initial screening procedure. Participants with a mean clinic blood pressure of ≥ 140 mmHg and/or ≥ 90 mmHg, who were infected with human immunodeficiency virus, previously diagnosed with a chronic disease, pregnant, or breastfeeding were excluded. All procedures were explained to the participants before measurements commenced. This study included cross-sectional baseline data from the first 827 participants of the African-PREDICT study, with complete ambulatory blood pressure recordings.

Cardiovascular measurements

Participants were fitted with a 24-hour ambulatory blood pressure and ECG apparatus (CardioXplore®, CE0120, Meditech, Budapest, Hungary) to measure ambulatory blood pressure, and determine MBPS, dipping status and heart rate variability (HRV). The apparatus was programmed to take blood pressure recordings every 30 minutes during the day (6am to 10pm) and every hour during the night (10pm to 6am). Over the 24-hour period that ambulatory devices were worn by participants, we achieved 87% successful mean inflation rate.

Morning blood pressure surge was quantified from ambulatory blood pressure measurements as previously described [24, 25]. Sleep-trough and dynamic morning surge were determined from valid 24-hour systolic blood pressure measurements. Sleep-trough surge was determined as the morning systolic blood pressure (2-hour average of four 30-minute blood pressure readings just after waking) minus the lowest nocturnal systolic blood pressure (1-hour average of the three blood pressure readings based on the lowest night-time reading) [8]. Dynamic surge was determined as the moving peak morning systolic blood pressure

(highest 1 hour moving average of consecutive systolic blood pressures between 6am and 10am) minus moving lowest night-time systolic blood pressure (lowest 1 hour moving average of consecutive systolic blood pressures between 1am and 6am) [26, 27]. The difference in the sample size for sleep-trough (n=323) and dynamic morning surge (n=827) is a consequence of missing data regarding waking and sleep times recorded by participants, which are essential for quantifying sleep-trough surge.

Individuals whose night-time blood pressure declined less than 10% were classified as non-dippers, [16, 18, 28] and those whose night-time blood pressure declined by $\geq 10\%$ were considered as dippers [16, 18, 28].

The 24-hour HRV was measured automatically with the Cardio Visions 1.15.2 Personal Edition (Meditech, Budapest, Hungary) software, and analysis were taken from frequency and geometric domains. The frequency domain analysis, determined by the fast Fourier transformation involve low frequency (a major index of sympathetic cardiac tone with a parasympathetic component) and high frequency (a major indicator of parasympathetic activity) [29]. Low frequency-to-high frequency ratio (reflector of sympatho-vagal autonomic balance) and HRV total power (global determinant of overall autonomic modulation) were also determined [29].

Continuous arterial blood pressure was recorded with the Finometer device (Finapres Medical Systems, Amsterdam, the Netherlands) with participants lying in the Fowler's position with their arm at heart level. The cardiovascular data collected with the Finometer was processed using the Beatscope v1.1 software (Finapres Medical Systems, Amsterdam, the Netherlands) to provide baroreceptor sensitivity. Baroreceptor sensitivity was calculated using the validated cross-correlation baroreflex sensitivity (xBRS) method [30]. This method was proven to yield lower within-patient variance than other methods and was recommended to be used in clinical and experimental settings [30]. This method quantifies the correlation between beat-to-beat systolic blood pressure and R-R interval, resampled at 1 Hz, over 10-sec sliding windows. This is a timespan sufficient to accommodate a 10-sec variability in rhythm, or several cycles at ventilatory frequencies [30].

Biochemical sampling and clinical procedures

Participants were required to fast overnight for a period of eight to ten hours. Blood samples were obtained with a sterile winged infusion set and syringes from the antebrachial vein. Samples were prepared according to standard procedures and stored at -80°C for future biochemical analyses. The γ -glutamyl transpeptidase (GGT), high sensitivity C-reactive protein (CRP), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined in serum and fasting glucose in fluoride plasma (Cobas Integra 400 plus Roche, Basel, Switzerland). Cortisol was quantified using the Roche e411 apparatus (Basel, Switzerland). Aldosterone levels were determined with the Radio Immuno Assay Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic) and cotinine was quantified using the chemiluminescence method on the Immulite (Siemens, Erlangen, Germany).

Body Composition

Anthropometric measurements included body height (SECA stadiometer, SECA), weight (SECA electronic scales, SECA, Birmingham, UK) and waist circumference (validated non-flexible tape measure (Holtain, Crymych, UK)), were measured in triplicate. Body mass index (BMI) was calculated. Body fat percentage and lean mass were obtained through bio-electrical impedance assessment (Biostat 1500, Biostat Ltd, Isle of Man, UK).

Questionnaires

Participants completed a standard general health and demographic questionnaire to obtain basic details regarding their medical history, lifestyle, socio-economic status score (SES) and traditional risk factors such as sex, age, smoking and alcohol consumption. The SES of participants were derived from three categories included in the questionnaire, including skills level, education, and household income. Points were awarded to each of these categories, and the total number of points determined whether a participant was classified into the low, middle, or high SES groups [31]. The Berlin questionnaire was also used to assess sleep apnea risk [32].

Statistical analyses

We used IBM® SPSS® Statistics, Version 25 software (IBM Corporation, Armonk, New York) for statistical analyses. All variables were tested for normality by visual inspection (QQ-plots) and skewness and kurtosis coefficients. In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable (24-hour HRV low frequency-to-high

frequency ratio, glucose, total cholesterol, LDL cholesterol, triglycerides, GGT, cotinine, CRP, aldosterone and cortisol). Interaction terms were determined with multiple regression analysis to test the main effects of dipping status on the association between MBPS and markers of autonomic function (HRV and baroreceptor sensitivity). We performed independent t-tests and Chi-square tests to compare characteristics of dippers and non-dippers. Partial correlations (with adjustments applied for age, sex and ethnicity) were used to assess the direction of association on markers of interest with MBPS. Multiple regression analyses were performed to confirm associations independent of potential confounders. Several variables were considered for entry into the regression models by using bivariate regression analyses. Finally, the following covariates were included in regression models: age, sex, ethnicity, SES, sleep apnea risk, 24-hour systolic blood pressure, CRP, LDL cholesterol and cortisol.

Results

Interaction terms (Table 1) indicated an interaction of dipping status on the relationship between dynamic morning surge and markers of autonomic function, namely low frequency and high frequency HRV as well as low frequency-to-high frequency ratio. The general characteristics of the study population are provided in Table 2, stratified by dippers and non-dippers. Body composition was comparable between dippers and non-dippers. The SES as well as the sleep-trough and dynamic morning surge (Figure 1) of non-dippers were lower compared to dippers (all $p < 0.001$). Furthermore, 70.4% of non-dippers were black, with no sex differences between dippers and non-dippers. Markers of autonomic nervous system activity including 24-hour HRV (total power, low frequency, high frequency and low frequency-to-high frequency), aldosterone and cortisol were comparable between the groups, except for baroreceptor sensitivity ($p = 0.021$) which was higher in non-dippers. As expected, night-time ambulatory blood pressure (Figure 1) (all $p < 0.001$) as well as night-time heart rate ($p = 0.028$) were higher in non-dippers, whereas daytime systolic and diastolic blood pressure (both $p < 0.001$) were lower in non-dippers. Non-dippers had lower total cholesterol ($p = 0.017$) and cotinine ($p = 0.003$) levels, whereas other biochemical variables (glucose, LDL cholesterol, HDL cholesterol, triglycerides and GGT) were comparable between dippers and non-dippers.

Table 1. Interaction terms of dipping status on the relationship between morning blood pressure surge and markers of autonomic function

Sleep-trough surge	Dipping status, <i>P</i>
Independent variables	
24-h HRV total power, ms ²	0.92
24-h HRV LF, n.u	0.35
24-h HRV HF, n.u	0.46
24-h HRV LF/HF ratio	0.72
BRS, ms/mmHg	0.61
Dynamic morning surge	
24-h HRV total power, ms ²	0.19
24-h HRV LF, n.u	<0.001
24-h HRV HF, n.u	<0.001
24-h HRV LF/HF ratio	<0.01
BRS, ms/mmHg	0.09

Abbreviations: 24-h – twenty-four hour; BRS – baroreceptor sensitivity; HF – high frequency; HRV – heart rate variability; LF – low frequency; LF/HF – low frequency-to-high frequency

Table 2. Characteristics of the total study population (N=827)

	Dippers n = 492	Non-dippers n = 335	P
Age, years	24.9 ± 3.13	24.5 ± 3.04	0.039
Ethnicity (black), n (%)	256 (52.0)	236 (70.4)	0.001
Sex (men), n (%)	223 (45.3)	171 (51.0)	0.77
Socio-economic status score	21.3 ± 6.33	19.6 ± 6.28	<0.001
Body composition			
Body mass index, kg/m ²	25.3 ± 5.54	25.2 ± 6.15	0.86
Waist circumference, cm	80.4 ± 12.6	79.6 ± 13.0	0.34
Waist-to-hip ratio	0.78 ± 0.08	0.77 ± 0.08	0.08
Body fat percentage, %	26.1 ± 10.1	27.3 ± 26.3	0.35
Lean mass, kg	52.2 ± 12.0	51.8 ± 11.8	0.62
Morning blood pressure surge			
Sleep-trough surge, mmHg	20.3 ± 9.13	12.3 ± 8.31	<0.001
Dynamic morning surge, mmHg	28.4 ± 10.1	18.1 ± 9.74	<0.001
Cardiovascular profile			
24-h HRV total power, ms ²	5798 ± 3431	5756 ± 3585	0.86
24-h HRV low frequency, n.u	62.1 ± 12.4	60.8 ± 13.4	0.14
24-h HRV high frequency, n.u	35.5 ± 11.4	36.3 ± 12.8	0.30
24-h HRV LF/HF ratio	2.09 (1.99; 2.19)	2.10 (1.92; 2.28)	0.26
Baroreceptor sensitivity, ms/mmHg	17.9 ± 9.60	19.9 ± 11.5	0.021
24-hour SBP, mmHg	116 ± 9.32	117 ± 9.41	0.13
24-hour DBP, mmHg	68.7 ± 5.62	68.6 ± 5.89	0.78
24-hour PP, mmHg	47.2 ± 6.95	48.3 ± 7.24	0.028
24-hour heart rate, bpm	74.7 ± 9.89	74.8 ± 10.9	0.94
Daytime SBP, mmHg	122 ± 9.73	119 ± 9.52	<0.001
Daytime DBP, mmHg	74.3 ± 6.07	71.7 ± 6.44	<0.001
Daytime PP, mmHg	47.6 ± 7.25	47.5 ± 7.45	0.89
Daytime heart rate, bpm	79.5 ± 10.5	78.8 ± 11.4	0.30
Night-time SBP, mmHg	105 ± 9.07	113 ± 10.2	<0.001
Night-time DBP, mmHg	60.0 ± 5.90	62.3 ± 7.18	<0.001
Night-time PP, mmHg	46.4 ± 7.12	50.3 ± 8.51	<0.001
Night-time heart rate, bpm	65.9 ± 10.6	67.5 ± 11.7	0.028
Percentage dipping	14.3 ± 3.24	5.56 ± 4.30	<0.001
Sleep apnea risk, n (%)	45 (9.15)	30 (8.96)	0.94
Biochemical analyses			
Glucose, mmol/L	4.70 (4.62; 4.78)	4.58 (4.48; 4.68)	0.16
Total cholesterol, mmol/L	4.31 (4.21; 4.41)	4.13 (4.03; 4.23)	0.017
Low density lipoprotein cholesterol, mmol/L	2.84 (2.74; 2.94)	2.71 (2.61; 1.39)	0.07
High density lipoprotein cholesterol, mmol/L	1.32 ± 0.39	1.29 ± 0.37	0.27
Triglycerides, mmol/L	0.97 (0.91; 1.03)	0.90 (0.84; 0.96)	0.44
γ-glutamyl transpeptidase, U/L	25.7 (23.8; 27.6)	27.0 (24.8; 29.2)	0.11
Cotinine, ng/ml	58.6 (48.5; 68.7)	43.2 (32.5; 53.9)	0.003
C-reactive protein, mg/L	2.72 (2.29; 3.15)	3.25 (2.23; 4.27)	0.80
Aldosterone, pg/ml	105 (93.1; 117)	105 (87.4; 123)	0.83
Cortisol, nmol/L	455 (433; 477)	458 (430; 486)	0.92
Health behaviour			
Self-reported smoking, n/total (%)	131/528 (24.8)	79/391 (20.2)	0.12
Self-reported alcohol consumption, n/total (%)	300/525 (57.1)	226/388 (58.3)	0.61

Abbreviations: DBP – diastolic blood pressure; HRV – heart rate variability; LF/HF – low frequency-to-high frequency; n.u – normalised unit; PP – pulse pressure; ROS – Reactive oxygen species; SBP – systolic blood pressure

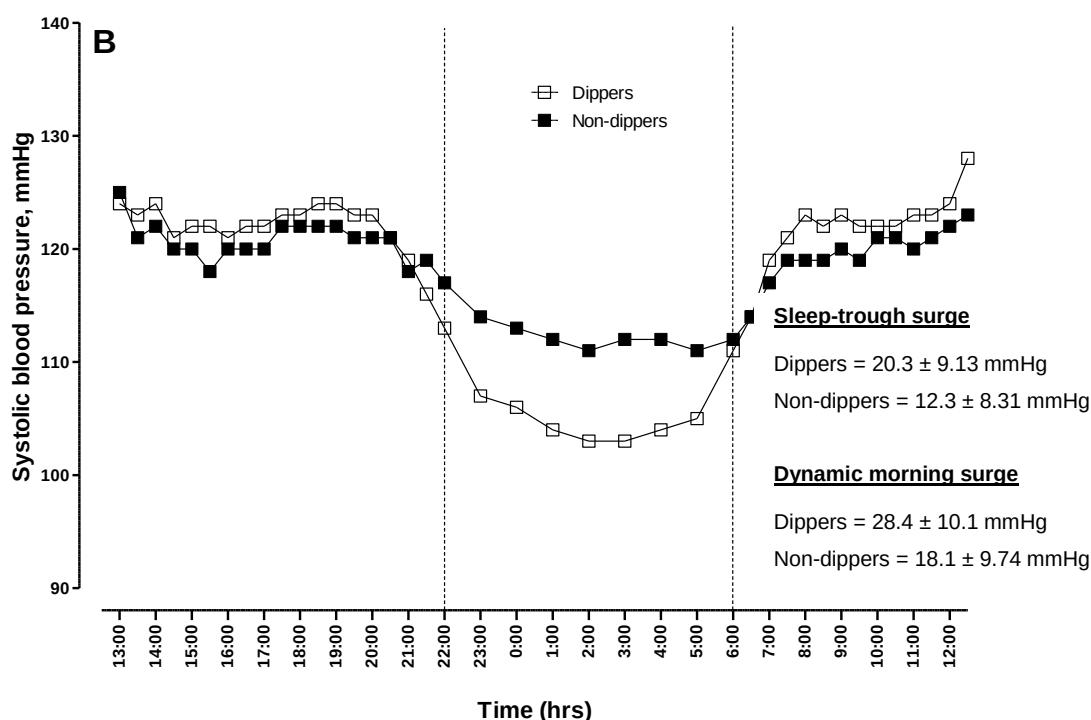


Figure 1. Twenty-four-hour systolic blood pressure profile of dippers and non-dippers

Partial correlations (Table 3) between MBPS measures and markers of autonomic nervous system activity were performed with adjustments applied for age, sex and ethnicity. In non-dippers only, 24-hour HRV total power correlated inversely with both sleep-trough ($r=-0.24$; $p=0.044$) and dynamic morning surge ($r=-0.28$; $p=0.018$).

Table 3. Partial correlations of morning blood pressure surge with measures of sympathetic nervous system activity

	Sleep-trough surge	
	Dippers n = 112	Non-dippers n = 211
24-h HRV total power, ms^2	$r = 0.11$; $p = 0.22$	$r = -0.24$; $p = 0.044$
24-h HRV low frequency, n.u	$r = -0.09$; $p = 0.93$	$r = 0.04$; $p = 0.77$
24-h HRV high frequency, n.u	$r = 0.04$; $p = 0.62$	$r = -0.06$; $p = 0.61$
24-h HRV LF/HF ratio	$r = -1.00$; $p = 0.26$	$r = -0.03$; $p = 0.79$
Baroreceptor sensitivity, ms/mmHg	$r = -0.01$; $p = 0.95$	$r = -0.05$; $p = 0.70$
	Dynamic morning surge	
	Dippers n = 335	Non-dippers n = 492
24-h HRV total power, ms^2	$r = -0.01$; $p = 0.87$	$r = -0.28$; $p = 0.018$
24-h HRV low frequency, n.u	$r = 0.01$; $p = 0.88$	$r = 1.00$; $p = 0.43$
24-h HRV high frequency, n.u	$r = 0.03$; $p = 0.71$	$r = -0.11$; $p = 0.38$
24-h HRV LF/HF ratio	$r = -0.002$; $p = 0.99$	$r = 0.09$; $p = 0.47$
Baroreceptor sensitivity, ms/mmHg	$r = -0.03$; $p = 0.74$	$r = -0.12$; $p = 0.34$

Adjustments applied for age, sex and ethnicity. Abbreviations: HRV – heart rate variability

Multiple regression analyses (Table 4) were performed to determine independent associations of sleep-trough and dynamic surge with HRV total power while taking appropriate covariates into account, namely age, sex, ethnicity, SES, sleep apnea risk, 24-hour systolic blood pressure, CRP, LDL cholesterol and cortisol. These analyses confirmed the inverse relationship of sleep-trough ($\beta=-0.25$; $p=0.039$) and dynamic morning surge ($\beta=-0.14$; $p=0.047$) with HRV total power in non-dippers only. Additionally, dynamic morning surge associated positively with SES ($\beta=0.19$; $p=0.016$) in non-dippers; 24-hour systolic blood pressure in non-dippers ($\beta=0.21$; $p=0.010$) and dippers ($\beta=0.17$; $p=0.010$) and LDL cholesterol in dippers ($\beta=0.14$; $p=0.017$).

Table 4. Multiple regression analysis of heart rate variability in dippers and non-dippers

Sleep-trough surge		
	Dippers n = 211	Non-dippers n = 112
R²	0.08	0.13
	Std β ($\pm 95\%$ CI)	Std β ($\pm 95\%$ CI)
24-h HRV total power, ms ²	0.07 (−4.80 ; 7.27)	−0.25 (−14.7 ; −0.05)*
Age, years	0.16 (−0.41 ; 0.73)	0.14 (−0.62 ; 0.90)
Sex	0.10 (−3.94 ; 4.41)	− 0.05 (−4.52 ; 4.42)
Ethnicity	0.03 (−3.67 ; 3.73)	0.04 (−4.19 ; 4.27)
Socio-economic score	−0.001 (−0.32 ; 0.31)	−0.11 (−0.48 ; 0.26)
Sleep apnea risk	0.05 (−5.34 ; 4.91)	−0.15 (−7.68 ; 7.38)
24-hour SBP, mmHg	0.11 (−0.11 ; 0.33)	0.02 (−0.20 ; 0.24)
C-reactive protein, mg/L	−0.05 (−2.62 ; 2.52)	−0.14 (−3.39 ; 3.11)
LDL cholesterol, mmol/L	0.08 (−11.3 ; 11.5)	0.04 (−13.8 ; 15.0)
Cortisol, nmol/L	−0.03 (−4.91 ; 4.85)	0.19 (−8.06 ; 8.44)
Dynamic morning surge		
	Dippers n = 335	Non-dippers n = 492
R²	0.10	0.09
	Std β ($\pm 95\%$ CI)	Std β ($\pm 95\%$ CI)
24-h HRV total power, ms ²	−0.07 (−6.08 ; 3.67)	−0.14 (−11.1 ; −0.05)*
Age, years	−0.02 (−0.41 ; 0.37)	−0.05 (−0.56 ; 0.46)
Sex	0.01 (−2.83 ; 2.85)	−0.08 (−3.55 ; 3.39)
Ethnicity	0.03 (−2.10 ; 2.16)	−0.01 (−3.19 ; 3.17)
Socio-economic score	0.09 (−0.13 ; 0.31)	0.19 (0.07 ; 0.45)*
Sleep apnea risk	0.07 (−4.01 ; 4.15)	−0.09 (−5.11 ; 4.93)
24-hour SBP, mmHg	0.17 (0.03 ; 0.31)*	0.21 (0.03 ; 0.39)*
C-reactive protein, mg/L	−0.05 (−1.99 ; 1.89)	−0.07 (−2.48 ; 2.34)
LDL cholesterol, mmol/L	0.14 (1.79 ; 18.3)*	0.06 (−10.1 ; 10.3)
Cortisol, nmol/L	0.05 (−4.40 ; 4.50)	0.02 (−6.41 ; 6.45)

Abbreviations: HRV – Heart rate variability; LDL – Low density lipoproteins; SBP – Systolic blood pressure.

* indicates $p<0.05$.

Discussion

With previous studies indicating that sympathetic nervous system activity is associated with exaggerated MBPS [7, 11, 33, 34], our study is the first to demonstrate an association of lower MBPS with autonomic function in young healthy adults with blunted night-time blood pressure dipping. We found inverse independent associations between HRV total power with two distinct estimates of MBPS in a large young adult population consisting of 112 (sleep-trough surge) and 492 (dynamic morning surge) non-dippers.

In our study population, 16 participants had an exaggerated sleep-trough surge whereas 129 had an exaggerated dynamic morning surge, defined by a surge greater than 37 mmHg [8]. Additionally, notwithstanding which measure of MBPS we used, non-dippers in our study had a lower surge compared to dippers, and is in accordance with previous findings [35]. When considering that both sleep-trough and dynamic morning surge are determined by the difference in morning blood pressure and night-time blood pressure, the lower MBPS observed in non-dippers is not surprising. A surge quantified by a difference in high nocturnal blood pressure (evident in non-dippers) from an increased morning blood pressure (associated with the process of waking), may result in low MBPS. Despite non-dippers having a lower MBPS, their higher night-time systolic blood pressure (Figure 1) profiles pose an increased risk for cardiovascular disease [36]. Previous studies proposed that a higher heart rate is associated with increased sympathetic nervous activity [37, 38]. Non-dippers in our study had a higher night-time systolic blood pressure and heart rate. These observations in our study population may reflect that non-dippers have increased nocturnal sympathetic activity which is also known to reflect increased risk for cardiovascular disease [39].

It is well established that an exaggerated MBPS has predictive value for cardiovascular events and mortality in hypertensive and general populations [2, 5, 7-9, 11-13, 35]. However, some longitudinal studies suggested that a lower MBPS (observed in non-dippers) also has a predictive value for a higher risk of morbidity and mortality [35, 40]. Furthermore, it is known that non-dippers have increased cardiovascular risk due to their high night-time blood pressure profile [36]. Individuals with clinic blood pressure in the hypertensive range were excluded from our study, and importantly, our study population reflects no cardiovascular disease. Importantly, our findings in young healthy non-dippers reflect a higher night-time blood pressure and a significantly lower MBPS than dippers, highlighting the significance of dipping status when interpreting results on MBPS. In young healthy non-dippers, the combination of blunted dipping of nocturnal blood pressure and suppressed MBPS may act as a potential risk factor for future cardiovascular disease and events.

In young non-dippers, we found that increased autonomic activity (HRV total power) was associated with a lower morning surge. This is contrary to what one would expect but is likely due to the high night-time blood pressures in non-dippers. Since HRV total power reflects a global determinant of overall autonomic modulation [29], it is unknown whether the driving force of these associations are increased sympathetic or decreased parasympathetic nervous system activity. In addition, we did not find any significant associations of MBPS with other HRV variables which give an indication of either sympathetic or parasympathetic activity such as low frequency HRV, high frequency and low frequency-to-high frequency ratio [29]. Based on previous findings and the increased night-time heart rate of the young non-dippers, we expect sympathetic nervous system over-activity to be the driving force behind this association as it is known to be linked to non-dipping [41, 42].

Differences in MBPS, night-time blood pressure and heart rate between the dippers and non-dippers in our study are supported by previous reports suggesting heightened nocturnal alpha-adrenergic receptor sensitivity of the sympathetic nervous system in normotensive and hypertensive non-dippers [41]. Mechanisms indicating the cause of low MBPS due to elevated sensitivity of the alpha-adrenergic receptors have not been fully described. However, a higher density of alpha-adrenergic receptors is responsible for higher peripheral vasoconstriction and blood pressure reactivity, and may lead to impaired decline in night-time blood pressure and possibly, lower MBPS in non-dippers [21].

This study has to be interpreted within the context of its limitations and strengths. Due to the cross-sectional study design, causality cannot be inferred. In addition, we did not assess sympathetic nervous system activity by means of microneurography. Since we did not find any significant associations of low frequency HRV, high frequency HRV or low frequency-to-high frequency ratio with MBPS, we cannot confirm the contribution of sympathetic nervous activity on MBPS. Overall, this study was conducted in highly controlled conditions in a well-equipped research facility. This was the first study to investigate the effect of autonomic neural activity on morning surge, with two methods of estimating surge, namely sleep-trough and dynamic morning surge among a young population.

Conclusions

In conclusion, we found a higher night-time blood pressure coupled with lower MBPS in young healthy non-dippers. Furthermore, this lower MBPS was independently and negatively associated with enhanced sympathetic activity. With studies indicating non-dipping status as a predictor of future cardiovascular complications [36], we need to determine if the suppressed

nocturnal dipping profile of young healthy individuals increases their risk for future cardiovascular disease.

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Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.

Conflicts of interest

There are no conflicts of interest.

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CHAPTER 6

Summary of main findings and conclusion

6.1 Introduction

Here, a summary of the main findings from three research manuscripts are stated. The results from each manuscript are also explained and compared to the original hypotheses (as detailed in Chapter 1) and the relevant literature. Conclusions are drawn and recommendations are made to researchers investigating morning blood pressure surge (MBPS) in young healthy adults.

6.2 Interpretation of main findings and comparison with the relevant literature

It is important to compare results of this study with those found in the literature regarding other population groups. Certain results in this thesis confirmed the findings of previous studies and others contribute to the existing literature. This study aimed to obtain a better understanding regarding the MBPS and how it relates to arterial function and subclinical hypertension-mediated organ damage in a healthy bi-ethnic South African population, aged between 20 and 30 years. The relevant findings reported in the three manuscripts of this thesis were:

6.2.1 Morning blood pressure surge in young black and white adults: The African-PREDICT Study (Chapter 3)

This sub-study explored MBPS quartiles, and also reflected whether the proportions of sex and ethnicity, as well as cardiovascular-related markers and health behaviours differ with increasing MBPS quartiles. We originally hypothesised that there will be an interaction of sex and ethnicity, and both cardiovascular and health behaviour markers will be augmented in higher MBPS categories, and that a larger proportion of white adults will present an exaggerated MBPS than black adults.

Results of this study indicated an interaction of ethnicity on the relationship between sleep-trough surge with cardiovascular markers, and health behaviours. Furthermore, interaction terms of ethnicity and sex were observed on the relationship between dynamic morning surge with cardiovascular markers and health behaviours. Moreover, higher 24-hour and daytime ambulatory systolic and diastolic blood pressure, as well as a higher proportion of masked hypertensive individuals were evident in the higher MBPS quartiles. Measures of health behaviours were similar between the MBPS categories. We also found a higher, but physiologically normal MBPS in white men only (Table 1, below). A larger proportion of white individuals presented with exaggerated sleep-trough surge (6.16% versus 3.42%) and dynamic morning surge (15.8% versus 14.6%) compared to their black counterparts. In this study, more black individuals were non-dippers, and were presented with a lower MBPS

(Table 1). In addition, our multiple regression analyses showed positive association of MBPS with night-time percentage dipping, both in black and white men and women.

The results of this study confirmed findings in the literature regarding higher MBPS that is evident in normotensive and hypertensive white individuals when compared to their black counterparts (1-3). Furthermore, a lower MBPS of black hypertensive patients was also confirmed by results of another study (4). Our findings also confirmed reports in the literature regarding the confounding determinants of MBPS which include 24-hour systolic blood pressure, and night-time dipping status (5-8). Furthermore, it was reported that sleep-trough surge is likely to be associated with night-time dipping (9). These reports were evident in our study as sleep-trough and dynamic morning surge of dippers were greater compared to non-dippers. In addition, we found independent associations between MBPS and night-time dipping status. Although numerous studies reported an association between health behaviours and MBPS, in our study none of the health behaviours (activity energy expenditure, cotinine and γ -glutamyl transpeptidase) indicated association with MBPS (10-12).

The hypotheses for this research manuscript are therefore partially accepted, since we found an interaction of ethnicity on the relationship between sleep-trough surge and an interaction of ethnicity and sex on the relationship between dynamic morning surge with cardiovascular markers and health behaviours. Furthermore, a larger proportion of white adults presented with an exaggerated sleep-trough and dynamic morning surge than black adults, and measures of health behaviour were comparable between the MBPS quartiles. The hypotheses are partially rejected because of lack of associations between MBPS and health behaviours.

Table 1. Morning blood pressure surge and night-time blood pressure profiles of the study population

Manuscript 1						
	Men			Women		
	Black (N=182)	White (N=177)	P	Black (N=257)	White (N=229)	P
Sleep-trough surge, mmHg	15.9 ± 9.90	20.6 ± 13.2	0.001	17.9 ± 10.6	18.2 ± 9.66	0.79
Dynamic morning surge, mmHg	23.4 ± 10.7	28.5 ± 12.3	<0.001	22.9 ± 12.2	25.2 ± 10.3	0.028
Night-time SBP, mmHg	111 ± 9.54	114 ± 9.26	<0.001	104 ± 8.64	103 ± 9.40	0.43
Night-time DBP, mmHg	59.4 ± 6.89	61.1 ± 7.43	<0.029	59.5 ± 6.00	58.2 ± 6.30	0.022
Night-time heart rate, bpm	51.0 ± 7.70	53.3 ± 7.91	0.008	44.6 ± 6.32	45.3 ± 6.67	0.28
Manuscript 2						
	Dippers (N=211)		Non-dippers (N=112)		P	
Sleep-trough surge, mmHg	20.3 ± 9.13		12.3 ± 8.31		<0.001	
Night-time SBP, mmHg	104 ± 8.25		112 ± 9.37		<0.001	
Night-time DBP, mmHg	58.3 ± 5.84		61.4 ± 6.99		<0.001	
Night-time heart rate, bpm	65.9 ± 9.94		66.1 ± 9.94		0.85	
Manuscript 3						
	Dippers		Non-dippers		P	
Sleep-trough surge, mmHg	20.3 ± 9.13 (N=112)		12.3 ± 8.31 (N=221)		<0.001	
Dynamic morning surge, mmHg	28.4 ± 10.1 (N=335)		18.1 ± 9.74 (N=492)		<0.001	
Night-time SBP, mmHg	105 ± 9.07		113 ± 10.2		<0.001	
Night-time DBP, mmHg	60.0 ± 5.90		62.3 ± 7.18		<0.001	
Night-time heart rate, bpm	65.9 ± 10.6		67.5 ± 117.		0.028	

Abbreviations: DBP – diastolic blood pressure; SBP – systolic blood pressure

6.2.2 Morning blood pressure surge and the vasculature: A comparison between young dippers and non-dippers from the African-PREDICT Study (Chapter 4)

The aim of this paper was to determine whether the MBPS is associated with retinal calibres and arterial stiffness. The hypothesis was that MBPS will relate positively to central retinal vein equivalent (CRVE) and arterial stiffness (pulse wave velocity, PWV), and negatively with central retinal artery equivalent (CRAE).

Measures of microvascular structure and macrovascular function were comparable between dippers and non-dippers. Night-time ambulatory blood pressures were higher in non-dippers (Table 1), except for daytime systolic and diastolic blood pressure, nocturnal percentage dipping and sleep-trough surge (Table 1) which were higher in dippers. A positive association between CRAE and sleep-trough surge was confirmed in dippers, after performing partial correlations with adjustments applied for age, sex and ethnicity. Following full adjustments, a positive relationship of CRAE with sleep-trough surge was confirmed in dippers only. The association between CRAE and sleep-trough surge lost significance in sensitivity analysis when 24-hour diastolic blood pressure was replaced with night-time diastolic blood pressure, indicating that the association between CRAE and sleep-trough surge is dependent on night-time blood pressure. However, we observed a negative association between CRAE and night-time diastolic blood pressure in dippers only.

To the best of our knowledge, no research has been conducted to investigate the relationships between MBPS and measures of microvascular structure and arterial stiffness. Our results offer a better understanding regarding the association of a normal MBPS (due to night-time dipping) with retinal artery diameter in healthy individuals. In the literature, it is clear that adverse changes in vessel calibres may provide prognostic information and were shown to relate to incident cardiovascular events and other related diseases (13-15). Although measures of microvascular structure and arterial stiffness were comparable between dippers and non-dippers in our study, we observed a positive association between CRAE and sleep-trough surge in dippers only. These findings suggest an essential role of a normal MBPS (due to night-time dipping) in preserving the retinal artery diameter of dippers. This association has not been previously reported by other studies.

Our hypothesis is therefore rejected since MBPS related positively with CRAE in dippers only and we observed no significant associations with pulse wave velocity or CRVE.

6.2.3 Morning blood pressure surge relates to autonomic neural activity in young non-dipping adults: The African-PREDICT Study (Chapter 5)

This study aimed to establish whether the MBPS is related to markers of autonomic neural activity (heart rate variability [HRV] and baroreceptor sensitivity) in dippers and non-dippers. The hypothesis was that MBPS will relate inversely with low frequency HRV and baroreceptor sensitivity.

The 24-hour HRV (total power, low frequency, high frequency and low frequency-to-high frequency) was comparable between dippers and non-dippers, whereas night-time blood pressure and baroreceptor sensitivity were higher in non-dippers. Multivariable-adjusted regression analyses confirmed a negative association of sleep-trough and dynamic morning surge with 24-hour HRV total power in non-dippers only. No associations were observed between MBPS and baroreceptor sensitivity as well as low or high frequency HRV or ratio variables in any of our study groups.

In the literature, it is evident that exaggerated MBPS is evident in hypertensive and general populations (1, 9, 16-18). Furthermore, notwithstanding which measure of MBPS we used, non-dippers in our study had a lower MBPS compared to dippers, and is in accordance with previous findings (4). In addition, previous studies indicated that sympathetic nervous system activity is associated with exaggerated MBPS (16, 19-21). On the other hand, our study demonstrated an association of lower MBPS with a marker of autonomic function (HRV total power).

To the best of our knowledge, the link between suppressed MBPS of healthy young non-dippers and autonomic function has never been reported in any young and healthy population. This is rather a distinct addition to current literature regarding the importance of dipping status on MBPS. Therefore, our hypothesis is rejected since a negative association existed between MBPS (sleep-trough and dynamic morning surge) and HRV total power in non-dippers.

6.3 Limitations, chance and confounding

This is the first study to investigate the MBPS profiles, and its association with arterial function and subclinical hypertension-mediated organ damage using two methods of estimating surge namely, sleep-trough and dynamic morning surge among a young South African bi-ethnic population. Overall, this was a highly controlled study following a strict protocol, however, it is important to reflect on some factors that might have affected the results in this study. One limitation was the lack of assessing sympathetic nervous system activity by means of gold standard method such as microneurography. Since we did not find any significant associations of low frequency HRV, high frequency HRV or low frequency-to-high frequency ratio with MBPS, we cannot confirm the contribution of sympathetic or parasympathetic nervous activity on the MBPS

in our study population. Furthermore, since we did not review the renin-angiotensin system, we could not investigate the low renin phenotype (typically observed in black populations) or high renin, and the association thereof with MBPS.

It is important to reflect on some factors that might have affected the results in this study. This study was a cross-sectional study and therefore one cannot infer causality. Sample size estimation indicated that a population of 800 participants is sufficient for 80% power to accurately assume probability for the posed research question. The results were obtained from a particular target population, only revealing the general health of a particular geographical area of South Africa, and do not necessarily reflect the status of the entire black and white populations of South Africa.

Moreover, the sample size for sleep-trough (N=650) is relatively small compared to that of dynamic morning surge (N=845), yet associations obtained were similar for both morning surge variables. The difference in sample size between sleep-trough and dynamic morning surge is a consequence of missing data (N=552) regarding waking and sleep times recorded by participants which are essential for quantifying sleep-trough surge. In addition, the difference in number of participants with an exaggerated morning blood pressure surge when using sleep-trough and dynamic morning surge should be taken into consideration. Since there is less information regarding the preferred method for quantifying 24-hour clock-based MBPS, dynamic morning surge was used as a reliable method for 24-hour clock-based MBPS. Future studies are however, recommended to investigate other improved techniques of quantifying MBPS.

With reference to the results, the possibility of chance findings should be considered. Also, the possible influence of confounders in the associations between MBPS, and various variables investigated in this study was kept to the minimum, with adjustments made where necessary in the statistical analyses. However, a rule was followed that only one covariate per 10 subjects was allowed in the final regression analysis. It was also necessary to interpret all the statistical results from a physiological perspective, which entails that all statistical significance does not necessarily indicate physiological significance and vice versa.

6.4 Summary of main findings

In all our research manuscripts, MBPS was higher (but in normal range) in white normotensive individuals who were mostly dippers, and associated positively with night-time blood pressure. These observations confirmed findings that were reported in normotensive and hypertensive white individuals (1-3, 9). Since we did not review the renin-angiotensin system, we speculated that these observations may be due to increased plasma renin activity that was reported to be increased in white individuals.

Adverse structural changes on microvascular structure were suggested to occur as a result of long-term elevated blood pressure (22). In our study, we found no association between MBPS and retinal vessel calibres in non-dippers who presented with a higher night-time blood pressure. However, in dippers, we obtained an association between MBPS and retinal artery diameter. This association was dependent on night-time blood pressure, further supporting the notion that night-time dipping plays an important role in MBPS. Our observed association suggests the essential role of normal night-time dipping on maintaining an adequate retinal artery diameter, and we consequently recommend this association to be investigated further to understand the potential of confounders not addressed in this study.

Elevated activity of the sympathetic nervous system was suggested to occur as a result of sleep apnea in hypertensive patients, thereby leading to exaggerated MBPS (23). In our study, we found no association between MBPS and sleep apnea. Considering the association between increased activity of sympathetic nervous system at night-time and elevated night-time blood pressure, and possibly lower MBPS evident in non-dippers (24), it is essential to confirm if this is also true in our study population. Our study results showed an association between HRV total power and MBPS in non-dippers who presented with a higher night-time blood pressure. Since non-dippers of our study population had a lower MBPS (associated with increased autonomic function) and higher night-time blood pressure, the concern is raised whether they are subjected to a significant increased risk for future cardiovascular events and disease. Although it is difficult to extend the results of this study to the general black population of South Africa, our findings provide a starting point (Figure 1) for larger scale prospective studies, with a study population consisting of randomly selected participants in order to address increased future cardiovascular events and disease in black populations.

Overall, although white individuals had a higher (but normal MBPS) compared to their black counterparts, this study included a healthy normotensive population. Non-dippers may not necessarily have an exaggerated MBPS since their suppressed night-time blood pressure pattern may mask the surge in the morning hours, hence it is important to take night-time blood pressure dipping into account when interpreting MBPS. This may be especially true for young dipping and non-dipping individuals (in our study population) who are still normotensive or pre-hypertensive with a normal MBPS. Whether this appears to be the same or otherwise in young hypertensives should still to be explored.

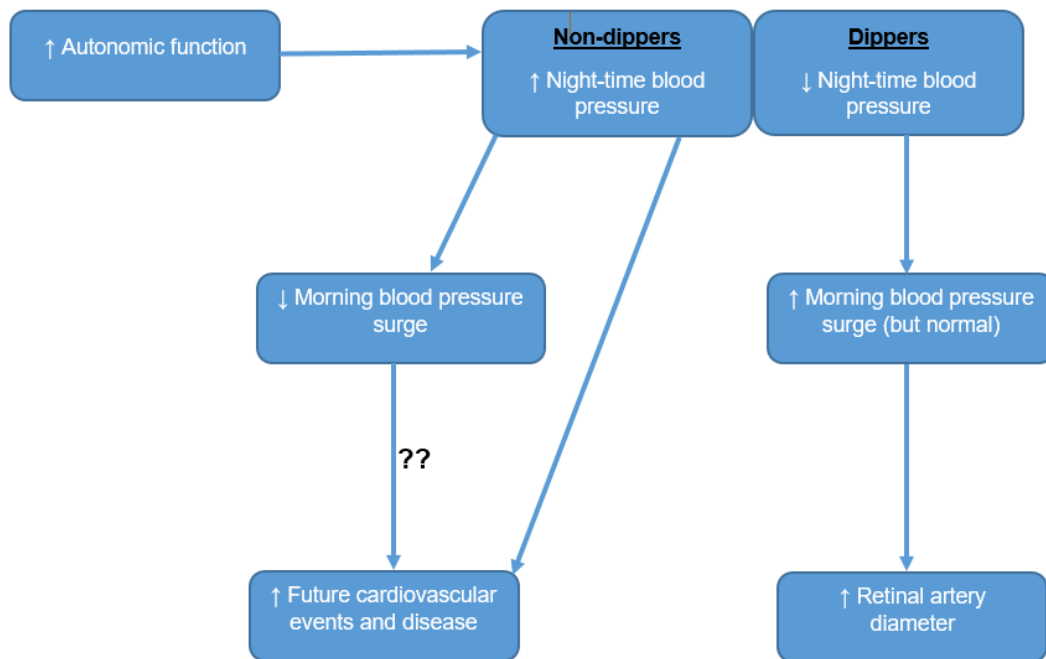


Figure 1. Flow diagram depicting the main findings in a young healthy population from this Ph.D. study

6.5 Final conclusion

In young, apparently healthy adults (aged 20-30 years), white men presented with a higher, but physiologically normal MBPS compared to their black counterparts. Furthermore, a higher proportion of white individuals had exaggerated sleep-trough surge compared to their black counterparts. Morning blood pressure surge associated positively with night-time blood pressure dipping in all ethnic groups, further supporting the notion that night-time blood pressure dipping patterns should be considered when interpreting MBPS. Although non-dippers had a lower MBPS, their suppressed night-time blood pressure pattern is associated with increased autonomic function, possibly increasing their risk for future cardiovascular disease and adverse outcomes. Moreover, the positive association established between retinal artery diameter and sleep-trough surge in dippers, indicated that their normal MBPS (due to normal night-time blood pressure dipping) may play an important role in preserving retinal artery diameter. Collectively, the results of this study suggest the importance of considering night-time dipping pattern in young and healthy normotensive individuals who presented with a normal MBPS.

6.6 Recommendations

The following recommendations are intended to offer a better understanding regarding MBPS:

- Future cross-sectional and longitudinal research studies should be conducted to investigate the preferred method and other techniques for quantifying 24-hour clock-based MBPS.
- The influence of renin-angiotensin-aldosterone-system activity could provide more insightful information on other factors (besides autonomic function) that result in elevated night-time blood pressure that is evident in non-dipping individuals.
- Longitudinal studies are recommended to investigate and suggest ethnic and sex specific MBPS cut-off points which are considered pathological and contributes to future cardiovascular events.
- Longitudinal studies are recommended to investigate and suggest a MBPS cut-off point which is considered pathological and contributes to future cardiovascular events in non-dippers.
- Assessment of sympathetic nervous system activity by means of microneurography is essential and to predetermine if MBPS is indeed driven by sympathetic tone or impaired parasympathetic activity.
- A call on clinical and/ or epidemiological studies are encouraged to explore various possible mechanisms to identify other risk factors, apart from night-time dipping that may shed light on the occurrence of future cardiovascular events in healthy and young non-dipping individuals. New knowledge on such additional contributions would be valuable in implementing cardiovascular prevention programs in young non-dipping adults.
- Studies are encouraged to explore various possible mechanisms to identify other risk factors, apart from night-time dipping that may shed light on the occurrence of future cardiovascular events.

6.7 References

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Appendix A: Ethics approval for the Ph.D. thesis



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Faculty of Health Sciences
Health Sciences Ethics Office for Research,
Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

30 March 2017

Prof R Kruger
Physiology-HART

Dear Prof Kruger

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00008-17-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: Morning blood pressure surge and its association with arterial function and subclinical target organ damage in 20-30 year old South Africans: The African-PREDICT study

Study leader/supervisor: Prof R Kruger

Student: GG Mokwatsi

Application type: Single study

Risk level: Minimal

You are kindly informed that your application was reviewed at the meeting held on 16/02/2017 of the HREC, Faculty of Health Sciences, and was approved on 30/03/2017.

The commencement date for this study is 30/03/2017 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApPLY@nwu.ac.za.

Yours sincerely



Prof Wayne Towers
HREC Chairperson



Prof Minnie Greeff
Ethics Office Head

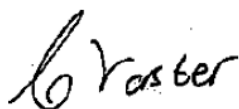
Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2017 Applications\Applications 01-16 February 2017\NWU-00008-17-S1(R Kruger-GG Mokwatsi)\NWU-00008-17-S1(R Kruger-GG Mokwatsi)-AL.docm
11 April 2017

File reference: 9.1.5.3

DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a thesis written by G Mkwatsi.

Title of the thesis: Morning blood pressure surge and its association with arterial function and subclinical target organ damage in young South Africans: The African-PREDICT study



19 November 2018

Appendix C: Turn-It-In originality report

Turnitin Originality Report

Thesis_GGM_turn-it-in.docx by GONTSE MOKWATSI



From Turnitin-External (Postgraduate Turn-it-in)

- Processed on 19-Nov-2018 13:32 SAST
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Appendix D: Solemn Declaration



Higher Degrees Administration

SOLEMN DECLARATION AND PERMISSION TO SUBMIT

1. Solemn declaration by student

I, **Gontse Gratitude Mokwatsi**
declare herewith that the thesis/dissertation/mini-dissertation/article entitled (exactly as registered/approved title),

Morning blood pressure surge and its association with arterial function and subclinical target organ damage in young South Africans: The African-PREDICT study

which I herewith submit to the North-West University is in compliance/partial compliance with the requirements set for the degree:

Doctor of Philosophy in Science (Physiology)

is my own work, has been text-edited in accordance with the requirements and has not already been submitted to any other university.

LATE SUBMISSION: If a thesis/dissertation/mini-dissertation/article of a student is submitted after the deadline for submission, the period available for examination is limited. No guarantee can therefore be given that (should the examiner reports be positive) the degree will be conferred at the next applicable graduation ceremony. It may also imply that the student would have to re-register for the following academic year.

My Ethics number is:

NWU-00008-17-S1

My ORCID is:

orcid.org/0000-0001-6203-3965

Signature of Student

**Gontse
Mokwatsi**

Digitally signed by Gontse Mokwatsi
DN: cn=Gontse Mokwatsi, o=North-
West University, ou=Physiology,
email=22368590@nwu.ac.za, c=ZA
Date: 2018.11.19 13:50:58 +02'00'

University Number **22368590**

Signed on this **19** day of **November** of 20**18**

2. Permission to submit and solemn declaration by supervisor/promoter

The undersigned declares that the thesis/dissertation/mini-dissertation complies with the specifications set out by the NWU and that:

- the student is hereby granted permission to submit his/her mini-dissertation/ dissertation/thesis:
☒ Yes ☐ No
- that the student's work has been checked by me for plagiarism (by making use of TurnItIn software for example) and a satisfactory report has been obtained:
☒ Yes ☐ No

Signature of Supervisor/Promoter

**Prof Ruan
Kruger**

Digitally signed by
Prof Ruan Kruger
Date: 2018.11.19
14:10:03 +02'00'

Date

19/11/2018