THE ASSOCIATION BETWEEN SERUM AND URINARY COTININE LEVELS AND ARTERIAL STIFFNESS IN THE BIRTH-TO-TWENTY AND AFRICAN-PREDICT COHORT STUDIES

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A dissertation submitted to the Faculty of Health Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science

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DECLARATION

I, Betty Nembulu, student number 1102731, declare that this Dissertation Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Dissertation Report.

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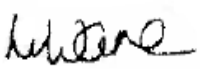
Signature of Primary Supervisor: 

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Article 1: Title: Association between tobacco exposure, blood pressure, and arterial stiffness in South African adults and children

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DEDICATION

I would like to thank my family for the support and my supervisors for the dedication and patience. A special vote of thanks to my parents (Albert Nembulu, Tshifhiwa Mukwevho) and grandmother (Annah Mukwevho).

PUBLICATIONS ARISING FROM THIS STUDY

The following manuscript that has risen from this work to date is currently under review:

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ABSTRACT

Cardiovascular disease (CVD) is the leading cause of mortality globally, resulting from various risk factors, including genes, environment, demographics and poor lifestyle behaviours, such as tobacco exposure, a preventable CVD risk factor. Arterial stiffness is a predictor of CVD, pulse wave velocity (PWV) is often used to stratify the risk for development of CVD. The aim of this study was to examine the relationship between tobacco exposure (own use and environmental tobacco smoke [ETS]), as assessed by the biomarker cotinine, and arterial stiffness in South African adults and children of African ancestry.

Two South African cohort studies of young adults, the African-PREDICT study (n=587 African adult men and women, 20-30 years) and Birth-to-Twenty-Plus study (n=95 African adult women, 28-68 years and n=47 African children, 4-10 years), were explored. Chemiluminescence method on the IMMULITE system was used to measure cotinine (serum and urinary). Cotinine values above 10 ng/ml were considered for tobacco exposed and PWV was measured using the SphygmoCor XCEL device.

Thirty one percent adults and forty five percent children were classified as tobacco exposed. Linear regression showed that, in both children and adults, tobacco exposure was associated with arterial stiffness in the univariate analysis but not in multivariate analysis. Blood pressure (BP) was higher in tobacco exposed individuals (adults and children) compared to the non-exposed counterparts. These findings highlight the need to stop tobacco use to maintain ideal cardiovascular (CV) health. Highlighting the need for a thorough assessment of the current guidelines on tobacco cessation strategies in South Africa.

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ABBREVIATIONS

ANCOVA- Analysis of covariance

AP- African Predict

baPWV- Brachial-ankle pulse wave velocity

BMI- Body mass index

BP- Blood pressure

Bt20- Birth to twenty

cf-PWV- Carotid-femoral pulse wave velocity

cr-PWV- Carotid-radial PWV

CV- Cardiovascular

CVD- Cardiovascular disease

cSBP- Central systolic BP

DBP- Diastolic blood pressure

ETS- Environmental tobacco smoke

EVA- Early vascular aging

FMO- Flavin-containing monooxygenase

GATS- Global adult tobacco survey

GPAQ- Global Physical Activity Questionnaire

IMT- Intima-media thickness

IQR- Interquartile range

ISAK- International Society for the Advancement of Kinanthropometry

LMICs- Low- and middle-income countries

MAP- Mean arterial pressure

PP- Pulse pressure

PWV- Pulse wave velocity

SBP- Systolic blood pressure

SD- Standard deviation

SSA- Sub-Saharan Africa

T2DM- Type 2 diabetes mellitus

UGT- UDP glucuronosyltransferase

WHO FCTC- WHO Framework Convention on Tobacco Control

WHtR- Waist to height ratio

CHAPTER 1: INTRODUCTION

1.1. Background

Cardiovascular disease (CVD) is a leading cause of mortality globally, causing approximately 17.9 million deaths annually (1). CVD results from poor vascular health due to various risk factors including genes, environment, demographics and poor lifestyle behaviours, such as tobacco use, a preventable CVD risk factor (2,3). Tobacco use and exposure are frequently assessed through self-reports, however, this method can be unreliable due to inaccuracy in measures of smoking exposure due to smoking denial or difficulty in recalling smoking (4). Another disadvantage is that adults and children who do not smoke, but are exposed to tobacco in their environment (through secondhand smoke), including smoking and smokeless tobacco may not realise the cardiovascular risk associated with their exposure (5). Nicotine from tobacco is metabolized in the liver by enzymes such as CYP2A6, UDP-glucuronosyltransfease (UGT), and flavin-containing monooxygenase (FMO) into cotinine (6). Cotinine, is a major metabolite of nicotine and a reliable biomarker of tobacco exposure (2,6). About 80% of nicotine produced in the body is rapidly converted into cotinine (2), detectable in both serum, saliva and urine (4). Cotinine has a longer half-life than nicotine and is detectable up to four days after exposure (7). Thus, using biomarkers such as cotinine overcomes the challenges related with recalling tobacco use and inaccuracy in self-reporting. Due to the longer half-life of cotinine, it is an ideal biomarker of tobacco exposure, meaning that its metabolism is slower than that of nicotine (6). Using cotinine as a biomarker of tobacco exposure, the South African National Health and Nutrition Examination Survey recommends a cut-off value of 10 ng/ml to distinguish no tobacco exposure and tobacco exposure in South African adults (7). Using this cutoff, the study showed that in 2019, 32% of adults and 45% of children either used or were exposed to tobacco. While tobacco use has declined globally, evidence has shown a relative increase in the African region (from 54 million tobacco users in 2010 to 57 million users in 2018) (8). Preventative strategies targeting tobacco exposure in adults and children are, therefore, needed for this region.

Tobacco exposure accelerates the process of vascular aging, contributing to early vascular aging (EVA) (9). EVA is the premature changes of arterial structure resulting in arterial stiffness

mimicking the effects of aging (10). A previous study has shown higher arterial stiffness (a marker of vascular ageing and independent risk factor for CVD) in the African population (11), including in children as young as 6-8 years (12). The importance of strategies for reducing tobacco exposure in this region is particularly salient (13) as it is a known contributor to EVA (9). The progression of EVA can be measured by arterial stiffness, which progressively increases with age (14). Identifying vascular ageing in children and adolescents may provide insight into cardiovascular risk later in life (15). However, it is not clear how tobacco exposure impacts vascular health, vascular ageing and arterial stiffness in South African adults and children when considered against a background of other common risk factors in sub-Saharan Africa (SSA) (16). Social characteristics such as, depression, racial discrimination, socioeconomic position, social participation, and cardiovascular outcomes may be linked with accelerated aging (17). Combining data from two South African cohort studies, this study aimed to examine the relationship between tobacco exposure, as assessed by the biomarker cotinine, and arterial stiffness in South African adults and children of African ancestry adjusting for other risk factors as part of a multiple linear regression model. This will help to inform communication strategies for tobacco cessation campaigns and intervention strategies in the South African region not to consider tobacco exposure as an isolated risk factor but also consider other risk factors.

1.2. Problem statement and justification

Tobacco use remains relatively high in the younger South African population aged 15 years and above. Amidst the multiple risk factors that contribute to CVD, the increase in tobacco exposure may be one of the driving factors in this region. However, data on the impact of risk factors such as tobacco exposure on EVA for both adults and children remains limited in this region.

1.3. Study aim and specific objectives

The aim of this study was to examine the relationship between tobacco exposure, as assessed by the biomarker cotinine, and arterial stiffness in South African adults and children of African ancestry adjusting for other risk factors.

The specific objectives were:

i. To compare a range of cardiovascular risk factors between tobacco exposed and non-exposed adults and children (based on detectable levels of the biomarker cotinine).
ii. To evaluate the relationship between tobacco exposure and arterial stiffness in adults and children, adjusting for these other risk factors.

1.4. Hypothesis

Tobacco exposed adults and children will exhibit increased arterial stiffness as shown by higher pulse wave velocity (PWV) and tobacco exposure will be a significant predictor of PWV.

1.5. Dissertation layout

The dissertation layout is as follows: chapter 1 the introductory chapter presents the background on CVD and vascular health in the South African context, problem statement and justification, study aim and objectives and the hypothesis. Chapter 2 presents a literature review expanding on the prevalence and the risk factors for CVD, the known impact of tobacco exposure on CVD, legislation, and policies to ameliorate tobacco use, measurements of tobacco use and exposure (the combination of use and environmental exposure), the pathophysiological impact of tobacco on the cardiovascular system (arterial stiffness). Chapter 3 presents the manuscript submitted to the Journal of Public Health in Africa, with the following sub-sections: abstract, background, methods which encompasses ethical considerations, measurements (health questionnaire, anthropometry, blood pressure measurements, pulse wave velocity (PWV) measurements and cotinine samples),

statistical analysis, results, discussion of the findings and conclusion. The last chapter (Chapter 4) is the concluding chapter, giving recommendations for future research and work.

CHAPTER 2: LITERATURE REVIEW

2.1. Prevalence and the risk factors for cardiovascular disease (CVD)

2.1.1. Prevalence of CVD

Cardiovascular disease (CVD) is the leading cause of mortality globally, the prevalence is on the rise in low- and middle-income countries (LMICs) (18,19). In 2019 17.9 million people died from CVDs, contributing towards 32% of all deaths globally, with over one third of these deaths occurring in LMICs (1). CVDs are one of the world's major burden, with rising rates of mortality and disability, its affecting working individuals adding to high economic cost (20). The prevalence of CVD is rising around the world, with a particularly high burden in Sub-Saharan Africa (SSA) and among people of African descent (21). CVD is known to result from a complex interaction of genetic predisposition and environmental factors, resulting in continuous degradation of cardiovascular structure and function (18). There are various risk factors for CVD, including modifiable (environmental, psychological and behavioural) and non-modifiable (biological such as genetics, sex, ethnicity and age) risk factors (18,19,22). The majority of cardiovascular illnesses can be avoided by addressing behavioural risk factors such as tobacco exposure (use and environmental tobacco smoke (ETS)), poor diet and obesity, physical inactivity, and alcohol use (1). Globally almost half of children are exposed to environmental tobacco exposure daily in public places, and as a result about 65 000 children die each year (23). Nonetheless, interventions are urgently needed to improve survival rates and reduce the burden of CVD in regions where incidence or death is rising. Having policies in place, as well as programs, and resources, and enforcing changes in behaviour, environment, and lifestyle, may contribute to a reduction in CVD (19).

2.1.2. CVD risk factors

Tobacco exposure is not an isolated CVD risk factor but is related to other risk factors such as increased mean arterial pressure, contraceptive use, alcohol consumption, obesity, sex, socioeconomic status and family history (18,19), (**Figure 2.1**). Although the use of antihypertensive medication reduces risk for CV events, the use is associated with increased CV risk attributable to factors associated with hypertension (24). Unhealthy lifestyle behaviours such as individuals' decisions to drink alcohol, smoke tobacco, or mix the two are influenced by socioeconomic and demographic factors (25). This implies that alcohol and tobacco reduction efforts in South Africa should include focus on public awareness campaigns that address the health, psychological, and economic consequences of using these substances (25). Programs have been put in place to promote healthy quitting behaviour in South Africa. Cessation aids introduced include counselling and nicotine replacement therapy/pharmacotherapy (nicotine patch, nicotine spray), however, the most utilised aid remains nicotine replacement (26). Despite high awareness of tobacco cessation aids the utilisation of these aids remains low (26). An approach that includes multiple sectors outside of the heath sector, healthy public policy and incorporation of health in all policies has been introduced (27). Health professionals are involved in giving quit smoking advise, though the implementation still requires strengthening (28). This is due to poor knowledge and skills of health professionals, health systems constraints, sociocultural barriers, misconceptions and misperceptions about the effectiveness of tobacco dependence treatments (28). However, a marginal increase in e-cigarette use has been noted between 2010 and 2017 in South Africa as a result of attempts to quit smoking (28). This emphasizes the importance of quit guidance in promoting healthy quitting behaviour and recommendation that it should be scaled up in South Africa. This is because the use of e-cigarettes is harmful to cardiovascular health (18). Despite the tobacco control regulations put in place, there is still a need for programs to help smokers who want to quit, and training programs to educate healthcare providers to help active smokers break their nicotine addiction. Socioeconomic status and unhealthy lifestyle behaviours such as binge drinking, physical inactivity, and unhealthy eating are among the factors that influence the success of smoking cessation efforts (29). The structural deterioration of the elastic components of the large arteries is only partially reversible with the current pharmacological treatment in place (30).

Therefore, it is critical to assess how preventative measures may reduce age-associated increases in arterial stiffness (30). Such measures include sodium limitation in the diet, regular physical activity, the cessation of smoking and reduction in alcohol consumption (30).

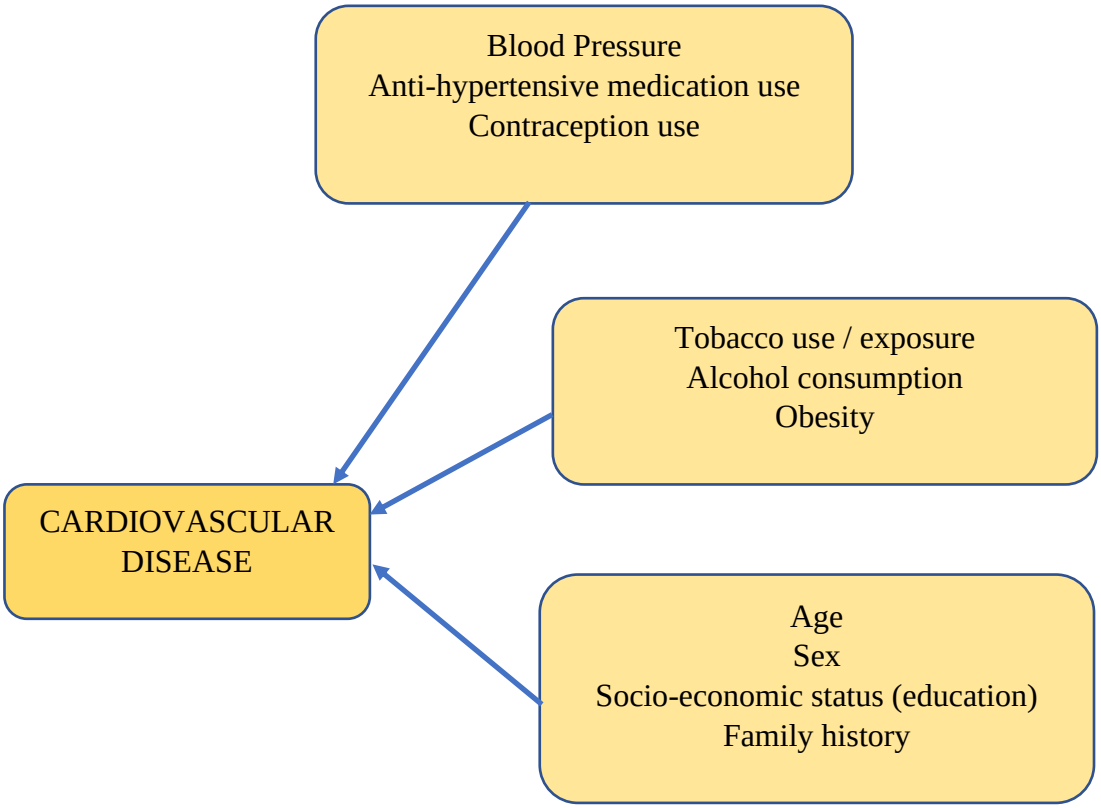


Figure 2.1. Cardiovascular risk factors

2.2. The known impact of tobacco exposure on CVD

Tobacco use continues to be one of the major preventable causes of deaths related to CVD, contributing to one third of mortality globally (31–33). The detrimental effects of tobacco exposure on cardiovascular health is due to both direct tobacco exposure and second-hand exposure (23). Tobacco products include harmful and potentially harmful ingredients, which contribute to cardiovascular damage (31). Thus, having smoke-free laws could help safeguard the health of non-smokers while also encouraging smokers to stop (23).

2.3. Legislation and policies to ameliorate tobacco use

Tobacco use is generally low in the SSA region, however, while it has gone down in other regions it is on the rise in this region (13). This is due to inadequate information on tobacco use (13). Tobacco is estimated to cause approximately 8 million deaths annually globally, with more than 7 million deaths attributed to direct tobacco exposure and approximately 1.2 million attributed to environmental tobacco exposure, also referred to as second-hand smoke (23). In 2015, approximately 24.9% of the global population aged over 15 years were users of tobacco (8). This rate is expected to decrease assuming all countries maintain the current efforts to control tobacco (8). The use of tobacco in the African region is on the rise compared to other regions, leading to an increase in tobacco-related conditions (34). In 2016 approximately 22% of the South African population aged 15 years and above were known to use tobacco products according to the South African demographic and health survey (35). Globally, strategies to accelerate tobacco control have been put in place, such as the world health organization (WHO) Framework Convention on Tobacco Control (the Convention or the WHO FCTC) (36). The legislations cover the following intervention measures: tax increase on tobacco, smoke free policy in all public places, health information and warnings about tobacco on packages and the ban on advertising, promotion and sponsorship of all tobacco products, prohibition of sale of tobacco to persons under the age of 16, prohibition of free distribution of tobacco or at discounted prices and restrictions on use of vending-machines (27,37). These have been adopted across countries including in South Africa, where legislation around tobacco control has been developed (27). However, the enforcement of policies and interventions to combat tobacco use in LMICs such as those in SSA still needs to be

explored further (13). In 2020, the focus of the World No Tobacco Day campaign was on promotion of protective measures to protect children and young people from being exploited by the tobacco industry, as a large number of adolescents aged 13 to 15 years (approximately 40 million) were reported to have initiated smoking in the year 2020 (38). Majority of adolescents who start smoking continue to smoke into adulthood (39). In comparison to other regions in the world, Africa has the lowest frequency of tobacco use, however, there appears to be an increase in tobacco industry marketing in Africa, with a large share of these efforts directed at adolescents (38). The use of media has been identified as a driver of tobacco use, there is a direct link between media use and tobacco use (39). Mass media education is more effective in developed countries due to better access to the media compared to LMICs (39). Regardless of the bans on advertisements individuals in LMICs continue to be exposed to tobacco products through the media imported from other countries (39). This highlights the importance of strengthening tobacco control efforts among young adolescents in LMICs. Mass media campaigns, focused on tobacco control programs, might be of great use in reducing the prevalence of tobacco use in SSA.

2.4. Measurement of tobacco exposure (use and environmental tobacco smoke)

Global adult tobacco survey (GATS) which is a national representative survey focusing on individuals aged 15 years and older is the frequently used mode for self-reported tobacco use (40). Self-reporting is commonly used to assess tobacco use and exposure; however, this method can be unreliable (4), as previously mentioned, individuals who are exposed to tobacco but are unaware of it may be missed. This method is subject to reporting bias. The reporting bias was reviewed in South African women of African ancestry as the group may fail to report their tobacco use due to the cultural unacceptability of smoking (7). Wastewater analysis which is the analysis of water from the sewage to determine the presence of nicotine is another method of assessing tobacco use (41). However, this is a community-based method (41) and would only be suitable in an integrated population. The use of cotinine, a major metabolite of nicotine has shown to be a reliable biomarker of tobacco exposure across different populations (2,6,8). About 80% of nicotine is rapidly converted into cotinine (2), detectable in both serum and urine (4). Cotinine has a longer half-life

than nicotine and is detectable up to four days after exposure (7). Various cut-offs have been proposed for the different types of tobacco exposure (exposed and not exposed) (7). A serum cut-off of 10 ng/ml improves sensitivity in South African women of African ancestry (7). Whereas, a urine cotinine cut-off of 300ng/ml best predicts tobacco use in South African adults of African ancestry (7).

Tobacco exposure including both tobacco use and environmental exposure remains harmful to cardiovascular health (42). Tobacco cessation has been shown to reduce the risk for CVD (42). An American report stated that the risk of CVD in individuals who quit smoking decreases to that of a person who has never smoked after 15 years of cigarette abstinence (43). Successful tobacco control efforts would result in lower tobacco consumption and reduced exposure to non-users as well as lower morbidity and mortality from tobacco-related CVD (42).

2.5. Pathophysiological impact of tobacco on the cardiovascular system (arterial stiffness)

This section describes the normal effects of ageing on the cardiovascular system focusing on the mechanisms that lead to stiffening of the major arteries, and the processes and risk factors for early vascular ageing (EVA). The final section explores the impact that tobacco use and exposure have on the vascular system as it relates to vascular ageing and arterial stiffness.

2.5.1. Arterial structure and function (physiology)

Arteries are made up of three layers (Figure 2.2), which include a single layer of endothelium, a layer of smooth muscles and an outer adventitia (44). The three different layers are made of different tissues. The outer layer, the tunica adventitia, is made of collagen tissue and elastic fibres (44). It is followed by the tunica media which is mainly made up of smooth muscle (44). The inner

layer, the tunica intima which lines the cavity (lumen) is made up of endothelial cells supported by an internal elastic lamina, known as the endothelium (44). Together the three layers work to maintain vascular tone.

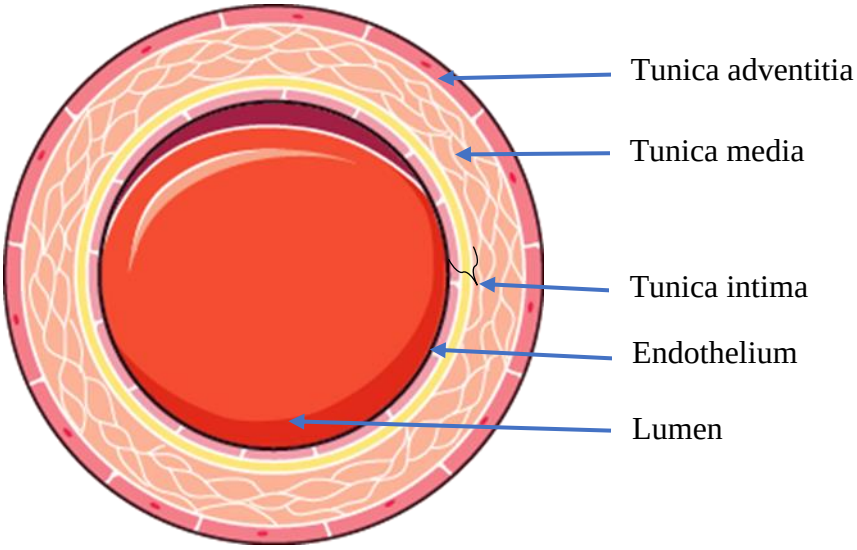


Figure 2.2. Arterial structure showing the three layers, the tunica adventitia, media and intima (43)

Adapted from https://smart.servier.com/ (open access)

Arteries are constantly subjected to forces exerted in the form of stretching and shear stress as a result of blood flow (45). Blood pressure is the main driving force that causes arteries to stretch (45). Endothelial cells are the main cells exposed to forces exerted as a result of blood flow (shear stress), resulting in changes in the intima-media (45). However, when shear stress continues overtime, the pressure created causes a loss of elasticity in the intima-media (measured through intima-media thickness) and leads to endothelial dysfunction (45). The endothelium is important in the vascular physiology as it acts by regulating the vascular tone and flow through the release of nitric oxide (30). The loss of arterial wall elasticity in the tunica media (Figure 2.2) due to shear stress causes the arteries to stiffen (46). This is due to the decrease in the media-to-lumen ratio (47). This lowers arterial storage capacity and increases the speed at which pulse is spread along the vessel wall (46). Due to the changes on the arterial wall to absorb pulse energy and how the wave spreads, it affects peripheral wave reflection (46).

As people age, arterial pulse in the central arteries causes changes in the vascular wall's build-up (47). These changes include decreased density of elastin fibres and a corresponding rise in collagen content in the tunica media (Figure 2.2.) (47). These changes, as well as the loss of arterial elasticity and compliance allows cholesterol to accumulate under the endothelium (44). The accumulation of the cholesterol molecules in the tunica intima (Figure 2.2) initiates an inflammatory response (44). This allows further infiltration of macrophages resulting in the formation of an atheromatous plaque (44). Over time, successive accumulation of lipids in the intima stimulates the formation of fibrocollagenous tissue, composed of collagen and smooth muscle cells, that engulf the atheromatous plaque resulting in fibro-atheromatous plaques (48) (Figure 2.3). This results in vascular changes in arteries, which then increase the media width and decrease the lumen diameter, resulting in a lower media-to-lumen ratio (47). This mechanism is linked to inflammatory processes, which result in extracellular matrix remodelling leading to enhanced new extracellular matrix deposition (47). At the same time, smooth muscle cells move from the tunica media to the tunica intima where they grow and deposit extracellular matrix leading to intimal thickening through vascular calcification (47). This entire process is responsible for arterial stiffening and is known as arterial stiffness.

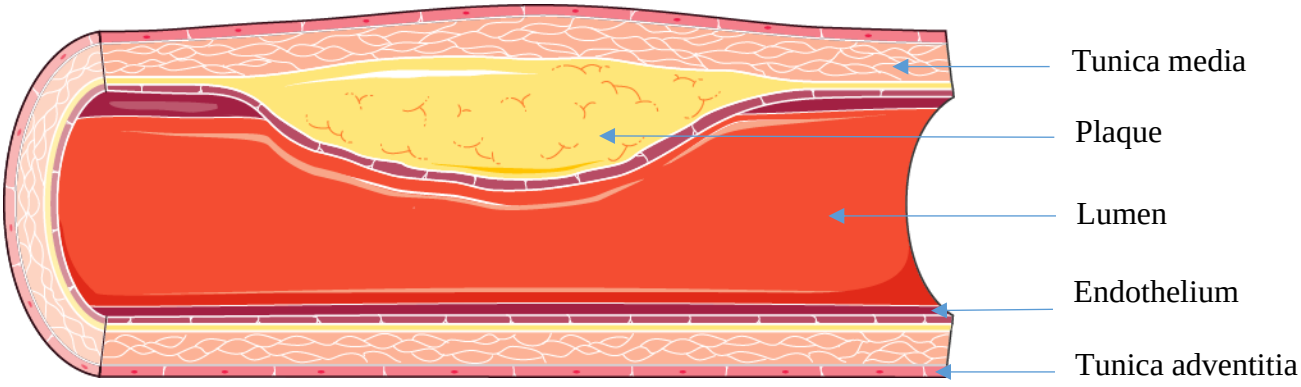


Figure 2.3. Atheromatous plaque

Adapted from https://smart.servier.com/ (open access)

2.5.2. Alterations in arterial structure and function (pathophysiology)

Arterial stiffening is a natural process which occurs with aging. It is due to the natural increase in elastic fragmentation and collagen accumulation, as a result of prolonged exposure to the pulsatile forces of blood flow, which leads to loss of elasticity and compliance of the arterial wall over time (9). A pulse wave is generated with each heart beat and travels along the arterial bed until it gets to a point of peripheral resistance of arterial bifurcation point where it is reflected back to heart (14). At a young age arteries are more elastic, thus, the pulse wave generated (i.e. the pulse wave velocity [PWV]), reaches the heart during diastole (14). Increasing the diastolic pressure and ultimately improving coronary perfusion (14). However, with older individuals with stiffer arteries, PWV increases, resulting in an early reflection of that wave, which then reaches the heart during systole (14). Raising the systolic blood pressure, which then causes an increase in cardiac workload and a decrease in coronary perfusion (14). Therefore, aortic stiffness is known to cause a rise in systolic blood pressure (SBP) and decreased diastolic blood pressure (DBP) (14,30). During the diastole, arterial stiffness results in greater post-load of the left ventricle and a decrease in mean coronary perfusion pressure (30).

Decreased complacency of the major arteries is one of the numerous phenomena associated with increased arterial stiffness (30). Increased arterial stiffness is mainly driven by the effects of aging (30). However, there are other driving factors, such as various medical conditions (e.g. hypertension; diabetes mellitus, dyslipidaemia (30)). Poor lifestyle factors (e.g. smoking, poor diet and exercise) and genetics are also known to increase the risk of developing arterial stiffness (14) (**Figure 2.4**). These alterations mimic the effect of aging resulting in arterial stiffness known as early vascular aging (EVA). Oxidative stress, free radical generation, neuroendocrine changes, and genetic alterations are some of the mechanisms involved in development of arterial stiffness (14).

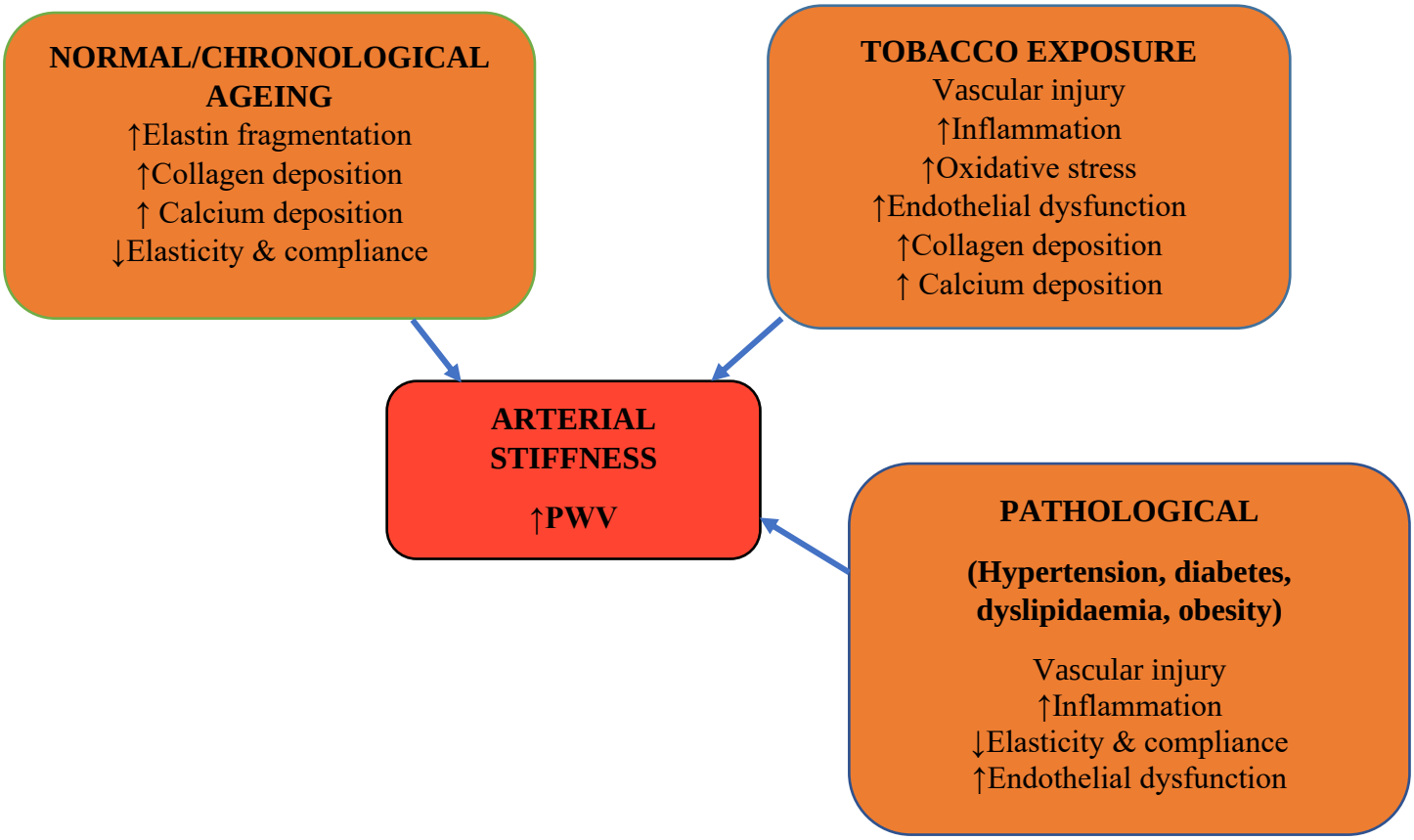
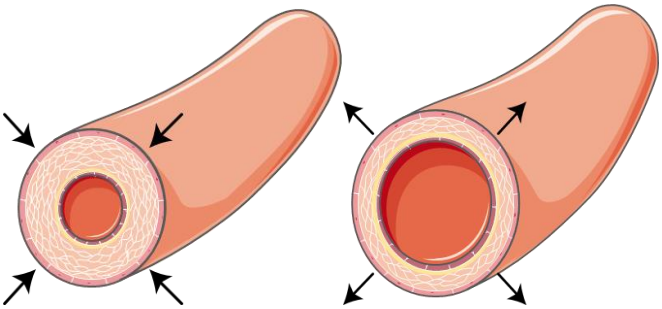


Figure 2.4. Determinants of arterial stiffness and early vascular ageing (14,22,30)

The endothelium is important in the vascular physiology as it acts by regulating the vascular tone and flow through the release of nitric oxide and endothelin (30). Nitric oxide regulates vasodilation whereas endothelin regulates vasoconstriction (49), (Figure2.5). Under homeostasis the endothelium maintains a normal vascular tone (50). However, CVD risk factors, trigger a chronic inflammatory response that includes a loss of vasodilator and anti-thrombotic factors resulting in increased vasoconstrictor and pro-thrombotic factors (50). As people age, arterial pulsation in the central arteries causes changes in the vascular wall's architecture, including rarefaction of elastin fibres and a corresponding rise in collagen content (47). These changes, as well as the loss of

arterial elasticity and compliance, are caused by inflammatory processes and vascular calcification (47). A review has found that cigarette smoke emits toxins that initiate inflammation which overtime lead to chronic inflammation (51), however, this phenomenon goes beyond the scope of this study.



Vasoconstriction ↔ Vasodilation

Figure 2.5. Vasoconstriction and vasodilation

Adapted from https://smart.servier.com/ (open access)

2.5.3. Risk factors of arterial stiffness - Tobacco use and other modifiable and non-modifiable risk factors

Tobacco exposure is an environmental factor that can accelerate the biological process of vascular aging, resulting in EVA (9,52,53). Tobacco exposure induces acute vascular changes which is a short-term phenomenon (54). However, overtime through inflammation and oxidative stress it impact the ability of the endothelium to cause vasodilation (5,31), resulting in endothelial dysfunction that causes arterial stiffness. Tobacco exposure increases expression of adhesion molecules which initiate a pro-coagulation and inflammatory environment that over a period of time results in atheromatous plaque development (33). Furthermore, tobacco exposure, through changes in arterial wall, induces tissue remodelling altering the structural integrity of the arterial wall, and further driving arterial stiffness (9,55). However, tobacco exposure is not an isolated risk

factor of arterial stiffness. Other risk factors include; ethnicity, blood pressure, obesity, age, sex and socio-economic status (18,19).

People of African descendent are reported to be more susceptible to early onset arterial stiffness than other racial groups (56). However, few studies have established the existence of arterial stiffness in African children (12). This area of research is important as these racial differences are driven to a large extent by risk factor connected to social exclusion and historical oppression throughout the life-course, which begins in the neonatal phases of development (56). Indeed, South African children of African ancestry as young as 6 to 8 years of age showed higher arterial stiffness compared to white children, confirming the early onset of vascular changes in populations of African ancestry and increasing the risk for developing hypertension in early adulthood (12).

This is further evidenced through rising levels of paediatric hypertension (increased systolic and diastolic pressures) (57). Elevated diastolic blood pressure is shown to relate to arterial stiffness in children (58). A growing body of evidence demonstrates that adversity in childhood raises the chance of poor physical health generally in adulthood, making them vulnerable to chronic diseases and illnesses, metabolic syndrome, accelerated aging, inflammation and premature mortality (56). Given the global decline in youth health, it is predictable that an increasing number of children and adolescents will have EVA, increasing their risk of CVD in early adulthood (15).

Evidence suggests that CVD risk factors emerge at a young age, necessitating the development of instruments to screen for CVD risk in children and adolescents, vascular ageing has been suggested as a useful tool for detecting early and asymptomatic symptoms of CVD burden (57). Although arterial stiffness develops with chronological age, many children and adolescents are subjected to the premature development of arterial stiffness, due to genetic or epigenetic predispositions, lifestyle and behavioural risk factors, and early life programming (21). From a public health perspective it can be argued that early childhood exposures are important modifiable risk factors

in the treatment and prevention of adult illness and disease (56). This warrants target of policy programming to include clinical interventions.

2.5.4. Measuring arterial stiffness

Arterial stiffness, particularly in the aortic tree, significantly contributes to CVD and is positively associated with coronary artery disease and stroke (59). Due to the potential life threating consequences of arterial stiffness non-invasive measurements are needed to assess arterial stiffness (14). This can be achieved by measuring PWV, a predictive value of aortic stiffness (60,61). The non-invasive gold standard method for measuring aortic stiffness is carotid-femoral pulse wave velocity (cf-PWV) (62). Other methods include carotid-radial PWV (cr-PWV) (62) and brachial-ankle pulse wave velocity (baPWV) (63). Using cf-PWV to measure arterial stiffness, the distance between the carotid and femoral arteries is measured, about 80% of the distance between the carotid pulse and femoral pulse, this is recommended by an expert consensus on the measurement of aortic stiffness in 2012 (61). PWV is then determined using the corrected distance to avoid inaccuracy in the PWV value and the pulse wave transit time (61). Studies have shown a direct relationship between arterial stiffness and cardiovascular mortality (64) with a PWV equal to and greater than 10 m/s predictive for cardiovascular events (61). With the correct techniques i.e. the use of cf-PWV as a measure of arterial stiffness has also been found to be reliable in children across different populations (65).

2.6. Summary

There is limited data on the impact of tobacco on vascular ageing in South Africa, especially for children and young adults to inform primary CVD prevention efforts. Furthermore, it is unclear if in this context, given the heavy burden of all other risk factors for CVD and EVA, whether tobacco would show an independent effect. Hence this study was conducted to examine the relationship

between tobacco exposure, as assessed by the biomarker cotinine, and arterial stiffness in South African adults and children of African ancestry adjusting for other risk factors. Ethics approval was obtained for both studies, refer to Appendix A and B. African-PREDICT obtained informed consent from participants before sample collection to analyse for cotinine, the Bt20 study did not. As such, all participants who had provided a urine sample were recontacted to obtain verbal permission to conduct the analysis, with dates and times of all conversations recorded. This revised process was approved by the University of the Witwatersrand HREC. No further data collection occurred as the research unit was closed due to COVID-19 lockdown restrictions and a revised informed consent form for cotinine analysis of urine was not needed.

CHAPTER 3: MANUSCRIPT

This manuscript has been submitted to the Journal of Public Health in Africa and is currently under review.

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This chapter will be structured as follows; title page followed by declaration of interest, funding, acknowledgements, background, methods, results, discussion, and conclusions. Tables and figures will be in the text, labelled as figure 3.1, table 3.1, 3.2 and 3.3. and not at the end as per the journal guidelines. I will have one combined reference list at the end for flow.

Association between tobacco exposure, blood pressure, and arterial stiffness in South African adults and children

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Declaration of interests

No conflicts of interest.

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Author's contributions

LJW, LFGM, AKR and BN conceptualized and designed the study. BN was responsible for data management, data analysis and wrote the original draft. BN, AKR, RK, YB, LFGM and LJW contributed to the interpretation of data and critical review of manuscript. All authors gave final approval of the version to be submitted.

3.1. Abstract

Introduction: Sociodemographic factors, health status and health behaviour have all been associated with arterial stiffness. We examined the association between tobacco use or exposure and pulse wave velocity (PWV, a marker of arterial stiffness) in black South African adults and children against a background of other known risk factors.

Methods: Two datasets were used: African-PREDICT (A-P; n=587 apparently healthy black adult men and women, 20-30 years) and Birth-to-Twenty-Plus (Bt20; n=95 black adult women, 28-68 years and n=47 black children, 4-10 years). A cotinine value >10 ng/ml in urine (Bt20) or serum (A-P) was considered as tobacco exposed and carotid-femoral PWV was measured using the SphygmoCor XCEL device. Regression analysis included cotinine and other known risk factors.

Results: One third of adults (32%) and almost half of all children (45%) were tobacco exposed with the prevalence of elevated blood pressure (BP) approximately twice as high as their non-exposed counterparts (adults, p=0.014; children, p=0.017). Cotinine was the only variable that significantly associated positively with PWV in both adults and children in univariate analysis (p<0.05), but only MAP remained significant for adults in multivariate analysis (p =0.001).

Conclusions: In this sample, tobacco exposure was adversely associated with vascular health in adults and children. BP was higher in the tobacco exposed adults and children compared to their non-exposed counterparts. These findings suggest tobacco cessation programs for adults should include screening for blood pressure and consider the impact of tobacco exposure on children's vascular health.

Keywords: arterial stiffness, cotinine, health behaviours, pulse wave velocity, tobacco exposure, vascular health.

3.2. Background

Sociodemographic factors, health status and health behaviour can all increase the risk of early vascular aging (EVA) and cardiovascular disease (CVD) (15). EVA is an independent predictor of target organ damage that indicates the deterioration in vascular structure and function (1). CVD is the leading cause of death globally, resulting in 17.9 million deaths annually (1). Identifying those at high risk for CVD remains a priority to prevent premature death. CVD results from vascular abnormalities as a result of complex interactions of different factors including genetics, environment, demographics and poor health behaviours such as tobacco use, which is a known preventable CVD risk factor (2,3,66). Tobacco use and exposure frequently coexist with other CVD risk factors, such that tobacco cessation efforts may present an opportunity to target multiple CVD risk reduction strategies if we know the associations with other risk factors. For example, healthy lifestyle choices such as cessation of tobacco use, regular physical activity and avoiding harmful use of alcohol have been shown to reduce CVD risk (1,67).

Tobacco is estimated to cause approximately 8 million deaths annually, with more than 7 million deaths attributed to direct tobacco use and approximately 1.2 million attributed to environmental tobacco exposure, also referred to as secondhand smoke (23). In 2015, approximately 24.9% of the global population aged over 15 years were users of tobacco (8). While tobacco use has declined globally, evidence has shown a relative increase in the African region (from 54 million tobacco users in 2010 to 57 million users in 2018) (8). Similar to the global prevalence, approximately 22% of the South African population aged 15 years and above are estimated to use tobacco products according to the 2016 South African demographic and health survey (35). Tobacco is frequently assessed through self-report, however, this method does not account for individuals who do not smoke but are exposed to environmental tobacco smoke (4). Thus, cotinine, a major metabolite of nicotine, has become a reliable biomarker of both tobacco use and exposure (2,8).

Tobacco exposure can contribute to pathophysiological processes, such as endothelial dysfunction (9,31) that leads to EVA and arterial stiffness. There is a direct relationship between arterial

stiffness and cardiovascular mortality (64). Vascular ageing, as commonly reflected by stiffening of large arteries, is a normal process that occurs with chronological ageing (9), due to increased elastin fragmentation and collagen accumulation leading to loss of elasticity and compliance of the arterial wall (9). However, increased arterial stiffness has been observed in South African children as early as 6 years of age (12). Arterial stiffness is determined by pulse wave velocity (PWV), a predictive value of aortic stiffness (60,64) whereby a PWV value ≥10 m/s in adults is a predictor of cardiovascular events (61). Evidence indicates that the risk for EVA starts earlier in life, however, limited evidence of vascular aging exist for children (15,21). As such, it is important to understand the impact of risk factors that are considered non-modifiable (age, sex, family history and socioeconomic status), as well as modifiable risk factors (tobacco exposure, excess adiposity, alcohol use, blood pressure and medication use) in the assessment of accelerated deterioration of the vasculature and to inform intervention strategies. Therefore, we examined the relationship between tobacco exposure, assessed by the biomarker cotinine, and PWV, a marker of arterial stiffness, against a background of other risk factors in black South African adults and children.

3.3. Methods

This was a cross-sectional analysis of secondary data from two different cohort studies in South Africa. The first cohort, from Soweto, Gauteng province, was an intergenerational study on the transmission of vascular health in families from the Birth-to-Twenty (Bt20) cohort with data collected between August 2019 to March 2020. The second cohort was from Potchefstroom, North West province, the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) with data collected between 2013 and 2017. Inclusion criteria for Bt20 were: BT20 index females, with both a biological child aged between 4-10 years and a biological mother still alive, to be able to determine the original aim of this study i.e. the degree to which vascular health is transmitted through generations. Individuals with apparent physical, mental, or congenital abnormalities were excluded. For this analysis, only participants with complete vascular health measurements who gave consent (adults) or assent in all the children aged 7-10 years for the collection of urine samples and analysis of urinary cotinine were included. Inclusion criteria for African-PREDICT were: 20-30 years old; male or female;

black or white ethnicity; and apparently healthy with a BP measurement <140/90 mmHg. Individuals with pre-existing cardiovascular disease were excluded. However, only black participants (female and male) with complete vascular health measures who gave consent for serum samples were included for this analysis. The total sample size for this analysis was 729 participants, inclusive of children (n=47; 4-10 years from Bt20) and adults (n= 682; 19-68 years), **(Figure 3.1)**.

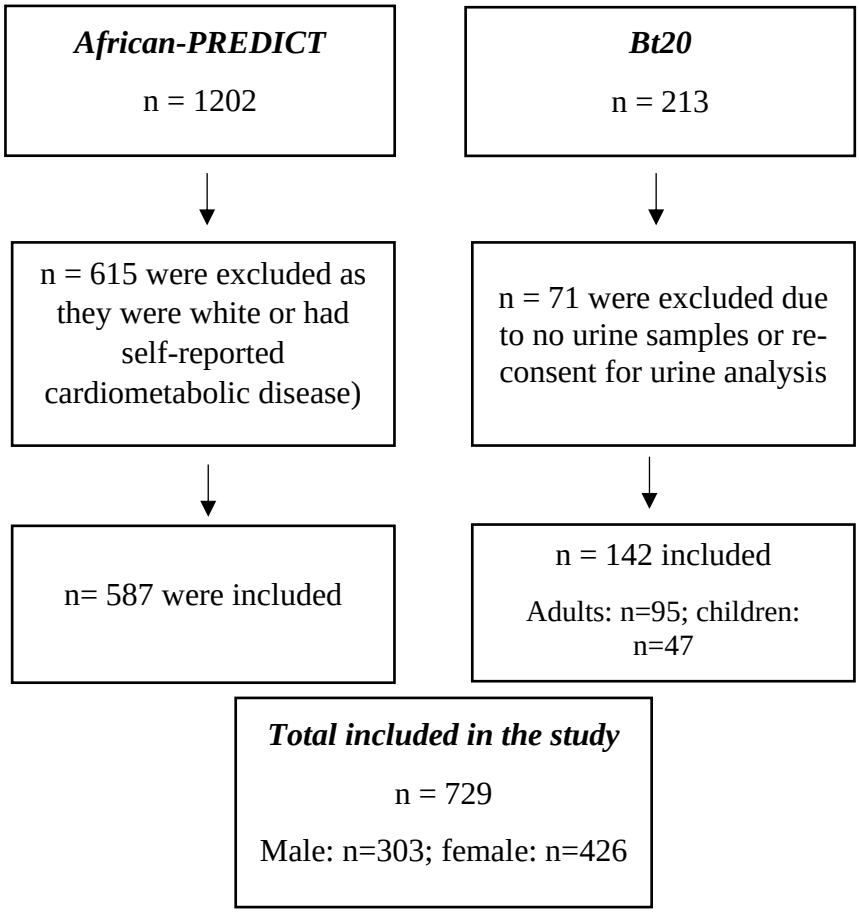

Figure 3.1. Study flow diagram

3.3.1. Ethical consideration

Ethical approval was obtained from the Witwatersrand Human Research Ethics Committee (Medical) (M190263) and the North-West University Health Research Ethics Committee (NWU-HREC) of the Faculty of Health Sciences (NWU-00001-12-A1). Informed consent was obtained from both Bt20 and African-PREDICT adults and assent in all the Bt20 children aged 7-10 years.

3.3.2. Measurements

3.3.2.1. Health questionnaire

In both studies a general health and demographic questionnaire was used to obtain self-reported health data and demographics, including age and sex. Socio-economic data, family history and alcohol use were recorded through a health questionnaire, while tobacco use was assessed using the Global Adult Tobacco Survey for Bt20 only (68). Self-reported medical history (type 2 diabetes mellitus (T2DM), hypertension, high cholesterol, or previous heart disease) and current medication use, including anti-hypertensive, diabetic and high cholesterol medication, was assessed by questionnaire in both studies.

3.3.2.2. Anthropometry

Height, weight and waist circumference, were measured in triplicate using the standardized World Health Organization (WHO) measurement protocol (69) and the International Society for the Advancement of Kinanthropometry protocol (70) for Bt20 and African-PREDICT studies respectively. Thereafter, waist to height ratio (WHtR) was calculated in both adults and children, where a WHtR 0.50-0.59 was classified as overweight and $\geq$ 0.60 as obese (71).

3.3.2.3. Blood pressure measurements

Brachial blood pressure (BP) was measured using automated devices (Bt20 – Omron MIT5 for adults; Omron HBP-1300 for children (72); Omron Healthcare, Kyoto, Japan; African-PREDICT BP - Dinamap® Procare BP monitor, Milwaukee, US). Three measurements were taken and the second and third measurements on the right arm averaged following the International Society of Hypertension measurement guidelines (73). Participants were seated and asked to rest for at least 5-minutes prior to measurement in a quiet room with the monitor facing away from the participant and a 2-minute rest interval between the measurements. Elevated BP was defined as ≥90th percentile for children (74) and SBP ≥130 mmHg and/or DBP ≥85 mmHg in adults (73). The right brachial mean arterial pressure (MAP) was calculated using the following equation: MAP = DBP + 1/3(SBP – DBP) (75).

3.3.2.4. Pulse wave velocity (PWV) measurements

All participants were asked to complete an overnight fast and to avoid tobacco use prior to measurements. Participants rested in a supine position for at least 10-minutes prior to PWV measurements. We used the SphygmoCor XCEL device (AtCor Medical, Sydney, New South Wales, Australia) to measure central BP and PWV. The distance between the carotid and femoral arteries was measured with a tape measure using the direct distance method, as recommended by an expert consensus on the measurement of aortic stiffness in 2012 (61), and using 80% of the distance from the carotid pulse to the femoral pulse. Peripheral BP was obtained by partially inflating a size appropriate cuff over the right brachial artery approximately midway between the shoulder and the elbow. This was needed as an input variable along with height for PWV measurement. Thereafter, to assess carotid-femoral PWV, following input of the participant's peripheral BP, height, sex and date of birth, carotid pulse waves were measured by applanation tonometry (high-fidelity micromanometer; Millar Instruments) while a partially inflated cuff

obtained femoral pulse waves over the femoral artery at the leg midway between the hip and the knee, simultaneously capturing carotid and femoral waveforms over a pre-set time of 10-seconds.

3.3.2.5. Cotinine samples

For Bt20, all participants were asked to produce spot urine on the same day the PWV measurements were performed. Three 1.5ml aliquots were taken for each participant and stored at -80^{o}C prior to biochemical analysis. For African-PREDICT, blood samples were drawn from the antebrachial vein, centrifuged in an onsite laboratory to obtain serum aliquots and stored at -80^{o}C until analysis (76). Urine and serum cotinine were analysed in different laboratories but using the same chemiluminescence method on the IMMULITE system (Siemens, Erlangen, Germany). For both urine and serum, samples above the analytical cotinine limit of detection (LOD; 10 ng/ml) were classified as tobacco exposed (either through use or environmental exposure) as previously shown to be a valid threshold in this population (77). When used as a continuous variable in the regression analysis, to avoid excluding concentrations below the LOD (<10 ng/ml), these were substituted by dividing the LOD by two (10/2=5 ng/ml), a method shown to reduce bias (78) and values were log transformed.

3.3.3. Statistical analysis

Normality of data was tested using visual inspection of histograms and the Shapiro-Wilks test, with continuous variables reported as mean ± standard deviation (SD) or median and interquartile range (IQR). Differences between tobacco-exposed and non-exposed adults and children were tested using Chi-square tests for categorical data, and paired T-tests (parametric) or Mann-Whitney U (nonparametric) for continuous data. Analysis of covariance (ANCOVA) was then used to adjust PWV for MAP to assess differences in study participants by tobacco exposure category. Linear regression analyses (univariate and multivariate) were used to explore the relationship between PWV, cotinine, and other known risk factors (MAP, antihypertensive medication use, contraception use, self-reported alcohol use, and WHtR, age, sex, family history, and education

level) in children and adults, and for adults with urine and serum cotinine separately. SPSS version 27.0 (IBM Corp, Armonk, NY) was used for all statistical analyses.

3.4. Results

3.4.1. Study participants characteristics

Table 3.1 summarizes participant characteristics by tobacco exposure group. In adults, 31% were classified as tobacco exposed. Overall, the tobacco exposed adult group were slightly older (p=0.018), more frequently male (p<0.001), with higher reported alcohol use (p<0.001) and increased prevalence of elevated blood pressure (p=0.014) and antihypertensive medication use (p=0.017). Prevalence of hypertension in the adults was low (<10%) as African-PREDICT excluded adults with elevated blood pressure at baseline. Tobacco exposed adults had significantly higher PWV when adjusted for MAP (p=<0.001). In children, 45% were classified as tobacco exposed. The tobacco exposed children had lower MAP adjusted PWV (p=0.046), but increased prevalence of elevated blood pressure (p=0.017) compared to the tobacco not exposed group.

3.4.2. Linear regression analysis

Results of the linear regression analysis in children and adults (**Table 3.2)** showed that in children, only cotinine was significant for predicting PWV in the univariate analysis but not in multivariate analysis. In adults, cotinine together with anti-hypertension medication, contraceptive use, WHtR, age, sex and education level were significant for predicting PWV in the univariate analysis but in the multivariate analysis only MAP was significant (p<0.001). In the multivariate analysis for adults, for each one unit (mmHg) increase in MAP, there was an increase in PWV of 0.040 m/s.

When the results for urine and serum cotinine in adults were analysed separately (**Table 3.3)**, the results showed that serum cotinine was significant (p<0.001) in the univariate analysis in the

prediction of PWV. While MAP was significant (p<0.001) in the multivariate analysis, with the model explaining 24% of the variance in PWV. In adults with urine cotinine, no significant association was found between cotinine and PWV in either the univariate (p=0.714) or multivariate (p=0.930) analysis, though the sample size for this group was much smaller (n=95 with urine cotinine; n=587 with serum cotinine).

Table 3.1. Characteristics of adults and children by tobacco exposure categories

	Adults (n=660)		Children (n=47)	
Characteristics	Tobacco exposed[#] (n=209)	Tobacco not exposed[#] (n=451)	Tobacco exposed# (n=21)	Tobacco not exposed# (n=26)
Socio-demographics				
Age (years)	26.0 (6.0)	25.0 (6.0)*	6.0 (3.0)	7.5 (2.3)
Females, n (%)	98 (46.9)	289 (64.1)***	12 (57.1)	16 (61.5)
Males, n (%)	111 (53.1)	162 (35.9)***	9 (42.9)	10 (38.5)
Socioeconomic status				
Education level (years)	8.0 (2.0)	8.0 (2.0)	-	-
Anthropometry				
Height (cm)	165.1±8.3	163.0±8.4	120.8±11.1	126.3±11.6
Weight (cm)	64.3 (18.5)	65.7 (18.0)	22.5 (9.6)	23.8 (13.4)
Waist circumference (cm)	77.0 (20.0)	77.6 (15.0)	54.7 (8.7)	55.3 (14.7)
WHtR	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)
WHtR class				
Obese (WHtR ≥0.60), n (%)	35 (16.7)	51 (11.3)	0 (0)	1 (3.8)
Overweight (WHtR 0.50-0.59), n (%)	46 (22.0)	118 (26.2)	5 (23.8)	5 (19.2)
Normal (WHtR < 0.50), n (%)	81 (38.8)	172 (38.1)	15 (71.4)	19 (73.1)
Medical history				
Self-report Hypertensive, n (%)	15 (7.2)	15 (3.3)*	-	-
Self-report high cholesterol, n (%)	5 (2.4)	7 (1.6)	-	-
Self-report diabetes mellitus, n (%)	1 (0.5)	2 (0.4)	-	-
Family history, n (%)	114 (54.5)	239 (53.0)	-	-
Antihypertensive medication use, n (%)	13 (6.2)	13 (2.9)*	-	-
Contraceptive use, n (%) [#]	7 (3.3)	48 (10.6)	-	-
Health behaviours				
Cotinine, ng/ml	170.0 (254.0)	<10***	18.0 (46.8)	<10***
Current alcohol consumption, n (%)	149 (71.3)	205 (45.5)***	-	-
Current tobacco use, n (%)	119 (56.9)	26 (5.8)***	-	-
Right seated office blood pressure				
Systolic BP (mmHg)	117 (17)	116 (18)	108 (16)	102 (10)
Diastolic BP (mmHg)	77 (14)	79 (12)	71 (11)	69 (10)
Pulse pressure (mmHg)	39 (11)	40 (11)	35 (11)	43 (24)
Right brachial MAP (mmHg)	92 (13)	78 (12)	83 (8)	81 (11)
BP status				
Elevated BP, n (%) [¥]	18 (8.6)	18 (4.0)*	18 (85.7)	12 (46.2)*
Normotensive BP, n (%)	188 (90.0)	431 (95.6)*	3 (14.3)	11 (42.3)*
SphygmoCor data				
Central systolic pressure (mmHg)	111 (17)	111 (13)	92 (9)	93 (12)
Central diastolic pressure (mmHg)	76 (10)	77 (11)	63 (7)	65 (5)
Central pulse pressure (mmHg)	34 (9)	33 (7)	29 (5)	26 (8)

PWV (m/s)	6.7 (1.4)	6.3 (1.1)***	4.0 (0.5)	4.3 (0.7)
PWV adjusted for MAP	6.7 (1.4)	6.3 (1.1)***	4.0 (0.5)	4.3 (0.7)*

Data presented as median and interquartile range or mean ± standard deviation. T-test was used on normally distributed variables, Mann Whitney u-test for non-normally distributed variables and Chi-squared for categorical variables. # Contraceptive use includes both hormone replacement therapy and contraceptives. ¥Elevated BP: ≥90th percentile for children (74) and SBP ≥ 130 mmHg and/or DBP ≥85 mmHg in adults (73). BP, blood pressure; MAP, mean arterial pressure; PWV, pulse wave velocity; WHtR, waist to height ratio. *p<0.05; **p<0.01; ***p<0.001

Table 3.2. Linear regression analysis to evaluate the relationship between tobacco exposure and pulse wave velocity taking into consideration current health status, current modifiable health behaviours and current non-modifiable risk factors in adults and children.

	Adults (n=682)					Children (n=47)				
	Pulse Wave Velocity									
Independent variables	Univariate		Multivariate			Univariate		Multivariate		
	β(SE)	R^2	β(SE)	R^2	*P* value	β(SE)	R^2	β(SE)	R^2	*P* value
Model										
logCotinine (ng/ml)	0.245 (0.041)***	0.053	0.166 (0.107)	0.237	0.001	-0.313 (0.153)*	0.085	-0.324 (0.171)	0.121	0.419
MAP	-0.047 (0.087)	0.000	0.040 (0.010)***			0.004(0.010)	0.003	0.003 (0.011)		
Anti-hypertension medication	2.072 (0.462)***	0.030	-			-	-	-		
Contraception use	0.402 (0.092)***	0.030	0.048 (0.145)			-	-	-		
Alcohol	-0.047 (0.087)	0.000	-0.101 (0.146)			-	-	-		
WHtR	2.072 (0.462)***	0.030	-0.190 (0.110)			-1.407 (1.425)	0.022	-0.125 (0.155)		
Age	0.073 (0.004)***	0.322	0.035 (0.026)			0.063 (0.046)	0.040	0.020 (0.055)		
Sex	0.373 (0.085)***	0.028	-			0.016 (0.149)	0.000	-0.026 (0.158)		
Family history	0.075 (0.087)	0.001	-0.165 (0.142)			-	-	-		
Education level	0.097 (0.020)***	0.036	-0.052 (0.064)			-	-	-		

R^2, R square; F (df), β(SE), Unstandardised beta (standard error); WHtR, waist to height ratio; MAP, mean arterial pressure. *p<0.05; **p<0.01; ***p<0.001.

Table 3.3. Linear regression analysis to evaluate the relationship between tobacco exposure and pulse wave velocity taking into consideration current health status, current modifiable health behaviours and current non-modifiable risk factors in adults

	Adults with urine cotinine (n=95)					Adults with serum cotinine (n=587)				
	Pulse Wave Velocity									
Independent variables	Univariate		Multivariate			Univariate		Multivariate		
	β(SE)	R^2	β(SE)	R^2	*P* value	β(SE)	R^2	β(SE)	R^2	*P* value
Model										
logCotinine (ng/ml)	0.064 (0.174)	0.001	-0.023 (0.256)	0.324	0.348	0.143 (0.039)***	0.025	0.166 (0.107)	0.237	0.001
MAP	0.061 (0.010)***	0.293	0.014 (0.017)			0.049 (0.004)***	0.224	0.040 (0.010)***		
Anti-hypertension medication	1.477 (0.605)*	0.175	0.181 (1.122)			-	-	-		
Contraception use	-	-	-			0.136 (0.150)	0.008	0.048 (0145)		
Alcohol	-0.857 (0.289)**	0.087	0.367 (0.589)			0.129 (0.078)	0.005	-0.101 (0.146)		
WHtR	3.177 (1.241)*	0.069	0.232 (0.445)			-2.453 (0.555)***	0.034	-0.190 (0.110)		
Age	0.080 (0.007)***	0.584	0.049 (0.040)			0.057 (0.012)***	0.039	0.035 (0.026)		
Sex	-	-	-			0.774 (0.070)***	0.181	-		
Family history	0.009 (0.327)	0.000	-0.922 (0.633)			-0.046 (0.078)	0.001	-0.165 (0.142)		
Education level	-0.127 (0.050)*	0.067	0.003 (0.078)			0.033 (0.027)	0.003	-0.052 (0.064)		

R^2, R square; F (df), β(SE), Unstandardised beta (standard error); WHtR, waist to height ratio; MAP, mean arterial pressure. *p<0.05; **p<0.01; ***p<0.001.

3.5. Discussion

Our aim was to evaluate the number of adults and children who used or were exposed to tobacco as indicated by the biomarker cotinine and the association between cotinine and arterial stiffness. We found that one third of adults had cotinine detected in either their urine or serum and almost half of children had cotinine detectable in their urine. In both adults and children classified as being exposed to tobacco, the frequency of elevated BP was doubled, while PWV was higher in adults only. Tobacco exposure was additionally a significant predictor of PWV in univariate analysis in both adults and children, while in multivariate analysis blood pressure (MAP) predicted arterial stiffness in adults only.

When considering the results from adults with either urinary or serum cotinine, prediction of PWV was stronger in the serum group. This may indicate that urine cotinine may not be as good to detect associations with markers of vascular health as serum cotinine although urinary cotinine has been established previously as a good detector of tobacco exposure in a South African adult population (7). This may be influenced by the metabolic enzymes in the liver responsible for oxidation of cotinine (such as CYP2A6 and a cytoplasmic aldehyde oxidase) (6). These enzymes can be influenced by age, sex, hormones, medication use and kidney disease (6).

Our results indicated that MAP was significant in the prediction of PWV in adults. These findings support previous research showing the association between blood pressure and tobacco exposure in the prediction of PWV in adults (79). One South African study found that, when compared to Caucasian smokers, African smokers showed higher arterial stiffness, indicating greater impact of tobacco use on cardiovascular dysfunction (80). The association between tobacco exposure and CVD has been well established in developed countries but there is still limited data in low- and middle-income countries (LMICs) like South Africa (80). However, more than 80% of the 1.3 billion tobacco users worldwide are based in LMICs (23).

A review in 2016 suggested that nearly 100,000 children transition to regular smoking daily, with 14,000–15,000 children per day in high income countries (HICs) and 68,000–84,000 in LMICs (81). To delay the onset of poor vascular health and deterioration such as arterial stiffness, health behaviours and exposures need to be changed early in life (66,79), indicating interventions are required to maintain ideal cardiovascular health. However, multi-pronged approaches are needed involving public health science, policy, and clinical practice targeted at the reduction of tobacco use; promoting cardiovascular health; and preventing tobacco-attributable deaths from CVD (81).

While the WHO Framework Convention on Tobacco Control (WHO FCTC) implemented as the Global Strategy to Accelerate Tobacco Control has been progressive over the years (36), challenges have been experienced in LMICs (81). These have been cited as, slow integration into national law, insufficient funds for implementation and tobacco industry interference with policy-making (36).

South Africa passed comprehensive national legislations on tobacco controls, which are compliant with the WHO FCTC (27). The legislations cover increasing tobacco prices, the use of health warnings on packaging, smoke free policies and anti-smoking messaging via mass media marketing, which have been effective in the reduction of tobacco use (23,37,81). Despite the tobacco control regulations put in place, there is still a need for programs to help smokers who want to quit, and training programs to educate healthcare providers to help active smokers break their nicotine addiction.

3.6. Limitations

A limitation of the study was the small sample of children (n= 47) as compared to adults (n= 682), with lower absolute cotinine values in children that may influence our findings related to arterial stiffness. In addition, the sample of adults with urinary cotinine was smaller than the sample with serum cotinine. In children, only urine cotinine was available. One other limitation of this study is

that urine and serum cotinine were not analysed in the same laboratory, although the same method and equipment were used. Furthermore, overall prevalence of hypertension in adults was low (<10%) as African-PREDICT aimed to evaluate change in cardiovascular health over time excluding adults with elevated blood pressure at baseline. In a representative sample of adults where hypertension prevalence would be higher, the impact of tobacco exposure on hypertension and vascular health may be greater. The strengths of this study were the large overall sample size (n=729), with measurements taken in controlled environments following the same standard procedures and analytical methods.

3.7. Conclusion

In this study, one third of black South African adults and almost half of the children aged 4-10 years were found to have cotinine in either their serum or urine, indicating use of tobacco and/or exposure to tobacco users. The prevalence of elevated blood pressure was approximately twice as high in those adults and children exposed to tobacco. In adults, blood pressure appeared to associate most closely with arterial stiffness. Our findings indicate that tobacco cessation strategies in South Africa should consider other cardiovascular risk factors, especially at younger ages and include blood pressure screening of those using tobacco and those exposed to tobacco use within their households.

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

This work addressed the relationship between tobacco exposure, assessed by the biomarker cotinine, and arterial stiffness in South African adults and children of African ancestry adjusting for other risk factors representing biological, pharmacological, behavioural, sociodemographic and economic risks. This was achieved through the comparison of a range of cardiovascular risk factors between tobacco exposed and non-exposed adults and children (based on detectable levels of the biomarker cotinine). The association between tobacco exposure and arterial stiffness in adults and children, was evaluated by adjusting for these other risk factors.

With respect to the proposed study hypothesis that tobacco exposed adults and children will exhibit increased arterial stiffness as shown by higher pulse wave velocity (PWV) and tobacco exposure will be a significant predictor of PWV, we did find that tobacco exposure is clearly related to BP which in turn relates to arterial stiffness. In both children and adults, although tobacco exposure was correlated with arterial stiffness in the univariate analysis, there was no independent association multivariate analysis. This is after we adjusted for MAP, antihypertensive medication use, contraception use, self-reported alcohol use, and WHtR, age, sex, family history, and education level as covariates and these variables are associated with arterial stiffness. The 6.7 m/s and 6.3 m/s although statistically significant, may not have clinical importance presently, however, may have significant implications for vascular ageing in future.

With the increased susceptibility to early vascular aging (EVA) previously shown in individuals of African ancestry, I have argued throughout this work that tobacco exposure is not an isolated risk factor, but the phenomenon of EVA occurs due to early exposure to multiple risk factors. This was shown by the difference observed between the exposed and non-exposed group. This study showed other risk factors may be important for EVA such as obesity, age and sex in adults. This heavy multiple burden may explain why, in multiple regression analysis adjusted for other risk factors, we did not find independent effects of tobacco exposure on arterial stiffness.

This work has also highlighted the lack of evidence that exists in South African adults and children of African ancestry. In this sample, serum seemed to be a better biomarker related to CVD than urine. To better understand the implications of these findings, future research could further evaluate the relationship between tobacco exposure and arterial stiffness in a larger sample size. There should be a shift of focus on children and young adults and more data should be collected around early exposure (in childhood or early adulthood). This study also recommends future research to look at how inflammation due to tobacco exposure changes overtime in individuals exposed to tobacco.

Worryingly, in this study, one third of South African adults of African ancestry and almost half of the children aged 4-10 years were found to have cotinine in either their serum or urine, indicating use of tobacco and/or exposure to tobacco users. The prevalence of tobacco exposure was higher than the population based self-reported prevalence determined by the South African demographic and health survey in 2016 (35).

Of concern, the prevalence of elevated blood pressure was approximately twice as high in those adults and children exposed to tobacco than their non-exposed counterparts, with mean arterial pressure predicting arterial stiffness. These findings hold important implications to also target BP in efforts targeted at addressing tobacco use. Children exposed to tobacco also exhibited elevated BP highlighting the need to also consider tobacco exposure impact on individuals exposed to second-hand smoke such as children. The findings indicate that tobacco cessation strategies in South Africa should consider other cardiovascular risk factors, especially at younger ages and include BP screening of those using tobacco and those exposed to tobacco use within their households. Given that early exposure to risk factors for CVD may drive the trajectory of vascular health, greater efforts are needed to reduce exposure for future cardiovascular health outcomes. This calls for a thorough assessment of the current guidelines on tobacco cessation strategies in South Africa.

In conclusion, exposure to tobacco, either through use or ETS in urban adults and children is higher than may be predicted. Most data collected on tobacco use is self-reported, this leaves gaps in our understanding of the true level of ETS in children. This study shows that about 45% of children were exposed to tobacco in this sample. This has significant implications for the future cardiovascular health of children and young adults particularly, requiring early intervention to sustain ideal CV health.

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APPENDICIES

APPENDIX A: Bt20 ethics certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

2020/07/09

Dr L Ware
School of Clinical Medicine
Department of Paediatrics and Child Health
Developmental Pathways for Health Research Unit
Chris Hani Baragwanath Academic Hospital

Sent by e-mail to: Lisa.Ware@wits.ac.za

Dear Dr Ware

Re: **Protocol Ref No: M190263**
Protocol Title: *Intergenerational transmission of vascular health in South Africans: three generations of the Birth-to-Twenty study*
Principal Investigator: Dr L Ware

Thank you for your e-mail of 2020/06/15.

We note your proposal to analyse Cotinine in urine spot samples for the purpose of validation of participant reported use of exposure to tobacco products. As discussed, we will approve this provided:

1. an explanation is given to and verbal consent is obtained from participants who have already donated samples; this may be done remotely by telephone as you propose, provided a rcord of the discussion is kept
2. in the case of future participants, the Information Sheet is modified as you propose, to include the necessary explanation

Thank you for keeping us informed.

Yours Sincerely

.....
Mr I Burns
For the Human Research Ethics Committee (Medical)

.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

APPENDIX B: African- PREDICT ethics certificate

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

Prof A Schutte

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: http://www.nwu.ac.za

Ethics Committee
Tel +27 18 299 4850
Fax +27 18 293 5329
Email Ethics@nwu.ac.za

2012/07/31

ETHICS APPROVAL OF PROJECT

The North-West University Ethics Committee (NWU-EC) hereby approves your project as indicated below. This implies that the NWU-EC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title : African Prospective study for the Early Detection and Identification of Cardiovascular disease and hyperTension. (African-PREDICT study)

Project Leader: Prof A Schutte

Ethics number: N W U - 0 0 0 0 1 - 1 2 - A 1
(Institution / Project Number / Year / Status)

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2012/04/12 **Expiry date:** 2017/04/11

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

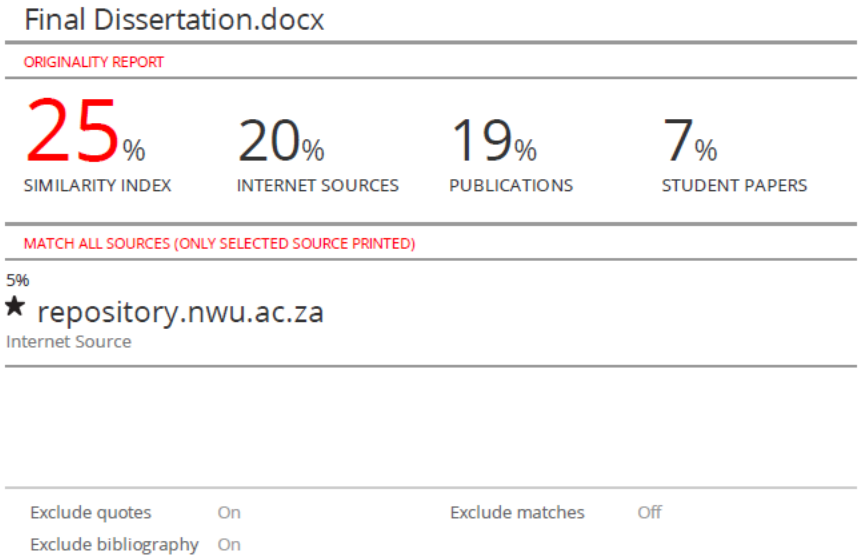
- *The project leader (principle investigator) must report in the prescribed format to the NWU-EC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-EC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-EC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-EC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-EC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

Yours sincerely

Prof Amanda Lourens
(chair NWU Ethics Committee)

APPENDIX C: Turn-it-in report



APPENDIX D: Motivation letter for Turn-it in report

DST-NRF Centre of Excellence
in Human Development
Individual and Society

Office 154, First Floor East Wing
Wits School of Public Health
Education Campus
University of the Witwatersrand,
27 St Andrew's Road
Parktown, Johannesburg
South Africa 2193
Email: lisa.ware@wits.ac.za

Dear Sir/Madam

Re: Turnitin Report for Ms Betty Nembulu – Student Number: 1102731

With reference to the Turnitin report showing a 25% similarity index for this student, as the primary supervisor of this student I have reviewed the report and can see that much of the similar content is due to standard funding statements and standard methodologies from previous publications. These are particularly for those that have used the same data source i.e. the African-PREDICT study conducted at North West University, with such papers and dissertations held in the NWU repository.

This is shown in the Turnitin index as 5% of the similar content is from the NWU repository alone.

I hope that this provides some clarity for this result.

Best regards,

Lisa Ware PhD
Senior Researcher DSI-NRF Centre of Excellence in Human Development
Associate Director Developmental Pathways for Health Research Unit.

Tel: +2711 717 2382

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG