Smoking and vascular dysfunction in African and Caucasian people from South Africa

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DECLARATIONS BY AUTHORS

The researchers listed below contributed in this study in ways explained in the table:

Researcher	Role in the study
Mr. MC Zatu (Physiologist)	Searched the literature; contributed to the data collection of another study (SABPA project) which involved similar research techniques; performed the statistical analyses; processed the data; planned, interpreted and responsible for final writing of the dissertation.
Prof. AE Schutte (Physiologist)	Supervisor. Supervised the writing of the manuscript, project leader of the SAfrEIC study, coordinated all data collection, responsible for collection of cardiovascular data, and also responsible for the planning and design of the dissertation.
Prof. JM van Rooyen (Physiologist)	Co-supervisor. Supervised the writing of the manuscript, responsible for collection of cardiovascular data, and also responsible for the planning and design of the dissertation.

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

I declare that I have approved the above-mentioned manuscript, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give consent that it may be published as part of the M.Sc dissertation of MC Zatu.

Prof. AE Schutte, PhD

Prof. JM van Rooyen, D.Sc

SUMMARY

Title: Smoking and vascular dysfunction in African and Caucasian people from South Africa: The SAfrEIC study

Motivation: Smoking is a health risk factor associated with vascular dysfunction world-wide. In South Africa, ethnic differences with regards to smoking exist between the African and Caucasian people. The few available studies in South Africa indicate a higher smoking prevalence in Caucasians over Africans. However, Africans are at a higher risk to develop smoking-related adverse effects on cardiovascular function. Limited data regarding the prevalence of smoking and its associated effects on vascular function in South Africa serves as the motivation for this study.

Objective: To determine the prevalence and association of smoking with vascular dysfunction in African and Caucasian people from South Africa.

Methodology: The manuscript is presented as Chapter 3 and makes use of the data from the SAfrEIC study (South African study on the influence of Sex, Age, and Ethnicity on Insulin sensitivity and Cardiovascular function). A group of 630 participants (258 Africans and 372 Caucasians), aged between 20 and 70 years were recruited from the urban areas of the North-West Province of South Africa. Anthropometric and cardiovascular measurements were taken and pulse wave velocity (PWV), high sensitivity C-reactive protein (hs-CRP), cotinine and lipid profiles (from blood samples drawn from participants) were determined with appropriate methods. The basic health, demographic and lifestyle questionnaires were completed and each participant was requested to indicate their income per month, duration of smoking and the type of smoke being used. An independent t-test and analysis of covariance (ANCOVA) were used to compare the variables between the two ethnic groups, and between smokers and non-smokers within each group whilst adjusting for certain confounders. The Chi-square test was used to determine if there were significant differences between categorical variables such as smoking and income. Correlations of smoking with cardiovascular and lipid variables were determined while also adjusting for age, body mass index (BMI) and waist circumference (WC). In addition, PWV measurements were taken while also adjusting for mean arterial pressure (MAP). The study was approved by the Ethics committee of the North-West University and all the subjects gave

informed consent in writing. The methods section of the manuscript in chapter 3 has a more detailed description of the experimental procedure used.

Results and conclusion: A higher prevalence of smoking was found in Africans over Caucasians. Africans showed a significantly lower weight, BMI, WC, higher blood pressure, favorable lipid profiles and lower socio economic status than Caucasians. Smokers in each ethnic group revealed significant differences when compared to non-smokers in most of the measured variables. For example, cardiac output and triglycerides were elevated in smokers of both ethnic groups. However, only African smokers showed increased higher density lipoprotein cholesterol (HDL-C) and arterial stiffness than their non-smokers as opposed to lower HDL-C levels in Caucasian smokers than non-smokers. Although correlations of smoking with the measured variables were generally weak, Africans revealed stronger associations than Caucasians, especially before adjustments were made. Metabolic syndrome components, arterial stiffness and peripheral resistance are conditions usually associated with increased cardiovascular risk in Africans, and, together with smoking, these are likely to be the best known risk factors for vascular dysfunction. HDL-C correlated weakly ($r=0.13$) but significantly ($p=0.035$) with chronic smoking in Africans - a finding that is not found in the literature. On the other hand, a negative correlation of smoke with HDL-C in Caucasian smokers (chronic: $r=-0.12$, $p=0.026$; acute: $r=-0.10$, $p=0.052$) was found. Cotinine correlated positively with smoking duration, throughout, indicating the persistently high blood levels of nicotine of smokers. Hs-CRP correlated weakly with smoking duration in Africans ($r=0.13$; $p=0.038$) and Caucasians ($r=0.13$; $p=0.015$). Based on the findings of the study, it is concluded that Africans smoke more than the Caucasians. Factors such as socio-economic status and westernized lifestyle (urbanization) seem to play an important role in this regard. In addition, Africans showed more correlations of smoking with other measures of cardiovascular risk than Caucasians, even though these associations were generally weak. This might indicate that smoking could be a larger cardiovascular risk factor in Africans than Caucasians.

Keywords: smoking, vascular dysfunction, socio-economic status, ethnicity, hs-CRP, Africans, Caucasians.

OPSOMMING

Titel: Rook en vaskulêre disfunksie in Kaukasiër en Afrika populasies van Suid Afrika: SAfrEIC-studie.

Motivering: Rook is 'n risikofaktor vir gesondheid wat wêreldwyd met vaskulêre disfunksie geassosieer word. In Suid-Afrika bestaan daar etniese verskille met betrekking tot die rookgewoonte tussen Kaukasiër en Afrika populasie groepe. Die beperkte beskikbare studies wat in Suid-Afrika oor die rookgewoonte beskikbaar is, dui daarop dat Kaukasiërs meer rook as Afrikane. Nietemin het Afrikane 'n groter risiko om rookverwante nadelige effekte rakende kardiovaskulêre funksie te ontwikkel. Beperkte data omtrent die voorkoms van die rookgewoonte en die verwante effekte daarvan op vaskulêre disfunksie in Suid-Afrika dien as motivering vir hierdie studie.

Doelstelling: Om die voorkoms en verwantskap tussen rook en vaskulêre disfunksie in Afrikane en Kaukasiërs van Suid-Afrika te bepaal.

Metodologie: Die manuskrip word in Hoofstuk 3 aangebied en maak gebruik van die data van die SAfrEIC studie (*South African study on the influence of Sex, Age, and Ethnicity on Insulin sensitivity and Cardiovascular function*). 'n Groep van 630 deelnemers (258 Afrikane en 372 Kaukasiërs), tussen die ouderdomme van 20 en 70 jaar, is gewerf vanuit verstedelikte gebiede van die Noordwes Provinsie van Suid-Afrika. Antropometriese en kardiovaskulêre metings is geneem en polsgolfsnelheid (PGS), hoë sensitiwiteit C-reaktiewe protein (hs-CRP), kotinien en lipiedprofiele (vanaf bloedmonsters wat by proefpersone geneem is) is met die geskikte metodes bepaal. Basiese gesondheids-, demografiese en lewenstylvraelyste is voltooi en elke proefpersoon is gevra om daarop aan te dui wat hul maandelikse inkomste, duur van die rookgewoonte en tipe tabakproduk. 'n Onafhanklike t-toets en analise van kovariansie (ANKOVA) is gebruik om veranderlikes tussen die twee etniese groepe te vergelyk, asook tussen rokers en nie-rokers binne elke groep terwyl daar aanpassings uitgevoer is vir sekere faktore wat die resultate kan beïnvloed. Die Chi-*square* toets is gebruik om te bepaal of daar betekenisvolle verskille tussen kategoriese veranderlikes soos rookgewoonte en inkomste was. Korrelasies van rook met kardiovaskulêre en lipied veranderlikes is bepaal terwyl daar vir ouderdom, liggaamsmassa indeks (LMI) en middelomtrek (MO) aangepas is. Polsgolfsnelheid (PGS) is gemeet terwyl

aanpassings vir gemiddelde arteriële bloeddruk uitgevoer is. Die studie is goedgekeur deur die Etiekkomitee van die Noordwes-Universiteit en al die proefpersone het geskrewe ingeligte toestemming gegee. Die Metodes-afdeling van die manuskrip in Hoofstuk 3 gee 'n meer volledige beskrywing van die eksperimentele prosedures.

Resultate en gevolgtrekking: Afrikane is die groep wat meer gerook het as Kaukasiërs. Dit word ondersteun deur 'n betekenisvolle laer liggaamsgewig, LMI, MO, hoër bloeddruk, gunstige lipiedprofiele en laer sosio-ekonomiese status in die Afrikane. Rokers in elke etniese groep het vir die meeste van die gemete veranderlikes betekenisvolle verskille getoon wanneer met nie-rokers vergelyk is. Afrikaan rokers was egter die enigste groep wat verhoogde hoë digtheids lipoproteïen cholesterol (HDL-C) en hoër arteriële styfheid as nie-rokers getoon het, waár Kaukasiër rokers laer HDL-C vlakke as nie-rokers getoon het.. Hoewel korrelasies van rook met die gemete veranderlikes oor die algemeen swak was het die Afrikane sterker assosiasies as die Kaukasiërs getoon, veral voordat gekorrigeer is. Metaboliese sindroom komponente, arteriële styfheid en perifere weerstand is toestande wat gewoonlik met verhoogde kardiovaskulêre risiko in Afrikane geassosieer is, en tesame met rook, is hierdie moontlik die mees bekende risikofaktore vir vaskulêre disfunksie. HDL-C het swak ($r=0.13$), maar tog betekenisvol ($p=0.035$) met chroniese rookgewoonte in Afrikane gekorreleer – 'n bevinding wat nie in die literatuur voorkom nie. Hierteenoor het Kaukasiërs 'n negatiewe korrelasie van die rookgewoonte met HDL-C getoon (chronies: $r=-0.12$; $p=0.026$; akuut: $r=-0.10$; $p=0.052$). Kotinien het deurgaans 'n positiewe korrelasie met die duur van die rookgewoonte getoon wat daarop dui dat daar voortdurend hoë vlakke van nikotien in rokers se bloed voorkom. Hs-CRP het 'n swak korrelasie met die duur van rookgewoonte in Afrikane ($r=0.13$; $p=0.038$) en Kaukasiërs ($r=0.13$; $p=0.015$) getoon. Op grond van die resultate van die studie is die gevolgtrekking dat binne hierdie proefgroep Afrikane meer as Kaukasiërs gerook het. Faktore soos sosio-ekonomiese status en 'n verwesterde lewenstyl (verstedeliking) kan moontlik 'n belangrike rol hier speel. Tweedens, Afrikane het meer korrelasies van rook met ander metings van kardiovaskulêre risiko getoon as Kaukasiërs, hoewel hierdie assosiasies oor die algemeen swak was. Dit kan daarop dui dat die rookgewoonte n groter kardiovaskulêre risikofaktor in Afrikane as Kaukasiërs kan wees.

Sleutelwoorde: rook, vaskulêre disfunksie, sosio-ekonomiese status, etnisiteit, hs-CRP, Afrikane, Kaukasiërs

PREFACE

The structure of this study follows that of an article format and each chapter has its references at the end. Chapter 1 consists of a general introduction to the dissertation, and also includes the aims and motivation of the study. Chapter 2 is the literature study, i.e. background information needed for the interpretation of the data. Chapter 3 consists of the manuscript to be submitted for publication (article). At the beginning of this chapter, there are instructions for the authors of the journal aimed for publication. The format of the article and the entire dissertation therefore comply with the instructions of the *American Journal of Hypertension.* Lastly, Chapter 4 is a summary and critical review of the study conclusions, and also includes recommendations for future studies.

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Chapter 3

LIST OF ABBREVIATIONS

AI:	Augmentation index
ANCOVA:	Analysis of covariance
BMI:	Body mass index
BP:	Blood pressure
CAD:	Coronary artery disease
CHD:	Coronary heart disease
CO:	Cardiac output
CRP:	C-reactive protein
CVD:	Cardiovascular disease
Cwk:	Windkessel compliance
DBP:	Diastolic blood pressure
FFA:	Free fatty acids
HART:	Hypertension in Africa Research Team
HDL-C:	High-density lipoprotein cholesterol
HIV:	Human immunodeficiency virus
HR:	Heart rate
Hs-CRP:	high-sensitivity C-reactive protein
IHD:	Ischemic heart disease
LDL-C:	Low-density lipoprotein cholesterol
MAP:	Mean aterial pressure
MI:	Myocardial infarction
NADPH:	Nicotinamide adenine dinucleotide phosphate oxidase
NO:	Nitric oxide
NOS:	Nitric oxide synthase
PAD:	Peripheral artery disease

PAF:	Platelet activating factor
PAI-1:	Plasminogen activator inhibitor-1
PWV:	Pulse wave velocity
PVR:	Peripheral vascular resistance
ROS:	Reactive oxygen species
SAfrEIC:	South African study on the influence of Sex, Age and Ethnicity on Insulin sensitivity.and Cardiovascular function
SBP:	Systolic blood pressure
SES:	Socio-economic status
SV:	Stroke volume
TF:	Tissue factor
TG:	Triglycerides
TPR:	Total peripheral resistance
WC:	Waist circumference

CHAPTER 1

General introduction, problem statement, aims, and motivation.

General introduction and problem statement

Cigarette smoking is the chief avoidable cause of illness and death in many developed and developing countries. Over 2 million people die from smoking every year in the world, half of them before the age of 70 years.[1,2,3] Besides causing many diseases such as cancer and pulmonary diseases,[2] the adverse effects of smoking on the cardiovascular system are well established.[4,5,6,7]

Despite the higher prevalence of smoking among whites compared to blacks,[8] blacks suffer more from smoking-related cardiovascular illness and death.[9,10,11] South Africa has made significant progress in the past decade with regards to tobacco smoke reduction. An estimated 2.5 million smokers stopped smoking during the last 10 years.[12,13] Age, gender, ethnicity, cultural and economic characteristics are some factors that promote smoking prevalence rates in South Africa.[8] Poorer smokers are more likely to quit than smokers that are more affluent.[8,12,13] According to available data, the white community in South Africa smokes most heavily, with a reported average of 18 cigarettes a day compared to only 7 cigarettes per day in Africans.[8] Whites and coloureds have almost 1:1 male to female ratio of smokers while Africans reveal one female smoker for every eight smoking males.[8,14]

Black smokers, reveal higher incidences of vascular diseases related to smoking than Caucasians.[15] Blacks prefer high-tar, high nicotine mentholated brands.[15] Because of higher nicotine yield and presence of menthol, fewer cigarettes would be needed to maintain daily blood nicotine levels.[16,17] Thus, although blacks smoke fewer cigarettes per day, their choices of brands may provide the tar yield and smoking pattern necessary to contribute to the smoking-related diseases observed among them.[15] Furthermore, smoking acts synergistically with other risk factors, substantially increasing the risk of cardiovascular diseases such as stroke and coronary heart disease.[5,11] Such risk factors may include obesity-related diseases and the metabolic syndrome, which are highly prevalent among Africans[18,19,20,21,22] and cause cardiovascular disease.[23]

Tobacco smoke has many potent chemical substances in the form of nicotine, tar, nitrosamines, hydrogen cyanide, formaldehyde and carbon monoxide.[3,6] Nicotine and

carbon monoxide are the most studied with regard to their damaging effects on the cardiovascular system.[4,5,6] Nicotine causes both immediate and longer term increases in blood pressure (BP), heart rate (HR) and cardiac output (CO).[3] Carbon monoxide binds to hemoglobin, a pigment in erythrocytes that carries oxygen from the lungs via the bloodstream, and therefore carbon monoxide reduces the oxygen carrying capacity of the blood.[4,5] Smoking also damages blood vessels, allowing cholesterol and other dangerous fatty material to build up, leading to atherosclerosis.[24,25]

Nicotine in smoke also correlates positively with markers of cardiovascular disease such as C-reactive protein (CRP), cholesterol and triglycerides (TG).[5,26] Smokers have significantly higher serum cholesterol, TG and low-density lipoprotein cholesterol (LDL-C) levels, but high-density lipoprotein cholesterol (HDL-C) levels are lower in smokers than in non-smokers.[26,27] Lipid levels are markers of hypertension and heart disease in black people of South Africa.[22,28,29] There is a significant body of evidence that links smoking with increased levels of CRP, a marker of inflammation with a half-life of 19 hours.[5,30]

One other main challenge facing many countries is the exposure of non-smokers to cigarette smoke. Second-hand smoke affects the cardiovascular and other body systems in ways similar to active smokers.[2,4] Protection of non-smokers through smoke-free environments leads to a decrease in heart and vascular disease mortality.[31] The optimal assessment of exposure to tobacco smoke would be by analysis of the concentration of a component of smoke in body fluids of a smoker, i.e. a biological marker.[32,33] Self-report measures in smokers are imprecise owing to individual differences in how cigarettes are smoked and proximity of non-smokers to smokers.[34,35] Cotinine, the metabolite of nicotine, has been widely used as the biomarker of tobacco smoke.[36,37] Plasma cotinine concentration correlates better with various measures of biologic effects of cigarette smoking than does self-reported cigarettes per day.[32,33] Despite smoking fewer cigarettes per day, blacks have higher levels of serum cotinine.[38] The measures of biological markers of smoking, mainly cotinine, in both Africans and Caucasians will form an important part of this study.

Due to the limited knowledge regarding the current smoking habits of African and Caucasian people of South Africa, as well as the effects of smoking on the

cardiovascular system, this study will seek to investigate the ethnic differences with regards to smoking prevalence between Africans and Caucasians, as well as the association between smoking and cardiovascular markers within the two ethnic groups.

Motivation

The effect of smoking on vascular function is quite extensively reported in the literature.[5,6,26,39] However, there is very limited information available from a South African perspective. This is one motivation for this study. Secondly, the limited studies conducted in South Africa on smoking in general were only based in a few areas, such as the Western Cape region.[14,40,41] Performing a study on smoking and health from the region of the North-West Province is therefore needed. Thirdly, despite the reported high prevalence of smoking in whites over blacks,[8,15] there is an observed speedy increase of smoking habit among young blacks in South Africa, following a high degree of urbanisation in blacks.[42,43,44] Data of this study, purely taken from an urban area of the city of Potchefstroom, would therefore be very informative, especially since published data is available only for 2002.

Aims

The aims of this study are to investigate if:

- there are ethnic differences that exist with regard to the prevalence of smoking habits between Africans and Caucasians of South Africa;
- differences exist with regard to the associations of smoking with cardiovascular markers, lipid markers, and CRP between African and Caucasian people.

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CHAPTER 2

LITERATURE STUDY

1. Introduction

Cigarette smoking predisposes one to several cardiovascular manifestations such as peripheral artery disease (PAD), coronary artery disease (CAD), sudden death and others.[1] Although the mechanisms by which smoking exerts its effects on the cardiovascular system are being extensively explored,[2] there are still a number of unknown areas requiring further research. Active smoking is one of the most important modifiable risk factors for coronary heart disease (CHD) and stroke, with many epidemiologic investigations carried out on smoking and its relationship to CHD.[3] Furthermore, many epidemiologic investigations have been carried out on second-hand smoke exposure and its relationship to cardiovascular diseases such as CHD and stroke.[3,4,5] Many studies revealed that exposure to second-hand smoke is as fatal as smoking itself.[4,6,7]

There are many studies in the literature that link tobacco smoke with cardiovascular disease.[1,6,8] Some of these studies provide clear mechanisms of the dangerous effects of tobacco smoke on the cardiovascular system.[6,9] In brief, smoking raises blood pressure (BP) and heart rate (HR).[10,11] Tobacco smoke also produces oxidant chemicals that damage the blood vessels, inviting inflammatory processes, which could cause further endothelial dysfunction.[1,6,12] Smokers reflect increased red and white blood cell count, high-sensitivity C-reactive protein (hs-CRP) levels, fibrinogen and low-density lipoprotein cholesterol (LDL-C) levels, all associated with vascular damage and CHD. Anti-oxidant chemicals and high-density lipoprotein cholesterol (HDL-C) concentrations are low in smokers.[1,6]

In South Africa, although there are studies on tobacco smoke and ethnicity,[13,14] there are few studies that link tobacco smoke with cardiovascular disease. This study will therefore attempt to encourage more research on this crucial topic of smoking and vascular dysfunction in South Africa. In this study, the prevalence of tobacco smoke in two ethnic groups, African and Caucasian populations, will be compared. Furthermore, the associated physiological effects of smoking on the cardiovascular system will be investigated.

2. Tobacco smoke

2.1 Chemical constituents of tobacco smoke

It is estimated that there are 4000 constituents in tobacco smoke. Of these about 400 have been measured or estimated in mainstream or sidestream smoke.[9,15] "Mainstream smoke" is the smoke emerging from the mouth end of a cigarette during puffing; "sidestream smoke" is primarily the smoke emerging into the environment from the lit end of the cigarette between puffs.[16] Combined with its vast array of toxic constituents is the addictive quality of tobacco, which is largely due to naturally occurring nicotine and related alkaloids. Some chemical constituents of tobacco such as ammonia, influence the toxicity of the smoke indirectly by increasing the pH of inhaled smoke and, thereby facilitating the absorption of nicotine in its unionised state.[16]

In addition to nicotine, tar and carbon monoxide, there are also other damaging chemical compounds in smoke. Tobacco smoke contains at least 10 of the 36 listed chemicals[17] that are known to cause cancer.[9] Thirty metals have been detected in tobacco smoke, including nickel, arsenic, cadmium, chromium and lead.[16,17] Arsenic levels in tobacco have been elevated in the past due to the use of arsenical pesticides.[16] The radioactive compounds found in highest concentration in cigarette smoke are polonium-210 and potassium-40.[16,18]

- Nicotine

Nicotine, a tertiary amine (Fig. 1), is the major addictive substance in cigarette tobacco.[16] Nicotine is distilled from burning tobacco and is carried on tar droplets which are inhaled.[17] Absorption of nicotine across biological membranes depends on pH. As a weak base, nicotine may exist in an ionised or a non-ionised form. The relative proportions of these two forms affect where nicotine is most readily absorbed into the body.[17,19] At the acidic pH of most cigarette smoke, absorption occurs predominantly in the lungs, but with the alkaline smoke produced by cigars and pipe tobacco, nicotine, being predominantly non-ionised, is absorbed mainly in the mouth.[17,19] Absorption into the blood stream is rapid, and concentrations of nicotine in the blood rise rapidly during smoking. Nicotine is metabolised, mainly in the liver, and the two major metabolites are cotinine and nicotine-N'-oxide.[17,20] Nicotine is an alkaloid found in the nightshade family of plants (*Solanaceae; Nicotiana tabacum*), predominantly in tobacco and cocoa, and in lower quantities in tomato, potato, eggplant, and green pepper.[16,17] It is primarily used

as an antiherbivore chemical, being a potent neurotoxin with particular specificity to insects.[16,17]

Nicotine increases HR , BP and blood glucose in various ways, and causes other cardiovascular effects. These mechanisms will be discussed later in this literature study.

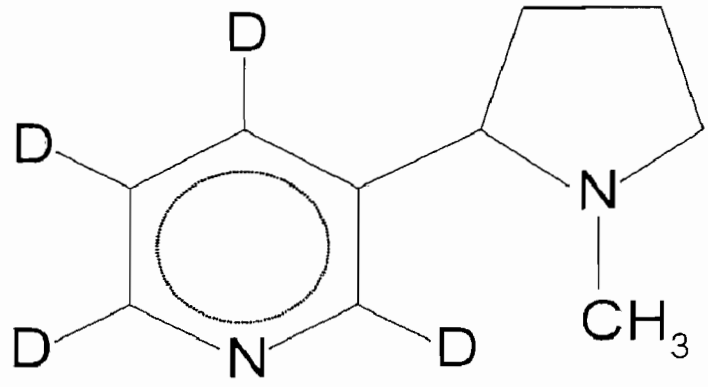

Figure 1. Chemical structure of nicotine (D4 structure).[17]

- Tar

Tar is defined as the dry particulate mass of tobacco smoke.[17] In its condensed form, tar is a sticky brown substance which can stain smokers' fingers and teeth yellow brown. It also stains the lung tissue.[16] The nature of the chemical components in tar and their toxicity vary widely across the tobacco from the various sources.[16,17] Therefore, measurement of tar, is only a crude measure of the relative toxic potential of tobacco combustion products.[16]

- Carbon monoxide

Carbon monoxide is a major constituent of cigarette smoke. Carboxyhemoglobin levels are higher in smokers than in nonsmokers.[6,20] Carbon monoxide binds readily to hemoglobin reducing the ability of hemoglobin to carry oxygen to the cells. Reduced oxygen levels in the cells trigger increased production of red blood cells by the bone marrow[6,21] to compensate for decreased oxygen delivery to body organs. Increased red blood cell mass contributes to increased blood viscosity, which is believed to contribute to the hypercoagulable state in smokers.[1,6]

The vessel wall damaging effects of chronic and acute carbon monoxide exposure have been demonstrated in humans.[22,23] Carbon monoxide causes endothelial cell and platelet release of nitric oxide (NO), and the formation of oxygen free radicals including peroxynitrite.[6] In the brain, this causes further mitochondrial dysfunction, capillary leakage, leukocyte sequestration, and apoptosis.[24] The end result is lipid peroxidation, and can lead to edema within the brain.[25] Carbon monoxide could also result to ischemic heart diseases (IHD) by mechanims illustrated in Figure 2.

2.2 Biochemical markers of smoking

Smoke contains nicotine, which is distilled from burning tobacco and is carried on tar droplets which are inhaled.[26] The absorption across epithelial cell membranes occur and the smoke is absorbed rapidly in the alveoli of the lungs as a result of the large surface area and the dissolution of nicotine in the fluid of pH 7.4 in the lungs.[16,17] The human stomach absorbs nicotine slowly because nicotine is ionized in the gastric juice. However, nicotine is well absorbed in the small intestine, which has a more alkaline pH and large surface area.[17]

After absorption, nicotine enters the bloodstream and is transported mostly in ionized form (more than two-thirds) to all body tissues.[17] The liver, kidneys, spleen and lungs have the highest affinity for nicotine while adipose tissue has the lowest affinity.[16] Blood nicotine concentrations in smokers range from 10 to 37 ng/ml, with peak concentrations reaching 50 ng/ml.[27] Blood levels peak after smoking and decline rapidly over the next 20 min due to tissue distribution. The distribution half-life averages about 8 min.[16]

The most important metabolite of nicotine in humans is the lactam derivative cotinine, to which about 70 to 80% of nicotine is converted.[28,29] Cotinine is a marker of smoking with a long half-life and has been used as a biomarker for daily intake of nicotine in many studies.[17,30] High concentrations of cotinine are found in the plasma, saliva, and urine of smokers and all these fluids can be used as markers of nicotine intake.[17,29]

Various factors influence the metabolism of nicotine to cotinine. First of all, mentholated cigarette smoking inhibits metabolism of nicotine to cotinine and nicotine glucuronidation when compared to nonmentholated cigarettes.[29,31] Secondly, clearance of nicotine is decreased in the elderly (age ≥ 65) compared to younger adults, a fact that could be explained by reduced hepatic blood flow in old age, as may also be the

case during sleep when nicotine metabolism is slow.[17] Gender-related differences in nicotine metabolism, however, reveal contradictory findings.[17] Many studies support the view that nicotine clearance is higher in women than men.[32,33,34] Pregnant smokers have a lower level of serum nicotine[35] while diseases such as kidney failure causes decreased nicotine clearance in the body.[17]

2.3 Use of tobacco products in the African culture

Ethnicity is increasingly recognized as often playing an important role in the prevalence of tobacco use.[36] Smoking in the African culture has been dominated by mainly adult males.[37] The chief motivators for smoking in both African men and women are associated with mood and anxiety symptoms, peer pressure and stress related events.[38] Furthermore, smoking has been seen as a transition stage in which a smoking male would regard himself as an adult, and as a result, smoking was more prevalent among the older members of the community.[13] However, since urbanization, this trend has changed, and increased numbers of young African smokers are now found.[39]

2.4 Addictiveness of nicotine

Nicotine is not a direct cause of all tobacco-related diseases, but it is highly addictive.[40] The addictiveness of nicotine is the cause of the continuing use of tobacco products, which, in turn, cause nicotine to be the leading cause of premature deaths worldwide.[17] Contrary to popular belief that smoking relieves stress, the same studies reveal that, on average, smokers have higher stress levels than non-smokers.[38]

Compared to nonsmokers, smokers' brain cells have been shown to have few dopamine receptors, which are believed to play a role in addiction.[41] Dopamine is normally released naturally while engaging in certain behaviors like eating, drinking and copulation.[17] The release of dopamine is believed to give one a sense of reward.[16] The mechanism towards addiction from nicotine starts with increased dopamine transmission on nicotine exposure, followed by subsequent decreases in dopamine receptor function and number.[16] The initial increase in dopamine activity from nicotine results initially in pleasant feelings for the smoker, but the subsequent decrease in dopamine leaves the smoker craving for more cigarettes.[17,38]

3. Smoking and cardiovascular disease

3.1 Smoking in coronary heart disease (CHD) and myocardial infarction (MI)

Mechanisms by which smoking causes cardiovascular events include thrombosis, endothelial dysfunction, and inflammation,[6] as will be discussed in detail later in this study. The higher risk of CHD in smokers can be explained by several mechanisms. Increased erythrocyte and leukocyte levels in the blood of smokers may contribute to hyperviscosity and increased risk of thrombosis.[42] Furthermore, fibrinogen levels are high in smokers, and, as a result, there is increased clotting potential in smokers.[43] These factors could contribute to the smoking-induced ischemic disease,[43,44] which may compromise the normal functioning of the heart muscles and may lead to IHD or development of infarcts.[44]

Smoking is one of the rsik factors for the development of MI. Changes in lipid metabolism caused by smoking is one mechanism that leads to MI in humans.[42,45] Smoking raises plasma free fatty acid (FFA) levels through enhanced lipolysis resulting from sympathoadrenal stimulation.[6,46] FFAs contribute to plaque formation in atherosclerosis leading to vessel lumen stenosis. This leads to decreased blood flow in the heart and may lead to ischemia of the myocardium and thus myocardial infaction.[6] Increased FFA together with decreased HDL-C in humans are the causes of atherosclerosis, one other risk factor for the IHD and MI.[9] Moreover, the vasoconstrictive effect of nicotine on the blood vessels leads to acute constriction or increased vascular resistance and may lead to ischemia and, again, IHD or MI.[9]

Although the mechanisms underlying the relationship between smoking habits and cardiovascular risks such as CHD and MI are clearly defined, few studies have demonstrated possible mechanisms among young people, where the effects of aging, and conditions like diabetes and hypertension are uncommon.[43]

3.2 Smokeless tobacco use in cardiovascular disease

Smokeless tobacco users are not exposed to oxidant gases, carbon monoxide and other combustion products as in smoking tobacco.[4] However, the nicotine levels in smokeless tobacco may be equal in concentration as in cigarette smoke.[6] Nicotine in cigarette smoke is inhaled rapidly and delivered in high concentrations into the blood and body organs. In contrast, there is a slow delivery of nicotine in smokeless

tobacco.[6,47] Studies of clinical cardiovascular disease indicate that smokeless tobacco use, such as snuff, is much less hazardous than cigarette smoking, and a conclusion can be made that nicotine, in a slow release form, is much less hazardous than cigarette smoking.[6,47,48]

Both smoking and smokeless tobacco have the same effect on HR and BP,[6,47] although smokeless tobacco does not appear to influence peripheral vascular resistance (PVR).[49] Furthermore, smokeless tobacco does not produce the inflammatory reactions seen in cigarette smokers, nor does it produce endothelial dysfunction.[47] Levels of hs-CRP and fibrinogen, antioxidant vitamin levels, and thromboxane A_2 metabolite excretion and lipid profiles are similar in smokeless tobacco users and in people who do not use tobacco.[47] Moreover, some studies in the literature [47,50] have found that snuff users, as opposed to smokers, do not have increased intima-media thickness or atherosclerotic lesions. Few studies in the literature have linked smokeless tobacco with the risk of MI, cardiac death[51] and CHD.[52] Furthermore, there are reports of an increased risk of type 2 diabetes mellitus in snuff users, confirming the findings that nicotine does cause insulin resistance.[6,53]

The prevalence of smokeless tobacco use is high among black South Africans compared to their white counterparts.[54] The majority of white smokers prefer cigarette smoke whereas almost half of black smokers use smokeless tobacco.[13] Moreover, the use of smokeless tobacco in South African blacks takes place at an earlier age of 13 years to 15 years of whites, and smokeless tobacco use and dependence is common among women,[47,55] although high prevalences of smokeless tobacco among men has been reported elsewhere in the world.[48]

3.3 Hypertension and cardiovascular disease in sub-Saharan Africa

Hypertension is one of the most common ailments in Africa with BP increases correlating with age.[56,57,58] Several studies[39,56,59,60] agree that environmental factors are likely to have some influence on the development of hypertension in sub-Saharan Africa. Urbanization and westernized lifestyle have shifted the dietary requirements of black Africans from carbohydrate and dietary fibre to increased fat and animal protein intakes.[39,61] A high fat and protein diet is associated with increased fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and obesity - risk factors for IHD, stroke and hypertension.[39,62] Hypertension is highly prevalent among black Africans,[63] and the

increase in the incidence of hypertension appears to be closely related to the aging of the population as well as with the growing number of overweight and obese persons, including the metabolic syndrome.[62,63,64]

There are well-described ethnic variations in the incidence and pathophysiology of hypertension between Africans and Caucasians in sub-Saharan Africa.[56,65,66] Hypertension is associated with a higher incidence of cerebrovascular and renal complications, and blacks have a higher tendency to develop complications such as left ventricular hypertrophy more often than non-blacks.[65,67] Hypertension in Africans presents several etiopathogenic particularities mainly with regard to dependence on sodium sensitivity and lower plasma renin activity.[68] Reduced sodium-potassium ATPase activity is also associated with hypertension in blacks, resulting in increased intracellular sodium and calcium concentrations.[59,66,68,69] Moreover, the effects of urbanization resulted in increases in weight and disturbances in urinary electrolyte balance, which contributed to the metabolic syndrome and consequently hypertension.[39,61] Another aspect of note, between Africans and Caucasians, is that proteinuria has been observed more frequently in Africans, a feature that may be related to the underlying ethnic differences in renal pathophysiology.[39]

Age is another factor that is a major determinant of hypertension. With increasing age, the systolic blood pressure (SBP) increases so that values greater than 140 mmHg become common, while diastolic blood pressure (DBP) remains constant.[70] This leads to increased pulse pressure due to large artery stiffening, which causes a decrease in large artery compliance and early reflected pulse waves.[70] The stiffened large arteries conduct the pulse wave faster in both directions, forward and backward, so that the characteristic abrupt increase and decrease of the pulse wave in the elderly occurs.[71] Age and hypertension affect all the ethnic groups in the world, and black South Africans are no exception because of their association with the metabolic syndrome and obesity.[63]

Despite the effect of smoking on cardiovascular disease[6], its relationship with hypertension remains unclear.[72] One study[73] in the literature exposes the indirect mechanisms that link smoking with hypertension. Cigarette smoking causes sympathetic activation and oxidative stress that increases markers of inflammation,[1,6,73]

which are linked with hypertension.[74] Moreover, smoking causes endothelial dysfunction, plaque progression[75] and arterial stiffness[76] that lead to the development of hypertension.[73] The association of BP with smoking is mentioned by some studies[58,73] in which blood pressure correlates positively with smoking. This could lead to the development of hypertension over time. Other studies, however, have reported lower BP levels among smokers, and increases in BP after smoking cessation.[77,78] Moreover, quitting smoking decreases inflammation and may lead to weight gain[6,78] that causes greater BP and increased rates of hypertension.[79] Thus, it remains unclear to what extent cigarette smoking is a risk factor for the development of hypertension.

4. The effects of tobacco smoke on vascular function

4.1 Smoking and blood pressure

BP is the force per unit area exerted on a vessel wall by the contained blood.[70,80] BP is expressed in millimetres of mercury (mmHg) and actually refers to the arterial blood pressure in the largest arteries.[80]

The acute haemodynamic responses to smoking include increases in HR, systolic and diastolic blood pressure and myocardial contractility, mediated by beta adrenergic effects of nicotine.[6,81,82] These acute responses occur within 1-2 minutes of the act of smoking and result in increased myocardial oxygen demand.[6] One mechanism involves the binding of nicotine to nicotinic receptors and, in the adrenal medulla nicotine increases flow of catecholamines into the bloodstream, which increases HR, BP and myocardial contractility.[6,11] The net result is an increase in myocardial work, which then requires an increase in myocardial blood flow (Figure 2). Coronary vasoconstriction through the effect of norepinephrine leads to reduced oxygen supply and as a result leads to ischemia and infarction of the myocardial wall in diseased states. The consequences may be fatal (Figure 2).[6]

Furthermore, the sensitivity of the baroreflex function is reduced by acute smoking. This impairment may be directly linked to smoking-related reduced arterial distensibility and the consequent loss of stretch receptor responsiveness, all which contribute in part to increases in BP variability and inhibits muscle nerve activity.[72,83]

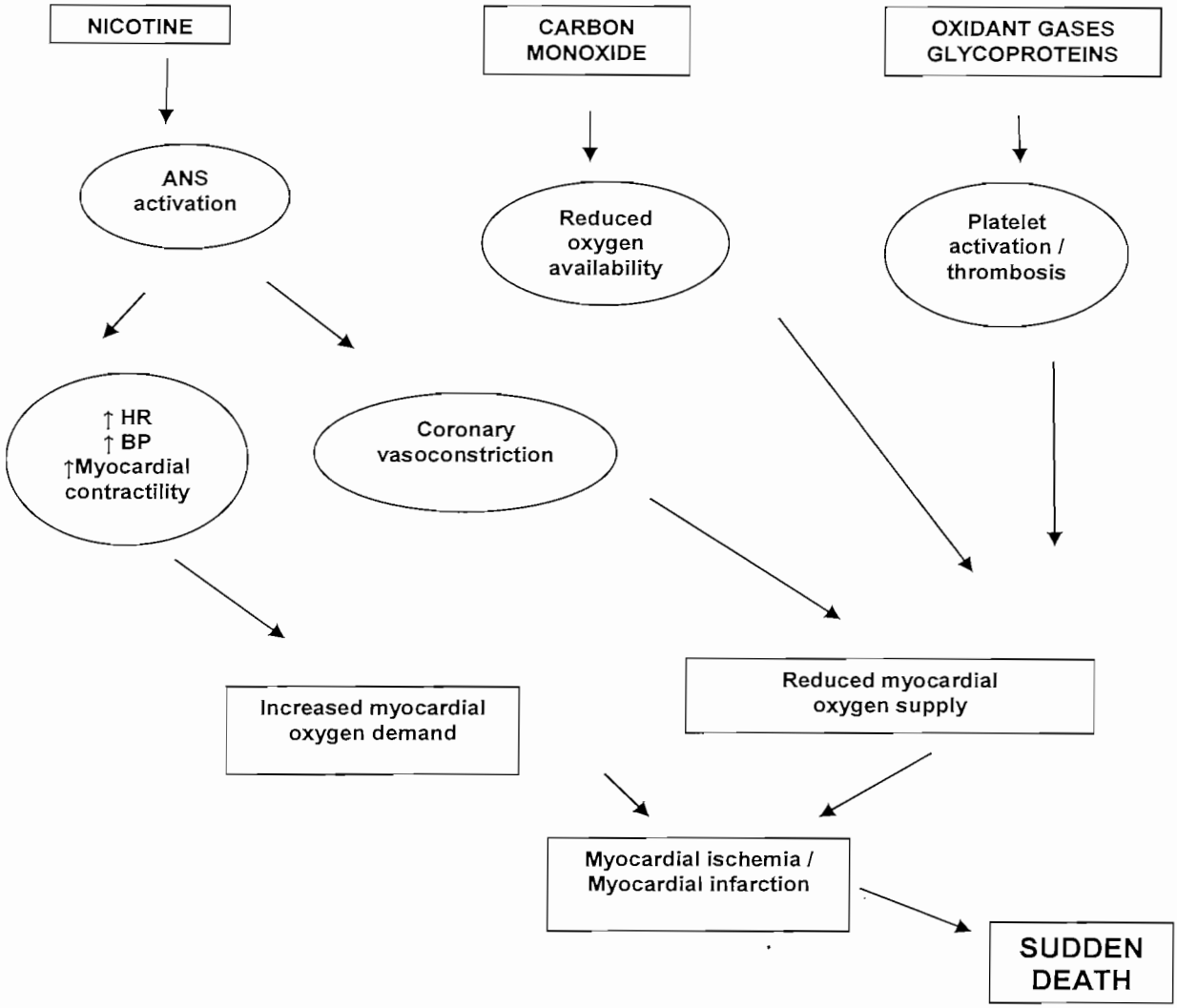

Figure 2. Cigarette smoking and acute coronary events.[6] ANS, autonomic nervous system; HR, heart rate; BP, blood pressure.

4.2 Smoking and atherosclerosis

Atherosclerosis (Figure 3) affects mainly the arteries and is characterized by the increased accumulation of lipids, cholesterol, CRP and other inflammatory components.[84,85] Atherosclerosis is a gradual process that begins in childhood with deposits of cholesterol and its esters in arterial intima, and macrophages and smooth muscle cells to form fatty streaks.[86,87,88] In young adulthood these fatty streaks are converted into raised lesions by accumulation of lipids and the formation of a fibromuscular intimal cap. At middle age, lesions increase in size by continued accumulation of lipids (Figure 3) and become susceptible to rupture of the fibromuscular cap and overlying endothelium.[85,86,87] Atherosclerosis is highly associated with the metabolic syndrome and is common in blacks.[43,86]

However, neither serum lipoproteins nor smoking accounts for the excess of fatty streaks in blacks.[86] Instead, a polymorphism in the apolipoprotein B gene was associated with fatty streaks in blacks but not in whites.[86] Smoking and LDL-C were associated positively with the extent of fatty streaks and raised lesions as the risk factors that affect atherosclerosis to the same degree in both sexes and both races.[86] The effects of smoking on the initiation of atherosclerosis have been reviewed in many studies[43,86,89] with the conclusion that tobacco smoke can initiate the atherosclerotic process, both in active and passive smokers. Blood endothelial cell counts and platelet aggregability are increased in smokers and non-smokers exposed to smoke.[1,6] This effect is initiated more by nicotine, although other chemicals in smoke, such as 1,3-butadiene, play some role.[88]

Furthermore, smoking destroys NO through the effect of oxidant chemicals, although endogenous production of NO seems a typical biological activity of plaque.[90] Smoking also results in high fibrinogen and lipid concentration, the key features of plaque formation.[6,91,92] Cigarette smoking stimulates immune system cells which become sources of reactive oxygen species (ROS), highly toxic substances for the vascular wall. Free radicals oxidize LDL-C, which are then taken up by macrophages leading to foam cells, a key step in atheromatic plaque formation.[1,93] All these effects act together and contribute to the initiation of plaque formation (Figures 3 & 4.)

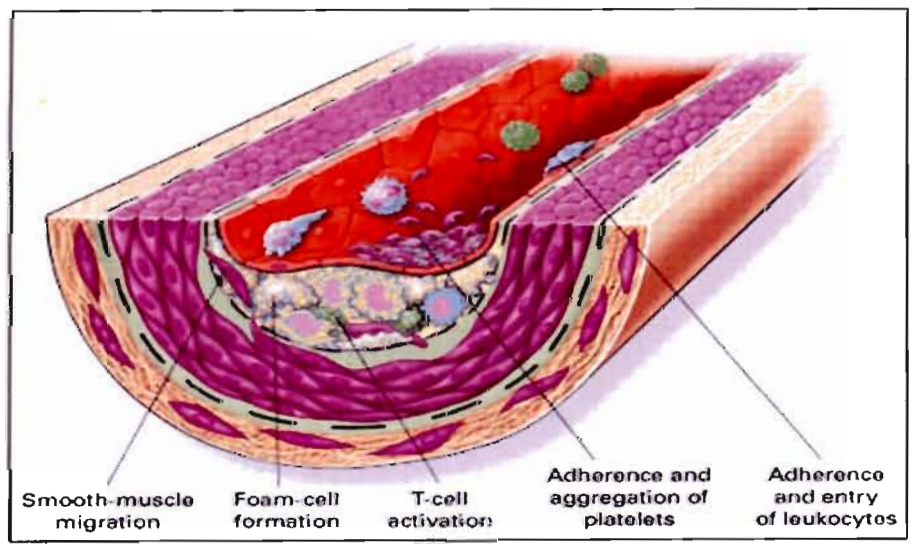

Figure 3. The various stages in the atherosclerosis process.[85]

4.3 Smoking and endothelial dysfunction

The endothelium is the innermost layer of a blood vessel that is in direct contact with the blood. It maintains vessel integrity and controls vascular tone and the vascular inflammatory process.[4,70] In response to increased blood flow and acetylcholine, the enzyme nitric oxide synthase (NOS) in the endothelial cells uses L-arginine to generate NO in the endothelium, leading to vasodilation.[57,70] Vasoconstriction by the endothelium is mediated by endothelin in response to epinephrine.[4,57] Disturbance of these normal endothelial physiological processes leads to endothelial dysfunction, which ultimately leads to cardiovascular diseases through a number of mechanisms that include thrombosis and atherosclerosis (Figure 4).[4,6,94]

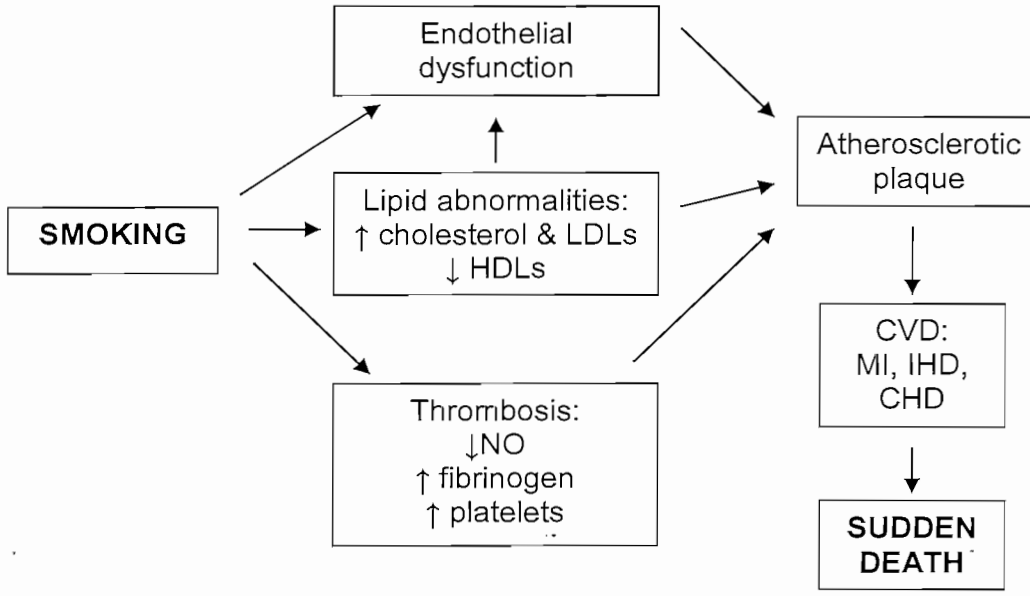

Figure 4. Mechanisms of smoking mediated atherosclerosis.[91] LDL's, low-density lipoproteins; HDL's, high-density lipoproteins; NO, nitric oxide; CVD, cardiovascular disease; MI, myocardial infarction; IHD, ischemic heart disease; CHD, coronary heart disease.

There is considerable evidence in the literature that cigarette smoking causes endothelial dysfunction. The consequence of endothelial dysfunction includes reduced NO release.[1,95,96] The mechanism by which smoking causes endothelial dysfunction is the effect of oxidant chemicals that are contained in smoke.[6] Cigarette smoke increases superoxide anion (O_2–) by stimulation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), which in turn reduces NO bioavailability, resulting in endothelial dysfunction. So, oxidant chemicals degrade NO, reducing NO availability and antagonizing its effect on the endothelium, leading to platelet and lipid aggregation, and

thrombosis.[4,6] Several other studies[4,97] also report the direct damage caused by nicotine on the endothelium, a desquamating effect by increased shear stress from increased blood viscosity, increased HR, BP and cardiac output (CO), and vasoconstriction induced by smoking.

As a result, increased levels of circulating endothelial cells have been observed in smokers.[6,97] Nicotine may also enhance the release of basic fibroblast growth factor and inhibit the production of transforming growth factor beta-1 and increases DNA synthesis, mitogenic activity, and endothelial proliferation.[98] Tobacco smoke also increases smooth muscle cell proliferation by inducing adherence to the injured endothelium, resulting in release of platelet-derived growth factor (PDGF).[99]

4.4 Smoking and arterial stiffness

Pulse wave velocity (PWV) is an indicator of arterial stiffness and a marker for vascular dysfunction.[70,100] Smoking correlates positively with arterial stiffness.[4,76,101] The effect of cigarette smoking on BP, HR and PWV in chronic smokers suggests that cigarette smoking can have deleterious effects on the cardiovascular system by stiffening arteries.[4,70] Another study reports that in habitual smokers, smoking one cigarette causes short-term increases in arterial wall stiffness that might be harmful to the artery and increase the risk for plaque rupture.[102] Smoking also increases plasma nicotine, which stimulates the release of catecholamines and alters arterial distensibility, consequently leading to arterial stiffness.[4,6]

Gender differences in arterial stiffness may underlie the variation in cardiovascular risk profile between men and women, in relation to age.[103] In men and women matched for mean blood pressures, the age-related stiffening of large arteries is more pronounced in postmenopausal women, compared to age-matched men.[103,104] Moreover, increased arterial stiffness and disturbed wave reflections are the basis for understanding reduced aortic elasticity and systolic hypertension, particularly in older people.[100,101,104] Smoking acutely increases the PWV and augmentation index (AI), a measure of arterial wave reflection.[4] This is more pronounced in Africans than in Caucasians. In Caucasians PWV may increase with increasing BP, a feature that was not observed in Africans in a South African study.[105] Increased PWV in the Africans point to vascular changes

independent of BP.[105] Differences in nicotine metabolism and beta-adrenergic sensitivity could explain these findings.[106]

4.5 Smoking and lipid abnormalities

Hypercholesterolemia refers to the presence of excessively high levels of cholesterol in the blood, while hyperlipidemia refers to the high levels of lipids in the blood.[4,107] Cigarette smoking is associated with a more atherogenic lipid profile that includes higher LDL-C and lower HDL-C levels compared to nonsmokers.[1,6,15] HDL-C mediates cholesterol efflux from macrophage cells, inhibit foam cell formation, and prevent the oxidation of LDL-C.[4,45] This way, HDL-C prevents endothelial dysfunction. The two mechanisms by which nicotine contributes to lipid abnormalities is by accelerating lipolysis and inducing insulin resistance,[1,6,99] resulting in increased FFA and TG in the blood.[99] The increasing lipid molecules in the blood may also be the pathway towards atherosclerosis and heart disease.[108] Smoking and hypercholesterolemia are also associated with decreased levels of antioxidants[109] and thus an increase in oxidative stress, which damages mitochondria in cardiovascular cells and leads to cardiovascular disease.[110]

Dyslipidemia is associated with the metabolic syndrome, which is highly prevalent in blacks and in whites.[111,112] Although black smokers have presented high concentrations of blood lipid molecules,[111,113] most studies report whites to have higher serum cholesterol than blacks, with blacks having higher HDL-C than whites.[4,39,71,86,114] Women were found, in the same studies, to have higher HDL-C levels than men. Serum cholesterol increases with age, with a more pronounced increase for whites than in blacks.[86]

4.6 Smoking and inflammation (C-reactive protein)

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[12] It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.[70,80] Through its mechanisms, inflammation results in increased lipids, platelets and CRP, which in the process, cause CHD,[115] thrombosis and atherosclerosis.[70,116] The simple association between smoking and inflammation is that smoking causes endothelial damage that sets the tone for the inflammatory process.[1,6,70] The

endothelium is the main interface between inflammation and plaque, and promotes the development of inflammatory processes. Loss of endothelial integrity involves a reduction in bioavailability of NO, loss of protective action and the development of atherosclerosis.[90]

Cigarette smoking is associated with a chronic inflammatory state that involves increased levels of circulating leukocytes, increased CRP and fibrinogen levels[39,45] by promoting recruitment and adhesion of leukocytes to blood vessel walls.[6,116,117] Smoking is believed to generate platelet activating factor (PAF)-like agonists (through lipid peroxidation products and oxidative stress), which enhance leukocyte adhesion and platelet aggregation.[1,6] Nicotine does also act on human monocyte-derived dendritic cells to stimulate an inflammatory response,[6,9,99] although nicotine introduction other than in smoke may result in significant decline in leucocyte count.[118]

There is a significant body of evidence that links smoking with increased levels of CRP, a marker of inflammation with a half-life of 19 hours.[6,45,119] The function of CRP is thought to stimulate tissue factor (TF), a contributor in blood coagulation, which may account for its important role in cardiovascular disease.[115,119] Smoking therefore increases CRP synthesis by hepatocytes. The TF produced by CRP release, promotes thrombosis and inflammation. The other explanation for the association between CRP and vascular dysfuction is as a marker that increases in inflammatory states associated with cardiovascular disease.[115] CRP is also seen as a culprit in atherogenesis acting as a pro-coagulant.[119,120] New data suggests CRP in serum mediates the uptake of LDL-C into macrophages, which then become foam cells.[121] Other studies point to its role in destabilizing plaques.[122] In another concept, CRP is a marker of vascular inflammation that is released from atherosclerotic sites.[115,119]

Ethnicity is reported by one study[123] as an important factor when considering CRP as a marker of atherothrombotic risk. Blacks are reported by a number of studies to reflect higher CRP concentrations than whites,[71,124,125] a marker highly associated with the metabolic syndrome.[112,113,126] In the metabolic syndrome, CRP levels are elevated and body mass index (BMI) accounts for much but not all of the ethnic differences in CRP concentrations.[124,127] In one study,[128] CRP levels were higher in women compared with men despite accounting for BMI and other common confounding variables.

4.7 Smoking and insulin resistance

Insulin resistance refers to a diminished ability of the tissues to respond to the action of insulin.[129] The pancreas will secrete more insulin to compensate for the resistance, and over time, the excess insulin secretion will lead to a drop in insulin production. A type 2 diabetic may, over time, therefore also become a type 1 diabetic.[80,129] Smokers are more likely to be insulin resistant, exhibit several aspects of the insulin resistance syndrome, and are at an increased risk for type 2 diabetes.[6,42] The mechanism involving smoking and the development of diabetes is unclear, but there is evidence that nicotine might play a role.[6,130] Nicotine reduces the secretion of insulin in type 2 diabetics.[42,131] One mechanism by which nicotine produces insulin resistance is the activation of the sympathetic nervous system.[6] Nicotine also increases release of corticosteroids and growth hormone, which may contribute to insulin resistance.[6] Furthermore, nicotine stimulates lipolysis, and smokers therefore have higher levels of FFA and TG, which has been associated with insulin resistance.[6,132,133]

Smoking and diabetes potentiates cardiovascular risk, both by increasing oxidative stress, producing endothelial dysfunction, and enhancing coagulation.[6,134] Diabetes is one of the components of the metabolic syndrome, which is highly prevalent among Africans.[112,135,136] Therefore, smoking acting together with the metabolic syndrome might have detrimental cardiovascular effects in both blacks and whites. Cigarette smoking increases this risk for diabetic nephropathy, retinopathy, and neuropathy, probably via its metabolic effects in combination with increased inflammation and endothelial dysfunction.[137]

4.8 *Aging, gender and smoking habit*

Structural changes in the cardiovascular system are evident with increasing age.[70,100] The most noticeable of such changes is the correlation of arterial stiffness with age.[100] The increased arterial stiffness will affect BP so that, with increasing age, SBP increases, while DBP would tend to decrease.[57,100] The stiffening of the arteries can be caused by decreased connective tissue elasticity, atherosclerosis and a decrease in smooth muscle relaxation,[57,138] features of arterial stiffness, that were found to be more prevalent in aging African males.[139] Large artery stiffening results in an increase in PWV and early reflected pulse waves.[71,100] Thus, it is clear that aging alone does cause damage to the cardiovascular system.

Smoking accelerates age-related cardiovascular conditions such as CAD, MI, and PAD leading to premature death[6]. In smoking, markers such as CRP and total cholesterol are elevated and these are risk factors of cardiovascular disease.[1] McGill et al[86] reported a lower smoking prevalence in men than in women of 15 to 19 years of age, while equal in men and women of 25 to 34 years of age, especially in Caucasians. In South Africa there has been a gradual rise from young to old age smoking and smoking related mortality at old age has been increasing.[140] With regards to gender, smoking is a major cause of cardiovascular diseases such as CHD in both men and women.[91,141]

A number of studies[91,142,143] report that although smoking prevalence is higher among males, this is followed by a likewise higher cessation rate in this gender, leaving more women smoking than males in many developed countries. Together with gender, socioeconomic status (SES) emerged as a determinant of smoking, with the habit starting among upper social classes and later extending to lower SES groups.[91] Rates in men are higher among the higher socioeconomic groups than women.[38,144] Prevalence of smoking then tends to decrease in both sexes towards the lower socioeconomic groups, with a higher degree of cessation in men than with women.[38,144] Cigarette use among women is more strongly associated with weight control and body image concerns, in addition to stressful life events.[38] Smoking prevalence is on a high in younger women worldwide, and this puts them on a higher risk for sudden cardiac death.[91,145] However, men are more likely to have MI compared to premenopausal women.[91,146] This is attributed to the beneficial effect on the CVS of hormones such as estrogen in females.[91,146]

Smoking has distinct mechanisms of plaque instability and sudden coronary death in women, which vary by menopausal status.[73] Men have a significantly higher risk of arterial thrombotic complications than women, with the risk of hypertension correlating positively with smoking in women.[73,91] Hormonal differences may explain these findings to some extent, leading to the notion that, an additional factor, such as smoking, may in many cases be needed for the development of thrombosis.[73,91]

To conclude, this review provides the general physiological mechanisms that link smoking to cardiovascular disease. The prevalence and ethnic differences of smoking between Africans and Caucasian people have been discussed. Furthermore, the differences in the cardiovascular markers between the two ethnic groups have been explained in order to expose their possible mechanisms.

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CHAPTER 3:

Manuscript to be submitted for publication

SMOKING AND VASCULAR DYSFUNCTION IN AFRICAN AND CAUCASIAN PEOPLE FROM SOUTH AFRICA: The SAfrEIC study

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INSTRUCTIONS FOR AUTHORS:

American Journal of Hypertension (Am J Hypertens)

- An abstract of a maximum of 250 words must follow the title.

- The abstract must be followed by about 3-10 key words which will assist the cross-indexing of the article.

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- Figures and tables must be labeled sequentially, numbered and cited in the text. Caption for a figure must be placed at the bottom of the figure, and tables must have their captions at the top.

- References are to be numbered in the order of citation within the article. Citations in the main text should appear as superscript Arabic numerals. All authors must be listed. Journal names must be abbreviated as in *Index Medicus.*

- Reference example:

- Lender D, Arauz-Pacheco C, Breen L, Mora-Mora P, Ramirez LC, Raskin P. A double-blind comparison of the effects of amlodipine and enalapril on insulin sensitivity in hypertensive patients. *Am J Hypertens* 1999; 12:298-303.
- Opie LH. *Heart physiology: From cell to circulation.* Lippincott Williams & Wilkins, USA, 2004, pp 452.

ABSTRACT

Background: Smoking causes vascular dysfunction. According to available literature Caucasians are the most prevalent smokers in South Africa while Africans are the most affected by smoking-related cardiovascular diseases. Smoking acts with metabolic syndrome components to cause vascular dysfunction in Africans. We aimed to determine the prevalence of smoking and its association with vascular function in Africans and Caucasians of South Africa.

Methods: About 602 participants of African and Caucasian origin from the North West province were recruited and their anthropometric, cardiovascular and lipid variables, cotinine and C-reactive protein measured. Socio-economic status (SES) was determined by questionnaire completion.

Results: Africans smoked more than Caucasians, a finding supported by significantly lower weight, body mass index, waist circumference, higher blood pressure and lower SES in Africans. All smokers showed increased cardiac output and triglycerides than non-smookers in both groups. However, only African smokers showed increased higher density lipoprotein cholesterol (HDL-C) and increased arterial stiffness than their non-smokers while Caucasian smokers had lower HDL-C levels than their non-smokers. Despite the generally weak correlations of smoking with the measured variables, Africans reflected more and stronger associations overall, including the surprisingly positive correlation of smoking with HDL-C. Cotinine correlated strongly with smoking duration throughout.

Conclusion: Africans smoked more than Caucasians. This is possibly due to the immense differences in SES. Smoking was related to vascular dysfunction in both ethnic groups, but indicated more associations with lipids and cardiovascular risk markers in Africans than Caucasians.

Key words: smoking, vascular dysfunction, socio-economic status, ethnicity, hs-CRP, Africans, Caucasians.

Introduction

It is well known that smoking has negative health consequences and it is the main avoidable cause of illness and death world-wide.[1] Smoking causes premature death of many people every year in the world.[1,2] Despite these negative effects of smoking, the continuous use of tobacco products is rising.[3] Globally, the prevalence of smoking-related cardiovascular diseases (CVDs) is higher in Africans than Caucasians.[4,5,6] In South Africa, factors such as age, gender, ethnicity, cultural and economic characteristics influence the prevalence of smoking.[7,8] There is a high prevalence of smoking in adults, mostly white males and those earning a low income.[7,9] However, it has been shown that poorer smokers are more likely to quit than smokers that are more affluent.[9]

Many studies have reported on the effect of smoking on the metabolic syndrome, which is a highly prevalent cluster of disorders that are quite common in Africans.[10,11,12] Smoking acts together with the metabolic syndrome and causes dyslipidemia, increased C-reactive protein (CRP) levels, and endothelial dysfunction.[11,13] Smokers are therefore characterized by high serum triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) with significantly lower high-density lipoprotein cholesterol (HDL-C) than non-smokers.[4]

Nicotine in cigarette smoke increases the heart rate and cardiac output through cardiac beta-adrenergic effects,[1] causing increases in blood pressure (BP). Carbon monoxide decreases the oxygen carrying capacity of the blood and may lead to ischemia and hypoxia of the tissues.[4,14] This stimulates increased red blood cell production, which contributes to increased viscosity and consequently inflammatory and coagulatory processes.[4] Inflammation and coagulation are associated with atherosclerosis and coronary heart disease (CHD).[15,16] All these factors therefore contribute negatively, in one way or the other, to increased risk for CVD.

Nicotine is broken down metabolically into various metabolites that include cotinine and nicotine-N-oxide.[3] The most important metabolite of nicotine is cotinine,[3,17] which is a vital biological marker of smoking and has been used to identify smokers.[3,18] Serum cotinine levels of smokers are consequently significantly higher compared to nonsmokers.[3]

The association of smoking with CVD has been well documented in developed countries.[1,4] However, limited data exists in low and middle-income countries such as South Africa. The aims of this study were firstly to investigate if ethnic differences exist with regards to the prevalence of smoking between Africans and Caucasians of South Africa, and secondly to determine if there are ethnic differences regarding the association between smoking and measures of cardiovascular function between African and Caucasian people of South Africa.

Methods

This study is a substudy and is based on data from the SAfrEIC study (South African study on the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function). The SAfrEIC study was a cross-sectional study with 602 participants (apparently healthy African and Caucasian men and women) from urban areas of the North-West Province of South Africa, aged 20 to 70 years. Exclusion criteria for this substudy were diabetes (type 1 and 2), or persons on diabetic medication, pregnant or breast feeding women, and those testing positive for the Human Immunodeficiency Virus (HIV).

The Ethics committee of the North-West University (Potchefstroom campus) approved this study. The participants all signed informed consent forms after all procedures were explained to them. An interpreter was available to relay the information to the African subjects in their home language.

Organizational procedures

For a period of seven weeks, ten to twenty participants visited the facility daily (consisting of 10 bedrooms, two bathrooms, a living room and kitchen) on the Potchefstroom campus of the North-West University. They arrived at 07:00 and the African participants were accompanied by four field workers. The participants were introduced to the set-up. Each subject received a 'participant sheet' which guided them through the different research stations where the various measurements were done. During the course of the morning, basic health, demographic and lifestyle questionnaires were completed. Participants were requested to indicate their income per month according to the codes in the questionnaire and also had to specify the

duration of smoking (in years) or use of tobacco products. A fasting blood sample was taken by a registered nurse from the antebrachial vein using sterile winged infusion sets and syringes, and anthropometric measurements were taken in a private room. BP and pulse wave velocity (PWV) measurements were also taken in a private bedroom. When all questionnaires were completed and all cardiovascular measurements taken, each participant received breakfast as well as a small financial compensation. In the event of a subject being identified with any abnormalities (such as hypertension or diabetes), the subject was referred to their local clinic, hospital or physician. Each subject received a short report containing their health information.

Anthropometric measurements

Body height, body mass, waist circumference (WC) and hip circumference of each subject were taken according to standard procedures.[19] The circumferences were measured in triplicate. Maximum height was measured to the nearest 0.1 cm using the Invicta Stadiometer (IP 1465, U.K). Weight was measured to the nearest 0.1 kg using a digital scale (Precision Health Scale, A & D Company, Japan). A flexible metallic measuring tape was used to measure the circumferences, taken with the subjects standing upright, with the face directed toward the observer and shoulders relaxed. The WC was measured at the thinnest visible point (below the last rib) of the trunk of the body. The hip circumference was measured at the broadest point over the gluteal muscles. Body mass index (BMI) was determined with the formula: body mass / body height^2.

Cardiovascular measurements

After a 10-minute rest in the sitting position, BP (systolic and diastolic) and heart rate (HR) were measured using the OMRON HEM-757 apparatus, with the BP cuff on the left upper arm. The appropriate cuff sizes were used for obese subjects. The test subjects were seated comfortably during measurements. Two measurements were taken, with a five- minute rest interval.

PWV (both carotid-radialis and carotid-dorsalis pedis) was measured using the Complior SP apparatus. The following two distances were measured on the left side of each subject: carotid-radialis (from the suprasternal notch to the radial artery, in the wrist) and carotid-dorsalis pedis (from the suprasternal notch, to the dorsalis pedis

artery, in the foot). The subtraction method was used, i.e – the distance from the carotid artery to the suprasternal notch was subtracted from the measurements to the dorsalis pedis or to the radialis. The PWV was measured on the left side of each participant, while the subject was in a supine position.

Cardiovascular parameters were monitored by making use of the Finometer™ device (FMS, Finapres Medical Systems, Amsterdam, Netherlands). This entailed a 5-minute continuous recording of each subject's cardiovascular parameters under resting, yet awake, conditions. After the first two minutes the upper arm pressure was calibrated with the finger pressure for each individual subject (i.e. return-to-flow systolic calibration). The last two minutes of each recording was used to calculate the average of the cardiovascular variables, namely stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and Windkessel arterial compliance (Cwk). Data files were extracted from the device's hard drive and were then processed with the Beatscope 1.1 software programme.

Biochemical analyses

Plasma and serum samples were prepared using standard methods and stored at -80°C until the time of analyses. High-sensitivity C-reactive protein (hs-CRP) and serum lipids were determined with a Konelab 20i (Labsystems Clinical Laboratory Division, Vantaa, Finland) clinical chemistry analyzer. Cotinine analyses were performed with the use of the IMMULITE 2000 Nicotine Metabolite assay (Siemens Medical Solutions Diagnostics Ltd., Los Angeles, CA, USA), a solid-phase competitive chemiluminescent immunoassay (Catalog Number L2KNM6). HIV status was determined immediately after blood sampling with a rapid test according to the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response Test and was repeated with Pareeshak test for confirmation.

Statistical analysis

All statistical analyses were performed by making use of Statistica version 8 (Statsoft, Inc., Tulsa, OK, 2007). Statistical results are presented as means, standard errors and 95% confidence intervals. Variables that were not normally distributed were logarithmically transformed, namely TG and hs-CRP. An independent t-test and analysis of covariance (ANCOVA) were performed to compare the variables between the two ethnic groups and to determine significant differences. Self-reported smokers were included in the smoking group for statistical analyses. Furthermore, the same tests were performed to compare the variables between smokers and non-smokers within each ethnic group, and also while adjusting for certain variables such as age, gender, BMI, and WC. Furthermore, mean arterial pressure (MAP) was included as an adjustment variable while comparing PWV between and within the two ethnic groups. The Chi-square test was used to determine if there were significant differences between categorical variables such as smoking and income. For the second aim of this study, correlations between smoking and cardiovascular variables such as systolic blood pressure (SBP), diastolic blood pressure (DBP), SV, CO, TPR, Cwk, PWV, and HR, together with lipid variables (HDL-C, LDL-C, TG) and hs-CRP were performed within each ethnic group. Partial correlations were then performed while adjusting for age, BMI and WC (and MAP for PWV data). The complete data for all variables were not available for all participants during the statistical analysis, hence small discrepancies in participant numbers in the tables.

Results

The specific characteristics of the African and Caucasian subject groups are compared in table 1. The table shows that many variables between the two groups differed significantly ($p \leq 0.001$). The African group had a higher proportion of smokers than the Caucasian group. Height, weight, BMI and WC levels were significantly higher in Caucasians compared to Africans, whereas Africans showed higher SBP, DBP, HR, PWV, TPR and lower Cwk values.

Caucasians showed significantly lower values for hs-CRP than Africans but Africans had significantly higher HDL-C and lower LDL-C and TG levels than Caucasians. Cotinine levels were significantly higher in Africans compared to their Caucasian counterparts, which was expected, due to higher numbers of reported smokers in

Africans. The results also revealed strong significant differences in socio-economic status (SES) ($p \leq 0.001$) in which the majority of African subjects were living on low income. A total of 90% of Africans earned less than R1000 per month. In contrast, the majority of the Caucasian group (65.6%) was living on more than R5000 per month. There were no Africans who were in the highest paid category of more than R5000 per month.

Table 1: Descriptive statistics of the population studied.

Variable	Africans (N=258)	Caucasians (N=372)	p-value
Age (yr)	41.6 ± 0.82 (39.9; 43,1)	40.4 ± 0.69 (39.0; 41.7)	0.287
Gender (men/women)	127 / 131	161 / 211	
Height (m)	1.63 ± 0.01 (1.62; 1.64)	1.72 ± 0.00 (1.71; 1.73)	**≤ 0.001**
Weight (kg)	63.5 ± 1.10 (61.3; 65.7)	82.2 ± 1.03 (80.2; 84.2)	**≤ 0.001**
BMI (kg/m^2)	24.0 ± 0.45 (23.1; 24.9)	27.7 ± 0.31 (27.1; 28.3)	**≤ 0.001**
WC (cm)	78.7 ± 0.85 (77.0; 80.4)	87.4 ± 0.79 (85.8; 88.9)	**≤ 0.001**
SBP (mmHg)	127 ± 1.39 (124; 129)	119 ± 0.84 (118; 121)	**≤ 0.001**
DBP (mmHg)	85 ± 0.87 (83; 87)	78 ± 0.52 (77; 79)	**≤ 0.001**
HR (beats/min)	70.0 ± 0.82 (68.3; 72.0)	68.0 ± 0.50 (67.0; 68.4)	**0.008**
SV (ml)	75.2 ± 1.39 (72.5; 77.9)	90.8 ± 1.27 (88.4; 93.3)	**≤ 0.001**
CO (L/min)	4.99 ± 0.09 (4.81; 5.17)	6.12 ± 0.09 (5.94; 6.31)	**≤ 0.001**
TPR (mmHg.s/mL)	1.39 ± 0.04 (1.31; 1.46)	1.06 ± 0.02 (1.02; 1.09)	**≤ 0.001**
Cwk (ml/mmHg)	1.60 ± 0.03 (1.54; 1.67)	2.10 ± 0.03 (2.04; 2.17)	**≤ 0.001**
C-R PWV (m/s)	8.67 ± 0.10 (8.47;8.87)	7.63 ± 0.07 (7.48; 7.77)	**≤ 0.001**
C-P PWV (m/s)	8.18 ± 0.10 (7.98; 8.38)	7.82 ± 0.06 (7.69; 7.94)	**≤ 0.001**
HDL-C (mmol/L)	1.57 ± 0.04 (1.49; 1.65)	1.39 ± 0.02 (1.35; 1.44)	**≤ 0.001**
LDL-C (mmol/L)	2.35 ± 0.06 (2.24; 2.46)	3.76 ± 0.07 (3.63; 3.89)	**≤ 0.001**
TG (mmmol/L)	1.38 ± 1.01 (1.36; 1.39)	1.46 ± 1.01 (1.44; 1.48)	**≤ 0.001**
hs-CRP (mg/L)	1.90 ± 1.03 (1.80; 2.01)	1.65 ± 1.02 (1.59; 1.72)	**≤ 0.001**
Smoking duration (years)	13.9 ± 0.82 (12.3;15.5)	12.7 ± 1.46 (9.82; 15.7)	0.490
Cotinine (ng/ml)	199 ± 10.8 (178; 221)	43.2 ± 5.02 (33.3; 53.1)	**≤ 0.001**
N smokers (%)	161 (62.4)	54 (14.5)	**≤ 0.001**
N male smokers (%)	96 (76.0)	34 (21.1)	**≤ 0.001**
N female smokers (%)	65 (50.0)	20 (9.48)	**≤ 0.001**
Income: ≤ R1000 p.m	232 (90.0%)	23 (6.18%)	**≤ 0.001**
R1000-R2000	22 (8.52%)	29 (7.79%)	**≤ 0.001**
R2000-R3000	3 (1.16%)	14 (3.76%)	**≤ 0.001**
R3000-R4000	0 (0.00 %)	23 (6.18%)	**≤ 0.001**
R4000-R5000	1 (0.39%)	26 (6.99%)	**≤ 0.001**
≥ R5000 p.m	0 (0.00%)	244 (65.6%)	**≤ 0.001**

Note: values are expressed as the mean ± standard error (95% confidence intervals). The mean values for TG and hs-CRP were logarithmically transformed and geometric means used. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; Cwk, windkessel compliance; C-R PWV, carotid-radialis pulse wave velocity; C-P PWV, carotid-dorsalis pedis pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high-sensitivity C-reactive protein.

Table 2 compares the African smokers and non-smokers. Age differed significantly, with smokers being older than non-smokers. Weight, BMI and WC also showed

significant differences between the two groups, with smokers having lower values for these variables.

Table 2: Comparison between African smokers and non-smokers.

Variable	African non-smokers (N=93)	African smokers (N=152)	p-value
Age (yr)	37.5 ± 1.36 (34.8; 40.2)	44.0 ± 0.98 (42.1; 46.0)	**≤ 0.001**
Gender (men/women)	31 / 66	96 / 65	
Height (m)	1.62 ± 0.01 (1.60; 1.63)	1.64 ± 0.01 (1.63; 1.65)	0.103
Weight (kg)	71.4 ± 1.99 (67.4; 75.4)	58.8 ± 1.15 (57.0; 61.0)	**≤ 0.001**
BMI (kg/m^2)	27.4 ± 0.81 (25.8; 29.0)	22.1 ± 0.47 (21.1; 23.0)	**≤ 0.001**
WC (cm)	83.0 ± 1.54 (79.9; 86.0)	76.3 ± 0.95 (74.4; 78.2)	**≤ 0.001**
SBP (mmHg)	124 ± 2.64 (119; 129)	129 ± 1.55 (126; 132)	0.079
DBP (mmHg)	84 ± 1.19 (81.0; 87.2)	86 ± 1.02 (84.0; 88.0)	0.393
HR (beats/min)	69.3 ± 1.15 (67.0; 72.0)	70.3 ± 1.13 (68.0; 73.0)	0.561
SV (ml)	80.1 ± 1.58 (75.6; 85.0)	72.1 ± 1.72 (69.0; 75.5)	**0.005**
CO (L/min)	5.31 ± 0.16 (4.99; 5.63)	4.80 ± 0.12 (4.57; 5.00)	**0.006**
TPR (mmHg.s/mL)	1.28 ± 0.05 (1.18; 1.37)	1.46 ± 0.05 (1.36; 1.57)	**0.016**
Cwk (ml/mmHg)	1.76 ± 0.04 (1.66; 1.86)	1.50 ± 0.04 (1.42; 1.58)	**≤ 0.001**
C-R PWV (m/s)	8.05 ± 0.16 (7.76; 8.35)	9.04 ± 0.12 (8.79; 9.30)	**≤ 0.001**
C-P PWV (m/s)	7.57 ± 0.13 (7.25; 7.89)	8.55 ± 0.10 (8.31; 8.79)	**≤ 0.001**
HDL-C (mmol/L)	1.33 ± 0.04 (1.25; 1.41)	1.72 ± 0.06 (1.61; 1.84)	**≤ 0.001**
LDL-C (mmol/L)	2.36 ± 0.09 (2.18; 2.53)	2.35 ± 0.07 (2.21; 2.50)	0.962
TG (mmmol/L)	1.35 ± 1.01 (0.27; 0.32)	1.40 ± 1.01 (0.32; 0.35)	**0.015**
hs-CRP (mg/L)	1.95 ± 1.04 (0.59; 0.75)	1.88 ± 1.04 (0.55; 0.70)	0.434
Cotinine (ng/ml)	43.7 ± 9.13 (25.5; 61.8)	296 ± 10.9 (274; 317)	**≤ 0.001**
Comparison after adjustment for age, gender, BMI and WC			
Variable	**African non-smokers (N=93)**	**African smokers (N=152)**	**p-value**
SBP (mmHg)	128 ± 2.05 (124; 132)	126 ± 1.55 (123; 129)	**0.050**
DBP (mmHg)	85 ± 1.37 (82.1; 87.5)	85 ± 1.03 (82.8; 87.0)	0.443
HR (beats/min)	70.1 ± 1.38 (67.4; 73.0)	70.0 ± 1.04 (68.0; 72.0)	0.539
SV (ml)	74.5 ± 1.94 (70.7; 78.3)	75.2 ± 1.48 (72.3; 78.2)	**≤ 0.001**
CO (L/min)	4.91 ± 0.13 (4.66; 5.17)	5.01 ± 1.00 (4.81; 5.20)	**≤ 0.001**
TPR (mmHg.s/mL)	1.40 ± 0.06 (1.29; 1.51)	1.39 ± 0.04 (1.30; 1.47)	**0.006**
Cwk (ml/mmHg)	1.59 ± 0.03 (1.54; 1.65)	1.60 ± 0.02 (1.56; 1.65)	**≤ 0.001**
C-R PWV (m/s)	8.42 ± 0.15 (8.12; 8.72)	8.81 ± 0.11 (8.59; 9.03)	**≤ 0.001**
C-P PWV (m/s)	8.00 ± 0.12 (7.77; 8.23)	8.29 ± 0.09 (8.12; 8.47)	**≤ 0.001**
HDL-C (mmol/L	1.44 ± 0.07 (1.31; 1.57)	1.64 ± 0.05 (1.54; 1.74)	**≤ 0.001**
LDL-C (mmol/L)	2.39 ± 0.09 (2.21; 2.58)	2.34 ± 0.07 (2.20; 2.48)	0.809
TG (mmmol/L)	1.35 ± 0.01 (0.27; 0.32)	1.40 ± 0.01 (0.32; 0.35)	**0.012**
hs-CRP (mg/L)	0.63 ± 0.04 (0.55; 0.71)	0.66 ± 0.03 (0.59; 0.72)	0.392
Cotinine (ng/ml)	53.9 ± 13.2 (27.8; 79.9)	288 ± 9.96 (269; 308)	**≤ 0.001**

Note: values are expressed as the mean ± standard error (95% confidence intervals). The mean values for TG and hs-CRP were logarithmically transformed and geometric means used. PWV was also adjusted for MAP. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; Cwk, windkessel compliance; C-R PWV, carotis-radialis pulse wave velocity; ; C-P PWV, carotis-dorsalis pedis pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high-sensitivity C-reactive protein.

Smokers showed significantly higher PWV, TG and cotinine than non-smokers. CO was significantly higher in smokers than non-smokers of the African group. Both measures of PWV, a measure of arterial stiffness, were significantly higher in smokers than non-smokers in Africans. Furthermore, HDL-C remained higher in African smokers over their non-smoker counterparts even after adjusting for age, gender, BMI and WC.

In table 3 the Caucasian smokers and non-smokers are compared. Caucasians had fewer smokers than non-smokers. HR and CO were higher in smokers while TPR and Cwk were lower in smokers after adjustments were made, followed by lower HDL-C (higher in non-smokers) and TG (higher in smokers). Hs-CRP was also significantly higher in smokers than non-smokers in non-smokers. Cotinine differed significantly throughout, with significantly higher values in smokers, as expected.

Table 3: Comparison between Caucasian smokers and non-smokers.

Variable	Caucasian non-smokers (N=303)	Caucasian smokers (N=53)	p-value
Age (yr)	41.4 ± 0.74 (39.9; 42.8)	35.0 ± 1.67 (31.6; 38.2)	**≤ 0.001**
Gender (men/women)	127 / 191	34 / 20	
Height (m)	1.71 ± 0.01 (1.71; 1.73)	1.73 ± 0.01 (1.70; 1.75)	0.444
Weight (kg)	82.0 ± 1.12 (79.8; 84.1)	84.1 ± 2.75 (78.6; 89.6)	0.453
BMI (kg/m^2)	27.7 ± 0.34 (27.0; 28.4)	28.0 ± 0.81 (26.4; 29.7)	0.686
WC (cm)	87.2 ± 0.85 (85.5; 88.9)	89.0 ± 2.19 (84.6; 93.4)	0.410
SBP (mmHg)	119 ± 0.93 (118; 121)	119 ± 1.98 (115; 123)	0.909
DBP (mmHg)	78 ± 0.56 (77.1; 79.3)	79 ± 1.42 (75.6; 81.3)	0.863
HR (beats/min)	67.0 ± 0.53 (66.0; 68.1)	71.0 ± 1.24 (68.0; 73.0)	**0.014**
SV (ml)	90.3 ± 1.36 (87.6; 93.0)	94.1 ± 3.43 (87.3; 101)	0.283
CO (L/min)	6.04 ± 0.10 (5.85; 6.24)	6.57 ± 0.28 (6.01; 7.14)	**0.046**
TPR (mmHg.s/mL)	1.07 ± 0.02 (1.03; 1.11)	0.96 ± 0.04 (0.89; 1.04)	**0.027**
Cwk (ml/mmHg)	2.07 ± 0.03 (2.00; 2.13)	2.33 ± 0.08 (2.17; 2.48)	**0.002**
C-R PWV (m/s)	7.62 ± 0.08 (7.46; 7.78)	7.62 ± 0.14 (7.34; 7.89)	0.982
C-P PWV (m/s)	7.82 ± 0.07 (7.69; 7.95)	7.82 ± 0.15 (7.52; 8.12)	0.983
HDL-C (mmol/L	1.42 ± 0.02 (1.37; 1.47)	1.22 ± 0.06 (1.10; 1.33)	**≤ 0.001**
LDL-C (mmol/L)	3.76 ± 0.07 (3.62; 3.90)	3.80 ± 0.16 (3.47; 4.13)	0.837
TG (mmol/L)	1.45 ± 0.01 (0.36; 0.39)	1.54 ± 0.02 (0.39; 0.47)	**0.005**
hs-CRP (mg/L)	1.63 ± 0.02 (0.45; 0.53)	1.80 ± 0.05 (0.49; 0.69)	0.060
Cotinine (ng/ml)	10.4 ± 0.78 (8.89; 11.9)	231 ± 18.5 (194; 268)	**≤ 0.001**

Comparison after adjustment for age, gender, BMI and WC

Variable	Caucasian non-smokers (N=303)	Caucasian smokers (N=53)	p-value
SBP (mmHg)	119 ± 0.74 (118; 121)	120 ± 1.84 (116; 123)	0.970
DBP (mmHg)	78 ± 0.46 (77.1; 78.9)	79 ± 1.14 (77.1; 81.6)	0.776
HR (beats/min)	67.0 ± 0.52 (66.0; 68.0)	71.0 ± 1.29 (68.2; 73.2)	**0.014**
SV (ml)	91.2 ± 1.04 (89.1; 93.2)	89.3 ± 2.59 (84.2; 94.4)	0.165
CO (L/min)	6.11 ± 0.08 (5.95; 6.26)	6.26 ± 0.19 (5.87; 6.64)	**0.012**
TPR (mmHg.s/mL)	1.06 ± 0.02 (1.03; 1.09)	1.04 ± 0.04 (0.96; 1.12)	**0.011**
Cwk (ml/mmHg)	2.11 ± 0.02 (2.08; 2.14)	2.05 ± 0.04 (1.97; 2.13)	**≤ 0.001**
C-R PWV (m/s)	7.62 ± 0.07 (7.48; 7.76)	7.65 ± 0.18 (7.29; 8.01)	0.423
C-P PWV (m/s)	7.79 ± 0.05 (7.69; 7.90)	7.98 ± 0.14 (7.71; 8.25)	0.423
HDL-C (mmol/L	1.41 ± 0.02 (1.37; 1.45)	1.29 ± 0.05 (1.20; 1.39)	**≤ 0.001**
LDL-C (mmol/L)	3.75 ± 0.07 (3.62; 3.88)	3.89 ± 0.17 (3.57; 4.22)	0.859
TG (mmol/L)	1.45 ± 0.01 (0.36; 0.38)	1.54 ± 0.02 (0.40; 0.47)	**0.002**
hs-CRP (mg/L)	1.62 ± 0.02 (0.45; 0.52)	1.84 ± 0.04 (0.52; 0.70)	**0.029**
Cotinine (ng/ml)	10.2 ± 3.06 (4.23; 16.3)	232 ± 7.47 (218; 247)	**≤ 0.001**

Note: values are expressed as the mean ± standard error (95% confidence intervals). The mean values for TG and hs-CRP were logarithmically transformed and geometric means used. PWV was also adjusted for MAP. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; Cwk, windkessel compliance; C-R PWV, carotid-radialis pulse wave velocity; C-P PWV, carotid-dorsalis pedis pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high-sensitivity C-reactive protein.

In order to address the second aim of this study further analyses were performed in which smoking was correlated with cardiovascular variables (SBP, DBP, HR, CO, Cwk,

PWV), hs-CRP and lipids (HDL-C, LDL-C, TG). To correlate smoking with these variables, smoking was viewed as either chronic exposure, using the duration of smoking of the subjects (obtained from questionnaires), or acute exposure, by using the measured cotinine levels in serum. Table 4 correlates smoking with the above-mentioned variables in Africans. The results showed that chronic exposure to smoking (smoking duration) had significant correlations with most cardiovascular variables in the whole African group before adjustments were made. This trend remained quite similar after dividing the African group according to gender. Arterial stiffness (PWV) (Figure 1a), CO and Cwk also showed strong significant correlations with smoking duration ($p \leq 0.001$).

The relationships between cotinine and cardiovascular variables were somewhat weaker in the whole African group. A positive correlation was found between cotinine and PWV, and weak correlations with TPR and Cwk. HDL-C correlated positively and significantly with smoking in the African group. On adjustment for age, BMI and WC, all correlations between smoking and the variables became weak and non significant. HR also remained significant when correlated with chronic smoking. Hs-CRP correlated weakly with increased chronic exposure in the whole group of Africans, while cotinine correlated positively with smoking duration in all cases in the African group, also after adjustments for age, BMI and WC.

Table 4: Correlations between smoking and measures of cardiovascular function and lipids in African participants.

	Men (N=127)				Women (N=131)				Whole group (N=258)			
	Smoking duration		Cotinine		Smoking duration		Cotinine		Smoking duration		Cotinine	
Variable	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
SBP (mmHg)	0.22	**0.012**	-0.05	0.593	0.17	**0.043**	0.02	0.795	0.23	**≤ 0.001**	0.03	0.680
DBP (mmHg)	0.21	**0.019**	-0.04	0.678	0.18	**0.039**	0.03	0.750	0.17	**0.006**	-0.08	0.901
HR (beats/min)	0.34	**≤ 0.001**	0.07	0.428	0.74	0.403	-0.05	0.586	0.18	**0.004**	-0.02	0.727
CO (L/min)	-0.13	0.141	-0.12	0.183	-0.28	**≤ 0.001**	-0.18	**0.046**	-0.20	**≤ 0.001**	-0.16	**0.011**
TPR (mmHg.s/mL)	0.15	0.104	0.13	0.153	0.33	**≤ 0.001**	0.15	0.096	0.21	**≤ 0.001**	0.01	**0.024**
Cwk (ml/mmHg)	-0.54	**≤ 0.001**	-0.19	**0.035**	-0.40	**≤ 0.001**	-0.23	**0.008**	-0.40	**≤ 0.001**	-0.17	**0.006**
C-R PWV (m/s)	0.36	**≤ 0.001**	0.15	0.102	0.22	**0.012**	0.30	**≤ 0.001**	0.36	**≤ 0.001**	0.27	**≤ 0.001**
C-P PWV (m/s)	0.46	**≤ 0.001**	0.16	0.077	0.24	**0.005**	0.26	**0.002**	0.41	**≤ 0.001**	0.25	**≤ 0.001**
HDL-C (mmol/L)	0.25	**0.005**	0.19	**0.039**	0.21	**0.019**	0.08	0.352	0.28	**≤ 0.001**	0.18	**0.005**
LDL-C (mmol/L)	0.08	0.400	0.04	0.641	0.12	0.162	0.12	0.186	0.35	0.578	0.04	0.502
TG (mmmol/L)	0.04	0.684	-0.00	0.995	0.21	**0.016**	0.22	**0.011**	0.74	0.239	0.11	0.096
hs-CRP (mg/L)	0.24	**0.006**	0.09	0.338	0.05	0.556	0.00	0.957	0.95	0.132	0.01	0.932
Cotinine (ng/ml)	0.44	**≤ 0.001**	-	-	0.49	**≤ 0.001**	-	-	0.48	**≤ 0.001**	-	-

After adjustment for age, BMI and WC

	Men (N=127)				Women (N=131)				Whole group (N=258)			
	Smoking duration		Cotinine		Smoking duration		Cotinine		Smoking duration		Cotinine	
Variable	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
SBP (mmHg)	0.08	0.357	-0.03	0.753	-0.04	0.624	-0.13	0.132	-0.00	0.968	-0.10	0.129
DBP (mmHg)	0.12	0.185	0.03	0.757	0.06	0.476	-0.05	0.589	0.07	0.270	-0.04	0.526
HR (beats/min)	0.13	0.153	-0.03	0.706	-0.00	0.996	-0.12	0.190	0.13	**0.047**	-0.08	0.202
CO (L/min)	-0.01	0.933	0.04	0.696	-0.09	0.317	0.01	0.950	0.02	0.788	0.03	0.588
TPR (mmHg.s/mL)	-0.00	0.963	0.07	0.432	0.10	0.258	-0.05	0.591	-0.02	0.812	-0.00	0.965
Cwk (ml/mmHg)	-0.16	0.075	0.05	0.588	-0.06	0.535	0.09	0.329	-0.10	0.119	0.11	0.095
C-R PWV (m/s)	0.05	0.656	0.01	0.911	0.09	0.444	0.14	0.279	0.15	0.071	0.07	0.408
C-P PWV (m/s)	0.06	0.585	-0.06	0.567	-0.17	0.199	0.29	0.025	0.04	0.588	0.09	0.246
HDL-C (mmol/L)	0.14	0.135	0.02	0.851	0.05	0.575	-0.05	0.551	0.13	**0.035**	0.04	0.523
LDL-C (mmol/L)	-0.02	0.810	0.07	0.441	0.05	0.607	0.07	0.441	-0.01	0.880	0.05	0.444
TG (mmmol/L)	-0.02	0.860	0.11	0.220	0.08	0.382	0.14	0.117	-0.02	0.811	0.12	0.062
hs-CRP (mg/L)	0.05	0.565	0.03	0.731	0.11	0.224	0.07	0.473	0.13	**0.038**	0.08	0.187
Cotinine (ng/ml)	0.31	**≤ 0.001**	-	-	0.40	**≤ 0.001**	-	-	0.35	**≤ 0.001**	-	-

PWV was additionally adjusted for MAP. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CO, cardiac output; TPR, total peripheral resistance; Cwk, windkessel compliance; C-R PWV, carotid-radialis pulse wave velocity; C-P PWV, carotid-dorsalis pedis pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high-sensitivity C-reactive protein; MAP, mean arterial pressure.

Table 5: Correlations between smoking and measures of cardiovascular function and lipids in Caucasian participants.

	Men (N=161)				Women (N=211)				Whole group (N=372)			
	Smoking duration		Cotinine		Smoking duration		Cotinine		Smoking duration		Cotinine	
Variables	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
SBP (mmHg)	0.05	0.556	-0.07	0.397	-0.03	0.694	-0.05	0.454	0.07	0.161	-0.01	0.889
DBP (mmHg)	0.04	0.597	-0.05	0.558	0.13	0.061	0.06	0.362	0.09	0.078	0.03	0.565
HR (beats/min)	0.18	**0.021**	0.18	**0.023**	0.20	**0.004**	0.17	**0.019**	0.15	**0.005**	0.15	**0.004**
CO (L/min)	0.03	0.751	0.09	0.226	0.10	0.137	0.11	0.118	0.09	0.095	0.13	**0.013**
TPR (mmHg.s/mL)	0.00	0.989	-0.10	0.199	-0.06	0.426	-0.09	0.156	-0.05	0.329	-0.12	**0.019**
Cwk (ml/mmHg)	-0.13	0.098	0.07	0.410	0.02	0.762	0.06	0.364	0.01	0.786	0.13	**0.016**
C-R PWV (m/s)	0.09	0.279	-0.12	0.154	0.05	0.444	0.04	0.588	0.01	0.870	-0.01	0.902
C-P PWV (m/s)	-0.01	0.902	-0.02	0.770	0.00	0.956	-0.02	0.779	0.03	0.558	0.01	0.921
HDL-C (mmol/L)	-0.16	**0.046**	-0.20	**0.014**	-0.03	0.702	-0.00	0.945	-0.17	**≤ 0.001**	-0.15	**0.004**
LDL-C (mmol/L)	0.07	0.379	0.06	0.493	0.03	0.716	-0.04	0.617	0.06	0.286	0.02	0.737
TG (mmmol/L)	0.15	**0.052**	0.10	0.194	0.08	0.237	-0.00	0.974	0.16	**0.003**	0.08	0.116
hs-CRP (mg/L)	0.14	0.073	0.14	0.087	0.09	0.191	0.07	0.303	0.09	0.102	0.08	0.113
Cotinine (ng/ml)	0.71	**≤ 0.001**	-	-	0.84	**≤ 0.001**	-	-	0.74	**≤ 0.001**	-	-
After adjustment for age, BMI and WC												
	Men (N=161)				Women (N=211)				Whole group (N=372)			
	Smoking duration		Cotinine		Smoking duration		Cotinine		Smoking duration		Cotinine	
Variables	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
SBP (mmHg)	0.02	0.854	-0.04	0.660	-0.00	0.970	0.01	0.924	-0.00	0.957	-0.01	0.813
DBP (mmHg)	0.14	0.863	-0.02	0.844	0.18	**0.013**	0.14	**0.050**	0.07	0.198	0.06	0.240
HR (beats/min)	0.20	**0.016**	0.17	**0.033**	0.20	**0.006**	0.17	**0.023**	0.18	**≤ 0.001**	0.17	**0.002**
CO (L/min)	0.09	0.296	0.06	0.482	0.13	0.066	0.16	**0.021**	0.09	0.102	0.11	**0.044**
TPR (mmHg.s/mL)	-0.07	0.406	-0.05	0.569	-0.05	0.477	-0.11	0.138	-0.05	0.327	-0.07	0.161
Cwk (ml/mmHg)	-0.07	0.364	-0.08	0.300	-0.02	0.807	0.22	0.755	-0.07	0.216	-0.02	0.649
C-R PWV (m/s)	-0.18	0.349	-0.23	0.226	-0.45	0.096	0.22	0.438	-0.27	0.056	0.02	0.901
C-P PWV (m/s)	-0.00	0.980	-0.11	0.557	-0.03	0.925	0.39	0.156	-0.11	0.457	0.10	0.490
HDL-C (mmol/L)	-0.20	**0.012**	-0.18	**0.027**	-0.03	0.652	-0.02	0.395	-0.12	**0.026**	-0.10	**0.052**
LDL-C (mmol/L)	0.06	0.470	0.07	0.393	0.04	0.539	0.00	0.985	0.04	0.413	0.04	0.459
TG (mmmol/L)	0.16	**0.050**	0.12	0.131	0.11	0.111	0.05	0.503	0.13	**0.012**	0.09	0.088
hs-CRP (mg/L)	0.17	**0.037**	0.14	0.096	0.11	0.106	0.12	0.090	0.13	**0.015**	0.13	**0.017**
Cotinine (ng/ml)	0.73	**≤ 0.001**	-	-	0.84	**≤ 0.001**	-	-	0.74	**≤ 0.001**	-	-

PWV was additionally adjusted for MAP. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CO, cardiac output; TPR, total peripheral resistance; Cwk, windkessel compliance; C-R PWV, carotid-radialis pulse wave velocity; C-P PWV, carotid-dorsalis pedis pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high-sensitivity C-reactive protein. MAP, mean arterial pressure.

The Caucasian group (Table 5) generally did not show strong correlations with smoking (both chronic and acute). However, CO (r=0.13), TPR (r=-0.12) and Cwk (r=0.13) did reflect statistically significant correlations with cotinine in the whole Caucasian group, though most correlations disappeared after adjusting for age, BMI and WC. Significant correlation could also be seen between cotinine and the duration of smoking in the Caucasian group. Moreover, HR correlated positively and significantly with smoke throughout in the Caucasian group.

A statistically significant negative correlation existed between smoking (both chronic and acute) and HDL-C in the male Caucasian group before and after adjustments. TG increased with chronic exposure to smoking in men, even after adjusting for age, BMI and WC. Hs-CRP correlated positively and significantly with smoking duration, only after adjustment for age and obesity markers (BMI, WC) in men.

Fig 1a

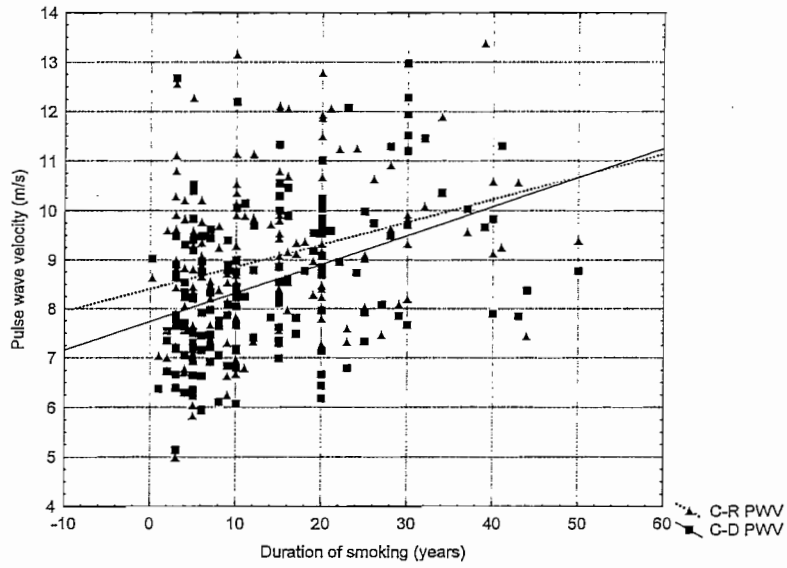

Fig 1b

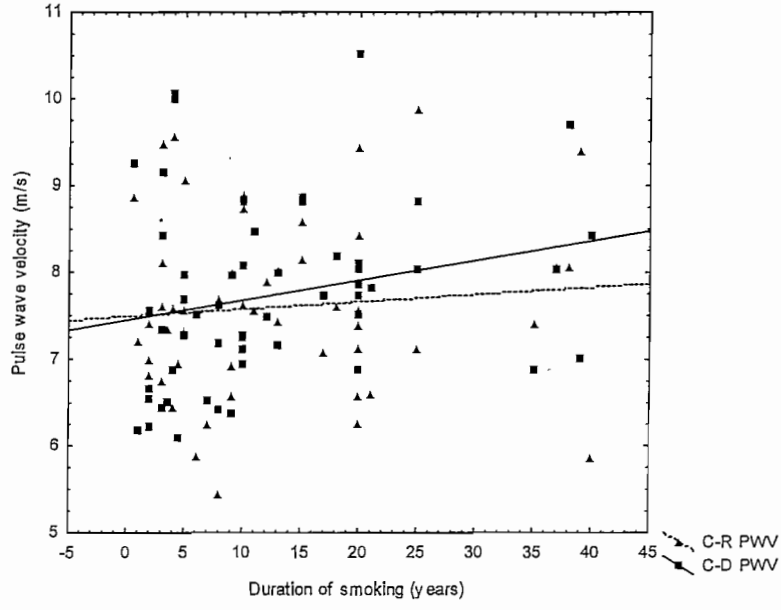

Figure 1: The relationship between duration of smoking and pulse wave velocity in Africans (1a) (C-R PWV: r=0.36 p < 0.001; C-P PWV: r=0.41 p < 0.001) and Caucasians (1b) (C-R PWV: r=0.01 p = 0.870; C-P PWV: r=0.03 p = 0.558). C-R PWV, carotid-radialis pulse wave velocity; C-P PWV, carotid-dorsalis pedis pulse wave velocity.

Discussion

Smoking is associated with vascular dysfunction[1,20,21] but studies regarding this topic are limited in South Africa. The first objective of this study was therefore to investigate the ethnic differences with regards to the prevalence of smoking between Africans and Caucasians, while the second was to compare associations of smoking with vascular dysfunction in these ethnic groups. The ethnic differences were indicated by a higher proportion of smokers in Africans over Caucasians in this South African sub-sample. These results contradict previous findings in South Africa which found Caucasians to smoke more.[9,22] However, our subsample consisted of African and Caucasian people who differed significantly with regards to SES, suggesting that one should be careful to extrapolate the results. Our results showed that African smokers were older than non-smokers, weighed less with lower BMI and WC values. These differences could be explained by the metabolic effects of nicotine in accelerating lipolysis,[1] with consequent reduction in weight and thus lean body mass in smokers. Many smokers, especially women, prefer smoking for the purpose of reducing weight,[23,24] including the more urbanized areas in South Africa.[25] However, the effect of advancement in medical techonology over the last decade has influenced the nutritional status and health in general. This might also have played some role in reducing the weights of the smoking group.

Moreover, the low SES confines Africans to cheaper mentholated nicotine packed brands of tobacco with longer half-life and more lipolytic effects while Caucasians can afford non-mentholated lighter brands.[5] Based on their SES and nicotine packed brands, Africans could afford to smoke less often while keeping significant plasma concentrations of nicotine. Furthermore, most African participants were in the lowest paid category, an indication of low SES, in which the majority of African smokers in this study were found. The higher prevalence of smoking in lower SES persons is due both to higher initiation rates and to lower cessation rates.[7,25] The low income African participants in this study were older and were long duration smokers. The cardiovascular markers related to smoking were generally higher in Africans, another indication of higher prevalence of smoking in Africans. For example, BP, PWV, hs-CRP and cotinine showed higher values in Africans. Low SES African people may also be less likely to benefit from increases in health-related knowledge for reducing risk to CVD.[21]

The mean serum cotinine of 199 ng/ml (95% CI: 178; 221) in Africans were much lower than the reported average of 250-300 ng/ml in African Americans,[3] probably due to lower SES of the South Africans. LDL-C and TG were higher in Caucasians compared to Africans, a feature supported by the literature.[26] Therefore, in addressing the first objective, in contrast to previous findings that the prevalence of smokers among Caucasians is higher,[22,27] our results indicate that the prevalence in Africans is higher in South Africa when also keeping in mind that random sampling did not take place in the present study.

The second objective was to investigate the ethnic differences with regards to the associations of smoking with cardiovascular and lipid markers. Africans showed more associations of cardiovascular risk factors with smoking than Caucasians, when smokers were compared with non-smokers, within each ethnic group. This trend is supported by literature findings.[21,28] Although the correlations between smoking and cardiovascular variables were not the replica of previous literature findings,[1,4,13] stronger correlations of PWV, HDL-C and hs-CRP were observed in Africans. Smoking acts with age and the metabolic syndrome to cause vascular dysfunction.[1,28] Cigarette smoking increases HR and CO both acutely as well as throughout the day with regular smoking,[1] a finding evident in both our groups to some degree, especially in Caucasian smokers. The increases in HR and CO are mediated by the beta adrenergic effects of nicotine.[1] Although nicotine does constrict some peripheral vascular beds,[4] it is likely that on increased HR and CO nicotine appears to dilate other vascular beds, through stimulation of epinephrine release,[1,4] thereby decreasing TPR, as found in our study groups.

The suggested factors that influenced the generally weak correlations in the study may include the relatively young age group of smokers in which stronger correlations may only be evident later in life. Weak correlations may also be affected by misclassification of chronic smoking by participants as it was determined only by self-reporting.[29] Low SES and poverty may cause Africans to suspend smoking on financial grounds,[24] or only smoking on occasions such as traditional ceremonies. Thus, chronic smoking in this way may be compromised. The weak correlations in Caucasians may be explained by the brands of smoke that have less effect on vascular function used by a better paid Caucasian population and the generally lower arterial stiffness and TPR compared to

Africans. The cotinine levels of the African and Caucasian smokers were not statistically different. However, the stronger correlation between cotinine and duration of smoking in Caucasians may be based on their higher SES, as they can more easily afford cigarette smoke at will. Thus, Caucasians may be expected to have persistently higher cotinine levels. Moreover, the total clearance of cotinine is generally lower in whites[3] leading to increased persistence of cotinine in their serum. Again, this might indicate that smoking in Africans acts with other cardiovascular risk factors to cause more associated effects than observed in Caucasians.

The relative risk of cardiovascular events is much greater in older versus younger smokers, indicating the important effect of smoking duration.[1,30] Thus, it would be interesting to observe whether there would be stronger correlations had other biological markers, in addition to cotinine, been investigated. Interestingly, African non-smokers showed quite high cotinine levels, possibly due to higher passive smoking prevalence in Africans. Most African non-smokers are exposed to smoking areas, such as spouses smoking in front of their partners and children[31] in small households, compared to more spacious houses of Caucasians, a fact that also points to the lower SES and low level of education in Africans.[5,27] Passive smoke exposure, like active smoke, activates platelets and produce endothelial dysfunction in exposed people.[31]

The differences in lipid profiles and hs-CRP between smokers and non-smokers are very significant in the literature.[4,30,32,33] Smokers generally revealed higher TG, LDL-C and hs-CRP levels, while HDL-C levels were significantly lower in smokers.[1,4] TG levels were significantly higher in smokers of both ethnic groups, a finding that is consistent with the literature.[1,4,30] The increased TG levels could be caused by nicotine in smoke, which directly accelerates lipolysis and also by increasing the release of corticosteroids and growth hormone, inducing insulin resistance,[1,4] especially in smokers who are also snuff users.[1] The raised TG levels in smokers is an independent risk factor for mortality in the form of stroke or myocardial infarction.[34] Differences in LDL-C were non-significant in both ethnic groups, though higher levels were observed in Caucasian smokers. Correlations between lipid profiles and smoking were generally weak in this study, a feature that was not expected, given the literature findings.[1,35]

HDL-C correlated positively and significantly with smoking in Africans. Literature is sparse regarding the high HDL-C level in smokers. However, a few studies from South Africa do mention significantly increased HDL-C levels in Africans even when smoking[28] and with cardiovascular disease.[33] Increased HDL-C production could possibly serve as a defense mechanism against oxidative stress caused by smoking, especially in Africans, known to generally have higher HDL-C levels than Caucasians.[26] Moreover, some studies[35,36,37] report no change in plasma concentrations of lipid profiles, including HDL-C when nicotine is delivered either in pharmaceutical forms or as snuff. It is likely that some African smokers in this study were also snuff users and therefore took cigarette smoke less often. This could reduce accumulation of more oxidant chemicals and thus less HDL-C destruction by oxidant gases from smoke.

However, higher HDL-C in African smokers than their non-smoker counterparts is a finding that needs further research for more clarity. Caucasian smokers, on the other hand, were less likely to be also snuff users as snuff use is more common in societies with low SES.[5,21] Low LDL-C and high HDL-C is more prevalent in black South Africans and protect them against some cardiovascular disorders such as ischemic heart disease (IHD),[33] and production of oxygen free radicals that deactivate nitric oxide (NO), especially in the presence of smoke.[15]

Hs-CRP, an inflammatory marker expressed by the liver,[38] was generally higher in smokers. This was followed by positive but relatively weak correlations of hs-CRP with smoking duration, in both groups. Hs-CRP is a marker that is increased even with acute smoking as seen in Caucasians (Table 5). Endothelial dysfunction caused by smoking promotes inflammation and leads to increased hs-CRP levels in smokers.[32,39] Age, gender, ethnicity, obesity, chronic infections and low SES are responsible for variations in hs-CRP levels.[40] As a result, the concentration of hs-CRP between smokers and non-smokers may be masked. This is reflected to some extent in this study, in which hs-CRP showed weak correlations with acute smoking throughout and also in the women participants.

As with all research studies, there are a number of limitations, and this study is no exception. One limitation is that the two ethnic groups were not matched for SES. Secondly, the study made use of an availability sample of subjects, and subjects were

therefore not selected on a random basis. This sampling method may also have influenced the difference in the number of smokers in the two ethnic groups. Thirdly, it would have strengthened the outcome of this study had coagulation markers been incorporated into the results, especially since it is known that Africans suffer from very high levels of fibrinogen,[20] and since smoking plays an important role with regards to coagulation.[1,4] Moreover, cotinine was the only biochemical marker used and this probably affected the correlations of smoking with cardiovascular variables.

In conclusion, this study showed that ethnic differences do exist with regards to the prevalence of smoking between our sub-sample of African and Caucasian people of South Africa, in which Africans smoked more than Caucasians. It seems as if SES plays an important role in this regard. Furthermore, our results showed more associations of smoking with vascular dysfunction through cardiovascular markers in Africans than Caucasians. A high degree of urbanization among Africans[41] coupled with higher smoking prevalence might be to blame for the high prevalence of cardiovascular diseases in the African population.

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Chapter 4

General findings and conclusions

INTRODUCTION

The main findings in the article will be presented, interpreted and compared to relevant literature in this chapter. Conclusions will be drawn and recommendations will be made regarding further research in smoking and vascular dysfunction in South African people.

SUMMARY OF MAIN FINDINGS

Smoking and vascular dysfunction in African and Caucasian people from South Africa: The SAfrEIC study (Chapter 3)

The aims of this study were to investigate if there are ethnic differences that exist with regards to the prevalence of the smoking habit between Africans and Caucasians of South Africa; and secondly, if there are associations between smoking and cardiovascular variables, and whether these associations differ between the two ethnic groups.

The African participants showed significantly higher prevalence with regards to the smoking habit compared to their Caucasian counterparts. Thus, in line with our first aim, there is a distinct difference in the prevalence of smoking between Africans and Caucasians of South Africa. Socio-economic status (SES), in addition to urbanization, could be important factors regarding the higher prevalence of smoking in Africans over Caucasians. Africans were from a low SES background, with low education and salaries. Low SES individuals are less likely to benefit from health-related knowledge for reducing the risk due to smoking.[1] It would have been interesting to see whether the finding would be consistent had the SES between the two groups been similar. Despite low cessation rate, low SES Africans are more likely to quit smoking.[2]

Significant differences in most cardiovascular and lipid variables were observed when smokers were compared with non-smokers within each ethnic group. This provided some evidence that smoking does affect vascular function. In African smokers, there were significantly higher levels of stroke volume (SV), cardiac output (CO), pulse wave velocity (PWV) and high-density lipoprotein cholesterol (HDL-C) compared to their non-smoker counterparts after adjustments were made. The higher PWV in smokers was only observed in Africans, which might indicate that smoking affects the elasticity of peripheral arteries more in Africans than Caucasians. Caucasian smokers, on the other hand, revealed higher heart rate (HR), CO, triglycerides (TG) and hs-CRP over the non-

smokers. Furthermore, there was a significant decrease in windkessel compliance (Cwk) and HDL-C in Caucasian smokers, a trend that supports literature findings.[3] Correlations between smoking and cardiovascular and lipid variables in both ethnic groups were generally weak. Significant correlations in Africans were only observed before adjusting for age, body mass index (BMI) and waist circumference (WC) in almost all groups. In Caucasians, except for HR, which showed a positive correlation with smoking throughout, smoking did not show correlations with most cardiovascular variables even after adjustments. Interestingly, a positive correlation was found between chronic smoking and hs-CRP in Caucasians after adjustments were made.

DISCUSSION OF MAIN FINDINGS

The ethnic differences in the prevalence of smoking and associated effects to cardiovascular dysfunction do exist between Africans and Caucasians.[2,4] Based on this study, the prevalence of smoking in black South Africans exceeds Caucasians. This could partly be the result of urbanization and westernized lifestyle in urbanized Africans.[5] Moreover, the metabolic syndrome is more prevalent in Africans.[6,7] The metabolic syndrome could act with chronic smoking to affect vascular function,[3] and probably bring forward the onset of most vascular conditions to an earlier age.

The findings of this study provide the basis for future studies on this topic and is not a complete representation of the entire South African population. Africans weighed less and had lower BMI values than Caucasians. Weight reduction could be the result of the lipolytic effect of nicotine in the body[3] in the African group. The more nicotine, the stronger its effect on fat metabolism, as could be the case with mentholated nicotine packed tobacco brands commonly used by lower SES Africans.[8,9]

The surprisingly weak correlations of smoking with cardiovascular and lipid variables may be attributed to a number of factors, which include the large gap in SES between the two ethnic groups and perhaps the less associated effect of snuff use on vascular function in African smokers.[10] Furthermore, the stronger catecholamine release in Africans leads to increased α-adrenergic effect and decreased β-adrenergic stimulation causing increased vasoconstriction and thus total peripheral resistance (TPR), especially in urbanized Africans,[11] even in non-smokers. This could also have been responsible for the weak correlations observed in Africans after adjustments were

made. PWV was significantly higher in African smokers and correlated positively with smoking only in African participants - a finding indicating the significant effect of smoking on arterial stiffness in Africans.[3] Increased PWV is due to a nicotine effect causing stiffening of the blood vessel walls.[3,12] HDL-C, a lipoprotein of importance, was higher in African smokers than non-smokers, a finding that is difficult to interpret and needs further investigation.

COMPARISON OF FINDINGS WITH THE LITERATURE

When the results from this study are compared to the literature, it is evident that some findings are consistent with those found in the literature, whereas others are not. Supporting findings from this study were the generally higher values of cardiovascular and lipid variables in Africans over Caucasians.[13,14] Moreover, there was a distinct difference in these markers between smokers and non-smokers within each ethnic group,[3,12] a feature possibly also showing the associated effects of smoking on vascular function. Most African smokers were from the low SES and were found to be more prevalent smokers in this study, supporting available evidence in literature, especially in South Africa.[15,16]

Contradictory findings, however, included the higher prevalence of smoking in Africans over Caucasians in South Africa. Studies on smoking prevalence in South Africa[2,17] reported a higher prevalence of smoking among Caucasians. Furthermore, the higher HDL-C values in smokers compared to non-smokers in Africans is a finding in this study which has not, to date, been supported by the literature, although smokeless tobacco use may have no effect on lipid profiles of smokers[10] but does not increase HDL-C of smokers. Smokers generally have low HDL-C levels because oxidant gases in smoke destroy these lipid variables, especially in cigarette smoke.[12,14] Smokeless tobacco users not using cigarette smoke are not exposed to oxidant chemicals, hence no decreased HDL-C in their blood.[10] Generally, lipid profiles are similar in smokeless tobacco users and in non-tobacco users[3,10], though it could be speculated that HDL-C levels may be elevated in smokers as a defense mechanism against oxidative stress.

Another contradictory finding was the weak correlations between smoking and cardiovascular (including lipid) variables (Tables 4 and 5 in Chapter 3), while previous studies show these variables to have a strong correlation with smoking.[3,18] The

associated effects of smoking on vascular dysfunction are generally more evident in older populations. Perhaps our study group, which had a mean age of 40 years, was not quite suitable to show any strong correlations between smoking and cardiovascular variables, especially chronic smoking. Moreover, fewer smokers in Caucasians and the additional use of smokeless tobacco by some African smokers in the study could have influenced the results and been responsible for the weak correlations in these ethnic groups, respectively.

CHANCE AND CONFOUNDING

It is important to point at some factors that may have influenced the results of this study. Some methodological issues may have caused weaknesses in the study and may therefore have influenced the outcomes of the study.

Although the number of participants (about 630 participants) used in this study were more than the required number by statistical power analysis, the entire population of the country cannot be represented by this group since this group was recruited only from the Potchefstroom region of the North-West Province. This study also included an availability sample of volunteers. In the African population these are typically people without employment who have the time to take part in research, whereas the Caucasian subjects were typically people who were concerned about their health and were absent from work in order to get a "free health check-up". This could provide the reason for the large gap in SES between the two ethnic groups.

Concerning the results, the possibility of chance should be taken into account. By using correlations, statistics indicate that one out of twenty significant correlations may be because of chance.

CONFOUNDERS

Although an inclusion criteria for this study was that subjects should be "apparently healthy", their health could not be guaranteed. Other confounding factors such as diet, physical activity level, alcohol intake and diabetes could have influenced the outcome of the study. However, in this study, age, obesity markers and HIV positive status were addressed, since HIV subjects were excluded and there were statistical adjustments for age and obesity markers.

WEAKNESSES OF THE STUDY

Weaknesses of the study included:

1. The exclusion of 146 (due to HIV positive status) and 8 subjects (due to incomplete datasets) could have affected the statistical significance of the various analyses.

2. Cotinine is the important biochemical marker and was used in this study. However, other markers, in addition to cotinine, in the form of carbon monoxide, urinary or salivary nicotine and other nicotine metabolites such as nicotine N´-oxide and nornicotine, could have provided stronger correlations between smoking and the cardiovascular variables in the study.

3. The study did not consider the associated effects of smoking on vascular dysfunction in passive smokers, which is as crucial as active smoking itself.

4. The gap in SES between the two ethnic groups was large, and because SES appeared to have played a vital role in prevalence of smoking, this could have had more influence on the outcome of the results of this study. It is nevertheless noteworthy that most Africans in South Africa are from a lower SES, and most Caucasians from a higher SES, which indicates that the results from this study are relevant to the South African situation to some extent.

5. The Caucasian group had far fewer smokers than the Africans, and this could have affected the significance of results of some analyses in the study. A possible explanation for the fewer Caucasian smokers could be that the Caucasian volunteers who participated were those who were concerned about their health. In many instances (but not all) smokers may not fall into this category of people looking after their health.

6. The study made use of an available sample of subjects, and perhaps the results are biased since subjects were not selected on a random basis.

7. Coagulation markers did not form part of this study. This could have strengthened the results of the study, especially since it is known that Africans

suffer from very high levels of fibrinogen,[19] and since it is known that smoking plays an important role with regards to coagulation.[3]

CONCLUSIONS

There is enough evidence to conclude that Africans smoked more than Caucasians in this sub-samples of South Africans. Although it was not directly brought into analyses, it is expected that SES is one of the main factors influencing the prevalence of smoking between the two groups. Concerning the second aim, our results showed more associations of smoking with vascular dysfunction through cardiovascular markers in Africans. A higher degree of urbanization among Africans coupled with higher smoking prevalence might be to blame for the high prevalence of cardiovascular diseases in the African population.

RECOMMENDATIONS

The following recommendations are proposed for future studies:

- Although the age range of the study group was quite wide (20 – 70 yrs), a follow-up study is recommended in which participants in the age range of 60 – 80 yrs are recruited. This could provide better associated correlations of smoking with vascular function, as some of the smoking effects are evident later in life, especially with chronic smoking. On the other hand, a study of smoking on a younger population (20 - 40 years) could provide more acutely associated affects of smoking on vascular dysfunction before the onset of age-related cardiovascular diseases (CVDs).

- The use of other biochemical markers of smoking such as carbon monoxide in both active and passive smokers, and other nicotine metabolites in the form of nicotine N´-oxide and nornicotine, should be included in order to provide more in-depth results from various perspectives.

- The SES of the participants should be considered when future studies are performed by matching participants from two ethnic groups for SES, i.e. the level

of education and income status should be evenly distributed in both groups during sampling.

- Further investigations on smoking and vascular dysfunction are recommended in which positive associations between smoking and HDL-C in African smokers and association of smoking and coagulation markers are studied.

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