

Cardiovascular disease and reduced pulmonary function in black South Africans: Investigating the interplay with markers of systemic inflammation

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“Be strong and courageous. Do not be afraid; do not be discouraged, for the Lord your God will be with you wherever you go.” ~ Joshua 1:9

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PREFACE

This thesis is presented in article-format and consists of three peer-reviewed published or submitted manuscripts (presented in chapters 3, 4 and 5), as approved by the North-West University's guidelines for postgraduate studies. The layout of this thesis is as follows:

Chapter 1: The introductory chapter offers a detailed literature review. The motivation, aim and hypotheses, formulated from the literature, are also included in this chapter.

Chapter 2: Describes in detail the PURE study protocol, methods of data collection and statistical analyses that were performed.

Chapter 3: The first manuscript entails the comparison of respiratory prediction values obtained from three different reference populations namely from Europe, the United States and South Africa, in a large sample of black South Africans and furthermore describes the association between lung function and blood pressure in these individuals. These results were published in the journal: *Heart, Lung and Circulation*, 2015.

Chapter 4: This manuscript explores the possible role of systemic inflammation as the mediator between lung function and arterial stiffness. These results were published in the journal: *Lung*, 2016.

Chapter 5: In a third manuscript, the contribution of lung function in predicting all-cause and cardiovascular mortality in Africans was investigated, while taking inflammatory markers into account. This manuscript was submitted to the *European Journal of Clinical Investigation*, 2016.

Chapter 6: A summary of the main findings is provided - all the presented results are critically discussed, conclusions are drawn and applicable recommendations are made.

The promoter and co-promoters were included as co-authors in each manuscript, together with collaborators who provided additional input regarding spirometry in the manuscripts and participated in the concept and design of the PURE study. The first author, namely the PhD candidate, was responsible for the initiation and all parts of this thesis, including literature searches, collection and cleaning of data, statistical analyses, interpretation of results, as well as writing of the manuscripts. All co-authors gave their consent that the manuscripts could be included in this thesis (pages vii-viii). The relevant references are provided at the end of each chapter. Each manuscript was prepared according to the instructions for authors of the individual journals (which was summarised before each manuscript). In order to ensure uniformity throughout the thesis, the Vancouver reference style was used throughout.

AUTHOR CONTRIBUTIONS

The researchers listed below contributed to this thesis in the following capacities:

Mrs. Y. Breet

Responsible for initial proposal of this study along with all extensive literature searches, critical evaluation of study protocol and methodology, data cleaning and composition of the spirometry data set, statistical analyses, design and planning of research articles and the thesis, interpretation of results and writing of all sections of this thesis.

Prof. J.M. van Rooyen (promoter), Prof. H.W. Huisman and Prof. A.E. Schutte (co-promoters)

Responsible for guidance, intellectual input, data collection and critical evaluation of statistical analyses and also the final product.

Prof. F.C. Eloff and Prof. J.L. du Plessis (co-authors of manuscripts)

Valued expert input and collection of spirometry data in the manuscripts presented in chapters 3, 4 and 5.

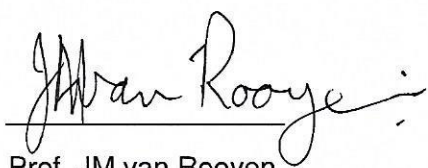
Prof. A. Kruger (co-author of manuscripts)

In her capacity as project leader of the South African leg of the PURE study, provided intellectual input in the manuscripts presented in chapters 3 and 4.

STATEMENT BY THE AUTHORS

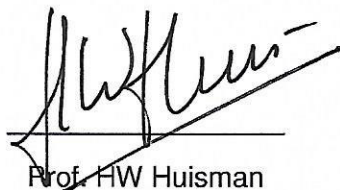
The following is a statement of the contributors verifying their individual contribution and involvement in this study and granting their permission that the relevant research articles may form part of this thesis:

Hereby, I declare that I approved the aforementioned manuscript and that my role in this thesis, as stated above, is representative of my actual contribution. I also give my consent that the manuscript may be published as part of the PhD thesis of Mrs Yolandi Breet.



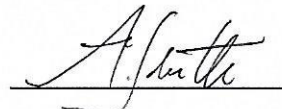
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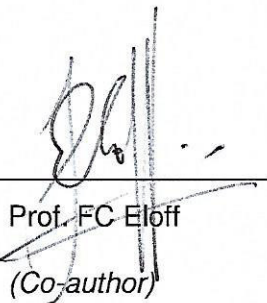
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SUMMARY

Motivation

In South Africa, the process of rapid urbanisation has led to a high prevalence of non-communicable diseases such as chronic respiratory and cardiovascular diseases which are accompanied by a high cardiovascular mortality rate. The identification of possible risk factors for this disease burden is essential for prevention and the allocation of healthcare and treatment regimens.

Lung function differs between different populations of the world and the use of appropriate reference data is important as inaccurate interpretation may lead to misdiagnosis. From international literature it is known that reduced lung function is associated with various cardiovascular variables such as blood pressure and arterial stiffness, and is also associated with an increased risk of cardiovascular-related mortality. The mechanism driving this association remains largely unexplained. It has been hypothesised that the changes in lung- and arterial function originate from the same pathophysiological process which could be mediated by systemic inflammation.

However, whether lung function plays a role in the development of cardiovascular disease and whether reduced lung function could predict all-cause and cardiovascular mortality among the understudied black South African population, remain to be established. In addition, the role of systemic inflammation in this regard needs to be explored.

Aim

The central aim of this study was to determine the potential role of lung function in the development of cardiovascular disease in a black South African population and to investigate whether inflammation is the mechanistic link between these two disease states. We therefore explored the associations between lung function and measures of cardiovascular function as well as between lung function and inflammatory markers. Finally, we determined the value of

lung function in predicting cardiovascular mortality over five years, whilst taking inflammatory markers into account.

Methodology

This sub-study, which is embedded in the international Prospective Urban and Rural Epidemiology (PURE) study, included an apparently healthy cohort of black South African volunteers of ages older than 35 years from the North-West Province, South Africa. Baseline data collection took place in 2005 during which 2010 men and women from urban and rural areas were included. The first follow-up took place in 2010 and a total of 218 participants had passed away over the five year follow-up period, with cardiovascular mortality contributing to 63 deaths and non-cardiovascular mortality to 155.

Standardised methods were used to capture all data and included health questionnaires (lifestyle factors, medication usage, disease status and history), cardiovascular and anthropometric measurements, spirometry as well as biochemical analyses of inflammatory markers (C-reactive protein, interleukin-6), HIV status and relevant metabolic markers. Verbal autopsies were performed to establish mortality outcome.

In preparation for statistical analyses, non-Gaussian variables were logarithmically transformed. We compared means and proportions with independent *t*-tests, analysis of variance, analysis of covariance (for adjustments) and Chi-square tests. We determined relationships between variables with Pearson's correlation coefficients. Independent relationships were determined with logistic regression, forward stepwise multiple regression and proportional Cox-regression analyses. Mortality rates were calculated using Kaplan-Meier survival function estimates and log-rank tests. In all cases, p values ≤ 0.05 were regarded as statistically significant.

Results and conclusions of each manuscript

Three manuscripts were written in order to achieve the main aim of this thesis. In the first manuscript we compared respiratory prediction values from three different reference populations namely from Europe, the United States and South Africa and secondly explored whether lung function is associated with blood pressure in a large sample of black South Africans. We showed that South African reference values displayed the highest percentages of the predicted values for forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), (87.9 and 99.7%, respectively.) Blood pressure increased with a reduction in lung function for both FEV₁ and FVC, (p for trend <0.001). After adjustment for potential confounders, the correlations remained significant (p<0.05). Our findings suggest that South African prediction equations may be more useful when investigating lung function in black South Africans. Furthermore, elevated blood pressure is related to reduced lung function, highlighting the importance in managing both respiratory- and cardiovascular disease.

In the second manuscript the possible role of systemic inflammation as the mediator between lung function and arterial stiffness in a sample of black South Africans was determined. An independent inverse association was found between interleukin-6 (IL-6) and FEV₁ ($\beta=-0.20$, p<0.001) and FVC ($\beta=-0.18$, p<0.001). Similar results were found for C-reactive protein (CRP). Pulse wave velocity (PWV) was inversely associated with FEV₁ ($\beta=-0.06$, p=0.037). No association was found between inflammatory markers, blood pressure or PWV, suggesting that inflammation may not be the mediating link between lung- and vascular function in this population.

In the third manuscript the contribution of lung function in predicting all-cause and cardiovascular mortality in Africans was investigated, while taking inflammatory markers into account. The cardiovascular mortality group had the lowest FEV₁ and FVC values when compared to the survivors and the non-cardiovascular mortality group. CRP did not significantly predict all-cause or cardiovascular mortality in any of the Cox-regression models, however IL-6

predicted all-cause mortality independent of potential confounders. Furthermore, FVC predicted cardiovascular mortality independent of several covariates (hazard ratio, 0.57 [0.35-0.94]), including C-reactive protein (CRP). When CRP was replaced by IL-6 in the model, the significance of FVC was lost (hazard ratio, 0.85 [0.55-1.30]). Our results suggest that FVC is a strong predictor of cardiovascular mortality in this population of black South Africans and that this association may be mediated by IL-6. However, further research is needed to establish the exact mechanism behind this association and this provides a strong motive for future research on the matter.

General conclusion

We showed for the first time that reduced lung function is independently associated with arterial stiffness and is prognostic of cardiovascular mortality in a large black population. In addition, elevated blood pressure is also related to reduced lung function. Although we further determined that reduced lung function is associated with increased inflammation in this population of black South Africans, the role of inflammation as the mediator for the relationship between lung function and CVD remains controversial. However, our findings showed that IL-6 seems to play a more significant role than CRP in this regard. This study underlines the importance of preserving normal lung function, both in an occupational- and household environment. Ultimately, our findings lend support to the consideration of reduced lung function as a risk factor for the high prevalence of cardiovascular morbidity and mortality in black South Africans.

Key words: blacks; epidemiology; lung function; inflammation; cardiovascular function; mortality; prognostic

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Figure 1 - Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), respectively.

LIST OF ABBREVIATIONS

ANCOVA	-	Analysis of covariance
ANOVA	-	Analysis of variance
BMI	-	Body mass index
BP	-	Blood pressure
bpm	-	Beats per minute
CI	-	Confidence interval
cm	-	Centimetre
COPD	-	Chronic obstructive pulmonary disease
CRP	-	C-reactive protein
CV	-	Cardiovascular
CVD	-	Cardiovascular disease
DBP	-	Diastolic blood pressure
Et al.	-	Et alia “and others”
FEV₁	-	Forced expiratory volume in one second
FVC	-	Forced vital capacity
GGT	-	Gamma-glutamyltransferase
HDL	-	High-density lipoprotein
HIV	-	Human immunodeficiency virus
HR	-	Hazard ratio
IL	-	Interleukin
kg	-	kilogram
L	-	Litre

LLN	-	Lower limit of normality
log	-	Logarithm
m	-	metre
MAP	-	Mean arterial pressure
mmHg	-	millimeters mercury
mmol/L	-	millimole per litre
m/s	-	metres per second
N	-	Number of
NCDs	-	Non-communicable diseases
NO	-	Nitric oxide
p	-	probability
pg/ml	-	picograms per millilitre
PP	-	Pulse pressure
PURE	-	Prospective Urban and Rural Epidemiology
PWV	-	Pulse wave velocity
r	-	regression coefficient
R²	-	Relative predictive power of a model
ROS	-	Reactive oxygen species
SBP	-	Systolic blood pressure
SD	-	Standard deviation
SE	-	Standard error
suPAR	-	Soluble urokinase plasminogen activator receptor
TB	-	Tuberculosis

- TC** - Total cholesterol
- UK** - United Kingdom
- U/L** - Units per litre
- USA** - United States of America
- TNF- α** - Tumor necrosis factor- α
- vs** - Versus
- WC** - Waist circumference
- WHO** - World Health Organization

CHAPTER 1

**INTRODUCTION, LITERATURE STUDY, AIMS,
OBJECTIVES AND HYPOTHESES**

1. GENERAL INTRODUCTION

There is an increasing burden of non-communicable diseases (NCDs) in sub-Saharan Africa (SSA) ^{1,2} and projections from the World Health Organization (WHO) state that NCDs will account for 46% of mortality in SSA by 2030.³

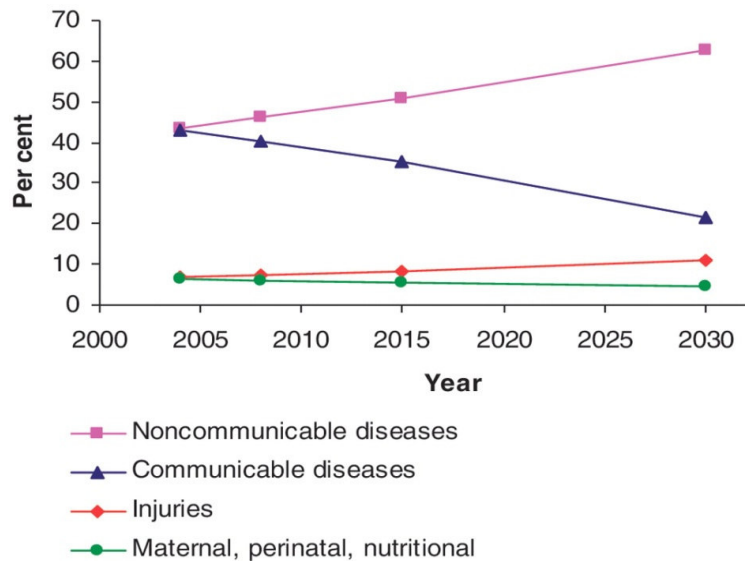


Figure 1: Estimated proportions of age-standardized mortality rates by cause in SSA. Mortality estimates were standardized to the WHO World Standard Population. Source: WHO. Global Burden of Disease. Projections of mortality and burden of disease, 2002-2030.³

This increase in the incidence of NCDs is likely due to rapid urbanisation, which is associated with a change in lifestyle factors^{1,4} such as an unhealthy diet, a decrease in physical activity, smoking, obesity and alcohol abuse.^{5,6} With regard to cardiovascular disease (CVD), the prevalence is twice as high in developing countries such as South Africa, when compared to developed countries and the relatively young age of CVD-related deaths are becoming an even greater concern.⁷ Therefore, there is an urgent need to identify possible risk factors contributing to the development of CVD to combat the high cardiovascular mortality rate.

Reduced lung function is associated with an increased risk for CVD as well as CVD-related mortality;⁸ however, the exact mechanism behind this association is not fully understood. It has been suggested that chronic low-grade inflammation may be mediating this association.⁸

Inflammation plays an integral role in CVD development^{9,10} and in general is a known risk factor for cardiovascular mortality.^{11,12} Reduced lung function is also associated with persistent low-grade inflammation¹³⁻¹⁵ with increased levels of acute phase proteins such as interleukin-6 (IL-6) and C-reactive protein (CRP).¹⁶ It is plausible that there are parallel physiological pathways leading to changes in elasticity in both the lungs and vasculature and inflammatory pathways may possibly link arterial elasticity and lung function.¹⁷

In the literature overview, the background of lung function and lung function measurement will be addressed, as well as the applicable respiratory pathophysiology. In addition, a broad overview will be provided regarding cardiovascular function and measures thereof. The role of inflammation in reduced lung function and cardiovascular disease development and mortality will also be discussed. Finally, this chapter includes a short motivation for each research article (chapters 3, 4 and 5) as well as the aims, objectives and hypotheses.

2. LITERATURE OVERVIEW

2.1 LUNG FUNCTION

Optimal gas exchange between the lungs and the pulmonary capillaries depend on efficient ventilation and involves the movement of the chest wall to create a pressure gradient that will allow the flow of air in and out of the lungs.¹⁸ Inhalation is dependent on contraction of the diaphragm and intercostals where expiration is predominantly a passive process, resulting from the elastic recoil of the chest wall and lungs.¹⁹ The mechanical properties of the respiratory system are evaluated by pulmonary function tests which are used to categorise the nature and severity of respiratory disorders and to establish response to therapy.²⁰

2.1.1 Measures of lung function

Spirometry

A widely used method for the measurement of lung function is spirometry – a physiological test that measures how a volume of air is inhaled and exhaled as a function of time and the primary signal measured is the volume or flow.²¹ The air volumes and air flow rates of the lung are influenced by the physical properties of the airways, lung parenchyma, pleura and chest wall as well as the strength of the respiratory muscles.^{22,23}

In order to determine the range of normal values of air volumes in different populations and detect abnormalities, it is necessary to reduce the variability of results and increase the accuracy of measurement.²¹ Adequate spirometry relies on factors such as competent operators, accurate equipment, standard operating procedures, quality control and patient co-operation.^{23,24} To aid in the achievement of these prerequisites, the American Thoracic Society (ATS) has issued statements on the standardization of spirometry.^{25,26} This initiative was also implemented by the European Community for Steel and Coal in 1983²⁷ which was then updated as the official statement of the European Respiratory Society (ERS) in 1993.²⁸

Most modern computerised spirometers that are commercially available are flow-type spirometers.²⁰ These spirometers make use of a flow-sensor to derive volumes and display expiratory and inspiratory efforts as flow-volume curves (Figure 2).²³ Two of the most important variables that are measured by spirometry are the forced vital capacity (FVC), which is the maximal volume of air that is exhaled with full effort after a maximal inhalation, and the forced expiratory volume in one second (FEV₁), which is the maximal volume of air exhaled in the first second of forced expiration.²¹

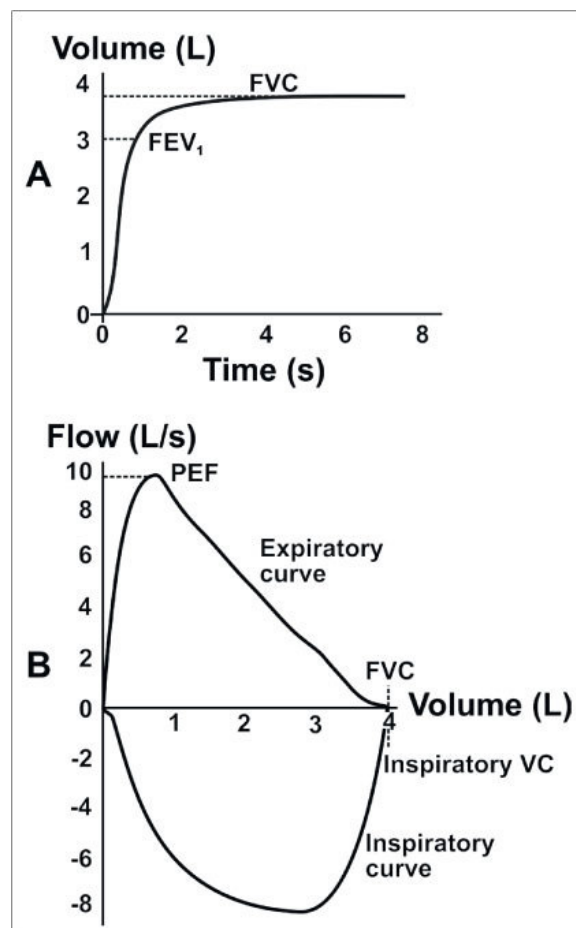


Figure 2. A: volume-time and B: flow-volume curves. When using a flow-type spirometer, FEV₁ is a derived value read from the flow-volume graph. FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow; VC, vital capacity.²⁰

Reliable interpretation of lung function results is based upon the comparison of observed results with appropriate reference data to aid in the identification of any abnormality and the assessment of any functional impairment that may be present.²⁹

Reference data for the assessment of lung function.

Lung and airway size in healthy individuals are largely determined by age, height and gender and predicted values for FVC and FEV₁ are calculated from equations that are based on these factors.^{28,30,31} These prediction equations are often derived from a sample of healthy individuals from a general population. However, some difficulties lie in the definition of health and it is of great importance that inclusion and exclusion criteria should be evaluated carefully depending on the specific use of the reference ranges.²⁹ The sample that is selected should be

generalizable and the characteristics of the reference population should first be evaluated by the user before implementation to ensure that the reference data is applicable to the study population.²⁹ Although population-specific equations are expensive and logistically problematic to determine, they may provide a more suitable representation of the specific population under investigation.²⁹ The use of predicted values that are not applicable to the study population, may lead to under- or over diagnosis.²⁰

It has been demonstrated that population groups indigenous to southern Africa show a FVC and FEV₁ up to 10% lower, when compared to Europeans.²³ There still remains a lack of appropriate prediction equations for ethnic groups other than those of European descent and ethnic-specific equations require large representative samples that are not easily accessible.²⁹ In South Africa there is also a lack of a large, all-inclusive study and therefore the European Community for Steel and Coal prediction equations are most widely used.²⁰ It has been suggested that a correction factor of 0.9 should be used to adjust predicted values for individuals of African or Asian ancestry.²³ To address the lack of suitable prediction equations for a population of African ancestry, studies have been conducted to develop normative lung function values.^{32,33} However, these prediction equations are rarely used as they are not implemented in software packages of commercially available spirometers.²⁰ It is therefore necessary for the interpreting clinician to consider the most appropriate reference equations for their practice and whether to incorporate ethnical adjustment.²⁰

Interpretation of spirometric results

Assessment of respiratory function is based on an algorithm (Figure 3) incorporating three variables namely the volume of air expired in one second as a percentage of the total expired volume (FEV₁/FVC %), the forced vital capacity as a percentage of the predicted value (FVC % predicted) and the forced expiratory volume in one second as a percentage of the predicted value (FEV₁ % predicted).

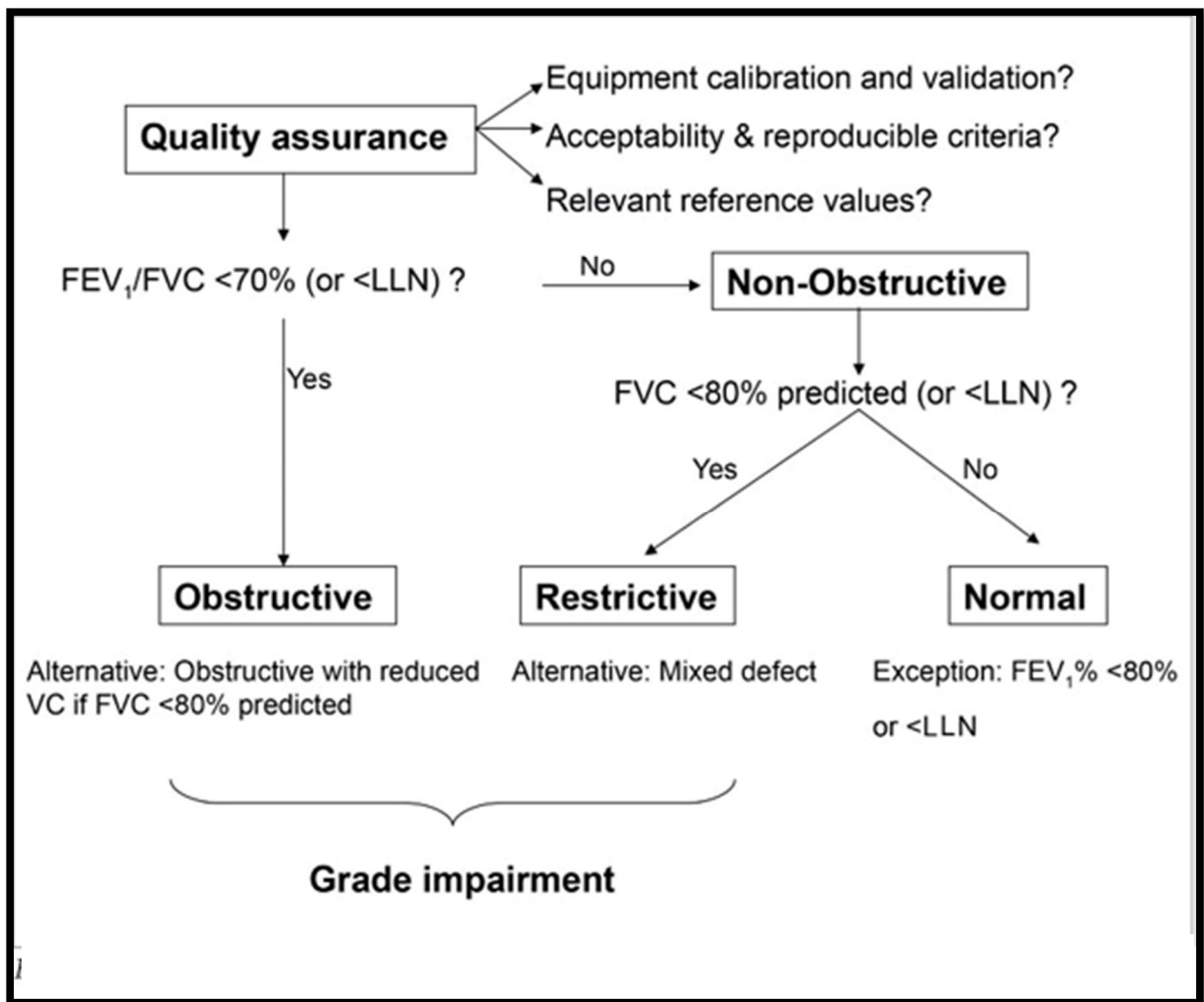


Figure 3: An algorithm for the categorization of spirometry. LLN = lower limit of normal.²⁰

An obstructive ventilatory defect, such as chronic obstructive pulmonary disease (COPD), is characterised by a reduction of maximal airflow from the lung in relation to the maximal volume that can be displaced from the lung (vital capacity).²⁰ This is indicative of narrowing of the airways during expiration and is described by a FEV₁/FVC ratio that is below the 5th percentile of the predicted value.³⁴ A restrictive defect is inferred when the FEV₁/FVC% is normal or high and the FVC is reduced, which may be caused by conditions such as interstitial fibrosis.²⁰ Both obstructive and restrictive lung diseases are important causes of morbidity and mortality.^{35,36}

2.1.2 Pathophysiology

Lung diseases in the South African context

South Africa is a country rich in minerals and mining of these minerals generates wealth for the country and is a major source of employment. The majority of those employed in the mining sector are black migrant workers from rural districts.³⁷ Mining poses several direct and indirect health risks, such as injury and occupational lung diseases.³⁷ The prevalence and severity of mining-related occupational lung diseases are influenced by several factors, including the commodities mined, airborne hazard exposure levels, the period of exposure and co-existing illnesses as well as lifestyle factors.³⁸ The commodities that are of major public and occupational health importance include asbestos, coal and silica, as exposure to these materials contribute significantly to the development of diseases such as asbestosis, silicosis and coal workers' pneumoconiosis.³⁸ The age-old relationship between silica exposure and tuberculosis (TB) has also acquired a renewed importance with the growing epidemic of Human Immunodeficiency Virus (HIV) in developing countries such as South Africa.³⁸

Apart from the mining industry, exposure to organic and inorganic dust particles in the agricultural sector may also increase the risk of developing respiratory diseases such as silicosis and lung cancer.³⁹ This is especially important in South Africa where there is a high rate of HIV infection, as silicosis in HIV-positive individuals increases the risk of contracting TB significantly.⁴⁰ Furthermore, a form of pneumoconiosis in rural African women termed "Transkei silicosis" has been thought to be due to silica particles inhaled while they are hand grinding maize between rocks.⁴¹ This activity is performed daily for 30-90 minutes by girls and women over the age of 9 years, so that by the fifth decade, when most cases are discovered, there has been significant exposure.⁴¹ Women and children are also at increased risk of exposure to respirable quartz due to a significant amount of time spent near smoky wood fuelled stoves in poorly ventilated dwellings, further increasing the risk for developing pneumoconiosis.⁴²

The pathogenesis of pneumoconiosis is centred on increased inflammation and pulmonary fibrosis.⁴³ The alveolar macrophages play an important role, as these cells release inflammatory growth and differentiation factors.^{44,45} Cells such as endothelial cells, epithelial cells and fibroblasts have been shown to be effectors, secreting and expressing various cytokines and molecules involved in inflammatory and fibrotic processes.^{46,47} The pneumoconiosis diseases may present as obstructive or restrictive in nature.³⁸

Restrictive lung disease

Restrictive lung disease includes a variety of conditions with the effect of a reduced total lung capacity and resting volume, yet often a normal resistance to airflow.⁴⁸ These diseases occur due to alterations in the lung parenchyma or abnormalities in the pleura, chest wall, or neuromuscular apparatus.⁴⁹

Obstructive lung disease

Obstructive lung disorders are mainly characterised by an increased resistance to airflow by conditions occurring either inside the lumen, in the wall of the airway, or in the peribronchial region.⁵⁰ Conditions which infer the inside of the lumen, such as bronchitis, cause the airway to be partially obstructed by excess secretions.⁵¹ The wall of the airway can be affected by conditions such as asthma, where there is contraction of the bronchial smooth muscle cells,⁵² or chronic bronchitis which causes hypertrophy of the mucous glands.⁵³ The peribronchial region may be affected by conditions such as emphysema which is characterised by a destruction of lung parenchyma.⁵⁴

Chronic obstructive pulmonary disease

Patients who either have chronic bronchitis or emphysema, or even a combination of both, are diagnosed as having COPD. This condition involves pathological changes in four different compartments of the lungs, namely the central airways, peripheral airways, lung parenchyma and the pulmonary vasculature.⁵⁵⁻⁵⁸ The main risk factor for COPD is tobacco use; however, exposure to other inhaled noxious particles may further increase the risk.⁵⁹ The inhalation of tobacco smoke and noxious particles causes an inflammatory response, which is responsible for the persistent airflow limitation that characterises COPD.¹⁶ An imbalance of proteinases and anti-proteinases in the lung as well as oxidative stress further contribute to the pathogenesis of COPD⁶⁰ and gives rise to the various physiological abnormalities (Figure 4) which includes cardiovascular compromise.⁵⁰

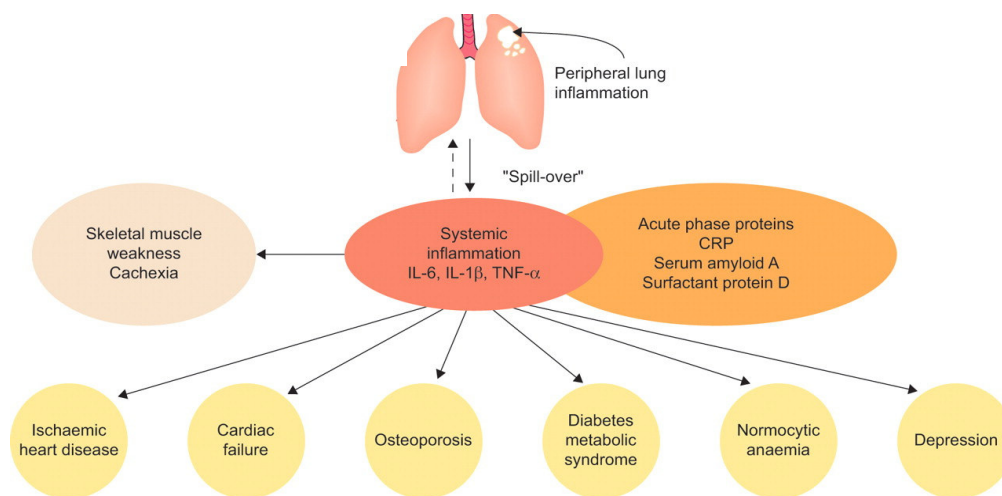


Figure 4: One of the systemic effects of COPD is cardiovascular compromise. Adapted from Barnes et al.¹⁶

2.2 THE CARDIOVASCULAR SYSTEM AND ITS RELATION TO LUNG FUNCTION

The cardiovascular system comprises the heart and blood vessels. A significant amount of mechanisms are involved for the homeostasis of important cardiovascular elements such as blood pressure (BP) and arterial tone. One of these mechanisms is the functioning of the endothelium, described by Gibbons and Dzau to be “a mechanoreceptor within the vasculature that senses flow or pressure and modulates vascular tone accordingly”.⁶¹

The endothelium, inflammation and atherosclerosis

Arteries consist out of three layers, namely the intima, media and adventitia.⁶² Within the intima is the endothelium, a single layer of cells which play an important role in the homeostasis of the circulation.⁶² The endothelium is responsible for the secretion of various vaso-active factors and under normal conditions the net-effect of these factors is to maintain normal vascular tone, blood fluidity and prevent vascular inflammation.⁶³ Damage to the endothelium may occur due to various factors which include mechanical stress associated with hypertension⁶⁴ and cigarette smoking.⁶⁵ This changes the phenotype of the endothelium to promote inflammation, thrombosis, vasoconstriction and atherosclerotic lesion formation.⁶⁶

Atherosclerosis was formerly regarded as a simple lipid storage disease; however, recent advances have illuminated the role of inflammation and the underlying cellular and molecular mechanisms that contribute to this disease.⁶⁷ During mechanical stress, arterial endothelial cells express selective adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) on their surface.⁶⁷ These adhesion molecules also serve as indirect markers of endothelial dysfunction,^{68,69} which is an early manifestation in the atherosclerotic process.⁶⁹ In response to these adhesion molecules, neutrophils and monocytes adhere to infected and damaged tissues and infiltrate the arterial wall where they differentiate into macrophages.⁷⁰ The macrophages express scavenger receptors for modified lipoproteins which permits these proteins to ingest lipids and become foam cells.⁶⁷ Furthermore, macrophages also have the ability to activate T-

cells through antigen presentation, leading to the production of a substantial amount of molecules downstream in the cytokine cascade such as CRP and IL-6.⁷¹⁻⁷³ As this inflammatory cascade continues, the activated leukocytes can release fibrogenic mediators which promote the replication of smooth muscle cells and contribute to the formation of a dense extracellular matrix, characteristic of the more advanced atherosclerotic lesion.⁷⁴ Thus, inflammation not only plays a role in the initiation and formation of atheroma, it actively contributes to the acute thrombotic complications associated with atheroma.⁶⁷ Hypertension is regarded as a risk factor for atherosclerosis and there is increasing evidence indicating that inflammation may play a role in hypertension.⁷⁵⁻⁷⁷ This may provide the pathophysiological link between these two conditions.

Inflammatory biomarkers and cardiovascular risk

Numerous former prospective epidemiological studies have described an association between inflammatory markers (such as white blood count and fibrinogen) and CVD.⁷⁸⁻⁸⁰ More recent investigations have examined markers such as CRP and IL-6 and these studies add consistency to the inflammation-CVD association. Large population-based studies such as the MONICA study (MONItoring trends and determinants in CArdiovascular disease),⁸¹ the Atherosclerosis Risk in Communities Study (ARIC)⁸² and the Women's Health Study⁸³ show an association between CRP levels and risk of incident coronary disease. In addition, an association with peripheral arterial disease has also been found⁸⁴ and studies utilizing IL-6 show similar results.⁸⁵ An important characteristic of the above-mentioned studies is their limitation to white North-American and European populations. There is limited data for populations of African descent known to have an increased risk for CVD.⁸⁶ This underlines the urgency to investigate inflammation alongside any factors that may contribute to inflammation as a possible risk factor for CVD in these populations.

2.2.1 Cardiovascular disease and a reduction in lung function

The burden of non-communicable diseases (NCDs) in South Africa

NCDs can be classified as diseases that are not transferable from person to person.⁸⁷ More than 36 million deaths each year are attributable to NCDs and nearly 80% of these deaths (29 million) occur in low- and middle-income countries.^{88,89} This is in part driven by urbanisation and changes in lifestyle such as an unhealthy diet, physical inactivity, smoking, obesity and alcohol abuse.^{5,6} NCDs can be divided into four main categories, namely CVD, respiratory disease, cancer and diabetes.

Cardiovascular disease

According to the Centres for Disease Control (CDC), CVD can be defined as a group of disorders of the heart and blood vessels and may include coronary heart disease, cerebrovascular disease, peripheral arterial disease and congenital heart disease. CVD is a major health concern that has reached near epidemic proportions in Africa.⁹⁰ Heart disease, diabetes, and stroke together constitute the second most dominant cause of mortality in adult South Africans.⁹¹ Ischemic heart disease (IHD) remains fairly uncommon in the African population of South Africa^{86,92} due to their favourable total cholesterol profile in association with high levels of protective high density lipoprotein cholesterol in more than 80% of Africans.^{92,93} However, the emergence of risk factors for atherosclerotic vascular disease in both urban and rural communities has increased.⁹⁴ Various risk factors for CVD are known with the most important one being elevated BP or hypertension.⁹⁵ Blood pressure regulation is essential to allow adequate perfusion to vital organs; however, an increased pressure poses negative effects such as damage to organs and blood vessels.⁹⁶ Hypertension contributes significantly to the global burden of disease and the prevalence has been increasing significantly in Sub-Saharan Africa.^{97,98} In a global analysis of blood pressure it was found that African countries displayed the highest mean blood pressure, even though, globally seen, blood pressure decreased during the last 30 years.⁹⁹ It has recently been described that 47.8 % of the participants in the South African leg of the PURE study were hypertensive at baseline and that a

further 24% with optimal BP at baseline, developed hypertension over 5 years.¹⁰⁰ Alberts et al. conducted a study to determine the prevalence and associated risk factors of CVD in a rural adult black population from South Africa and reported that approximately 25% of the study population presented with elevated BP.¹⁰¹ This burden of hypertension could have severe consequences as a large portion of those with hypertension could be undiagnosed and untreated.¹⁰² This was illustrated in a 1-year follow-up study of newly diagnosed hypertensive patients in South Africa which showed that, despite referral, 63% had uncontrolled hypertension and that 27% claimed to be unaware of their hypertension.¹⁰³ These data highlight the importance of hypertension in contributing to CVD and ultimately to the NCD burden in Africa.

Respiratory disease

Apart from CVD contributing substantially to the NCD burden in Africa, respiratory diseases including COPD, asthma, occupational lung diseases and lung cancer⁹¹ also play a key role. The burden of these diseases in South Africa is not well documented,⁹⁰ however, data released by Statistics South Africa for 1999-2006 showed that by 2003 premature adult deaths due to COPD increased by 23%.⁹⁰ COPD is also the fourth leading cause of mortality world-wide⁹⁰ and the burden of this disease is predicted to increase in the coming decades.¹⁰⁴ South Africa is undergoing rapid industrialization and occupational exposures contribute substantially to the increase in respiratory diseases.⁹¹ Although industrial hygiene control and regulation of the mining industry have greatly improved, many miners that relocated to their village of origin have had significant morbidity from respiratory disorders such as TB.¹⁰⁵ This is of concern especially in those who are also infected with HIV-1, as it greatly increases the risk of active TB.¹⁰⁶ More than 70% of the 36.1 million infected with HIV-1 worldwide live in sub-Saharan Africa, and a high proportion of these are co-infected with TB.¹⁰⁷

2.2.2 Chronic obstructive pulmonary disease as cardiovascular risk factor

Chronic obstructive pulmonary disease poses significant extra pulmonary effects, with one of the best recognised manifestations being cardiovascular complications.¹⁶ Due to the anatomical and functional association between the heart and lungs, any factor that impacts one of these organs is bound to have an effect on the other. COPD in itself also poses as a significant cardiovascular risk factor and it has been found that the leading cause of hospitalization and mortality among COPD patients are due to cardiovascular events.¹⁰⁸ Data from the Lung Health Study, in which more than 5 800 patients with mild to moderate COPD were studied, indicated that 42% to 48% of all hospitalizations that occurred over the 5-year follow-up period were related to cardiovascular complications.¹⁰⁹

Epidemiology of chronic obstructive pulmonary disease

The most plausible explanation for the high incidence of cardiovascular morbidity and mortality associated with COPD is the high prevalence of tobacco use in this group as well as other known risk factors for CVD such as an unhealthy diet, physical inactivity and socio-economic status.¹¹⁰ However, a number of large population-based studies have shown that a decrease in lung function is positively associated with cardiovascular risk, independent of risk factors such as age, sex, smoking and socio-economic status.¹¹¹⁻¹¹⁴

While previously regarded as purely a lung disease, COPD is now recognised as having important systemic effects which play a role in the severity of this condition.¹¹⁰ A hypothesis has been formulated that the relationship between lung function and cardiovascular risk may be due to a specific COPD effect (Figure 5). Patients with COPD have increased systemic inflammation, oxidative stress and hypoxia.¹¹⁰ Increased incidences of hemodynamic abnormalities are also present and any one of these factors may increase the risk for CVD.¹¹⁰

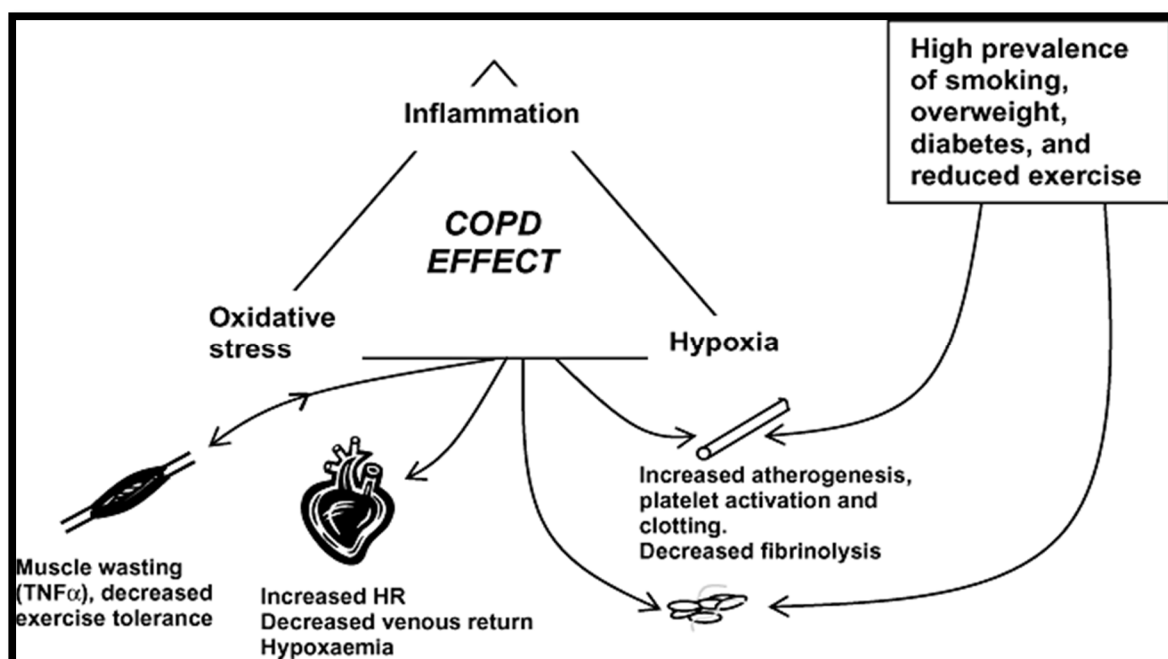


Figure 5: A possible mechanism for the cardiovascular risk associated with COPD. COPD, chronic obstructive pulmonary disease; HR, heart rate; TNF- α , tumour necrosis factor alpha.¹¹⁰

Systemic inflammation in COPD and CVD

Patients with COPD display evidence of systemic inflammation, especially when the disease is severe or during exacerbations.¹⁶ The mechanism behind this phenomenon remains unclear, although several possible explanations exist such as a spill-over of local lung inflammation and hypoxia-induced production of inflammatory mediators.¹⁵ This can be measured either as an enhanced number of circulating cytokines, chemokines and acute phase proteins, or as abnormalities in circulating cells.^{50,115,116} Various inflammatory markers are elevated in COPD such as interleukin-6 (IL-6) and interleukin-8 (IL-8),^{115,117,118} as well as CRP.¹⁶

Interleukin-6

Human IL-6 is a compound existing of 184 amino acids with two potential N-glycosylation sites and four cysteine residues.¹¹⁹ It is produced by various types of cells, including T cells, B cells, monocytes and endothelial cells.¹²⁰ In healthy individuals IL-6 is usually expressed at low levels with concentrations increasing during infection, trauma or other stress.¹²¹ It has been implicated

to play a role in the pathogenesis of various diseases such as obesity, CVD and atherosclerosis.^{122,123}

In the heart activation of the cardiac IL-6 system has been found in cases of advanced heart failure with IL-6 being increased in both left ventricular dysfunction and congestive heart failure.¹²⁴ Regarding the vasculature, activation of the inflammatory cytokine cascade challenges the homeostatic state of the vascular wall which may in turn lead to accumulation of cells and fatty deposits.¹²¹ Although atherosclerosis is a complex process involving several cell types and mechanisms, one of the key underlying factors is inflammation.¹²¹ IL-6 has been implicated in the development of atherogenesis by initiating the cascade of events leading to atherosclerosis. IL-6 is responsible for the hepatic synthesis of CRP¹²⁵ which in turn induces the secretion of cellular adhesion molecules and tissue factors.¹²⁶ During basal conditions IL-6 has an effect on various tissues, influencing cell growth and differentiation including angiogenesis and re-vascularisation.¹²¹ In addition, exposure of vascular smooth muscle cells to IL-6 significantly enhances the cell's response to angiotensin II by increasing the expression of angiotensin II type 1 receptor.¹²⁷ This, in turn, stimulates the production of reactive oxygen species (ROS) and ultimately leads to endothelial dysfunction.¹²⁷ IL-6 is also involved in the induction of matrix metalloproteinases and thus plays an important role in the instability of atherosclerotic plaque.¹²⁸

Furthermore, there is a positive association between the level of IL-6 and arterial blood pressure in healthy adults;^{129,130} however, the mechanism underlying this association largely remains unclear. It has been described that there is a relationship between sympathetic nervous system activity and IL-6 production with a subcutaneous injection of epinephrine into rats causing a significant increase in plasma IL-6 concentration.¹³¹ These data suggests that the sympathetic nervous system stimulates the production of IL-6 in the periphery, which may be of vital importance especially in African populations known to have a higher sympathetic nervous system activity compared to their Caucasian counterparts.¹³² A recent study also indicated that

IL-6 independently predicts both all-cause and cardiovascular mortality in a population of Africans.¹³³

C-reactive protein

CRP is a phylogenitcally highly conserved plasma protein that participates in the systemic response to inflammation.¹³⁴ This protein is primarily produced by hepatocytes in response to IL-6 stimulation;¹¹⁰ however, CRP is also found in atheromatous lesions and may therefore play a causal role in atherogenesis.¹³⁵ Based on findings from numerous prospective epidemiological studies, CRP has emerged as a powerful independent predictor of CVD.^{85,136,137} CRP predicts incident myocardial infarction and risk of ischemic stroke,¹³⁸ sudden cardiac death¹³⁹ and peripheral arterial disease.⁸⁴

CRP elicits a variety of effects on the vascular endothelium favouring a pro-inflammatory and pro-athersclerotic phenotype (Figure 6).¹⁴⁰ These effects are overall very similar to those of IL-6, but the longer plasma half-life of CRP compared to IL-6 may enable CRP to represent a more reliable indicator of chronic inflammation.¹⁴¹ CRP inhibits endothelial nitric oxide synthase and consequently the production of nitric oxide (NO).¹¹⁰ Furthermore, CRP stimulates endothelin-1 and IL-6 release from endothelial cells¹⁴² and decreases the production of the vasodilator prostacyclin¹⁴³ leading to endothelial dysfunction and unopposed vasoconstriction. By promoting endothelial dysfunction, CRP initiates endothelial activation and plaque formation.¹⁴⁰ A key characteristic of endothelial activation is the expression of adhesion molecules on the surface of these cells which recruit monocytes to the vascular surface.¹⁴⁰ Migration of these monocytes into the arterial wall leads to lipid oxidation which sustains this cycle, enhancing plaque formation.¹⁴⁰ CRP also has a direct effect on atherogenesis by increasing the number of angiotensin I receptors in vascular smooth muscle cells.¹⁴⁴ All of these cumulative effects of the inflammation-sensitive plasma proteins may herald the changes in the arterial wall that characterises hypertension¹⁴⁵⁻¹⁴⁸ and is shown that these proteins may predict a future increase in systolic BP.¹⁴⁹

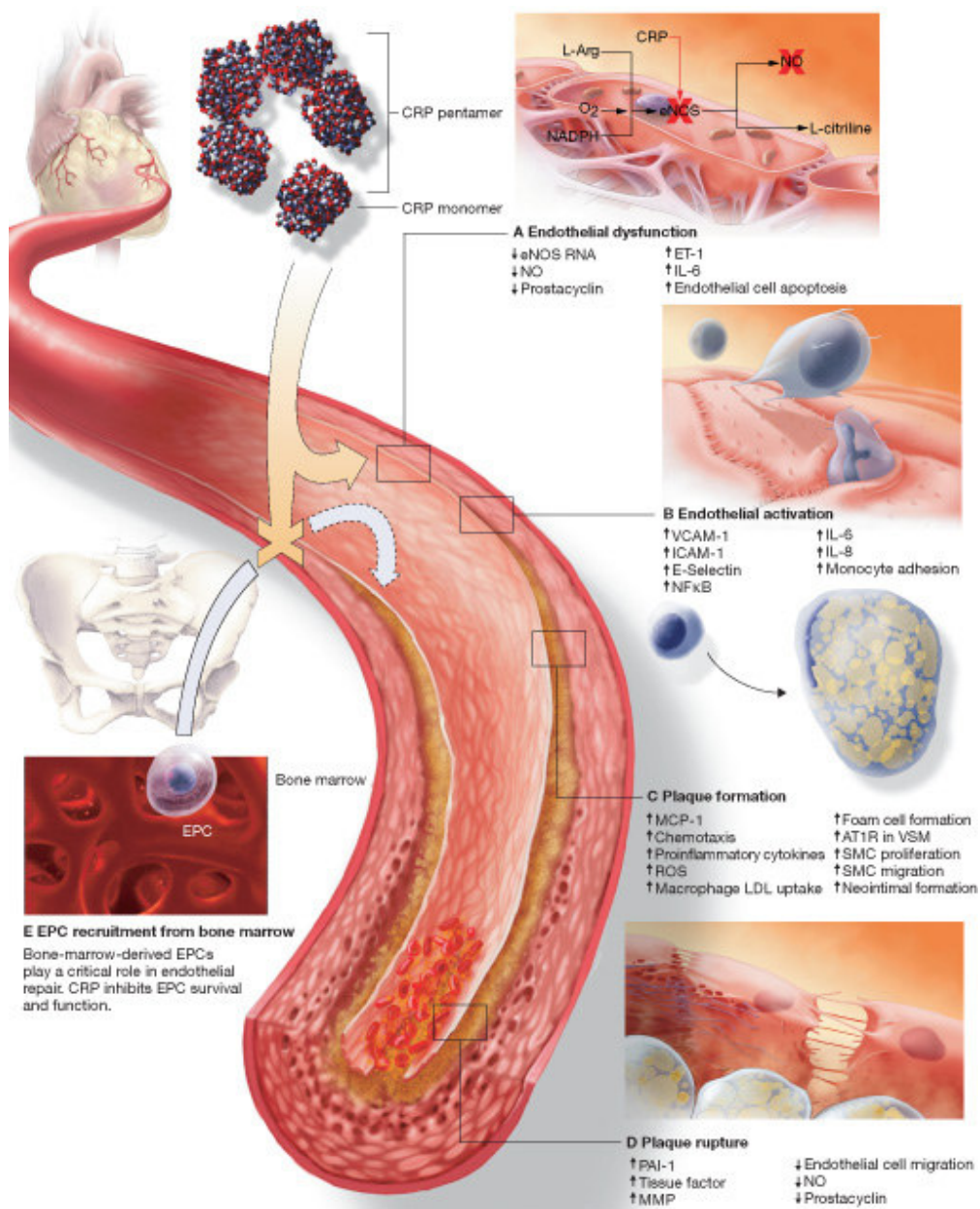


Figure 6: The role of C-reactive protein in atherogenesis

(A) Endothelial dysfunction. (B) Endothelial cell activation. (C) Plaque formation. (D) Plaque rupture. (E) Inhibition of EPC survival and function. AT1R, angiotensin type I receptor; CRP, C-reactive protein; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cells; ET-1, endothelin-1; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin 6; IL-8, interleukin 8; MCP, monocyte chemotactic protein-1; MMP, matrix metalloproteinase; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor κB; NO, nitric oxide; PAI-1, plasminogen activator inhibitor type I; ROS, reactive oxygen species; SMC, smooth-muscle cells; VCAM-1, vascular cell adhesion molecule-1; VSM, vascular smooth muscle.¹⁴⁰

Oxidative stress in COPD and cardiovascular disease

Oxidative stress is an imbalance between the production of reactive oxygen species and protective antioxidants, such as superoxide dismutase and glutathione peroxidase.¹¹⁰ Excess oxidation causes apoptosis, cell destruction and necrosis and also enhances inflammation.¹¹⁰

To date no studies have described that increased oxidative stress in COPD increases cardiovascular risk, however, numerous studies investigated oxidative stress in CVD,^{150,151} as well as oxidative stress in COPD.^{152,153} Regarding COPD, pulmonary- as well as systemic oxidative stress occurs¹¹⁰ due to the lungs being exposed to exogenous oxidants in tobacco products and air pollutants and inflammatory leukocytes producing oxidants endogenously.¹⁵⁴ Increased levels of hydrogen peroxide, suspected to be released from alveolar macrophages, are present in the exhaled breath condensate of COPD patients and smokers, indicating an oxidative burden.¹⁵⁵

Similar to inflammation, the oxidative stress associated with COPD poses systemic implications.¹¹⁰ The neutrophils within the peripheral circulation of COPD patients have been shown to produce an increased amount of ROS compared to healthy subjects and this is associated with increased plasma levels of lipid peroxidation products.^{152,156}

On the cardiovascular side, several traditional risk factors for CVD, including hypertension, hypercholesterolemia, smoking and diabetes are associated with increased production of free oxygen radicals from the vascular endothelium.^{152,157-159} ROS precipitates atherosclerosis by various mechanisms, including up-regulation of adhesion molecules, proliferation of vascular smooth muscle cells, apoptosis of the endothelium and lipid peroxidation.¹⁶⁰⁻¹⁶²

Hypoxia and cardiovascular disease

Patients with COPD are subjected to intermittent hypoxia during exercise or exacerbations and in advanced stages of the disease, sustained hypoxia.¹¹⁰ Hypoxia has a pronounced effect on the cardiovascular system and it has been shown to have a multifactorial influence on atherogenesis. These include increased inflammation, oxidative stress, upregulation of adhesion molecules and hemodynamic stress.¹⁶³⁻¹⁶⁸ Hypoxia also influences the renal circulation, causing reduced renal blood flow which in turn activates the renin-angiotensin system causing peripheral vasoconstriction.¹⁶⁹

2.3. Factors affecting both lung- and cardiovascular function

There are several determinants that could play a role in varying lung function, the most well-known being sex, age, height, ethnicity and general health.¹⁷⁰ Reference values are usually adjusted for age, sex, height and ethnicity. Additional factors which should be considered when investigating lung function include physical activity, body composition, smoking and pollutants as well as socio-economic status, as these factors are also considered to influence lung function.¹⁷⁰ Many of these factors also have various cardiovascular effects.¹⁷¹⁻¹⁷⁵

Physical activity

The extent to which regular physical activity could reduce or prevent the occurrence of lung disease is not completely known, but epidemiological and experimental studies have supported this hypothesis.^{176,177} A large prospective study conducted by Garcia-Aymerich and colleagues, showed that a level of physical activity equivalent to walking or cycling 2 hours/week or more was associated with a 30–40% reduction in the risk of both hospital admission due to COPD and respiratory mortality.¹⁷⁸ The physiological mechanisms underlying the potential beneficial effects of regular physical activity in lung function are not fully understood, although some evidence does exist which may give support to its biological plausibility. Physical activity improves peripheral muscle function¹⁷⁹ and has various anti-inflammatory and anti-oxidant

effects,^{180,181} all of which may reduce the symptoms and morbidities associated with reduced lung function.

With regard to CVD, physical activity is shown to be beneficial as chronic intermittent increases in shear stress during exercise improve endothelial function and attenuates pathological vasoconstriction.^{182,172} The mechanism behind the improved endothelial function is suggested to be an increase in the expression of the endothelial nitric oxide synthase enzyme, leading to increased NO bio-availability.¹⁸³ Physical inactivity is a known modifiable risk factor for CVD¹⁷⁵ and this is supported by a number of studies showing a reduction in cardiovascular mortality risk with regular physical activity.¹⁸⁴⁻¹⁸⁷

Body composition

Several studies have reported that both underweight and overweight individuals present with decreased lung function.¹⁸⁸⁻¹⁹² Obesity is associated with decreased compliance of the chest wall, reduced lung volume, impaired airway function, dysfunction of the thoracic skeletal muscle and arterial hypoxemia.¹⁹³ The decrease in chest wall compliance may be caused by abdominal and thoracic adipose tissue encasing the chest and abdomen, limiting movement of the diaphragm.¹⁹⁴ In addition, obesity has been shown to be associated with markers of systemic and vascular inflammation, such as CRP,¹⁹⁵ which may exert local effects within the lung tissue leading to a reduction in the airway diameter.¹⁹⁶

Obesity has numerous adverse effects on cardiovascular health as well, including insulin resistance, hypertension, dyslipidemia, endothelial dysfunction and increased systemic inflammation.¹⁹⁷ The adipocyte acts as an endocrine organ and plays a key role in the pathogenesis of obesity.¹⁷⁴ These cells are capable of synthesizing and releasing into the circulation a variety of compounds that may play a role in cardiovascular homeostasis.¹⁷⁴ Adipose tissue is a significant source of IL-6 and it is estimated that, in vivo, up to 30% of the total circulating concentrations of IL-6 originate from adipose tissue.¹⁹⁸ This may be important in

the modulating effect of IL-6 on CRP production in the liver, as CRP represents a chronic inflammatory state which may lead to coronary artery disease⁸⁴ and is related to body mass index (BMI).¹⁹⁸

Smoking and pollutants

It has been established that cigarette smokers display a higher prevalence of lung function abnormalities as well as a greater rate of decline in FEV₁ when compared to non-smokers.¹⁰⁴ Passive exposure to tobacco smoke increases the burden of inhaled harmful particles and may also contribute to a decrease in lung function.¹⁹⁹⁻²⁰¹ With regard to occupational exposures, a prolonged exposure to dusts and chemicals may lead to a reduction in lung function even in the absence of cigarette smoking.²⁰² Airway hyper responsiveness is increased when exposure to particulate matter, irritants organic dust and sensitizing agents occur.²⁰³

Furthermore, the indoor burning of biomass fuels for cooking and heating in dwellings that are not well ventilated has shown to be a risk factor for reduced lung function.²⁰⁴⁻²⁰⁸ This is very concerning as human exposure to air pollution is greatly attributable to the indoor environment.²⁰⁹ Solid fuels such as dung, wood, coal and agricultural residues still remain the primary source for cooking and heating in poverty-stricken communities.²⁰⁹ According to data released by Statistics South Africa in 2001, although 70% of South African households implemented electricity for lighting purposes, only half used electricity for cooking and heating. One-third of the households in South Africa made use of solid fuels and 95% of these households were of African-descent. Poor-quality stoves and open fires used for burning solid fuels are responsible for exposure to significant amounts of pollutants and carcinogenic substances.²⁰⁹ The limited ventilation in dwellings, especially in low socio-economic areas, further increases exposure, especially in women and children who spend a great deal of time indoors.²⁰⁹



Figure 7: *Living conditions in the informal settlements of South Africa. These conditions may contribute to the burden of chronic lung diseases. Exposure to the burning of biomass fuels used for cooking and heating in a closed environment, as well as residence in areas with poor air quality are significantly related to reduced lung function.*²¹⁰⁻²¹²

The detrimental effects of smoking on the cardiovascular system have been extensively described and numerous epidemiological studies have stated that tobacco smoke increases the incidence of myocardial infarction and coronary artery disease.²¹³⁻²¹⁷ Even environmental tobacco exposure is associated with a 30% increase in cardiovascular risk. Although this association is clear, the mechanisms responsible have not been fully described. One of the most important observations is that smoking predisposes an individual to aortic and peripheral atherosclerosis by affecting various components of the atherosclerotic process, such as vasomotor dysfunction, inflammation and modification of the lipid profile.¹⁷¹

2.4 Summary

South Africa is a country of great diversity, undergoing different stages of urbanisation. It extends from highly industrialized cities with an urban lifestyle, to remote rural regions where a more traditional lifestyle is still being implemented. This process of urbanisation has had the effect of an increase in the prevalence of NCDs,²¹⁸ especially CVD.⁶ Reduced lung function is considered a risk factor for CVD, independent of age, sex and tobacco use.²¹⁹ Although the association between reduced lung function and CVD is clear, the mechanistic link between these two disease states remains uncertain. International literature exists indicating that inflammatory pathways may be mediating this relationship.^{14,17,195} Inflammation and endothelial

dysfunction may act as a common physiological pathway for elastic changes in the vasculature and lung tissue.¹⁷ Data regarding this phenomenon in African populations is scant. The quantification of the extent of CVD in South Africa and the identification of possible risk factors may be essential for effective treatment and action, especially in vulnerable populations where health-care resources are limited. An investigation into the relationship of lung function and its possible contribution to CVD might therefore be of importance in the understanding of CVD in South Africa and might aid in minimizing the associated morbidity and mortality.

3. MOTIVATION AND PROBLEM STATEMENT

The central aim of this study was to determine the potential role of lung function, as measured by FEV₁ and FVC, in CVD development in a black South African population from the PURE study. Furthermore the aim was to establish whether inflammation is the mechanistic link between these two disease states. The associations between lung function and inflammatory markers, which may play a role in the development of hypertension, were explored. In addition, the predictive value of lung function for cardiovascular mortality within this population was determined.

Motivation, aims and hypotheses of each manuscript:

3.1 CHAPTER 3 - *South African and international reference values for lung function and its relationship with blood pressure in Africans.*

Motivation

Lung function differs significantly between different populations of the world.²²⁰ There are several challenges in estimating the respiratory disease burden in South Africa, including the use of appropriate reference data, as inaccurate interpretation may lead to misdiagnosis.²⁹ South African prediction equations are available^{32,33} but are seldom used since it is not included in the software packages of commercially available spirometers. Instead, European reference

equations have largely been used in South Africa. However, it has been described that populations from Southern Africa display a lower forced vital capacity (FVC) as well as forced expiratory volume (FEV₁) when compared to Europeans.²³ Furthermore, an inverse relationship between lung function and blood pressure has been reported in several studies,²²¹⁻²²³ however information on this association lacks in African populations.

Aims

- To compare the prediction equations from three different reference populations namely European, US and South Africa, in a large sample of black South Africans.
- To establish whether lung function is associated with blood pressure in these participants.

Hypotheses

- The prediction values from the three different reference populations, namely European, US and South Africa, differ significantly in a large sample of black South Africans, with the South African reference equations showing the highest percentage of the predicted values.
- Lung function is inversely associated with blood pressure in this population, independent of confounders.

3.2 CHAPTER 4 - *Inflammation as possible mediator for the relationship between lung- and arterial function.*

Motivation

Numerous studies have reported an inverse relationship between lung function and arterial elasticity. Jankowich and colleagues studied the association between pulse pressure (PP) and FEV₁ in a large cohort of men and women and demonstrated a significant inverse association in those aged >40 years.²²⁴ In addition, an inverse association between PWV and lung function has also been reported, even after adjustment for known confounders.²²⁵⁻²²⁷ The mechanisms behind these associations remain unclear, but it is plausible that the changes in lung- and

arterial function originate from the same pathophysiological process.¹⁷ This process could possibly involve systemic inflammation, as a significant association between lung function and markers of systemic inflammation has been reported. In comparison, inflammation causes vascular dysfunction and sustains the atherosclerotic process.¹⁷ However, this mechanism requires further investigation, especially to determine if the findings from international studies are also applicable to African populations, known to be at increased risk for atherosclerotic vascular disease.²²⁸

Aims

To investigate the three-way relationship between:

- lung function (FEV₁ and FVC) and markers of inflammation (IL-6 and CRP);
- inflammation and arterial stiffness by using pulse wave velocity (PWV);
- lung function and arterial stiffness in a sample of Africans to determine whether there is an association between lung- and vascular function, and whether this association is mediated through inflammation.

Hypotheses

- Reduced lung function is independently associated with markers of inflammation (IL-6 and CRP).
- Reduced lung function is independently associated with arterial stiffness (PWV).
- Both IL-6 and CRP are independently associated with PWV.
- The relationship between reduced lung function and arterial stiffness is mediated by inflammation.

3.3 CHAPTER 5 - Lung function, inflammation and cardiovascular mortality in a population of black South Africans

Motivation

Cardiovascular events are the second leading cause of mortality in South Africa, highlighting the current burden of CVD.²²⁹ Furthermore, occupational exposures and the living conditions in the informal settlements of South Africa may contribute to an increase in chronic lung diseases.^{211,212,230-233} International literature describes that reduced lung function is associated with an increased risk of CVD-related mortality,^{8,112,234} however, whether this is applicable to African populations remains largely unexplored. In addition, the mechanism facilitating the association between reduced lung function and CVD is also not known. Reduced lung function is associated with elevated circulating inflammatory markers^{14,235} known to play a role in endothelial dysfunction and arterial stiffening and this could be the factor leading to an increase in cardiovascular (CV) risk. Although it is evident that there is a significant CVD burden in South Africa, data concerning the possible contribution of reduced lung function to cardiovascular mortality is limited. Identifying factors which contribute to mortality risk is valuable in order to implement proper treatment and action.

Aim

- To establish the contribution of lung function, measured by FEV₁ and FVC, in predicting all-cause and cardiovascular mortality over five years in black South Africans, whilst taking inflammatory markers into account.

Hypothesis

- Lung function and markers of inflammation predict all-cause and cardiovascular mortality over five years in a population of Africans

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CHAPTER 2

STUDY DESIGN AND RESEARCH METHODOLOGY

1. STUDY DESIGN, PARTICIPANTS AND EXPERIMENTAL PROTOCOL

1.1 *Overarching Prospective Urban Rural Epidemiology Study*

This sub-study forms part of the South African leg of a global research study, namely the Prospective Urban Rural Epidemiology Study (PURE). The PURE study was designed to investigate how societal transitions with urbanisation cause changes in the lifestyles of populations that predispose them to non-communicable diseases, such as cardiovascular disease (CVD). The framework is based on the assumption that the “causal” pathways for the development of CVD involve influences at multiple levels. The characteristics of countries influence the characteristics of communities and households. These in turn influence individual lifestyle behaviours which are modified by the individual's attitude, culture and awareness of health behaviours. Adverse changes in these health behaviours lead to the development of biological risk factors such as high blood pressure, diabetes and obesity and ultimately the development of CVD.

A total of 156,424 adults from 628 communities in 17 low-, middle-, and high-income countries were prospectively recruited (Figure 1). Within each country urban and rural communities were selected according to prescribed guidelines¹ to provide substantial variations in health determinants and outcomes. Important contributions regarding the understanding of societal transitions and the effect thereof on population lifestyles have already been made from the PURE study. Among a sample of 7519 patients with a coronary heart disease or stroke event from countries with varying income levels, the prevalence of healthy lifestyle behaviours was low, with even lower levels in poorer countries.² It has recently also been established that secondary prevention medicines are unavailable and unaffordable for a large proportion of communities and households in upper middle-income, lower middle-income and low-income countries.³

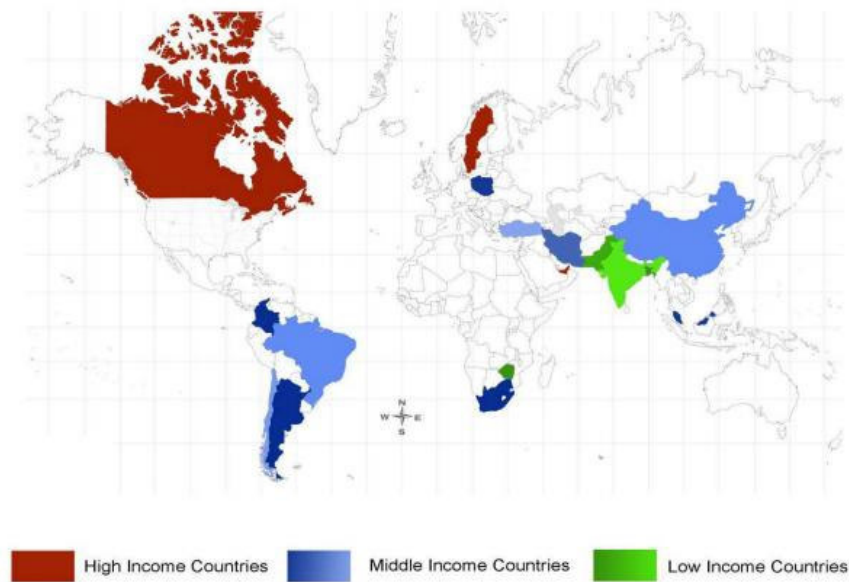


Figure 1: The countries involved in the international PURE study, stratified by income.¹

1.2 The South African leg of the Prospective Urban Rural Epidemiology Study

Within South Africa the main selection criteria were that the participating communities had to show migration stability. All the baseline data were collected during August to November 2005. Four urban and rural communities were identified in the North-West Province (Figure 2). The rural community (A), namely Ganyesa, was identified 450 km west of Potchefstroom towards Botswana. A deep rural community (B), Tlaskgameng, 35 km east from community A and only accessible with a gravel road, was also included. Both communities are still under tribal law. The urban communities (C and D) were chosen around the city of Potchefstroom. Community C was selected from the established part of the Ikageng township next to Potchefstroom and D from the informal settlements surrounding Ikageng.

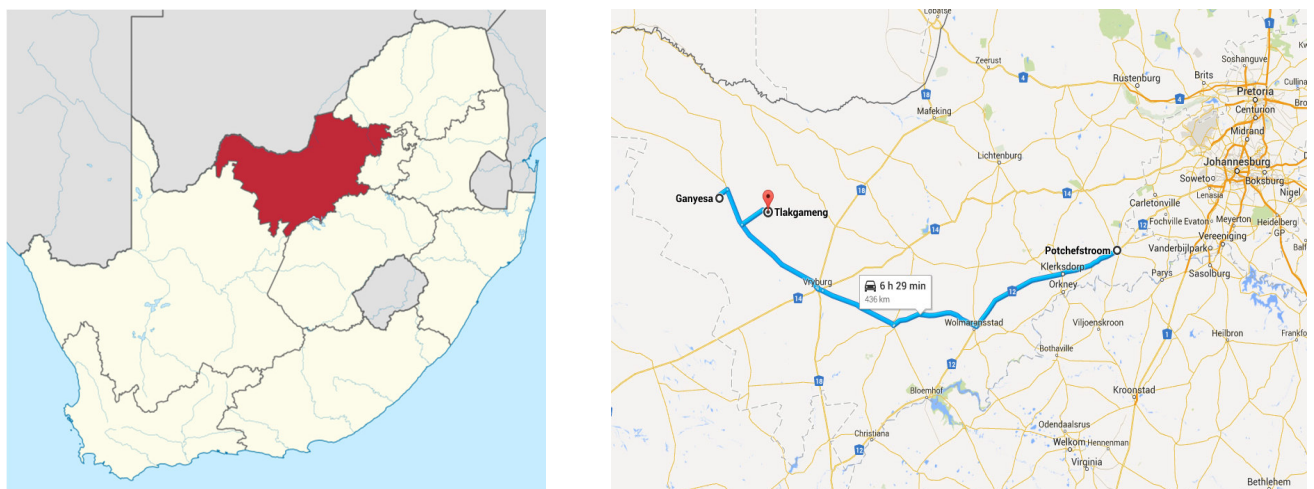


Figure 2: A map of South Africa, indicating the North West Province and the locations from where the participants were selected for this sub-study.

A household census regarding the number of people, their ages and health profile was done in 6 000 houses (1 500 in each community) and the head of each household signed written informed consent to fill out the questionnaire. In the case of refusal of participation or if no one was home, a non-complier questionnaire was filled out.

The data obtained from the above-mentioned census was used for a paper-based selection of possible subjects based on the following inclusion criteria: An apparently healthy cohort of men and women older than 35 years, who were not pregnant, intoxicated and did not have any cognitive impairment.

Based on the above-mentioned criteria, approximately 1 000 subjects from each community were selected, thus 4000 in total. These subjects were visited at home by a trained field worker and at the time of base-line data collection (2005), a total of 2010 subjects agreed to participate in the study (approximately 500 from each community). Further specific exclusion criteria were used for the sub-study which are described in detail in the individual research articles (chapters 3-5).

2. ETHICAL CONSIDERATIONS

2.1 Legal authorisation

The study protocol complies with the Declaration of Helsinki and approval was obtained from the North-West Department of Health, and the Ethics Committee of the North-West University, Potchefstroom, South Africa for the period January 2005 – December 2009 (Ethics approved number 04M10) as well as for the period January 2010 – January 2015 (Ethics approved number NWU-0016-10-A1).

2.2 Goodwill permission

The principal investigator of the PURE-SA study consulted with the mayors of both Potchefstroom and Ganyesa where they were informed of the aims of the study as well as

possible outcomes and benefits. The research procedures were also explained and permission was granted to proceed with the study. The *inkosi* (tribal chief) of the rural communities in Ganyesa and the community leaders in the urban areas were also approached and verbal permission was granted from the gatekeepers to perform the study within these communities.

2.3 *Expertise and skills*

All of the researchers and assistants (nurses, anthropometrists, counsellors and students) are experienced in their fields. All field workers were trained in all the procedures of the study in order to give participants a comprehensive overview of what was expected from them on the day of participation.

2.4 *Privacy and confidentiality*

Privacy of the participants was ensured by using either private rooms or dedicated areas that was closed off from the general research area. In the case where a measurement required the participant to partially undress, only the researcher was present in the room or designated area with the participant. This was also applicable to the completion of questionnaires.

Each participant was assigned a unique participant number during the baseline data collection in 2005 and this number was used in all stages of data collection. All of the data captured was done using this number and was entered into a computer locally (at each site) by utilizing a customized database in EpiInfo (version 3.2).

2.5 *Benefits*

a) Direct benefits

There was a direct benefit to the participants from the measurements conducted on the study day. Feedback was given regarding results that were immediately available, which include blood pressure, blood glucose levels, ECG, carotid plaque scores, lung function and HIV status.

In the case of any abnormalities, the lead researcher provided the participant with a referral letter to the local clinic or hospital.

b) Indirect benefits

Through participating in the PURE study, the participants help to provide South Africa with a direct estimate of the disease burden attributable to established and emerging risk factors for CVD. Findings from the PURE study are being utilised to facilitate the development of effective public health policies which may aid in decreasing the disease burden, hence indirectly benefiting the overall population.

2.6 Incentives and reimbursement

Participants were provided with transport by the North-West University to limit travelling expenses. They were also financially compensated to the amount of R100 per day for any expenses or loss of income on the day of their attendance. In the case of participants who were employed full-time, daily remuneration was confirmed with the employer and they were compensated for loss of income for one day.

3. ORGANISATIONAL PROCEDURES

Data collection took place in 2005 and 2010. The participants were transported by the research team from their communities and arrived at the dedicated rural and urban research facilities at approximately 08:00. The participants were first familiarised with the research setup and the procedures were explained to them in their native language by trained fieldworkers from the communities. Written informed consent was obtained from each participant and they were free to withdraw at any time. In the case where a participant was illiterate, the right thumb print was taken as a substitute for a signature. Participants were requested to fast for at least eight hours and to refrain from smoking and exercise at least 30 minutes prior to the measurements.

4. QUESTIONNAIRES

The questionnaires used in this study were validated for this specific population and the data were collected by trained field workers in each participant's home language.

The PURE South Africa Adult Questionnaire (Annexure A), was used in 2005 and 2010 to collect data which included socio-economic and demographic data, current health status, medical and family history, medication and tobacco use, as well as alcohol consumption. For the purpose of this sub-study, nominal coding variables were created for tobacco use (0: never used tobacco products; 1: formerly or currently using tobacco products). The adapted BAECKE questionnaire was used to determine the physical activity index.⁴

The PURE Household Questionnaire (Annexure B) was used to collect data regarding house structure, amenities, access to water and sanitation, the primary fuel used for food preparation as well as the primary heating source during the cold/rainy season.

5. ANTHROPOMETRIC MEASUREMENTS

Standardised procedures were used to obtain all anthropometric measurements as prescribed by the guidelines adopted at the National Institutes of Health sponsored Arlie Conference⁵ and the International Society for the Advancement of Kinanthropometry (ISAK).⁶ All measurements were done under the supervision of a trained anthropometrist and included waist circumference (Holtain stretchable metal tape, Crosswell, Wales), height (Invicta Stadiometer, IP 1465, Invicta, London, UK) and weight (Precision Health Scale, A&D Company, Tokyo, Japan). Body mass index ($BMI = \text{weight (kg)} / \text{height (m)}^2$) was also calculated.⁶

6. CARDIOVASCULAR MEASUREMENTS

Brachial systolic- (bSBP) and diastolic blood pressure (bDBP) were taken by cardiovascular physiologists on the upper right arm using a validated automated digital BP monitor (OMRON HEM-757, Omron Healthcare, Kyoto, Japan) with the participant in a sitting position. The measurement was repeated after a five minute resting interval and the second BP reading was

used for statistical analyses. Pulse wave velocity (PWV) was measured on the left side of each participant while in a supine position. Cardiovascular physiologists used the Complior SP device (Artech-Medical, Pantin, France) to measure the segment over the carotid to radial artery.

7. SPIROMETRY

Lung function was assessed according to the American Thoracic Society recommendations⁷ using a mobile Micro GP Spirometer (Micro Medical Ltd, Kent, UK). A minimum of three acceptable recordings and a maximum of five were obtained from each participant while in the standing position and with at least one minute between repeat measurements. Each participant was supplied with a disinfected mouthpiece. Measurements were taken by trained occupational hygienists who demonstrated the procedure to the participants with a dummy nose-clip and mouthpiece. Variables that were recorded included forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and peak expiratory flow rate (PEFR). Analysis was based on the maximal effort for both FEV₁ and FVC from all measurements obtained from each participant. Spirograms were regarded as acceptable when they were free from cough during exhalation, early termination or cut-off, variable effort, leaks or an obstructed mouthpiece.

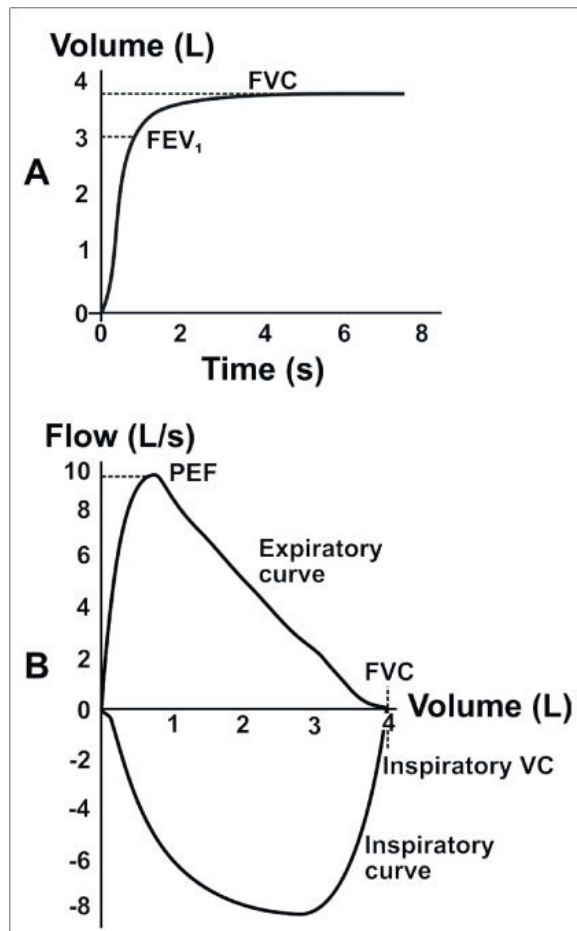


Figure 3: (A) A volume-time curve is an alternative way of expressing lung function, indicating the forced expiratory volume, where 80% of total volume is expired in the first second. (B) Lung function was determined by a flow-volume curve which is a representation of the rate of airflow as a function of lung volume during a complete respiratory cycle consisting of a forced inspiration followed by a forced expiration.⁸

8. BLOOD SAMPLING

Fasting blood samples were taken from the antebraichial vein branches by a registered nurse, in order to minimise risk to the participant. The blood was centrifuged on-site for 15 minutes at 2000g at 4°C. Aliquots of plasma and serum were frozen on dry ice, stored in the field at -18°C for two to four days after which the samples were transported to a storage facility where the samples were kept at -80°C until analysis.

9. BIOCHEMICAL ANALYSIS

Serum γ -glutamyltransferase (GGT), total cholesterol (TC) and C-reactive protein (CRP) were analysed by particle enhanced turbidimetric assays with the Konelab 20i™ auto-analyser

(Thermo Fisher Scientific Oy, Vantaa, Finland). Interleukin-6 (IL-6) was analysed by the Elecsys apparatus using ultra-sensitive enzyme immunoassays (Elecsys 2010, Roche, Basel, Switzerland). Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the Clinical and Laboratory Standards Institute: two runs per day in duplicate, each for 21 days (n=84). The repeatability (within-run precision) was 6% and the intermediate precision (between run precision) 8.5%. Glucose levels were determined in sodium fluoride tubes by an enzymatic reference method with hexokinase (Vitros DT6011 Chemistry Analyzer; Ortho-Clinical Diagnostics, Rochester, New York, USA). To determine glycated haemoglobin (HbA1c) levels from ethyl-enediamine-tetra-acetic acid plasma samples, the D-10 Haemoglobin testing system from Bio-Rad (#220-0101) was used. The Human Immunodeficiency Virus (HIV) status was determined by a First Response Rapid HIV card test (PMC Medical, Daman, India). In the case of a positive outcome, the result was confirmed with the Pareeshak card test (BHAT, Bio-tech, India).

10. MORTALITY OUTCOME ASSESSMENT

Participants were contacted by trained fieldworkers in three month intervals over the five year period (2005-2010) to ensure maximal retainment of study participants. In cases of deceased participants, verbal autopsies and family death certificates were used to obtain cause of death. A physician coded the immediate and underlying causes according to the International Classification of Diseases codes. Cardiovascular mortality was classified as death due to cardiovascular reasons, which included cardiac failure, heart failure, heart attack, myocardial infarction, stroke or cerebral vascular incident.

11. STATISTICAL ANALYSES

All statistical analyses were performed using Statistica version 12 software (Statsoft, Inc., Tulsa, OK, 2010) and Graphpad Prism version 5.03 for Windows (Graphpad Software, San Diego, California, USA) was used to analyse and plot results. Analyses of covariance were performed to determine differences between lung function in different groups of the primary fuel used for

food preparation as well as the primary heating source during the cold/rainy season. No significant differences were found between these groups and therefore this data was not utilized further. Details of the various statistical analyses and covariates that were included are described in the individual research articles (chapters 3-5).

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CHAPTER 3

South African and international reference values for lung function and its relationship with blood pressure in Africans.

South African and International Reference Values for Lung Function and its Relationship with Blood Pressure in Africans



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Title:	Concise and informative. Avoid abbreviations and formulae where possible.
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References:	<ul style="list-style-type: none"> • Ordlijn TM, Shainoff JR, Lawrence SO, and Simpson-Haidaris PJ. Thrombin cleavage enhances exposure of heparin binding domain in the N-terminus of the fibrin beta chain. <i>Blood</i> 1996;88:2050-61. • Blomback B. Fibrinogen to fibrin transformation. In: Seegers WH, editor. <i>Blood Clotting Enzymology</i>. New York-London: Academic Press; 1967. p. 143-215.
Sections:	Introduction, Material and Methods, Results, Discussion and Conclusion
Ethical considerations:	<p>Research Protocol Authors must state that the protocol has been approved by the appropriate Ethics Committee (state which).</p> <p>Human Investigation All work should conform to the 'Statement on Human Experimentation' by the National Health and Medical Research Council of Australia, or the equivalent in other countries. The ethical guidelines that were followed by the investigators must be included in the Methods section of the manuscript. State clearly that the subject gave informed consent. Anonymity should be preserved.</p>
Other:	--

(**Note: Some of the format was changed to ensure uniformity throughout the thesis.)

ABSTRACT

Background: In South Africa respiratory diseases are highly prevalent, with cardiovascular disease being a manifestation. However, international reference values for lung function are commonly used, which may not be appropriate to correctly identify reduced lung function. An inverse relationship exists between lung function and blood pressure (BP) but is not investigated extensively in black South Africans. **Methods:** We included 2010 Africans from the PURE (Prospective Urban Rural Epidemiology) study (aged > 35 years) in the North West Province. Spirometry was performed and predicted values for forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were calculated from South African, European and United States prediction equations. **Results:** With the exception of the European predicted values, all other predicted mean FEV₁ and FVC were above 80%. South African reference values displayed the highest percentages of the predicted values for FEV₁ and FVC (87.9 and 99.7%, respectively.) BP increased from quintiles five to one for both FEV₁ and FVC, (p for trend <0.001). After adjustment the differences remained (p<0.05). **Conclusions:** South African reference values yielded higher percentages of predicted FEV₁ and FVC values than European and US equations suggesting that South African prediction equations may be more useful when investigating lung function in black South Africans. Elevated BP is related to reduced lung function, highlighting the importance in managing both respiratory- and cardiovascular disease.

Key words: cardiovascular disease, ethnicity, respiratory diseases, hypertension, lung function

Word count: 224

INTRODUCTION

The prevalence of non-communicable diseases (NCDs) including cardiovascular disease (CVD) and respiratory diseases in South Africa is high and account for 11% and 3% respectively, of the total NCD mortality in South Africa [1]. The burden of NCDs is also predicted to increase in South Africa if preventive measures are not taken [2].

There are several challenges in estimating the respiratory disease burden in South Africa. Appropriate reference data is vital and unsuitable reference equations as well as inaccurate interpretation may lead to over- or under diagnosis [3]. In South Africa where the mining industry is prominent and a large number of the population still use open fires for food preparation and as a heating source, the accurate assessment and prediction of lung function should be highlighted. South African prediction equations are available [4,5] but are seldom used since it is not included in the software packages of commercial spirometers. Instead, European reference equations have largely been used in South Africa. However, it has been described that populations from southern Africa display a lower forced vital capacity (FVC) as well as forced expiratory volume (FEV_1) when compared to Europeans [6] and a recent study conducted by Quanjer et al., [7] confirmed these findings. To account for this, the South African Thoracic Society (SATS) proposed that a correction factor of 0.9 be utilized for black South Africans when European prediction equations are being used [8].

Reduced lung function poses significant extra pulmonary effects, with one of the best recognised manifestations being accompanying cardiovascular disease [9]. This is especially notable in patients with chronic obstructive pulmonary disease (COPD) where it has been shown that even moderate reductions in expiratory flow volumes elevate the risk for CVD two to three fold [10-13]. An inverse relationship between lung function and blood pressure has been reported in several studies [14-17]. Although the mechanism underlying this relationship remains unclear, it has been shown that individuals with reduced lung function have higher levels of C-reactive protein, fibrinogen and other systemic inflammatory markers when

compared to those with normal lung function [18,19]. Since low-grade systemic inflammation is associated with vascular dysfunction, reduced lung function poses as a risk factor for cardiovascular morbidity and mortality [20].

We therefore aimed firstly to compare the prediction values from three different reference populations namely European, US and South Africa, in a large sample of black South Africans (n=2010); and secondly to establish whether lung function is associated with blood pressure in these participants.

MATERIAL AND METHODS

Study design and subject selection

This study is embedded in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study, taking place in the North West Province. This is a prospective study of which baseline data was collected in 2005. PURE was designed to investigate lifestyle changes and health status of populations from numerous developing countries [21,22] .

In the North-West province of South Africa, a total of 2010 volunteers aged ≥ 35 years were included from 6000 randomly selected households – equal numbers from rural and urban settings.

Approval was obtained from the Ethics Committee of the North-West University, Potchefstroom, South Africa. Permission to conduct the study in the above-mentioned communities was granted by the Provincial Department of Health, community leaders, tribal chiefs, and mayors. The protocol was explained to the subjects in their home language by field workers and they were given the opportunity to ask questions. Confidentiality and anonymity of all the results were assured by making use of anonymised numbers. Participants received remuneration for travelling expenses during the study and were referred to clinics if any pathology was noticed.

Questionnaires

The subjects were interviewed by extensively trained field workers using structured demographic, socio-economic, lifestyle and physical activity questionnaires, developed and standardized for the international PURE study and adjusted for each country [21]. Lifestyle data included tobacco use, alcohol intake, health history, and medication use.

Anthropometric and cardiovascular measurements

Anthropometric measurements were done under the supervision of a level three anthropometrist and included height (Invicta Stadiometer, IP 1465, Invicta, London, UK) and weight (Precision Health Scale, A&D Company, Tokyo, Japan). The body mass index ($\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$) was also calculated [23].

Cardiovascular measurements

Brachial blood pressure (BP) was taken by cardiovascular physiologists on the right arm using an automated digital BP monitor (OMRON HEM-757, Omron Healthcare, Kyoto, Japan) after a ten minute resting period. The subject was sitting in a comfortable position with the right arm rested on a stable surface. The BP measurement was repeated after a five minute resting interval and the mean BP was used for statistical analyses.

Spirometry

Lung function was assessed using a mobile Micro GP Spirometer (Micro Medical Ltd, Kent, UK) according to the American Thoracic Society recommendations [24]. The protocol was explained to participants prior to the assessment. A minimum of three acceptable recordings and a maximum of five were obtained from each participant while in the standing position and with at least 1 minute between repeat measurements. The variables that were analysed were FEV_1 (Forced Expiratory Volume in 1 sec) and FVC (Forced Vital Capacity). Analysis was based on the best measurement for both FEV_1 and FVC out of all measurements obtained from each

participant. Published prediction equations from European [7], American [25], and South African [8] populations were used to calculate predicted FEV₁ and FVC values for each participant.

Blood sampling and biochemical analysis

Blood samples were taken by a registered nurse from the antebrachial vein branches. The subjects were asked to fast overnight (eight to ten hours with no food or beverage, excluding water). The blood was centrifuged for 15 minutes at 2000g at 4°C. Aliquots of plasma and serum were frozen on dry ice, stored in the field at -18°C for two to four days after which the samples were transported to a storage facility where the samples were kept at - 80°C until analysis.

Fluoride plasma glucose, serum γ -glutamyl transferase (GGT) and total cholesterol (TC) were measured using the Konelab 20i (Thermo Scientific, Vantaa, Finland) and the Cobas Integra 400 Plus (Roche, Indianapolis, United States of Indiana) instruments. The HIV status was determined by a First Response Rapid HIV card test (PMC Medical, Daman, India). In the case of a positive outcome, the result was confirmed with the Pareeshak card test (BHAT, Bio-tech, India). All participants also received pre- and post HIV-test counselling by trained counsellors.

Data processing

Statistical analyses were performed using Statistica version 12 (Statsoft, Inc., Tulsa, OK, 2010). Variables with a non-Gaussian distribution were logarithmically transformed (fasting glucose and GGT). Independent t-tests and Chi-Square tests were used to compare means and proportions between men and women. We also selected a group of apparently healthy men, fitting the criteria of an age between 30 and 50 years, with no history of tobacco use, HIV uninfected, a BMI between 20 and 25 kg/m² and with blood pressure <140/90 mmHg. We compared the lung function of the healthy men to the rest of the men. We further compared the lung function of men with no history of tobacco use to men making use of tobacco products, formerly or currently. The subjects were divided into quintiles of the FEV₁ and FVC with Quintile

1 (Q1) being the lowest lung function. An analysis of covariance was performed to determine differences between the quintile groups. We performed single and partial regression analyses to determine relationships between blood pressure and measures of lung function. The covariates that were included were age, gender, waist circumference, height and a history of tobacco use.

RESULTS:

Characteristics of participants

In total 2010 participants were included in this study with the majority being women (63%, n=1264). Although men and women had similar ages of approximately 50 years ($p=0.15$) the women displayed a higher weight (66.4 ± 18.6 vs. 58.3 ± 12.0 ; $p < 0.001$) and BMI (27.0 ± 7.36 vs. 20.7 ± 4.01 ; $p < 0.001$). Men displayed significantly higher values for both FEV₁ (2.62 ± 0.76 vs. 2.04 ± 0.54 ; $p < 0.001$) and FVC (3.14 ± 0.84 vs. 2.37 ± 0.62 ; $p < 0.001$), but the FEV₁/FVC ratio for the men and women were well within the normal range of >70-80% [8]. Based on all the reference values included (i.e. US reference values, the reference values from the European Community as well as South African reference values), almost all the mean FEV₁ and FVC for both the men and women were above 80% of the predicted value, indicative of normal lung function. Based on the South African reference values, 27% (n=203) of the men displayed a FEV₁ that was below 80% of the predicted value and 37% (n=278) had a FVC that was below 80%. With regard to the women, 16% (n=206) had a FEV₁ below 80% of the predicted value and 40% (n=507) had a FVC that was lower than 80%.

Table 1: Characteristics of the African men and women.

	Men	Women	p-value
N	746	1264	
Age (years)	50.3 ± 10.3	49.6 ± 10.4	0.154
<i>Anthropometric measurements</i>			
Height (m)	1.67 ± 0.07	1.57 ± 0.06	< 0.001
Weight (kg)	58.3 ± 12.0	66.4 ± 18.6	< 0.001
Body mass index (kg/m ²)	20.7 ± 4.01	27.0 ± 7.36	< 0.001
<i>Biochemical markers</i>			
TC (mmol/L)	4.81 ± 1.33	5.13 ± 1.39	< 0.001
Fasting glucose (mmol/L)	4.80 (3.4;6.1)	4.88 (3.5;6.9)	< 0.001
<i>Cardiovascular measurements</i>			
bSBP (mmHg)	135.3 ± 23.2	132.2 ± 24.8	0.008
bDBP (mmHg)	86.7 ± 14.6	88.2 ± 14.4	0.024
<i>Spirometry variables</i>			
FEV ₁ (L)	2.62 ± 0.76	2.04 ± 0.54	< 0.001
FVC (L)	3.14 ± 0.84	2.37 ± 0.62	< 0.001
FEV ₁ / FVC (%)	83.7 ± 11.9	86.8 ± 10.2	< 0.001
US reference values^a			
FEV ₁ predicted value (L)	3.19 ± 0.55	2.46 ± 0.29	< 0.001
FEV ₁ % predicted value	82.5 ± 20.9	82.9 ± 18.7	0.640
FVC predicted value (L)	3.90 ± 0.65	2.95 ± 0.34	< 0.001
FVC % predicted value	80.8 ± 19.0	80.2 ± 18.4	0.482
European reference values^b			
FEV ₁ predicted value (L)	3.26 ± 0.44	2.35 ± 0.38	< 0.001
FEV ₁ % predicted value	80.3 ± 19.9	87.3 ± 20.0	< 0.001
FEV ₁ LLN	1.88 ± 0.44	1.34 ± 0.38	< 0.001
FEV ₁ Correction factor [†]	2.93 ± 0.40	2.12 ± 0.35	< 0.001
FVC predicted value (L)	4.01 ± 0.50	2.76 ± 0.41	< 0.001
FVC % predicted value	78.2 ± 18.1	86.0 ± 19.6	< 0.001
FVC LLN	2.37 ± 0.50	1.60 ± 0.41	< 0.001
FVC Correction factor [†]	3.61 ± 0.50	2.49 ± 0.37	< 0.001
South African reference values^c			
FEV ₁ predicted value (L)	2.96 ± 0.36	2.07 ± 0.39	< 0.001
FEV ₁ % predicted value	87.9 ± 22.4	99.7 ± 24.2	< 0.001
FEV ₁ LLN	1.73 ± 0.36	1.02 ± 0.38	< 0.001
FVC predicted value (L)	3.76 ± 0.43	2.87 ± 0.39	< 0.001
FVC % predicted value	83.0 ± 19.6	82.4 ± 19.0	0.513
FVC LLN	2.30 ± 0.43	1.78 ± 0.39	< 0.001
<i>Lifestyle factors</i>			
γ-glutamyl transferase (U/L)	70.8 (22.7;436)	47.9 (17.9;296)	< 0.001
Tobacco usage, n (%)	496 (66.9)	623 (49.5)	< 0.001
Physical activity index	7.30 ± 2.16	7.29 ± 1.73	0.891
HIV infected, n (%)	115 (15.5)	207 (16.5)	0.532
TB diagnosis, n (%)	36 (4.88)	26 (2.08)	0.001
<i>Medication usage</i>			

Anti-hypertensive, <i>n</i> (%)	7 (0.94)	32 (2.53)	0.012
Anti-TB, <i>n</i> (%)	4 (0.54)	5 (0.40)	0.648

Data are presented as mean \pm SD. Data with a non-Gaussian distribution presented as geometric mean and confidence intervals (5% - 95%). Abbreviations: DBP, diastolic blood pressure; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; LLN, lower limit of normality; SBP, systolic blood pressure; TB, tuberculosis; TC, total cholesterol;. The p-value is the statistical test of difference in effects between men and women with $p \leq 0.05$ regarded as significant. ^a Knudson 1976, ^b Quanjer 1993, ^c Mokoetle 1994, Louw 1996. [†]Correction factor of 0.9 applied.

A comparison between the lung function of the apparently healthy men (n=45) and the rest of the men (n=701) in Table 2 indicates - as expected - that the apparently healthy men displayed a more favourable lung function compared to the rest of the men (n=701). In the group of apparently healthy men, the predicted values based on the South African reference values displayed the highest percentages both for FEV₁ (97.5 ± 20.5) and FVC (89.7 ± 20.6). The FEV₁ and the FVC percentage of the predicted value were higher in the apparently healthy group of men compared with the rest of the men, this finding being present in all three sets of reference values.

When comparing men with no history of tobacco use to those with current or former tobacco use (Table 3), the men with no history of tobacco use, generally displayed more favourable lung function with a borderline higher FEV₁ (2.69 ± 0.75 vs. 2.59 ± 0.77 ; $p = 0.089$) and a significantly higher FVC (3.23 ± 0.86 vs. 3.09 ± 0.82 ; $p = 0.038$). The FEV₁ and FVC percentage of the predicted value were significantly higher ($p < 0.001$) in the men with no history of tobacco use, for each of the three sets of reference values.

Table 2: Lung function of the total group of men compared to the apparently healthy group of men.

	Total group of men	Apparently healthy men	p-value
N	701	45	
Age (years)	50.8 ± 10.4	42.6 ± 4.68	< 0.001
Height (m)	1.67 ± 0.07	1.67 ± 0.08	0.651
<i>Spirometry variables</i>			
FEV ₁ (L)	2.58 ± 0.75	3.10 ± 0.81	< 0.001
FVC (L)	3.11 ± 0.81	3.57 ± 1.09	< 0.001
FEV ₁ / FVC (%)	83.4 ± 12.1	88.2 ± 8.19	0.010
US reference values			
FEV ₁ % predicted value	81.9 ± 21.0	90.4 ± 17.1	0.009
FVC % predicted value	80.5 ± 19.1	85.6 ± 17.6	0.082
European reference values			
FEV ₁ % predicted value	79.7 ± 20.0	88.8 ± 17.9	0.003
FVC % predicted value	77.8 ± 17.9	84.1 ± 18.9	0.023
South African reference values			
FEV ₁ % predicted value	87.2 ± 22.4	97.5 ± 20.5	0.003
FVC % predicted value	82.6 ± 19.4	89.7 ± 20.6	0.017

Data are presented as mean ± SD. Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

The p-value is the statistical test of difference in effects between the total group of men and the healthy group of men with $p \leq 0.05$ regarded as significant.

Table 3: Basic characteristics and lung function of the men with no history of tobacco use compared to men with tobacco use.

	Men		% Δ	p-value
	No Tobacco use	Tobacco use		
N	236	465		
Age (years)	51.8 ± 11.0	49.5 ± 9.84		0.004
Height (m)	1.67 ± 0.07	1.68 ± 0.07		0.299
<i>Spirometry variables</i>				
FEV ₁ (L)	2.69 ± 0.75	2.59 ± 0.77	-3.72	0.089
FVC (L)	3.23 ± 0.86	3.09 ± 0.82	-4.33	0.038
FEV ₁ / FVC (%)	83.5 ± 10.6	83.9 ± 12.4	0.48	0.714
US reference values				
FEV ₁ % predicted value	86.5 ± 20.7	80.6 ± 20.6	-6.82	< 0.001
FVC % predicted value	84.7 ± 19.3	79.0 ± 18.5	-6.73	< 0.001
European reference values				
FEV ₁ % predicted value	84.3 ± 19.2	78.4 ± 19.9	-6.99	< 0.001
FVC % predicted value	82.0 ± 18.2	76.4 ± 17.6	-6.83	< 0.001
South African reference values				
FEV ₁ % predicted value	92.4 ± 21.0	85.8 ± 22.7	-7.14	< 0.001
FVC % predicted value	87.1 ± 19.1	81.1 ± 19.5	-6.89	< 0.001

Data are presented as mean ± SD. Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; % Δ, percentage change between control and effect. A p value of ≤ 0.05 was regarded as significant.

Unadjusted and adjusted analyses

Figure 1 demonstrates the unadjusted relationship between the SBP and DBP with both FEV₁ and FVC in the entire study sample. FEV₁ was negatively associated with SBP ($r=0.095$; $p<0.001$) and DBP ($r=0.110$; $p<0.001$). FVC also displayed a negative association with SBP ($r=0.063$; $p=0.006$) and DBP ($r=0.083$; $p<0.001$). After adjusting for the covariates age, gender, waist circumference, height and a history of tobacco use, none of these associations remained significant.

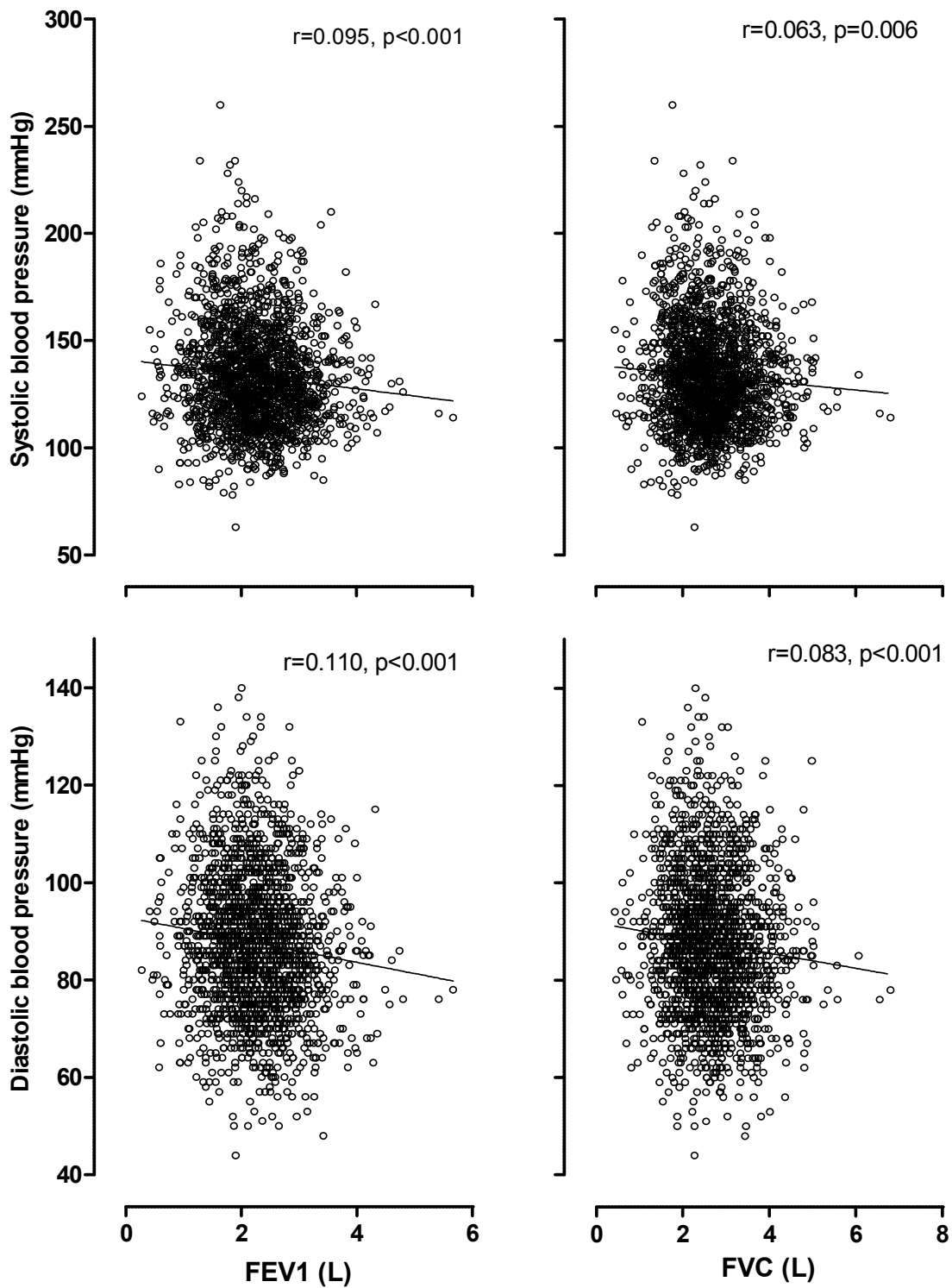


Figure 1: The unadjusted relationship between blood pressure, FEV₁ and FVC in 2010 African men and women. Abbreviations: FEV₁, forced expiratory volume in one second; FVC, forced vital capacity.

Figure 2 represents the unadjusted and adjusted difference in SBP and DBP between the different quintiles of FEV₁. Before and after adjustments both SBP and DBP are lower in the higher quintiles of FEV₁ and that this trend is highly significant (p<0.001). With the differences in BP between the FEV₁ quintiles adjusted for age, gender, waist circumference, height and a history of tobacco use as covariates, this trend no longer remains significant although BP between the quintiles still differed significantly from the reference quintile 5.

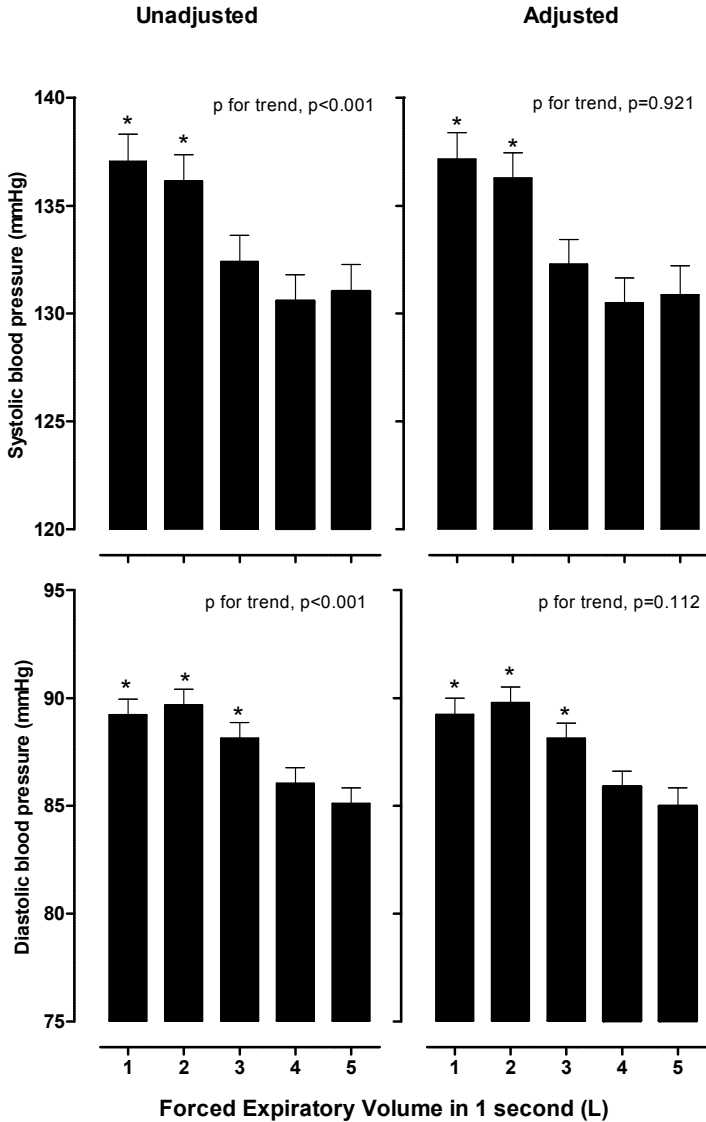


Figure 2: The unadjusted and adjusted differences in systolic- and diastolic blood pressure between quintiles of FEV₁ in 2010 African men and women. Bars with an asterisk differ significantly from Q5. Adjusted for age, gender, waist circumference, height and a history of tobacco use.

In Figure 3 similar results are shown for FVC. Both SBP and DBP decrease in the higher quintiles of FVC and this trend is significant for SBP ($p=0.042$) and highly significant for DBP ($p<0.001$). After adjusting for covariates, the trends were no longer significant, but Q5 showed significant lower BP than Q1 in all instances ($p<0.05$).

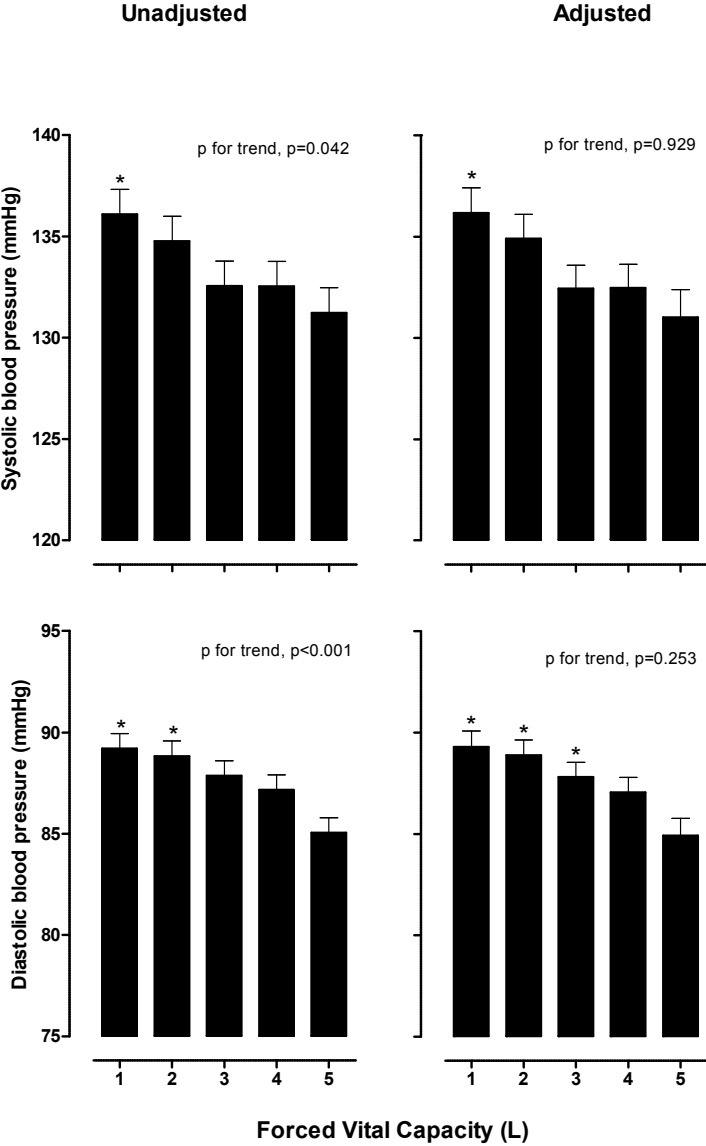


Figure 3: The unadjusted and adjusted differences in systolic- and diastolic blood pressure between quintiles of FVC in 2010 African men and women. Bars with an asterisk differ significantly from Q5. Adjusted for age, gender, waist circumference, height and a history of tobacco use.

Sensitivity analyses

Due to the known effects of inflammation as a consequence of HIV infection [26], we compared SBP and DBP as well as FEV₁ and FVC between HIV infected and uninfected groups to determine whether the BP and lung function differ. No significant differences were found and based on these findings HIV status was not included as a covariate in statistical analyses and the HIV infected participants were not excluded from the analyses.

DISCUSSION:

Our aims were to compare South African, European and U.S. prediction equations for lung function in a large sample of black South Africans. We also wanted to establish whether an association exists between lung function and blood pressure in this population.

In addressing our first aim we found that the implementation of the South African and international prediction equations yielded different results, underlying the challenge in selecting appropriate reference values in lung function assessment [3]. This is underscored by evidence that lung function varies significantly between different regions of the world [22]. Overall, the highest predicted values were seen for the South African reference values. These prediction equations yielded higher predicted values when compared to the European reference values even after the correction factor of 0.9, which was suggested by the South African Thoracic Society (SATS), was applied. Also when comparing the group of apparently healthy men with the rest of the men, the South African reference values displayed the highest percentages for FEV₁ (97.5 ± 20.5) and FVC (89.7 ± 20.6). Despite the lack of a large all-inclusive study of the South African population [27], there are smaller studies that have aimed to establish reference values for South African population groups [4,5]. These reference values are based on a study population comprised of Sesotho, isiZulu and Setswana speaking individuals [5], which is comparable with our Setswana speaking population. Although not reported formally, body composition and size of different black ethnic groups is perceived to be different. This requires further investigation but may explain measured as well as predicted values in our study for FEV₁

and FVC being considerably lower when compared to data from other South African studies [4,5] even for the group of healthy men. Louw et al.,[4] and Mokoetle et al.,[5] reported a mean FEV₁ of 3.41 L and 3.14 L respectively, which is considerably higher than the 2.58 L of the men in our study population. With regards to the FVC, these authors reported a mean of 4.26 L and 4.19 L respectively, which is also higher than the 3.11 L from our data. Urbanization could play a key role in these findings due to an increase in environmental and occupational exposures [2]. South Africa is undergoing rapid industrialization and occupational exposures contribute substantially to the increase in respiratory and cardiovascular diseases [2]. Although occupational health and hygiene control and regulation of the mining industry has greatly improved, many miners that relocated to their village of origin have had significant morbidity from respiratory disorders such as tuberculosis [28].

Our most prominent finding was a significant inverse association between BP and lung function evident in the total group according to FEV₁ and FVC quintiles. In a pooled analysis by Sin et al., that categorized FEV₁ into quintiles, it was shown that persons in the lowest FEV₁ quintile had a 75% increase in the risk for cardiovascular mortality [20]. An inverse relationship between BP and lung function (measured by FEV₁ and FVC) has also been reported in several studies, which is confirmed by our results [13-16].

Although it is clear that a relationship exists between lung function and BP, the mechanisms underlying this association remain unclear. It has been hypothesized that the association can be explained by the confounding effect of age, since lung function decreases and BP increases with age [17]. However, our results remained significant after adjustment for age. Another proposed mechanism is that impaired lung function can give rise to increased systemic levels of CRP and other acute phase proteins leading to inflammation [29]. In animal models it has been found that induction of airway inflammation can provoke and increase systemic inflammation which may contribute to the progression of vascular endothelial dysfunction [30]. Endothelial

dysfunction is evident in patients with reduced lung function and may predispose them to systemic hypertension [31].

Our study has several strengths and limitations. This study presents results in a large population-based study in South Africa on lung function, the use of different reference values and the relationship of BP with lung function. Limitations of our study are that we included a large proportion of unemployed individuals with sampling being done in poverty-stricken communities. The participants were also selected from Setswana-speaking rural and urban areas chosen for stability to make the follow-up of this longitudinal study possible. Our results may therefore not be applicable to other population groups.

CONCLUSIONS:

In conclusion, our investigation into the accurate assessment of lung function in this population of black South Africans shows that the previously published South African reference values [4,5] are more useful when compared to reference values from Europe and the US. Furthermore, SBP and DBP are higher when lung function is impeded. It is important to select appropriate reference values to quantify lung function and to extensively study the mechanistic link between reduced lung function and cardiovascular comorbidities.

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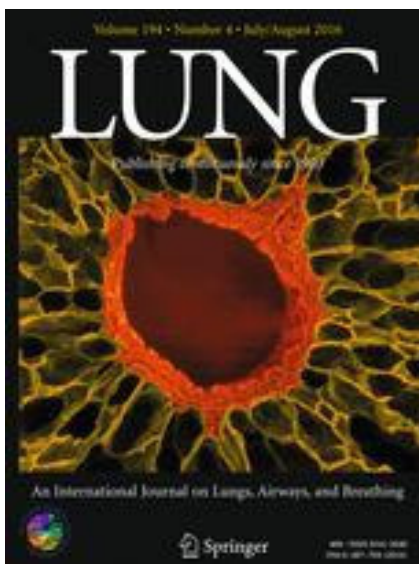
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CHAPTER 4

Inflammation as possible mediator for the relationship between lung- and arterial function.

Inflammation as Possible Mediator for the Relationship Between Lung and Arterial Function

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SUMMARY OF INSTRUCTIONS TO AUTHORS

JOURNAL DETAILS	
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(**Note: Some of the format was changed to ensure uniformity throughout the thesis.)

ABSTRACT:

Introduction: Reduced lung function is associated with a risk for the development of cardiovascular disease. This association may be due to chronic inflammation which is often present in those with reduced lung function. **Purpose:** We investigated the possible role of systemic inflammation as the mediator between lung function and arterial stiffness in 1534 black South Africans. **Methods:** Spirometric data including forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) was obtained. C-reactive protein (CRP), interleukin-6 (IL-6), blood pressure (BP) and carotid-radial pulse wave velocity (PWV) were determined. **Results:** In multivariable-adjusted models an independent inverse association was found between IL-6 and FEV₁ ($\beta=-0.20$, $p<0.001$) and FVC ($\beta=-0.18$, $p<0.001$). Similar results were found for CRP. PWV was inversely associated with FEV₁ ($\beta=-0.06$, $p=0.037$). No association was found between inflammatory markers, BP or PWV. **Conclusion:** Reduced lung function was associated with increased inflammation and arterial stiffness. The lack of association between arterial stiffness and inflammatory markers suggests that inflammation may not be the mediating link between lung- and vascular function in this population.

Key words: pulmonary function, blood pressure, C-reactive protein, Interleukin-6, arterial stiffness, African

Word count: 172

INTRODUCTION:

Reduced lung function, as measured by forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), is associated with an increased risk for the development of hypertension [1-3] and cardiovascular disease (CVD) [4]. Although the association between lung function and CVD is clear, the mechanistic underpinnings of this association remain largely unexplained.

Reduced lung function is associated with persistent low-grade inflammation [5-7] with increased levels of cytokines such as interleukin-6 (IL-6) as well as the acute phase protein, C-reactive protein (CRP) [8]. On the cardiovascular side, both of these markers were also shown to play a role in endothelial dysfunction [9] and arterial stiffening [10,11]. With arterial stiffness being one of the risk factors for CVD [12-15], it can be asserted that one of the possible mechanisms linking reduced lung function and CVD may be chronic low-grade inflammation leading to arterial stiffness.

Inflammation as the possible link between lung- and cardiovascular function has not been investigated extensively in population-based studies, especially in black populations, known to have a high prevalence of hypertension [16]. We therefore aimed to investigate the three-way relationship between: (a) lung function (FEV₁ and FVC) and markers of inflammation (IL-6 and CRP); (b) inflammation and arterial stiffness by using pulse wave velocity (PWV); and (c) lung function and arterial stiffness in a sample of Africans to determine whether there is an association between lung- and vascular function, and whether this association is mediated through inflammation.

MATERIALS AND METHODS:

Study design and subject selection

This study is embedded in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study. This longitudinal study was designed to investigate urban and rural lifestyle changes and health status of populations from numerous countries, with the details described elsewhere [17,18]. The main inclusion criteria for participating South African communities were that they had to show migration stability and also had to be part of the North West Province. The baseline data used in this study was collected in 2005, during which four different resident areas were identified for participation. A household census regarding the number of people, their ages and health profile was done in 6000 houses (1500 in each community) starting from a specific point. Every head of household signed written informed consent to fill out the questionnaire. If a person refused or was not at home, the next house was taken and a non-complier questionnaire was filled out. From the data obtained from the census a paper-based selection of possible subjects was made based on the following inclusion criteria: An apparently healthy cohort of men and women older than 35 years, who were not pregnant, intoxicated, and did not have any cognitive deficits. A total of 2010 volunteers were recruited from the 6000 randomly selected households. Our specific sub-study excluded those infected with the human immunodeficiency virus (HIV) (n=337), those making use of anti-inflammatory medication (n=55) and those with missing spirometry data (n=84). The final study population consisted of 1534 participants.

The study protocol complies with the Declaration of Helsinki as revised in 2000 and approval was obtained from the Ethics Committee of the North-West University, Potchefstroom, South Africa (Ethics number 04M10). Permission to conduct the study in the above-mentioned communities was granted by the Provincial Department of Health, community leaders, tribal chiefs, and mayors. The recruited participants were visited at their homes where the protocol

was explained to them in their home language by field workers. They were given the opportunity to ask questions prior to giving informed consent.

Questionnaires

The subjects were interviewed by trained field workers using structured demographic, lifestyle and physical activity questionnaires, developed and standardised for the international PURE study and adjusted for each country [18]. Lifestyle data included tobacco use, alcohol intake, health history, and medication use.

Anthropometric measurements

Anthropometric measurements included waist circumference (Holtain unstretchable metal tape), height (Invicta Stadiometer, IP 1465, Invicta, London, UK) and weight (Precision Health Scale, A&D Company, Tokyo, Japan). We also calculated body mass index ($\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$) [19].

Cardiovascular measurements

Brachial systolic- (bSBP) and diastolic blood pressure (bDBP) were measured on the right arm using an automated digital BP monitor (OMRON HEM-757, Omron Healthcare, Kyoto, Japan) after a ten minute resting period. The subject was sitting in a comfortable position with the right arm rested on a stable surface. The BP measurement was repeated after a five minute resting interval and the second BP reading was used for statistical analyses.

Pulse wave velocity (PWV) was measured on the left side of each participant while in a supine position by using the Complior SP device (Artech-Medical, Pantin, France) in a segment over the carotid-radialis.

Spirometry

Lung function was assessed according to the American Thoracic Society recommendations [20] using a mobile Micro GP Spirometer (Micro Medical Ltd, Kent, UK). A minimum of three acceptable recordings and a maximum of five were obtained from each participant while in the standing position and with at least 1 minute between repeat measurements. Measurements were taken by trained occupational hygienists. Analysis was based on the maximal effort for both FEV₁ and FVC of all measurements obtained from each participant.

Blood sampling

Blood samples were taken by a registered nurse from the antebachial vein branches. The subjects were asked to fast overnight (eight to ten hours with no food or beverage, excluding water). The blood was centrifuged for 15 minutes at 2000g at 4°C. Aliquots of plasma and serum were frozen on dry ice, stored in the field at -18°C for two to four days after which the samples were transported to a storage facility where the samples were kept at -80°C until analysis.

Biochemical analysis

Serum γ -glutamyltransferase (GGT), total cholesterol (TC) and high sensitivity C-reactive protein (CRP) were analysed by particle enhanced turbidimetric assays with the Konelab 20i™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). IL-6 was analysed by the Elecsys apparatus using ultra-sensitive enzyme immunoassays (Elecsys 2010, Roche, Basel, Switzerland).

Glucose levels were determined by an enzymatic reference method (Vitros DT6011 Chemistry Analyzer; Ortho-Clinical Diagnostics, Rochester, New York, USA). To determine glycated haemoglobin (HbA1c) levels the D-10 Haemoglobin testing system from Bio-Rad (#220-0101) was used. The HIV status was determined by a First Response Rapid HIV card test (PMC Medical, Daman, India). In the case of a positive outcome, the result was confirmed with the

Pareeshak card test (BHAT, Bio-tech, India). All participants also received pre- and post HIV-test counselling by trained counsellors.

Statistical analysis

Statistical analyses were performed using Statistica version 12 (Statsoft, Inc., Tulsa, OK, USA). We tested for the interaction of sex on the relationships between lung function (FEV₁ and FVC), inflammation (IL-6 and CRP) and cardiovascular function (SBP and PWV). The interaction tests with sex were not significant (all $p > 0.394$) and therefore we pooled the data for men and women. Variables with a non-Gaussian distribution were logarithmically transformed (HbA1c, IL-6, CRP and GGT) and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Single and partial correlations were performed to determine if associations exist between measures of lung function (FEV₁ and FVC), cardiovascular function (SBP and PWV) and markers of inflammation (IL-6 and CRP). Furthermore, by using either a marker of inflammation (IL-6 or CRP) or measure for cardiovascular function (SBP or PWV) as dependent variable, we determined whether an independent association exists with lung function as main independent variable (FEV₁ or FVC) in forward stepwise multiple regression analysis. Results from the different regression models are shown as standardized beta-values (β) (Table 3). Covariates included in all models were age, sex, height, physical activity index, tobacco use, GGT (log), HbA1c (log), TC and anti-hypertensive medication, with mean arterial pressure and heart rate included additionally in the models with PWV as the dependent variable.

RESULTS:

The characteristics of the study population (n=1534) are outlined in Table 1. The population consisted of 62.4% women and had a mean age of 51.2 ± 10.5 years. With regards to BP, 41.6% were hypertensive (SBP and/or DBP $\geq 140/90$ mmHg). The mean FEV₁ and FVC were 2.22 ± 0.70 L and 2.62 ± 0.81 L, respectively. The mean CRP level was 3.17 mg/L, exceeding the 3.0 mg/L cut-point for a high risk of future CV events [21]. A total of 54.4% of the participants reported previous or current use of tobacco products. A very low percentage (0.78%) of the population indicated use of anti-hypertensive medication.

Table 1: Characteristics of the study population

	Total population
N	1534
Women, N (%)	957 (62.4)
Age (years)	51.2 ± 10.5
<i>Anthropometric measurements</i>	
Height (m)	1.61 ± 0.08
Body mass index (kg/m ²)	25.1 ± 10.2
Waist circumference (cm)	80.7 ± 13.4
<i>Cardiovascular measurements</i>	
Systolic blood pressure (mmHg)	135.6 ± 24.2
Diastolic blood pressure (mmHg)	88.5 ± 14.2
Pulse wave velocity (m/s)	11.1 ± 2.34
<i>Lung function measurements</i>	
Forced expiratory volume in one second (L)	2.22 ± 0.70
Forced vital capacity (L)	2.62 ± 0.81
<i>Biochemical markers</i>	
Total cholesterol (mmol/L)	5.13 ± 1.37
Glycated haemoglobin (%)	5.63 (4.80; 6.60)
C-reactive protein (mg/L)	3.17 (0.25; 37.8)
Interleukin-6 (pg/mL)	2.82 (0.75; 16.8)
<i>Lifestyle factors</i>	
γ -glutamyltransferase (U/L)	55.8 (19.0; 361)
Smoking, N (%)	834 (54.4)
Physical activity index	7.29 ± 1.87
<i>Medication usage</i>	
Anti-hypertensive, N (%)	12 (0.78)

Data expressed as arithmetic mean \pm SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. N, number of participants.

Figure 1 illustrates the single regression analyses between inflammation (IL-6), cardiovascular function (SBP and PWV) and lung function (FEV₁). We found negative associations between FEV₁ and IL-6 ($r=-0.27$, $p<0.001$) as well as FEV₁ and CRP ($r=-0.23$, $p<0.001$; not shown). The correlations between SBP and IL-6 ($r=0.11$, $p<0.001$) and PWV and IL-6 ($r=0.06$, $p=0.030$) were weak, although significant. Similar results were found with CRP (not shown). We also found a negative association between SBP and FEV₁ ($r=-0.10$, $p<0.001$), but no association between PWV and FEV₁ ($r=0.02$, $p=0.523$). We repeated these analyses and adjusted for age, sex, height, physical activity, tobacco use and GGT (Table 2). We found a significant negative association between FEV₁ and IL-6 ($r=-0.19$, $p<0.001$) as well as between FEV₁ and CRP ($r=-0.19$, $p<0.001$). FVC also associated negatively with IL-6 ($r=-0.17$, $p<0.001$) and with CRP ($r=-0.16$, $p<0.001$). None of the cardiovascular variables (SBP, DBP and PWV) associated with either IL-6 or CRP after adjustments. Furthermore, we found negative associations between FEV₁ and PWV ($r=-0.06$, $p<0.001$; not shown) and between FVC and PWV ($r=-0.05$, $p<0.001$; not shown). Neither SBP nor DBP was associated with either FEV₁ or FVC.

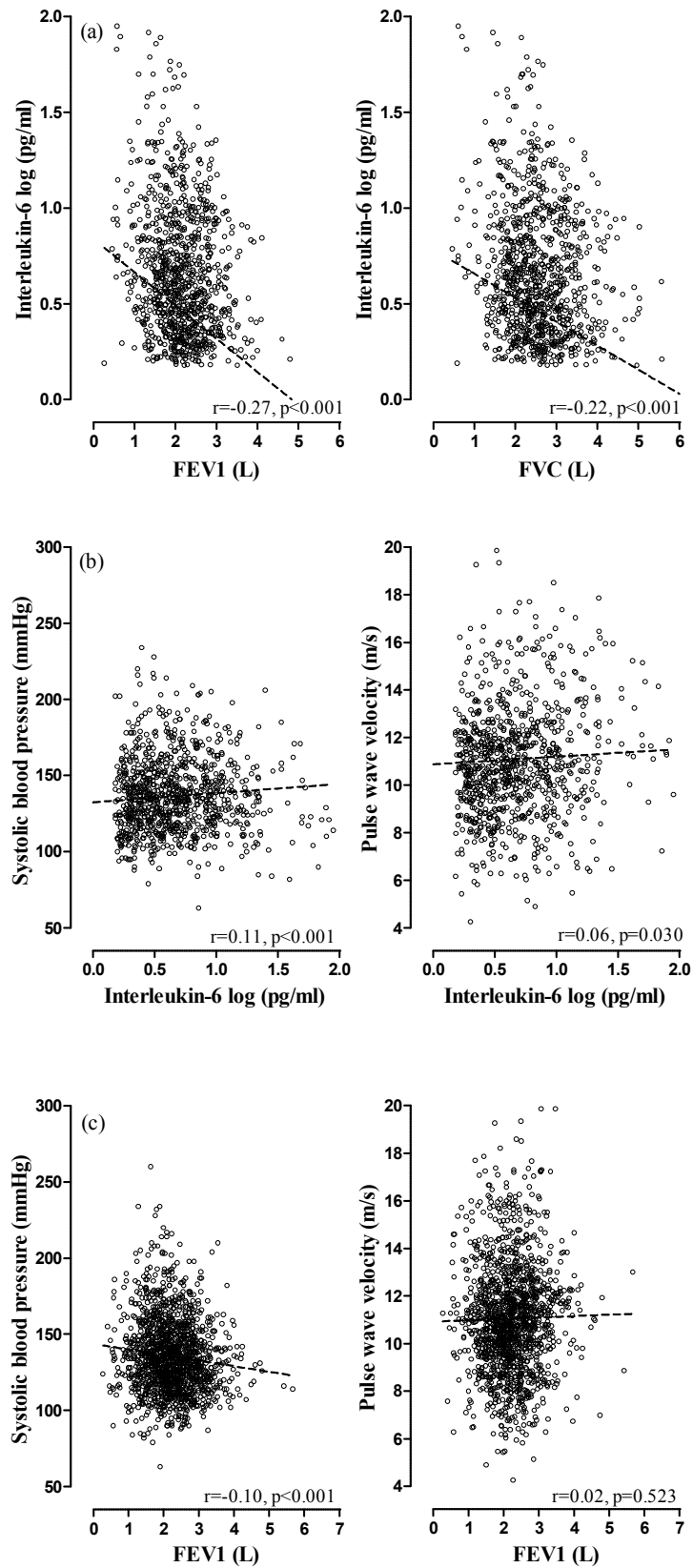


Figure 1: Single regression analyses between (a) inflammation and lung function, (b) cardiovascular function and inflammation and (c) cardiovascular function and lung function. FEV₁, forced expiratory volume in one second; FVC, forced vital capacity

Finally we performed forward stepwise multiple regression analyses (Table 3) with IL-6 (log) or CRP (log) as dependent variable. We found an independent negative association between IL-6 and FEV₁ in Model 1 ($p < 0.001$) as well as between IL-6 and FVC in Model 2 ($p < 0.001$). Similar results were found between CRP and FEV₁ in Model 1 ($p < 0.001$) and FVC in Model 2 ($p < 0.001$).

Table 2: Partial correlations of inflammatory markers with lung function and cardiovascular measures

	Interleukin-6 (pg/mL)		C-reactive protein (mg/L)	
	<i>r</i>	<i>p-value</i>	<i>r</i>	<i>p-value</i>
Lung function markers				
FEV ₁ (L)	-0.19	<0.001	-0.19	<0.001
FVC (L)	-0.17	<0.001	-0.16	<0.001
Cardiovascular variables				
Systolic blood pressure (mmHg)	-0.01	0.925	-0.01	0.778
Diastolic blood pressure (mmHg)	-0.01	0.994	-0.01	0.771
Pulse wave velocity (m/s)	-0.04	0.127	-0.04	0.159

Adjusted for age, sex, height, physical activity, tobacco use and GGT. FEV₁, forced expiratory volume in one second; FVC, forced vital capacity.

In further forward stepwise multiple regression analyses; we included SBP or PWV as dependent variable, with either IL-6 or a marker of lung function as the main independent variable. SBP was not significantly associated with IL-6 or either of the lung function markers in any of the models. In the models with PWV as the dependent variable, there was also no significant association between PWV and IL-6 (Model 3), but PWV was significantly associated with FEV₁ in Model 4 ($p = 0.037$), and with borderline significance with FVC (Model 5) ($p = 0.078$). When entering both IL-6 and a marker of lung function into Models 6 or 7, PWV was not associated with IL-6, but with both FEV₁ ($p = 0.045$) and FVC ($p = 0.045$). We repeated all models with SBP and PWV as dependent variables, replacing IL-6 with CRP and found similar results.

Table 3: Forward stepwise multiple regression analyses with IL-6 (log), CRP (log), systolic blood pressure and pulse wave velocity, respectively, as dependent variable and lung function as well as inflammatory markers as independent variables

Independent variable(s)	IL-6 (pg/ml)		CRP (mg/L)	
	β (SE)	<i>p</i> -value	β (SE)	<i>p</i> -value
Model 1	<i>R</i>²=0.18		<i>R</i>²=0.17	
FEV ₁ (L)	-0.20 (0.03)	<0.001	-0.21 (0.03)	<0.001
Model 2	<i>R</i>²=0.17		<i>R</i>²=0.16	
FVC (L)	-0.18 (0.03)	<0.001	-0.19 (0.03)	<0.001
	Systolic blood pressure (mmHg)		Pulse wave velocity (m/s)	
	β (SE)	<i>p</i> -value	β (SE)	<i>p</i> -value
Model 3	<i>R</i>²=0.16		<i>R</i>²=0.22	
IL-6 (pg/ml)	-	-	-	-
Model 4	<i>R</i>²=0.16		<i>R</i>²=0.23	
FEV ₁ (L)	-	-	-0.06 (0.03)	0.037
Model 5	<i>R</i>²=0.16		<i>R</i>²=0.23	
FVC (L)	-	-	-0.05 (0.03)	0.078
Model 6	<i>R</i>²=0.16		<i>R</i>²=0.23	
FEV ₁ (L)	-	-	-0.06 (0.03)	0.045
IL-6 (pg/ml)	-	-	-	-
Model 7	<i>R</i>²=0.16		<i>R</i>²=0.23	
FVC (L)	-	-	-0.05 (0.03)	0.045
IL-6 (pg/ml)	-	-	-	-

Regression coefficients shown are standardized beta coefficients. *R*² values represent adjusted *R*² values of the whole model. SE, standard error. Covariates included in all models: age, sex, height, physical activity, systolic blood pressure, tobacco use, GGT (log), HbA1c (log), TC and anti-hypertensive medication usage. Heart rate and mean arterial pressure included as additional covariates in models with pulse wave velocity as dependent variable. *p*-values ≤ 0.05 regarded as significant.

DISCUSSION:

We investigated the possible role of systemic inflammation as the mediator between lung function and arterial stiffness. We found an independent inverse association between lung function and inflammation. In accordance with recent studies [22-24], our study indicates that higher levels of both CRP and IL-6 are associated with reduced pulmonary function (FEV₁ and FVC), independent of potential confounders. This supports the notion that inflammatory processes are involved in the pathogenesis of various pulmonary diseases [25]. The comorbidities associated with reduced lung function, such as CVD, are reported to be due to a systemic spill-over of inflammation occurring within the lungs [8]; however more evidence is needed to support this hypothesis.

There is a limited amount of information regarding the association between lung function and arterial stiffness; however our finding, namely an inverse association between lung function and arterial stiffness, supports existing findings that also show this association independent of confounding factors [26-28]. Several possible causes for this association exist, such as genetic susceptibility or inherited factors that may both influence lung function as well as arterial stiffness [29]. Alternatively, lung function and arterial stiffness could both be influenced by external factors such as metabolic disruption, environmental factors such as smoking or inflammatory processes [27]. Duprez et al.,[22] recently stated that inflammation acts as a parallel physiological pathway for a simultaneous loss of elasticity in both the connective tissue of the alveoli and arterial wall which may lead to reduced lung function and arterial stiffness.

Based on these associations between lung function and inflammation as well as between lung function and arterial stiffness, also observed in our study, it is reasonable to assume that lung function and arterial stiffness could be linked by inflammation as the mediator. However, we found no association between markers of inflammation and BP or measures for arterial stiffness in our black population. These results are surprising especially when viewed in light of findings from another South African study that showed that Africans have significantly higher levels of

CRP when compared to white counterparts [30]. It remains controversial whether CRP is merely a marker [31,32] or indeed causal [33-35] in the development of CVD, with strong evidence supporting either statement. Notwithstanding this controversy, CRP remains an important marker to include when investigating CVD development. Various studies report an association between CRP and PWV [10,36-38] but in some, these associations disappear after adjustment for conventional risk factors for CVD [37,39,40]. There are also studies that have reported no association between CRP and PWV [41,42], and it is possible that CRP does not have a causal role in the development of arterial stiffness but may only act as a marker of vascular damage [43]. Contradictory to previous studies which showed a positive association between IL-6 and PWV [11,44], this association was not evident in our population. A recent study also reported no association between IL-6, CRP and small or large artery elasticity in women, while in men IL-6 and CRP only associated with small artery elasticity [22]. The lack of association between inflammation, BP and arterial stiffness in our study may be due to the variety of conventional and behavioural risk factors for CVD that have been identified in Africans and may have a confounding effect on this association. These factors include insufficient physical activity [45], dietary factors [46], smoking [47] and alcohol abuse [48], all of whom are known to play a role in arterial stiffening [49-52]. Furthermore, other inflammation-sensitive plasma proteins may be more sensitive markers of inflammation than those used in our study and could have a greater contribution to arterial stiffening and CV risk [4].

In a population already afflicted by a significant burden of hypertension [16,48] and a low percentage of anti-hypertensive medication usage - which is indicative of poor diagnosis and control - it is important to investigate and understand all of the factors that may lead to the development of CVD. Arterial stiffness is one of the risk factors of CVD and an independent predictor of cardiovascular risk [53-55]. Several studies have also reported that arterial stiffness is more common in those of African ancestry when compared to those of European descent [56-59]. This may have an important impact in South Africa as the country is undergoing rapid industrialisation and occupational exposures are contributing substantially to the increase in

respiratory- and CVD [60]. Occupational health and hygiene control as well as the regulation of the mining industry have greatly improved; however, many miners that relocated to their village of origin have had significant morbidity from respiratory disorders such as silicosis [61] and tuberculosis [62]. Furthermore, with arterial stiffness already being elevated even in young Africans [63], the association between lung function and arterial stiffness found in our study warrants the consideration of lung function in global risk factor evaluation for CVD. However, the lack of association between arterial stiffness and markers of inflammation in this study population suggests that mechanisms other than inflammation are at play.

Some limitations of our study are that we included a large proportion of unemployed individuals with sampling being done in poverty-stricken communities. The participants were also selected from mainly Setswana-speaking rural and urban areas and results may therefore not be generalized to other populations in South Africa. Due to the cross-sectional design, cause and effect could not be inferred. Furthermore, PWV was assessed in an upper-limb muscular artery segment over the carotid-radialis and not the large conduit vessels (carotid-femoral PWV) which is the gold standard for assessing aortic stiffness. However, arterial stiffness of the peripheral arteries may be more important in black than white populations as CV risk factor [63] and was also found to predict CV events independent of age [64]. Strengths of our study include a large population-based study with several measures of lung function, inflammation and arterial stiffness in Africans for which limited information is available.

CONCLUSION:

Reduced lung function was associated with increased inflammation and arterial stiffness, but we found no association between inflammation and arterial stiffness in this black population. Our findings suggest that inflammation may not be mediating the link between lung function and arterial stiffness, and that inflammation may be involved in other pathophysiological mechanisms relating to CVD development.

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CHAPTER 5

**Lung function, inflammation and cardiovascular mortality in a
population of black South Africans**

Lung function, inflammation and cardiovascular mortality in a population of black South Africans

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SUMMARY OF INSTRUCTIONS TO AUTHORS

JOURNAL DETAILS	
Title:	<i>European Journal of Clinical investigation</i> (Eur J Clin Invest)
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<p>Aim & Scope: The EJCI publishes reports of high-quality research that pertain to the genetic, molecular, cellular, or physiological basis of human biology and disease, as well as research that addresses prevalence, diagnosis, course, treatment, and prevention of disease. We are primarily interested in studies directly pertinent to humans, but submission of robust in vitro and animal work is also encouraged. Interdisciplinary work and research using innovative methods and combinations of laboratory, clinical, and epidemiological methodologies and techniques is of great interest to the journal. Several categories of manuscripts (for detailed description see below) are considered: editorials, original articles (also including randomized clinical trials, systematic reviews and meta-analyses), reviews (narrative reviews), opinion articles (including debates, perspectives and commentaries); and letters to the Editor.</p>	
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Tables & Figures:	max 5
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References:	± 40
	1 Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.
Sections:	Background, Materials and methods, Results, Conclusions
Ethical considerations:	Formal review and approval by an appropriate institutional review board or ethics committee is required and should be described in the 'Materials and methods' section. Reports on biomedical research involving human subjects must include a statement that informed consent was obtained from each subject or subject's guardian.
Other:	---

(**Note: Some of the format was changed to ensure uniformity throughout the thesis.)

ABSTRACT:

Background: The link between impaired lung function and cardiovascular outcome is well established in European and American populations. It is possible that this association may be driven by a systemic spill-over of inflammation occurring within the lungs. Since several studies have found an increased level of inflammatory markers in African populations, we aimed to establish the contribution of lung function in predicting all-cause and cardiovascular mortality in Africans, whilst taking inflammatory markers into account. **Methods:** We followed 1 442 black South Africans from the North West Province participating in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study, over a five year period. Spirometry, cardiovascular and metabolic measures were performed and cardiovascular mortality as well as all-cause mortality used as endpoints. **Results:** In univariate Cox regression models, both forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) predicted all-cause (P=0.022; P<0.001) and cardiovascular mortality (P=0.004; P<0.001). In multivariate adjusted standardized Cox regression analyses, only FVC predicted cardiovascular mortality independent of several covariates (hazard ratio, 0.57 [0.35-0.94]), including C-reactive protein (CRP). When CRP was replaced by interleukin-6 in the model, the significance of FVC was lost (hazard ratio, 0.85 [0.55-1.30]). **Conclusion:** FVC, but not FEV₁, is a strong predictor of both all-cause and CV mortality in black South Africans, which may be mediated by inflammation.

Keywords: pulmonary function, African, death, South Africa

Word count: 221

INTRODUCTION:

South Africa is undergoing a health transition that is characterised by an increase in epidemic infectious diseases as well as non-communicable diseases such as cardiovascular disease (CVD) and chronic lung disease.¹ Rapid industrialization has contributed to occupational exposure, increasing the incidence of respiratory diseases.² Occupational hygiene control especially in the mining industry has greatly improved; however, there is still a significant burden of silicosis and asbestos-related diseases.³ Apart from occupational exposure, living conditions in the informal settlements of South Africa may also contribute to the burden of chronic lung diseases. Exposure to the burning of biomass fuels used for cooking and heating in a closed environment, as well as residence in areas with poor air quality are significantly related to reduced lung function.⁴⁻⁷ Reduced lung function is associated with an increased risk for CVD as well as CVD-related mortality.⁸ European and American studies have found that both a reduction in forced expiratory volume (FEV₁) and forced vital capacity (FVC) are associated with increased incidences of myocardial infarction and cardiovascular mortality.^{8,9} The exact mechanism behind this association is not fully understood. It remains unclear whether reduced lung function and CVD merely share similar risk factors such as increased age, smoking and sedentary behaviour or whether reduced lung function has a causal role in the development of CVD.¹⁰ One of the mechanisms suggesting a causal role is chronic low-grade inflammation leading to vascular dysfunction.⁹ Reduced lung function is associated with higher levels of C-reactive protein (CRP) and other systemic inflammatory markers, such as interleukin-6 (IL-6).^{11,12} Increased levels of both CRP and IL-6 are associated with endothelial dysfunction and arterial stiffening and may therefore increase the risk for CV morbidity and mortality.⁹ Although it is evident that South Africa has a significant burden of both cardiovascular- and respiratory disease, data concerning the possible contribution of reduced lung function to cardiovascular mortality in South Africa is still limited. The identification of markers for the detection of mortality risk is essential to implement proper treatment and action. We aimed to establish the contribution of lung function, measured by FEV₁ and FVC, in predicting all-cause and

cardiovascular mortality over five years in a study sample of 1442 black South Africans, whilst taking inflammatory markers into account.

MATERIALS AND METHODS:

Study design and subject selection

This sub-study is embedded in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study which was designed to investigate lifestyle changes and health status of populations from numerous countries.¹³⁻¹⁵ The main inclusion criteria for participating South African communities were that they had to show migration stability and also had to be part of the North West Province. The baseline data used in this study was collected in 2005, during which four different resident areas were identified for participation. A household census regarding the number of people, their ages and health profile was done in 6000 houses (1500 in each community) starting from a specific point. Every head of household signed written informed consent to fill out the questionnaire. If a person refused or was not at home, the next house was taken and a non-complier questionnaire was filled out. From the data obtained from the census, a paper-based selection of possible subjects was made based on the following inclusion criteria: An apparently healthy cohort of men and women older than 35 years, who were not pregnant, intoxicated, and did not have any cognitive impairment. The exclusion criteria were participants who were older than 65 years, had a history of tuberculosis or Human Immunodeficiency Virus (HIV) infection, had an ear temperature exceeding 37°C, who were blood donors or were vaccinated within the previous three months. The first follow-up took place in 2010. Our specific sub-study excluded 568 participants: those with missing spirometry data (n=119) and those who were lost to follow-up (n=449). The final study population consisted of 1442 participants. Of the 1442 participants, a total of 218 passed away over the five year follow-up period, with cardiovascular mortality contributing to (n=63) deaths and non-cardiovascular mortality to (n=155).

Ethical considerations

The study protocol complies with the Declaration of Helsinki as revised in 2008 and approval was obtained from the Ethics Committee of the North-West University, Potchefstroom Campus, South Africa. The protocol was explained to the subjects in their home language by field workers and they were given the opportunity to ask questions prior to giving informed consent. Confidentiality and anonymity of all the results were assured by making use of anonymised numbers.

Questionnaires

The participants were interviewed by trained field workers using structured demographic, socio-economic, lifestyle and physical activity questionnaires, developed and standardised for the international PURE study and adjusted for each country.¹⁴ Lifestyle data included tobacco use, alcohol intake, health history and medication use.

Anthropometric and cardiovascular measurements

Anthropometric measurements were done by a trained anthropometrist and included waist circumference (Holtain stretchable metal tape, Croswell, Wales), height (Invicta Stadiometer, IP 1465, Invicta, London, UK) and weight (Precision Health Scale, A&D Company, Tokyo, Japan). Body mass index ($BMI = \text{weight (kg)} / \text{height (m)}^2$) was also calculated.¹⁶

Cardiovascular measurements

Brachial systolic- (bSBP) and diastolic blood pressure (bDBP) were measured on the right upper-arm using an automated digital BP monitor (OMRON HEM-757, Omron Healthcare, Kyoto, Japan) with the participant in a sitting position. The measurement was repeated after a five minute resting interval and the second BP reading was used for statistical analyses.

Pulse wave velocity (PWV) was measured on the left side of each participant while in a supine position. We used the Complior SP device (Artech-Medical, Pantin, France) to measure the segment over the carotid to radial artery.

Spirometry

Lung function was assessed according to the American Thoracic Society recommendations¹⁷ using a mobile Micro GP Spirometer (Micro Medical Ltd, Kent, UK). A minimum of three acceptable recordings and a maximum of five were obtained from each participant while in the standing position and with at least 1 minute between repeat measurements. Measurements were taken by trained occupational hygienists. Analysis was based on the maximal effort for both FEV₁ and FVC from all measurements obtained from each participant.

Blood sampling

Fasting blood samples were taken by a registered nurse from the antebrachial vein branches. The blood was centrifuged for 15 minutes at 2000g at 4°C. Aliquots of plasma and serum were frozen on dry ice, stored in the field at -18°C for two to four days after which the samples were transported to a laboratory storage facility where the samples were kept at -80°C until analysis.

Biochemical analysis

Serum γ -glutamyltransferase (GGT), total cholesterol (TC) and C-reactive protein (CRP) were analysed by particle enhanced turbidimetric assays with the Konelab 20i™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Interleukin-6 (IL-6) was analysed by the Elecsys apparatus using ultra-sensitive enzyme immunoassays (Elecsys 2010, Roche, Basel, Switzerland). Glucose was determined in sodium fluoride tubes by an enzymatic reference method with hexokinase (Vitros DT6011 Chemistry Analyzer; Ortho-Clinical Diagnostics, Rochester, New York, USA). To determine glycated haemoglobin (HbA1c) from ethylenediamine-tetra-acetic acid plasma samples, the D-10 Haemoglobin testing system from Bio-Rad (#220-0101) was used. The HIV status was determined by a First Response Rapid HIV card test (PMC Medical, Daman, India). In the case of a positive outcome, the result was confirmed with the Pareeshak card test (BHAT, Bio-tech, India). All participants also received pre- and post HIV-test counselling by trained counsellors.

Mortality outcome assessment

Participants were contacted by trained fieldworkers in three month intervals over the five year period (2005-2010). Verbal autopsies and family death certificates were used to obtain cause of death. A physician coded the immediate and underlying causes according to the International Classification of Diseases codes. Cardiovascular mortality was classified as death due to cardiovascular reasons, which included cardiac failure, heart failure, heart attack, myocardial infarction, stroke or cerebral vascular incident.

Statistical analysis

Statistical analyses were performed using Statistica version 12 (Statsoft, Inc., Tulsa, OK, 2010) and Graphpad Prism version 5.03 for Windows (Graphpad Software, San Diego, California, USA) was used to analyse and plot results. Continuous data were presented as arithmetic mean \pm standard deviation and categorical data as proportions. Variables with a non-Gaussian distribution were logarithmically transformed (fasting glucose and GGT) and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals.

We compared means and proportions of baseline data between survivor and non-survivor groups with analysis of variance (ANOVA) and Chi-square tests, respectively. We used Kaplan-Meier survival function estimates and the log-rank test to compare incidence rates across tertiles of FEV₁ and FVC.

Furthermore, we applied univariate and multivariable-adjusted Cox regression to compute standardised hazard ratios, which expresses the risk for a 1-standard deviation increase in the independent variable (Models 1-3). We examined FEV₁ and FVC in separate models as the main independent variable. We also included two models with either CRP (Model 4) or IL-6 (Model 5) as additional independent variable. Covariates were included in the models based on exploratory partial regression analyses and included age, sex, HIV status, height, waist

circumference, physical activity index, systolic blood pressure, heart rate, GGT, tobacco use, fasting glucose, TC and anti-hypertensive medication. Statistical significance was set at $P \leq 0.05$.

Results

The baseline characteristics are stratified by mortality and are reported in Table 1. Of the 1442 participants, 218 died during the five-year follow-up period, with 63 deaths attributable to cardiovascular causes. The cardiovascular mortality group was the oldest, with a mean age of 59 years ($P < 0.001$) and had the lowest spirometric volumes, both for FEV₁ (2.06ℓ, $P = 0.018$) and FVC (2.19ℓ, $P < 0.001$). This group also had the lowest level of physical activity ($P < 0.001$), the highest IL-6 level ($P < 0.001$) and included the highest number of individuals on anti-hypertensive medication (36.5%). The non-cardiovascular mortality group consisted out of 52.3% men from rural areas (52.9%) with the highest percentage of HIV infection (44.4%).

Table 1: Baseline characteristics of the study population stratified by five year mortality

	Survivors (n=1224)	Cardiovascular mortality (n=63)	Non- Cardiovascular mortality (n=155)	P
<i>Socio-demographic profile</i>				
Age (years)	50 ± 10 ^a	59 ± 12 ^{ab}	50 ± 11 ^b	< 0.001
Sex, men (%)	411/1224 (33.6) ^a	24/63(38.1)	81/155 (52.3) ^a	< 0.001
Locality, rural (%)	697/1224 (56.9) ^a	26/63 (41.3) ^a	82/155 (52.9)	0.037
Education, none (%)	454/1191 (38.1)	23/61 (37.7)	58/150 (38.7)	0.989
HIV status, positive (%)	142/1220 (11.6) ^a	8/62 (12.9) ^{ab}	68/153 (44.4) ^b	< 0.001
<i>Anthropometric measurements</i>				
Body mass index (kg/m ²)	25.0 ± 7.06 ^a	24.4 ± 7.52 ^b	21.6 ± 5.58 ^{ab}	< 0.001
Waist circumference (cm)	79.9 ± 12.9 ^a	81.4 ± 16.7 ^b	76.3 ± 10.9 ^{ab}	0.002
Height (m)	1.60 ± 0.08 ^a	1.59 ± 0.09 ^b	1.63 ± 0.09 ^{ab}	< 0.001
<i>Cardiovascular measurements</i>				
Systolic blood pressure (mmHg)	131 ± 20	135 ± 22	131 ± 22	0.489
Diastolic blood pressure (mmHg)	87 ± 12	89 ± 14	87 ± 14	0.287
Heart rate (beats per minute)	72 ± 14 ^a	76 ± 16	77 ± 15 ^a	< 0.001
Pulse pressure (mmHg)	44 ± 12	46 ± 14	44 ± 13	0.461
Pulse wave velocity (m/s)	10.8 ± 2.01	11.3 ± 1.94	11.1 ± 2.04	0.062
<i>Lung function measurements</i>				
FEV ₁ (ℓ)	2.27 ± 0.56 ^a	2.06 ± 0.47 ^a	2.22 ± 0.63	0.018
FVC (ℓ)	2.58 ± 0.67 ^a	2.19 ± 0.65 ^{ab}	2.52 ± 0.71 ^b	< 0.001
FEV ₁ / FVC (%)	86.8 ± 9.04	85.9 ± 9.06	86.7 ± 9.74	0.753
<i>Biochemical markers</i>				
Fasting glucose (mmol/ℓ)	4.78 (3.5;6.4)	4.74 (3.4;5.9)	4.63 (3.8;6.0)	0.152
Total cholesterol (mmol/ℓ)	5.00 ± 1.20 ^a	4.89 ± 1.23 ^b	4.42 ± 1.09 ^{ab}	< 0.001
<i>Inflammatory markers</i>				
C-reactive protein (mg/ ℓ)	2.64 (0.25;22.2)	2.60 (0.28;16.1)	3.63 (0.44;29.1)	0.051
Interleukin-6 (pg/mℓ)	2.78 (0.75;18.0) ^{ab}	5.90 (0.75;48.5) ^a	5.15 (0.75;43.0) ^b	< 0.001
<i>Lifestyle</i>				
Gamma-glutamyltransferase (U/ℓ)	50.9 (19.0;274) ^{ab}	67.4 (24.3;364) ^a	68.8 (23.0;381) ^b	< 0.001
Tobacco use, n (%)	654/1219 (53.7)	38/63 (60.3)	95/154 (61.7)	0.112
Physical activity index	7.39 ± 1.81 ^a	6.12 ± 1.83 ^{ab}	7.04 ± 1.83 ^b	< 0.001
<i>Medication use</i>				
Anti-hypertensive, n (%)	218/1224 (17.8) ^a	23/63 (36.5) ^{ab}	27/155 (17.4) ^b	0.001

Data expressed as arithmetic mean ± standard deviation, geometric mean (5th and 95th percentile boundaries) or % of n. CV, cardiovascular; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity.

^{ab} Values with the same superscript letter differ significantly (P ≤ 0.05).

Kaplan-Meier survival function estimates are indicated in Figure 1. In the case of all-cause mortality, log-rank tests were significant across tertiles of FEV₁ (P = 0.002) and FVC (P = 0.006), with the lowest tertile of FEV₁ and FVC having the highest mortality percentage. With regard to cardiovascular mortality, the log-rank tests were also significant for both FEV₁ (P = 0.003) and FVC (P < 0.001).

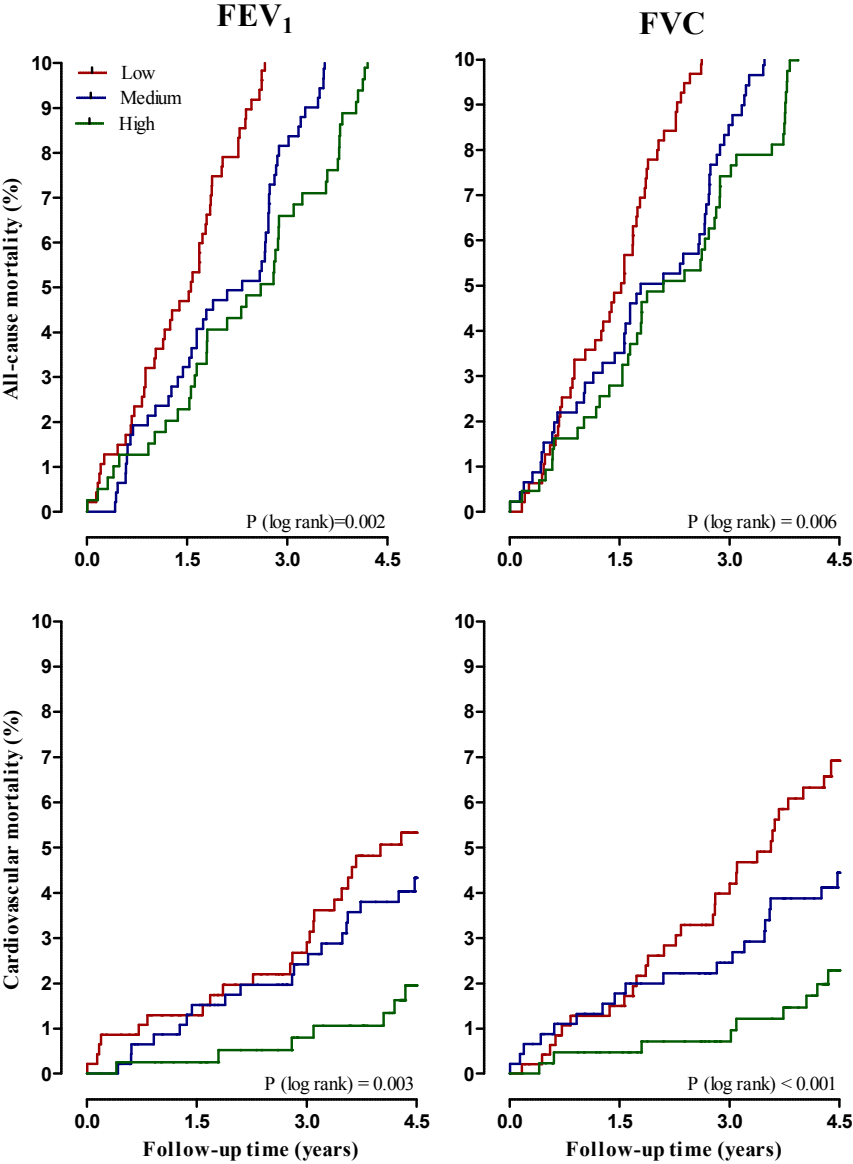


Figure 1: Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), respectively.

We further performed standardized Cox-regression models (Table 2). We executed five models for which we included either FEV₁ or FVC as the main independent variable, using different known risk factors as covariates for each of the models. In univariate models both FEV₁ (P = 0.022 and P = 0.004) and FVC (both P < 0.001) predicted all-cause and CV mortality. In multivariate models FEV₁ and FVC predicted all-cause mortality in Models 1-3 (all P < 0.001). FVC also predicted all-cause mortality in Model 4 (P = 0.021). Furthermore, in Model 5, IL-6 predicted all-cause mortality when included with FEV₁ (P = 0.036) and FVC (P = 0.048). With regards to CV mortality, FEV₁ did not independently predict the outcome in any of the adjusted models. FVC independently predicted cardiovascular mortality in Model 1 (HR = 0.56, P < 0.001), Model 2 (HR = 0.59, P < 0.001), Model 3 (HR = 0.60, P = 0.002) and Model 4 (HR = 0.57, P = 0.027). When replacing CRP with IL-6 in Model 5, FVC no longer predicted cardiovascular mortality (HR = 0.85, P = 0.452).

Table 2: Standardized Cox proportional hazard ratios of either FEV₁ or FVC with all-cause and cardiovascular mortality.

Univariate	All-cause mortality			Cardiovascular mortality		
	HR	95% CI	P	HR	95% CI	P
FEV ₁	0.84	(0.72-0.97)	0.022	0.64	(0.47-0.86)	0.004
FVC	0.78	(0.68-0.90)	< 0.001	0.52	(0.39-0.68)	< 0.001
Multivariate models with either FEV₁ or FVC as main independent variable						
<i>Model 1 (Adjusted for age, sex)</i>						
FEV ₁	0.74	(0.33-0.62)	< 0.001	0.72	(0.51-1.21)	0.067
FVC	0.68	(0.32-0.59)	< 0.001	0.56	(0.41-0.76)	< 0.001
<i>Model 2 (Adjusted for age, sex and height)</i>						
FEV ₁	0.71	(0.60-0.85)	< 0.001	0.77	(0.53-1.12)	0.176
FVC	0.66	(0.56-0.78)	< 0.001	0.59	(0.43-0.81)	< 0.001
<i>Model 3 (Adjusted for age, sex, height and tobacco use)</i>						
FEV ₁	0.72	(0.60-0.86)	< 0.001	0.80	(0.55-1.16)	0.239
FVC	0.67	(0.57-0.78)	< 0.001	0.60	(0.43-0.83)	0.002
<i>Model 4 (Adjusted for age, sex, height, waist circumference, physical activity index, systolic blood pressure, heart rate, tobacco use, gamma-glutamyltransferase, fasting glucose, total cholesterol, HIV status and anti-hypertensive medication)</i>						
FEV ₁	0.86	(0.68-1.11)	0.265	0.87	(0.51-1.50)	0.617
CRP	1.19	(0.98-1.45)	0.085	0.91	(0.58-1.41)	0.661
FVC	0.76	(0.60-0.96)	0.021	0.57	(0.35-0.94)	0.027
CRP	1.14	(0.94-1.39)	0.180	0.82	(0.53-1.26)	0.360
<i>Model 5 (Similar to Model 4, with C-reactive protein replaced by interleukin-6)</i>						
FEV ₁	0.85	(0.67-1.07)	0.167	0.93	(0.57-1.53)	0.777
IL-6	1.25	(1.01-1.55)	0.036	1.48	(0.95-2.29)	0.083
FVC	0.84	(0.67-1.04)	0.107	0.85	(0.55-1.30)	0.452
IL-6	1.23	(1.00-1.53)	0.048	1.39	(0.90-2.14)	0.135

CI= confidence interval, CRP= C-reactive protein, FEV₁ = forced expiratory volume in one second, FVC= forced vital capacity, HR= hazard ratio, IL-6= interleukin-6. P ≤ 0.05 regarded as significant.

Discussion

We investigated the contribution of lung function in predicting all-cause and CV mortality over five years in a sample of black South Africans, whilst taking the inflammatory markers, CRP and IL-6, into account. We found reduced lung function in the CV mortality group when compared to survivors and the non-CV mortality group. The CV mortality group also had the highest IL-6 level, confirming the findings from previous studies that reduced lung function is associated with increased inflammation, including IL-6^{18,19} and an increased risk for CV mortality.^{12,20} Even modest reductions in FEV₁ can increase the risk 2- to 3-fold.^{8,21}

Several possible explanations have been given for the relationship between reduced lung function and CVD, some being that there is a common offending agent or that the relationship is

confounded by other measured or unmeasured factors.⁹ Another promising explanation is that processes leading to reduced lung function may be causally related to CVD⁹ and this is where chronic low-grade inflammation comes into play. In conditions relating to reduced lung function, CRP and other acute-phase proteins may increase due to an over expression of certain cytokines and growth factors in the lung tissue.^{22,23} Furthermore, in animal models, induction of airway inflammation can propagate systemic inflammation.²⁴ In turn, systemic inflammation is associated with many forms of CVD such as atherothrombosis, atherosclerosis and ischemic heart disease.²⁵⁻²⁷ This is supported by findings that certain inflammatory markers have a direct effect on the pathogenesis of endothelial dysfunction²⁸ and atherosclerosis.^{29,30}

We found that FVC significantly and independently predicted CV mortality in differently adjusted models; however the association between FVC and CV risk was eliminated with the inclusion of IL-6 into the multivariate Cox regression model. We suggest that a reduction in either FEV₁ or FVC and an increase in IL-6, is so closely correlated that the inclusion of IL-6 into our multivariate Cox regression model eliminated the association between FVC and CV risk. We have previously shown a highly significant inverse association between IL-6 and both FEV₁ and FVC (both $P < 0.001$) in this population.³¹ This finding supports previous studies that showed a stronger relationship between IL-6 and lung function, when compared to other inflammation markers such as CRP and TNF- α .³² IL-6 also predicted all-cause mortality when included with FEV₁ ($P = 0.036$) and FVC ($P = 0.048$) and with regards to CV mortality, had a borderline predicative value included with FEV₁ ($P = 0.083$) and FVC ($P = 0.135$). In contrast, CRP did not predict all-cause or CV mortality. These results are also in accordance with that of previous studies where IL-6 predicted both all-cause and CV mortality.³³⁻³⁷ IL-6, a pro-inflammatory cytokine, is in part found in the peripheral vascular bed³⁸ and thus exerts a direct inflammatory effect on the peripheral circulation and the heart. This effect is proposed to be stronger than that of CRP³⁹ and also in haemodialysis patients; IL-6 is a stronger predictor of all-cause and cardiovascular mortality than CRP.⁴⁰

Infectious diseases have until recently, been the main contributors to morbidity and mortality in sub-Saharan Africa. However, due to rapid urbanization, non-communicable diseases, such as CVD, have become more prevalent.⁴¹ In South Africa, cardiovascular mortality is the second leading cause of mortality⁴² and this reflects the current burden of CVD in this country. Data regarding chronic diseases and associated risk factors in black populations in South Africa is scant and understanding the interplay between reduced lung function, inflammation and CV mortality may aid in a global risk factor evaluation for CVD.

This study has important strengths as it is based on prospective data, with several measures of lung function and inflammation which is limited in the South African research setting. Some limitations do however exist. We included a large proportion of unemployed individuals with sampling being done in poverty-stricken communities. The participants were also selected from mainly Setswana-speaking rural and urban areas and results may therefore not be generalized to other populations in South Africa.

Conclusion

In conclusion, we showed that FVC is a strong predictor of both all-cause and CV mortality in black South Africans and may be mediated by inflammation, especially IL-6. Our data support the use of both lung function and IL-6 as screening tools for the prediction of CV mortality. Future research is needed to identify the exact mechanism of this association which will aid in the allocation of healthcare resources.

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CHAPTER 6

CONCLUDING REMARKS AND FINDINGS

1. INTRODUCTION

In this concluding chapter a summary of the main findings of the three manuscripts reported in this thesis will be presented. The results from the manuscripts will be discussed, interpreted, explained and compared to the relevant literature. Conclusions will be drawn and recommendations made to the reader with regards to the role of lung function and inflammation in cardiovascular disease development in black South Africans.

2. SUMMARY OF MAIN FINDINGS

The main findings of the three manuscripts reported in this thesis (chapters 3, 4 and 5) are as follows:

2.1 South African and international reference values for lung function and its relationship with blood pressure in Africans

In this article the prediction values from three different reference populations namely from Europe, the United States and South Africa, in a large sample of black South Africans were compared. It was also established whether lung function is associated with blood pressure in this population. The hypotheses put forward in chapter 1 stated firstly that the prediction values from the three different reference populations namely from Europe, the United States and South Africa differ significantly in this sample of black South Africans, with the South African reference equations showing the highest percentage of the predicted values. The second hypothesis was that lung function is inversely associated with blood pressure in this population, independent of confounders.

In this cross-sectional analysis, the South African reference values displayed the highest percentages of the predicted values for FEV₁ and FVC and therefore the first hypothesis is accepted. Furthermore, with a reduction in lung function there was a significant increase in blood pressure and this remained even after adjusting for the appropriate confounders. Hence, the second hypothesis is also accepted.

2.2 Inflammation as possible mediator for the relationship between lung- and arterial function

The possible role of systemic inflammation as the mediator between lung function and arterial stiffness in a sample of black South Africans was investigated. In chapter 1 the hypothesis was stated that reduced lung function is independently associated with markers of inflammation (IL-6 and CRP). The second hypothesis was that reduced lung function is independently associated with arterial stiffness (PWV). Thirdly, we hypothesised that both IL-6 and CRP are independently associated with PWV. Our final hypothesis was that the relationship between reduced lung function and arterial stiffness is mediated by inflammation.

We found an independent inverse association between IL-6 and FEV₁ and FVC. Similar results were found for CRP. Therefore, our first hypothesis is accepted. PWV was inversely associated with FEV₁ but no association was found with FVC. Thus, our second hypothesis is accepted in part. The inflammatory markers did not associate with PWV as expected and our third hypothesis was rejected. The lack of association between arterial stiffness and inflammatory markers suggests that inflammation may not be the mediating link between lung- and vascular function in this population hence rejecting our final hypothesis.

2.3 Lung function, inflammation and cardiovascular mortality in a population of black South Africans

The link between impaired lung function and cardiovascular outcome is well established in international literature.^{1,2} It is possible that this association may be driven by a systemic spill-over of inflammation occurring within the lungs.^{3,4} Therefore, we investigated the contribution of lung function in predicting all-cause and cardiovascular mortality in Africans, whilst taking inflammatory markers into account. Our hypothesis was that lung function and markers of inflammation predict all-cause and cardiovascular mortality over five years in a population of Africans.

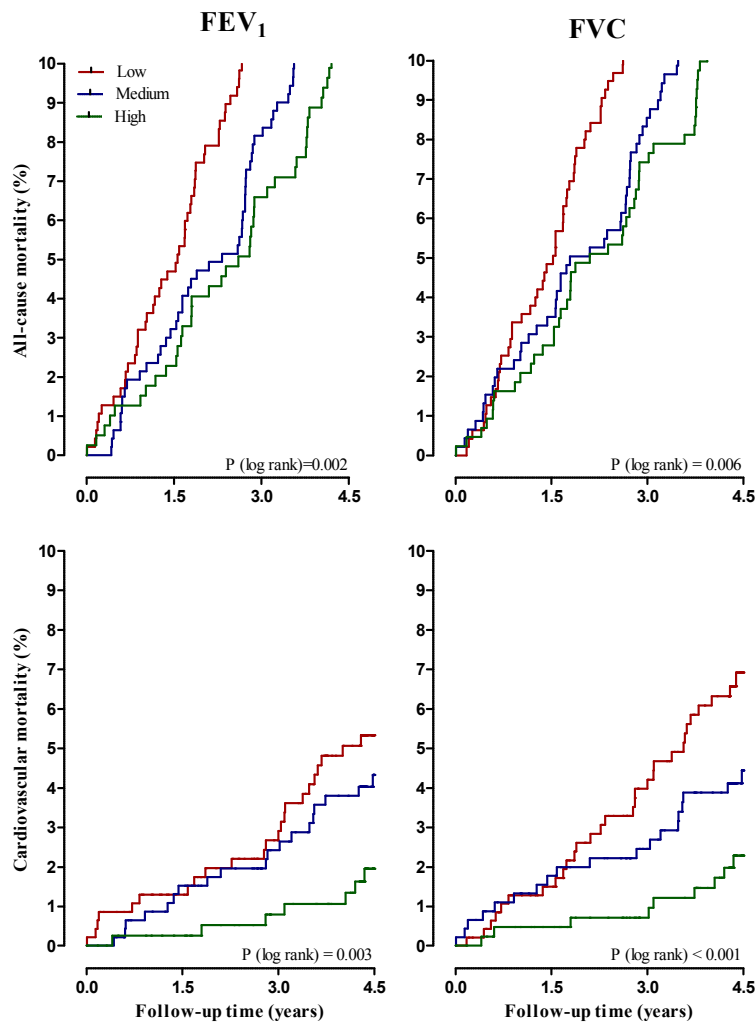


Figure 1: Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), respectively.

We found that the CV mortality group had the lowest FEV₁ and FVC values when compared to the survivors and the non-CV mortality group. Kaplan-Meier survival function estimates (Figure 1) indicated that log-rank tests were significant across tertiles of FEV₁ and FVC for CV mortality with the lowest tertile of FEV₁ and FVC having the highest mortality rate. CRP did not significantly predict all-cause or cardiovascular mortality in any of the Cox-regression models, however, IL-6 predicted all-cause mortality independent of potential confounders. Furthermore, FVC significantly and independently predicted CV mortality in differently adjusted models; the

association between FVC and CV mortality, however, was eliminated with the inclusion of IL-6 into the multivariate Cox regression model. Our hypothesis is therefore only accepted in part.

3. DISCUSSION AND COMPARISON OF MAIN FINDINGS TO THE LITERATURE

It is important to compare the findings from this study to that of the literature and to interpret the results within that context. Some of our findings confirmed those of previous studies or contributed to the existing body of knowledge while some were contradictory.

Reference values for lung function and the relationship of lung function with blood pressure in Africans

There is an increasing burden of NCDs in sub-Saharan Africa, which includes respiratory diseases.⁵⁻⁷ COPD is regarded as one of the leading causes of morbidity and mortality worldwide and the Burden of Obstructive Lung Disease (BOLD) study indicated that South Africa has the highest prevalence of clinically significant COPD compared to various other countries.⁸ This high prevalence of COPD can be attributed to an increase in the relevant risk factors.⁹ The most important risk factor remains exposure to tobacco products⁹ and in our study population 66.9% of men and 49.5% of women reported current or previous tobacco use. Additional risk factors include occupational exposure to dust and chemicals and indoor air pollution¹⁰ which entails the burning of biomass and fossil fuels (wood, animal dung, crop residues and coal) in open fires or poorly-functioning stoves in poorly-ventilated spaces. This is of significant importance in South Africa where more than 20 million families use biomass and coal as their main source of energy for cooking and heating.^{11,12}

These findings highlight the importance of the accurate assessment of lung function in South Africa as well as the appropriate interpretation of these findings by use of the correct reference values. When comparing three different sets of reference values we found significantly different results, underlying the challenge in selecting appropriate reference values for lung function assessment.¹³ The highest percentages of the predicted values were seen for the South African

reference values. European reference values¹⁴ have been suggested for general use,¹⁵ but their suitability, particularly for black populations have not been fully established. We found that the South African prediction equations yielded a higher percentage of the predicted values compared to the European and American equations, even after a prescribed correction factor¹⁶ was applied. Data regarding the comparison of reference values for lung function in South Africa is scant. However, our findings correspond with those of Hnizdo et al.,¹⁷ who aimed to estimate lung function prediction equations and to identify appropriate normal reference values for a population of black and white South African gold miners. Their study indicated that European and American equations provided the best fit to the data for white miners, whereas the South African equations by Louw et al.,¹⁸ provided the best fit to the data for black miners.¹⁷ Our findings also contribute to the literature, as we included African women, who are overall an understudied group.

Another prominent finding was the inverse association between BP and lung function according to the FEV₁ and FVC quintiles. In this regard our findings support the current international literature. Data from a nested case-control study of 1,031 incident cases of essential hypertension indicated that FVC was independently predictive.¹⁹ Furthermore, the longitudinal Normative Aging Study found that the age-adjusted incidence of hypertension during a 10 year follow-up period was inversely related to FVC.²⁰ As far as could be established, our study is the first to report these findings in black South Africans and contributes to the body of literature regarding lung function and blood pressure. This is especially important in a South African setting, as Africans are known to have a high prevalence of hypertension.^{21,22} Although it is clear that a relationship exists between lung function and BP, the mechanisms underlying this association remain unclear.

Lung function, inflammation and arterial stiffness in Africans

Following on from our first analyses on lung function and BP, we investigated the possible role of systemic inflammation as the mediator between lung function and arterial stiffness. We found

an independent inverse association between lung function and inflammation and this confirms previous findings from the literature. Data from a large-scale prospective study which included over 1 500 men and women showed that higher baseline serum levels of CRP and IL-6 were strongly associated with lower FVC and FEV₁, independent of potential confounders.²³ In this regard our findings also contributed to the literature, as data on the relationship between lung function and inflammation are limited in African populations. Furthermore, our findings, in addition to the literature, support the notion that inflammatory processes are involved in the pathogenesis of various pulmonary diseases.²⁴ Moreover, our data enforces the hypothesis that the comorbidities associated with reduced lung function, such as CVD, may be due to a systemic spill-over of inflammation occurring within the lungs.²⁵

Our second prominent finding was an inverse association between lung function and arterial stiffness. Bolton and colleagues²⁶ also found that both FEV₁ and FVC in mid-life and later life were inversely associated with PWV in men. Reduced lung function was also found to be associated with increased central arterial stiffness in a sample of French men, even after adjusting for cardiovascular risk factors such as weight, smoking habits, hypercholesterolemia, diabetes, and hypertension.²⁷ In this regard our findings confirm those in international literature but also add to the body of knowledge by including data from African men and women. The literature describes various possible causes for the association between reduced lung function and arterial stiffness, one being that there could be a genetic susceptibility or inherited factors that may both influence lung function as well as arterial stiffness.²⁸ Lung function and arterial stiffness could both be influenced by external factors such as metabolic disruption or environmental factors such as smoking;²⁶ however we adjusted for these variables as covariates to eliminate a confounding effect. The most promising explanation is that inflammation acts as a parallel physiological pathway for a simultaneous loss of elasticity in both the connective tissue of the alveoli and the arterial wall and this may lead to reduced lung function and arterial stiffness.²⁹

Based on the above-mentioned possibility and having confirmed the associations between lung function and inflammation as well as between lung function and arterial stiffness, we were surprised to find that markers of inflammation were not associated with BP or measures of arterial stiffness in our black population. Various studies have found an association between CRP and PWV³⁰⁻³³ and in others the association disappears after adjustment for conventional risk factors for CVD.^{32,34,35} There are also studies that have reported no association between CRP and PWV^{36,37} and it remains controversial whether CRP is merely a marker^{38,39} or indeed causal^{40,41} in the development of CVD, with strong evidence supporting either statement. With regard to IL-6, our findings were also controversial, with some previous studies showing a positive association between IL-6 and PWV.^{42,43} In contradiction, a recent study reported no association between IL-6, CRP and small or large artery elasticity in women, while in men IL-6 and CRP only associated with small artery elasticity.²⁹

The lack of association between inflammation markers and arterial stiffness in our study could be due to other confounding factors not considered in this investigation. Tobacco use has a significant effect on arterial function⁴⁴ and measurement of cotinine levels could have been more accurate in determining tobacco exposure than self-reported tobacco use. Furthermore, other inflammation-sensitive plasma proteins may be more sensitive markers of inflammation than those used in our study and could have a greater contribution to arterial stiffening and CV risk.¹ Our findings ultimately suggest that there is an association between lung function and arterial stiffness, but that inflammation is possibly not mediating this link in our population of black South Africans. The findings we reported here were based on cross-sectional data and did not allow for the assessment of any disease progression. Further exploration is therefore needed on the matter.

Lung function, inflammation and cardiovascular mortality

It can be inferred from the above evidence that a proven link between reduced lung function and cardiovascular function indeed exists in this population, which is further justified by the rest of

our findings. The CV mortality group presented with the lowest volumes for FEV₁ and FVC, when compared to the survivors and the non-CV mortality group. This finding corresponds to those from various other studies which describe that even modest reductions in expiratory flow volumes elevate the risk of ischemic heart disease, stroke, and sudden cardiac death 2- to 3-fold, independent of other risk factors.^{1,45-48} In addition, we also found that FVC significantly and independently predicted CV mortality in differently adjusted models. To the best of our knowledge our study is the first to report prospective findings regarding lung function and CV mortality in black South Africans and in this regard contributes to the field.

Apart from our confirmatory finding above, we included a novel approach namely to review the possible contribution of inflammatory markers to the prognostic value of lung function measurements. In following this approach, we found unexpectedly that the association between FVC and CV mortality was eliminated with the inclusion of IL-6 into our multivariate Cox-regression model. Based on the highly significant inverse association between IL-6 and both FEV₁ and FVC from our previous findings, we hypothesise that a reduction in either FEV₁ or FVC and an increase in IL-6, is so closely correlated that the inclusion of IL-6 into the models eliminated the association between FVC and CV risk. This hypothesis is in part supported by previous findings from a prospective study conducted on the elderly that showed a stronger relationship between IL-6 and markers of lung function, when compared to other inflammation markers such as CRP and TNF- α .⁴⁹ Furthermore we found that IL-6 predicted all-cause mortality when included with FEV₁ and FVC and had a borderline predictive value with regard to CV mortality. In contrast, CRP did not predict all-cause or CV mortality. The literature regarding CRP and CV mortality is controversial. Other researchers have reported that CRP has continuous associations with the risk of coronary heart disease, ischaemic stroke and vascular mortality.^{50,51} However, our findings support those of a recent study conducted on the same population of black South Africans that found that the predictive value for cardiovascular mortality was evident in the case of IL-6, and a novel inflammatory marker named soluble urokinase plasminogen activator receptor (suPAR), only.⁵² In addition, Mendall and colleagues⁵³

found no association between CRP and ischaemic heart disease mortality in a Welsh study involving 1 395 men. Furthermore, in haemodialysis patients, IL-6 is a stronger predictor of all-cause and CV mortality than CRP.⁵⁴ Interleukin-6 is in part found in the peripheral vascular bed and exerts a direct inflammatory effect on the peripheral circulation⁵⁵ which is proposed to be stronger than that of CRP.⁵⁶

Notwithstanding the controversy in our findings regarding different inflammation markers, it is clear that FVC is a strong predictor of both all-cause and CV mortality in this population of black South Africans and that this association may be mediated by IL-6. However, the exact mechanism behind this association is still not clear and provides a strong motive for future research on the matter. Our data support the use of lung function and IL-6 as a screening tool for the prediction of CV mortality in Africans. In South Africa, CV mortality is the second leading cause of mortality⁵⁷ and data regarding chronic diseases and associated risk factors in Africans is scant. This highlights the contribution of our findings.

4. CHANCE AND CONFOUNDING

It is important to critically reflect on some of the factors that may have confounded the results of this study. Some methodological issues are of relevance.

We recruited a sample of black participants from the North-West province of South Africa who were mainly Setswana-speaking and this study is therefore not representative of the entire black South African population. We included a large proportion of unemployed individuals with sampling being done in poverty-stricken communities which may have biased our results. Cause and effect could not be inferred as we only implemented regression analyses for this investigation. We did not measure body temperature or determine leukocyte count or test for any opportunistic infections (this was only reported in questionnaires) and can therefore not exclude underlying infections which could have influenced inflammatory marker concentrations. A history of diseases of lifestyle, TB or HIV infection (as reported in the PURE South Africa

Adult Questionnaire - Appendix A) formed part of the exclusion criteria for the PURE study. In addition to the questionnaire, the HIV status was determined, allowing exclusion of these participants in order to avoid confounding, especially regarding the inflammatory markers. However, clinical testing for TB and COPD were not performed and consequently, undiagnosed participants could have been included in the study sample which could have a confounding effect. Furthermore, PWV was assessed in an upper-limb muscular artery segment over the carotid-radialis and not the large conduit vessels (carotid-femoral PWV) which is the gold standard for assessing aortic stiffness. However, it has been described that arterial stiffness of the peripheral arteries has merit as a cardiovascular risk factor in black populations.^{58,59} Some of the data were self-reported (such as the tobacco use, physical activity and the household data) which may induce a lack of sensitivity that may have diluted the result. We did not determine cotinine levels, which could have confirmed tobacco product exposure. The effect of the primary fuel used for food preparation and the primary heating source during the cold season was not confirmed by measuring exposure to harmful respirable particles in the households. Although these non-measured factors may have weakened the study, this study is of a prospective nature and the opportunity exists to incorporate these factors in the scheduled follow-up studies. With reference to the results, the possibility of chance should also be considered. By adjusting for appropriate covariates such as age, sex, HIV status, height, waist circumference, physical activity index, systolic blood pressure, heart rate, GGT, tobacco use, fasting glucose, TC and medication use, it is possible that these covariates or potential confounders could have influenced the results by causing over- or underestimation of the associations between the various variables investigated in this study. It was also necessary to interpret all the statistical results from a physiological perspective. We included a relatively large sample of the black South African population which provided adequate statistical power.

5. RECOMMENDATIONS

In light of improving cardiovascular health of black South Africans, the following is recommended for future research regarding lung function and CVD in this population:

The analysis of other biochemical variables such as markers of oxidative stress, TNF- α , plasma fibrinogen, blood leukocytes, platelets, additional interleukins and extracellular matrix proteins could be of value. Furthermore, other measures of cardiovascular structure and endothelial function including intima-media thickness, plaque scores, carotid-femoral pulse wave velocity and flow-mediated dilation, as well as ambulatory blood pressure could be performed. These factors could contribute to the understanding of the mechanisms behind the association of lung function and inflammation with CVD development and mortality.

Given the living conditions in the informal settlements in South Africa, it could be beneficial to measure levels of exposure to noxious particles from open fires used for cooking and heating purposes in poorly-ventilated dwellings, to ultimately consider these factors as possible contributors to reduced lung function in African populations.

The longitudinal nature of this study has provided valuable contributions to the field of cardiovascular research and further follow-up studies may be able to resolve some of the controversies and questions that have surmounted from our study as well as the literature. Therefore, given the complex interaction between lung function, inflammation and CVD, this field remains open to further study of the black South African population in the years to come.

6. FINAL CONCLUSIONS AND PERSPECTIVES

We showed for the first time that reduced lung function is independently associated with arterial stiffness and prognostic of cardiovascular mortality in a large black population. Evidence from this thesis indicates that South African prediction equations may be more useful when investigating lung function in black South Africans and that elevated BP is related to reduced

lung function. In addition, reduced lung function is associated with increased inflammation in this population of black South Africans. The role of inflammation as the mediator for the relationship between lung function and CVD in this population remains controversial but it appears that IL-6 may contribute more significantly than CRP in this regard. Our findings provide concerning evidence of the effect of reduced lung function on the CVD burden. This study underlines the importance of preserving normal lung function, both in an occupational- and household environment. This may aid in providing early preventative and therapeutic strategies to combat the high prevalence of cardiovascular morbidity and mortality in this population of black South Africans.

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ANNEXURE A

PURE South Africa Adult Questionnaire

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Adult Questionnaire

INSTRUCTIONS

Please answer EACH question by marking an X in ONE BOX on each line:
(unless otherwise instructed)



OR

By writing number(s) in the spaces provided:



OR

By specifying the answer on the line(s) provided

Adult Questionnaire

Subject Initials- **F**= first letter of first name
M= first letter of middle name
L= first letter of last name

3. National I.D#

If not applicable please mark the N/A box

Ethnicity Codes

- 01 - South Asian (India, Sri Lanka, Pakistan, Bangladesh)
- 02 - Chinese (China, Hong Kong, Taiwan)
- 03 - Japanese
- 04 - Malays
- 05 - Other Asian (Korea, Malaysia, Papua New Guinea, Thailand, Philippines, Indonesia, Nepal, Vietnam, Cambodia, Laos, Myanmar/Burma, Bhutan, Singapore)
- 06 - Persian
- 07 - Arab
- 08 - Black African
- 09 - Coloured African (Subsaharan African only)
- 10 - European
- 11 - Native North/South American or Australian Aborigine
- 12 - Latin American (Latino)
- 13 - Bantu/Semi Bantu
- 14 - Hemitic/Semi Hemitic
- 15 - Nilotic/Hausa
- 16 - Pygmie
- 17 - Swahili
- 18 - Other (any other ethnoracial group not listed above)

Adult Questionnaire

11. Occupation

Group 1: Legislators, senior officials and managers

Legislators and senior officials
Corporate managers
General managers
Businessman

Group 2: Professionals

Physical, mathematical and engineering science professionals
Life science and health professionals
Teaching professionals
Other professionals

Group 3: Technicians and associate professionals

Physical, mathematical and engineering-
science associate professionals/technicians
Life science and health associate professionals/technicians
Teaching associate professionals/technicians
Other associate professionals/technicians

Group 4: Clerks

Clerks
Customer service clerks

Group 5: Service workers and shop and market sales workers

Personal and protective services workers
Models, salespersons and demonstrators

Group 6: Skilled agricultural and fishery workers

Market-oriented skilled agricultural and fishery workers
Subsistence agricultural and fishery workers

Group 7: Craft and related trade workers

Extraction and building trade workers
Metal, machinery and related trades workers
Precision, handicraft, printing and
related trades workers
Other craft and related trades workers

Group 8: Plant and machine operators and assemblers

Stationary plant and related operators
Machine operators and assemblers
Drivers and mobile plant operators

Group 9: Elementary occupations

Sales and services elementary occupations
Agricultural, fishery and related labourers
Labourers in mining, construction,
manufacturing and transport

Group 10: Armed forces

Armed forces

Group 11: Homemaker

Housewife/Househusband

Subject ID

Centre #

Community#

Household #

Subject #

Subject Initials

F M L

10. Not applicable in South Africa

11a) Not applicable in South Africa

b) Please indicate which group best describes your main occupation.

(Please refer to facing page for definitions of groups and instruction manual for detailed definitions)

 Group 1 Group 2 Group 3 Group 4 Group 5 Group 6 Group 7 Group 8 Group 9 Group 10 Group 11

c) Not applicable in South Africa

d) What is your main source of income? _____

If occupation is group 11 (homemaker) go to question 13

12. Are you currently employed?

No → (answer 12a - 12b) Yes → Go to #13

a) Are you retired/stopped work from your primary occupation due to old age? No Yes

b) Have you stopped working due to illness? No Yes

Subject ID

Centre #		Community#		Household #		Subject #	

Subject Initials

F	M	L

13. CURRENT DISABILITY:

- | | No | Yes |
|--|--------------------------|--------------------------|
| a) Do you have any problems using your fingers to grasp or handle? | <input type="checkbox"/> | <input type="checkbox"/> |
| b) Do you have any trouble walking about? | <input type="checkbox"/> | <input type="checkbox"/> |
| c) Do you have any trouble bending down and picking up an object from the floor? | <input type="checkbox"/> | <input type="checkbox"/> |
| d) Do you require a walking stick cane/walker to move about? | <input type="checkbox"/> | <input type="checkbox"/> |
| e) Do you have any trouble reading or seeing the individual grains of rice/corn on your plate? (with glasses worn) | <input type="checkbox"/> | <input type="checkbox"/> |
| f) Do you have trouble seeing a person from across the room? (12 feet/3.5 meters) (with glasses worn) | <input type="checkbox"/> | <input type="checkbox"/> |
| g) Do you have trouble speaking and being understood? | <input type="checkbox"/> | <input type="checkbox"/> |
| h) Do you have any trouble hearing what is said in a normal conversation? | <input type="checkbox"/> | <input type="checkbox"/> |

Subject Medical History

14. Have you experienced any of the following in the last six months?

- | | No | Yes | | No | Yes |
|---|--------------------------|--------------------------|-------------------------------------|--------------------------|--------------------------|
| a) Chest pain or tightness with usual activity | <input type="checkbox"/> | <input type="checkbox"/> | i) Vomiting | <input type="checkbox"/> | <input type="checkbox"/> |
| If Yes, —→ does the pain spread to the back, neck or inner border of arm | <input type="checkbox"/> | <input type="checkbox"/> | j) Loss of appetite | <input type="checkbox"/> | <input type="checkbox"/> |
| b) Breathlessness with usual activity | <input type="checkbox"/> | <input type="checkbox"/> | k) Painful or bleeding teeth/gums | <input type="checkbox"/> | <input type="checkbox"/> |
| c) Cough for at least 2 weeks | <input type="checkbox"/> | <input type="checkbox"/> | l) Jaundice | <input type="checkbox"/> | <input type="checkbox"/> |
| d) Any sputum while coughing | <input type="checkbox"/> | <input type="checkbox"/> | m) Burning while passing urine | <input type="checkbox"/> | <input type="checkbox"/> |
| e) Blood in sputum | <input type="checkbox"/> | <input type="checkbox"/> | n) Swelling of feet | <input type="checkbox"/> | <input type="checkbox"/> |
| f) Wheezing or whistling in the chest | <input type="checkbox"/> | <input type="checkbox"/> | o) Swelling of face | <input type="checkbox"/> | <input type="checkbox"/> |
| g) Early morning cough with chest tightness | <input type="checkbox"/> | <input type="checkbox"/> | p) Blood in urine | <input type="checkbox"/> | <input type="checkbox"/> |
| h) Loose stools/diarrhea for at least 3 days | <input type="checkbox"/> | <input type="checkbox"/> | q) Involuntary weight loss of > 3kg | <input type="checkbox"/> | <input type="checkbox"/> |

15. Not applicable in South Africa

- 16a) Do you use glasses/spectacles/contact lenses at present? No Yes
- b) Do you use a hearing aid? No Yes

Adult Questionnaire

Cancer Sites

- 1= Mouth
- 2= Esophagus
- 3= Stomach
- 4= Small intestine
- 5= Large intestine including rectum
- 6= Pancreas
- 7= Liver
- 8= Lung
- 9= Breast
- 10= Cervical/uterine/ovarian
- 11= Prostate
- 12= Head and neck
- 13= Other, specify

Subject ID

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<i>Centre #</i>	<i>Community#</i>	<i>Household #</i>	<i>Subject #</i>

Subject Initials

F M L

17. Have you ever been diagnosed with any of the following?(check all that apply)

	No	Yes	#of yrs since diagnosis		No	Yes	#of yrs since diagnosis
a) Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	i) COPD	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
b) Hypertension/ high blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	j) Asthma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
c) Stroke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	k) Tuberculosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
d) Angina/heart attack/ Coronary artery disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	l) Malaria	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
e) Heart failure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	m) Chagas	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
f) Other heart disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	n) HIV/AIDS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>
g) Cancer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	Not answered	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>

Please refer to facing page for cancer sites *site* *other, specify*

18. Have you been taking any medications regularly (ie. at least once per week) in the last month? No → go to 19 Yes

a) If yes, for what conditions:

	No	Yes	
Blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	
Cholesterol lowering drugs	<input type="checkbox"/>	<input type="checkbox"/>	
Stroke	<input type="checkbox"/>	<input type="checkbox"/>	
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	
Asthma	<input type="checkbox"/>	<input type="checkbox"/>	
Chinese medicine	<input type="checkbox"/>	<input type="checkbox"/>	
Others	<input type="checkbox"/>	<input type="checkbox"/>	→ If Yes, specify _____
Unknown	<input type="checkbox"/>	<input type="checkbox"/>	

Adult Questionnaire

18b) If name of medication is unknown, please list as unknown.

Subject ID

Centre #

Community#

Household #

Subject #

Subject Initials

F M L

18b) List all the medications you are currently consuming at least once a week for the last month?

i) _____ ii) _____

iii) _____ iv) _____

v) _____ vi) _____

vii) _____ viii) _____

Men go to question #23

For Women Only (Questions 19 - 22)

19. Are you currently pregnant ? No Yes → Go to #21

20. Do you still have periods? No → (answer 20a) Yes → Go to #21

a) How many years since you stopped menstruating? years

21. Have you ever used an oral/ injectable contraceptive? No Yes

22a) How many live children have you given birth to? Boys Girls

b) Did you breast feed any of your children? No Yes

Adult Questionnaire

23. Accidents and Injuries

Location of Injury

- 1= Factory/industrial place
- 2= Office
- 3= Agriculture field/farm
- 4= Home
- 5= Road
- 6= Sport/game e.g. track, court, field, etc.
- 7= Public building
- 8= Mine/quarry
- 9= Construction site e.g. building, road-works, etc.
- 10 = Other

Type of Injury

- 1= Burns
- 2= Scalds
- 3= Fractures
- 4= Muscle and ligament sprains/tears
- 5= Cuts and lacerations
- 6= Bruises and abrasions
- 7= Suffocation
- 8= Head injury (where person did not lose consciousness)
- 9= Head injury (where person lost consciousness for some time)

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

23. During the past 12 months, have you had any injuries that were serious enough to limit your normal activities? (check all that apply)

No → **Go to #24**
 Yes → **(answer 23a - 23s)**

If yes, please provide details:

Please refer to facing page for Location and Type Codes
 Absence from work or usual activities (Days)

Cause of injury				Location	Type	Absence from work or usual activities (Days)
a) Motor vehicle accident (as a passenger)	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
b) Motor vehicle accident (as a pedestrian)	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
c) Struck by an object	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
d) Explosion	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
e) Natural/environmental factors (gales/cyclones/lightning, etc.)	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
f) Suffocation	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
g) Poisoning	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
h) Snake/scorpion bite	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
i) Fall	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
j) Fire/flames, resultant fumes	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
k) Physical assault (gun, kidnapping, etc.)/violent crime	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
l) Domestic violence (beaten by a family member)	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
m) Drowning/submersion	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
n) Hot or corrosive liquids/floods/substances	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
o) Crush injuries (boulders, building materials, etc.)	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
p) Accident caused by machinery	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
q) Attempted suicide	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
r) Armed conflict	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>
s) Other(specify) _____	<input type="checkbox"/> No	<input type="checkbox"/> Yes	→	<input type="text"/> <input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>

Adult Questionnaire

Location of Fractures

- 1= Hip/pelvis
- 2= Thigh
- 3= Leg
- 4= Forearm
- 5= Wrist
- 6= Hand/finger
- 7= Vertebrae (back)
- 8= Other

Fractures: In situations where subjects are in a cast and cannot differentiate between ligament tear or fracture, include as fracture only if doctor confirmed it as a broken bone

25c) Tobacco: Regular use is defined as consuming at least one tobacco product per day.

Duration of use:

For those that have consumed tobacco for <1 year, please enter "0"

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

24. Have you ever fractured a bone? No (go to #25) Yes (if yes, answer a),b) and c)

a) Number of fractures

b) Years since last fracture

(yrs)

c) Bone (s) broken in the most recent fracture(if more than 3, list most severe sites) (location) If other, specify

→ _____

Please refer to facing page for fracture locations

→ _____

→ _____

Tobacco

25. Which best describes your history of tobacco use?

a) Formerly used tobacco products Currently use tobacco products Never used tobacco products → Go to #26

b) At what age did you start? yrs

c) Have you ever regularly used any of the following tobacco products? (check all that apply)

Past users only

	Average amount/day	Duration (years)	When Stopped (years ago)	If less than 1 yr (months ago)
(i) Cigarettes (all kinds)	<input type="text"/> <input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(ii) Beedies	<input type="text"/> <input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(iii) Cigars	<input type="text"/> <input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(iv) Pipes	<input type="text"/> <input type="text"/> <input type="text"/> number	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(v) Sheesha/water pipe Hookah	<input type="text"/> <input type="text"/> <input type="text"/> # of times	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(vi) Chewing tobacco	<input type="text"/> <input type="text"/> <input type="text"/> # of times	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(vii) Snuff	<input type="text"/> <input type="text"/> <input type="text"/> # of times	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>

(x) Other _____

Specify

Subject ID

Centre #

Community#

Household #

Subject #

Subject Initials

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Question 26 to be answered by non-smokers and former smokers only

26. During the past 12 months, have you been regularly (at least once per week) exposed to other people's tobacco smoke?

("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke.)

No → **Go to #27** Yes → Please answer questions **26a**

a) Over the past 12 months, what has been your typical exposure to other peoples smoke?

("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other peoples smoke)

Select **ONE** only

1-2 times/week 3-6 times/week at least once a day 2-3 times/day 4 or more times/day

27. Not applicable in South Africa

Adult Questionnaire

28c) Alcoholic Beverage: Regular use is defined as at least once a month.

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

28. Which best describes your history of alcohol use?

a) Formerly used alcohol products
 Currently use alcohol products
 Never used alcohol products → **Go to #29**

b) At what age did you start? yrs

c) What forms of alcohol have you regularly used? (check all that apply)

Form of Alcohol	Approx. size of one "drink"	Frequency			Average # of drinks	Duration (years)	Past users only
		Daily	Weekly	Monthly			When Stopped (years ago)
(i) Spirits(rum,whisky, gin,vodka etc)	30ml	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(ii) Wine	125ml	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(vi) Beer	375ml	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
(vii) Country liquor/arrack/ sugar cane spirit	30ml	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>

d) **At least once a month, do you consume >5 alcoholic drinks/day?** No → **Go to #29** Yes

↓ **If yes,(i,ii)**

i) How many times per month do you consume >5 alcoholic drinks in a day?

ii) What is the average number of drinks that you consume each time?

29 a) **During your longest or nocturnal sleep period, what time do you normally go to bed?** :

(00:00-23:59)

b) **During your longest or nocturnal sleep period, what time do you normally wake up?** :

(00:00-23:59)

c) **Do you usually take naps/siestas?** No Yes → *Total nap duration* → mins

Adult Questionnaire

33. **Civic organization**: are defined as non-profit, voluntary organization societies, self help groups and clubs.

Religious organization: are defined as different types of formal and informal groups set up on a religious basis.

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

30. Are you a member of any of the following:

How often do you participate in the activities of this group?

			Per Month	OR	Per Year
(i) Self help group, Co-operative, Social club, Sports club,	<input type="checkbox"/> No	<input type="checkbox"/> Yes →	<input type="text"/>		<input type="text"/>
(ii) Religious Group (e.g: church group, etc.)	<input type="checkbox"/> No	<input type="checkbox"/> Yes →	<input type="text"/>		<input type="text"/>
(iii) Other _____ <i>Specify</i>	<input type="checkbox"/> No	<input type="checkbox"/> Yes →	<input type="text"/>		<input type="text"/>

31. Please answer the following: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
(i) People are generally honest and want to help others.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(ii) If I do nice things for someone, I can anticipate that they will respect me and treat me just as well as I treat them.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

32a) The television, radio, newspaper or magazine advertisements help me decide to buy the type of: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree	Not Applicable
(i) Cooking oil	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(ii) Flour	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(iii) Rice/ Maize meal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

b) The television, radio, newspaper or magazine advertisements influence whether I buy: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree	Not Applicable
(i) Soft drinks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(ii) Snacks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(iii) Cigarettes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
(iv) Alcohol	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

33. In a difficult situation, whose help can you count on from?(Please see facing page for definitions)

(i) Civic organizations: specify _____

none
 little
 moderate/average
 a great deal

(ii) Religious organizations: specify _____

none
 little
 moderate/average
 a great deal

Subject ID

Centre #		Community#		Household #		Subject #	

Subject Initials

F	M	L

34. Have you experienced any of the following events during the last 12 months?

	No response	No	Yes	
(i) Loss of job	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(ii) Retirement	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(iii) Loss of crop/business failure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(iv) Household break in	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(v) Marital separation/divorce	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(vi) Other major intra-family conflict	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	→ Please specify _____
(vii) Major personal injury or illness	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(viii) Violence	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(ix) Armed conflict/war	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(x) Death of a spouse	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(xi) Death/major illness of another close family member	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(xii) Other major stress	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	→ Please specify _____
(xiii) Wedding of family member	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(xiv) New job	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(xv) Birth in the family	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(xvi) Separation from family	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
(xvii) Unavailability of food/ food insecurity	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

35. Please answer the following: (Choose only one option for each)

For the following question, stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home.

No response Never Experienced Stress Some Period of Stress Several Periods of Stress Permanent Stress

- a) How often have you felt stress at work in the last 12 months?
 (Mark here if not applicable: i.e. no longer working)
- b) How often have you felt stress at home in the last 12 months?

36. What level of financial stress have you felt in the last 12 months?

No response
 Little/none
 Moderate
 High/severe

37. During the past twelve months, was there ever a time when you felt sad, blue, or depressed for two weeks or more in a row?

No Yes
 → **If yes, during those times, did you:**

No response No Yes

- | | | | |
|--|--------------------------|--------------------------|--------------------------|
| a) Lose interest in most things like hobbies, work or activities that usually give you pleasure? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b) Feel tired or low on energy? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c) Gain or lose weight? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| d) Have more trouble falling asleep than you usually do? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| e) Have more trouble concentrating than usual? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| f) Think a lot about death (either your own, someone else's, or death in general) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| g) Feel down on yourself, no good or worthless? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Subject ID

Centre #

Community#

Household #

Subject #

Subject Initials

F M L

38. Please answer the following: (Choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
a) I can do most of my regular shopping (food, household necessities, etc.) at stores within easy walking distance (less than 15 minutes) of my home.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) Walking or bicycling in my neighbourhood is difficult because of the speed and/or amount of traffic.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c) My neighbourhood is generally free from pollution (litter, air pollution and noise pollution).	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d) My neighbourhood streets are well lit at night.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e) I can see other people when I am walking in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f) I can speak to other people when I am walking in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g) There is a high crime rate in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
h) There is a problem with unattended dogs in my neighbourhood.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Subject ID

Centre #

Community#

Household #

Subject #

Subject Initials

F M L

38a) Please answer the following: (Please check all that apply)

i) Has your household been a victim of the following crime(s) in the last 12 months?

	No	Yes
1. Armed robbery	<input type="checkbox"/>	<input type="checkbox"/>
2. Violent attacks	<input type="checkbox"/>	<input type="checkbox"/>
3. Murder	<input type="checkbox"/>	<input type="checkbox"/>
4. Vehicle hijacking	<input type="checkbox"/>	<input type="checkbox"/>
5. House breaking	<input type="checkbox"/>	<input type="checkbox"/>
6. Theft	<input type="checkbox"/>	<input type="checkbox"/>
7. Rape	<input type="checkbox"/>	<input type="checkbox"/>
8. Women abuse eg. (beat,swear-words,sexual) please specify _____	<input type="checkbox"/>	<input type="checkbox"/>
9. Child abuse eg. (burn,swear-words,rejection) please specify _____	<input type="checkbox"/>	<input type="checkbox"/>
10. Child sexual abuse	<input type="checkbox"/>	<input type="checkbox"/>
11. Other, please specify _____	<input type="checkbox"/>	<input type="checkbox"/>

ii) Do you think that crime in your area has increased in the past 5 years? No Yes

if yes, which of the following crime(s)?

- Armed robbery
- Violent attacks
- Murder
- Vehicle hijacking
- House breaking
- Theft
- Rape
- Women abuse
- Child abuse
- Child sexual abuse
- Other, please specify _____

Subject ID

Centre #

Community#

Household #

Subject #

Subject Initials

F M L

38b) Questions on HIV:

i) Do you know people who have HIV/AIDS? No Yes

if yes, which of these people: (please mark all that apply)

- Your children
- Your grandchildren
- Your spouse
- Your family members
- Your friends
- People in the community

ii) What would you consider the mean age of the people who are ill/have died of HIV/AIDS?

- Younger than 10 years
- Between 11-20 years
- Between 21-30 years
- Between 31-40 years
- Between 41-50 years
- Over 50 years

iii) If someone in your household is HIV positive, who is the primary caregiver?

- Spouse
- Parents
- Family member
- Child.children
- Friends
- Volunteer

38c) Do you care for any orphans in your family? No Yes

Adult Questionnaire

40b) Health History:

Cancer Sites

- 1= Mouth
- 2= Esophagus
- 3= Stomach
- 4= Small intestine
- 5= Large intestine including rectum
- 6= Pancreas
- 7= Liver
- 8= Lung
- 9= Breast
- 10= Cervical/uterine/ovarian
- 11= Prostate
- 12= Head and neck
- 13= Other, specify

Subject ID

<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>
Centre #	Community#	Household #	Subject #

Subject Initials
 F M L

39. How long would it take you to get from your house to the nearest facility if you walked?

	Minutes	Don't know		Minutes	Don't know
i) grocery/convenience store	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/>	iv) video store	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/>
ii) bank	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/>	v) non-fast food restaurant	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/>
iii) post office	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/>	vi) fast food restaurant	<input type="text"/> <input type="text"/> <input type="text"/>	<input type="checkbox"/>

40a) Total number of siblings

b) Health History: Complete for all parents and siblings, alive or dead

	Father			Mother			Siblings			# of siblings with the condition
	Unknown	No	Yes	Unknown	No	Yes	Unknown	No	Yes	
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Coronary Heart Disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
High Blood Pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Stroke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Cancer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
		if Yes, indicate site <input type="text"/> <input type="text"/>			if Yes, indicate site <input type="text"/> <input type="text"/>			if Yes, indicate site <input type="text"/> <input type="text"/>		
		Other, Specify			Other, Specify			Other, Specify		

Please refer to facing page for cancer sites

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of “0” into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

Subject ID

Centre # Community# Household # Subject #

Subject Initials
F M L

c) Biceps skinfold

#1 . mm

#2 . mm

#3 mm

d) Subscapular skinfold

#1 . mm

#2 . mm

#3 mm

e) Supra spinal skinfolds

#1 . mm

#2 . mm

#3 mm

44 a) Humerous breadth . cm

b) Femur breadth . cm

45. Grip Strength (Maximal contraction):

a) Non-dominant hand: #1 kg.

b) Dominant hand: #1 kg.

#2 kg.

#2 kg.

#3 kg.

#3 kg.

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of “0” into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

46. Spirometry:

**American Thoracic Society criteria for acceptable spirometers:
Spirometers are acceptable if they are free from:**

- 1. Cough during exhalation**
- 2. Early termination or cut-off**
- 3. Variable effort**
- 4. Leaks**
- 5. Obstructed mouth piece**

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

46. Spirometry:

a) FEV1 (Litre): #1 . #2 . #3 .

b) Does FEV1 obtained meet ATS criteria?

No → (answer (i) to (iii))
 Yes → Go to **c)**

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough
- ii) Values not within 0.2L of each other
- iii) Less than 3 values

c) FVC (Litre): #1 . #2 . #3 .

d) Does FVC obtained meet ATS criteria?

No → (answer (i) to (iii))
 Yes → Go to **e)**

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough
- ii) Values not within 0.2L of each other
- iii) Less than 3 values

e) PEF (Litre/min): #1 #2 #3

f) Does PEF obtained meet ATS criteria?

No → (answer (i) to (ii))
 Yes → Go to **Q#47**

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough
- ii) Less than 3 values

Subject ID

Centre # Community# Household # Subject #

Subject Initials

F M L

47. Not applicable in South Africa

48. ECG obtained? No → Go to #49 Yes

a)

year month day

Place ECG :File Label Here

b) Please print ECG label #:

49 a) Blood sample obtained? No → Go to #50 Yes

b) Fasting sample Non-fasting sample

c)

year month day Time : → Hours since any food/beverage consumed (excluding water)

(00:00-23:59)

Place Blood label here

d) Please print Blood label #:

50 a) Urine sample obtained? No → Go to #51 Yes

b) Fasting sample Non-fasting sample

c) Please print Urine label #:

Place Urine label here

51. Name of Interviewer: _____
(please print) First Initial Last Name

Interviewer Code:

ANNEXURE B

PURE Household Questionnaire

PURE

To be completed by a knowledgeable household member

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Household Questionnaire

INSTRUCTIONS

Please answer **EACH** question by marking
an **X** in **ONE BOX** on each line:
(unless otherwise instructed)



OR

By writing number(s) in the spaces provided:



OR

By specifying the answer on the line(s) provided

Household Questionnaire

Type of House

UAE, Kuwait (following questions not relevant): Q 2b,c and 3a,b

Use of Mosquito nets: If the subjects uses a mosquito net for atleast a week

Not applicable in Canada

Questions- 1, 2, 3, 4 and 5a) and b)

4a Roof of house

Russia: Exclude → Thatch, Tiles

Kuwait/UAE: Exclude → Thatch, Tiles, Slate, Galvanized iron sheet and Other

India: Exclude → Fibrocement/carton sheets

4b Walls of house

Russia: Exclude → Bamboo, Packed mud, Sun dried brick

Kuwait/UAE: Exclude → Bamboo, Packed mud, Sun dried brick, Wood, Burnt brick, Thatch and Stone

Chile: Exclude → Bamboo

India: Exclude → Fibrocement/carton sheets

4c Floor surfaces of house

Russia: Exclude → Dirt, Tile, Carpet

5a Exclude in Kuwait/UAE

5b Type of Fuel

Russia: Exclude → Charcoal, Agriculture/crop, Animal Dung, Shrub/grass

Chile: Exclude → Shrub/grass

Kuwait/UAE: Exclude → Charcoal, Agriculture/crop, Animal Dung, Shrub/grass, Coal, Wood, Kerosene

Exclude "Gobar gas" for all countries except India

5c Primary heating source

Russia: Exclude → Coal open fire, Wood open fire

Kuwait/UAE: Exclude → Coal open fire, Wood open fire, Furnace and None

Household ID

Centre # Community # Household #

Subject Initials

Today's date: 20

year month day

Type of House

1. Total number of rooms (including bedroom/sleeping areas)

2a) Number of sleeping rooms/bedrooms

b) Total number of windows in the sleeping areas/bedrooms

c) Number of household members that sleep with mosquito nets at any time of year.

3a) Is the cooking area/kitchen inside the house? No Yes

b) Do people sleep in the same room that is used for cooking? No Yes

4a) Type of roof on the main house

Thatch Tiles Reinforced concrete Slate Fibrocement sheets/carton sheets
 Galvanized iron sheets Asbestos sheets Other _____

b) Type of walls on the main house

Burnt brick Bamboo Thatch Stone Reinforced concrete/cement block
 Sun dried brick Packed mud Wood Galvanized iron sheets
 Fibrocement sheets/carton sheets Other _____

c) What material are most floor surfaces of the house made of?

Dirt Wood Concrete/brick Tile Carpet
 Reinforced concrete Other _____

5a) Does the house have electricity? No Yes

b) Primary fuel used for cooking

Kerosene Charcoal Coal Gas Wood Agriculture/crop Gobar gas
 Electricity Animal dung Shrub/grass Other _____

c) Primary heating source during the cold/rainy season

Coal open fire Wood open fire Furnace Portable heater
 None Electricity Other _____

Household Questionnaire

6 Sanitary facilities

Kuwait/UAE: Exclude question #6

Russia: Exclude → Public Latrine, Open field, Blair toilet

India: Exclude → Blair toilet

7a Primary drinking water source

Kuwait/UAE: Exclude → Household well, Community well, Natural Lake, River, Collected Rainwater, Bore well, Hand pump

Russia: Exclude → Natural Lake, River, Collected Rainwater, Borewell, Artificial Tanks

In all countries except India: Exclude → Water tankers

7b & c

Not relevant in **Kuwait/UAE and Canada**

Q8 Labour and Time Saving Devices

Kuwait/UAE: Exclude → Other four-wheeler/tractor, Livestock cart

Household income(financial assistance)

Q9a: Members are considered to be in the same household if they consume food from the same kitchen

Q9b

Not relevant in **Kenya**

Thailand: Definition of subsidy/assistance for individual/household would be:

- 1) Low interest loan
- 2) Partial/Full welfare
- 3) Monthly welfare payroll

9c) for income document only gross income (the entire amount of income before any deductions are made) for employed subjects.

Household Questionnaire

Question # 11 not applicable in Canada

11 Livestock

Other countries except Kuwait/UAE: Exclude → Camels

Kuwait/UAE: Exclude → Pigs

Russia: Exclude → Bulls

Household ID

Centre # Community # Household #

Subject Initials

10. Please read the following statements and mark the most appropriate choice

a) In the last 5 years, how often did it happen that you did not have enough food for you and your family's needs?

Most of the time
 Sometimes
 Rarely/Never
 Unable to answer

b) In the last 5 years, how often did it happen that you had difficulties paying bills or loans (for housing, electricity, heating etc)?

Most of the time
 Sometimes
 Rarely/Never
 Unable to answer

Questions 11 to 16 to be answered by rural subjects only

11. Do you own any livestock? No → Go to #12 Yes → If yes, indicate the numbers currently owned of the following:

Cows/Buffalo Chickens Goats Sheep

Bulls Camels Ducks Pigs

Geese Other animals _____ Specify
 Other animals _____ Specify

Household Questionnaire

Questions 12 to 16 not applicable in Canada

12a Agriculture

Kuwait/UAE: Exclude → Acres, Hectares

12b Type of land: definitions

Wet land: This is land that perennially (all through the year) receives water from a river/canal/lake that is located nearby. The land may also be fed by a bore-well, which yields enough quantity of water to sustain the usual cropping pattern of the household. This means that the farmer does not need any other source of water for agriculture.

Dry land: This is land that does not have a continuous source of water in the form of a river, lake or canal. In such cases the farmer depends solely on the rains for water.

Irrigated land: This is land where the water source is led by means of channels to all parts of the property, thereby feeding all the crops in the area. The water may be received from any of the perennial sources i.e. river/canal/lake/well, that is located nearby.

14a Crops

China: Exclude → Ragi

Chile: Exclude → Ragi, cotton, Groundnut, Sugarcane

Kuwait/UAE: Exclude → Rice, Ragi, Sugarcane, Soya, Groundnut, Wheat, Cotton, Cocoa, Tobacco, Tropical fruits, Bean, Sweet/naked Oats

Russia: Exclude → All except Vegetables, Fruit, Sweet/naked Oats, Wheat

All countries except Kuwait/UAE: Exclude → Animal feed

14b Animal/animal products

Kuwait/UAE: Exclude → Pigs

Russia: Exclude → Bulls

All countries except Kuwait/UAE: Exclude → Camels

Household ID

Centre # Community # Household #

Subject Initials

Agriculture

12. Does the household own any cultivable land?

No → (go to #16) Yes → (answer 12a,b&c)

a) How much cultivable land does the household own?:

acres

hectares Sq meters

b) What type of land is it?

please refer to definitions on facing page

Wet Dry Mixed

c) Is the land irrigated?

No Yes

13. Number of harvests/crops cycles per year?

14a) Do you use fertilizer (organic/inorganic) on your crop?

No

Yes

if yes →

specify:

(i) _____

(ii) _____

b) Do you use pesticides on your crop?

No

Yes

if yes →

specify:

(i) _____

(ii) _____

15a) On average what percent of the annual household agricultural yield is sold

%

b) Indicate which of the following provides the most net income?

(net income is the amount of money left over after all of the expenses incurred with growing the crop, have been paid (eg. seeds, fertilizer)) (select one only)

Rice Corn/maize Ragi Sugarcane Soya Groundnut Dates
 Vegetables Fruits Wheat Cotton Coffee Cocoa Tobacco
 Bean Sweet/naked Oats Animal feed Other _____ Don't sell crops

16. Indicate which of the following livestock/animal products provides the most net income?

(net income is the amount of money left over after all of the expenses incurred with raising the animals, have been paid. (eg. feed)) (select one only)

Cows Bulls Goats Sheep Pig Chicken Fish
 Ducks Geese Camels Other _____ Don't sell livestock/animal products

17. Name of Interviewer: _____
(please print)

Last Name

First Initial

Interviewer Code:

ANNEXURE C

Declaration of language editing

DECLARATION

I, Clarina Vorster (ID: 710924 0034 084), Language editor and Translator, and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of the thesis of ms Y Breet, student of the North-West University, Potchefstroom Campus (student number 21195706).

Title of the thesis: Cardiovascular disease and reduced pulmonary function in black South Africans: Investigating the interplay with markers of systemic inflammation

C Vorster

11 April 2016

C Vorster

Date

9 Lanyon Street

Potchefstroom

2520

082 440 4102

ANNEXURE D

Turn-it-in reports



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ANNEXURE E

Published manuscript of research article 1

South African and International Reference Values for Lung Function and its Relationship with Blood Pressure in Africans



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Background

In South Africa respiratory diseases are highly prevalent, with cardiovascular disease being a manifestation. However, international reference values for lung function are commonly used, which may not be appropriate to correctly identify reduced lung function. An inverse relationship exists between lung function and blood pressure (BP) but is not investigated extensively in black South Africans.

Methods

We included 2010 Africans from the PURE (Prospective Urban Rural Epidemiology) study (aged > 35 years) in the North West Province. Spirometry was performed and predicted values for forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were calculated from South African, European and United States prediction equations.

Results

With the exception of the European predicted values, all other predicted mean FEV₁ and FVC were above 80%. South African reference values displayed the highest percentages of the predicted values for FEV₁ and FVC (87.9 and 99.7%, respectively.) BP increased from quintiles five to one for both FEV₁ and FVC, (p for trend <0.001). After adjustment the differences remained (p<0.05).

Conclusions

South African reference values yielded higher percentages of predicted FEV₁ and FVC values than European and US equations suggesting that South African prediction equations may be more useful when investigating lung function in black South Africans. Elevated BP is related to reduced lung function, highlighting the importance in managing both respiratory- and cardiovascular disease.

Keywords

Cardiovascular disease • Ethnicity • Respiratory diseases • Hypertension • Lung function

Introduction

The prevalence of non-communicable diseases (NCDs) including cardiovascular disease (CVD) and respiratory diseases in South Africa is high and accounts for 11% and 3%

respectively, of the total NCD mortality in South Africa [1]. The burden of NCDs is also predicted to increase in South Africa if preventive measures are not taken [2].

There are several challenges in estimating the respiratory disease burden in South Africa. Appropriate reference data is

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vital and unsuitable reference equations as well as inaccurate interpretation may lead to over- or under diagnosis [3]. In South Africa where the mining industry is prominent and a large number of the population still use open fires for food preparation and as a heating source, the accurate assessment and prediction of lung function should be highlighted. South African prediction equations are available [4,5] but are seldom used since it is not included in the software packages of commercial spirometers. Instead, European reference equations have largely been used in South Africa. However, it has been described that populations from southern Africa display a lower forced vital capacity (FVC) as well as forced expiratory volume (FEV₁) when compared to Europeans [6] and a recent study conducted by Quanjer *et al.*, [7] confirmed these findings. To account for this, the South African Thoracic Society (SATS) proposed that a correction factor of 0.9 be utilised for black South Africans when European prediction equations are being used [8].

Reduced lung function poses significant extra pulmonary effects, with one of the best recognised manifestations being accompanying cardiovascular disease [9]. This is especially notable in patients with chronic obstructive pulmonary disease (COPD) where it has been shown that even moderate reductions in expiratory flow volumes elevate the risk for CVD two- to three-fold [10–13]. An inverse relationship between lung function and blood pressure has been reported in several studies [14–17]. Although the mechanism underlying this relationship remains unclear, it has been shown that individuals with reduced lung function have higher levels of C-reactive protein, fibrinogen and other systemic inflammatory markers when compared to those with normal lung function [18,19]. Since low-grade systemic inflammation is associated with vascular dysfunction, reduced lung function poses as a risk factor for cardiovascular morbidity and mortality [20].

We therefore aimed firstly to compare the prediction values from three different reference populations namely European, US and South Africa, in a large sample of black South Africans (n=2010); and secondly to establish whether lung function is associated with blood pressure in these participants.

Material and Methods

Study Design and Subject Selection

This study is embedded in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study, taking place in the North West Province. This is a prospective study of which baseline data was collected in 2005. PURE was designed to investigate lifestyle changes and health status of populations from numerous developing countries [21,22].

In the North-West province of South Africa, a total of 2010 volunteers aged ≥ 35 years were included from 6000 randomly selected households – equal numbers from rural and urban settings.

Approval was obtained from the Ethics Committee of the North-West University, Potchefstroom, South Africa. Permission to conduct the study in the above-mentioned communities was granted by the Provincial Department of Health, community leaders, tribal chiefs, and mayors. The protocol was explained to the subjects in their home language by field workers and they were given the opportunity to ask questions. Confidentiality and anonymity of all the results were assured by making use of anonymised numbers. Participants received remuneration for travelling expenses during the study and were referred to clinics if any pathology was noticed.

Questionnaires

The subjects were interviewed by extensively trained field workers using structured demographic, socio-economic, lifestyle and physical activity questionnaires, developed and standardised for the international PURE study and adjusted for each country [21]. Lifestyle data included tobacco use, alcohol intake, health history, and medication use.

Anthropometric and Cardiovascular Measurements

Anthropometric measurements were done under the supervision of a level three anthropometrist and included height (Invicta Stadiometer, IP 1465, Invicta, London, UK) and weight (Precision Health Scale, A&D Company, Tokyo, Japan). The body mass index (BMI = weight (kg) / height (m)²) was also calculated [23].

Cardiovascular Measurements

Brachial blood pressure (BP) was taken by cardiovascular physiologists on the right arm using an automated digital BP monitor (OMRON HEM-757, Omron Healthcare, Kyoto, Japan) after a 10 minute resting period. The subject was sitting in a comfortable position with the right arm rested on a stable surface. The BP measurement was repeated after a five minute resting interval and the mean BP was used for statistical analyses.

Spirometry

Lung function was assessed using a mobile Micro GP Spirometer (Micro Medical Ltd, Kent, UK) according to the American Thoracic Society recommendations [24]. The protocol was explained to participants prior to the assessment. A minimum of three acceptable recordings and a maximum of five were obtained from each participant while in the standing position and with at least 1 minute between repeat measurements. The variables that were analysed were FEV₁ (Forced Expiratory Volume in 1 sec) and FVC (Forced Vital Capacity). Analysis was based on the best measurement for both FEV₁ and FVC out of all measurements obtained from each participant. Published prediction equations from European [7], American [25], and South African [8] populations were used to calculate predicted FEV₁ and FVC values for each participant.

Table 1 Characteristics of the African men and women.

	Men	Women	p-value
N	746	1264	
Age (years)	50.3 ± 10.3	49.6 ± 10.4	0.154
<i>Anthropometric measurements</i>			
Height (m)	1.67 ± 0.07	1.57 ± 0.06	< 0.001
Weight (kg)	58.3 ± 12.0	66.4 ± 18.6	< 0.001
Body mass index (kg/m ²)	20.7 ± 4.01	27.0 ± 7.36	< 0.001
<i>Biochemical markers</i>			
TC (mmol/L)	4.81 ± 1.33	5.13 ± 1.39	< 0.001
Fasting glucose (mmol/L)	4.80 (3.4;6.1)	4.88 (3.5;6.9)	< 0.001
<i>Cardiovascular measurements</i>			
bSBP (mmHg)	135.3 ± 23.2	132.2 ± 24.8	0.008
bDBP (mmHg)	86.7 ± 14.6	88.2 ± 14.4	0.024
<i>Spirometry variables</i>			
FEV ₁ (L)	2.62 ± 0.76	2.04 ± 0.54	< 0.001
FVC (L)	3.14 ± 0.84	2.37 ± 0.62	< 0.001
FEV ₁ / FVC (%)	83.7 ± 11.9	86.8 ± 10.2	< 0.001
US reference values^a			
FEV ₁ predicted value (L)	3.19 ± 0.55	2.46 ± 0.29	< 0.001
FEV ₁ % predicted value	82.5 ± 20.9	82.9 ± 18.7	0.640
FVC predicted value (L)	3.90 ± 0.65	2.95 ± 0.34	< 0.001
FVC % predicted value	80.8 ± 19.0	80.2 ± 18.4	0.482
European reference values^b			
FEV ₁ predicted value (L)	3.26 ± 0.44	2.35 ± 0.38	< 0.001
FEV ₁ % predicted value	80.3 ± 19.9	87.3 ± 20.0	< 0.001
FEV ₁ LLN	1.88 ± 0.44	1.34 ± 0.38	< 0.001
FEV ₁ Correction factor [†]	2.93 ± 0.40	2.12 ± 0.35	< 0.001
FVC predicted value (L)	4.01 ± 0.50	2.76 ± 0.41	< 0.001
FVC % predicted value	78.2 ± 18.1	86.0 ± 19.6	< 0.001
FVC LLN	2.37 ± 0.50	1.60 ± 0.41	< 0.001
FVC Correction factor [†]	3.61 ± 0.50	2.49 ± 0.37	< 0.001
South African reference values^c			
FEV ₁ predicted value (L)	2.96 ± 0.36	2.07 ± 0.39	< 0.001
FEV ₁ % predicted value	87.9 ± 22.4	99.7 ± 24.2	< 0.001
FEV ₁ LLN	1.73 ± 0.36	1.02 ± 0.38	< 0.001
FVC predicted value (L)	3.76 ± 0.43	2.87 ± 0.39	< 0.001
FVC % predicted value	83.0 ± 19.6	82.4 ± 19.0	0.513
FVC LLN	2.30 ± 0.43	1.78 ± 0.39	< 0.001
<i>Lifestyle factors</i>			
γ-glutamyl transferase (U/L)	70.8 (22.7;436)	47.9 (17.9;296)	< 0.001
Tobacco usage, n (%)	496 (66.9)	623 (49.5)	< 0.001
Physical activity index	7.30 ± 2.16	7.29 ± 1.73	0.891
HIV infected, n (%)	115 (15.5)	207 (16.5)	0.532
TB diagnosis, n (%)	36 (4.88)	26 (2.08)	0.001
<i>Medication usage</i>			
Anti-hypertensive, n (%)	7 (0.94)	32 (2.53)	0.012
Anti-TB, n (%)	4 (0.54)	5 (0.40)	0.648

Data are presented as mean ± SD. Data with a non-Gaussian distribution presented as geometric mean and confidence intervals (5% - 95%). Abbreviations: DBP, diastolic blood pressure; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; LLN, lower limit of normality; SBP, systolic blood pressure; TB, tuberculosis; TC, total cholesterol. The p-value is the statistical test of difference in effects between men and women with p ≤ 0.05 regarded as significant. ^a Knudson 1976, ^b Quanjer 1993, ^c Mokoetle 1994, Louw 1996. [†] Correction factor of 0.9 applied.

Blood Sampling and Biochemical Analysis

Blood samples were taken by a registered nurse from the antebraichial vein branches. The subjects were asked to fast overnight (8 to 10 hours with no food or beverage, excluding water). The blood was centrifuged for 15 minutes at 2000 g at 4 °C. Aliquots of plasma and serum were frozen on dry ice, stored in the field at -18 °C for two to four days after which the samples were transported to a storage facility where the samples were kept at -80 °C until analysis.

Fluoride plasma glucose, serum γ -glutamyl transferase (GGT) and total cholesterol (TC) were measured using the Konelab 20i (Thermo Scientific, Vantaa, Finland) and the Cobas Integra 400 Plus (Roche, Indianapolis, United States of Indiana) instruments. The HIV status was determined by a First Response Rapid HIV card test (PMC Medical, Daman, India). In the case of a positive outcome, the result was confirmed with the Pareeshak card test (BHAT, Bio-tech, India). All participants also received pre- and post HIV-test counselling by trained counsellors.

Data Processing

Statistical analyses were performed using Statistica version 12 (Statsoft, Inc., Tulsa, OK, 2010). Variables with a non-Gaussian distribution were logarithmically transformed (fasting glucose and GGT). Independent t-tests and Chi-Square tests were used to compare means and proportions between men and women. We also selected a group of apparently healthy men, fitting the criteria of an age between 30 and 50 years, with no history of tobacco use, HIV uninfected, a BMI between 20 and 25 kg/m² and with blood pressure <140/90 mmHg. We compared the lung function of the healthy men to the rest of the men. We further compared the lung function of men with no history of tobacco use to men making use of tobacco products, formerly or currently. The subjects were divided into quintiles of the FEV₁ and FVC with Quintile 1 (Q1) being the lowest lung function. An analysis of covariance was performed to determine differences between the quintile groups. We performed single and partial regression analyses to determine relationships between blood pressure and measures of lung function. The covariates that were included were age, gender, waist circumference, height and a history of tobacco use (Table 1).

Results

Characteristics of Participants

In total 2010 participants were included in this study with the majority being women (63%, n=1264). Although men and women had similar ages of approximately 50 years ($p=0.15$) the women displayed a higher weight (66.4 ± 18.6 vs. 58.3 ± 12.0 ; $p < 0.001$) and BMI (27.0 ± 7.36 vs. 20.7 ± 4.01 ; $p < 0.001$). Men displayed significantly higher values for both FEV₁ (2.62 ± 0.76 vs. 2.04 ± 0.54 ; $p < 0.001$) and FVC (3.14 ± 0.84 vs. 2.37 ± 0.62 ; $p < 0.001$), but the FEV₁/FVC ratio for the

men and women were well within the normal range of >70-80% [8]. Based on all the reference values included (i.e. US reference values, the reference values from the European Community as well as South African reference values), almost all the mean FEV₁ and FVC for both the men and women were above 80% of the predicted value, indicative of normal lung function. Based on the South African reference values, 27% (n=203) of the men displayed a FEV₁ that was below 80% of the predicted value and 37% (n=278) had a FVC that was below 80%. With regard to the women, 16% (n=206) had a FEV₁ below 80% of the predicted value and 40% (n=507) had a FVC that was lower than 80%.

A comparison between the lung function of the apparently healthy men (n=45) and the rest of the men (n=701) in Table 2 indicates - as expected - that the apparently healthy men displayed a more favourable lung function compared to the rest of the men (n=701). In the group of apparently healthy men, the predicted values based on the South African reference values displayed the highest percentages both for FEV₁ (97.5 ± 20.5) and FVC (89.7 ± 20.6). The FEV₁ and the FVC percentage of the predicted value were higher in the apparently healthy group of men compared with the rest of the men, this finding being present in all three sets of reference values.

When comparing men with no history of tobacco use to those with current or former tobacco use (Table 3), the men with no history of tobacco use, generally displayed more favourable lung function with a borderline higher FEV₁ (2.69 ± 0.75 vs. 2.59 ± 0.77 ; $p = 0.089$) and a significantly higher FVC (3.23 ± 0.86 vs. 3.09 ± 0.82 ; $p = 0.038$). The FEV₁ and FVC percentage of the predicted value were significantly higher ($p < 0.001$) in the men with no history of tobacco use, for each of the three sets of reference values.

Unadjusted and Adjusted Analyses

Figure 1 demonstrates the unadjusted relationship between the SBP and DBP with both FEV₁ and FVC in the entire study sample. FEV₁ was negatively associated with SBP ($r=0.095$; $p < 0.001$) and DBP ($r=0.110$; $p < 0.001$). FVC also displayed a negative association with SBP ($r=0.063$; $p=0.006$) and DBP ($r=0.083$; $p < 0.001$). After adjusting for the covariates age, gender, waist circumference, height and a history of tobacco use, none of these associations remained significant.

Figure 2 represents the unadjusted and adjusted difference in SBP and DBP between the different quintiles of FEV₁. Before and after adjustments both SBP and DBP are lower in the higher quintiles of FEV₁ and this trend is highly significant ($p < 0.001$). With the differences in BP between the FEV₁ quintiles adjusted for age, gender, waist circumference, height and a history of tobacco use as covariates, this trend no longer remains significant although BP between the quintiles still differed significantly from the reference quintile 5. In Figure 3 similar results are shown for FVC. Both SBP and DBP decrease in the higher quintiles of FVC and this trend is significant for SBP ($p=0.042$) and highly significant for DBP ($p < 0.001$). After adjusting for covariates, the trends were no longer significant, but Q5 showed significant lower BP than Q1 in all instances ($p < 0.05$).

Table 2 Lung function of the total group of men compared to the apparently healthy group of men.

	Total group of men	Apparently healthy men	p-value
N	701	45	
Age (years)	50.8 ± 10.4	42.6 ± 4.68	< 0.001
Height (m)	1.67 ± 0.07	1.67 ± 0.08	0.651
<i>Spirometry variables</i>			
FEV ₁ (L)	2.58 ± 0.75	3.10 ± 0.81	< 0.001
FVC (L)	3.11 ± 0.81	3.57 ± 1.09	< 0.001
FEV ₁ / FVC (%)	83.4 ± 12.1	88.2 ± 8.19	0.010
US reference values			
FEV ₁ % predicted value	81.9 ± 21.0	90.4 ± 17.1	0.009
FVC % predicted value	80.5 ± 19.1	85.6 ± 17.6	0.082
European reference values			
FEV ₁ % predicted value	79.7 ± 20.0	88.8 ± 17.9	0.003
FVC % predicted value	77.8 ± 17.9	84.1 ± 18.9	0.023
South African reference values			
FEV ₁ % predicted value	87.2 ± 22.4	97.5 ± 20.5	0.003
FVC % predicted value	82.6 ± 19.4	89.7 ± 20.6	0.017

Data are presented as mean ± SD. Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity. The p-value is the statistical test of difference in effects between the total group of men and the healthy group of men with $p \leq 0.05$ regarded as significant.

Sensitivity analyses

Due to the known effects of inflammation as a consequence of HIV infection [26], we compared SBP and DBP as well as FEV₁ and FVC between HIV infected and uninfected groups to determine whether the BP and lung function differ. No

significant differences were found and based on these findings HIV status was not included as a covariate in statistical analyses and the HIV infected participants were not excluded from the analyses.

Table 3 Basic characteristics and lung function of the men with no history of tobacco use compared to men with tobacco use.

	Men		% Δ	p-value
	No Tobacco use	Tobacco use		
N	236	465		
Age (years)	51.8 ± 11.0	49.5 ± 9.84		0.004
Height (m)	1.67 ± 0.07	1.68 ± 0.07		0.299
<i>Spirometry variables</i>				
FEV ₁ (L)	2.69 ± 0.75	2.59 ± 0.77	-3.72	0.089
FVC (L)	3.23 ± 0.86	3.09 ± 0.82	-4.33	0.038
FEV ₁ / FVC (%)	83.5 ± 10.6	83.9 ± 12.4	0.48	0.714
US reference values				
FEV ₁ % predicted value	86.5 ± 20.7	80.6 ± 20.6	-6.82	< 0.001
FVC % predicted value	84.7 ± 19.3	79.0 ± 18.5	-6.73	< 0.001
European reference values				
FEV ₁ % predicted value	84.3 ± 19.2	78.4 ± 19.9	-6.99	< 0.001
FVC % predicted value	82.0 ± 18.2	76.4 ± 17.6	-6.83	< 0.001
South African reference values				
FEV ₁ % predicted value	92.4 ± 21.0	85.8 ± 22.7	-7.14	< 0.001
FVC % predicted value	87.1 ± 19.1	81.1 ± 19.5	-6.89	< 0.001

Data are presented as mean ± SD. Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; % Δ, percentage change between control and effect. A p value of ≤ 0.05 was regarded as significant.

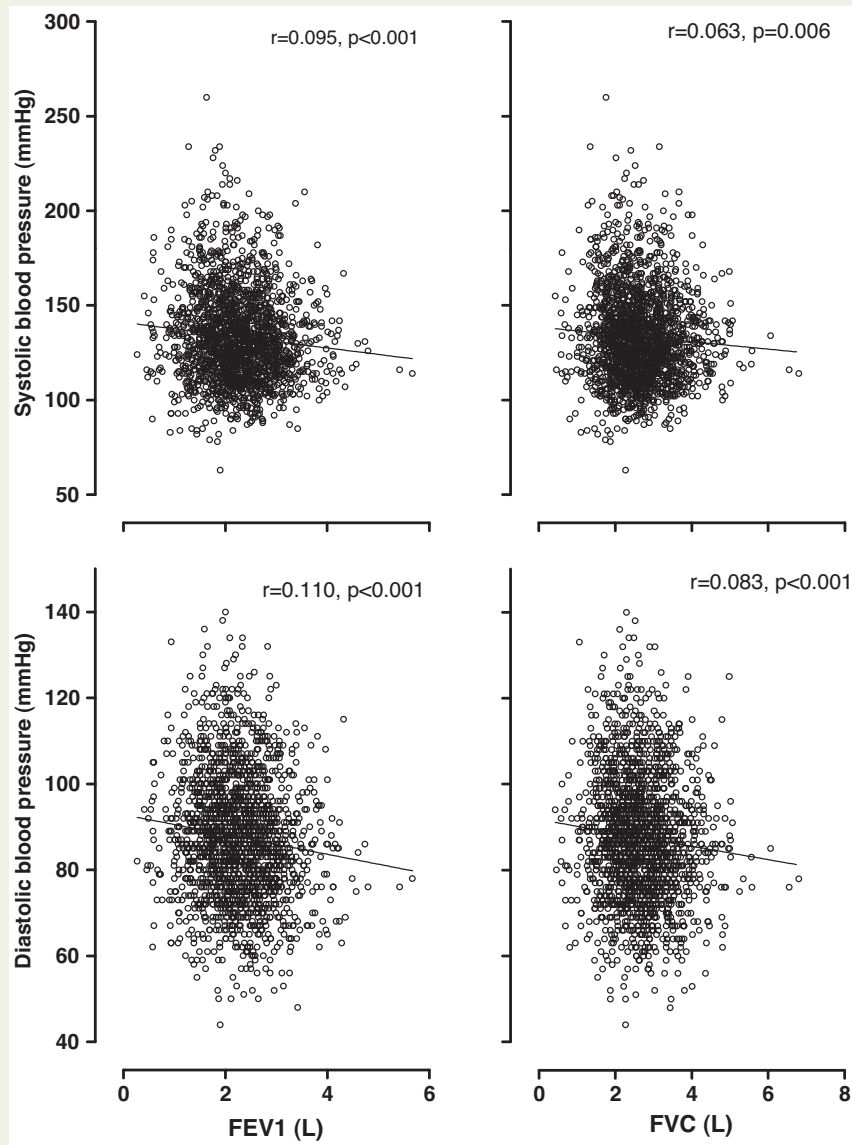


Figure 1 The unadjusted relationship between blood pressure, FEV₁ and FVC in 2010 African men and women. Abbreviations: FEV₁, forced expiratory volume in one second; FVC, forced vital capacity.

Discussion

Our aims were to compare South African, European and US prediction equations for lung function in a large sample of black South Africans. We also wanted to establish whether an association exists between lung function and blood pressure in this population.

In addressing our first aim we found that the implementation of the South African and international prediction equations yielded different results, underlying the challenge in selecting appropriate reference values in lung function assessment [3]. This is underscored by evidence that lung function varies significantly between different regions of the world [22]. Overall, the highest predicted values were seen for the South African reference values. These prediction

equations yielded higher predicted values when compared to the European reference values even after the correction factor of 0.9, which was suggested by the South African Thoracic Society (SATS), was applied. Also when comparing the group of apparently healthy men with the rest of the men, the South African reference values displayed the highest percentages for FEV₁ (97.5 ± 20.5) and FVC (89.7 ± 20.6). Despite the lack of a large all-inclusive study of the South African population [27], there are smaller studies that have aimed to establish reference values for South African population groups [4,5]. These reference values are based on a study population comprised of Sesotho, isiZulu and Setswana speaking individuals [5], which is comparable with our Setswana speaking population. Although not reported formally, body composition and size of different black ethnic

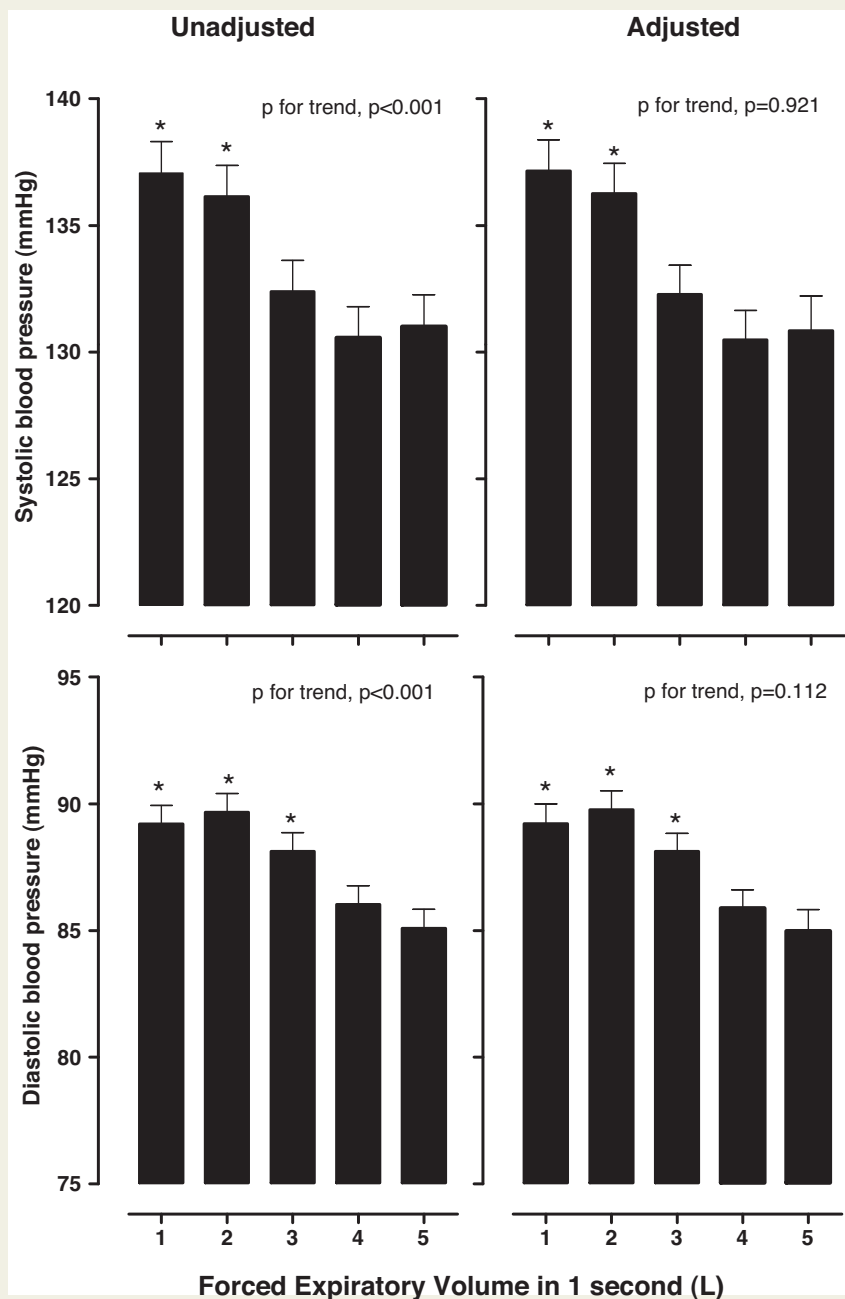


Figure 2 The unadjusted and adjusted differences in systolic- and diastolic blood pressure between quintiles of FEV₁ in 2010 African men and women. Bars with an asterisk differ significantly from Q5. Adjusted for age, gender, waist circumference, height and a history of tobacco use.

groups is perceived to be different. This requires further investigation but may explain measured as well as predicted values in our study for FEV₁ and FVC being considerably lower when compared to data from other South African studies [4,5] even for the group of healthy men. Louw et al., [4] and Mokoetle et al., [5] reported a mean FEV₁ of 3.41 L and 3.14 L respectively, which is considerably higher than the 2.58 L of the men in our study population. With regards to the FVC, these authors reported a mean of 4.26 L

and 4.19 L respectively, which is also higher than the 3.11 L from our data. Urbanisation could play a key role in these findings due to an increase in environmental and occupational exposures [2]. South Africa is undergoing rapid industrialisation and occupational exposures contribute substantially to the increase in respiratory and cardiovascular diseases [2]. Although occupational health and hygiene control and regulation of the mining industry has greatly improved, many miners that relocated to their village of

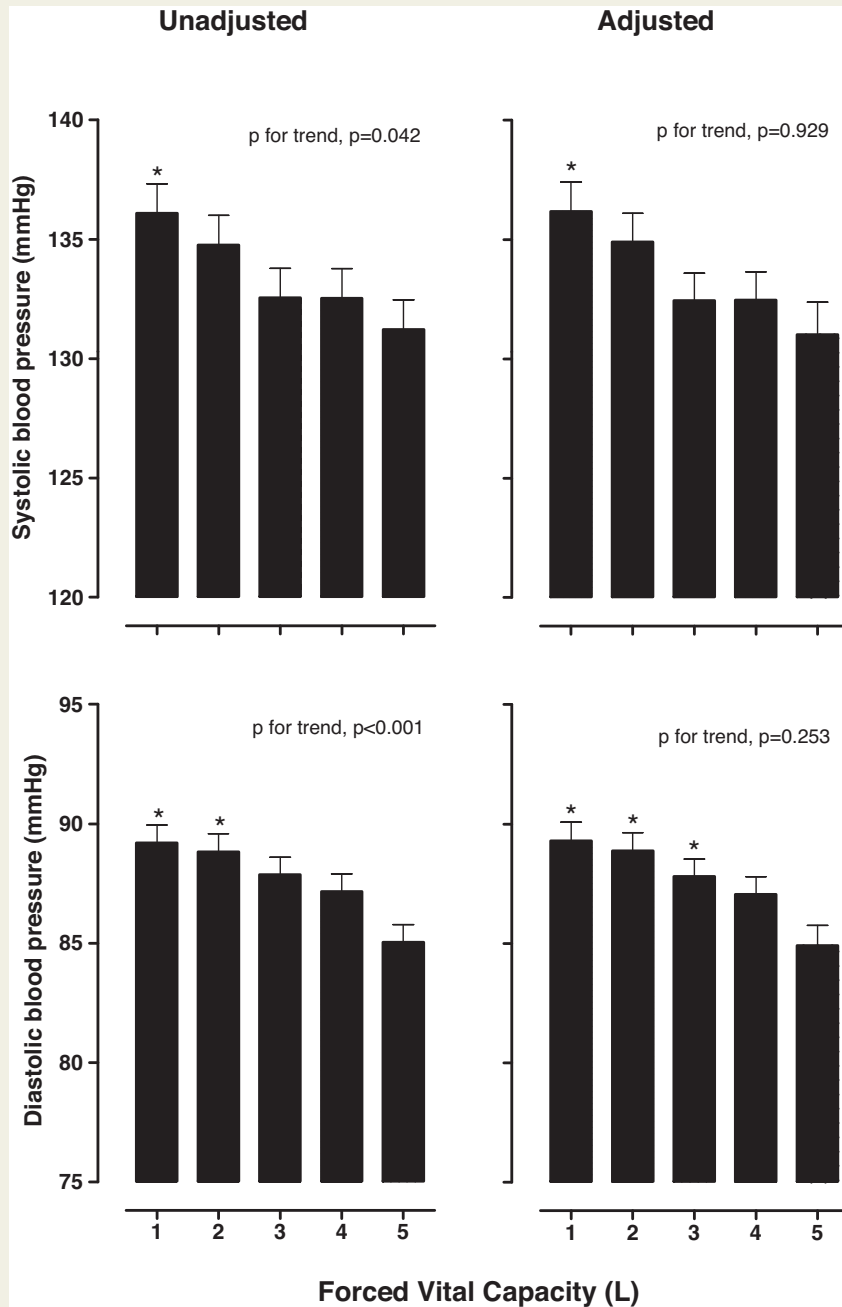


Figure 3 The unadjusted and adjusted differences in systolic- and diastolic blood pressure between quintiles of FVC in 2010 African men and women. Bars with an asterisk differ significantly from Q5. Adjusted for age, gender, waist circumference, height and a history of tobacco use.

origin have had significant morbidity from respiratory disorders such as tuberculosis [28].

Our most prominent finding was a significant inverse association between BP and lung function evident in the total group according to FEV₁ and FVC quintiles. In a pooled analysis by Sin *et al.*, that categorised FEV₁ into quintiles, it was shown that persons in the lowest FEV₁ quintile had a 75% increase in the risk for cardiovascular mortality [20]. An inverse relationship between BP and

lung function (measured by FEV₁ and FVC) has also been reported in several studies, which is confirmed by our results [13–16].

Although it is clear that a relationship exists between lung function and BP, the mechanisms underlying this association remain unclear. It has been hypothesised that the association can be explained by the confounding effect of age, since lung function decreases and BP increases with age [17]. However, our results remained significant after adjustment for age.

Another proposed mechanism is that impaired lung function can give rise to increased systemic levels of CRP and other acute phase proteins leading to inflammation [29]. In animal models it has been found that induction of airway inflammation can provoke and increase systemic inflammation which may contribute to the progression of vascular endothelial dysfunction [30]. Endothelial dysfunction is evident in patients with reduced lung function and may predispose them to systemic hypertension [31].

Our study has several strengths and limitations. This study presents results in a large population-based study in South Africa on lung function, the use of different reference values and the relationship of BP with lung function. Limitations of our study are that we included a large proportion of unemployed individuals with sampling being done in poverty-stricken communities. The participants were also selected from Setswana-speaking rural and urban areas chosen for stability to make the follow-up of this longitudinal study possible. Our results may therefore not be applicable to other population groups.

Conclusions

In conclusion, our investigation into the accurate assessment of lung function in this population of black South Africans shows that the previously published South African reference values [4,5] are more useful when compared to reference values from Europe and the US. Furthermore, SBP and DBP are higher when lung function is impeded. It is important to select appropriate reference values to quantify lung function and to extensively study the mechanistic link between reduced lung function and cardiovascular comorbidities.

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ANNEXURE F

Published manuscript of research article 2

Inflammation as Possible Mediator for the Relationship Between Lung and Arterial Function

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Abstract

Introduction Reduced lung function is associated with a risk for the development of cardiovascular disease. This association may be due to chronic inflammation which is often present in those with reduced lung function.

Purpose We investigated the possible role of systemic inflammation as the mediator between lung function and arterial stiffness in 1534 black South Africans.

Methods Spirometric data including forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were obtained. C-reactive protein (CRP), interleukin-6 (IL-6), blood pressure (BP) and carotid-radial pulse wave velocity (PWV) were determined.

Results In multivariable-adjusted models, an independent inverse association was found between IL-6 and FEV₁ ($\beta = -0.20, p < 0.001$) and FVC ($\beta = -0.18, p < 0.001$). Similar results were found for CRP. PWV was inversely associated with FEV₁ ($\beta = -0.06, p = 0.037$). No association was found between inflammatory markers, BP or PWV.

Conclusion Reduced lung function was associated with increased inflammation and arterial stiffness. The lack of association between arterial stiffness and inflammatory markers suggests that inflammation may not be the mediating link between lung and vascular function in this population.

Keywords Pulmonary function · Blood pressure · C-reactive protein · Interleukin-6 · Arterial stiffness · African

Introduction

Reduced lung function, as measured by forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC), is associated with an increased risk for the development of hypertension [1–3] and cardiovascular disease (CVD) [4]. Although the association between lung function and CVD is clear, the mechanistic underpinnings of this association remain largely unexplained.

Reduced lung function is associated with persistent low-grade inflammation [5–7] with increased levels of cytokines such as interleukin-6 (IL-6) as well as the acute phase protein, C-reactive protein (CRP) [8]. On the cardiovascular side, both of these markers were also shown to play a role in endothelial dysfunction [9] and arterial stiffening [10, 11]. With arterial stiffness being one of the risk factors for CVD [12–15], it can be asserted that one of the possible mechanisms linking reduced lung function and CVD may be chronic low-grade inflammation leading to arterial stiffness.

Inflammation as the possible link between lung- and cardiovascular function has not been investigated extensively in population-based studies, especially in black populations, known to have a high prevalence of

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hypertension [16]. We therefore aimed to investigate the three-way relationship between: (a) lung function (FEV₁ and FVC) and markers of inflammation (IL-6 and CRP); (b) inflammation and arterial stiffness by using pulse wave velocity (PWV); and (c) lung function and arterial stiffness in a sample of Africans to determine whether there is an association between lung- and vascular function, and whether this association is mediated through inflammation.

Materials and Methods

Study Design and Subject Selection

This study is embedded in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study. This longitudinal study was designed to investigate urban and rural lifestyle changes and health status of populations from numerous countries, with the details described elsewhere [17, 18]. The main inclusion criteria for participating South African communities were that they had to show migration stability and also had to be part of the North West Province. The baseline data used in this study were collected in 2005, during which four different resident areas were identified for participation. A household census regarding the number of people, their ages and health profile was done in 6000 houses (1500 in each community) starting from a specific point. Every head of household signed written informed consent to fill out the questionnaire. If a person refused or was not at home, the next house was taken and a non-complier questionnaire was filled out. From the data obtained from the census, a paper-based selection of possible subjects was made based on the following inclusion criteria: An apparently healthy cohort of men and women older than 35 years, who were not pregnant, intoxicated and did not have any cognitive deficits. A total of 2010 volunteers were recruited from the 6000 randomly selected households. Our specific sub-study excluded those infected with the human immunodeficiency virus (HIV) ($n = 337$), those making use of anti-inflammatory medication ($n = 55$) and those with missing spirometry data ($n = 84$). The final study population consisted of 1534 participants.

The study protocol complies with the Declaration of Helsinki as revised in 2000 and approval was obtained from the Ethics Committee of the North-West University, Potchefstroom, South Africa (Ethics Number 04M10). Permission to conduct the study in the above-mentioned communities was granted by the Provincial Department of Health, community leaders, tribal chiefs, and mayors. The recruited participants were visited at their homes where the protocol was explained to them in their home language by

field workers. They were given the opportunity to ask questions prior to giving informed consent.

Questionnaires

The subjects were interviewed by trained field workers using structured demographic, lifestyle and physical activity questionnaires, developed and standardized for the international PURE study and adjusted for each country [18]. Lifestyle data included tobacco use, alcohol intake, health history and medication use.

Anthropometric Measurements

Anthropometric measurements included waist circumference (Holtain unstretchable metal tape), height (Invicta Stadiometer, IP 1465, Invicta, London, UK) and weight (Precision Health Scale, A&D Company, Tokyo, Japan). We also calculated body mass index (BMI = weight (kg)/height (m)²) [19].

Cardiovascular Measurements

Brachial systolic (bSBP) and diastolic blood pressure (bDBP) were measured on the right arm using an automated digital BP monitor (OMRON HEM-757, Omron Healthcare, Kyoto, Japan) after a 10 min resting period. The subject was sitting in a comfortable position with the right arm rested on a stable surface. The BP measurement was repeated after a 5 min resting interval and the second BP reading was used for statistical analyses.

Pulse wave velocity (PWV) was measured on the left side of each participant while in a supine position by using the Complior SP device (Artech-Medical, Pantin, France) in a segment over the carotid-radialis.

Spirometry

Lung function was assessed according to the American Thoracic Society recommendations [20] using a mobile Micro GP Spirometer (Micro Medical Ltd, Kent, UK). A minimum of three acceptable recordings and a maximum of five were obtained from each participant while in the standing position and with at least 1 min between repeat measurements. Measurements were taken by trained occupational hygienists. Analysis was based on the maximal effort for both FEV₁ and FVC of all measurements obtained from each participant.

Blood Sampling

Blood samples were taken by a registered nurse from the antecubital vein branches. The subjects were asked to fast

overnight (8–10 h with no food or beverage, excluding water). The blood was centrifuged for 15 min at $2000\times g$ at 4 °C. Aliquots of plasma and serum were frozen on dry ice, stored in the field at -18 °C for 2–4 days after which the samples were transported to a storage facility where the samples were kept at -80 °C until analysis.

Biochemical Analysis

Serum γ -glutamyltransferase (GGT), total cholesterol (TC) and high sensitivity CRP were analysed by particle-enhanced turbidimetric assays with the Konelab 20iTM auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). IL-6 was analysed by the Elecsys apparatus using ultra-sensitive enzyme immunoassays (Elecsys 2010, Roche, Basel, Switzerland).

To determine glycated haemoglobin (HbA1c) levels, the D-10 Haemoglobin testing system from Bio-Rad (#220-0101) was used. The HIV status was determined by a First Response Rapid HIV card test (PMC Medical, Daman, India). In the case of a positive outcome, the result was confirmed with the Pareeshak card test (BHAT, Bio-tech, India). All participants also received pre- and post HIV-test counselling by trained counsellors.

Statistical Analysis

Statistical analyses were performed using Statistica version 12 (Statsoft, Inc., Tulsa, OK, USA). We tested for the interaction of sex on the relationships between lung function (FEV₁ and FVC), inflammation (IL-6 and CRP) and cardiovascular function (SBP and PWV). The interaction tests with sex were not significant (all $p > 0.394$) and therefore we pooled the data for men and women. Variables with a non-Gaussian distribution were logarithmically transformed (HbA1c, IL-6, CRP and GGT) and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Single and partial correlations were performed to determine if associations exist between measures of lung function (FEV₁ and FVC), cardiovascular function (SBP and PWV) and markers of inflammation (IL-6 and CRP). Furthermore, by using either a marker of inflammation (IL-6 or CRP) or measure for cardiovascular function (SBP or PWV) as dependent variable, we determined whether an independent association exists with lung function as main independent variable (FEV₁ or FVC) in forward stepwise multiple regression analysis. Results from the different regression models are shown as standardized beta-values (β) (Table 3). Covariates included in all models were age, sex, height, physical activity index, tobacco use, GGT (log), HbA1c (log), TC and anti-hypertensive medication, with

Table 1 Characteristics of the study population

<i>N</i>	Total population 1534
Women, <i>N</i> (%)	957 (62.4)
Age (years)	51.2 ± 10.5
Anthropometric measurements	
Height (m)	1.61 ± 0.08
Body mass index (kg/m ²)	25.1 ± 10.2
Waist circumference (cm)	80.7 ± 13.4
Cardiovascular measurements	
Systolic blood pressure (mmHg)	135.6 ± 24.2
Diastolic blood pressure (mmHg)	88.5 ± 14.2
Pulse wave velocity (m/s)	11.1 ± 2.34
Lung function measurements	
Forced expiratory volume in 1 s (L)	2.22 ± 0.70
Forced vital capacity (L)	2.62 ± 0.81
Biochemical markers	
Total cholesterol (mmol/L)	5.13 ± 1.37
Glycated haemoglobin (%)	5.63 (4.80; 6.60)
C-reactive protein (mg/L)	3.17 (0.25; 37.8)
Interleukin-6 (pg/mL)	2.82 (0.75; 16.8)
Lifestyle factors	
γ -Glutamyltransferase (U/L)	55.8 (19.0; 361)
Smoking, <i>N</i> (%)	834 (54.4)
Physical activity index	7.29 ± 1.87
Medication usage	
Anti-hypertensive, <i>N</i> (%)	12 (0.78)

Data expressed as arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables

N number of participants

mean arterial pressure and heart rate included additionally in the models with PWV as the dependent variable.

Results

The characteristics of the study population ($n = 1534$) are outlined in Table 1. The population consisted of 62.4 % women and had a mean age of 51.2 ± 10.5 years. With regards to BP, 41.6 % were hypertensive (SBP and/or DBP $\geq 140/90$ mmHg). The mean FEV₁ and FVC were 2.22 ± 0.70 and 2.62 ± 0.81 L, respectively. The mean CRP level was 3.17 mg/L, exceeding the 3.0 mg/L cut-point for a high risk of future CV events [21]. A total of 54.4 % of the participants reported previous or current use of tobacco products. A very low percentage (0.78 %) of the population indicated use of anti-hypertensive medication.

Figure 1 illustrates the single regression analyses between inflammation (IL-6), cardiovascular function (SBP and PWV) and lung function (FEV₁). We found negative associations between FEV₁ and IL-6 ($r = -0.27$, $p < 0.001$) as well as FEV₁ and CRP ($r = -0.23$, $p < 0.001$; not shown). The correlations between SBP and IL-6 ($r = 0.11$, $p < 0.001$) and PWV and IL-6 ($r = 0.06$, $p = 0.030$) were weak, although significant. Similar results were found with CRP (not shown). We also found a negative association between SBP and FEV₁ ($r = -0.10$, $p < 0.001$), but no association between PWV and FEV₁ ($r = 0.02$, $p = 0.523$). We repeated these analyses and adjusted for age, sex, height, physical activity, tobacco use and GGT (Table 2). We found a significant negative association between FEV₁ and IL-6 ($r = -0.19$, $p < 0.001$) as well as between FEV₁ and CRP ($r = -0.19$, $p < 0.001$). FVC also associated negatively with IL-6 ($r = -0.17$, $p < 0.001$) and with CRP ($r = -0.16$, $p < 0.001$). None of the cardiovascular variables (SBP, DBP and PWV) associated with either IL-6 or CRP after adjustments. Furthermore, we found negative associations between FEV₁ and PWV ($r = -0.06$, $p < 0.001$; not shown) and between FVC and PWV ($r = -0.05$, $p < 0.001$; not shown). Neither SBP nor DBP was associated with either FEV₁ or FVC.

Finally, we performed forward stepwise multiple regression analyses (Table 3) with IL-6 (log) or CRP (log) as dependent variable. We found an independent negative association between IL-6 and FEV₁ in Model 1 ($p < 0.001$) as well as between IL-6 and FVC in Model 2 ($p < 0.001$). Similar results were found between CRP and FEV₁ in Model 1 ($p < 0.001$) and FVC in Model 2 ($p < 0.001$).

In further forward stepwise multiple regression analyses, we included SBP or PWV as dependent variable with either IL-6 or a marker of lung function as the main independent variable. SBP was not significantly associated with IL-6 or either of the lung function markers in any of the models. In the models with PWV as the dependent variable, there was also no significant association between PWV and IL-6 (Model 3), but PWV was significantly associated with FEV₁ in Model 4 ($p = 0.037$), and with borderline significance with FVC (Model 5) ($p = 0.078$). When entering both IL-6 and a marker of lung function into Models 6 or 7, PWV was not associated with IL-6, but with both FEV₁ ($p = 0.045$) and FVC ($p = 0.045$). We repeated all models with SBP and PWV as dependent variables, replacing IL-6 with CRP and found similar results.

Discussion

We investigated the possible role of systemic inflammation as the mediator between lung function and arterial stiffness. We found an independent inverse association between lung

function and inflammation. In accordance with recent studies [22–24], our study indicates that higher levels of both CRP and IL-6 are associated with reduced pulmonary function (FEV₁ and FVC), independent of potential confounders. This supports the notion that inflammatory processes are involved in the pathogenesis of various pulmonary diseases [25]. The comorbidities associated with reduced lung function, such as CVD, are reported to be due to a systemic spill-over of inflammation occurring within the lungs [8]; however more evidence is needed to support this hypothesis.

There is a limited amount of information regarding the association between lung function and arterial stiffness; however our finding, namely an inverse association between lung function and arterial stiffness, supports existing findings that also show this association independent of confounding factors [26–28]. Several possible causes for this association exist, such as genetic susceptibility or inherited factors that may both influence lung function as well as arterial stiffness [29]. Alternatively, lung function and arterial stiffness could both be influenced by external factors such as metabolic disruption, environmental factors such as smoking or inflammatory processes [27]. Duprez et al. [22], recently stated that inflammation acts as a parallel physiological pathway for a simultaneous loss of elasticity in both the connective tissue of the alveoli and arterial wall which may lead to reduced lung function and arterial stiffness.

Based on these associations between lung function and inflammation as well as between lung function and arterial stiffness, also observed in our study, it is reasonable to assume that lung function and arterial stiffness could be linked by inflammation as the mediator. However, we found no association between markers of inflammation and BP or measures for arterial stiffness in our black population. These results are surprising especially when viewed in light of findings from another South African study that showed that Africans have significantly higher levels of CRP when compared to white counterparts [30]. It remains controversial whether CRP is merely a marker [31, 32] or indeed causal [33–35] in the development of CVD, with strong evidence supporting either statement. Notwithstanding this controversy, CRP remains an important marker to include when investigating CVD development. Various studies report an association between CRP and PWV [10, 36–38] but in some, these associations disappear after adjustment for conventional risk factors for CVD [37, 39, 40]. There are also studies that have reported no association between CRP and PWV [41, 42], and it is possible that CRP does not have a causal role in the development of arterial stiffness but may only act as a marker of vascular damage [43]. Contradictory to previous studies which showed a positive association between IL-6

Fig. 1 Single regression analyses between (a) inflammation and lung function, (b) cardiovascular function and inflammation and (c) cardiovascular function and lung function. *FEV₁* forced expiratory volume in 1 s, *FVC* forced vital capacity

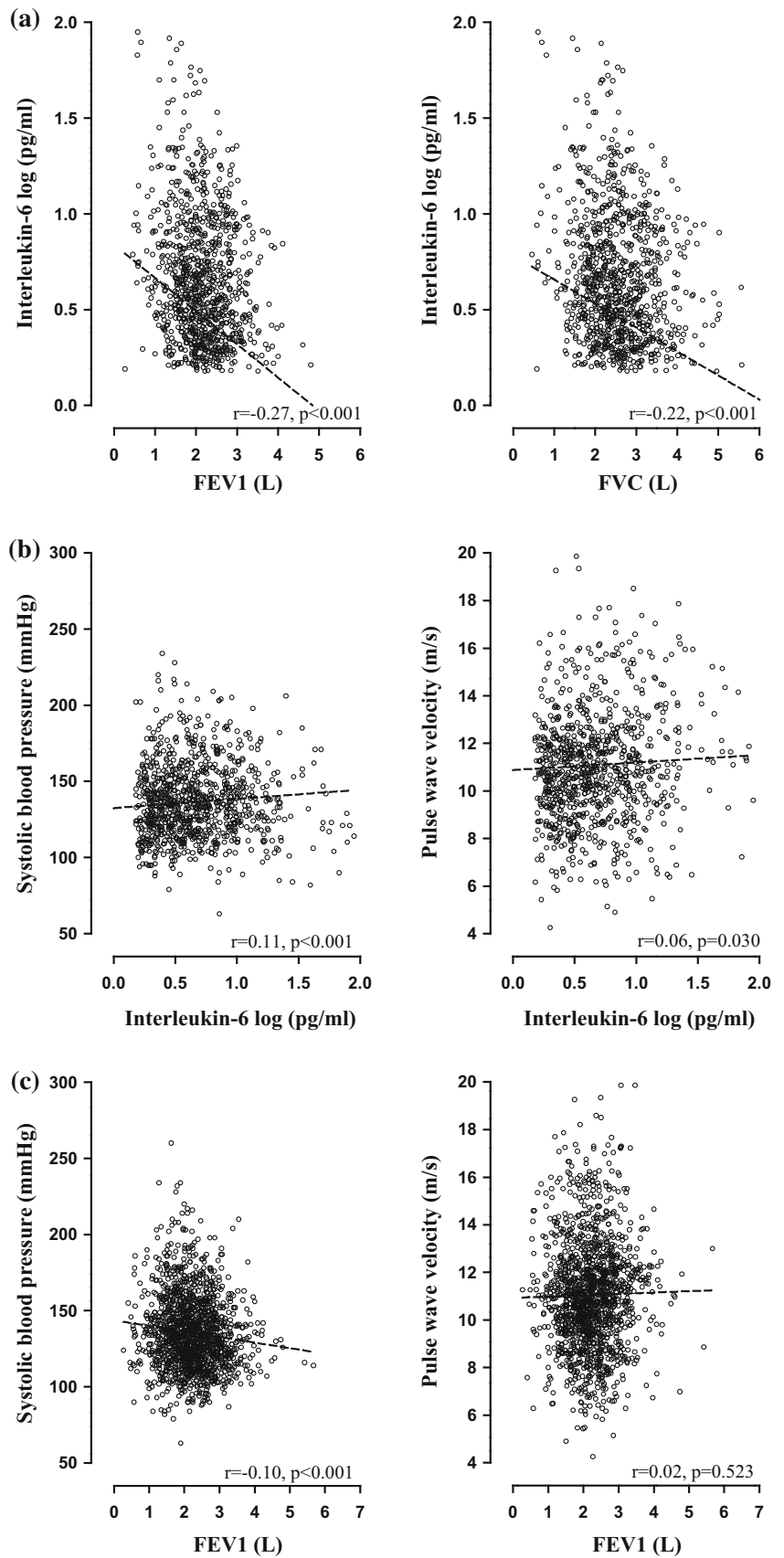


Table 2 Partial correlations of inflammatory markers with lung function and cardiovascular measures

	Interleukin-6 (pg/mL)		C-reactive protein (mg/L)	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Lung function markers				
FEV ₁ (L)	−0.19	<0.001	−0.19	<0.001
FVC (L)	−0.17	<0.001	−0.16	<0.001
Cardiovascular variables				
Systolic blood pressure (mmHg)	−0.01	0.925	−0.01	0.778
Diastolic blood pressure (mmHg)	−0.01	0.994	−0.01	0.771
Pulse wave velocity (m/s)	−0.04	0.127	−0.04	0.159

Adjusted for age, sex, height, physical activity, tobacco use and GGT

FEV₁ forced expiratory volume in 1 s, FVC forced vital capacity

Table 3 Forward stepwise multiple regression analyses with IL-6 (log), CRP (log), systolic blood pressure and pulse wave velocity, respectively, as dependent variable and lung function as well as inflammatory markers as independent variables

Independent variable(s)	IL-6 (pg/mL)		CRP (mg/L)	
	β (SE)	<i>p</i> value	β (SE)	<i>p</i> value
Model 1	$R^2 = 0.18$		$R^2 = 0.17$	
FEV ₁ (L)	−0.20 (0.03)	<0.001	−0.21 (0.03)	<0.001
Model 2	$R^2 = 0.17$		$R^2 = 0.16$	
FVC (L)	−0.18 (0.03)	<0.001	−0.19 (0.03)	<0.001
	Systolic blood pressure (mmHg)		Pulse wave velocity (m/s)	
	β (SE)	<i>p</i> value	β (SE)	<i>p</i> value
Model 3	$R^2 = 0.16$		$R^2 = 0.22$	
IL-6 (pg/mL)	−	−	−	−
Model 4	$R^2 = 0.16$		$R^2 = 0.23$	
FEV ₁ (L)	−	−	−0.06 (0.03)	0.037
Model 5	$R^2 = 0.16$		$R^2 = 0.23$	
FVC (L)	−	−	−0.05 (0.03)	0.078
Model 6	$R^2 = 0.16$		$R^2 = 0.23$	
FEV ₁ (L)	−	−	−0.06 (0.03)	0.045
IL-6 (pg/mL)	−	−	−	−
Model 7	$R^2 = 0.16$		$R^2 = 0.23$	
FVC (L)	−	−	−0.05 (0.03)	0.045
IL-6 (pg/mL)	−	−	−	−

Regression coefficients shown are standardized beta coefficients. R^2 values represent adjusted R^2 values of the whole model

Covariates included in all models: age, sex, height, physical activity, systolic blood pressure, tobacco use, GGT (log), HbA1c (log), TC and anti-hypertensive medication usage. Heart rate and mean arterial pressure included as additional covariates in models with pulse wave velocity as dependent variable. *p* values ≤ 0.05 regarded as significant

SE standard error

and PWV [11, 44], this association was not evident in our population. A recent study also reported no association between IL-6, CRP and small or large artery elasticity in women, while in men IL-6 and CRP only associated with small artery elasticity [22]. The lack of association between inflammation, BP and arterial stiffness in our study may be due to the variety of conventional and behavioural risk

factors for CVD that have been identified in Africans and may have a confounding effect on this association. These factors include insufficient physical activity [45], dietary factors [46], smoking [47] and alcohol abuse [48], all of whom are known to play a role in arterial stiffening [49–52]. Furthermore, other inflammation-sensitive plasma proteins may be more sensitive markers of inflammation

than those used in our study and could have a greater contribution to arterial stiffening and CV risk [4].

In a population already afflicted by a significant burden of hypertension [16, 48] and a low percentage of anti-hypertensive medication usage—which is indicative of poor diagnosis and control—it is important to investigate and understand all of the factors that may lead to the development of CVD. Arterial stiffness is one of the risk factors of CVD and an independent predictor of cardiovascular risk [53–55]. Several studies have also reported that arterial stiffness is more common in those of African ancestry when compared to those of European descent [56–59]. This may have an important impact in South Africa as the country is undergoing rapid industrialisation and occupational exposures are contributing substantially to the increase in respiratory and CVD [60]. Occupational health and hygiene control as well as the regulation of the mining industry have greatly improved; however, many miners that relocated to their village of origin have had significant morbidity from respiratory disorders such as silicosis [61] and tuberculosis [62]. Furthermore, with arterial stiffness already being elevated even in young Africans [63], the association between lung function and arterial stiffness found in our study warrants the consideration of lung function in global risk factor evaluation for CVD. However, the lack of association between arterial stiffness and markers of inflammation in this study population suggests that mechanisms other than inflammation are at play.

Some limitations of our study are that we included a large proportion of unemployed individuals with sampling being done in poverty-stricken communities. The participants were also selected from mainly Setswana-speaking rural and urban areas and results may therefore not be generalized to other populations in South Africa. Due to the cross-sectional design, cause and effect could not be inferred. Furthermore, PWV was assessed in an upper-limb muscular artery segment over the carotid-radialis and not the large conduit vessels (carotid-femoral PWV) which is the gold standard for assessing aortic stiffness. However, arterial stiffness of the peripheral arteries may be more important in black than white populations as CV risk factor [63] and was also found to predict CV events independent of age [64]. Strengths of our study include a large population-based study with several measures of lung function, inflammation and arterial stiffness in Africans for which limited information is available.

Conclusion

Reduced lung function was associated with increased inflammation and arterial stiffness, but we found no association between inflammation and arterial stiffness in this

black population. Our findings suggest that inflammation may not be mediating the link between lung function and arterial stiffness, and that inflammation may be involved in other pathophysiological mechanisms relating to CVD development.

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Compliance with Ethical Standards

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Conflict of interest All authors have declared no conflict of interest.

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