Soluble Urokinase Plasminogen Activator Receptor: Exploring its potential as a marker of cardiovascular disease development in black South Africans of the PURE study

S Botha

BSc Hons, MSc (Physiology)

20695241

Thesis submitted in fulfilment of the requirements for the degree *Philosophiae Doctor* in Physiology at the Potchefstroom Campus of the North-West University

Promoter: Dr CMT Fourie

Co-promoters: Prof AE Schutte

Prof R Schutte

April 2015

It all starts here ™

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

ACKNOWLEDGEMENTS

I would like to extend my gratitude to the following people who contributed to make this study possible:

- To my promoter and co-promoters for whom I have the utmost respect and endless appreciation as they have shaped me as a scientific researcher:
  - Dr. Carla Fourie, who has been my promoter since the beginning of my post-graduate studies, for her exceptional mentorship, input and commitment in this study. She believed in me and I will always be grateful for the unparalleled role that her guidance and support, on all levels, has played in my career.
  - Prof. Alta Schutte, my co-promoter, for her outstanding scientific input and guidance in this study. She is an example of what it means to be the best you can be and she will always remain an incredible inspiration to me.
  - Prof. Rudolph Schutte, my co-promoter, whose extraordinary insight and advice in this study have encouraged me to always aim towards high quality work. In sharing his statistical knowledge, he has taught me the value of science.
- To all the participants, researchers, field workers and supporting staff of the PURE study.
- To the National Research Foundation (DAAD-NRF) for the financial support for this study.
- There are no words to describe my gratitude towards my parents, grandparents and family for their infinite and incomparable encouragement, prayers, motivation and unconditional love during this study.
- To my sister for her immense inspiration, understanding and never-ending love. I dedicate this thesis to you!
- Thank you to my closest friends for their moral support, for being there and for standing by me.
- A special thanks to God for giving me the opportunity, talent, determination and endurance to complete my studies and for giving me the will to make a difference. I know that “I can do all things through Christ which strengtheneth me” (Philippians 4:13).

TABLE OF CONTENTS

Acknowledgements ..... **i**
Preface iv
Summary v
Opsomming ix
Affirmation by the authors xiii
List of tables xv
List of figures xvi
List of abbreviations xix

CHAPTER 1: INTRODUCTION AND LITERATURE OVERVIEW 1
1. General introduction 2
2. Literature overview 4
   2.1. The South African population 4
   2.2. The uPA-uPAR system and suPAR 7
   2.3. SuPAR as a marker of CVD 9
      2.3.1. SuPAR and modifiable risk factors for CVD 10
      2.3.2. SuPAR, the inflammatory process and CVD 15
      2.3.3. SuPAR as prognostic marker of CVD and mortality 21
   2.4. Summary 22
3. Motivation and problem statement 24
   Motivation, aim and hypothesis of each manuscript 24
4. References 27

CHAPTER 2: STUDY DESIGN AND METHODOLOGY 52
1. Study design, participants and experimental protocol 53
2. Questionnaires 56
3. Mortality outcome assessment 57
4. Anthropometric measurements 57
5. Cardiovascular measurements 58
6. Blood sampling and biochemical analyses 58
7. Statistical analyses 60
8. References 61

CHAPTER 3: Associations of suPAR with lifestyle and cardiometabolic risk factors **... 63**

CHAPTER 4: SuPAR and hypertension among black South Africans after 5 years**..... 84**

CHAPTER 5: SuPAR as a prognostic marker of all-cause and cardiovascular mortality in a black population **..... 107**

CHAPTER 6: CONCLUDING REMARKS AND FINDINGS 128

1. Introduction 129
2. Summary of main findings 129
3. Discussion and comparison of main findings to the literature 132
4. Chance and confounding 136
5. Recommendations 137
6. Final conclusions and perspectives 139
7. References 140

PREFACE

This thesis is presented in article-format and consists of three published and 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 overview of the literature which supports the focused literature backgrounds presented in each of the manuscripts. The motivation, aim and hypotheses are also included in this chapter.
- **Chapter 2** elaborates in detail on the PURE study protocol, methods of data collection and statistical analyses that were performed.
- In **Chapter 3**, the first manuscript describes the cross-sectional relationship of an unhealthy lifestyle and cardiometabolic risk profile with suPAR. These results were published in the *European Journal of Clinical Investigation*, 2014.
- **Chapter 4** explores the role of suPAR in hypertension development over five years. This manuscript has been accepted for publication by *Hypertension Research*, 2015.
- In **Chapter 5**, the third manuscript investigates the prognostic value of suPAR in all-cause and cardiovascular mortality. These results were published in the *International Journal of Cardiology*, 2015.
- In the final chapter, **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 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 xiii-xiv).

The relevant references are provided at the end of each chapter. Each manuscript was prepared according to the instructions of the individual journals to authors (which was summarised after each manuscript). In order to ensure uniformity throughout the thesis, the Vancouver reference style was used throughout.

SUMMARY

Motivation

In South Africa, various transitional changes parallel detrimental modifications in lifestyle behaviour of especially the lower socio-economic communities. We are currently double-burdened by a high prevalence of communicable and non-communicable diseases such as diabetes, chronic respiratory and cardiovascular diseases, which is accompanied by a high cardiovascular mortality rate. Healthcare and treatment resources are limited and low-cost intervention strategies to lower this burden are urgently needed.

Unhealthy lifestyle behaviours, such as excessive alcohol consumption and tobacco use, are known to augment inflammation as reflected by inflammatory markers such as C-reactive protein and interleukin-6, which are well-known risk factors for cardiovascular disease and mortality.

Several studies showed the prognostic value of soluble urokinase plasminogen activator receptor (suPAR) in advanced disease states and that suPAR associates with different types of cancers, infectious diseases, diabetes, coronary artery disease and all-cause mortality. Since the discovery of suPAR in 1991, the role of this less known inflammatory marker in various diseases has been under debate. It was further reported that black individuals have higher suPAR levels than whites.

However, whether an unhealthy lifestyle and cardiometabolic risk factors are related to suPAR, whether suPAR plays a role in the development of cardiovascular disease such as hypertension, and whether suPAR could predict all-cause and cardiovascular mortality, especially among the understudied black South African population, remain to be established.

Aim

The central aim of this thesis was to determine if suPAR associates with cardiovascular disease development in a black South African population. We therefore explored whether suPAR relates to lifestyle and cardiometabolic risk factors, associates with the development of hypertension and has prognostic value for cardiovascular and all-cause mortality over five years.

Methodology

This five-year prospective sub-study, which is embedded in the international Prospective Urban and Rural Epidemiology study, included 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 2 010 men and women from urban and rural areas were examined. A total of 1 292 participants returned for examination and were followed-up for the first time in 2010. Of these participants, 214 were newly identified as being infected with the human immunodeficiency virus (HIV), while 233 died during the five year period.

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

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, while dependent *t*-tests and McNemar tests were used for analysis of longitudinal data within individual groups. 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, $p \leq 0.05$ were used to indicate statistical significance.

Results and conclusions of each manuscript

Three manuscripts were written in order to achieve the aim of this thesis. In the first manuscript we explored the cross-sectional relationships of suPAR with lifestyle and cardiometabolic risk factors in a black South African population. We showed that suPAR was independently associated with lifestyle behaviours, including alcohol consumption, as indicated by gamma-glutamyltransferase levels (β=0.24; p<0.001), tobacco use (β=0.13; p<0.001) and unemployment (β=0.07; p=0.039), despite no direct links with cardiometabolic factors such as blood pressure, dyslipidaemia, glycaemia or adiposity. These findings emphasise the important need to address lifestyle behaviours in order to limit the detrimental effect of modifiable risk factors on the health and mortality rate of this population.

Secondly, we determined whether suPAR was associated with the development of hypertension over five years. We found that suPAR was higher and increased more prominently (14.2% vs. 6.94%; p=0.007) in participants that developed hypertension than in those that remained normotensive. Change in systolic blood pressure was independently associated with baseline suPAR (β=0.14; p=0.043), while becoming hypertensive was associated with an increase in suPAR (odds ratio=1.41; p=0.015). Whether inflammation leads to the development of hypertension or *vice versa*, remains unclear. Our findings emphasise the need to acknowledge the role of inflammation in hypertension and may permit further investigation of the use of suPAR as a potential marker for early risk identification and intervention.

The third manuscript investigated the prognostic value of suPAR, compared to other inflammatory markers C-reactive protein and interleukin-6, in all-cause and cardiovascular mortality. We showed for the first time in a black population that suPAR predicted both all-cause (hazard ratio=1.27; p=0.003) and cardiovascular mortality (hazard ratio=1.40; p=0.026), independent of interleukin-6. Future research is needed to clarify the mechanisms behind the association of suPAR with cardiovascular mortality and to explore the possibility of a suPAR cut-off value for early identification of those with increased risk for cardiovascular morbidity and mortality in this population.

General conclusion

In this thesis we showed for the first time that suPAR has potential as a marker of cardiovascular disease development in black South Africans. SuPAR associated with hypertension and independently predicted all-cause and cardiovascular mortality over five years. Our findings, that suPAR is independently associated with adverse health behaviours such as alcohol and tobacco use, lend support for the use of suPAR as a novel approach for early risk identification and intervention strategies, which may be effective in combatting the high cardiovascular disease burden among the black South African community.

Keywords: blacks; epidemiology; hypertension; inflammation; lifestyle; mortality; prognostic

AFRIKAANSE TITEL: Oplosbare urokinase plasminogeen aktiveerder reseptor (suPAR): Ondersoek van sy potensiaal as ’n merker van kardiovaskulêre siekte ontwikkeling in swart Suid-Afrikaners van die PURE studie

OPSOMMING

Motivering

In Suid-Afrika gaan verskeie transformasieveranderinge gepaard met nadelige modifikasies in leefstyl gedrag van spesifiek die laer sosio-ekonomiese gemeenskappe. Ons word tans dubbeld belas deur ’n hoë voorkoms van oordraagbare en nie-oordraagbare siektes soos diabetes, chroniese respiratoriese en kardiovaskulêre siektes, wat gepaard gaan met ’n hoë kardiovaskulêre mortaliteitsyfer. Gesondheidsorg en behandelingshulpbronne is beperk en lae-koste intervensie strategieë, om hierdie las te verlaag, word dringend benodig.

Ongesonde leefstyl gedrag, soos oormatige alkoholgebruik en die gebruik van tabak, is bekend om inflammasie te vermeerder, soos uitgewys deur inflammatoriese merkers soos C-reaktiewe proteïen en interleukin-6, wat welbekende risikofaktore vir kardiovaskulêre siekte en mortaliteit is.

Verskeie studies het die prognostiese waarde van oplosbare urokinase plasminogeen aktiveerder reseptor (suPAR) in gevorderde siektetoestande aangetoon en dat suPAR assosieer met verskillende tipes kankers, aansteeklike siektes, diabetes, koronêre arteriële siekte en alle-oorsaak mortaliteit. Sedert die ontdekking van suPAR in 1991, is die rol van hierdie minder bekende inflammatoriese merker in verskeie siektes onder debat. Dit is verder berig dat swart individue hoër suPAR vlakke as blankes het.

Maar, of 'n ongesonde leefstyl en kardiometaboliese risikofaktore verwant is aan suPAR, of suPAR 'n rol speel in die ontwikkeling van kardiovaskulêre siekte soos hipertensie, en of suPAR alle-oorsaak en kardiovaskulêre mortaliteit kan voorspel, veral onder die onder-nagevorste swart Suid-Afrikaanse bevolking, moet steeds bepaal word.

Doelstelling

Die sentrale doel van hierdie tesis was om te bepaal of suPAR assosieer met kardiovaskulêre siekte-ontwikkeling in 'n swart Suid-Afrikaanse bevolking. Ons het daarom ondersoek of suPAR verband hou met leefstyl en kardiometaboliese risikofaktore, assosieer met die ontwikkeling van hipertensie; en prognostiese waarde vir kardiovaskulêre en alle-oorsaak mortaliteit oor vyf jaar het.

Metodologie

Hierdie vyf jaar prospektiewe sub-studie, wat ingesluit is in die internasionale Prospektiewe Stedelike en Landelike Epidemiologie studie, het swart Suid-Afrikaanse vrywilligers van ouderdomme ouer as 35 jaar van die Noordwes-provinsie, Suid-Afrika ingesluit. Basislyn data-insameling het in 2005 plaasgevind waartydens 2 010 mans en vroue van stedelike en landelike gebiede ondersoek is. 'n Totaal van 1 292 deelnemers het teruggekeer vir ondersoek en is vir die eerste keer in 2010 opgevolg. Van hierdie deelnemers, is 214 nuut geïdentifiseer as geïnfekteer met die menslike immuniteitsgebreksvirus (MIV), terwyl 233 gedurende die vyf jaar tydperk gesterf het.

Gestandaardiseerde metodes is gebruik om al die data op te neem wat gesondheidsvraelyste (lewenstyl faktore, medikasie-gebruik, siekte-status en geskiedenis, mortaliteit uitkoms), kardiovaskulêre en antropometriese metings, sowel as biochemiese analises van inflammatoriese merkers (suPAR, C-reaktiewe proteïen, interleukin -6), MIV status en relevante metaboliese merkers ingesluit het.

Ter voorbereiding vir statistiese ontledings, is nie-Gaussiese veranderlikes logaritmies getransformeer. Ons het gemiddelde en proporsies met onafhanklike *t*-toetse, analise van variansie, analise van kovariansie (vir aanpassings) en Chi-kwadraat toetse gebruik, terwyl afhanklike *t*-toetse en McNemar toetse vir ontleding van longitudinale data binne individuele groepe, gebruik is. Ons het verhoudings tussen veranderlikes met Pearson se korrelasiekoëffisiënte bepaal. Onafhanklike verhoudings is bepaal met logistieke regressie, voorwaarts stapsgewyse meervoudige regressie en proporsionele Cox-regressie analises. Mortaliteitsyfers is bereken met behulp van Kaplan-Meier oorlewingsfunksie beramings en log-rang toetse. In alle gevalle, is $p \leq 0.05$ gebruik om statistiese betekenisvolheid aan te dui.

Resultate en gevolgtrekkings van elke manuskrip

Drie manuskripte is geskryf om sodoende die doel van hierdie tesis te bereik. In die eerste manuskrip het ons die dwarsdeursnit-verhoudings van suPAR met lewenstyl en kardiometaboliese risikofaktore in 'n swart Suid-Afrikaanse bevolking ondersoek. Ons het gewys dat suPAR onafhanklik assosieer met lewenstyl gedrag , insluitende alkohol verbruik, soos aangedui deur gamma-glutamiel transferase vlakke (β=0.24; p<0.001), tabakgebruik (β=0.13; p<0.001) en werkloosheid (β=0.07; p=0,039), ten spyte van geen direkte verbande met kardiometaboliese faktore soos bloeddruk, dislipidemie, glisemie of adipositeit. Hierdie bevindinge beklemtoon die belangrike behoefte om lewenstyl-gedrag aan te spreek, ten einde die nadelige effek van veranderbare risikofaktore op die gesondheid en mortaliteitsyfer van hierdie bevolking te beperk.

Tweedens het ons vasgestel of suPAR met die ontwikkeling van hipertensie oor vyf jaar geassosieer het. Ons het gevind dat suPAR hoër was en meer prominent toegeneem het (14.2% vs. 6,94%; p=0.007) in deelnemers wat hipertensie ontwikkel het as in diegene wat normotensief gebly het. Verandering in sistoliese bloeddruk was onafhanklik geassosieer met basislyn suPAR (β=0,14; p=0.043), terwyl om hipertensief te word, geassosieer was met 'n toename in suPAR (kans ratio=1.41; p=0,015). Of inflammasie lei tot die ontwikkeling van hipertensie of *vice versa*, is steeds onduidelik. Ons bevindinge beklemtoon die behoefte om die rol van inflammasie in hipertensie te erken en mag verdere ondersoek van die gebruik van suPAR as 'n potensiële merker vir vroeë identifikasie en intervensie toelaat.

Die derde manuskrip het die prognostiese waarde van suPAR, in vergelyking met ander inflammatoriese merkers C-reaktiewe proteïen en interleukin-6, in alle-oorsaak en kardiovaskulêre mortaliteit ondersoek. Ons het vir die eerste keer in 'n swart bevolking gewys dat suPAR beide alle-oorsaak (gevaar ratio=1.27; p=0,003) en kardiovaskulêre mortaliteit (gevaar ratio=1.40; p=0.026) voorspel, onafhanklik van interleukin-6. Toekomstige navorsing is nodig om die meganismes agter die assosiasie van suPAR met kardiovaskulêre mortaliteit te verklaar en om die moontlikheid van 'n suPAR afsnywaarde vir vroeë identifisering van diegene met 'n verhoogde risiko vir kardiovaskulêre morbiditeit en mortaliteit in hierdie bevolking te ondersoek.

Algemene gevolgtrekking

In hierdie tesis het ons vir die eerste keer gewys dat suPAR potensiaal as 'n merker van kardiovaskulêre siekte-ontwikkeling in swart Suid-Afrikaners het. SuPAR het geassosieer met hipertensie en het onafhanklik alle-oorsaak en kardiovaskulêre mortaliteit oor vyf jaar voorspel. Ons bevindinge, dat suPAR onafhanklik geassosieer is met skadelike gesondheid-gedrag soos alkohol- en tabakverbruik, verleen ondersteuning aan die gebruik van suPAR as 'n nuwe benadering vir vroeë risiko-identifiserings- en intervensiestrategieë, wat effektief mag wees om die hoë kardiovaskulêre siekte las onder die swart Suid-Afrikaanse gemeenskap te bestry.

Sleutelwoorde: epidemiologie; hipertensie; inflammasie; leefstyl; mortaliteit; prognosties; swartes

AFFIRMATION BY THE AUTHORS

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

Miss. S Botha

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

Dr. CMT Fourie (promoter), Prof. AE Schutte and Prof. R Schutte (co-promoters)

Supervised the design, planning and writing of the thesis, as well as data collection, provided intellectual input on statistical analyses and writing of the manuscripts presented in Chapters 3, 4 and 5.

Prof. A Kruger

In her capacity as project leader of the South African leg of the PURE study, provided intellectual input in the manuscript presented in Chapter 3.

Prof. J Eugen-Olsen

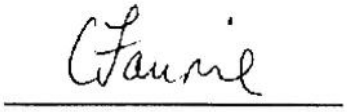
Performed biochemical analysis of serum suPAR and provided supportive and intellectual input in the manuscripts presented in Chapters 4 and 5.

Dr. R Pretorius

Provided financial support for the biochemical analysis of serum interleukin-6 and intellectual input in the manuscript presented in Chapter 5.

The following is a statement of the co-authors 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 Shani Botha.

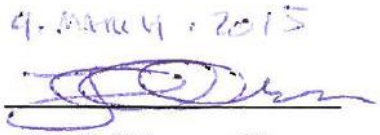


Dr. CMT Fourie

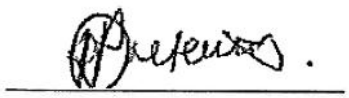
Prof. AE Schutte

Prof. R Schutte

Prof. A Kruger



Prof. J Eugen-Olsen



Dr. R Pretorius

LIST OF TABLES

CHAPTER 3 **p. 63**

Table 1 - Characteristics of a black South African study population, stratified by tertiles of suPAR.

Table 2 - Independent associations with suPAR and CRP.

Table S1 - Blood pressure and the lipid profile of a black South African study population, adjusted for medication use.

CHAPTER 4 **p. 84**

Table 1 - Characteristics of black South African participants showing differences in baseline (2005) and follow-up (2010) of participants that remained normotensive (*n* = 238) and those that developed hypertension (*n* = 191) after 5 years, respectively.

Table 2 - Change in characteristics of black South African participants over five years.

Table 3 - Independent associations with percentage change in systolic blood pressure.

Table 4 - Logistic regression with hypertension status as dependent variable.

Table S1 - Relationship between suPAR and clinical characteristics in the total normotensive group at baseline (*n* = 429).

CHAPTER 5 **p. 107**

Table 1 - Baseline characteristics of black South African participants.

Table 2 - Cox proportional hazard ratios of suPAR, CRP and IL-6 with all-cause and cardiovascular mortality.

Table S1 - Cox proportional hazard ratios of suPAR, CRP and IL-6 with all-cause and cardiovascular mortality in HIV uninfected participants.

LIST OF FIGURES

CHAPTER 1 **p. 1**

Figure 1 - Global prevalence of raised blood pressure for 2008 in ages higher than 25 years. Age standardised and for both sexes.

Figure 2 - The three-domain uPAR structure, the cleavage of the structure and the formation of suPAR.

Figure 3 - The average soluble urokinase plasminogen activator receptor (suPAR) levels in men and women according to the combined cardiovascular disease (CVD) and lifestyle model, with regard to smoking status, as proposed by Haupt and colleagues.

Figure 4 - A summary of risk factors, including central obesity, predisposing to coronary heart disease and cardiovascular disease.

Figure 5 - The uPA-uPAR system and foam cell formation.

Figure 6 - The uPA-uPAR system and smooth muscle cells in vascular remodelling.

Figure 7 - The cellular composition of atherosclerotic plaque.

Figure 8 - Atherosclerotic lesion in a human artery.

Figure 9 - Summary of the role of risk factors in the development of cardiovascular disease with regard to the inflammatory response.

CHAPTER 2 **p. 52**

Figure 1 - Causal pathway of community influences on individual risk.

Figure 2 - Countries involved in the international PURE study, categorised by income.

Figure 3 - Areas involved in this sub-study from the North West province, South Africa.

Figure 4 - Data collection in the Ganyesa and Tlakgameng areas in the North West Province, South Africa.

CHAPTER 3 **p. 63**

Figure 1 - Pearson correlation between suPAR and C-reactive protein.

CHAPTER 4 **p. 84**

Figure 1 - Single linear regression analyses of the percentage change in systolic blood pressure and baseline soluble urokinase plasminogen activator receptor (suPAR) in participants that remained normotensive and those that developed hypertension over five years, respectively.

Figure S1 - Layout of the study population.

Figure S2 - Percentage change in systolic blood pressure, stratified by tertiles of baseline soluble urokinase plasminogen activator receptor (suPAR) in participants that developed hypertension and in those that remained normotensive over five years, respectively.

CHAPTER 5 **p. 107**

Figure 1 - Layout of the study population.

Figure 2 - Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of soluble urokinase plasminogen activator receptor (suPAR), C-reactive protein (CRP) and interleukin-6 (IL-6), respectively.

Figure 3 - Absolute five-year risk of cardiovascular mortality in relation to soluble urokinase plasminogen activator receptor (suPAR) at different levels of interleukin-6 (IL-6).

Figure S1 - Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of soluble urokinase plasminogen activator receptor (suPAR) in human immunodeficiency virus (HIV) infected and -uninfected groups, respectively.

Figure 1 - Single linear regression analyses of the percentage change in SBP and baseline suPAR (A) and the percentage change in SBP, stratified by tertiles of baseline suPAR (adjusted for age, gender and baseline SBP) (B), in participants who remained normotensive and developed hypertension over five years.

Figure 2 - A: Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of suPAR. B: Absolute 5-year risk of cardiovascular mortality in relation to suPAR at different levels of IL-6.

LIST OF ABBREVIATIONS

%SBP - Percentage change in systolic blood pressure

%suPAR - Percentage change in soluble urokinase plasminogen activator receptor

CRP - C-reactive protein

CVD - Cardiovascular disease

DBP - Diastolic blood pressure

ECM - Extracellular matrix

GGT - Gamma-glutamyltransferase

HDL - High-density lipoprotein cholesterol

HIV - Human immunodeficiency virus

IL-6 - Interleukin-6

LDL - Low-density lipoprotein cholesterol

MMP - Matrix metalloproteinase

NCD - Non-communicable disease

PURE - Prospective Urban and Rural Epidemiology

SBP - Systolic blood pressure

SMC - Smooth muscle cell

suPAR - Soluble urokinase plasminogen activator receptor

TNF-α - Tumour necrosis factor-α

uPA - Urokinase plasminogen activator

uPAR - Urokinase plasminogen activator receptor

CHAPTER 1

Introduction and Literature overview

1. GENERAL INTRODUCTION

The international Prospective Urban and Rural Epidemiology study, on which this study was based, was designed to examine the impact of societal influences on chronic non-communicable diseases (NCDs) in low-, middle-, and high-income countries.[1] According to the World Health Organization, 36 million (63%) of global deaths in 2008 were due to NCDs, including cardiovascular disease.[2] In the African region, it is projected that NCDs will be the cause of around 3.9 million deaths by 2020.[3] Unfortunately, South Africa currently suffers from the highest double disease burden globally, where the 5.6 million people that are living with the human immunodeficiency virus (HIV),[4] are paralleled by a hypertension prevalence of 10% for participants aged ≥15 years, 40% in adults aged ≥25 years[5] and 78% in people older than 50 years.[6]

The South African society is that of a multicultural kind, with a multitude of customs and traditions, all influencing the way people live and bring up their children. However, transition and urbanisation are rapidly changing human behaviour, traditional ways of life and value systems.[7] The high prevalence of destructive behaviour are impacting the health of many South Africans in a detrimental way, thereby increasing the already high burden on limited healthcare resources in the country and increasing the risk of developing cardiovascular disease (CVD) in the process. This is the founding reason for the urgent need to identify low-cost prognostic markers for the early detection of cardiovascular disease in order to combat the current high cardiovascular mortality rate.

Inflammation plays a pivotal role in the mechanisms involved in cardiovascular and metabolic disease development[8-10] and inflammation in general is a known risk factor for cardiovascular mortality.[11,12] Soluble urokinase plasminogen activator receptor (suPAR) is a relative novel, general marker of low-grade inflammation,[13,14] even in healthy subjects.[14] Over the past 25 years, many laboratories have done intensive research on the urokinase plasminogen activator receptor and its ligands, however, the importance of this system in various biological processes, and especially in CVDs, has been underscored. It is known that suPAR associates with sub-clinical organ damage[13] and plays a role in endothelial dysfunction and later occurring processes in the development of CVD, such as the presence of atherosclerotic plaques.[13,15-17] This marker further associates with a higher risk for new-onset cardiovascular disease in patients with chronic kidney disease[18] and predicts cardiovascular events and mortality,[14,16,19]

independent of other markers of sub-clinical organ damage and traditional risk factors.[13] Studies have shown how effective treatment of cancer[20] and infectious diseases[21,22] leads to a commensurate decrease in plasma suPAR concentrations, underlining the importance to explore the physiological role of suPAR in various processes and to determine the value thereof in the prognostic identification of high-risk patients and therapeutic interventions.

In this chapter, I will provide a broad overview of the literature, mainly focussing on the molecular background of suPAR, its role in the inflammatory process and hypertension development, the influence of modifiable risk factors, including lifestyle and cardio-metabolic risk factors, as well as on the role of suPAR in CVD and mortality risk, specifically within the context of black populations.

2. LITERATURE OVERVIEW

2.1. The South African population

The South African nation comprises African, European and Asian cultures,[7] with a population size of 54 million people,[23] that speak 11 official languages,[24] and with different distributions in socio-economic classes, urban and rural settings, gender and age groups.[25] South Africa consists of land with sufficient resources and therefore offers modern and westernised ways of life to its inhabitants.[7] It is a country in transition. Indeed, by 1994, more than half of the population was already urbanised.[24]

The World Health Organization has projected that the global disease burden, attributable to NCD in Africa, may increase by 27% over the next 10 years,[2] while over the next 20 years, the number of deaths attributable to hypertension may substantially exceed the number resulting from HIV and associated disease.[26] In November 2003, the South African government introduced antiretroviral treatment roll-out programmes, granting HIV infected people access to free treatment for the first time.[27] Even though these changes may have contributed to the latest increase in life expectancy, which is currently estimated at 59 years for males and 63 years for females,[23] the prevalence of both infectious and NCDs, including stroke, heart disease, diabetes and cancer, has unfortunately also risen.[28]

The epidemiological transition in South Africa has further caused African lifestyles to be replaced by westernised behaviour,[7,29] thereby changing traditional ways of life, values systems and dietary patterns. It was assumed that, albeit slowly, living conditions would be improving as access to water, sanitation and electricity increased, which is essential for good health.[25] However, other factors that play a fundamental role in health seem to be worsening. Socio-economic status and working conditions, especially in the mining industry, are deteriorating and unemployment is particularly high among Africans, the unskilled and the young.[25] Furthermore, less than third of the adult population currently has a matric or higher qualification.[25]

There is a notable escalation in behavioural risk factors (such as substance abuse and binge drinking) which, together with other underlying metabolic and physiological processes, impact the health of this population in a detrimental manner by increasing the CVD risk even more.[7,25,30] Annually, approximately 2.3 million people die from

alcohol abuse (3.8% of all deaths), 2.8 million from being overweight or obese, 2.6 million from having high cholesterol and almost 6 million from direct and second-hand tobacco use, while smoking causes 10% of CVDs.[2,31] In fact, research indicates that behavioural risk factors such as heavy drinking,[32] smoking[33] and chronic stress[34] may result in chronic inflammation, which in turn is implicated in the aetiology and pathogenesis of CVD. Further, the detrimental effect of alcohol were highlighted when health behaviour explained the excess burden of sub-clinical vascular disease in Africans.[35]

The detrimental impact of the transition, urbanisation and accompanying changes on the South African healthcare system may be most evident in the very high and ever-increasing prevalence of hypertension in South Africa[30,36,37] (Figure 1). A review, including 25 studies (during 1987-2004) from 10 sub-Saharan African countries, reported a hypertension prevalence ranging from 13% to 48%, indicating a higher prevalence in urban than in rural communities.[38] Moreover, results from the South African National Health and Nutrition Examination Survey showed that more than half of South Africans over 55 years had high systolic blood pressures,[39] while 33% of black South Africans from the Soweto township near Johannesburg displayed hypertension.[36]

Hypertension is more common in black than in white people,[40,41] despite the fact that black South Africans have a more favourable lipid profile,[30] which could possibly be linked to a genetic basis.[40] In Africans, hypertension seems to occur at a younger age, resulting in earlier damage to vital organs and adverse outcome.[42,43] Studies from as early as 1946,[44] 1958[45] and 1963[46] estimated that African men and women had a low rate of atherosclerotic heart disease, but a high hypertension rate. In these subjects, deaths related to hypertension were more than that related to vascular lesions, while notably, all of these trends were reversed for Asians and Coloureds.[46] Unfortunately, the picture of hypertension has not changed much since. According to Poulter *et al.*,[47] the prevalence of hypertension among sub-Sahara Africans were 16.2% (74.7 million) in 2008 and is projected to rise to 17.4% (125.5 million) in 2025. A recent systematic review of data from 33 surveys involving over 110 000 participants, provides an even worse picture with a pooled hypertension prevalence of 30% in sub-Saharan Africa.[48]

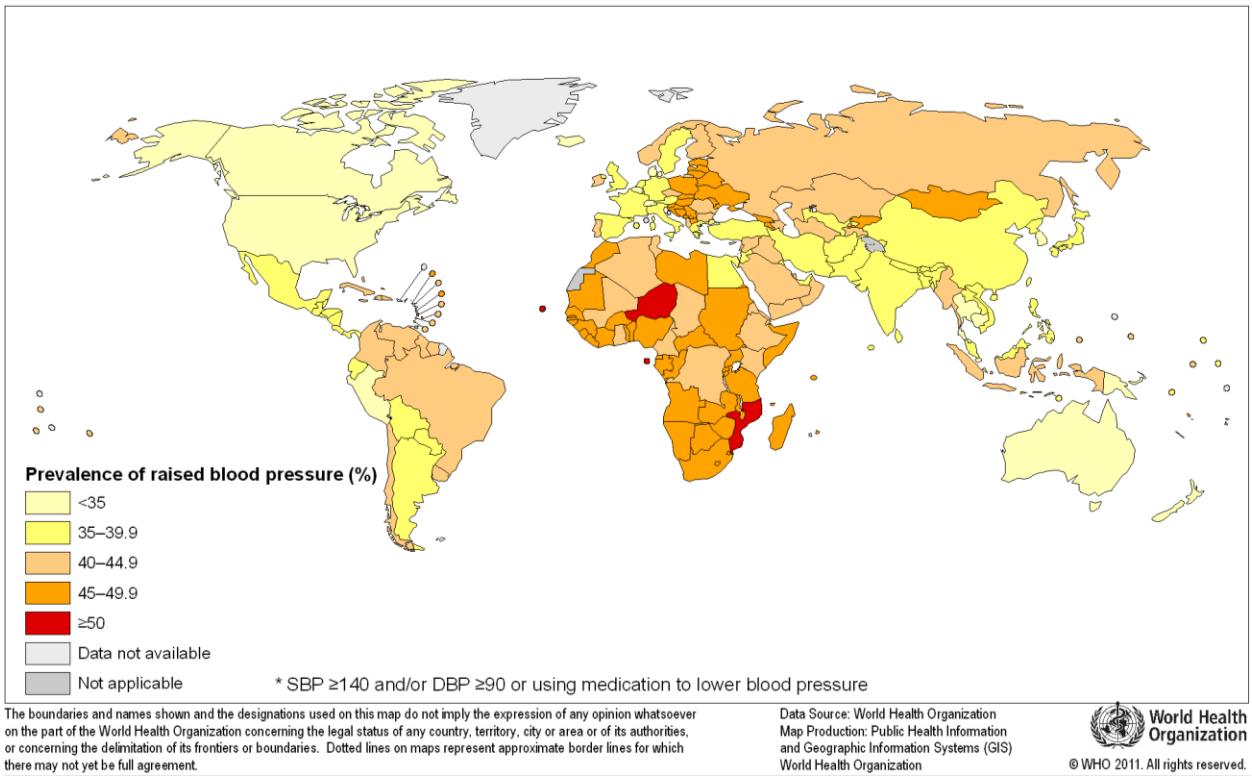


Figure 1 *Global prevalence of raised blood pressure for 2008 in ages higher than 25 years. Age standardised and for both sexes.*[49] *SBP, systolic blood pressure; DBP, diastolic blood pressure.*

According to the South African Declaration on the Prevention and Control of Non-Communicable Diseases, the Department of Health declared targets for reducing NCDs in South Africa by 2020, which included to "reduce prevalence of hypertension by 20% through lifestyle modification and medication", as well as to "increase the proportion of people receiving treatment for the control of hypertension, diabetes, and asthma by 30%".[50] However, despite the supposedly highly effective and cost-effective blood pressure lowering interventions in the country,[39,50] the prevalence of hypertension keeps rising, while the levels of knowledge about the condition does not.[50] This may contribute to the many undiagnosed, untreated or uncontrolled cases[30,48,50,51] and could lead to advanced forms of cardiovascular disease.[37]

These facts flag up a very clear message that programmes, adapted to the African context, should urgently be developed in order to evaluate the current size of the hypertension burden and to instigate preventive strategies to truncate the almost inevitable increase in hypertension among the vulnerable South African communities.[37,47,48] Interventions to prevent CVD should be targeted at Africans with a high CVD risk, including the unemployed, those with low education levels and those

living in rural areas.[29] Such strategies may include the development of rather helpful screening tools to assist in the early identification of processes that could lead to hypertension development.

2.2. The uPA-uPAR system and suPAR

Inflammation, which can be augmented by adverse lifestyle choices,[52,53] has previously received attention as a factor that mediates both the genesis and development of hypertension.[9,10,54,55] Within that context, inflammation is often seen as a non-specific phenomenon that involves an elevation of inflammatory marker levels including C-reactive protein (CRP), interleukin-6 (IL-6) and the less known marker soluble urokinase plasminogen activator receptor (suPAR).[8,9,56-58]

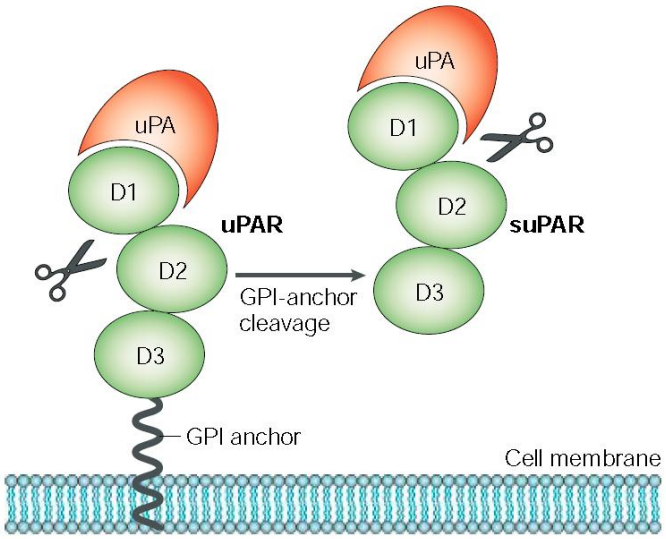


Figure 2 *The three-domain uPAR structure, the cleavage of the structure and the formation of suPAR.[59] D1-3, the three homologous domains; uPAR, urokinase plasminogen activator receptor; suPAR, soluble urokinase plasminogen activator receptor; GPI, glycosylphosphatidylinositol.*

Urokinase plasminogen activator receptor (uPAR), also referred to as CD87, was first cloned in 1990[60] and, together with the urokinase plasminogen activator (uPA) ligand and plasminogen activator inhibitors-1 and 2, forms part of the uPA-uPAR system.[19] UPA is an extracellular serine protease enzyme,[61,62] secreted as a 411 amino acids zymogen form (pro-uPA) and consists out of two-chains, held together by a single disulphide bond.[62] Once activated, it binds to its receptor with a high affinity,[63] thereby causing a conformational change of the uPAR.[63,64] As shown in Figure 2, uPAR is a

three domain membrane protein, of which uPAR(I) is the amino-terminal, uPAR(II) the kringle and uPAR(III) the carboxy-terminal domain, all bound by single disulphide bonds.[59] Intact uPAR is attached to the cell surface via a glycosylphosphatidylinositol anchor at the uPAR(III) domain.[59]

UPAR can be endocytosed rapidly and recycled to the cell surface,[65] but can also be hydrolysed by two different processes, called “shedding” or “cleavage”. During shedding, the whole uPAR protein is released from the cell surface. This occurs either by proteolytic cleavage of the polypeptide chain close to the glycosylphosphatidylinositol anchor,[66] or through the action of a phospholipase,[67] generating soluble uPAR in the process. SuPAR can be detected in both plasma and serum or in other organic fluids such as urine, saliva, semen and cerebrospinal fluid.[13,68,69] Another possibility is cleavage, where a proteolytic event occurs within the linker region between uPAR(I) and uPAR(II), releasing the uPAR(I) from the cell surface, while the uPAR(II-III) domains can either remain on the cell surface, or can be shed as described above.[66]

The sources of suPAR may differ between stable healthy individuals and those who are seriously ill. In order for suPAR to be released, cell-cell contact between for instance endothelial cells and monocytes is needed.[70] UPAR and suPAR production is thus stimulated by the presence of inflammation, which involves certain pro-inflammatory cytokines and growth factors such as interleukin-1 and vascular endothelial growth factor.[15,17] The uPA-uPAR system has been detected on a variety of cell types, including monocytes or macrophages, neutrophils, T-lymphocytes, keratinocytes, vascular endothelial and smooth muscle cells,[14,19,65,66,70-75] as well as in various tissues such as the lungs, kidneys, spleen, vessels, uterus, bladder, thymus, heart, liver and testis.[66] It is however striking how uPAR expression increases aberrantly in pathological conditions, such as cancer, inflammation, infection and vascular disease,[14,17,59,76-80] and how, in many cases, uPAR has been found in metastatic tumour cells and symptomatic atherosclerotic plaques.[20,72,73]

Although uPAR and suPAR have similar extracellular functions when it comes to processes such as cell migration and tissue remodelling,[81] suPAR is known to compete with uPAR for binding to extracellular ligands and to scavenge uPAR, thereby inhibiting its activity.[82] In 1991, Ploug *et al.*[83] first identified suPAR in the ascetic fluid of ovarian cancer patients. Since then, research suggested several suPAR concentrations under

various conditions. For example, a normal plasma suPAR level was said to range from 1-10 ng/mℓ.[17] Other studies cited a mean suPAR level of 2.74 ng/mℓ in 2 878 patients without and 2.96 ng/mℓ in 2 288 patients with carotid plaque,[79] while the suPAR concentration was 7.3 ng/mℓ in 902 patients with systemic inflammatory response syndrome.[78] However, as in the case of most research on this topic, these studies were performed in European population groups, while only few studies focused on suPAR in black South Africans. Blacks seem to have higher levels of inflammatory markers than whites.[84,85] In our group, Fourie et al.[86] found a mean suPAR level of 3.42 ng/mℓ in 154 black South Africans, uninfected by the human immunodeficiency virus. In addition, Schutte *et al.*[87] showed that black South Africans had higher suPAR levels than white South Africans (3.01 ng/mℓ, *n*=209 vs. 2.27 ng/mℓ, n=314) and, similarly, Kruger *et al.*[88] found higher suPAR levels in black South African men than in their white counterparts (2.95 ng/mℓ, *n*=117 vs. 2.02 ng/mℓ, n=116).

Compared to other inflammatory markers, suPAR is produced and released over a long time period and has a low clearance rate.[89] SuPAR is known to remain relatively stable in response to acute inflammatory stimuli, both in blood and urine[68] and both *in vivo*[90] and *in vitro*,[91] whereas the more familiar inflammatory marker, CRP, could increase up to 10 000-fold during a non-specific acute phase response.[92] Moreover, in contrast to alternative markers, such as IL-6 and tumour necrosis factor-α (TNF-α), which are subject to substantial circadian fluctuations,[69] suPAR has limited diurnal[93] and circadian changes and is not influenced by fasting or sampling schedules.[19] Plasma concentrations of this marker are not affected by storage or freeze-thaw procedures either,[91,94] making it an attractive clinical biomarker of low-grade inflammation,[68] especially when it comes to the prognosis of pathological conditions such as CVD.

2.3. suPAR as a marker of CVD

SuPAR is a novel, general marker of low-grade inflammation,[13,14] even in healthy subjects,[14] and links with subclinical organ damage[13] and new-onset CVD in chronic kidney disease patients.[18] To date, much research has been done on suPAR as a potential biomarker for intervention in various diseases.[61,62,95,96] However, in the process, many questions have arisen with regards to suPAR as inflammatory marker, its role in the development of hypertension and CVD, the influence of cardiovascular

risk factors on that process, as well as the predictive value of suPAR in cardiovascular mortality.

2.3.1. SuPAR and modifiable risk factors for CVD

The European Health Report has identified seven risk factors (tobacco and alcohol use, high overweight, physical inactivity, high cholesterol and low fruit and vegetable intake), which contribute to the major disease burden in Europe.[97] In South Africa, a comparative risk assessment has identified a cluster of risk and lifestyle factors which contribute considerably to the country's chronic disease burden, including cancers, respiratory and CVDs.[25] Such factors include a lack of physical activity, tobacco use and an unbalanced diet, which could lead to a lack of weight maintenance. This is supported by many studies which shows that black South Africans present multiple CVD risk factors.[98-102] Indeed, Africans use more tobacco products and have higher gamma-glutamyltransferase (GGT) levels,[87] while self-reported alcohol use is strongly associated with a blood pressure increase in Africans with a low socio-economic status.[103] Obesity prevalence is also high among urban South African communities, especially among women, where a prevalence of 49-59% is seen in different ethnic groups.[104-107] Although the average South African diet is energy dense, micro-nutrient intake is sub-optimal for many.[39,108] As a consequence, these and other adverse lifestyle conditions may lead to alterations in various metabolic and hemodynamic processes and could cause inflammation to become unavoidable.[52,53,109]

Over the centuries, alcohol has been the most socially accepted addictive drug globally.[110] However, several beneficial effects of light to moderate alcohol consumption have been postulated.[111] In fact, it was suggested that 1-2 drinks per day (15-30 g alcohol) is the amount associated with the lowest all-cause mortality[111] and that a history of moderate alcohol use relates to survival in a cohort of patients following complicated myocardial infarction.[112] Moderate alcohol consumption, especially red wine, has an anti-oxidant[113] and anti-inflammatory effect,[32,114] which include the inhibition of IL-6 production or its action on hepatocytes,[115] the lowering of interleukin-1 and CRP levels[114] and the decrease of adhesion molecule expression,[114] thereby protecting against atherosclerosis.[116] Light to moderate drinkers also seem to display a lower risk of diabetes,[117] hypertension,[118] coronary- and peripheral artery disease,[116,119]

myocardial infarction,[120,121] and stroke,[116,122] highlighting the cardio-protective effect of using alcohol in that manner.

On the other hand, long-term or excessive alcohol consumption (also referred to as binge drinking), which is accompanied by a severe increase in GGT levels, could be a life-threatening health hazard.[111] When ethanol is degraded, alcoholic metabolite products are formed, such as acetaldehyde and polyphenols,[110,114,123] which have the ability to cause detrimental changes in the production of biomarkers and mediators of the vascular system.[111,124] Indeed, excessive alcohol use is accompanied by an increase in adhesion molecule and pro-inflammatory biomarker expression,[111,115,125] which include CRP, IL-6 and TNF-α levels.[126,127] This is supported by several studies, as GGT levels are independently associated with CRP in black South Africans.[35] CRP concentrations were at a minimum in the case of daily alcohol consumptions of less than 16 g[124] or, in another study, of 20-40 g in men and 40-60 g in women.[32] There is further strong evidence to suggest that suPAR levels increase with heavy alcohol consumption,[128] as suPAR is up-regulated and higher in alcoholic liver disease, compared to other aetiologies.[95,128] SuPAR also strongly predicts unfavourable outcome in patients with cirrhosis,[95] associates with liver function in intensive care unit patients[129] and with the stage of liver fibrosis,[95] and it was shown that liver cirrhosis could be reverted by uPA gene therapy.[130]

An increase in oxidative stress via the inhibition of endothelial nitric oxide synthase, peroxynitrite and reactive oxygen species formation is seen during high alcohol use, which could result in protein oxidation, lipid peroxidation and a decrease in nitric oxide bioavailability.[111,123,125,131,132] Alcoholism also associates with endothelial dysfunction, even in healthy former alcoholics.[133] Previous evidence suggests that the vascular response to alcohol occurs in two phases. During the first hours, arterial dilation is accompanied by hypotension, but 11-13 hours after alcohol consumption, higher than baseline blood pressure levels are detected.[134-136] In normotensive men, four doses of alcohol per day increase systolic blood pressure with 2 mmHg,[137] while self-reported alcohol intake (but not GGT or percentage carbohydrate deficient transferrin) predict a five-year change in the blood pressure of black South Africans.[103] Heavy alcohol consumption can thus be classified as an estimated risk factor for atherosclerotic plaque formation and hypertension.[103,111,124]

Smoking status influences the degree of low-grade inflammation,[138] and is associated with both the inhibition and release of anti-inflammatory and pro-inflammatory markers, respectively.[33] In fact, in a study on 2 006 men and women from Germany, it was found that CRP was the highest (1.33 mg/ℓ) in smokers, lower (1.09 mg/ℓ) in ex-smokers and the lowest (0.97 mg/ℓ) in those that had never smoked.[32] Even though the association of suPAR with CVD showed to be independent of smoking status,[139] various studies had found that smokers had higher suPAR levels[13,69,139,140] (Figure 3). It was further shown that cessation from smoking could positively influence suPAR,[141] regardless of the serious and non-serious adverse cardiovascular events that accompanied some pharmacotherapies for smoking cessation.[142]

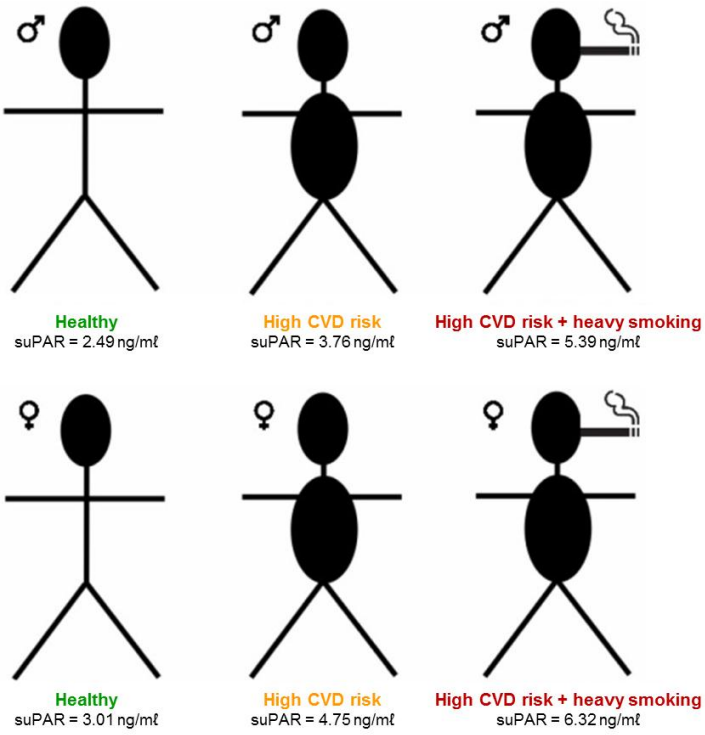


Figure 3 *The average soluble urokinase plasminogen activator receptor (suPAR) levels in men and women according to the combined cardiovascular disease (CVD) and lifestyle model, with regard to smoking status, as proposed by Haupt and colleagues.*[141]

During long-term smoking, exposure to the chemicals in tobacco products causes oxidative stress, thereby influencing the immune system in many ways.[33] For instance, nicotine was shown to suppress apoptosis and enhance endothelial cell growth, thereby mediating endothelial cell proliferation and invasion and up-regulating angiogenesis.[31,143-146] Previous reports further suggested that smoking facilitated the progression of atheroma to fibroatheroma,[147] associated with plaque progression and

with thicker, more fibrous atherosclerotic lesions[148] while, in 1 320 male decedents, smokers had much more aortic and coronary atherosclerosis than non-smokers.[149]

Underweight and very obese individuals, who are sometimes found in the same households,[150] have significantly shorter lifespans.[151] It is known that in obese individuals the number and activity of macrophages (specifically type M1 macrophages) in adipose tissue are increased.[61,152-154] These cells have pro-inflammatory characteristics, which explain the increased production of cytokines, such as IL-6 and TNF-α, together with the resultant increase in CRP production by the liver, that are often seen in obese individuals.[153-155] Indeed, research showed that intra-abdominal and visceral fat depots have the strongest impact on inflammatory markers.[156,157] Indices of body composition, such as waist circumference, body mass index and waist-to-hip ratio, all have similar strength of association with CVD risk[158] and associate with inflammatory markers,[159] including IL-6 and CRP,[160] in non-hispanic whites, African Americans, Mexican Americans,[161] as well as people from Europe and China.[162] Such a release of the products may create a chronic inflammatory state,[55] thereby contributing to the development of insulin resistance, dyslipidaemia and hypertension[55,153,157,163] (Figure 4).

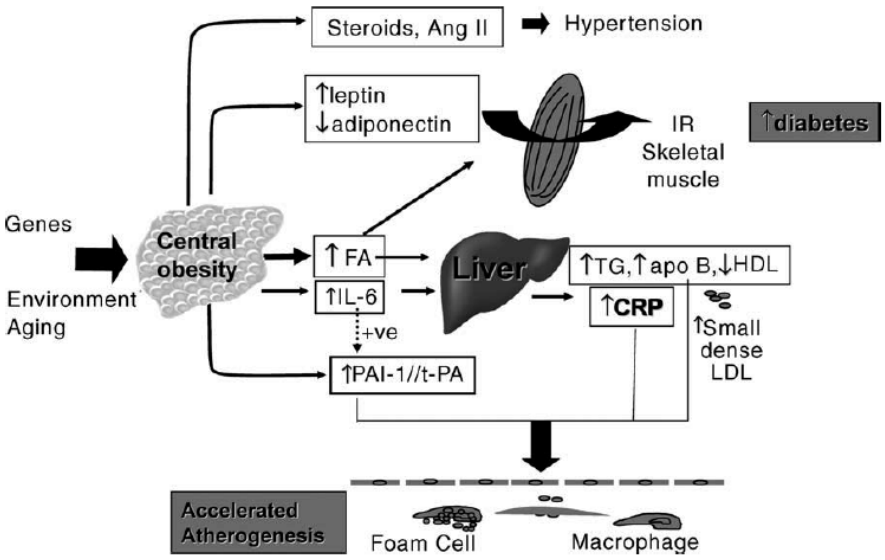


Figure 4 *A summary of risk factors, including central obesity, predisposing to coronary heart disease and cardiovascular disease.[163] Ang II, angiotensin II; IR, insulin resistance; FA, fatty acids; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue plasminogen activator; TG, triglycerides; apo B, apolipoprotein B; HDL, high-density lipoprotein cholesterol; CRP, C-reactive protein; LDL, low-density lipoprotein cholesterol.*

In contrast to the findings of Sehestedt *et al.*,[13] where suPAR levels were higher among women with a higher waist-to-hip ratio, other studies have found no association between suPAR and measures of overweight and obesity, such as waist circumference and body mass index.[13-15] It should be noted that, while CRP is more related to anthropometric measures such as body mass index and waist circumference,[160] this relationship has not been seen for suPAR.[15] Evidently, among many other, a study of 1 238 men and women from Germany (55-75 years),[159] as well as the third National Health and Nutrition Examination Survey (NHANES III), where 15 341 American adults older than 18 years were studied,[164] both found an association between elevated CRP levels and anthropometric parameters that characterised a dysmetabolic phenotype such as body mass index and waist circumference. In contrast, investigations showed that suPAR was less related to such anthropometric parameters[14] and therefore did not associate with overweight and obesity, as reflected by body mass index and waist circumference.[13-15] These studies have led to recent hypotheses that the pathological processes which occur in adipose tissue and cause an elevation in CRP concentrations, do not result in an increase in suPAR as well.[15] SuPAR may thus reflect pathology of other tissues than adipose tissue and may rather play a role in later occurring processes in the development of CVD.[15] Therefore, CRP and suPAR may represent different aspects of inflammation and other pathophysiological mechanisms that lead to atherosclerosis and CVD,[14,165] regardless of the significant positive relationship between these two markers that are often seen.[166]

Evidence indicates that adverse lifestyle choices can have a substantial impact on an individual's health. The World Health Organization previously suggested that a large percentage of NCDs can be prevented through the improvement of behavioural risk factors.[2] In accordance, a prospective study in more than 20 000 people found that a combination of four health behaviours (non-smoker, moderate alcohol intake, physically active and high fruit intake) predicted a four-fold reduction in all-cause mortality.[167] In another study, a much healthier diet at five-year follow-up also associated with the improvement of self-reported mental health.[168] Therefore, lifestyle modification remains a plausible non-pharmacological approach to treat hypertension and thereby lower CVD risk.[169]

2.3.2. SuPAR, the inflammatory process and CVD

It seems unclear whether suPAR *per se* adds to the inflammatory state, by exerting pro-inflammatory actions and thereby playing a causative role in disease development, or just reflects a disease promoting mechanism, such as inflammation in general.[14,89]

There is a complex interplay between hypertension and inflammation which is believed to occur in both directions,[170] the latter being the root of yet another controversy that exists in the literature. A possible hypothesis may be that of Harrison *et al.*[9] where a pre-hypertensive condition, involving inflammation, may initiate a more severe hypertensive state. Results from other studies, where inflammatory markers such as CRP and TNF-α were already increased in pre-hypertensive subjects,[171,172] also supported this hypothesis. A variety of physiological mechanisms are involved in maintaining normal blood pressure. Research has shown that when these mechanisms are deranged, as in the case of inflammation and alterations in the immune response, they could play a role in both the genesis and the development of hypertension. This strengthens the concept of hypertension being an inflammatory disease.[9,40,173-175]

During mechanical stress,[176] as in the case of hypertension, vascular endothelial cells secrete adhesion molecules (chemoattractants), such as soluble vascular cell adhesion molecule-1, soluble interstitial cell adhesion molecule-1 and von Willebrand factor, which are known to resemble a pro-inflammatory state of the inflamed tissue.[177-179] Such adhesion molecules also serve as indirect markers of endothelial dysfunction,[180,181] an early event in the atherosclerotic process,[180] where higher circulating concentrations of these biomarkers reflect greater dysfunction.[182] In response to adhesion molecules, cells such as neutrophils and monocytes adhere to sites of infected and damaged tissues, then migrate into the sub-endothelial space and differentiate into macrophages.[61,183,184] Various studies investigated monocytes and macrophages with regard to hypertension and found that monocytes were activated in hypertensive humans and rats compared to their normotensive controls.[185-187] Hypertension both induces and promotes the infiltration of such cells into the vascular wall,[9,176,187,188] as well as into target tissues such as the kidney[189] and the heart.[190] Furthermore, macrophages, or dendritic cells of the macrophage lineage, have the ability to activate neighbouring T-cells through antigen presentation, as in the case of

atherosclerotic lesions, thereby causing the production of large amounts of molecules downstream in the cytokine cascade, such as CRP and IL-6.[52,191-193]

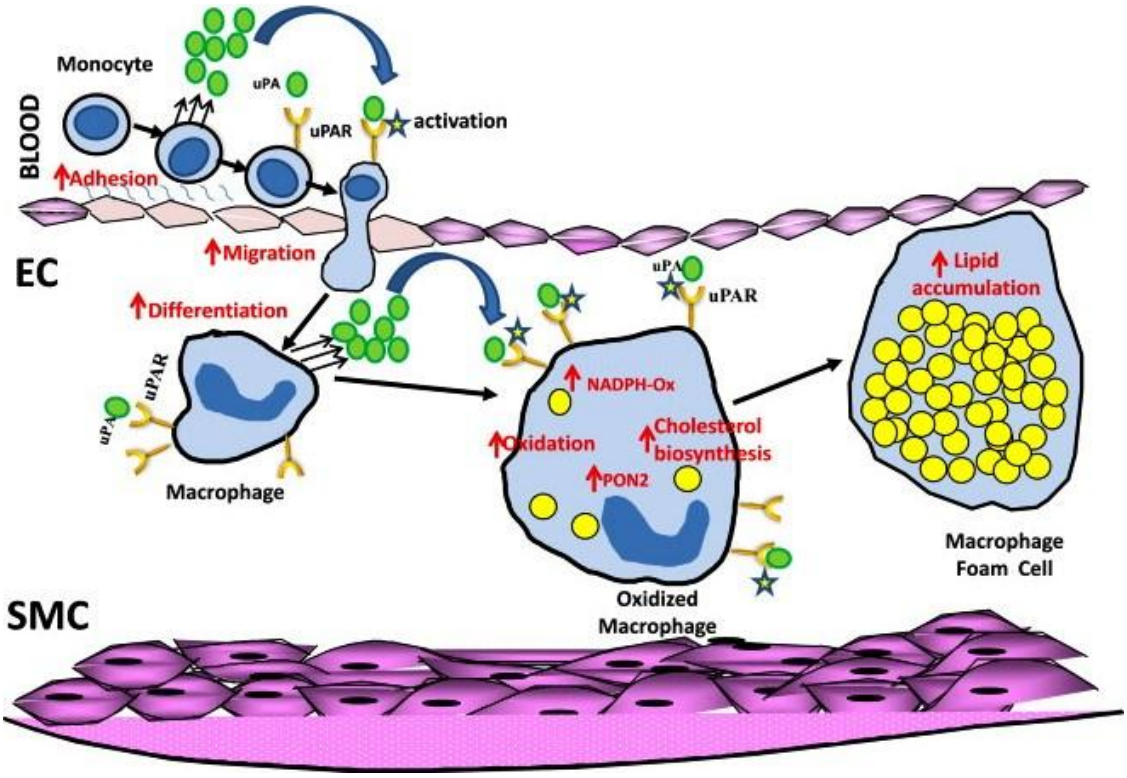


Figure 5 *The uPA-uPAR system and foam cell formation.[61] EC, endothelial cells; SMC, smooth muscle cells; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; NADPH-Ox, nicotinamide adenine dinucleotide phosphate oxidase; PON2, paraoxonase 2.*

SuPAR may also play a role in atherogenesis (Figure 5). The suPAR molecule is able to interact with a wide variety of ligands,[194] it is biologically active, functions in a remote paracrine manner[66] and especially the suPAR(II-III) fragment displays potent chemotactic properties.[195-197] UPA, uPAR and suPAR have been shown to regulate monocyte adhesion and migration,[61,66,75,197] by for instance forming complexes with integrins.[61] Circulating monocytes are able to express uPAR,[75] while cells present in the atherosclerotic arterial wall, such as endothelial cells and macrophages, have the ability to express and secrete uPA[198-200] and surface uPAR.[201] Even though Steyns *et al.*[72] found that in atherosclerotic lesions only 20-25% of the uPAR in the intima was occupied by uPA, uPA expression by macrophages remained an important determinant of atherosclerotic lesion growth in atherosclerotic apolipoprotein E-deficient mice.[202] Also, May *et al.*[203] found that uPAR expression was elevated on monocytes and contributed to enhanced cell adhesion in 20 patients with acute myocardial infarction, while in monocytes isolated from human blood, the uPA-uPAR system seemed to play a

role in monocyte-to-macrophage differentiation, which had both physiological and pathological consequences.[61]

During monocyte-to-macrophage differentiation, lipid accumulation occurs and macrophage foam cells form,[204] which is stimulated by the binding of uPA to uPAR and the activation of macrophage uPAR.[204,205] This was proven when macrophage unesterified cholesterol content increased upon incubation of macrophages with uPA.[205] During this process, these cells take up triglycerides and oxidised low-density lipoprotein cholesterol (LDL) at a gradual increasing rate, resulting in cell death.[206-208] Usually, macrophage foam cells undergo apoptosis and are phagocytised by efferocytosis in the atheroma.[61] However, inefficient efferocytosis may promote the release of LDL by the apoptotic cells, which can accumulate extracellularly, thereby forming the necrotic core of the plaque.[61] In this regard, Sørensen *et al.*[77] found an independent positive association between suPAR and coronary artery calcification score. UPA-uPAR binding can further increase cellular oxidative stress, through the activation of nicotinamide adenine dinucleotide phosphate oxidase,[209] and thereby increases the volume of the lipid core.[205]

In both early and progressive stages of atherosclerosis, lipid-rich macrophages are known to interact with intimal smooth muscle cells (SMC).[61] SMC are the main component of the arterial media layer[210] and novel research has recently been done on the ability of vascular SMC to shift from a contractile- to a synthetic phenotype.[210,211] Under normal circumstances, contractile vascular SMC are anchored to the extracellular matrix (ECM) via cell surface receptors such as integrins.[210] These strong attachments ensure the necessary compensatory transmission of contractile force in the vessel wall during an increase in blood pressure.[210] However, under pathological conditions, such as vessel injury, proteolytic processes occur where the ECM is degraded, a shift towards the synthetic vascular SMC phenotype is initiated and vascular SMC proliferate rapidly and migrate towards the intima.[210,212] During the migration of SMC, old connections between these cells and the ECM are broken down and new connections are simultaneously built up in order to ensure the motility of SMC.[75,213,214] In the end, SMC form the fibrous cap of the plaque with the intent to stabilise the plaque, thereby founding vascular remodelling (Figure 6).[61,210,213,214]

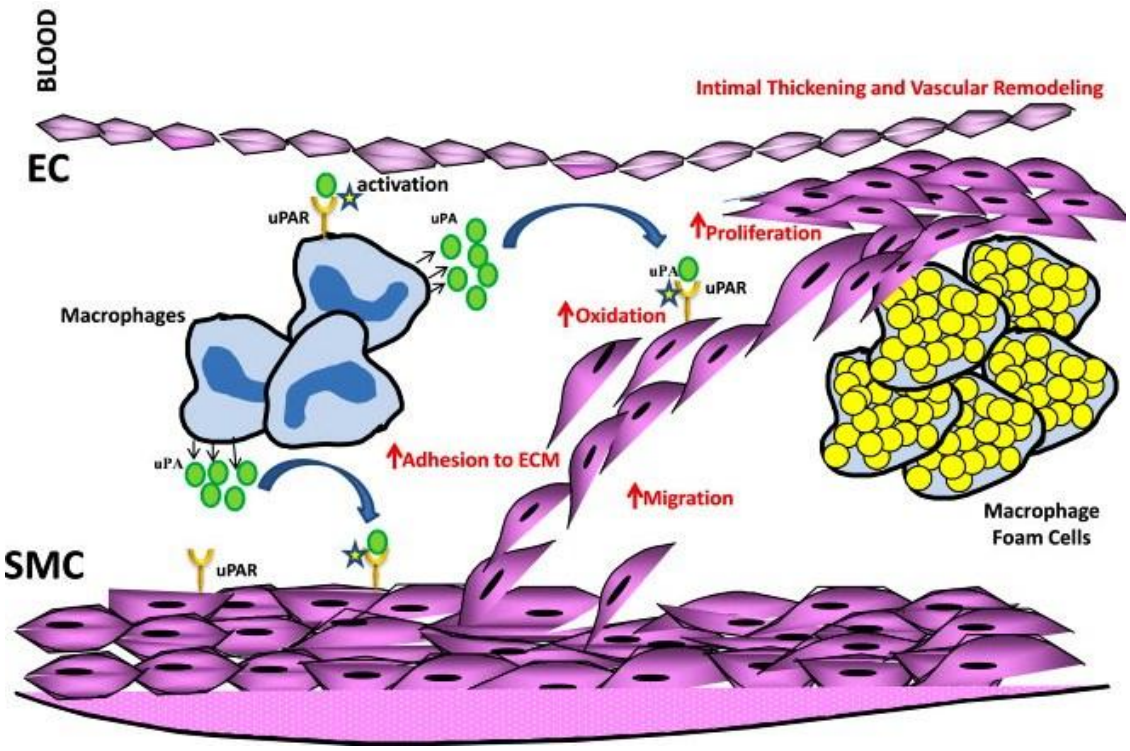


Figure 6 *The uPA-uPAR system and smooth muscle cells in vascular remodelling.*[61] *EC, endothelial cells; SMC, smooth muscle cells; ECM, extracellular matrix; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor.*

UPA was shown to stimulate such vascular SMC migration and proliferation processes[215,216] through second messenger systems,[217] even independent of binding to uPAR.[216] UPAR and integrin may also form new cell-cell, cell-matrix connections in order to facilitate cell migration.[75,213,214,218] In addition, the uPA-uPAR system regulates pericellular proteolytic activation cascades, such as matrix metalloproteinase (MMP),[219] through its involvement in the plasminogen cascade.[62,75,219,220] Interestingly, the receptors for both plasminogen and uPA are expressed on the same cells,[62] therefore activated uPA is able to catalyse the process where cell surface bound plasmin is formed from plasminogen.[62,219] Plasmin then activates pro-MMPs to form MMPs, which causes the degradation of collagen and fibrin in the ECM,[221-225] thereby destabilising the plaque.

Some controversy exists with regards to the stance of suPAR in atherosclerotic plaque. On the one hand, uPA-uPAR was shown to stabilise plaque by maintaining cellularity and collagen content,[226] suggesting that suPAR may not be a useful clinical biomarker of atherosclerotic plaque vulnerability and therefore is a less promising marker of plaque inflammation.[227] On the other hand, the work of Lijnen *et al.*[224,225] showed that uPA and uPAR had the ability to destabilise plaque. It is also believed that uPAR plays a role in

plaque rupture, as it is co-localised with macrophages, predominantly in ruptured plaque segments.[73] Another study reported that uPAR expression on circulating monocytes associated with the number of uPAR positive macrophages in plaques, which correlated with atherosclerosis formation and progression in mice.[75] Moreover, analyses of human coronary arteries demonstrated the high expression of uPAR in atherosclerotic plaques[72,228] and that the expression level of uPAR increased with the severity of atherosclerotic lesions.[75] Nonetheless, uPA over expression, uPAR content and suPAR seem to be associated with the progression and severity of atherosclerosis,[72,199,200,229] providing a possible explanation why suPAR is higher in organs undergoing extensive tissue remodelling.[218]

Human atherosclerotic plaques contain blood-borne immune and inflammatory cells, including mainly T-cells and macrophages, as well as lipids, vascular endothelial cells, SMC, ECM, and acellular lipid-rich debris (Figure 7).[52] During instability of the plaque, the fibrous cap covering the lipid-rich core may rupture and thereby expose the thrombogenic core to the blood (Figure 8).[52,192] This could result in a sudden thrombotic occlusion of the artery,[192] which is often the cause of many strokes, sudden onset of myocardial infarction and acute limb ischemia.[52]

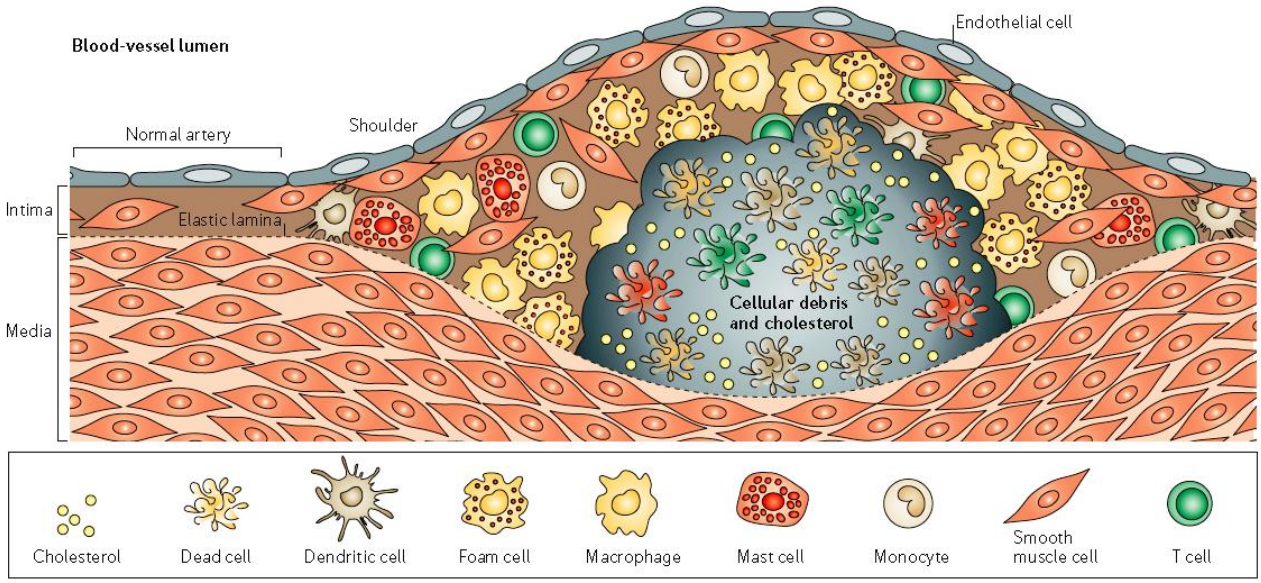


Figure 7 *The cellular composition of atherosclerotic plaque.*[52]

Endothelial cell signalling, vascular SMC tone and structural changes all affect arterial stiffness,[223,230] causing atherosclerosis and arteriosclerosis to often co-exist.[223,231] In fact, the constant elastin and collagen turnover in the ECM of the vascular wall is a fundamental determinant of arterial stiffness,[221] although wall thickening alone, even

without ECM protein changes, can also increase stiffness.[232] Subclinical low-grade inflammation is associated with impaired arterial elastic properties,[10,182,231] and according to biomarker scores, the combination of inflammation and endothelial dysfunction is associated with increased stiffness.[182,223] Indeed, pulse wave velocity, a measure of aortic stiffness,[233] is associated with inflammatory markers such as CRP, TNF-α and IL-6[233] and Mekonnen *et al.*[8] showed that suPAR independently predicted coronary micro-vascular function, as was measured by coronary flow reserve. However, the association between pulse wave velocity and suPAR seemed to be weak or even absent in Danish[13] and African[87] population groups, even though pulse wave velocity was shown to be higher in blacks than in whites[87] and to be an independent marker of cardiovascular risk.[234]

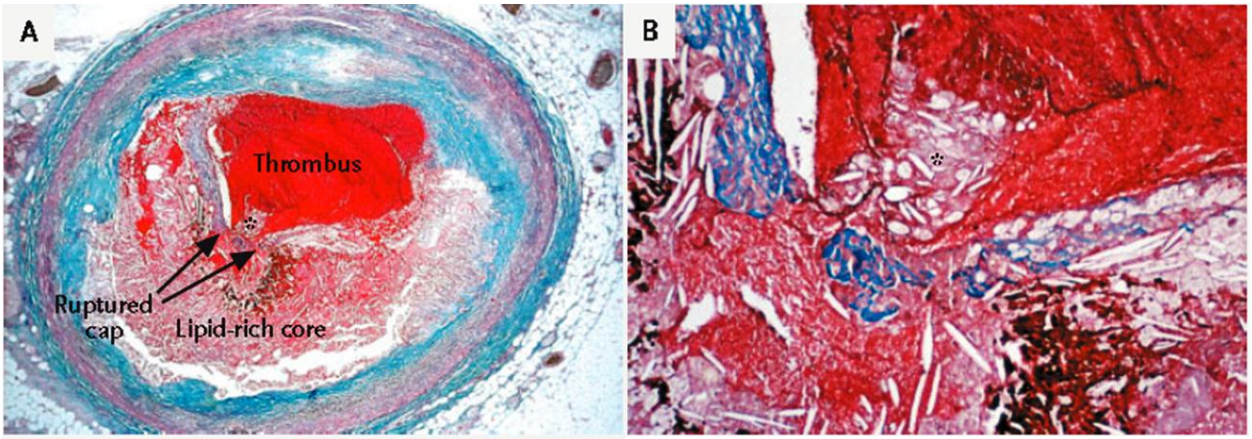


Figure 8 *Atherosclerotic lesion in a human artery. (A: A cross-sectioned coronary artery from a patient who died of a myocardial infarction. B: A high-power micrograph of the area in A, indicated by the asterisk which shows cholesterol crystals).*[192]

It is therefore clear that inflammation, particularly the uPA-uPAR system, induces changes in the vascular structure an plays a role in endothelial dysfunction by exerting pro-inflammatory actions (such as cytokine release, cell adhesion, migration, differentiation and oxidation).[9,61,231,235,236] This includes processes such as foam cell formation and extracellular matrix degradation, plaque formation and destabilisation, vascular remodelling, thrombus formation and vasodysregulation.[61,173,231,235] These processes ultimately lead to alterations in vascular elasticity, an increase in vascular stiffness and peripheral resistance, aggravating hypertension and increasing the risk of organ injury, CVD and mortality.[170,174,176] The value of the uPA-uPAR system and suPAR as a possible target for therapeutic strategies can therefore not be ignored and can be utilised to improve the high cardiovascular risk burden among the black South

African population. However, there exists an inevitable relationship between these factors and the development of CVD. Therefore, in order to optimise such strategies, the modification of cardiovascular risk factors is deemed necessary.

2.3.3. SuPAR as prognostic marker of CVD and mortality

CVD is one of the leading causes of NCD deaths in 2008, with numbers as high as 17 million (48% of NCD deaths),[2] and it is alarming how more than 80% of cardiovascular and diabetes deaths occur in low- and middle-income countries.[2] Statistics South Africa has estimated that the annual number of registered deaths has increased from 320 000 in 1997 to 612 000 in 2006[237] and is expected to nearly double by 2030.[238,239]

Recent evidence has led to the conclusion that suPAR may not be an early marker of CVD, but is rather an unspecific, more potent predictor of severe underlying diseases, such as CVD and respiratory diseases, where disease progression has already taken place.[87,165,240] This, in part, explains why suPAR was found to be the second strongest marker of all-cause mortality after age.[69]

In addition to the relationship between suPAR and diabetes,[14,241,242] suPAR also associates with cancer,[14,93,139,243] other critical illnesses (including sepsis, chronic liver disease, prosthetic joint infection, HIV infection, bacteraemia and where renal replacement therapy is needed),[19,95,129,166,244-248] as well as with outcome (such as organ dysfunction and case fatality) in intensive care patients.[245,248] Except for the findings of Lindqvist *et al.*[249] where suPAR does not appear to be a useful biomarker for abdominal aortic aneurysms, other studies found that a 1 ng/mℓ increase in suPAR was related to a 15.4% increase in risk of developing CVD[69] and that suPAR associated with the development of cardiovascular disease.[14,241] Results from a study in Danish patients showed that suPAR was higher in 302 patients who had an event (4.53 ng/mℓ) than in 2 013 patients that did not (3.93 ng/mℓ) and that suPAR therefore predicted CVD and mortality better in a population with a higher CVD risk than in those with known ischemic heart disease.[165] More studies found that suPAR associated with a higher incidence of CVD, especially in the elderly (mean age 65.5 years), independent of traditional risk factors[139] and independent of the Framingham risk score variables.[165] In addition, there

is much evidence to support that suPAR is a predictor of mortality[69,71] in the general population,[14] among the elderly,[129] in healthy subjects[250] and in healthy men between the ages of 63 to 68 years old who participated in prostate cancer screening,[139] but not in trauma patients, even though suPAR levels were higher in non-survivors.[251] Lyngbæk and colleagues[252] further discovered that patients with baseline suPAR in the highest quartile had a worse outcome of 41.5% mortality after six years than those with suPAR levels in the lowest quartile who had a mortality rate of 8.2%. Around the same time, other studies established that a suPAR cut-off value of 9.25 ng/mℓ,[253] as well as a value greater than 10 ng/mℓ[19] were predictive of mortality.

2.4. Summary

There is currently a “time bomb of cardiovascular risk factors”[36] that contribute to the poor cardiovascular profile of black South Africans, and through processes such as inflammation, could contribute substantially to the development of CVD and mortality risk (Figure 9). UPA has already been exploited for the treatment of acute myocardial infarction, ophthalmic clot and haemorrhage, pulmonary embolism and peripheral arterial occlusion.[64] However, more knowledge with regards to the role of suPAR in processes that lead to the development of specifically CVD and mortality is needed. Such knowledge may provide insight and assist in the development of early prevention strategies, especially in understudied black South Africans, where the high prevalence of CVD and mortality rates are currently taking its toll on the country’s health profile.

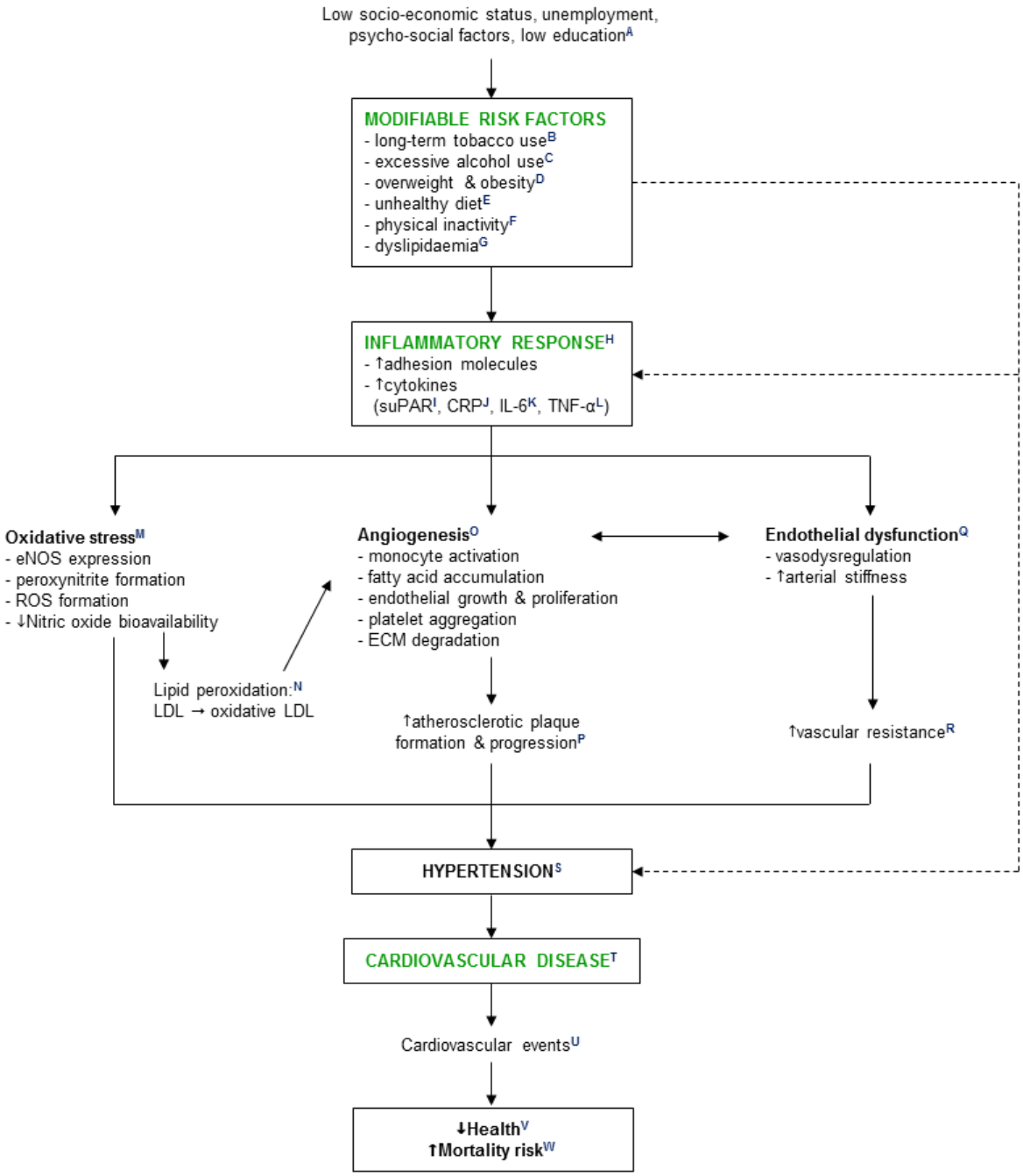


Figure 9 *Summary of the role of risk factors in the development of cardiovascular disease with regard to the inflammatory response. suPAR, soluble urokinase plasminogen activator receptor; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-α; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; ECM, extracellular matrix.*

References: A[25,103,254]; B[33,145,255,256]; C[111,114,256]; D[61,152-154,257]; E[2,7,24,108,258,259]; F[35,260-263]; G[207,208,264-269]; H[33,155,159,207,208,269,270]; I[13,69,95,128,139,141,145]; J[32,35,55,124,126,138,159,160,262,271]; K[115,153,154,160]; L[111,153,154]; M[33,111,131,144,145,272]; N[111,125,205-208]; O[95,125,144,145,265,273]; P[147,148]; Q[133,274,275]; R[276]; S[2,39,55,103,124,153,276]; T[111,158,258,263,277,278]; U[264,279-282]; V[25,254,261]; W[277,279,280,282,283].

3. MOTIVATION AND PROBLEM STATEMENT

This thesis consists of three research articles submitted to peer reviewed journals for publication. Since the relevant literature background and methods for each manuscript are discussed in the literature overview, methods and manuscript chapters, only a concise motivation, together with aims and hypotheses for each manuscript will be conveyed within this section.

The central aim of this study was to determine the potential of suPAR as a marker of CVD development in a black South African population from the PURE study. We therefore explored the associations of suPAR, with lifestyle and cardiometabolic risk factors, which might play a role in the development of CVDs, such as hypertension, and the prediction thereof for cardiovascular mortality among this population.

Motivation, aim and hypothesis of each manuscript

3.1. Associations of suPAR with lifestyle and cardiometabolic risk factors

Motivation

The epidemiological transition in South Africa has brought about changes in human behaviour and traditional ways of life[7] which are accompanied by a high prevalence of destructive health behaviours among South Africans, including heavy episodic alcohol consumption (>40%) and smoking (14%).[284,285] An unhealthy lifestyle is known to augment inflammation,[109] and may thereby contribute to the development of CVD.[69,77] Our group has previously shown that suPAR, as indicator of low-grade inflammation,[69] is higher in a black compared to a white population.[87] However, whether there is a relationship between suPAR and such lifestyle and cardiometabolic risk factors, especially in a black South African population, remains unexplored.

Aim

To explore the relationship of suPAR with lifestyle and cardiometabolic risk factors in a black South African population.

Hypothesis

Higher suPAR levels are associated with an unhealthy lifestyle and cardiometabolic risk factors in black South Africans.

3.2. SuPAR and hypertension among black South Africans after five years

Motivation

In addition to the association of suPAR with type 1 and 2 diabetes, incident cancer and total mortality, suPAR also relates to the presence and severity of coronary artery disease, predicts myocardial infarction, and is thus seen as a biomarker that links inflammation with cardiovascular risk.[14,18,77,242,286,287] There is however a paucity of information regarding the role of suPAR in the development of hypertension, especially among the understudied black South African population, where a high prevalence of hypertension is evident.[6]

Aim

To investigate suPAR in black South Africans that have developed hypertension over five years.

Hypothesis

Because of the known association of suPAR with CVD, black South Africans have higher baseline suPAR levels if they were to develop hypertension than if they remained normotensive, and suPAR is associated with the five-year development of hypertension among these individuals.

3.3. SuPAR as a prognostic marker of all-cause and cardiovascular mortality in a black population

Motivation

In South Africa, cardiovascular events are the second leading cause of death,[237] reflecting the current high burden of cardiovascular disease in the country. Healthcare resources in South Africa are limited and identification of low-cost prognostic markers, for the detection of mortality risk would be valuable. Elevated inflammatory markers such as CRP and IL-6, are well-known risk factors for cardiovascular mortality.[288-291] The less familiar marker, suPAR, is known to predict cancer,[14,292,293] infections[240,244,248,251] and all-cause mortality,[129,247,253] as well as to provide additional prognostic information over and beyond the Framingham Risk Score, especially when combined with CRP.[165] However, whether suPAR could predict all-cause and cardiovascular mortality in black South Africans, independent of traditional risk factors, HIV infection and other well-known inflammatory markers, remains to be established.

Aim

To determine whether suPAR, compared to CRP and IL-6 as well-known inflammatory markers, predicts all-cause and cardiovascular mortality among a black South African population, highly burdened by cardiovascular disease and HIV infection.

Hypothesis

SuPAR independently predicts all-cause and cardiovascular mortality among black South Africans.

4. REFERENCES

1. Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S. The Prospective Urban Rural Epidemiology (PURE) study: Examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. *Am Heart J.* 2009;158(1):1-7.e1.
2. Alwan A. Global status report on noncommunicable diseases 2010. Italy: World Health Organization; 2011.
3. Mathers C, Fat DM, Boerma J. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization; 2008.
4. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2010. Geneva, Switzerland: Joint United Nations Programme on HIV/AIDS; 2010.
5. Day C, Groenewald P, Laubscher R*, et al.* Monitoring of non-communicable diseases such as hypertension in South Africa: challenges for the post-2015 global development agenda: research. *S Afr Med J.* 2014;104(9):680-687.
6. Lloyd-Sherlock P, Beard J, Minicuci N, Ebrahim S, Chatterji S. Hypertension among older adults in low-and middle-income countries: prevalence, awareness and control. *Int J Epidemiol.* 2014;43(1):116-128.
7. Vorster HH, Margetts BM, Venter CS, Wissing MP. Integrated nutrition science: from theory to practice in South Africa. *Public Health Nutr.* 2005;8(6a):760-765.
8. Mekonnen G, Corban MT, Hung OY*, et al.* Plasma Soluble Urokinase-Type Plasminogen Activator Receptor Level is Independently Associated with Coronary Microvascular Function in Patients with Non-Obstructive Coronary Artery Disease. *Atherosclerosis.* 2015;239:55-60.
9. Harrison DG, Guzik TJ, Lob HE*, et al.* Inflammation, immunity, and hypertension. *Hypertension.* 2011;57(2):132-140.
10. Aznaouridis KA, Stefanadis CI. Inflammation and arterial function. *Artery Res.* 2007;1(1):32-38.
11. O Hartaigh B, Bosch JA, Carroll D*, et al.* Evidence of a synergistic association between heart rate, inflammation, and cardiovascular mortality in patients undergoing coronary angiography. *Eur Heart J.* 2013;34(12):932-941.
12. Tripepi G, Mallamaci F, Zoccali C. Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the

best risk marker by multivariate modeling. *J Am Soc Nephrol.* 2005;16(3, suppl. 1):S83-S88.

13. Sehestedt T, Lyngbæk S, Eugen-Olsen J*, et al.* Soluble urokinase plasminogen activator receptor is associated with subclinical organ damage and cardiovascular events. *Atherosclerosis.* 2011;216(1):237-243.
14. Eugen-Olsen J, Andersen O, Linneberg A*, et al.* Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. *J Intern Med.* 2010;268(3):296-308.
15. Lyngbæk S, Sehestedt T, Marott JL*, et al.* CRP and suPAR are differently related to anthropometry and subclinical organ damage. *Int J Cardiol.* 2013;167:781–785.
16. Kofoed K, Eugen-Olsen J, Petersen J, Larsen K, Andersen O. Predicting mortality in patients with systemic inflammatory response syndrome: an evaluation of two prognostic models, two soluble receptors, and a macrophage migration inhibitory factor. *Eur J Clin Microbiol Infect Dis.* 2008;27(5):375-383.
17. Chavakis T, Willuweit AK, Lupu F, Preissner KT, Kanse SM. Release of soluble urokinase receptor from vascular cells. *Thromb Haemost.* 2001;86(2):686-693.
18. Meijers B, Poesen R, Claes K*, et al.* Soluble urokinase receptor is a biomarker of cardiovascular disease in chronic kidney disease. *Kidney Int.* 2015;87(1):210-216.
19. Donadello K, Scolletta S, Covajes C, Vincent JL. suPAR as a prognostic biomarker in sepsis. *BMC Med.* 2012;10(2):2-10.
20. Mustjoki S, Sidenius N, Sier CF*, et al.* Soluble urokinase receptor levels correlate with number of circulating tumor cells in acute myeloid leukemia and decrease rapidly during chemotherapy. *Cancer Res.* 2000;60(24):7126-7132.
21. Eugen-Olsen J, Gustafson P, Sidenius N*, et al.* The serum level of soluble urokinase receptor is elevated in tuberculosis patients and predicts mortality during treatment: a community study from Guinea-Bissau. *Int J Tuberc Lung Dis.* 2002;6(8):686-692.
22. Ostrowski SR, Katzenstein TL, Piironen T*, et al.* Soluble urokinase receptor levels in plasma during 5 years of highly active antiretroviral therapy in HIV-1–infected patients. *J Acquir Immune Defic Syndr.* 2004;35(4):337-342.
23. Statistics South Africa. Mid-year population estimates. Pretoria, SA: Stats SA; 2014.
24. Steyn N, Bradshaw D, Norman R*, et al.* Dietary changes and the health transition in South Africa: implications for health policy. The Double burden of Malnutrition.

Case studies from six developing countries. FAO Food Nutr. Vol 84. Rome, Italy: Food and Agricultural Organization; 2006:259-304.

25. Bradshaw D. Determinants of health and their trends. In: Barron P, Roma-Reardon J, eds. Durban, SA: Health Systems Trust; 2008.
26. Lloyd-Sherlock P, Ebrahim S, Grosskurth H. Is hypertension the new HIV epidemic? *Int J Epidemiol.* 2014;43(1):8-10.
27. Karim SSA, Churchyard GJ, Karim QA, Lawn SD. HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response. *Lancet.* 2009;374(9693):921-933.
28. Kautzky K, Tollman SM. A perspective on primary health care in South Africa. In: Barron P, Roma-Reardon J, eds. South African health review 2008. Durban, SA: Health Systems Trust; 2008.
29. Pisa P, Behanan R, Vorster H, Kruger A. Social drift of cardiovascular disease risk factors in Africans from the North West Province of South Africa: the PURE study. *Cardiovasc J Afr.* 2012;23(7):371-378.
30. Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation.* 2005;112(23):3562-3568.
31. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation cellular and molecular mechanisms. *J Dent Res.* 2012;91(2):142-149.
32. Imhof A, Froehlich M, Brenner H*, et al.* Effect of alcohol consumption on systemic markers of inflammation. *Lancet.* 2001;357(9258):763-767.
33. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. *J Autoimmun.* 2010;34(3):J258-J265.
34. Black PH, Garbutt LD. Stress, inflammation and cardiovascular disease. *J Psychosom Res.* 2002;52(1):1-23.
35. Hamer M, Malan L, Schutte AE*, et al.* Conventional and behavioral risk factors explain differences in sub-clinical vascular disease between black and Caucasian South Africans: The SABPA study. *Atherosclerosis.* 2011;215(1):237-242.
36. Tibazarwa K, Ntyintyane L, Sliwa K*, et al.* A time bomb of cardiovascular risk factors in South Africa: Results from the Heart of Soweto Study "Heart Awareness Days". *Int J Cardiol.* 2009;132(2):233-239.
37. Stewart S, Libhaber E, Carrington M*, et al.* The clinical consequences and challenges of hypertension in urban-dwelling black Africans: Insights from the Heart of Soweto Study. *Int J Cardiol.* 2011;146(1):22-27.

38. Addo J, Smeeth L, Leon DA. Hypertension in Sub-Saharan Africa a systematic review. *Hypertension.* 2007;50(6):1012-1018.
39. Shisana O, Labadarios D, Rehle T, et al. South African National Health and Nutrition Examination Survey (SANHANES-1). Cape Town, SA: HSRC Press; 2013.
40. Beevers G, Lip GY, O'Brien E. ABC of hypertension: The pathophysiology of hypertension. *Br Med J.* 2001;322(7291):912-916.
41. Danaei G, Finucane MM, Lin JK*, et al.* National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet.* 2011;377(9765):568-577.
42. Johnson RJ, Segal MS, Sautin Y*, et al.* Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr.* 2007;86(4):899-906.
43. McLarty DG, Pollit C, Swai ABM. Diabetes in South Africa. *Diabet Med.* 1990;7:670–684.
44. Becker B. Cardiovascular disease in the Bantu and coloured races of South Africa. *S Afr J Med Sci.* 1946;11(1):107-120.
45. Schwartz M, Schamroth L, Seftel H. The pattern of heart disease in the urbanized Johannesburg African. *Med Proc.* 1958;4:275-281.
46. Adelstein AM. Some Aspects of Cardio-vascular Mortality in South Africa. *Br J Prev Soc Med.* 1963;17(1):29-40.
47. Poulter NR. Current and projected prevalence of arterial hypertension in sub-Saharan Africa by sex, age and habitat: an estimate from population studies. *J Hypertens.* 2011;29(7):1281-1282.
48. Ataklte F, Erqou S, Kaptoge S, Taye B, Echouffo-Tcheugui JB, Kengne AP. Burden of Undiagnosed Hypertension in Sub-Saharan Africa A Systematic Review and Meta-Analysis. *Hypertension* 2015; 65: Article in Press. DOI: 10.1161/HYPERTENSIONAHA.114.04394
49. World Health Organization. Prevalence of raised blood pressure. 2011. http://gamapserver.who.int/mapLibrary/Files/Maps/Global_BloodPressurePrevalence_BothSexes_ 2008.png Date of access: 04/01/2015.
50. Mayosi BM, Lawn JE, van Niekerk A*, et al.* Health in South Africa: changes and challenges since 2009. *Lancet.* 2012;380(9858):2029-2043.

51. Maseko M, Norton G, Majane O, Molebatsi N, Woodiwiss A. Global cardiovascular risk profiles of untreated hypertensives in an urban, developing community in Africa. *Cardiovasc J Afr.* 2011;22(5):261-267.
52. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol.* 2006;6(7):508-519.
53. Kwong J, Bouchard-Miller K. Smoking Cessation for Persons Living With HIV: A Review of Currently Available Interventions. *J Assoc Nurses AIDS Care.* 2010;21(1):3-10.
54. Sesso HD, Buring JE, Rifai N*, et al.* C-reactive protein and the risk of developing hypertension. *JAMA.* 2003;290(22):2945-2951.
55. Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension.* 2007;49(2):304-310.
56. Patterson CC, Smith AE, Yarnell JWG*, et al.* The associations of interleukin-6 (IL-6) and downstream inflammatory markers with risk of cardiovascular disease: The Caerphilly Study. *Atherosclerosis.* 2010;209(2):551-557.
57. Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. *Thromb Haemost.* 2009;102(2):215-222.
58. Hage F. C-reactive protein and hypertension. *J Hum Hypertens.* 2013;28:410-415.
59. Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. *Nat Rev Mol Cell Biol.* 2002;3(12):932-943.
60. Roldan AL, Cubellis MV, Masucci MT*, et al.* Cloning and expression of the receptor for human urokinase plasminogen activator, a central molecule in cell surface, plasmin dependent proteolysis. *EMBO J.* 1990;9(2):467-474.
61. Fuhrman B. The urokinase system in the pathogenesis of atherosclerosis. *Atherosclerosis.* 2012;222(1):8-14.
62. Alfano D, Franco P, Immacolata Vocca NG*, et al.* The urokinase plasminogen activator and its receptor. *Thromb Haemost.* 2005;93:205-211.
63. Estreicher A, Mühlhauser J, Carpentier J-L, Orci L, Vassalli J-D. The receptor for urokinase type plasminogen activator polarizes expression of the protease to the leading edge of migrating monocytes and promotes degradation of enzyme inhibitor complexes. *J Cell Biol.* 1990;111(2):783-792.

64. Crippa MP. Urokinase-type plasminogen activator. *Int J Biochem Cell Biol.* 2007;39(4):690-694.
65. Nykjær A, Conese M, Christensen EI*, et al.* Recycling of the urokinase receptor upon internalization of the uPA: serpin complexes. *EMBO J.* 1997;16(10):2610-2620.
66. Blasi F, Sidenius N. The urokinase receptor: Focused cell surface proteolysis, cell adhesion and signaling. *FEBS Lett.* 2010;584(9):1923-1930.
67. Wilhelm OG, Wilhelm S, Escott GM*, et al.* Cellular glycosylphosphatidylinositol-specific phospholipase D regulates urokinase receptor shedding and cell surface expression. *J Cell Physiol.* 1999;180(2):225-235.
68. Thunø M, Macho B, Eugen-Olsen J. suPAR: The molecular crystal ball. *Dis Markers.* 2009;27(3):157-172.
69. Eugen-Olsen J, Haugaard SB, Andersen O. Soluble urokinase plasminogen activator receptor (suPAR) as diagnostic marker for low-grade inflammation. Patent nr. EP2500730AI. Paris, FR: European Patent Office; 2012.
70. Mustjoki S, Sidenius N, Vaheri A. Enhanced release of soluble urokinase receptor by endothelial cells in contact with peripheral blood cells. *FEBS Lett.* 2000;486(3):237-242.
71. Yilmaz G, Köksal I, Karahan SC, Mentese A. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systemic inflammatory response syndrome. *Clin Biochem.* 2011;44(14–15):1227-1230.
72. Steins MB, Padró T, Schwaenen C*, et al.* Overexpression of urokinase receptor and cell surface urokinase-type plasminogen activator in the human vessel wall with different types of atherosclerotic lesions. *Blood Coagul Fibrinolysis.* 2004;15(5):383-391.
73. Svensson P-A, Olson FJ, Hägg DA*, et al.* Urokinase-type plasminogen activator receptor is associated with macrophages and plaque rupture in symptomatic carotid atherosclerosis. *Int J Mol Med.* 2008;22(4):459-464.
74. Intzilakis T, Hartmann G, Mouridsen MR*, et al.* Soluble urokinase plasminogen activator receptor, C-reactive protein and triglyceride are associated with heart rate variability in non-diabetic Danes. *Eur J Clin Invest.* 2013;43(5):457-468.
75. Chen W, Jin W-Q, Chen L-F*, et al.* Urokinase receptor surface expression regulates monocyte migration and is associated with accelerated atherosclerosis. *Int J Cardiol.* 2012;161(2):103-110.

76. Tkachuk VA, Plekhanova OS, Parfyonova YV. Regulation of arterial remodeling and angiogenesis by urokinase-type plasminogen activator. *Can J Physiol Pharmacol.* 2009;87(4):231-251.
77. Sørensen MH, Gerke O, Eugen-Olsen J*, et al.* Soluble urokinase plasminogen activator receptor is in contrast to high-sensitive C-reactive-protein associated with coronary artery calcifications in healthy middle-aged subjects. *Atherosclerosis.* 2014;237:60-66.
78. Raggam R, Wagner J, Prüller F*, et al.* Soluble urokinase plasminogen activator receptor predicts mortality in patients with systemic inflammatory response syndrome. *J Intern Med.* 2014;276:651-658.
79. Persson M, Östling G, Smith G*, et al.* Soluble Urokinase Plasminogen Activator Receptor A Risk Factor for Carotid Plaque, Stroke, and Coronary Artery Disease. *Stroke.* 2014;45(1):18-23.
80. Langkilde A, Hansen TW, Ladelund S*, et al.* Increased plasma soluble uPAR level is a risk marker of respiratory cancer in initially cancer-free individuals. *Cancer Epidemiol Biomarkers Prev.* 2011;20(4):609-618.
81. Chavakis T, Kanse SM, Yutzy B, Lijnen HR, Preissner KT. Vitronectin concentrates proteolytic activity on the cell surface and extracellular matrix by trapping soluble urokinase receptor-urokinase complexes. *Blood.* 1998;91(7):2305-2312.
82. Wilhelm O, Weidle U, Höhl S*, et al.* Recombinant soluble urokinase receptor as a scavenger for urokinase-type plasminogen activator (uPA): Inhibition of proliferation and invasion of human ovarian cancer cells. *FEBS Lett.* 1994;337(2):131-134.
83. Ploug M, Rønne E, Behrendt N*, et al.* Cellular receptor for urokinase plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. *J Biol Chem.* 1991;266(3):1926-1933.
84. Wong TY, Islam FA, Klein R*, et al.* Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). *Invest Ophthalmol Vis Sci.* 2006;47(6):2341-2350.
85. Schutte AE, van Vuuren D, van Rooyen JM*, et al.* Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: the POWIRS study. *J Hum Hypertens.* 2006;20(11):850-859.

86. Fourie C, Rooyen J, Kruger A*, et al.* Soluble Urokinase Plasminogen Activator Receptor (suPAR) is Associated with Metabolic Changes in HIV-1-Infected Africans: A Prospective Study. *Inflammation.* 2012;35(1):221-229.
87. Schutte AE, Myburgh A, Olsen MH, Eugen-Olsen J, Schutte R. Exploring soluble urokinase plasminogen activator receptor and its relationship with arterial stiffness in a bi-ethnic population: the SAfrEIC-study. *Thromb Res.* 2012;130(2):273-277.
88. Kruger R, Schutte R, Huisman HW*, et al.* NT-proBNP, C-Reactive Protein and Soluble uPAR in a Bi-Ethnic Male Population: The SAfrEIC Study. *PLoS One.* 2013;8(3):e58506.
89. Gustafsson A, Ljunggren L, Bodelsson M, Berkestedt I. The prognostic Value of supAR compared to Other Inflammatory Markers in Patients with Severe Sepsis. *Biomark Insights.* 2012;7:39-44.
90. Andersen O, Eugen-Olsen J, Kofoed K, Iversen J, Haugaard SB. Soluble urokinase plasminogen activator receptor is a marker of dysmetabolism in HIV-infected patients receiving highly active antiretroviral therapy. *J Med Virol.* 2008;80(2):209-216.
91. Kofoed K, Schneider UV, Scheel T, Andersen O, Eugen-Olsen J. Development and validation of a multiplex add-on assay for sepsis biomarkers using xMAP technology. *Clin Chem.* 2006;52(7):1284-1293.
92. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* 2003;111(12):1805-1812.
93. Thurison T, Christensen IJ, Lund IK, Nielsen HJ, Høyer-Hansen G. Circulating intact and cleaved forms of the urokinase-type plasminogen activator receptor: Biological variation, reference intervals and clinical useful cut-points. *Clin Chim Acta.* 2015;439(0):84-90.
94. Riisbro R, Christensen IJ, Høgdall C, Brünner N, Høgdall E. Soluble urokinase plasminogen activator receptor measurements: influence of sample handling. *Int J Biol Markers.* 2001;16(4):233-239.
95. Zimmermann HW, Koch A, Seidler S, Trautwein C, Tacke F. Circulating soluble urokinase plasminogen activator is elevated in patients with chronic liver disease, discriminates stage and aetiology of cirrhosis and predicts prognosis. *Liver Int.* 2012;32(3):500-509.
96. Pillay V, Dass CR, Choong PFM. The urokinase plasminogen activator receptor as a gene therapy target for cancer. *Trends Biotechnol.* 2007;25(1):33-39.

97. World Health Organization. The European health report 2005: Public health action for healthier children and populations. Geneva, Switzerland: World Health Organization; 2005.
98. Lammertyn L, Schutte AE, Schutte R. Blood glucose and nocturnal blood pressure in African and Caucasian men: The SABPA study. *Diabetes Res Clin Pract.* 2011;93(2):235-242.
99. Schutte AE, Schutte R, Huisman HW*, et al.* Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. *Int J Epidemiol.* 2012;41(4):1114-1123.
100. Fourie CMT, Van Rooyen JM, Huisman HW*, et al.* Dyslipidemia and lipodystrophy in newly diagnosed HIV-1 (subtype C) black South Africans. *J Clin Lipidol.* 2008;2(5, suppl. 1):S162.
101. Fourie CMT, Van Rooyen JM, Kruger A, Schutte AE. Lipid abnormalities in a never-treated HIV-1 subtype C-infected African population. *Lipids.* 2010;45(1):73-80.
102. Fourie CMT, van Rooyen JM, Schutte AE. HIV infection and cardiovascular risk in black South Africans. *Cardiovasc J Afr.* 2011;22(3):134–140.
103. Zatu MC, van Rooyen JM, Wentzel-Viljoen E, Greeff M, Schutte AE. Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low resource settings: the PURE study. *J Hypertens.* 2014;32(4):749-755.
104. Goedecke JH, Jennings CL, Lambertc EV. Obesity in South Africa. In: Steyn K, Fourie J, Temple N, eds. Chronic diseases of lifestyle in South Africa: 1995-2005. MRC Technical Report. Cape Town, South Africa: Medical Research Council; 2006.
105. Majane OH, Woodiwiss AJ, Maseko MJ*, et al.* Impact of age on the independent association of adiposity with pulse-wave velocity in a population sample of African ancestry. *Am J Hypertens.* 2008;21(8):936-942.
106. Norton GR, Majane OH, Libhaber E*, et al.* The relationship between blood pressure and left ventricular mass index depends on an excess adiposity. *J Hypertens.* 2009;27(9):1873-1883.
107. Hoebel S, Malan L, De Ridder H. Differences in MetS marker prevalence between black African and Caucasian teachers from the North West Province: Sympathetic

Activity and Ambulatory Blood Pressure in Africans (SABPA) Study: original research. *JEMDSA.* 2011;16(1):49-56.

108. Academy of Science of South Africa. HIV/AIDS, TB and nutrition: Scientific enquiry into the nutritional influences on human immunity with special reference to HIV infection and active TB in South Africa. Pretoria, SA: Academy of Science of South Africa, 2007.
109. Egger G, Dixon J. Non-nutrient causes of low-grade, systemic inflammation: support for a 'canary in the mineshaft' view of obesity in chronic disease. *Obes Rev.* 2011;12(5):339-345.
110. Guo R, Ren J. Alcohol and acetaldehyde in public health: from marvel to menace. *Int J Environ Res Public Health.* 2010;7(4):1285-1301.
111. Carnevale R, Nocella C. Alcohol and cardiovascular disease: Still unresolved underlying mechanisms. *Vascul Pharmacol.* 2012;57:69–71.
112. Brügger-Andersen T, Pönitz V, Snapinn S, Dickstein K. Moderate alcohol consumption is associated with reduced long-term cardiovascular risk in patients following a complicated acute myocardial infarction. *Int J Cardiol.* 2009;133(2):229-232.
113. Mudnic I, Modun D, Rastija V*, et al.* Antioxidative and vasodilatory effects of phenolic acids in wine. *Food Chem.* 2010;119(3):1205-1210.
114. Estruch R, Sacanella E, Badia E*, et al.* Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial: Effects of wine on inflammatory markers. *Atherosclerosis.* 2004;175(1):117-123.
115. McCarty MF. Interleukin-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline. *Med Hypotheses.* 1999;52(5):465-477.
116. Agarwal D, Srivastava L. Does moderate alcohol intake protect against coronary heart disease? *Indian Heart J.* 2001;53(2):224-230.
117. Ajani UA, Hennekens CH, Spelsberg A, Manson JE. Alcohol consumption and risk of type 2 diabetes mellitus among US male physicians. *Arch Intern Med.* 2000;160(7):1025-1030.

118. Thadhani R, Camargo CA, Stampfer MJ*, et al.* Prospective study of moderate alcohol consumption and risk of hypertension in young women. *Arch Intern Med.* 2002;162(5):569-574.
119. Shaper A, Wannamethee S. Alcohol intake and mortality in middle aged men with diagnosed coronary heart disease. *Heart.* 2000;83(4):394-399.
120. Mukamal KJ, Conigrave KM, Mittleman MA*, et al.* Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. *N Engl J Med.* 2003;348(2):109-118.
121. Beulens JW, Rimm EB, Ascherio A*, et al.* Alcohol consumption and risk for coronary heart disease among men with hypertension. *Ann Intern Med.* 2007;146(1):10-19.
122. Elkind MSV, Sciacca R, Boden-Albala B*, et al.* Moderate Alcohol Consumption Reduces Risk of Ischemic Stroke : The Northern Manhattan Study. *Stroke.* 2006;37:13-19.
123. Zhang X, Li S-Y, Brown RA, Ren J. Ethanol and acetaldehyde in alcoholic cardiomyopathy: from bad to ugly en route to oxidative stress. *Alcohol.* 2004;32(3):175-186.
124. Bau PFD, Bau CHD, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. *Alcohol.* 2007;41(7):479-488.
125. Hannuksela ML, Liisanantti MK, Savolainen MJ. Effect of alcohol on lipids and lipoproteins in relation to atherosclerosis. *Crit Rev Clin Lab Sci.* 2002;39(3):225-283.
126. Villegas R, Xiang YB, Cai H*, et al.* Lifestyle determinants of c-reactive protein in middle-aged, urban Chinese men. *Nutr Metab Cardiovasc Dis.* 2012;22(3):223-230.
127. An L, Wang X, Cederbaum AI. Cytokines in alcoholic liver disease. *Arch Toxicol.* 2012;86(9):1337-1348.
128. Tuomi H, Kultti J, Danielsson J*, et al.* Serum soluble urokinase plasminogen activator receptor in alcoholics: relation to liver disease severity, fibrogenesis and alcohol use. *J Gastroenterol Hepatol.* 2014;29:1991-1995.
129. Koch A, Voigt S, Kruschinski C*, et al.* Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the

intensive care unit and predicts mortality in critically ill patients. *Crit Care.* 2011;15(1):R63.

130. Salgado S, Garcia J, Vera J*, et al.* Liver cirrhosis is reverted by urokinase-type plasminogen activator gene therapy. *Mol Ther.* 2000;2(6):545-551.
131. Pacher P, Szabó C. Role of peroxynitrite in the pathogenesis of cardiovascular complications of diabetes. *Curr Opin Pharmacol.* 2006;6(2):136-141.
132. Deng X-S, Deitrich RA. Ethanol metabolism and effects: nitric oxide and its interaction. *Curr Clin Pharmacol.* 2007;2(2):145-153.
133. Di Gennaro C, Biggi A, Barilli AL*, et al.* Endothelial dysfunction and cardiovascular risk profile in long-term withdrawing alcoholics. *J Hypertens.* 2007;25(2):367-373.
134. Abe H, Kawano Y, Kojima S*, et al.* Biphasic effects of repeated alcohol intake on 24-hour blood pressure in hypertensive patients. *Circulation.* 1994;89(6):2626-2633.
135. Bau PFD, Bau CHD, Naujorks AA, Rosito GA. Early and late effects of alcohol on blood pressure and endothelial function. *Alcohol.* 2005;37:53-58.
136. Rosito GA, Fuchs FD, Duncan BB. Dose-dependent biphasic effect of ethanol on 24-h blood pressure in normotensive subjects. *Am J Hypertens.* 1999;12(2):236-240.
137. Zilkens RR, Burke V, Hodgson JM*, et al.* Red wine and beer elevate blood pressure in normotensive men. *Hypertension.* 2005;45(5):874-879.
138. Plaisance EP, Grandjean PW. Physical Activity and High-Sensitivity C-Reactive Protein. *Sports Med.* 2006;36(5):443-458.
139. Persson M, Engström G, Björkbacka H, Hedblad B. Soluble urokinase plasminogen activator receptor in plasma is associated with incidence of CVD. Results from the Malmö Diet and Cancer Study. *Atherosclerosis.* 2012;220(2):502-505.
140. Heraclides A, Jensen T, Rasmussen S*, et al.* The pro-inflammatory biomarker soluble urokinase plasminogen activator receptor (suPAR) is associated with incident type 2 diabetes among overweight but not obese individuals with impaired glucose regulation: effect modification by smoking and body weight status. *Diabetologia.* 2013;56(7):1542-1546.
141. Haupt TH, Kallemose T, Ladelund S*, et al.* Risk Factors Associated with Serum Levels of the Inflammatory Biomarker Soluble Urokinase Plasminogen Activator Receptor in a General Population. *Biomark Insights.* 2014;9:91-100.

142. Sharma A, Thakar S, Lavie CJ*, et al.* Cardiovascular Adverse Events Associated with Smoking-Cessation Pharmacotherapies. *Curr Cardiol Rep.* 2015;17(1):1-7.
143. Lee C-H, Huang C-S, Chen C-S*, et al.* Overexpression and activation of the α9-nicotinic receptor during tumorigenesis in human breast epithelial cells. *J Natl Cancer Inst.* 2010;102:1-14.
144. Conklin BS, Surowiec SM, Ren Z*, et al.* Effects of Nicotine and Cotinine on Porcine Arterial Endothelial Cell Function. *J Surg Res.* 2001;95(1):23-31.
145. Khoi PN, Park JS, Kim NH, Jung YD. Nicotine stimulates urokinase-type plasminogen activator receptor expression and cell invasiveness through mitogen-activated protein kinase and reactive oxygen species signaling in ECV304 endothelial cells. *Toxicol Appl Pharmacol.* 2012;259:248-256.
146. Dasgupta P, Kinkade R, Joshi B*, et al.* Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. *Proceedings of the National Academy of Sciences.* 2006;103(16):6332-6337.
147. Zieske AW, McMahan CA, McGill HC*, et al.* Smoking is associated with advanced coronary atherosclerosis in youth. *Atherosclerosis.* 2005;180(1):87-92.
148. Sharrett AR, Ding J, Criqui MH*, et al.* Smoking, diabetes, and blood cholesterol differ in their associations with subclinical atherosclerosis: The Multiethnic Study of Atherosclerosis (MESA). *Atherosclerosis.* 2006;186(2):441-447.
149. Tracy RE, Toca VT, Strong JP, Richards ML. Relationship of raised atherosclerotic lesions to fatty streaks in cigarette smokers. *Atherosclerosis.* 1981;38(3–4):347-357.
150. Popkin BM. An overview on the nutrition transition and its health implications: the Bellagio meeting. *Public Health Nutr.* 2002;5(1):93-103.
151. Henderson RM. The bigger the healthier: Are the limits of BMI risk changing over time? *Econ Hum Biol.* 2005;3(3):339-366.
152. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr.* 2006;83(2):461S-465S.
153. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res.* 2005;96(9):939-949.
154. Morris AA, Vaccarino V. Evidence Linking Mental Health with Obesity and Metabolic Syndrome: The Role of Inflammation. *Curr Nutr Rep.* 2013;2(4):181-188.

155. Ahima RS, Flier JS. Adipose Tissue as an Endocrine Organ. *Trends Endocrinol Metab.* 2000;11(8):327-332.
156. Forouhi N, Sattar N, McKeigue P. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. *Int J Obes Relat Metab Disord.* 2001;25(9):1327-1331.
157. Grundy SM, Brewer Jr HB, Cleeman JI, Smith Jr SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb Vasc Biol.* 2004;24(2):e13-e18.
158. The Emerging Risk Factors Collaboration. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet* 2011; 377(9771): 1085-1095.
159. Thorand B, Baumert J, Döring A*, et al.* Sex differences in the relation of body composition to markers of inflammation. *Atherosclerosis.* 2006;184(1):216-224.
160. Nagel G, Rapp K, Wabitsch M*, et al.* Prevalence and cluster of cardiometabolic biomarkers in overweight and obese schoolchildren: results from a large survey in southwest Germany. *Clin Chem.* 2008;54(2):317-325.
161. Festa A, D'Agostino Jr R, Williams K*, et al.* The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord.* 2001;25(10):1407-1415.
162. Lear SA, Chen MM, Birmingham CL, Frohlich JJ. The relationship between simple anthropometric indices and c-reactive protein: ethnic and gender differences. *Metabolism.* 2003;52(12):1542-1546.
163. Forouhi NG, Sattar N. CVD risk factors and ethnicity—A homogeneous relationship? *Atherosclerosis Suppl.* 2006;7(1):11-19.
164. Miller M, Zhan M, Havas S. High attributable risk of elevated C-reactive protein level to conventional coronary heart disease risk factors: the Third National Health and Nutrition Examination Survey. *Arch Intern Med.* 2005;165(18):2063-2068.
165. Lyngbæk S, Marott JL, Sehestedt T*, et al.* Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *Int J Cardiol.* 2013;167:2904-2911.

166. Galliera E, Drago L, Marazzi MG*, et al.* Soluble urokinase-type plasminogen activator receptor (suPAR) as new biomarker of the prosthetic joint infection: Correlation with inflammatory cytokines. *Clin Chim Acta.* 2015;441:23-28.
167. Khaw K-T, Wareham N, Bingham S*, et al.* Combined impact of health behaviours and mortality in men and women: the EPIC-Norfolk prospective population study. *PLoS Med.* 2008;5(1):e12.
168. Pisinger C, Ladelund S, Glümer C*, et al.* Five years of lifestyle intervention improved self-reported mental and physical health in a general population: The Inter99 study. *Prev Med.* 2009;49(5):424-428.
169. Ohta M, Nanri H, Matsushima Y, Sato Y, Ikeda M. Blood pressure-lowering effects of lifestyle modification: possible involvement of nitric oxide bioavailability. *Hypertens Res.* 2005;28(10):779-786.
170. Pruijm M, Vollenweider P, Mooser V*, et al.* Inflammatory markers and blood pressure: sex differences and the effect of fat mass in the CoLaus Study. *J Hum Hypertens.* 2013;27:169-175.
171. Stumpf C, John S, Jukic J*, et al.* Enhanced levels of platelet P-selectin and circulating cytokines in young patients with mild arterial hypertension. *J Hypertens.* 2005;23(5):995-1000.
172. Chrysohoou C, Pitsavos C, Panagiotakos DB, Skoumas J, Stefanadis C. Association between prehypertension status and inflammatory markers related to atherosclerotic disease: The ATTICA Study. *Am J Hypertens.* 2004;17(7):568-573.
173. Bautista LE. Inflammation, endothelial dysfunction, and the risk of high blood pressure: epidemiologic and biological evidence. *J Hum Hypertens.* 2003;17(4):223-230.
174. Bautista L, Vera L, Arenas I, Gamarra G. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-α) and essential hypertension. *J Hum Hypertens.* 2005;19(2):149-154.
175. Li JJ, Fang CH, Hui RT. Is hypertension an inflammatory disease? *Med Hypotheses.* 2005;64(2):236-240.
176. Hilgers KF. Monocytes/macrophages in hypertension. *J Hypertens.* 2002;20(4):593-596.
177. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med.* 1999;340(2):115-126.

178. Aird WC. Phenotypic heterogeneity of the endothelium I. Structure, function, and mechanisms. *Circ Res.* 2007;100(2):158-173.
179. Aird WC. Phenotypic heterogeneity of the endothelium II. Representative vascular beds. *Circ Res.* 2007;100(2):174-190.
180. Stenvinkel P, Wanner C, Metzger T*, et al.* Inflammation and outcome in end-stage renal failure: does female gender constitute a survival advantage? *Kidney Int.* 2002;62(5):1791-1798.
181. Corti R, Hutter R, Badimon JJ, Fuster V. Evolving concepts in the triad of atherosclerosis, inflammation and thrombosis. *J Thromb Thrombolysis.* 2004;17(1):35-44.
182. van Bussel BC, Schouten F, Henry RM*, et al.* Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period. *Hypertension.* 2011;58(4):588-595.
183. Moore Kathryn J, Tabas I. Macrophages in the Pathogenesis of Atherosclerosis. *Cell.* 2011;145(3):341-355.
184. Pliyev BK, Antonova OA, Menshikov M. Participation of the urokinase-type plasminogen activator receptor (uPAR) in neutrophil transendothelial migration. *Mol Immunol.* 2011;48(9–10):1168-1177.
185. Chen N-G, Abbasi F, Lamendola C*, et al.* Mononuclear cell adherence to cultured endothelium is enhanced by hypertension and insulin resistance in healthy nondiabetic volunteers. *Circulation.* 1999;100(9):940-943.
186. Ohki R, Yamamoto K, Mano H*, et al.* Identification of mechanically induced genes in human monocytic cells by DNA microarrays. *J Hypertens.* 2002;20(4):685-691.
187. Schmid-Schönbein G, Seiffge D, DeLano FA, Shen K, Zweifach BW. Leukocyte counts and activation in spontaneously hypertensive and normotensive rats. *Hypertension.* 1991;17(3):323-330.
188. Clozel M, Kuhn H, Hefti F, Baumgartner HR. Endothelial dysfunction and subendothelial monocyte macrophages in hypertension. Effect of angiotensin converting enzyme inhibition. *Hypertension.* 1991;18(2):132-141.
189. Johnson RJ, Alpers CE, Yoshimura A*, et al.* Renal injury from angiotensin II-mediated hypertension. *Hypertension.* 1992;19(5):464-474.
190. Haller H, Behrend M, Park JK*, et al.* Monocyte infiltration and c-fms expression in hearts of spontaneously hypertensive rats. *Hypertension.* 1995;25(1):132-138.

191. Hansson GK. Immune mechanisms in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2001;21(12):1876-1890.
192. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 2005;352(16):1685-1695.
193. Hansson GK. Atherosclerosis—An immune disease: The Anitschkov Lecture 2007. *Atherosclerosis.* 2009;202(1):2-10.
194. Huai Q, Mazar AP, Kuo A*, et al.* Structure of human urokinase plasminogen activator in complex with its receptor. *Science.* 2006;311(5761):656-659.
195. Pliyev BK. Activated human neutrophils rapidly release the chemotactically active D2D3 form of the urokinase-type plasminogen activator receptor (uPAR/CD87). *Mol Cell Biochem.* 2009;321(1-2):111-122.
196. Montuori N, Visconte V, Rossi G, Ragno P. Soluble and cleaved forms of the urokinase-receptor: degradation products or active molecules. *Thromb Haemost.* 2005;93(2):192-198.
197. Furlan F, Orlando S, Laudanna C*, et al.* The soluble D2D388-274 fragment of the urokinase receptor inhibits monocyte chemotaxis and integrin-dependent cell adhesion. *J Cell Sci.* 2004;117(14):2909-2916.
198. Lupu F, Heim DA, Bachmann F*, et al.* Plasminogen activator expression in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol.* 1995;15(9):1444-1455.
199. Padró T, Emeis JJ, Steins M, Schmid KW, Kienast J. Quantification of plasminogen activators and their inhibitors in the aortic vessel wall in relation to the presence and severity of atherosclerotic disease. *Arterioscler Thromb Vasc Biol.* 1995;15(7):893-902.
200. Kienast J, Padró T, Steins M*, et al.* Relation of urokinase-type plasminogen activator expression to presence and severity of atherosclerotic lesions in human coronary arteries. *Thromb Haemost.* 1998;79:579-586.
201. Shen GX. Vascular cell-derived fibrinolytic regulators and atherothrombotic vascular disorders (Review). *Int J Mol Med.* 1998;1(2):399-807.
202. Krishnan R, Kremen M, Hu JH*, et al.* Level of macrophage uPA expression is an important determinant of atherosclerotic lesion growth in Apoe−/− mice. *Arterioscler Thromb Vasc Biol.* 2009;29(11):1737-1744.
203. May AE, Schmidt R, Kanse SM*, et al.* Urokinase receptor surface expression regulates monocyte adhesion in acute myocardial infarction. *Blood.* 2002;100(10):3611-3617.

204. Ohwaki K, Bujo H, Jiang M*, et al.* A secreted soluble form of LR11, specifically expressed in intimal smooth muscle cells, accelerates formation of lipid-laden macrophages. *Arterioscler Thromb Vasc Biol.* 2007;27(5):1050-1056.
205. Fuhrman B, Nitzan O, Karry R*, et al.* Urokinase plasminogen activator (uPA) stimulates cholesterol biosynthesis in macrophages through activation of SREBP-1 in a PI3-kinase and MEK-dependent manner. *Atherosclerosis.* 2007;195(2):e108-e116.
206. Fuhrman B, Koren L, Volkova N*, et al.* Atorvastatin therapy in hypercholesterolemic patients suppresses cellular uptake of oxidized-LDL by differentiating monocytes. *Atherosclerosis.* 2002;164(1):179-185.
207. Aronis A, Madar Z, Tirosh O. Mechanism underlying oxidative stress-mediated lipotoxicity: exposure of J774. 2 macrophages to triacylglycerols facilitates mitochondrial reactive oxygen species production and cellular necrosis. *Free Radic Biol Med.* 2005;38(9):1221-1230.
208. Aronis A, Madar Z, Tirosh O. Lipotoxic effects of triacylglycerols in J774.2 macrophages. *Nutrition.* 2008;24(2):167-176.
209. Fuhrman B, Khateeb J, Shiner M*, et al.* Urokinase plasminogen activator upregulates paraoxonase 2 expression in macrophages via an NADPH oxidase-dependent mechanism. *Arterioscler Thromb Vasc Biol.* 2008;28(7):1361-1367.
210. Heidari M, Mandato CA, Lehoux S. Vascular smooth muscle cell phenotypic modulation and the extracellular matrix. *Artery Res* 2015;9:14-18
211. Cecchettini A, Rocchiccioli S, Boccardi C, Citti L. Chapter Two - Vascular Smooth-Muscle-Cell Activation: Proteomics Point of View. In: Kwang WJ, ed. International Review of Cell and Molecular Biology. Vol 288. USA: Academic Press; 2011.
212. Beamish JA, He P, Kottke-Marchant K, Marchant RE. Molecular regulation of contractile smooth muscle cell phenotype: implications for vascular tissue engineering. *Tissue Eng Part B Rev.* 2010;16(5):467-491.
213. Rabbani S, Mazar A. The role of the plasminogen activation system in angiogenesis and metastasis. *Surg Oncol Clin N Am.* 2001;10(2):393-415.
214. Binder BR, Mihaly J, Prager GW. uPAR-uPA-PAI-1 interactions and signaling: a vascular biologist's view. *Thromb Haemost.* 2007;97(3):336-342.
215. Goncharova EA, Vorotnikov AV, Gracheva EO*, et al.* Activation of p38 MAP-kinase and caldesmon phosphorylation are essential for urokinase-induced human smooth muscle cell migration. *Biol Chem.* 2002;383(1):115-126.

216. Kanse SM, Benzakour O, Kanthou C*, et al.* Induction of vascular SMC proliferation by urokinase indicates a novel mechanism of action in vasoproliferative disorders. *Arterioscler Thromb Vasc Biol.* 1997;17(11):2848-2854.
217. Nicholl SM, Roztocil E, Davies MG. Urokinase-induced smooth muscle cell responses require distinct signaling pathways: A role for the epidermal growth factor receptor. *J Vasc Surg.* 2005;41(4):672-681.
218. Gorrasi A, Santi AL, Amodio G*, et al.* The Urokinase Receptor Takes Control of Cell Migration by Recruiting Integrins and FPR1 on the Cell Surface. *PLoS One.* 2014;9(1):e86352.
219. Lacroix R, Sabatier F, Mialhe A*, et al.* Activation of plasminogen into plasmin at the surface of endothelial microparticles: a mechanism that modulates angiogenic properties of endothelial progenitor cells in vitro. *Blood.* 2007;110(7):2432-2439.
220. Mazzieri R, Masiero L, Zanetta L*, et al.* Control of type IV collagenase activity by components of the urokinase–plasmin system: a regulatory mechanism with cell-bound reactants. *EMBO J.* 1997;16(9):2319-2332.
221. Yasmin SW, McEniery CM, Dakham Z*, et al.* Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol.* 2005;25(2):372-378.
222. Stossel TP. On the crawling of animal cells. *Science.* 1993;260(5111):1086-1094.
223. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol.* 2005;25(5):932-943.
224. Lijnen H. Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb Haemost.* 2001;86(1):324-333.
225. Lijnen H. Elements of the fibrinolytic system. *Ann N Y Acad Sci.* 2001;936(1):226-236.
226. Dellas C, Schremmer C, Hasenfuss G, Konstantinides SV, Schafer K. Lack of urokinase plasminogen activator promotes progression and instability of atherosclerotic lesions in apolipoprotein E-knockout mice. *Thromb Haemost.* 2007;98(1):220-227.
227. Olson FJ, Thurison T, Ryndel M, Høyer-Hansen G, Fagerberg B. Soluble urokinase-type plasminogen activator receptor forms in plasma as markers of atherosclerotic plaque vulnerability. *Clin Biochem.* 2010;43(1–2):124-130.
228. Noda-Heiny H, Daugherty A, Sobel BE. Augmented urokinase receptor expression in atheroma. *Arterioscler Thromb Vasc Biol.* 1995;15(1):37-43.

229. Falkenberg M, Tom C, DeYoung MB*, et al.* Increased expression of urokinase during atherosclerotic lesion development causes arterial constriction and lumen loss, and accelerates lesion growth. *Proceedings of the National Academy of Sciences.* 2002;99(16):10665-10670.
230. Mahmud A, Feely J. Arterial stiffness and the renin-angiotensinaldosterone system. *J Renin Angiotensin Aldosterone Syst.* 2004;5(3):102-108.
231. Schillaci G, Pirro M. C-reactive protein in hypertension: clinical significance and predictive value. *Nutr Metab Cardiovasc Dis.* 2006;16(7):500-508.
232. Wagenseil JE, Mecham RP. Elastin in Large Artery Stiffness and Hypertension. *J Cardiovasc Transl Res.* 2012;5(3):264-273.
233. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension.* 2005;46(5):1118-1122.
234. Asmar R, Rudnichi A, Blacher J, London GM, Safar ME. Pulse pressure and aortic pulse wave are markers of cardiovascular risk in hypertensive populations. *Am J Hypertens.* 2001;14(2):91-97.
235. Boos CJ, Lip GYH. Elevated high-sensitive C-reactive protein, large arterial stiffness and atherosclerosis: a relationship between inflammation and hypertension? *J Hum Hypertens.* 2005;19(7):511-513.
236. Stocker R, Keaney J. New insights on oxidative stress in the artery wall. *J Thromb Haemost.* 2005;3(8):1825-1834.
237. Bradshaw D, Pillay-Van Wyk V, Laubscher R, et al. Cause of death statistics for South Africa: Challenges and possibilities for improvement. South African MRC Burden of Disease Research Unit; 2010.
238. Kengne AP, Ntyintyane LM, Mayosi BM. A systematic overview of prospective cohort studies of cardiovascular disease in sub-Saharan Africa: review article. *Cardiovasc J Afr.* 2012;23(2):103-112.
239. Ibrahim MM, Damasceno A. Hypertension in developing countries. *Lancet.* 2012;380(9841):611-619.
240. Wrotek A, Jackowska T. The role of the soluble urokinase plasminogen activator (suPAR) in children with pneumonia. *Respir Physiol Neurobiol* 2015: Article in Press. DOI: 10.1016/j.resp.2014.12.018
241. Hillman M, Landin-Olsson M. Soluble urokinase-plasminogen activator receptor (suPAR) and natural phosphorylcholine IgM antibodies in patients at clinical onset of diabetes mellitus. *J Diabetes Mellitus.* 2011;1(4):96-103.

242. Theilade S, Lyngbæk S, Hansen TW, et al. Soluble urokinase plasminogen activator receptor levels are elevated and associated with complications in patients with type 1 diabetes. *J Intern Med* 2015; 277(3): 362-371.
243. de Bock CE, Wang Y. Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. *Med Res Rev.* 2004;24(1):13-39.
244. Sidenius N, Sier CFM, Ullum H*, et al.* Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. *Blood.* 2000;96(13):4091-4095.
245. Huttunen R, Syrjänen J, Vuento R*, et al.* Plasma level of soluble urokinase-type plasminogen activator receptor as a predictor of disease severity and case fatality in patients with bacteraemia: a prospective cohort study. *J Intern Med.* 2011;270(1):32-40.
246. Jalkanen V, Yang R, Linko R*, et al.* SuPAR and PAI-1 in critically ill, mechanically ventilated patients. *Intensive Care Med.* 2013;39:489-496.
247. Jalkanen V, Vaahersalo J, Pettilä V*, et al.* The predictive value of soluble urokinase plasminogen activator receptor (SuPAR) regarding 90-day mortality and 12-month neurological outcome in critically ill patients after out-of-hospital cardiac arrest. Data from the prospective FINNRESUSCI study. *Resuscitation.* 2014;85(11):1562-1567.
248. Donadello K, Scolletta S, Taccone FS*, et al.* Soluble urokinase–type plasminogen activator receptor as a prognostic biomarker in critically ill patients. *J Crit Care.* 2014;29(1):144-149.
249. Lindqvist M, Wallinder J, Henriksson AE. Soluble urokinase plasminogen activator receptor in patients with abdominal aortic aneurysm. *Thromb Res.* 2012;130(3):511-513.
250. Kjellman A, Akre O, Gustafsson O*, et al.* Soluble urokinase plasminogen activator receptor as a prognostic marker in men participating in prostate cancer screening. *J Intern Med.* 2011;269(3):299-305.
251. Timmermans K, Vaneker M, Scheffer GJ, et al. SuPAR levels are related to plasma cytokine levels but have low predictive value for mortality in trauma patients. *J Crit Care* 2015: Article in Press. DOI: 10.1016/j.jcrc.2015.01.006
252. Lyngbæk S, Marott JL, Møller DV*, et al.* Usefulness of Soluble Urokinase Plasminogen Activator Receptor to Predict Repeat Myocardial Infarction and

Mortality in Patients with ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Intervention. *Am J Cardiol.* 2012;110:1756-1763.

253. Mölkänen T, Ruotsalainen E, Thorball C, Järvinen A. Elevated soluble urokinase plasminogen activator receptor (suPAR) predicts mortality in Staphylococcus aureus bacteremia. *Eur J Clin Microbiol Infect Dis.* 2011;30(11):1417-1424.
254. Doolan K, Ehrlich R, Myer L. Experience of violence and socioeconomic position in South Africa: a national study. *PLoS One.* 2007;2(12):e1290.
255. Hoffmann D, Hoffmann I, El-Bayoumy K. The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. *Chem Res Toxicol.* 2001;14(7):767-790.
256. Sasazuki S, Sasaki S, Tsugane S. Cigarette smoking, alcohol consumption and subsequent gastric cancer risk by subsite and histologic type. *Int J Cancer.* 2002;101(6):560-566.
257. Thorand B, Baumert J, Döring A*, et al.* Association of cardiovascular risk factors with markers of endothelial dysfunction in middle-aged men and women. Results from the MONICA/KORA Augsburg Study. *Thromb Haemost.* 2006;95:134-141.
258. Toft U, Kristoffersen LH, Lau C, Borch-Johnsen K, Jørgensen T. The Dietary Quality Score: validation and association with cardiovascular risk factors: the Inter99 study. *Eur J Clin Nutr.* 2007;61(2):270-278.
259. Oldewage-Theron WH, Kruger R. Food variety and dietary diversity as indicators of the dietary adequacy and health status of an elderly population in Sharpeville, South Africa. *J Nutr Elder.* 2008;27(1-2):101-133.
260. Pérez AB. Exercise as the Cornerstone of Cardiovascular Prevention. *Rev Esp Cardiol (Engl Ed).* 2008;61(5):514-528.
261. Pisinger C, Toft U, Aadahl M, Glümer C, Jørgensen T. The relationship between lifestyle and self-reported health in a general population: The Inter99 study. *Prev Med.* 2009;49(5):418-423.
262. Kasapis C, Thompson PD. The Effects of Physical Activity on Serum C-Reactive Protein and Inflammatory MarkersA Systematic Review. *J Am Coll Cardiol.* 2005;45(10):1563-1569.
263. Miller TL, Horgan S, Lipshultz SE. Exercise rehabilitation of pediatric patients with cardiovascular disease. *Prog Pediatr Cardiol.* 2005;20(1):27-37.

264. Touboul P-J, Labreuche J, Bruckert E*, et al.* HDL-C, triglycerides and carotid IMT: A meta-analysis of 21,000 patients with automated edge detection IMT measurement. *Atherosclerosis.* 2014;232(1):65-71.
265. Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. *Clin Immunol.* 2010;134(1):33-46.
266. Stemme S, Faber B, Holm J*, et al.* T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proceedings of the National Academy of Sciences.* 1995;92(9):3893-3897.
267. Binder CJ, Chang M-K, Shaw PX*, et al.* Innate and acquired immunity in atherogenesis. *Nat Med.* 2002;8(11):1218-1226.
268. Lim J, Kim YS, Kim SH*, et al.* Triglyceride enhances susceptibility to TNF-α-induced cell death in THP-1 cells. *Genes Genomics.* 2014;36(1):87-93.
269. Kastelein JJ, van der Steeg WA, Holme I*, et al.* Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. *Circulation.* 2008;117(23):3002-3009.
270. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* 2004;25(1):4-7.
271. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol.* 1999;19(4):972-978.
272. Higashi Y, Yoshizumi M. Exercise and endothelial function: Role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther.* 2004;102(1):87-96.
273. Numminen H, Syrjälä M, Benthin G, Kaste M, Hillbom M. The effect of acute ingestion of a large dose of alcohol on the hemostatic system and its circadian variation. *Stroke.* 2000;31(6):1269-1273.
274. Urbina EM, Khoury PR, McCoy CE*, et al.* Triglyceride to HDL-C ratio and increased arterial stiffness in children, adolescents, and young adults. *Pediatr.* 2013;131(4):e1082-e1090.
275. Rocchini AP. Obesity hypertension. *Am J Hypertens.* 2002;15(2):50S-52S.
276. Chapman MJ, Sposito AC. Hypertension and dyslipidaemia in obesity and insulin resistance: Pathophysiology, impact on atherosclerotic disease and pharmacotherapy. *Pharmacol Ther.* 2008;117(3):354-373.

277. Gambardella I, Blair PH, McKinley A, Baker R, Harkin DW. Triglyceride to HDL Ratio is a Reliable Predictor of Adverse Outcomes in Risk Stratification for Candidates Undergoing Abdominal Aortic Surgery. *Eur J Vasc Endovasc Surg.* 2011;41(2):249-255.
278. de Giorgis T, Mohn A. Could the triglyceride: HDL cholesterol ratio be considered a new marker of cardiovascular risk in obese children? *Clin Lipidol.* 2014;9(1):1-3.
279. Di Castelnuovo A, Costanzo S, Bagnardi V*, et al.* Alcohol dosing and total mortality in men and women - An updated meta-analysis of 34 prospective studies. *Arch Intern Med.* 2006;166:2437-2445.
280. White IR, Altmann DR, Nanchahal K. Alcohol consumption and mortality: modelling risks for men and women at different ages. *Br Med J.* 2002;325(7357):191-194.
281. Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, de Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. *Circulation.* 2002;105(24):2836-2844.
282. Ebrahimi-Mamaeghani M, Mohammadi S, Arefhosseini SR, Fallah P, Bazi Z. Adiponectin as a potential biomarker of vascular disease. *Vasc Health Risk Manag.* 2015;11:55-70.
283. Pischon T, Boeing H, Hoffmann K*, et al.* General and abdominal adiposity and risk of death in Europe. *N Engl J Med.* 2008;359(20):2105-2120.
284. World Health Organization. Global status report on alcohol and health. Geneva, Switzerland: World Health Organization; 2011.
285. Ando K, Kawarazaki H, Miura K*, et al.* Report of the Salt Reduction Committee of the Japanese Society of Hypertension. (1) Role of salt in hypertension and cardiovascular diseases. *Hypertens Res.* 2013;36(12):1009-1019.
286. Harskamp RE, Roe MT. Soluble Urokinase-Type Plasminogen Activator Receptor: A Useful Biomarker for Coronary Artery Disease and Clinical Outcomes? *J Am Heart Assoc.* 2014;3(5):e001431.
287. Eapen DJ, Manocha P, Ghasemzedah N*, et al.* Soluble Urokinase Plasminogen Activator Receptor Level Is an Independent Predictor of the Presence and Severity of Coronary Artery Disease and of Future Adverse Events. *J Am Heart Assoc.* 2014;3(5):e001118.

288. Panichi V, Maggiore U, Taccola D*, et al.* Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-reactive protein in haemodialysis patients. *Nephrol Dial Transplant.* 2004;19(5):1154-1160.
289. Rao M, Guo D, Perianayagam MC*, et al.* Plasma interleukin-6 predicts cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis.* 2005;45(2):324-333.
290. Volpato S, Guralnik JM, Ferrucci L*, et al.* Cardiovascular disease, interleukin-6, and risk of mortality in older women the women's health and aging study. *Circulation.* 2001;103(7):947-953.
291. Harris TB, Ferrucci L, Tracy RP*, et al.* Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med.* 1999;106(5):506-512.
292. Begum FD, HØGdall CK, Kjaer SK*, et al.* The prognostic value of plasma soluble urokinase plasminogen activator receptor (suPAR) levels in stage III ovarian cancer patients. *Anticancer Res.* 2004;24(3B):1981-1986.
293. Dass K, Ahmad A, Azmi AS, Sarkar SH, Sarkar FH. Evolving role of uPA/uPAR system in human cancers. *Cancer Treat Rev.* 2008;34(2):122-136.

CHAPTER 2

Study design and Methodology

1. STUDY DESIGN, PARTICIPANTS AND EXPERIMENTAL PROTOCOL

The international Prospective Urban and Rural Epidemiology (PURE) study is a multi-national study which was designed to examine changes in lifestyle and causes of the development of cardiovascular risk factors and chronic diseases through periodic standardised data collection.[1,2]

The PURE study framework is based on the assumption that the 'causal' pathways for the development of cardiovascular disease involve influences at multiple levels, as indicated in Figure 1. This gave rise to two main, overall objectives of PURE, namely (i) "to examine the relationship between societal influences and prevalence of risk factors and chronic non-communicable diseases measured at baseline" and (ii) "to examine the relationship between societal determinants and incidence of chronic non-communicable disease events and on changes in rates of selected risk factors".[1]

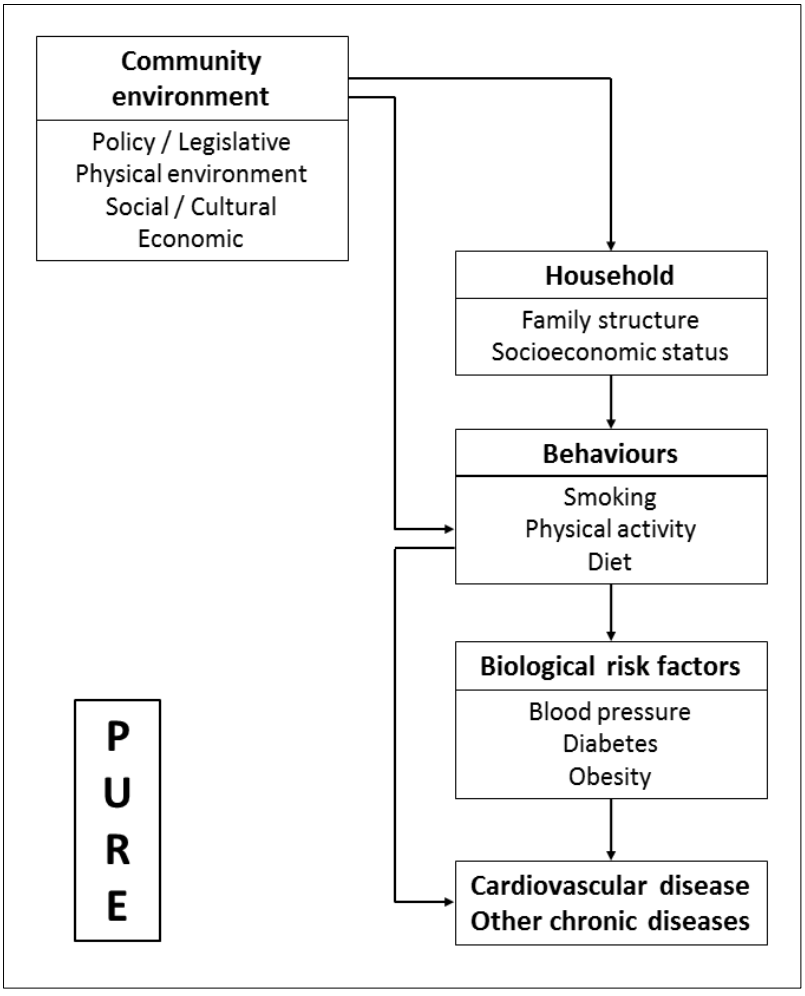


Figure 1 *Causal pathway of community influences on individual risk.*[1]

A minimum follow-up period of 10 years was planned, targeting 628 communities in urban and rural areas from more than 17 different countries around the world, including South Africa, that are at varying stages of epidemiologic transition (Figure 2).[1-3]

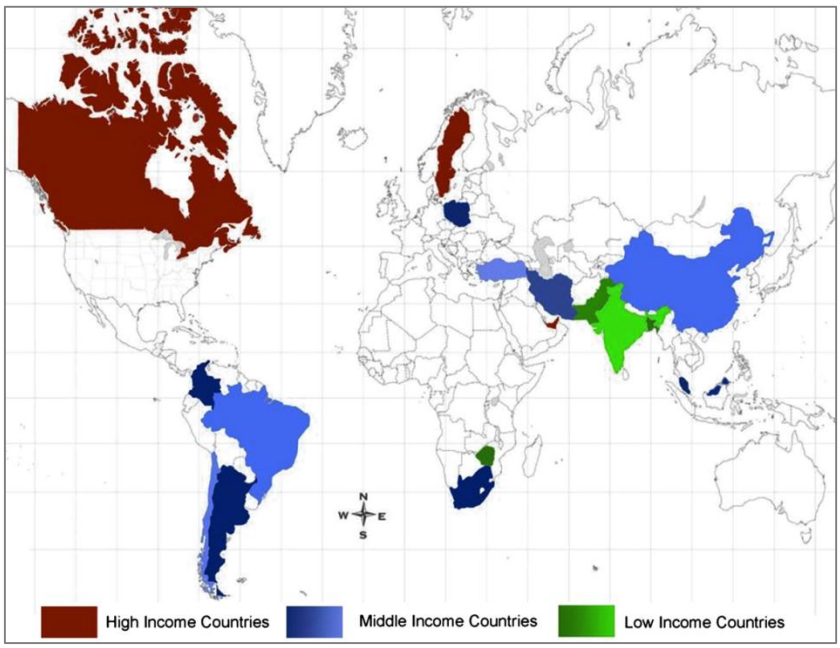


Figure 2 *Countries involved in the international PURE study, categorised by income.*[1]

For this sub-study, which is embedded in the South African leg of the international PURE study, participants were randomly recruited (door-to-door) from the Potchefstroom, Ganyesa and Tlakgameng areas in the North West Province, South Africa (Figure 3).

Figure 3 *Areas involved in this sub-study from the North West province, South Africa.*

Data collection took place twice, namely in 2005 and 2010. During these events, participants arrived at the rural and urban research facilities at approximately 08:00 after a 10-15 minute drive from their communities, which was provided by the research team. The participants were first introduced to the research setup after which the procedures were explained to them and informed consent was obtained from each participant. In each case, information was conveyed in each participant's native language by field workers from the communities. Participants were also free to withdraw at any time. Notably, participants were asked to fast for at least eight hours and not to smoke, exercise or climb the stairs at least 30 minutes prior to the measurements. After data collection, individual post-counselling was provided to each participant with regards to his or her general health (including HIV status, blood pressure levels and fasting glucose levels) and, where necessary, referrals were made to the local clinic or hospital if irregularities were identified.

Figure 4 *Data collection in the Ganyesa and Tlakgameng areas in the North West Province, South Africa.*

In total, 746 healthy black men (50.3 ± 10.3 years) and 1 264 women (49.6 ± 10.4 years) from urban (*n*=1 004) and rural (*n*=1 006) areas were examined during baseline data collection in 2005. Of these 2 010 participants, 322 were newly identified as being HIV infected. The first follow-up took place in 2010 where 1 292 participants returned for examination, of which 214 were tested as being HIV infected. However, 233 (33%) of the 718 participants that were lost to follow-up died during the five year period.

Permission for the execution of this study was obtained from the provincial Department of Health, the local authorities, as well as the tribal Chief from each specific rural area. The study protocol was approved by the Health Research Ethics Committee of the North-West University in Potchefstroom, South Africa, and complied with the Declaration of Helsinki (as revised in 2004).

A detailed layout of the experimental protocol and data collection procedures was previously described, and was consistent for baseline and follow-up.[1,4] The methodology appropriate to this sub-study will be discussed.

2. QUESTIONNAIRES

Questionnaires that were used in this study were validated for this population. All data were collected by specially trained field workers in each participant's home language, which was predominantly Setswana.

We used the PURE South Africa Adult Questionnaire, which was designed specifically for the PURE study, for the collection of data in 2005 and 2010 (Appendix A). Such data 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 education (0: no education; 1: a primary school qualification and higher), for alcohol consumption (0: never or formerly used alcohol products; 1: currently using alcohol products), as well as for tobacco use (0: never or formerly used tobacco products; 1: currently using tobacco products).

The Adapted BAECKE questionnaire was used to determine the physical activity index.[5] In order to obtain psychological data, the Mental Health Continuum-Short Form (which measures the degree of emotional well-being; social well-being and psychological well-being)[6] and the General Health Questionnaire (which measures the opposite of well-being, namely the degree of somatic symptoms, anxiety and insomnia, social dysfunction and severe depression)[7] were used. A quantitative food-frequency questionnaire, which was designed and validated for this population, was used to obtain dietary information,[8] whereas nutrient analysis was performed based on the South African food consumption tables[9] from the Medical Research Council of South Africa (Tygerberg, South Africa).

3. MORTALITY OUTCOME ASSESSMENT

After baseline data collection, trained fieldworkers visited participants at their homes every three months with the aim of retaining these participants and to determine their vital status. This was done under supervision of the senior researcher. To obtain cause of death data, verbal autopsy and family death certificates were used. The immediate and underlying causes of death were coded by a physician according to the International Classification of Diseases codes. For this sub-study, we classified cardiovascular mortality as death due to cardiovascular reasons, including cardiac failure, myocardial infarction and stroke.

4. ANTHROPOMETRIC MEASUREMENTS

We used standardised procedures to obtain all anthropometric measurements, as prescribed by the guidelines adopted at the National Institutes of Health sponsored Arlie Conference[10] and the International Society for the Advancement of Kinanthropometry (ISAK).[11] Participants were measured with minimal clothing, barefoot and in the correct prescribed position, followed by the recording of obtained values in the Adult Questionnaire.

A Holtain unstretchable flexible 7 mm wide metal tape (Crosswell, Wales) was used to determine waist- and hip circumference. We also used these values to calculate the waist-to-hip ratio.

Each participant's weight was measured with a standardised Precision Health Scale (A & D Company, Tokyo, Japan), while an Invicta Stadiometer (IP 1465, London, UK) was used to determine their height. Body mass index, which is used as another indirect measure of human adiposity,[12] was calculated from the obtained values by using the following formula: Body mass index (kg/m^2) = weight (kg) / height (m^2)

5. CARDIOVASCULAR MEASUREMENTS

A trained observer measured brachial systolic and diastolic blood pressure with the validated OMRON HEM-757 device (Omron Healthcare, Kyoto, Japan) at baseline and follow-up. After a ten minute rest period, measurements were taken in duplicate on the participant's right upper-arm, over the brachial artery; with a five minute rest period in between, while using an appropriate cuff size. During these measurements, each participant was in the sitting position, with his/her right arm in a relaxed position and supported at heart level. The second value was used for statistical analyses. We used these values to define hypertension according to the 2013 ESH/ESC guidelines as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg.[13]

6. BLOOD SAMPLING AND BIOCHEMICAL ANALYSES

A research nurse drew fasting blood samples from each participant's antebrachial vein with a sterile winged infusion set and syringes. Serum was prepared by centrifugation (within two hours after collection) at a speed of 2000 X *g* for 15 minutes at 10°C. The prepared serum was then transferred to micro-fuge tubes, samples were snap frozen on dry ice, and stored at -80°C in the laboratory until analysis. In the case where samples were obtained in rural areas, serum was snap frozen on dry ice and stored at -18°C (for a maximum of five days) until it could be transported to the laboratory facility, where it was stored at -80°C.

Soluble urokinase plasminogen activator receptor was determined from plasma (EDTA) samples with the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark)[14] at both baseline and follow-up, while interleukin-6 was determined in plasma with the electrochemiluminescence immunoassay method using an Elecsys 2010 (Roche, Basel, Switzerland) apparatus.

Using serum samples, a Konelab20i™ auto-analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland) was used in 2005 and a Cobas Integra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN) in 2010 to determine high-sensitivity C-reactive protein levels (by means of a particle enhanced turbidimetric assay), as well as gamma-glutamyltransferase, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and creatinine (by means of an enzymatic colorimetric method). By using whole blood (EDTA) samples, glycated haemoglobin was determined in 2005 and 2010 with the D-10 Haemoglobin testing system from Bio-Rad (Bio-Rad Laboratories Ltd., Hercules, CA, USA, #220-0101). This method was based on ion-exchange high-performance liquid chromatography. We further determined glucose in sodium fluoride tubes by means of an enzymatic reference method with hexokinase, using a Vitros DT6011 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA) in 2005 and a Cobas Integra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN) in 2010.

We determined HIV status from whole blood according to the protocol of the South African Department of Health, using the First Response rapid HIV card test (Premier Medical Corporation Limited, Daman, India). In the case of a positive result, the test was confirmed with the Pareeshak card test (BHAT Bio-tech, India) in 2005 and the SD BIOLINE HIV 1/2 3.0 card test (Standard Diagnostics, INC, Korea) in 2010. Upon confirmation of a positive result, whole blood samples were sent to a health clinic for CD4 cell count analysis by the National Health Laboratory service, where a flow cytometric method (Beckman COULTER® EPICS® XL™, Fullerton, USA) was used.

Finally, low-density lipoprotein cholesterol was calculated with the Friedewald formula[15] and estimated creatinine clearance with the Cockcroft-Gault formula.[16]

7. STATISTICAL ANALYSES

Variables with a non-Gaussian distribution were logarithmically transformed, including soluble urokinase plasminogen activator receptor, C-reactive protein, interleukin-6, glycated haemoglobin, glucose and gamma-glutamyltransferase. Percentage change was calculated as the difference between follow-up and baseline values, divided by the baseline value and multiplied by 100. In all cases, $p<0.05$ was regarded as statistically significant.

Independent *t*-tests, analyses of variance and Chi-square tests were performed to compare means and proportions, while analyses of covariance were used for comparison of groups while adjusting for covariates. Differences in means and proportions between baseline and follow-up within groups were determined with dependent *t*-tests and McNemar tests, respectively.

I established unadjusted relationships between variables with Pearson's correlation coefficients. To establish independent relationships partial correlations, multiple linear regression analyses (forward stepwise) and logistic regression analyses, to determine odds ratios, were performed.

Multivariable adjusted Cox regression analyses were applied to compute standardised hazard ratios, which expressed the risk for a 1-standard deviation increase in the independent variable. For comparison of mortality rates, I used Kaplan-Meier survival function estimates and the log-rank test. Finally, the five year risk for cardiovascular mortality in relation to both suPAR and IL-6 was plotted, as described in detail in Chapter 5.

Where applicable, the measure in which data were expressed was specified in the footnote and legend of each table and figure, while variables that were entered into models were also listed were applicable. In each case, covariates that were entered into the models were chosen, based on the literature[13,17] and on exploratory Pearson's and partial correlation analyses.

The programmes Statistica version 12.0 (Statsoft Inc., Tulsa, OK), IBM® SPSS® Statistics version 21.0 (IBM Corporation) and GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA) were used for all statistical analyses and preparation of graphs.

8. REFERENCES

1. Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S. The Prospective Urban Rural Epidemiology (PURE) study: Examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. *Am Heart J.* 2009;158(1):1-7.e1.
2. Yusuf S, Islam S, Chow CK*, et al.* Use of secondary prevention drugs for cardiovascular disease in the community in high-income, middle-income, and low-income countries (the PURE Study): a prospective epidemiological survey. *Lancet.* 2011;378(9798):1231-1243.
3. Yusuf S, Rangarajan S, Teo K*, et al.* Cardiovascular risk and events in 17 low-, middle-, and high-income countries. *N Engl J Med.* 2014;371(9):818-827.
4. Schutte AE, Schutte R, Huisman HW*, et al.* Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. *Int J Epidemiol.* 2012;41(4):1114-1123.
5. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr.* 1982;36(5):936-942.
6. Keyes CLM, Wissing M, Potgieter JP*, et al.* Evaluation of the mental health continuum–short form (MHC–SF) in setswana-speaking South Africans. *Clin Psychol Psychother.* 2008;15(3):181-192.
7. Goldberg DP, Hillier VF. A scaled version of the General Health Questionnaire. *Psychol Med.* 1979;9(1):139-145.
8. Wentzel-Viljoen E, Laubscher R, Kruger A. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. *S Afr J Clin Nutr.* 2011;24(3):143-148.
9. Wolmarans P, Danster N, Dalton A, Rossouw K, Schönfeldt H. Condensed food composition tables for South Africa. Cape Town, South Africa: Medical Research Council, 2010.
10. Lohman TG, Roche AF, Martorell R. Anthropometric Standardisation Reference Manual. Champaign, Illinois: Human Kinetic Books; 1988.

11. Marfell-Jones M, Olds T, Stewart A, Carter L. International standards for anthropometric assessment. Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry (ISAK); 2006.
12. Henderson RM. The bigger the healthier: Are the limits of BMI risk changing over time? *Econ Hum Biol.* 2005;3(3):339-366.
13. Mancia G, Fagard R, Narkiewicz K*, et al.* 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens.* 2013;31(7):1281-1357.
14. suPARnostic®. Enzyme immunoassay for quantitative determination of soluble urokinase plasminogen activator receptor in human plasma. Copenhagen, Denmark: ViroGates; 2008.
15. Johnson R, McNutt P, MacMahon S, Robson R. Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. *Clin Chem.* 1997;43(11):2183-2210.
16. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16(1):31-41.
17. Seedat YK, Rayner BL. South African Hypertension Guideline 2011. *S Afr Med J.* 2011;102(1):57-84.

CHAPTER 3

Associations of suPAR with lifestyle and cardiometabolic risk factors

DOI: 10.1111/eci.12278

ORIGINAL ARTICLE

Associations of suPAR with lifestyle and cardiometabolic risk factors

Shani Botha*, Carla M. T. Fourie*, Rudolph Schutte*, Annamarie Kruger† and Aletta E. Schutte*

*Hypertension in Africa Research Team (HART), †Africa Unit for Transdisciplinary Health Research (AUTHeR), North-West University, Potchefstroom, South Africa

This manuscript was published in the
European Journal of Clinical Investigation,
Volume 44, Issue 7, Pages 619-626, July 2014
(Appendix B)

ABSTRACT

Background Soluble urokinase Plasminogen Activator Receptor (suPAR), a novel indicator of low-grade inflammation, is associated with cardiovascular disease and mortality in the general population, while an unhealthy lifestyle influences inflammatory status. We aimed to explore the relationship of suPAR with lifestyle and cardiometabolic risk factors in a black South African population.

Design This cross-sectional study includes 1 068 men and women (56.4±10.1 years) from the North West province who took part in the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study in 2010. Captured data included a detailed lifestyle profile (tobacco use, alcohol consumption, physical activity, psychological and dietary intake status), biochemical analyses (suPAR, C-reactive protein (CRP), glucose and lipids), as well as cardiovascular and anthropometric measurements.

Results In exploratory analyses, we observed positive relationships between suPAR and lifestyle factors, such as tobacco use (p-trend<0.001), both alcohol consumption (p-trend=0.001) and gamma-glutamyltransferase (p-trend<0.001) and unemployment (p-trend=0.002). SuPAR and CRP correlated significantly (r=0.23; p<0.001). These relationships were confirmed in multiple regression analyses as suPAR independently associated with tobacco use (β=0.13; p<0.001), gamma-glutamyltransferase (β=0.24; p<0.001) and unemployment (β=0.07; p=0.039). SuPAR did not associate with the cardiometabolic factors glucose, lipids, blood pressure or measures of adiposity.

Conclusion SuPAR was independently associated with unhealthy lifestyle behaviours, but not with cardiometabolic risk factors suggesting that suPAR, as known predictor of cardiovascular disease and mortality, is augmented by modifiable cardiovascular risk factors. These findings emphasise the need for a healthy lifestyle to decrease the burden of cardiovascular disease in Africans.

Keywords African, C-reactive protein, cardiovascular disease, inflammation, lifestyle, South Africa

INTRODUCTION

An unhealthy lifestyle is known to be associated with low-grade inflammation [1]. Soluble urokinase plasminogen activator receptor (suPAR) is a relatively new general marker of low-grade inflammation [2-4] and can be used in combination with other inflammatory markers such as C-reactive protein (CRP) in order to improve the prediction of absolute cardiovascular risk [5].

The urokinase plasminogen activator receptor (uPAR) is a three domain protein [4] and is expressed on several immune cells, as well as on endothelial cells, smooth muscle cells and malignant cells [4, 6, 7]. The soluble version, suPAR, forms when uPAR is cleaved from the cell surface [4] and then functions in a paracrine manner [8]. SuPAR relates to the development of cardiovascular disease, even in healthy individuals [9, 10], as well as with cardiovascular risk factors such as increased blood pressure [11] and heart rate [12]. SuPAR is further associated with subclinical organ damage, predicts cardiovascular events [13] and predicts all-cause mortality in the general population [9, 14]. Studies have shown that CRP independently associates with gamma-glutamyltransferase (GGT) and physical activity in black South Africans [15], as well as with systolic and diastolic blood pressure levels [11], smoking [16], overweight and obesity [17, 18] in other population groups.

Evidence from Caucasian populations show that inflammation associates with unhealthy lifestyle factors such as smoking [19, 20], excessive alcohol consumption [16], physical inactivity [20, 21], malnutrition [22] and psychological conditions [23].

South Africa is currently undergoing a health transition where traditional African lifestyles are being replaced by westernised behaviour [15, 24]. Even though the South African government has made important changes over the last three years in order to address health challenges [24], heavy episodic alcohol consumption (>40%) and daily adult smoking prevalence (14%) remain high [25, 26]. Since our group has previously shown that suPAR levels are higher in Africans compared to Caucasians [27], we aimed to explore the relationship of suPAR with lifestyle and cardiometabolic risk factors in a black South African population.

MATERIALS AND METHODS

Study design and participants

The international Prospective Urban and Rural Epidemiology (PURE) study is an epidemiological, multi-national prospective study, assessing factors that could play a role in the development of cardiovascular disease [28]. This cross-sectional sub-study is embedded in the PURE study and includes 1 068 healthy black men (56.4±10.1 years) and women (56.3±10.2 years) from urban and rural areas in the North West province, South Africa. Data collection for this sub-study took place in 2010 and HIV infected participants (*n*=216) were excluded due to known effects on suPAR [29].

Experimental protocol

The detailed experimental protocol for data collection was previously described [30]. The study protocol was approved by the Ethics Committee of the North-West University in Potchefstroom, South Africa and complies with the Declaration of Helsinki (as revised in 2008). Reporting of the study conforms to STROBE statement along with references to STROBE [31] and the broader EQUATOR guidelines [32].

Measurements

Standardised procedures were used to measure height, weight, hip and waist circumference of each participant with a stadiometer (Invicta Stadiometer, IP 1465, London, UK); a scale (Precision Health Scale, A & D Company, Tokyo, Japan) and a measuring tape (Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales) [33].

Brachial systolic and diastolic blood pressure measurements were taken in duplicate on the right arm with an OMRON HEM-757 device (Omron Healthcare, Kyoto, Japan), while the participant was in the sitting position, with a five minute rest in-between. The last value was used.

Biochemical analyses

Fasting blood samples were obtained and serum as well as plasma was prepared according to the appropriate methods. Samples were snap frozen on dry ice, followed by storage at -80°C in the laboratory until analysis. SuPAR levels were determined from

plasma (EDTA) samples with the suPARnostiC® ELISA kit (ViroGates, Copenhagen, Denmark), while glycated haemoglobin levels were determined from plasma (EDTA) samples using the D-10 Haemoglobin testing system from Bio-Rad (#220-0101), which is based on ion-exchange high-performance liquid chromatography. All other biochemical analyses were performed (using serum samples) with the Cobas Integra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN) as follow: High-sensitivity CRP levels were analysed by particle enhanced turbidimetric assay, glucose levels (collected in sodium fluoride tubes) were determined by means of an enzymatic reference method with hexokinase, while high-density lipoprotein cholesterol, triglyceride and GGT levels were analysed by an enzymatic colorimetric assay method.

Questionnaires

All questionnaire data, including demographic and lifestyle information, were obtained by specially trained field workers in the participants' home language. Nominal coding variables were created for education (0: no education; 1: a primary school qualification and higher), for alcohol consumption (0: never used alcohol products; 1: currently using alcohol products), as well as for tobacco use (0: never or formerly used tobacco products; 1: currently using tobacco products). The Adapted BAECKE questionnaire was used to determine the physical activity index [34]. The Mental Health Continuum–Short Form (which measures the degree of emotional well-being; social well-being and psychological well-being) [35] and the General Health Questionnaire (which measures the opposite of well-being, namely the degree of somatic symptoms, anxiety and insomnia, social dysfunction and severe depression) [36] were used to obtain psychological data and are mentioned in our results as an "index of psychological well-being" and "mental health problems", respectively. A quantified food-frequency questionnaire, which was designed and validated for this population, was used to obtain dietary information [37], whereas nutrient analysis was performed based on the South African food-consumption tables [38] by the Medical Research Council of South Africa (Tygerberg, South Africa).

Statistical analyses

Statistical analyses and preparation of graphs were performed by using Statistica version 11.0 (Statsoft Inc., Tulsa, OK) and GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA). Skewed variables were logarithmically transformed

(suPAR, CRP, glycated haemoglobin, glucose and GGT). We compared means between tertiles of suPAR with analyses of variance tests (for continuous data) and with Chi-square tests (for categorical data). Additional analyses of covariance were performed on blood pressure and the lipid profile, while adjusting for medication use (including anti-hypertensive, anti-inflammatory, anti-diabetic and statin medication).

Additionally, we investigated independent associations of suPAR or CRP with lifestyle and cardiometabolic risk factors by using forward stepwise multiple regression analyses. Based on the literature as well as on univariate analyses, covariates considered for entry into the model included CRP (or suPAR), gender, age, locality, employment, GGT, current/former tobacco use, systolic blood pressure, resting heart rate, triglycerides, body mass index and energy intake.

RESULTS

Table 1 shows the characteristics of the study population, stratified by suPAR tertiles. There were fewer men (34.6%) than women, whereas the distribution between urban and rural areas was relatively equal (45.5% vs. 54.5%). The mean age of the total group was 56.4±10.1 years and increased significantly with suPAR (p-trend<0.001). Both resting heart rate and CRP also increased with increasing suPAR (p-trend<0.001) (Figure 1). Regarding lifestyle factors, 85.7% of the participants were unemployed and suPAR were higher in those participants that were unemployed (p-trend=0.002) (Table 1). Current tobacco use and alcohol consumption were highest, while the amount that have never used tobacco were lowest in the third suPAR tertile (p-trend=0.001). However, no differences in physical activity index, dietary intake or in the psychological profile were seen (Table 1). In the case of body mass index and waist-to-hip ratio, no significant differences were found between the first and third tertiles of suPAR. Cardiometabolic risk factors such as blood pressure, glycated haemoglobin, glucose and lipids did not increase with increasing suPAR levels. Medication use was higher in the third tertile of suPAR (Table 1); however, adjustments for medication use did not change the above mentioned results (Table S1).

Table 1 Characteristics of a black South African study population, stratified by tertiles of suPAR.

		Soluble urokinase plasminogen activator receptor			
	Total group (*n* = 1068)	1st tertile (< 3.24 ng/mℓ) (*n* = 333)	2nd tertile (3.24-4.27 ng/mℓ) (*n* = 361)	3rd tertile (> 4.27 ng/mℓ) (*n* = 337)	*P*-trend
Socio-demographic profile					
Age (years)	56.4 ± 10.1	54.3 ± 0.55	57.1 ± 0.53*	57.6 ± 0.55*	< 0.001
Gender, men (%)	370/1068 (34.6)	121/333 (36.3)	116/361 (32.1)	126/337 (37.4)	0.304
Locality, rural (%)	582/1068 (54.5)	192/333 (57.7)	197/361 (54.6)	173/337 (51.3)	0.259
Education, *n* (%)	644/1033 (62.3)	212/328 (64.6)	224/350 (64.0)	189/322 (58.7)	0.227
Unemployment, *n* (%)	873/1019 (85.7)	261/324 (80.6)	296/342 (86.8)*	290/321 (90.3)*	0.002
Anthropometric measurements					
Body mass index (kg/m^2)	26.1 ± 7.63	25.8 ± 0.41	27.1 ± 0.40	25.1 ± 0.41	0.002
Waist circumference (cm)	82.7 ± 13.4	81.9 ± 0.73	83.9 ± 0.71	81.9 ± 0.74	0.069
Waist-to-hip ratio	0.88 ± 0.08	0.89 ± 0.01	0.87 ± 0.01*	0.89 ± 0.01	0.034
Cardiometabolic measurements					
Systolic blood pressure (mmHg)	137 ± 24.3	137 ± 1.35	137 ± 1.30	138 ± 1.34	0.573
Diastolic blood pressure (mmHg)	89 ± 13.7	89 ± 0.76	89 ± 0.74	90 ± 0.76	0.604
Resting heart rate (bpm)	65 ± 17.6	62 ± 1.00	63 ± 0.99	69 ± 1.03*	< 0.001
suPAR (ng/mℓ)	3.81 (2.29-6.92)	2.70 (2.06-3.20)	3.70 (3.29-4.22)*	5.51 (4.32-8.81)*	< 0.001
C-reactive protein (mg/ℓ)	3.54 (0.31-32.4)	2.49 (2.16-2.88)	3.61 (3.15-4.15)*	4.84 (4.20-5.59)*	< 0.001
Glycated haemoglobin (%)	6.08 (5.25-7.59)	6.04 (5.94-6.14)	6.15 (6.05-6.25)	6.05 (5.95-6.15)	0.258
Glucose (mmol/ℓ)	5.15 (3.98-7.76)	5.13 (4.99-5.27)	5.14 (5.01-5.28)	5.16 (5.03-5.30)	0.943
Triglyceride:HDL-cholesterol ratio	5.71 ± 21.9	1.29 ± 0.48	1.08 ± 0.46	1.93 ± 0.47	0.411
HDL-cholesterol (mmol/ℓ)	1.46 ± 0.60	1.49 ± 0.03	1.46 ± 0.03	1.44 ± 0.03	0.650
Triglycerides (mmol/ℓ)	1.31 ± 0.82	1.31 ± 0.05	1.29 ± 0.04	1.32 ± 0.05	0.930
Lifestyle					
GGT (U/ℓ)	44.4 (12.0-302)	35.6 (32.1-39.3)	40.6 (36.9-43.7)	60.9 (55.1-67.4)*	< 0.001
Alcohol consumption, *n* (%)	366/1005 (36.4)	97/322 (30.1)	119/337 (35.3)	139/315 (44.1)*	0.001
Tobacco use					
Never, *n* (%)	434/1029 (42.2)	176/325 (54.2)	144/350 (41.1)	98/321 (30.5)	< 0.001
Formerly, *n* (%)	105/1029 (10.2)	29/325 (8.92)	38/350 (10.9)	36/321 (11.2)	0.586
Currently, *n* (%)	490/1029 (47.6)	120/325 (36.9)	168/350 (48.0)	187/321 (58.3)	< 0.001
Physical activity index	6.43 ± 1.84	6.47 ± 0.12	6.40 ± 0.11	6.47 ± 0.11	0.870

Table 1 Characteristics of a black South African study population, stratified by tertiles of suPAR *cont.*

		Soluble urokinase plasminogen activator receptor			
	Total group (*n* = 1068)	1st tertile (< 3.24 ng/mℓ) (*n* = 333)	2nd tertile (3.24-4.27 ng/mℓ) (*n* = 361)	3rd tertile (> 4.27 ng/mℓ) (*n* =337)	*P*-trend
Dietary intake					
Energy (kJ/day)	11526 ± 7159	11275 ± 396	11796 ± 380	11675 ± 397	0.616
Saturated fat (g/day)	22.2 ± 0.62	22.3 ± 1.11	23.0 ± 1.06	21.6 ± 1.11	0.646
Polyunsaturated fat (g/day)	23.6 ± 0.68	22.4 ± 1.22	25.0 ± 1.17	23.7 ± 1.22	0.314
Total protein (g/day)	86.0 ± 63.2	84.2 ± 3.50	86.2 ± 3.36	89.1 ± 3.50	0.609
Total available carbohydrates (g/day)	387 ± 221	382 ± 12.2	396 ± 11.7	389 ± 12.2	0.704
Psychological profile					
Psychological well-being index	46.6 ± 11.7	46.5 ± 0.82	46.0 ± 0.77	47.3 ± 0.80	0.548
Mental health problems index	9.96 ± 6.40	10.2 ± 0.45	9.77 ± 0.43	9.84 ± 0.44	0.776
Medication use					
Anti-hypertensive, *n* (%)	300/1068 (28.1)	81/333 (24.3)	102/361 (28.3)	109/337 (32.3)*	0.070
Anti-inflammatory, *n* (%)	73/1068 (6.84)	23/333 (6.91)	21/361 (5.82)	27.337 (8.01)	0.520
Anti-diabetic, *n* (%)	46/1068 (4.31)	11/333 (3.30)	16/361 (4.43)	17/337 (5.04)	0.527
Statins, *n* (%)	13/1068 (1.22)	1/333 (0.30)	5/361 (1.39)	7/337 (2.08)*	0.116
Co-morbidities					
Metabolic syndrome, *n* (%)	301/988 (30.5)	85/317 (26.8)	116/343 (33.8)	98/320 (30.6)	0.148
Hypertension, *n* (%)	672/1030 (65.2)	209/322 (64.9)	221/347 (63.7)	215/326 (66.0)	0.828

suPAR, soluble urokinase plasminogen activator receptor, HDL, high-density lipoprotein; GGT: gamma-glutamyltransferase. Data are expressed as arithmetic mean ± standard error, geometric mean (95% confidence intervals) or % of *n*. *P*-values for the comparison between groups were obtained with analysis of variance and Chi-square tests. $P \leq 0.05$ regarded as statistically significant. *Statistically different from first suPAR tertile.

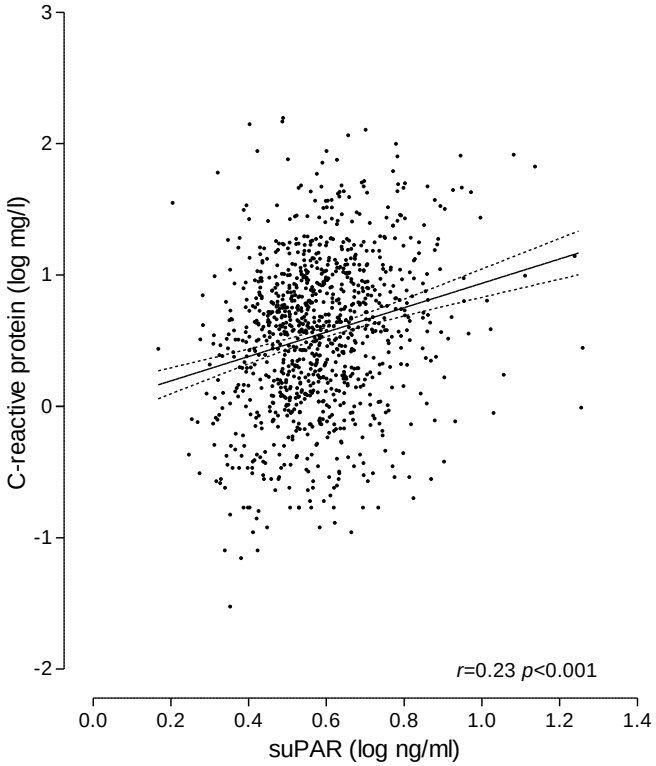


Figure 1 *Pearson correlation between suPAR and C-reactive protein. suPAR, soluble urokinase plasminogen activator receptor. Linear regression lines indicate mean ± 95% confidence intervals.*

In multiple regression analyses (Table 2), the associations with lifestyle were confirmed, as suPAR was significantly associated with unemployment (β=0.07; p=0.039), GGT (β=0.24; p<0.001) and tobacco use (β=0.13; p<0.001). When suPAR was replaced with CRP as dependent variable, CRP was significantly associated with suPAR (β=0.20; p<0.001), age (β=0.08; p=0.011), unemployment (β=0.09; p=0.005), resting heart rate (β=0.08; p=0.008) and body mass index (β=0.35; p<0.001).

During a sensitivity analysis, we repeated the aforementioned multiple regression analysis and replaced body mass index with either waist circumference or waist-hip-ratio. SuPAR was not associated with waist circumference or waist-to-hip ratio, as neither entered the regression model.

Table 2 Independent associations with suPAR and CRP.

	suPAR (log ng/mℓ)		CRP (log mg/ℓ)	
n = 889	*β (95% CI)*	*P*	*β (95% CI)*	*P*
R^2	0.170		0.182	
Adjusted R^2	0.161		0.176	
suPAR (log ng/mℓ)			0.20 (0.14;0.27)	< 0.001
CRP (log mg/ℓ)	0.19 (0.13;0.26)	< 0.001		
Gender, male (1,0)	-0.04 (-0.11;0.03)	0.223	--	--
Age (years)	0.09 (0.02;0.15	0.009	0.08 (0.02;0.15)	0.011
Locality, rural (1,0)	-0.03 (-0.10;0.03)	0.303	--	--
Unemployment (0,1)	0.07 (0.01;0.13)	0.039	0.09 (0.03;0.15)	0.005
GGT (log U/ℓ)	0.24 (0.17;0.31)	< 0.001	--	--
Tobacco use (1,0)	0.13 (0.07;0.20)	< 0.001	0.06 (-0.00;0.12)	0.065
Systolic blood pressure (mmHg)	--	--	-0.03 (-0.09;0.03)	0.313
Resting heart rate (bpm)	0.10 (0.03;0.16)	0.004	0.08 (0.02;0.15)	0.008
Triglycerides (mmol/ℓ)	-0.05 (-0.11;0.01)	0.092	--	--
Body mass index (kg/m^2)	--	--	0.35 (0.29;0.42)	< 0.001
Energy intake (kJ)	--	--	--	--

β: partial regression coefficient; 95% CI: 95% confidence intervals of *β*; suPAR: soluble urokinase Plasminogen Activator Receptor; CRP: C-reactive protein; GGT: gamma-glutamyltransferase. $P \leq 0.05$ regarded as statistically significant.

DISCUSSION

We investigated the relationship of suPAR with lifestyle and cardiometabolic risk factors. The main finding was that suPAR significantly and independently associated with lifestyle factors, including alcohol consumption, tobacco use and unemployment, but not with cardiometabolic risk factors such as blood pressure, dyslipidaemia, glycaemia or measures of adiposity.

On average, 36.4% of the participants in our study reported consumption of alcohol, and resultantly suPAR also showed a strong relationship with GGT. Although moderate alcohol consumption could have a systemic anti-inflammatory effect [16], severely elevated GGT, accompanying excessive alcohol consumption, could play a role in oxidative stress and inflammation [39] leading to cardiovascular disease development. Zimmerman *et al.* found that suPAR levels are highest in alcoholic compared to other liver disease aetiologies [40], which further supports the link that we found between suPAR, alcohol consumption and GGT.

Tobacco use can influence the immune system in many ways. Research shows that long-term smoking can result in inflammatory reactions due to the oxidative stress

caused by the chemicals in tobacco products [41]. This may explain why suPAR was related to tobacco use, which was relatively high (57.8%) in our study. Similarly, Persson *et al.* recently found higher suPAR levels in smokers from a Swedish population group, but suggested that smoking does not completely explain the relationship between suPAR and cardiovascular disease [42]. Even though we confirmed a significant relationship between suPAR and CRP [20], the strong link that were observed between inflammation and alcohol and tobacco use with suPAR as dependent variable, disappeared when we replaced suPAR with CRP as dependent variable. These findings confirm recent suggestions that the novel marker suPAR and the well-known marker CRP represent different biological processes with regard to vascular inflammation [5]. SuPAR, which is secreted by endothelial cells, smooth muscle cells and malignant cells [4, 6, 7], may play a role as biomarker of inflammation in the vascular wall [20]. Contrary to suPAR, CRP is synthesised by the liver in response to interleukin-6, of which 30% originates from adipose tissue [18, 20], and is rather associated with inflammation related to adipose tissue [20] and conditions such as overweight and obesity [17, 18].

Apart from the link between suPAR and both alcohol consumption and tobacco use, we also found that unemployment related to higher suPAR and CRP levels. Unemployment often coincides with self-destructive behaviours such as alcohol and substance abuse [43]. A recent study reported an increased risk of cardiovascular disease among the unemployed, despite their prudent dietary intake [24]. With regard to other lifestyle factors, evidence exist that a western diet (which includes high intakes of energy-dense foods) promotes secretion of inflammatory biochemical signals [22]. Another factor, regular physical activity, improves endothelial function and decrease cytokine production, thereby reducing CRP [21]. Additionally, chronic psychological stress causes endothelial injury, leading to recruitment, adhesion and activation of monocytes and lymphocytes [23]. However, despite this evidence, we failed to see a relationship between suPAR and dietary intake, physical activity and psychological status. Literature on this subject is very limited and the reasons for the lack of associations are yet to be elucidated.

Cardiometabolic risk factors, such as moderate elevations in blood pressure, result in an inflammatory response while inflammation in turn initiates a more severe hypertensive state [21]. In addition, an increase in systemic inflammation and

endothelial dysfunction are associated with an increase in heart rate [44], while a faster resting heart rate in turn, strongly amplifies the effects of inflammation [12]. Although we found no associations between suPAR and blood pressure, we did find a significant relationship between both inflammatory markers (suPAR and CRP) and resting heart rate. An inflammatory state and the oxidation of low-density lipoprotein cholesterol play a role in the development of cardiovascular disease. Hansson *et al.* reported that cholesterol alone could not explain the pathogenesis of atherosclerosis [45]. In our study, suPAR was not associated with dyslipidaemia. Furthermore, associations between inflammation and measures of adiposity could be impacted by glucose metabolism disturbances [46], but we found no associations between suPAR and either glucose or measures of adiposity. Black South Africans have a more favourable lipid profile, which may help in coronary protection, and it is known that coronary heart disease is relatively uncommon in sub-Saharan Africa [47]. In addition, previous studies suggested that suPAR may be linked closer to the inflammatory process in the later stages of atherosclerotic disease development [27] and in patients with severe underlying diseases [5].

This study needs to be interpreted within the context of its strengths and limitations. As only 14.3% were employed and participants were recruited from one of the nine provinces of South Africa, this study is not representative of the entire black South African population. Even though suPAR is mostly regarded as a prognostic marker, we could not determine cause-effect relationships due to the cross-sectional nature of the study. One may further argue that, because some of the variables were self-reported (such as the alcohol consumption and tobacco use), a lack of sensitivity may exist and may have diluted the result. However, a correlation existed between alcohol consumption and GGT, indicating a degree of reliability. The strengths of this study lies within the novel nature of suPAR, as most studies used CRP as inflammatory marker. Secondly, this is a relatively large sample, consisting of a much understudied black South African population and involved detailed information on a variety of lifestyle factors.

CONCLUSIONS

We showed that suPAR, a novel inflammatory marker, is independently associated with lifestyle choices (such as alcohol consumption and tobacco use) and unemployment, despite no direct links with cardiometabolic factors such as blood pressure, dyslipidaemia, glycaemia or adiposity. These findings emphasise the important need to address lifestyle awareness in order to limit the detrimental effect of modifiable risk factors on the health and mortality rate of black South Africans.

Conflicts of interest

None of the authors has a conflict of interest in relation to this work.

Acknowledgements

We are grateful towards the participants of this study, the PURE-SA research team, the field workers and supporting staff in the Africa Unit for Transdisciplinary Health Research (AUTHeR), North-West University, South Africa, as well as Dr S Yusuf (PURE-International) and the PURE project staff at the PHRI, Hamilton Health Sciences and McMaster University, ON, Canada.

This work was financially supported by SANPAD (South Africa - Netherlands Research Program on Alternatives in Development), PHRI (Population Health Research Institute), the MRC (Medical Research Council) of South Africa, the South African NRF (National Research Foundation) (GUN numbers 2069139 and FA2006040700010), the North-West University, Roche Diagnostics and the DAAD-NRF. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the DAAD-NRF.

REFERENCES

1 Egger G and Dixon J. Non-nutrient causes of low-grade, systemic inflammation: support for a ‘canary in the mineshaft’ view of obesity in chronic disease. Obes Rev 2011;12:339-45.

2 Yilmaz G, Köksal I, Karahan SC and Mentese A. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systemic inflammatory response syndrome. Clin Biochem 2011;44:1227-30.

3 Haugaard SB, Andersen O, Hansen TW, Eugen-Olsen J, Linneberg A, Madsbad S et al. The immune marker soluble urokinase plasminogen activator receptor is associated with new-onset diabetes in non-smoking women and men. Diabet Med 2012;29:479-87.

4 Eugen-Olsen J, Haugaard SB, Andersen O. Soluble urokinase plasminogen activator receptor (suPAR) as diagnostic marker for low-grade inflammation. Patent nr. EP2500730AI. Paris, FR: European Patent Office; 2012.

5 Lyngbæk S, Marott JL, Sehestedt T, Hansen TW, Olsen MH, Andersen O et al. Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. Int J Cardiol 2013;167:2904-11.

6 Chavakis T, Kanse SM, Yutzy B, Lijnen HR and Preissner KT. Vitronectin concentrates proteolytic activity on the cell surface and extracellular matrix by trapping soluble urokinase receptor-urokinase complexes. Blood 1998;91:2305-12.

7 Wittenhagen P, Kronborg G, Weis N, Nielsen H, Obel N, Pedersen SS et al. The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality. Clin Microbiol Infect 2004;10:409-15.

8 Blasi F and Sidenius N. The urokinase receptor: Focused cell surface proteolysis, cell adhesion and signaling. FEBS Lett 2010;584:1923-30.

9 Eugen-Olsen J, Andersen O, Linneberg A, Ladelund S, Hansen TW, Langkilde A et al. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. J Intern Med 2010;268:296-308.

10 Olson FJ, Thurison T, Ryndel M, Høyer-Hansen G and Fagerberg B. Soluble urokinase-type plasminogen activator receptor forms in plasma as markers of atherosclerotic plaque vulnerability. Clin Biochem 2010;43:124-30.

11 Pruijm M, Vollenweider P, Mooser V, Paccaud F, Preisig M, Waeber G et al. Inflammatory markers and blood pressure: sex differences and the effect of fat mass in the CoLaus Study. J Hum Hypertens 2013;27:169-75.

12 O Hartaigh B, Bosch JA, Carroll D, Hemming K, Pilz S, Loerbroks A et al. Evidence of a synergistic association between heart rate, inflammation, and cardiovascular mortality in patients undergoing coronary angiography. Eur Heart J 2013;34:932-41.

13 Sehestedt T, Lyngbæk S, Eugen-Olsen J, Jeppesen J, Andersen O, Hansen TW et al. Soluble urokinase plasminogen activator receptor is associated with subclinical organ damage and cardiovascular events. Atherosclerosis 2011;216:237-43.

14 Koch A, Voigt S, Kruschinski C, Sanson E, Duckers H, Horn A et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care 2011;15:R63.

15 Hamer M, Malan L, Schutte AE, Huisman HW, van Rooyen JM, Schutte R et al. Conventional and behavioral risk factors explain differences in sub-clinical vascular disease between black and Caucasian South Africans: The SABPA study. Atherosclerosis 2011;215:237-42.

16 Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB and Koenig W. Effect of alcohol consumption on systemic markers of inflammation. Lancet 2001;357:763-7.

17 Villegas R, Xiang YB, Cai H, Elasy T, Cai Q, Zhang X et al. Lifestyle determinants of c-reactive protein in middle-aged, urban Chinese men. Nutr Metab Cardiovasc Dis 2012;22:223-30.

18 Fontana L, Eagon JC, Trujillo ME, Scherer PE and Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 2007;56:1010-3.

19 Liakos CI, Vyssoulis GP, Michaelides AP, Chatzistamatiou EI, Theodosiades G, Toutouza MG et al. The effects of angiotensin receptor blockers vs. calcium channel blockers on the acute exercise-induced inflammatory and thrombotic response. Hypertens Res 2012;35:1193-200.

20 Lyngbæk S, Sehestedt T, Marott JL, Hansen TW, Olsen MH, Andersen O et al. CRP and suPAR are differently related to anthropometry and subclinical organ damage. Int J Cardiol 2013;167:781–5.

21 Kasapis C and Thompson PD. The Effects of Physical Activity on Serum C-Reactive Protein and Inflammatory MarkersA Systematic Review. J Am Coll Cardiol 2005;45:1563-9.

22 Myers JL and Allen JC. Nutrition and Inflammation Insights on Dietary Pattern, Obesity, and Asthma. Am J Lifestyle Med 2012;6:14-7.

23 Black PH and Garbutt LD. Stress, inflammation and cardiovascular disease. J Psychosom Res 2002;52:1-23.

24 Pisa P, Behanan R, Vorster H and Kruger A. Social drift of cardiovascular disease risk factors in Africans from the North West Province of South Africa: the PURE study. Cardiovasc J Afr 2012;23:371-8.

25 World Health Organization. Global status report on alcohol and health. Geneva, Switzerland: World Health Organization; 2011.

26 Ando K, Kawarazaki H, Miura K, Matsuura H, Watanabe Y, Yoshita K et al. Report of the Salt Reduction Committee of the Japanese Society of Hypertension. (1) Role of salt in hypertension and cardiovascular diseases. Hypertens Res 2013;36:1009-19.

27 Schutte AE, Myburgh A, Olsen MH, Eugen-Olsen J and Schutte R. Exploring soluble urokinase plasminogen activator receptor and its relationship with arterial stiffness in a bi-ethnic population: the SAfrEIC-study. Thromb Res 2012;130:273-7.

28 Teo K, Chow CK, Vaz M, Rangarajan S and Yusuf S. The Prospective Urban Rural Epidemiology (PURE) study: Examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. Am Heart J 2009;158:1-7.e1.

29 Fourie C, Rooyen J, Kruger A, Olsen M, Eugen-Olsen J, Schutte R et al. Soluble Urokinase Plasminogen Activator Receptor (suPAR) is Associated with Metabolic Changes in HIV-1-Infected Africans: A Prospective Study. Inflammation 2012;35:221-9.

30 Fourie CMT, Van Rooyen JM, Kruger A and Schutte AE. Lipid abnormalities in a never-treated HIV-1 subtype C-infected African population. Lipids 2010;45:73-80.

31 Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC and Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Prev Med 2007;45:247-51.

32 Simera I, Moher D, Hoey J, Schulz K and Altman D. A catalogue of reporting guidelines for health research. Eur J Clin Invest 2010;40:35-53.

33 Marfell-Jones M, Olds T, Stewart A, Carter L. International standards for anthropometric assessment. Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry (ISAK); 2006.

34 Baecke JA, Burema J and Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36:936-42.

35 Keyes CLM, Wissing M, Potgieter JP, Temane M, Kruger A and van Rooy S. Evaluation of the mental health continuum–short form (MHC–SF) in setswana-speaking South Africans. Clin Psychol Psychother 2008;15:181-92.

36 Goldberg DP and Hillier VF. A scaled version of the General Health Questionnaire. Psychol Med 1979;9:139-45.

37 Wentzel-Viljoen E, Laubscher R and Kruger A. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. S Afr J Clin Nutr 2011;24:143-8.

38 Wolmarans P, Danster N, Dalton A, Rossouw K, Schönfeldt H. Condensed food composition tables for South Africa. Cape Town, South Africa: Medical Research Council, 2010.

39 Yamada J, Tomiyama H, Yambe M, Koji Y, Motobe K, Shiina K et al. Elevated serum levels of alanine aminotransferase and gamma glutamyltransferase are markers of inflammation and oxidative stress independent of the metabolic syndrome. Atherosclerosis 2006;189:198-205.

40 Zimmermann HW, Koch A, Seidler S, Trautwein C and Tacke F. Circulating soluble urokinase plasminogen activator is elevated in patients with chronic liver disease, discriminates stage and aetiology of cirrhosis and predicts prognosis. Liver Int 2012;32:500-9.

41 Arnson Y, Shoenfeld Y and Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010;34:J258-J65.

42 Persson M, Engström G, Björkbacka H and Hedblad B. Soluble urokinase plasminogen activator receptor in plasma is associated with incidence of CVD. Results from the Malmö Diet and Cancer Study. Atherosclerosis 2012;220:502-5.

43 Bradshaw D. Determinants of health and their trends. In: Barron P, Roma-Reardon J, eds. Durban, SA: Health Systems Trust; 2008.

44 Nanchen D, Stott DJ, Gussekloo J, Mooijaart SP, Westendorp RGJ, Jukema JW et al. Resting heart rate and incident heart failure and cardiovascular mortality in older adults: role of inflammation and endothelial dysfunction: the PROSPER study. Eur J Heart Fail 2012;15:581-8.

45 Hansson GK. Atherosclerosis—An immune disease: The Anitschkov Lecture 2007. Atherosclerosis 2009;202:2-10.

46 Thorand B, Baumert J, Döring A, Herder C, Kolb H, Rathmann W et al. Sex differences in the relation of body composition to markers of inflammation. Atherosclerosis 2006;184:216-24.

47 Opie LH and Seedat YK. Hypertension in sub-Saharan African populations. Circulation 2005;112:3562-8.

SUPPLEMENTARY INFORMATION

Table S1 Blood pressure and the lipid profile of a black South African study population, adjusted for medication use.

		Soluble urokinase plasminogen activator receptor			
	Total group (*n* = 1068)	1st tertile (< 3.24 ng/mℓ) (*n* = 333)	2nd tertile (3.24-4.27 ng/mℓ) (*n* = 361)	3rd tertile (> 4.27 ng/mℓ) (*n* = 337)	*P*-trend
Systolic blood pressure (mmHg)	137 ± 24.3	137 ± 1.35	137 ± 1.30	138 ± 1.34	0.620
Diastolic blood pressure (mmHg)	89 ± 13.7	89 ± 0.77	89 ± 0.74	90 ± 0.76	0.641
Triglyceride:HDL-cholesterol ratio	5.71 ± 21.9	1.33 ± 0.48	1.08 ± 0.46	1.90 ± 0.47	0.443
HDL-cholesterol (mmol/ℓ)	1.46 ± 0.60	1.49 ± 0.03	1.46 ± 0.03	1.45 ± 0.03	0.720
Triglycerides (mmol/ℓ)	1.31 ± 0.82	1.31 ± 0.05	1.29 ± 0.04	1.31 ± 0.05	0.941

HDL, high-density lipoprotein. Data are expressed as arithmetic mean ± standard error. *P*-values for the comparison between groups were obtained with analyses of covariance. *P* ≤ 0.05 regarded as statistically significant. *Statistically different from first suPAR tertile.

European Journal of Clinical Investigation ESCI

SUMMARY OF INSTRUCTIONS TO AUTHORS

JOURNAL DETAILS				
Title:	*European Journal of Clinical investigation* (Eur J Clin Invest)			
Impact factor:	3.365			
Publisher:	Wiley-Blackwill			
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.				
JOURNAL GUIDELINES				
Author guidelines:	http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2362			
Title:	70 char (incl. spaces)		**Language:**	English (U.K.)
Keywords:	max 6		**Spacing:**	double (all)
Manuscript (words):	max 2700 (text only)		**Font:**	12
Abstract (words):	max 250		**Margins:**	2.5cm
Conflict of interest:	Yes		**Page nr:**	From title page
Acknowledgements:	Yes		**Alignment:**	Not justified
Tables & Figures:	max 5			
	Headings: <15 words			
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.

CHAPTER 4

SuPAR and hypertension among black South Africans after 5 years

Hypertension Research (2015) 38, 439–444
© 2015 The Japanese Society of Hypertension All rights reserved 0916-9636/15
www.nature.com/hr

ORIGINAL ARTICLE

Soluble urokinase plasminogen activator receptor and hypertension among black South Africans after 5 years

Shani Botha[1], Carla MT Fourie[1], Rudolph Schutte[1], Jesper Eugen-Olsen[2] and Aletta E Schutte[1]

This manuscript was published in
Hypertension Research,
Volume 38, Pages 439-444, March 2015
(Appendix B)

ABSTRACT

Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker that links inflammation with cardiovascular risk. However, studies linking suPAR and hypertension are scant. We firstly determined whether baseline suPAR is elevated in normotensive black South Africans that developed hypertension over five years, compared to those that remained normotensive; and secondly whether hypertension is associated with suPAR. This sub-study is embedded in the South African leg of the Prospective Urban and Rural Epidemiology study, performed in the North West Province. We investigated 429 normotensive individuals, of which 191 developed hypertension and 238 remained normotensive over five years. We determined suPAR from plasma (EDTA) samples with the suPARnostic® ELISA kit and blood pressure with an OMRON HEM-757 device (Omron Healthcare, Kyoto, Japan). Despite similar mean baseline suPAR levels (p=0.43), suPAR increased more in the group that developed hypertension compared to those that remained normotensive (14.2% vs. 6.94%; p=0.007). Five-year percentage change in systolic blood pressure correlated positively (r=0.23; p=0.002) and associated independently with baseline suPAR (β=0.14; p=0.043), only in participants that developed hypertension. Participants were 1.41 times more likely (p=0.015) to develop hypertension with one standard deviation increase in percentage change in suPAR levels over five years. Change in systolic blood pressure was associated with baseline suPAR in hypertensive participants and change in suPAR with hypertensive status. This study highlights the need for more research on the role of suPAR in hypertension and cardiovascular disease development in black South Africans.

Keywords: African, epidemiology, hypertension, inflammation, South Africa.

INTRODUCTION

Inflammation forms an integral part of the mechanisms involved in the development of cardiovascular and metabolic diseases and has received attention as a factor mediating both the genesis and the development of hypertension.[1-3]

In a hypertensive milieu, factors such as excessive mechanical stress increase oxidative stress, impair endothelial function and lead to atherosclerosis.[4] It is further known that, in hypertensive patients, vascular endothelial cells, monocytes and activated T-cells are especially activated and produce various cytokines.[2, 5] The urokinase plasminogen activator receptor, for example, is expressed by such cell types[6] and soluble urokinase plasminogen activator receptor (suPAR), a marker of inflammation,[7] is formed during inflammatory stimuli when the receptor is cleaved from the cellular membrane.[8]

SuPAR is associated with type 1[9] and 2 diabetes, incident cancer, cardiovascular disease and total mortality in the general population.[7] More recently, a large study involving 3 367 subjects (67% with coronary artery disease) showed that elevated levels of plasma suPAR are associated with the presence and severity of coronary artery disease, as well as that suPAR is an independent predictor of death and myocardial infarction.[10, 11] SuPAR further predicts cardiovascular events[10, 11] and provides additional prognostic information over and beyond the Framingham Risk Score, especially when combined with C-reactive protein (CRP).[12] There is however a paucity of information regarding the role of suPAR in hypertension, especially among the understudied black South African population in which the prevalence of hypertension is very high.[13]

We firstly investigated baseline and change in suPAR levels in black South Africans that developed hypertension and in those that remained normotensive after five years; and secondly, whether hypertension is associated with suPAR.

METHODS

Study design and participants

This sub-study is embedded in the international Prospective Urban and Rural Epidemiology (PURE) study.[14] We investigated 1 068 HIV-uninfected black men and women from urban and rural areas in the North West province, South Africa. Data collection for this sub-study took place twice, namely in 2005 (where 520 normotensive participants were included (Figure S1) and in 2010. From these normotensive participants, 191 (36.7%) developed hypertension over five years and 238 remained normotensive (excluding 73 participants that were using anti-hypertensive, anti-inflammatory, anti-diabetic and statin medication, and excluding 18 that had incomplete data at follow-up). The exact date on which participants became hypertensive during the five year interval, could however not be determined.

The study protocol was approved by the Ethics Committee of the North-West University in South Africa and complied with the Declaration of Helsinki (as revised in 2004). Informed consent was obtained by each participant. A detailed layout of the experimental protocol for data collection was previously described.[15]

Questionnaires

Specially trained field workers obtained questionnaire data in the participants' home language. Such data included demographic and lifestyle information such as locality, gender, age, education, employment, alcohol consumption, tobacco and medication use.

Anthropometric and cardiovascular measurements

We used standardised procedures to obtain anthropometric measurements, including height (Invicta Stadiometer, IP 1465, London, UK), weight (Precision Health Scale, A & D Company, Tokyo, Japan), hip and waist circumference (Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales).[16]

Using an appropriate cuff size, a trained observer took brachial systolic and diastolic blood pressure (SBP and DBP) with an OMRON HEM-757 device (Omron Healthcare, Kyoto, Japan), in duplicate, five minutes apart, while the participant was in the sitting

position. The second value was used. We defined hypertension according to the 2013 ESH/ESC guidelines as a SBP ≥140mmHg and/or DBP ≥90 mmHg.[17]

Biochemical analyses

After we obtained fasting blood samples, serum and plasma were prepared according to appropriate methods. These samples were snap frozen on dry ice and then stored at -80°C in the laboratory until analysis. SuPAR was determined from plasma (EDTA) samples with the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark).[18] A Konelab20i™ auto-analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland) was used in 2005 and a Cobas Integra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN) in 2010 to determine total cholesterol, high-density lipoprotein cholesterol, triglycerides, gamma-glutamyltransferase (GGT) and creatinine (by means of an enzymatic colorimetric method), as well as high-sensitivity CRP (by means of a particle enhanced turbidimetric assay) from serum samples. Glucose was determined in sodium fluoride tubes by means of an enzymatic reference method with hexokinase (Vitros DT6011 Chemistry Analyzer; Ortho-Clinical Diagnostics, Rochester, New York, USA in 2005 and Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN in 2010). Glycated haemoglobin was determined from whole blood (EDTA) samples, based on ion-exchange high-performance liquid chromatography, with the D-10 Haemoglobin testing system from Bio-Rad (Bio-Rad Laboratories Ltd., Hercules, CA, USA, #220-0101). Intra- and intercoefficients of variation for all assays were below 10%. We determined estimated creatinine clearance using the Cockcroft-Gault formula.[19]

Statistical analyses

Variables with a skewed distribution were logarithmically transformed (suPAR, CRP, glycated haemoglobin and glucose). We compared means and proportions with independent *t*-tests and Chi-square tests, respectively. Adjusted differences were determined with analyses of covariance, while differences in means and proportions between baseline and follow-up within each group were determined with dependent *t*-tests and McNemar tests, respectively. We established relationships between variables with Pearson correlations, while performing partial correlations and multiple linear regression analyses (forward stepwise) to establish independent relationships. In the latter, we used percentage change in SBP (%SBP) as the dependent variable, with

baseline and percentage change in suPAR (%suPAR) as the main independent variables. Other covariates that were entered into the models were chosen based on the literature[17, 20] and on exploratory partial correlation analyses. We further performed a logistic regression analysis to determine odds ratios, with hypertension status as dependent variable and %suPAR as independent variable. The analysis was repeated, while adjusting for other covariates. Percentage change was calculated as the difference between follow-up and baseline values, divided by the baseline value and multiplied by 100. Statistical analyses were performed with Statistica version 12.0 (Statsoft Inc., Tulsa, OK).

RESULTS

Of the 520 black South Africans that were normotensive at baseline, we included 429, of which 35% were men, 61% were situated in rural areas, 21% were employed and 58% had at least a primary school education. In these participants, suPAR related strongest to age, C-reactive protein, GGT and tobacco use (all $p<0.009$) (Table S1). Table 1 summarises the baseline and follow-up characteristics according to hypertensive status. Anthropometric measurements, systolic and diastolic blood pressure, glycated haemoglobin, creatinine clearance and tobacco use increased (all $p\leq0.002$), while heart rate and measures of alcohol consumption decreased (all $p\leq0.042$) in both those that remained normotensive and those that became hypertensive after the five years. At follow-up, the 191 participants that became hypertensive were older ($p=0.021$), had a higher alcohol consumption ($p=0.042$), GGT levels ($p=0.003$), body mass index ($p=0.006$) and waist circumference measure ($p<0.001$), while no differences in tobacco use ($p=0.72$) were seen. SBP increased 18% more ($p<0.001$) in participants that became hypertensive than those that remained normotensive (Table 2). Of the 191 hypertensives at follow-up, 27% were hypertensive despite being treated with anti-hypertensive medication (Table 1).

Table 1 Characteristics of black South African participants showing differences in baseline (2005) and follow-up (2010) of participants that remained normotensive (*n* = 238) and those that developed hypertension (*n* = 191) after 5 years, respectively.

	Normotensive (baseline) - Normotensive (follow-up)			Normotensive (baseline) - Hypertensive (follow-up)		
	Baseline	Follow-up	*P*-value	Baseline	Follow-up	*P*-value
Demographic profile						
Age (years)	48.4 ± 0.58 [a]	52.9 ± 0.58 [b]	< 0.001	50.5 ± 0.69 [a]	55 ± 0.69 [b]	< 0.001
Gender, men (%)	90/238 (37.8)			64/191 (33.5)		
Anthropometric measurements						
Body mass index (kg/m^2)	23.3 ± 0.39 [a]	24.6 ± 0.44 [b]	< 0.001	25.5 ± 0.50 [a]	26.5 ± 0.53 [b]	< 0.001
Waist circumference (cm)	76.1 ± 0.74 [a]	78.5 ± 0.77 [b]	< 0.001	81.1 ± 0.91 [a]	83.6 ± 0.98 [b]	< 0.001
Waist-to-hip ratio	0.81 ± 0.01 [a]	0.88 ± 0.01	< 0.001	0.83 ± 0.01 [a]	0.88 ± 0.01	< 0.001
Cardiovascular measurements						
Systolic blood pressure (mmHg)	115 ± 0.75 [a]	119 ± 0.77 [b]	< 0.001	121 ± 0.79 [a]	147 ± 1.18 [b]	< 0.001
Diastolic blood pressure (mmHg)	76 ± 0.54 [a]	79 ± 0.47 [b]	< 0.001	80 ± 0.52 [a]	95 ± 0.66 [b]	< 0.001
Heart rate (bpm)	70 ± 0.93 [a]	63 ± 0.77	< 0.001	74 ± 1.10 [a]	65 ± 1.01	< 0.001
Biochemical variables						
suPAR (ng/mℓ)	3.42 (2.21-6.13)	3.56 (2.18-6.29) [b]	0.016	3.51 (2.30-5.96)	3.97 (2.32-7.06) [b]	< 0.001
C-reactive protein (mg/ℓ)	2.46 (0.20-42.5)	2.90 (0.21-32.6)	0.12	3.33 (0.33-39.5)	2.89 (0.37-18.0)	0.15
Glycated haemoglobin (%)	5.57 (5.00-6.30)	5.92 (5.30-6.80)	< 0.001	5.58 (4.90-6.60)	5.95 (5.20-7.10)	< 0.001
Total cholesterol:HDL-cholesterol ratio	3.58 ± 0.08	3.62 ± 0.09	0.55	3.63 ± 0.10	3.86 ± 0.11	0.005
Triglycerides:HDL-cholesterol ratio	0.90 ± 0.04	0.92 ± 0.04	0.66	0.89 ± 0.05	1.05 ± 0.06	0.001
Creatinine clearance (mℓ/min)	89.3 ± 2.34	96.7 ± 2.45	0.002	92.6 ± 3.47	102 ± 2.77	0.001
Lifestyle						
Gamma-glutamyltransferase (U/ℓ)	40.6 (19.2-178) [a]	33.6 (21.1-344) [b]	< 0.001	52.8 (11.9-168) [a]	43.9 (12.0-229) [b]	< 0.001
Alcohol consumption, n (%)	70/238 (29.4) [a]	60/217 (27.7) [b]	< 0.001	74/188 (39.4) [a]	67/180 (37.2) [b]	< 0.001
Tobacco use, n (%)	130/237 (54.9)	139/226 (61.5)	0.007	103/189 (54.5)	110/184 (59.8)	0.042

Table 1 Characteristics of black South African participants showing differences in baseline (2005) and follow-up (2010) of participants that remained normotensive (*n* = 238) and those that developed hypertension (*n* = 191) after 5 years, respectively *cont.*

	Normotensive (baseline) - Normotensive (follow-up)			Normotensive (baseline) - Hypertensive (follow-up)		
	Baseline	Follow-up	*P*-value	Baseline	Follow-up	*P*-value
Medication use						
Anti-hypertensive, *n* (%)					52/191 (27.2)	
Anti-inflammatory, *n* (%)					12/191 (6.28)	
Anti-diabetic, *n* (%)					8/191 (4.19)	
Statins, *n* (%)					1/191 (0.52)	

Abbreviations: suPAR, soluble urokinase plasminogen activator receptor; HDL, high-density lipoprotein. Data are expressed as arithmetic mean ± standard error, geometric mean (5^{th} and 95^{th} percentile boundaries) or % of *n*. *P*-values for comparison between groups were obtained with dependent *t*-tests and McNemar tests. [a]Statistical significant difference between groups at baseline. [b] Statistical significant difference between groups at follow-up. $P \leq 0.05$ regarded as statistically significant.

Although baseline suPAR did not differ (p=0.43) between those that became hypertensive and remained normotensive, suPAR increased more in hypertensive participants (14.2% vs. 6.94%; p=0.007) and was higher at follow-up compared to the normotensive group (p=0.002). This was not the case for CRP at baseline (p=0.070) or follow-up (p=0.84) (Table 1 and Table 2).

Table 2 Change in characteristics of black South African participants over five years.

	Normotensive at follow-up (n = 238)	Hypertensive at follow-up (n = 191)	P-value
Percentage change (%)			
Waist circumference	3.26 ± 0.43	3.38 ± 0.57	0.86
Systolic blood pressure	3.63 ± 0.78	22.0 ± 1.15	< 0.001
Diastolic blood pressure	4.92 ± 0.92	19.4 ± 1.10	< 0.001
suPAR	6.94 ± 1.76	14.2 ± 2.05	0.007
C-reactive protein	132 ± 23.2	73.1 ± 18.4	0.055
Glycated haemoglobin	6.46 ± 0.49	7.60 ± 0.79	0.21
Triglycerides:HDL-cholesterol ratio	18.2 ± 5.00	27.1 ± 5.77	0.25
Gamma-glutamyltransferase	-9.48 ± 3.09	-8.10 ±3.74	0.77

Abbreviations: suPAR, soluble urokinase plasminogen activator receptor; HDL, high-density lipoprotein. Data are expressed as arithmetic mean ± standard error. P-values for comparison between normotensive and hypertensive groups were obtained with independent t-tests. $P \leq 0.05$ regarded as statistically significant.

In univariate analyses, %SBP correlated positively with baseline suPAR, but only in the hypertensive group (r=0.23; p=0.002) (Figure 1). %SBP did however not correlate with %suPAR in either the hypertensive (r=-0.11; p=0.14) or the normotensive (r=-0.06; p=0.42) groups (not shown). %SBP increased with an increase in baseline suPAR tertiles in those that developed hypertension (p-trend=0.030), but not in those that remained normotensive (p-trend=0.98) (Figure S1). Again, this was not the case when tertiles of %suPAR was used. We confirmed these findings in multivariate adjusted analyses (Table 3) where %SBP correlated positively with baseline suPAR only in the hypertensive group (β=0.14; p=0.043).

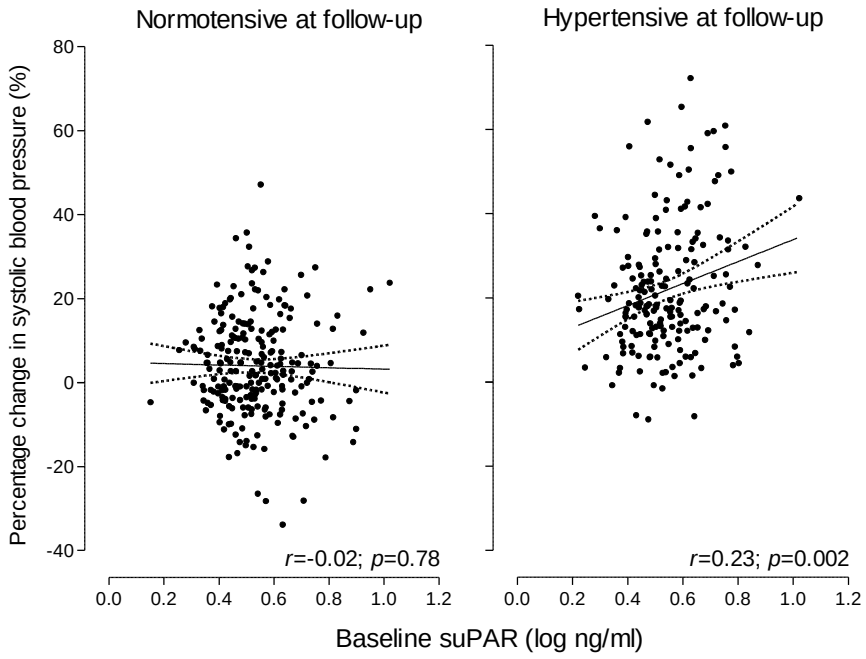


Figure 1 *Single linear regression analyses of the percentage change in systolic blood pressure and baseline soluble urokinase plasminogen activator receptor (suPAR) in participants that remained normotensive and those that developed hypertension over five years, respectively.*

Table 3 Independent associations with percentage change in systolic blood pressure.

	Normotensive at follow-up		Hypertensive at follow-up	
Multiple R^2; Adjusted R^2	0.40; 0.38		0.39; 0.36	
	β (95% CI)	*P*-value	*β* (95% CI)	*P*-value
Baseline SBP (mmHg)	-0.62 (-0.73;-0.51)	< 0.001	-0.56 (-0.69;-0.43)	< 0.001
Baseline suPAR (ng/mℓ)	-0.07 (-0.18;0.05)	0.27	0.14 (0.01;0.28)	0.043
Change in suPAR (%)	-0.10 (-0.22;0.02)	0.10	-0.06 (-0.19;0.07)	0.36
Age (years)			0.15 (0.00;0.29)	0.050
Gender, male (1,0)	0.13 (0.01;0.24)	0.030	0.11 (-0.03;0.25)	0.12
Waist circumference (cm)	0.14 (-0.01;0.28)	0.067	-0.14 (-0.33;0.06)	0.18
Triglyceride:HDL-cholesterol ratio	0.13 (0.02;0.25)	0.026	0.17 (0.03;0.31)	0.020
Gamma-glutamyltransferase (U/ℓ)			-0.09 (-0.23;0.05)	0.20
Creatinine clearance (mℓ/min)	-0.10 (-0.23;0.04)	0.17	-0.03 (-0.22;0.16)	0.74

Abbreviations: *β,* partial regression coefficient; 95% CI, 95% confidence intervals of *β*; SBP, systolic blood pressure; suPAR, soluble urokinase plasminogen activator receptor; HDL, high-density lipoprotein. Associations were determined by forward stepwise multiple regression analyses. Other co-variates entered included in the model: C-reactive protein, glycated haemoglobin, tobacco use. $P \leq 0.05$ regarded as statistically significant.

We determined standardised odds ratios with hypertension status as dependent variable and %suPAR as independent variable. In bivariate analyses we found that participants were 1.32 times (p=0.008) more likely to become hypertensive over five

years with each standard deviation increase in suPAR. These results also remained significant (odds ratio=1.41; *p*=0.015) in a multivariate analysis (Table 4).

Table 4 Logistic regression with hypertension status as dependent variable.

	Hypertension status	
	Odds ratio (95% CI)	*P*-value
Unadjusted analysis		
Percentage change in suPAR (%)	1.32 (1.08;1.63)	0.008
Baseline suPAR (ng/mℓ)	1.08 (0.89;1.32)	0.43
Adjusted analysis		
Percentage change in suPAR (%)	1.41 (1.07;1.86)	0.015
Baseline suPAR (ng/mℓ)	1.20 (0.92;1.55)	0.18
Baseline systolic blood pressure (mmHg)	1.82 (1.40;2.37)	<0.001
Age (years)	1.12 (0.85;1.47)	0.43
Gender, male (1,0)	0.68 (0.40;1.16)	0.16
Waist circumference (cm)	1.42 (1.00;2.03)	0.050
C-reactive protein (mg/ℓ)	0.79 (0.61;1.02)	0.068
Glycated haemoglobin (log %)	1.05 (0.81;1.37)	0.70
Triglyceride:HDL-cholesterol ratio	1.06 (0.81;1.37)	0.69
Creatinine clearance (mℓ/min)	1.08 (0.76;1.53)	0.68
Gamma-glutamyltransferase (U/ℓ)	1.39 (1.08;1.81)	0.012
Tobacco use, yes (1,0)	0.88 (0.53;1.46)	0.62

Abbreviations: 95% CI, 95% confidence intervals; suPAR, soluble urokinase plasminogen activator receptor; HDL, high-density lipoprotein. Associations were determined by logistic regression analyses. $P \leq 0.05$ regarded as statistically significant.

DISCUSSION

We investigated the inflammatory marker suPAR and associations thereof with hypertension in black South Africans. SuPAR increased over five years in both groups, but more prominently in those that developed hypertension. %SBP independently associated with baseline suPAR, only in the group that developed hypertension while, after multivariate adjustment, a one standard deviation increase in %suPAR was associated with a 41% more likely risk to be hypertensive.

Even though suPAR relates to renal disease,[21] cancer, type 1 and 2 diabetes,[7, 9] cardiovascular disease and mortality,[7] limited supportive evidence exist with regard to the positive relationship of suPAR with hypertension *per se*.

Hypertension is currently well known as an inflammatory disease[2] and increased levels of various inflammatory biomarkers precede the development of hypertension.[3, 22-24] We found that, even though both suPAR and blood pressure increased over five years,

%suPAR did not directly associate with change in systolic blood pressure, while baseline suPAR did. SuPAR levels were however similar for both groups at baseline and %suPAR did associate with being hypertensive. The controversy in our findings give rise to the question whether suPAR is involved in the process of hypertension development, or whether the hypertensive state may rather result in an increase in suPAR levels.

Inflammation affects multiple elements of the vascular wall, including the composition of the sub-endothelial matrix[25] by mechanisms such as elastin and collagen turnover.[26, 27] Urokinase plasminogen activator controls basement membrane and extracellular matrix degradation,[28] while CRP is involved in the process of vascular remodelling after injury.[29] Inflammation may therefore play a role to decrease large artery vascular elasticity[30, 31] and alter endothelial function.[32] The latter could lead to a decrease in nitric oxide bioavailability,[33, 34] impaired vasodilation and result in hypertension.[34]

On the other hand, higher blood pressure may be a stimulus for inflammation.[35] Hypertension is associated with a prothrombotic state, which includes abnormal endothelial and platelet function,[36] and evidently, treatment with anti-hypertensive drugs may have anti-fibrotic effects.[37] Hypertension further links to vascular changes, which could lead to an imbalance of decreased nitric oxide production or increased reactive oxygen species production, promoting endothelial dysfunction[4, 38] and ultimately result in elevated suPAR.[39] This is in concert with our findings where suPAR levels increased more in the group that became hypertensive than in those that remained normotensive.

It has been shown that CRP, a more familiar inflammatory marker, is correlated with systolic blood pressure and that hypertension is associated with increased CRP where diabetes mellitus is apparent.[40] On the contrary, in a study on 2 432 apparently healthy subjects, Bautista et al.[23] found that tumour necrosis factor-α and interleukin-6 could be independent risk factors for hypertension, but did not find any association between CRP and hypertensive status. This was also the case in our study where the association with hypertension remained for suPAR, but not for CRP when both markers were entered into the model. We further did not find any difference in CRP levels between hypertensives and normotensives at baseline or follow-up. Lyngbæk and colleagues[39] showed that suPAR and CRP represent different biological processes in vascular inflammation. Where suPAR is secreted by endothelial and smooth muscle cells,[6] CRP

originates from adipose tissue and is synthesised by the liver.[39] This supports the association of CRP with inflammation related to adipose tissue, while suPAR may rather play a role as biomarker of inflammation in the vascular wall.[39] Furthermore, Andersen et al.[41] have indicated that suPAR may be a more stable marker of the immune state than CRP, since suPAR has a high stability in plasma samples,[42, 43] has limited circadian changes[41] and is not affected by repeated freeze-thaw cycles of samples and sample schedule.[43] These properties make suPAR an attractive clinical marker of inflammation[42] which could be of value in prognostic algorithms.[44]

This study adds information to the growing body of evidence that link inflammation to hypertension, also in Africans.[22, 23, 45, 46] However, to our knowledge, this is the first study to report a link between suPAR and hypertension in Africans. We included a relatively large sample of the understudied black South African population, and other studies on suPAR were mostly conducted on European populations. Nevertheless, this study must also be interpreted within the context of its potential limitations. As participants were recruited from one of the nine provinces of South Africa and only 21% were employed at baseline, this study may not be representative of the entire black South African population. We did not measure body temperature or leukocyte count and can therefore not exclude underlying infections. As blood pressure was measured five years apart, we could not calculate the exact duration that each participant was hypertensive, and reverse causality should therefore be taken into consideration. However, the longitudinal nature of this study allowed for the evaluation of those that developed hypertension.

In conclusion, we showed that suPAR, an inflammatory marker, was higher and increased more prominently in participants that developed hypertension over five years than in those that remained normotensive. %SBP was independently associated with baseline suPAR, while becoming hypertensive was associated with an increase in suPAR. Whether inflammation leads to the development of hypertension or vice versa, remains to be established. However, it is known that suPAR is closely linked to cardiovascular disease. Our findings emphasize the important need to acknowledge the role of inflammation in hypertension and may permit the further investigation of the use of suPAR as a potential marker for early risk identification and intervention.

Conflict of interest

Jesper Eugen-Olsen is a founder, shareholder and board member of ViroGates A/S, Denmark, the company that produces the suPARnostic® assay. Jesper Eugen-Olsen is an inventor on a patent on suPAR and risk. Copenhagen University Hospital Hvidovre, Denmark, owns the patent, which is licensed to ViroGates A/S.

Acknowledgements

We are grateful towards the participants of this study, the PURE-SA research team, the field workers and supporting staff in the Africa Unit for Transdisciplinary Health Research (AUTHeR), North-West University, South Africa, as well as Dr S Yusuf (PURE-International) and the PURE project staff at the PHRI, Hamilton Health Sciences and McMaster University, ON, Canada.

This work was financially supported by SANPAD (South Africa - Netherlands Research Program on Alternatives in Development), PHRI (Population Health Research Institute), the MRC (Medical Research Council) of South Africa, the South African NRF (National Research Foundation) (GUN numbers 2069139 and FA2006040700010), the North-West University, Roche Diagnostics and the German Academic Exchange Service (DAAD)-NRF. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the DAAD-NRF.

REFERENCES

1. Aznaouridis KA, Stefanadis CI. Inflammation and arterial function. *Artery Res* 2007; **1**(1): 32-38.
2. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM. Inflammation, immunity, and hypertension. *Hypertension* 2011; **57**(2): 132-140.
3. Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. *JAMA* 2003; **290**(22): 2945-2951.
4. Higashi Y, Kihara Y, Noma K. Endothelial dysfunction and hypertension in aging. *Hypertens Res* 2012; **35**: 1039-1047.
5. Chen N-G, Abbasi F, Lamendola C, McLaughlin T, Cooke J, Tsao P, Reaven G. Mononuclear cell adherence to cultured endothelium is enhanced by hypertension and insulin resistance in healthy nondiabetic volunteers. *Circulation* 1999; **100**(9): 940-943.
6. Intzilakis T, Hartmann G, Mouridsen MR, Eugen-Olsen J, Kumarathurai P, Madsbad S, Almdal TP, Haugaard SB, Sajadieh A. Soluble urokinase plasminogen activator receptor, C-reactive protein and triglyceride are associated with heart rate variability in non-diabetic Danes. *Eur J Clin Invest* 2013; **43**(5): 457-468.
7. Eugen-Olsen J, Andersen O, Linneberg A, Ladelund S, Hansen TW, Langkilde A, Petersen J, Pielak T, Møller LN, Jeppesen J, Lyngbæk S, Fenger M, Olsen MH, Hildebrandt PR, Borch-Johnsen K, Jørgensen T, Haugaard SB. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. *J Intern Med* 2010; **268**(3): 296-308.
8. Huai Q, Mazar AP, Kuo A, Parry GC, Shaw DE, Callahan J, Li Y, Yuan C, Bian C, Chen L. Structure of human urokinase plasminogen activator in complex with its receptor. *Science* 2006; **311**(5761): 656-659.
9. Theilade S, Lyngbæk S, Hansen TW, et al. Soluble urokinase plasminogen activator receptor levels are elevated and associated with complications in patients with type 1 diabetes. *J Intern Med* 2015; 277(3): 362-371.

10. Harskamp RE, Roe MT. Soluble Urokinase-Type Plasminogen Activator Receptor: A Useful Biomarker for Coronary Artery Disease and Clinical Outcomes? *J Am Heart Assoc* 2014; **3**(5): e001431.
11. Eapen DJ, Manocha P, Ghasemzedah N, Patel RS, Al Kassem H, Hammadah M, Veledar E, Le NA, Pielak T, Thorball CW. Soluble Urokinase Plasminogen Activator Receptor Level Is an Independent Predictor of the Presence and Severity of Coronary Artery Disease and of Future Adverse Events. *J Am Heart Assoc* 2014; **3**(5): e001118.
12. Lyngbæk S, Marott JL, Sehestedt T, Hansen TW, Olsen MH, Andersen O, Linneberg A, Haugaard SB, Eugen-Olsen J, Hansen PR, Jeppesen J. Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *Int J Cardiol* 2013; **167**: 2904-2911.
13. Lloyd-Sherlock P, Beard J, Minicuci N, Ebrahim S, Chatterji S. Hypertension among older adults in low-and middle-income countries: prevalence, awareness and control. *Int J Epidemiol* 2014; **43**(1): 116-128.
14. Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S. The Prospective Urban Rural Epidemiology (PURE) study: Examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. *Am Heart J* 2009; **158**(1): 1-7.e1.
15. Schutte AE, Schutte R, Huisman HW, van Rooyen JM, Fourie CMT, Malan NT, Malan L, Mels CMC, Smith W, Moss SJ. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. *Int J Epidemiol* 2012; **41**(4): 1114-1123.
16. Marfell-Jones M, Olds T, Stewart A, Carter L. International standards for anthropometric assessment. Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry (ISAK); 2006.
17. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A. 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013; **31**(7): 1281-1357.

18. suPARnostic®. Enzyme immunoassay for quantitative determination of soluble urokinase plasminogen activator receptor in human plasma. Copenhagen, Denmark: ViroGates; 2008.
19. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**(1): 31-41.
20. Seedat YK, Rayner BL. South African Hypertension Guideline 2011. *S Afr Med J* 2011; **102**(1): 57-84.
21. Pawlak K, Ulazka B, Mysliwiec M, Pawlak D. Vascular endothelial growth factor and uPA/suPAR system in early and advanced chronic kidney disease patients: a new link between angiogenesis and hyperfibrinolysis? *Transl Res* 2012; **160**: 346-354.
22. Pruijm M, Vollenweider P, Mooser V, Paccaud F, Preisig M, Waeber G, Marques-Vidal P, Burnier M, Bochud M. Inflammatory markers and blood pressure: sex differences and the effect of fat mass in the CoLaus Study. *J Hum Hypertens* 2013; **27**: 169-175.
23. Bautista L, Vera L, Arenas I, Gamarra G. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-α) and essential hypertension. *J Hum Hypertens* 2005; **19**(2): 149-154.
24. Heijnen BF, Van Essen H, Schalkwijk CG, Janssen BJ, Struijker-Boudier HA. Renal inflammatory markers during the onset of hypertension in spontaneously hypertensive rats. *Hypertens Res* 2014; **37**(2): 100-109.
25. Stehouwer C, Henry R, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia* 2008; **51**(4): 527-539.
26. Yasmin SW, McEniery CM, Dakham Z, Pusalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR, Wilkinson IB. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005; **25**(2): 372-378.
27. Johnson C, Baugh R, Wilson C, Burns J. Age related changes in the tunica media of the vertebral artery: implications for the assessment of vessels injured by trauma. *J Clin Pathol* 2001; **54**(2): 139-145.
28. Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003; **9**(6): 685-693.
29. Hage F, McCrory M, Szalai A. C-reactive protein and cardiovascular disease: Lessons learned from studying genetically engineered mice. In: Nagasawa S, ed.

C-Reactive Protein–New Research. Hauppauge NY: Nova Publishers; 2008:83-116.

30. Schillaci G, Pirro M. C-reactive protein in hypertension: clinical significance and predictive value. *Nutr Metab Cardiovasc Dis* 2006; **16**(7): 500-508.
31. van Bussel BC, Schouten F, Henry RM, Schalkwijk CG, de Boer MR, Ferreira I, Smulders YM, Twisk JW, Stehouwer CD. Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period. *Hypertension* 2011; **58**(4): 588-595.
32. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005; **25**(5): 932-943.
33. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension* 2005; **46**(5): 1118-1122.
34. Bautista LE. Inflammation, endothelial dysfunction, and the risk of high blood pressure: epidemiologic and biological evidence. *J Hum Hypertens* 2003; **17**(4): 223-230.
35. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. *Hypertension* 2001; **38**(3): 399-403.
36. Sakamoto T, Kudoh T, Sakamoto K, Matsui K, Ogawa H. Antithrombotic effects of losartan in patients with hypertension complicated by atrial fibrillation: 4A (Angiotensin II Antagonist of platelet Aggregation in patients with Atrial fibrillation), a pilot study. *Hypertens Res* 2014; **37**(6): 513-518.
37. Mikami D, Kimura H, Kamiyama K, Torii K, Kasuno K, Takahashi N, Yoshida H, Iwano M. Telmisartan activates endogenous peroxisome proliferator-activated receptor-δ and may have anti-fibrotic effects in human mesangial cells. *Hypertens Res* 2014; **37**: 422-431.
38. Ceravolo R, Maio R, Pujia A, Sciacqua A, Ventura G, Costa MC, Sesti G, Perticone F. Pulse pressure and endothelial dysfunction in never-treated hypertensive patients. *J Am Coll Cardiol* 2003; **41**(10): 1753-1758.
39. Lyngbæk S, Sehestedt T, Marott JL, Hansen TW, Olsen MH, Andersen O, Linneberg A, Madsbad S, Haugaard SB, Eugen-Olsen J, Jeppesen J. CRP and suPAR are differently related to anthropometry and subclinical organ damage. *Int J Cardiol* 2013; **167**: 781–785.
40. Rabkin SW, Langer A, Ur E, Calciu C-D, Leiter LA. Inflammatory biomarkers CRP, MCP-1, serum amyloid alpha and interleukin-18 in patients with HTN and

dyslipidemia: impact of diabetes mellitus on metabolic syndrome and the effect of statin therapy. *Hypertens Res* 2013; **36**: 550-558.

41. Andersen O, Eugen-Olsen J, Kofoed K, Iversen J, Haugaard SB. Soluble urokinase plasminogen activator receptor is a marker of dysmetabolism in HIV-infected patients receiving highly active antiretroviral therapy. *J Med Virol* 2008; **80**(2): 209-216.
42. Thunø M, Macho B, Eugen-Olsen J. suPAR: The molecular crystal ball. *Dis Markers* 2009; **27**(3): 157-172.
43. Kofoed K, Schneider UV, Scheel T, Andersen O, Eugen-Olsen J. Development and validation of a multiplex add-on assay for sepsis biomarkers using xMAP technology. *Clin Chem* 2006; **52**(7): 1284-1293.
44. Haupt TH, Petersen J, Ellekilde G, Klausen HH, Thorball CW, Eugen-Olsen J, Andersen O. Plasma suPAR levels are associated with mortality, admission time, and Charlson Comorbidity Index in the acutely admitted medical patient: a prospective observational study. *Crit Care* 2012; **16**: R130.
45. Smith GD, Lawlor DA, Harbord R, Timpson N, Rumley A, Lowe GD, Day IN, Ebrahim S. Association of C-reactive protein with blood pressure and hypertension life course confounding and Mendelian randomization tests of causality. *Arterioscler Thromb Vasc Biol* 2005; **25**(5): 1051-1056.
46. Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension* 2007; **49**(2): 304-310.

SUPPLEMENTARY INFORMATION

Table S1 Relationship between suPAR and clinical characteristics in the total normotensive group at baseline (*n* = 429).

	suPAR (ng/mℓ)	
	r	*P*-value
Age (years)	0.12	0.009
Gender, male (1,0)	-0.04	0.35
Waist circumference (cm)	-0.06	0.21
Systolic blood pressure (mmHg)	-0.07	0.13
C-reactive protein (mg/ℓ)	0.26	< 0.001
Glycated haemoglobin (%)	0.05	0.33
Triglyceride:HDL-cholesterol ratio	0.12	0.008
Creatinine clearance (mℓ/min)	-0.05	0.33
Gamma-glutamyltransferase (U/ℓ)	0.22	< 0.001
Tobacco use, yes	0.23	< 0.001

Abbreviations: suPAR, soluble urokinase plasminogen activator receptor; HDL, high-density lipoprotein. *P*-values between baseline variables were obtained with Pearson correlations. $P \leq 0.05$ regarded as statistically significant.

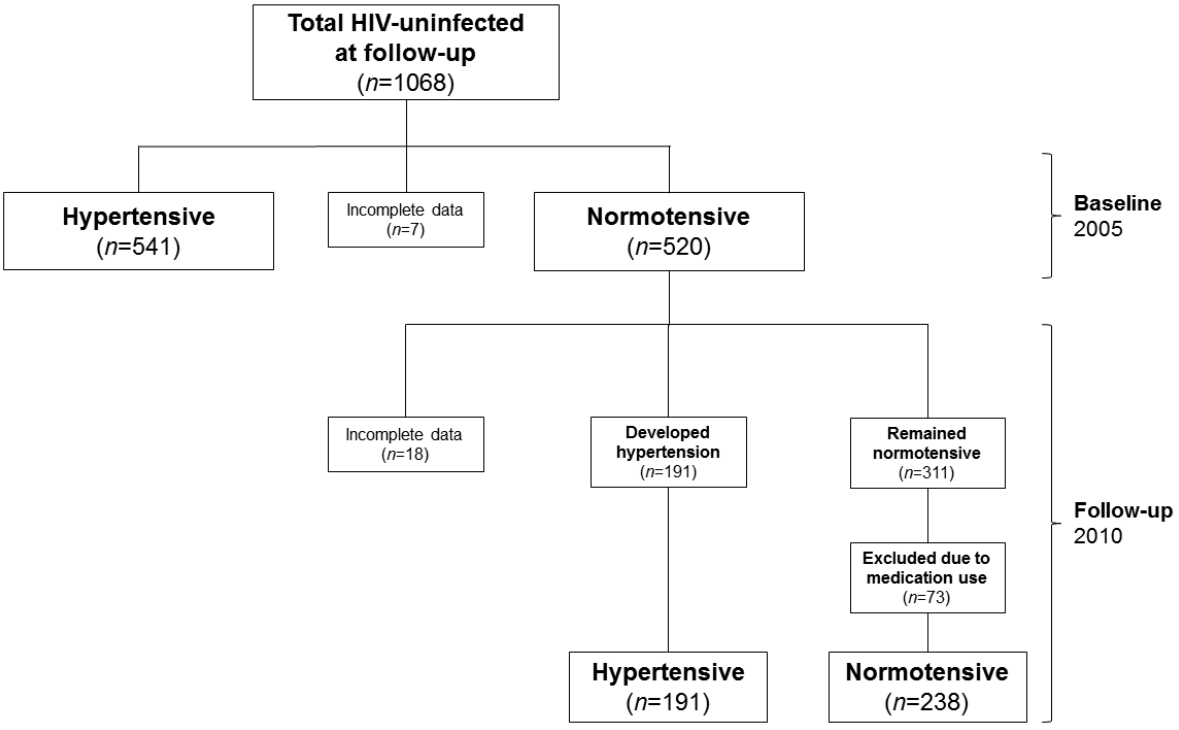


Figure S1 *Layout of the study population.*

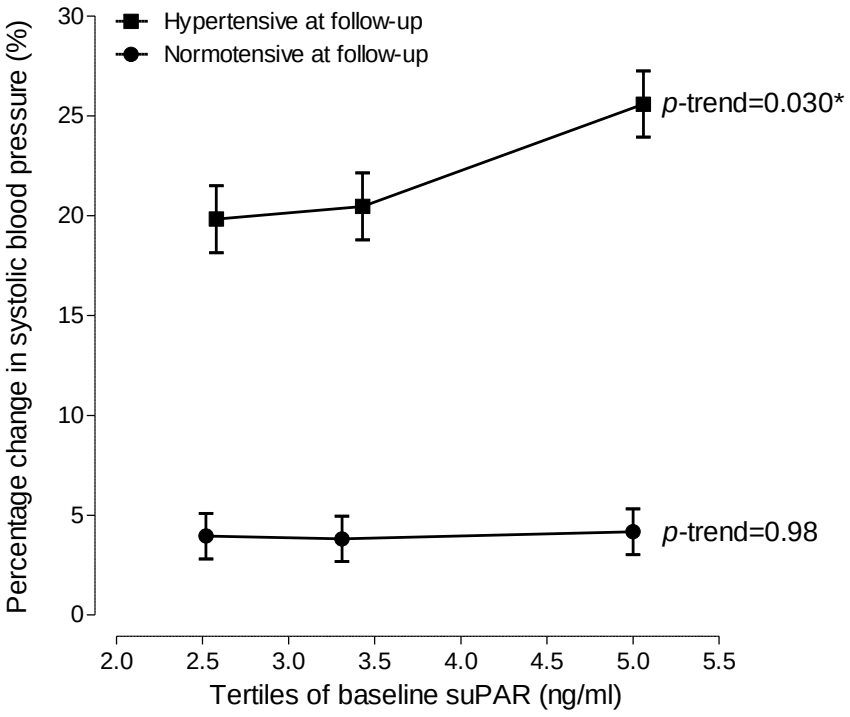


Figure S2 *Percentage change in systolic blood pressure, stratified by tertiles of baseline soluble urokinase plasminogen activator receptor (suPAR) in participants that developed hypertension and in those that remained normotensive over five years, respectively.*

Hypertension Research

SUMMARY OF INSTRUCTIONS TO AUTHORS

JOURNAL DETAILS				
Title:	*Hypertension Research* (Hypertens Res)			
Impact factor:	2.936			
Publisher:	Nature Publishing Group			
Aim & Scope: The journal publishes papers reporting original clinical and experimental research that contribute to the advancement of knowledge in the field of hypertension and related cardiovascular diseases.				
JOURNAL GUIDELINES				
Author guidelines:	http://mc.manuscriptcentral.com/societyimages/htr/HR_GTA.pdf			
Title:	---		**Language:**	Oxford
Keywords:	3-5 (alphabetical)		**Spacing:**	double
Manuscript (words):	max 5000 (incl. abstract)		**Font:**	---
Abstract (words):	max 250		**Margins:**	wide
Conflict of interest:	Yes		**Page nr:**	---
Acknowledgements:	Yes		**Alignment:**	---
Tables & Figures:	max 6			
	e.g. Table 1; at least two columns e.g. Figure 1; refer to Artwork Guidelines			
References:	▪ Glodny B, Pauli G. Medullopressin: a new pressor activity from the renal medulla. Hypertens Res 2005; 28: 827–836. ▪ Morrow DA, Gersch BJ, Braunwald E. Chronic coronary artery disease. In Zipes DP, Libby P, Bonow RO, Braunwald E (eds), Heart Disease, 7th edn. WB Saunders: Philadelphia, PA, USA, 2005, 1281–1341.			
Sections:	Title, abstract and keywords, text (introduction, methods, results and discussion), references, tables and figure captions			
Ethical considerations:	Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964 and Declaration of Tokyo, 1975, as revised in 2008).			
Other:	▪ There should be fewer than 10 co-authors ▪ Please provide a running title of no more than 50 characters including spaces.			

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

CHAPTER 5

SuPAR as a prognostic marker of all-cause and cardiovascular mortality in a black population

International Journal of Cardiology 184 (2015) 631–636

Contents lists available at ScienceDirect

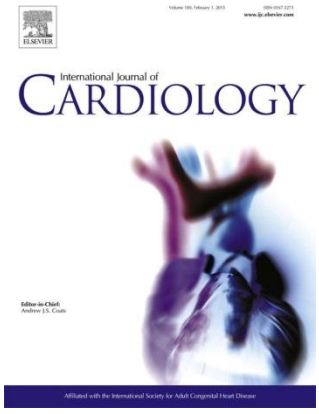
International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Soluble urokinase plasminogen activator receptor as a prognostic marker of all-cause and cardiovascular mortality in a black population ☆

Shani Botha [a,*], Carla M.T. Fourie [a], Rudolph Schutte [a,b], Jesper Eugen-Olsen [c], Ronel Pretorius [d], Aletta E. Schutte [a,b]

[a] Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa
[b] Medical Research Council, Research Unit for Hypertension and Cardiovascular Disease, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa
[c] Clinical Research Centre 136, Copenhagen University Hospital, Hvidovre, Denmark
[d] Research to Advance Quality of Nursing & Midwifery (INSINQ), North-West University, Potchefstroom, South Africa



This manuscript was published in the
International Journal of Cardiology,
Volume 184, Pages 631-636, March 2015
(Appendix B)

ABSTRACT

Background: Elevated inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) are well-known risk factors for cardiovascular mortality. The less familiar marker, soluble urokinase plasminogen activator receptor (suPAR), is known to predict cancer, infections and all-cause mortality. We determined whether suPAR, CRP and IL-6 are predictive of both all-cause and cardiovascular mortality in a black population, highly burdened by cardiovascular disease and HIV infection.

Methods: We included 1 425 black South Africans, of which 208 died within five years after baseline data collection. EDTA plasma biomarker levels were determined, while all-cause and cardiovascular mortality were used as endpoints.

Results: At baseline suPAR, CRP and IL-6 were higher in non-survivors than in survivors ($P<0.001$). SuPAR (HR 1.27, 95% CI 1.09-1.48), IL-6 (HR 1.49, 95% CI 1.24-1.78) and CRP (HR 1.39, 95% CI 1.17-1.65) predicted all-cause mortality, while only suPAR (HR 1.40, 95% CI 1.04-1.87) and IL-6 (HR 1.61, 95% CI 1.10-2.35) predicted cardiovascular mortality. The prognostic value of suPAR was independent of IL-6 and CRP ($P\leq0.015$).

Conclusion: SuPAR predicted both all-cause and cardiovascular mortality, independent of traditional risk factors, HIV and other inflammatory markers, underlining the prognostic value of suPAR in a black population.

Keywords: C-reactive protein; interleukin-6; predict; death; inflammation; South Africa.

INTRODUCTION

Inflammation is a known risk factor for cardiovascular mortality [1, 2]. Within the context of cardiovascular disease, inflammation is often seen as a nonspecific phenomenon which, for example, involves the presence of macrophages in tissue [3], as well as elevated inflammatory markers such as interleukin-6 (IL-6) [4] and C-reactive protein (CRP) [3].

Soluble urokinase plasminogen activator receptor (suPAR), a relatively new biomarker, is produced during inflammatory stimuli when the urokinase plasminogen activator receptor (uPAR) is cleaved from the cell membrane of endothelial cells, macrophages and other cells and becomes soluble [5]. Previous studies showed an association between suPAR and cardiovascular disease [6-9], type 1 [10] and type 2 diabetes [7], several types of cancer [7, 11, 12] and infectious diseases such as human immunodeficiency virus (HIV) [13]. Furthermore, suPAR provides prognostic information of cardiovascular disease risk in the general population beyond the Framingham risk score [14] and also predicts mortality in critically ill patients [15-17], independently of other inflammatory markers such as CRP [7]. SuPAR and CRP seem to reflect different pathophysiological pathways of cardiovascular disease risk. While CRP is closely related to anthropometric measures, suPAR is more closely linked with atherosclerosis and endothelial dysfunction [18] and less dependent on e.g. body mass index.

The predictive value of CRP and IL-6 on both all-cause and cardiovascular mortality is well established [19-22], however, information with regard to the prognostic value of suPAR on cardiovascular mortality *per se*, is scant, especially amongst Africans.

In South Africa, cardiovascular events are the second leading cause of death [23] reflecting the current high burden of cardiovascular disease in the country. Healthcare resources in South Africa are limited and identification of low cost prognostic markers for the detection of mortality risk would be valuable. We therefore investigated the prognostic value of suPAR in a cohort of 1 425 black South Africans.

METHODS

Study design and participants

This sub-study is embedded in the South African leg of the international Prospective Urban and Rural Epidemiology (PURE) study, which was designed to examine underlying determinants of chronic diseases in populations at varying stages of epidemiologic transition [24]. Baseline data collection of the South African leg in the North West province took place in 2005 and the first follow-up in 2010. A detailed layout of the study design and data collection procedures was previously described [25].

As shown in Figure 1, we included 2 010 randomly selected black South African men and women, aged 35 years and older from urban and rural areas. Of these 2 010 participants, we excluded 585 that were either lost to follow-up (444) or had missing data (141). From the remaining 1 425 participants, 208 (14.6%) died during the five years.

The study protocol was approved by the Health Research Ethics Committee of the North-West University in Potchefstroom, South Africa, and complied with the Declaration of Helsinki (as revised in 2004). Informed consent was obtained by each participant, with information conveyed in his or her native language and they were allowed to withdraw at any time.

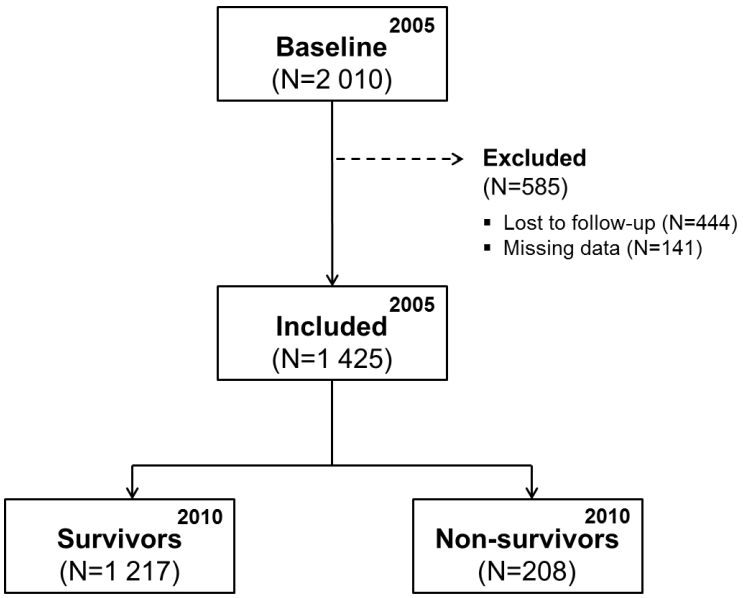


Figure 1 *Layout of the study population.*

Questionnaires, anthropometric and cardiovascular measurements

Specially trained field workers obtained questionnaire data, which included demographic and lifestyle information such as age, locality, employment, education, physical activity, alcohol consumption, tobacco and medication use.

Using standardised procedures, we obtained anthropometric measurements, including height (Invicta Stadiometer, IP 1465, London, UK), weight (Precision Health Scale, A & D Company, Tokyo, Japan), and hip and waist circumference (Holtain unstretchable flexible 7 mm wide metal tape, Crosswell, Wales) [26].

After a ten minute rest period, a trained observer measured systolic and diastolic blood pressure on the right upper arm with an OMRON HEM-757 device (Omron Healthcare, Kyoto, Japan). Measurements were taken in duplicate, five minutes apart, with an appropriate cuff size, while the participant was in the sitting position. The second blood pressure measurement was used.

Biochemical analyses

We obtained fasting blood samples and prepared serum and plasma according to appropriate methods. Samples were snap frozen on dry ice. Where samples were obtained in rural areas, serum was stored at -18°C (for a maximum of five days) until it could be transported to the laboratory. All samples were stored at -80°C in the laboratory until further analysis. SuPAR was determined with the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark) from plasma (EDTA) samples. We determined high-sensitivity CRP (by means of a particle enhanced turbidimetric assay), as well as total cholesterol, high-density lipoprotein cholesterol, triglycerides, gamma-glutamyltransferase and creatinine (by means of an enzymatic colorimetric method), using a Konelab20i™ auto-analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland). IL-6 was determined in plasma using an Elecsys 2010 (Roche, Basel, Switzerland) apparatus with the electrochemiluminescence immunoassay method. Glycated haemoglobin was determined from whole blood (EDTA) samples, based on ion-exchange high-performance liquid chromatography, with the D-10 Haemoglobin testing system from Bio-Rad (Bio-Rad Laboratories Ltd., Hercules, CA, USA, #220-0101). We determined glucose by means of an enzymatic reference method with hexokinase (Vitros DT6011 Chemistry Analyzer; Ortho-Clinical Diagnostics, Rochester, New York,

USA) in sodium fluoride tubes. We also determined HIV status with whole blood, using the First Response (PMC Medical, India) rapid HIV card test and repeated the test for confirmation with the Pareeshak (BHAT Bio-tech, India) card test. Creatinine clearance was estimated with the Cockcroft-Gault formula [27].

Mortality outcome assessment

Trained fieldworkers contacted participants every three months over the five-year period. Verbal autopsy 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. We classified cardiovascular mortality as death due to cardiovascular reasons, including cardiac failure, myocardial infarction or stroke.

Statistical analyses

Variables with a skewed distribution were logarithmically transformed (suPAR, CRP, IL-6, glycated haemoglobin, glucose and gamma-glutamyltransferase). Continuous data were presented as arithmetic mean ± standard deviation or geometric mean (5^{th} and 95^{th} percentile boundaries) and categorical data as proportions. In all cases, $P<0.05$ was regarded as statistically significant.

We compared means and proportions of baseline data between survivor and non-survivor groups with independent t-tests and Chi-square tests, respectively, and established relationships between suPAR, CRP and IL-6 with Pearson's correlation coefficients. We used Kaplan-Meier survival function estimates and the log-rank test to compare mortality rates across tertiles of suPAR, CRP and IL-6. This was repeated for HIV infected and uninfected groups by tertiles of suPAR.

We applied Cox regression to compute standardised hazard ratios, which express the risk for a 1-standard deviation increase in the independent variable. We first examined suPAR, CRP and IL-6 in separate models and, in order to isolate the predictive value of each marker independently of the other, then included two of the markers at a time in the same model. Other covariates that were entered into the models were chosen based on the literature and exploratory partial regression analyses. Finally, we plotted the 5-year risk for cardiovascular mortality in relation to both suPAR and IL-6. Covariates that were entered into models included age, gender, body mass index, systolic blood pressure, heart rate, gamma-glutamyltransferase, tobacco use, glycated

haemoglobin, triglyceride-to-high-density lipoprotein cholesterol ratio, creatinine clearance, HIV status, anti-hypertensive, anti-inflammatory and antiretroviral medication use.

RESULTS

Baseline characteristics according to mortality status are reported in Table 1. Compared to the survivor group, the non-survivors were older (P=0.001), consisted of more men (P<0.001) and of more HIV infected cases (P<0.001). They also had lower mean body mass index (P<0.001), waist circumference (P=0.002), glycated haemoglobin (P=0.042), creatinine clearance (P=0.003) and physical activity indices (P<0.001), while their heart rate (P<0.001) and gamma-glutamyltransferase levels (P<0.001) were higher.

The mean suPAR, CRP and IL-6 values of non-survivors were 4.29 ng/mℓ, 5.27 mg/ℓ and 5.09 pg/mℓ, respectively and were higher (all P<0.001) than in those who survived (Table 1). As expected, suPAR and IL-6 (r=0.39, P<0.001), suPAR and CRP (r=0.30, P<0.001), as well as CRP and IL-6 (r=0.56, P<0.001) correlated in those who did not survive. This was also the case in the survivors (data not shown).

Figure 2 indicates Kaplan-Meier survival function estimates. In the case of all-cause mortality, log-rank tests were significant across tertiles of suPAR (P<0.001), CRP (P=0.008) and IL-6 (P<0001), but only suPAR (P=0.004) and IL-6 (P=0.001) were significant for cardiovascular mortality.

The multivariable-adjusted standardised hazard ratios for all-cause and cardiovascular mortality in relation to suPAR, CRP and IL-6 are presented in Table 2 and Table S1. SuPAR predicted both all-cause (P=0.003) and cardiovascular (P=0.026) mortality, independent of traditional risk factors. Both CRP and IL-6 were predictive of all-cause mortality, however, only IL-6 (P=0.014) predicted cardiovascular mortality (Table 2).

Additional analyses showed that suPAR remained predictive of all-cause mortality, independent of CRP (HR 1.20, 95% CI 1.02-1.41, P=0.024) and IL-6 (HR 1.24, 95% CI 1.04-1.46, P=0.014), as well as of cardiovascular mortality, independent of CRP (HR 1.37, 95% CI 1.01-1.85, P=0.040) and IL-6 (HR 1.39, 95% CI 0.99-1.94, P=0.052).

Table 1 Baseline characteristics of black South African participants.

	Survivors (*N* = 1 217)	Non-survivors (*N* = 208)	*P*
Socio-demographic profile			
Age (years)	50.4 ± 10.1	53.0 ± 11.8	0.001
Gender, men (%)	426/1217 (35.0)	102/208 (49.0)	< 0.001
Locality, rural (%)	671/1217 (55.1)	99/208 (47.6)	0.044
Employed, *N* (%)	107/583 (18.4)	11/107 (10.3)	0.042
Educated, *N* (%)	736/1187 (62.0)	125/202 (61.9)	0.97
HIV status, positive (%)	152/1217 (12.5)	71/207 (34.3)	< 0.001
Anthropometric measurements			
Body mass index (kg/m^2)	24.9 ± 6.93	22.0 ± 5.96	< 0.001
Waist circumference (cm)	79.9 ± 12.8	77.0 ± 12.5	0.002
Cardiovascular measurements			
Systolic blood pressure (mmHg)	133 ± 23.5	135 ± 27.8	0.29
Diastolic blood pressure (mmHg)	88 ± 14.0	88 ± 16.1	0.85
Heart rate (bpm)	74 ± 15.5	80 ± 17.8	< 0.001
Inflammatory variables			
SuPAR (ng/mℓ)	3.55 (2.23-6.14)	4.29 (2.56-9.72)	< 0.001
C-reactive protein (mg/ℓ)	3.08 (0.25-38.2)	5.27 (0.32-53.6)	< 0.001
Interleukin-6 (pg/mℓ)	2.71 (0.75-16.7)	5.09 (0.75-39.3)	< 0.001
Biochemical variables			
Glycated haemoglobin (%)	5.64 (4.90-6.60)	5.53 (4.70-6.40)	0.042
Glucose (mmol/ℓ)	4.85 (3.50-6.90)	4.70 (3.40-6.10)	0.059
HDL (mmol/ℓ)	1.54 ± 0.62	1.45 ± 0.72	0.058
Triglycerides (mmol/ℓ)	1.30 ± 0.73	1.25 ± 0.65	0.35
Triglycerides:HDL ratio	1.03 ± 0.89	1.31 ± 1.49	< 0.001
Creatinine clearance (mℓ/min)	99.8 ± 34.9	91.3 ± 34.5	0.003
Lifestyle			
Gamma-glutamyltransferase (U/ℓ)	53.6 (19.0-350)	70.3 (21.8-442)	< 0.001
Alcohol consumption, *N* (%)	540/1352 (39.9)	23/57 (40.4)	0.95
Tobacco use, *N* (%)	623/1213 (51.4)	117/207 (56.5)	0.17
Physical activity index	7.39 ± 1.84	6.72 ± 1.88	< 0.001
Medication use			
Anti-hypertensive, *N* (%)	23/1217 (1.89)	7/208 (3.37)	0.17
Anti-inflammatory, *N* (%)	67/1217 (5.51)	15/208 (7.21)	0.33
Antiretroviral, *N* (%)	3/1217 (0.25)	1/208 (0.48)	0.56

Data are expressed as arithmetic mean ± standard deviation, geometric mean (5th and 95th percentile boundaries) or % of *N*. *P*-values for comparison between groups were obtained with independent t-tests and Chi-square tests. HIV=human immunodeficiency virus; suPAR=soluble urokinase plasminogen activator receptor; HDL=high-density lipoprotein cholesterol

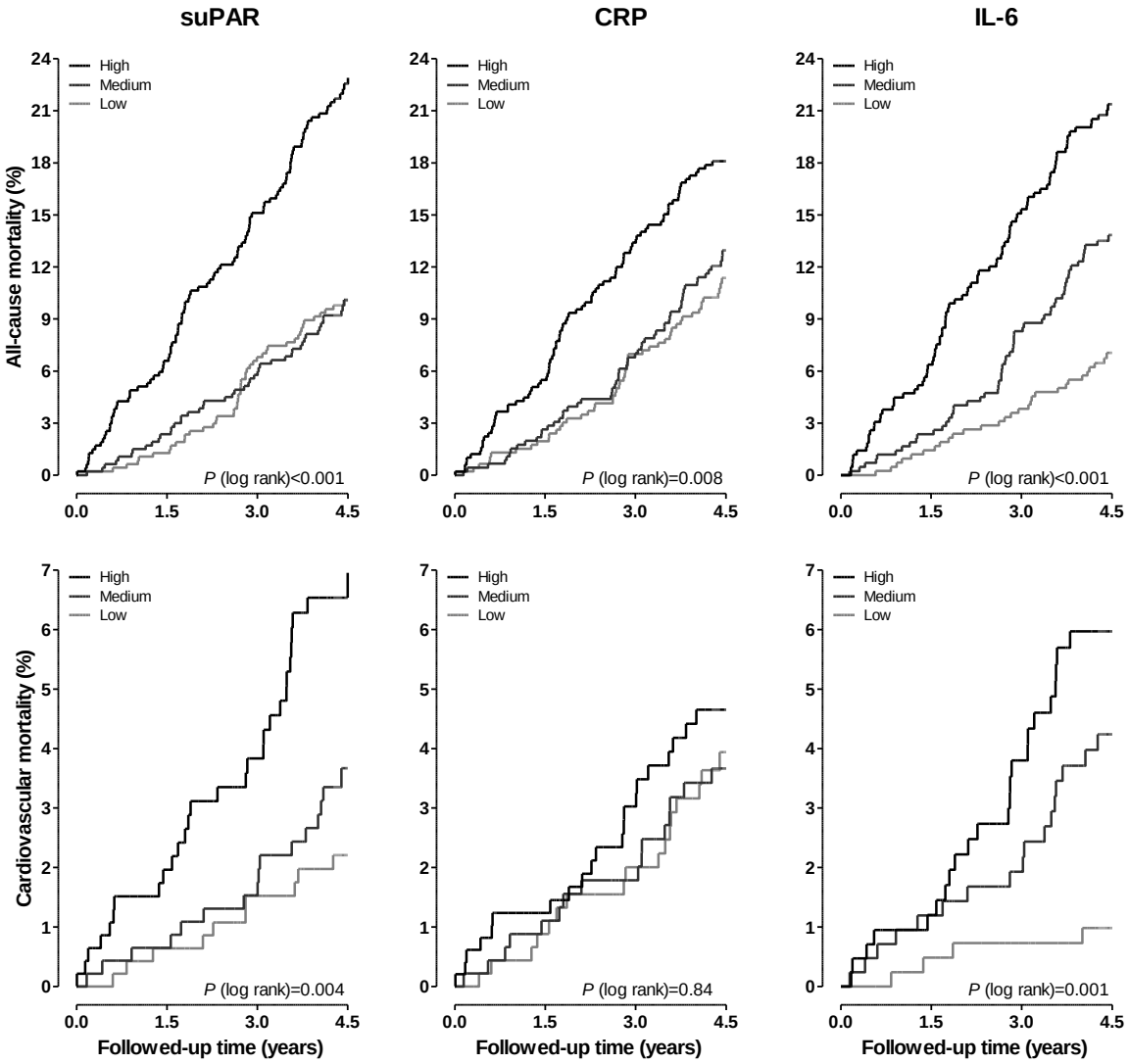


Figure 2 *Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of soluble urokinase plasminogen activator receptor (suPAR), C-reactive protein (CRP) and interleukin-6 (IL-6), respectively.*

In order to account for the effect of HIV infection, we performed the same analyses for HIV uninfected participants (Table S1) and obtained similar results. SuPAR was predictive of both all-cause (P<0.001) and cardiovascular (P=0.017) mortality in the HIV uninfected group, also independent of IL-6.

As illustrated in Figure 3, we computed the 5-year absolute risk of cardiovascular mortality associated with suPAR and IL-6, and confirmed the predictive value of suPAR (P=0.015) for cardiovascular mortality, independent of IL-6.

Table 2 Cox proportional hazard ratios of suPAR, CRP and IL-6 with all-cause and cardiovascular mortality.

	Univariate			Multivariate		
	HR (95% CI)	R^2	*P*	HR (95% CI)	R^2	*P*
All-cause mortality						
suPAR	1.63 (1.45-1.84)	0.24	<0.001	1.27 (1.09-1.48)	0.58	0.003
CRP	1.46 (1.26-1.69)	0.12	<0.001	1.39 (1.17-1.65)	0.60	<0.001
IL-6	1.74 (1.51-2.00)	0.27	<0.001	1.49 (1.24-1.78)	0.61	<0.001
Cardiovascular mortality						
suPAR	1.57 (1.24-1.97)	0.21	<0.001	1.40 (1.04-1.87)	0.68	0.026
CRP	1.30 (0.99-1.71)	0.06	0.06	1.19 (0.85-1.67)	0.66	0.31
IL-6	1.86 (1.41-2.46)	0.34	<0.001	1.61 (1.10-2.35)	0.77	0.014

Other covariates included in multivariate models: age, gender, body mass index, systolic blood pressure, heart rate, gamma-glutamyltransferase, tobacco use, glycated haemoglobin, triglyceride-to-high-density lipoprotein cholesterol ratio, creatinine clearance, HIV status, and anti-hypertensive, anti-inflammatory and antiretroviral medication use. suPAR=soluble urokinase plasminogen activator receptor; CRP=C-reactive protein; IL-6 =interleukin-6; HR=hazard ratio; R^2=goodness of fit.

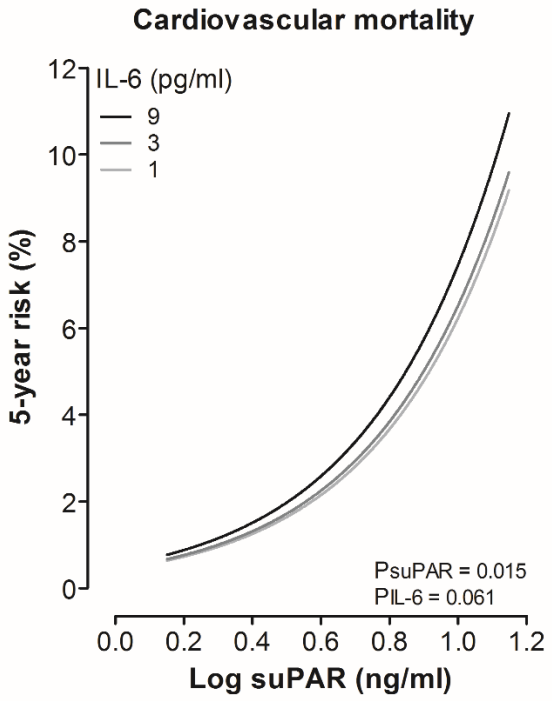


Figure 3 *Absolute five-year risk of cardiovascular mortality in relation to soluble urokinase plasminogen activator receptor (suPAR) at different levels of interleukin-6 (IL-6).*

Mean suPAR x-axis covers the 5th to 95th percentile interval. IL-6 is presented by three risk functions corresponding to 1, 3 and 9 units (approximate tertile midpoints). The risk functions were standardised to the distributions (mean or ratio) of age, gender, body mass index, systolic blood pressure, heart rate, gamma-glutamyltransferase, tobacco use, glycated haemoglobin, triglyceride-to-high-density lipoprotein cholesterol ratio, creatinine clearance, HIV status, and anti-hypertensive, anti-inflammatory and antiretroviral medication use. Among the 208 participants, 57 cardiovascular-related deaths occurred. PsuPAR and PIL-6 denote the significance of suPAR and IL-6 independent of each other.

DISCUSSION

In this study, we present rare prospective data on a black population from South Africa, a country which is double burdened by the highest rates of hypertension and HIV infection globally [28, 29]. SuPAR was previously investigated in white critically ill patients, especially with regard to cancer [7, 11, 12], infectious diseases [13] and all-cause mortality [15, 16]. However, this is the first study in blacks to demonstrate that suPAR is a significant predictor of both all-cause and cardiovascular mortality, independent of traditional risk factors, HIV infection and the well-known inflammatory markers CRP and IL-6.

We found higher suPAR concentrations in the non-survivor group than in those who survived. Similarly, in patients with HIV infection [30], bacterial infections [17, 31] and in those who were admitted to an acute medical unit [32], suPAR was higher in the people who died. We further found that suPAR predicted all-cause mortality, which supports previous studies where suPAR predicted renal replacement therapy and mortality [33], as well as mortality during intensive care unit admission [15].

It is known that suPAR levels are higher in black compared to Caucasian South African men and women [34-36]. However, data on the prognostic value of suPAR, with specific regard to cardiovascular mortality, remains limited. It was suggested that a suPAR cut-off of ≥3.5 ng/mℓ may predict future risk of myocardial infarction and cardiac death [9] and, notably, the mean suPAR in the non-survivors of our study was 4.29 ng/mℓ. In the Malmö Diet and Cancer Study, Persson *et al.* [6] found higher suPAR levels in Swedish men and women who had a cardiovascular event. Also, suPAR was predictive of cardiovascular events, independent of traditional risk factors, CRP and subclinical organ damage in 2 038 apparent healthy Danish participants [37]. More recently, Eapen *et al.* [9] found that elevated suPAR independently associated with the presence and severity of coronary artery disease in 3 367 American (83% Caucasian) subjects. These studies are however limited to white populations, underlining the importance of our findings where suPAR predicted cardiovascular mortality in Africans.

With regard to other inflammatory markers, IL-6 was predictive of both all-cause and cardiovascular mortality in this population, confirming the findings of previous studies [20-22, 38]. However, in contrast to suPAR and IL-6, CRP was predictive of all-cause mortality only. Mendall *et al.* [39] studied 1 395 men from Wales and also found that,

although CRP was associated with all-cause mortality, the association with all-incident ischaemic heart disease and ischaemic heart disease death was not significant. In contrast, other studies found that CRP was in fact predictive of both cardiovascular and all-cause mortality [22, 40].

Controversy still exists regarding the different mechanisms through which each of these markers plays a role in the pathology leading to cardiovascular mortality. It is known that the expression of uPAR by endothelial cells, macrophages and neutrophils substantially increases where organs undergo extensive tissue remodelling [41, 42]. UPAR further relates to processes in the extracellular matrix [42], endothelial dysfunction [43], the development of atherosclerosis [44] and plaque rupture [45, 46]. The pro-inflammatory cytokine [47], IL-6, is in part found in the peripheral vascular bed [47] and is released from monocytes, macrophages and the affected vasculature, in response to reactive oxygen species, vasoactive peptides and other cytokines [48]. CRP on the other hand, is secreted by hepatocytes in response to IL-6 stimulation and seems to rather be linked to anthropometric measures and metabolic inflammation [18, 49]. Nevertheless, we found that suPAR predicted all-cause and cardiovascular mortality, independent of both CRP and IL-6, which is similar to the findings of Sehestedt *et al.* [37] where the association between suPAR and cardiovascular risk remained after adjusting for CRP.

Finally, in parallel to the hypertension prevalence of 78% in people older than 50 years [29], an estimated 5.6 million South Africans are living with HIV [28], which are both the highest rates globally [28, 29]. HIV infection is associated with inflammation and endothelial dysfunction in black South Africans [50] and suPAR is known to predict mortality in participants with HIV infection [51]. Regardless, suPAR remained predictive of all-cause and cardiovascular mortality, independent of HIV infection and antiretroviral treatment in our study. Such research may, in the long term, contribute to the development of a low cost prognostic marker and benefit the healthcare resources in the country.

A limitation of this study is that we recruited black participants from the North West province of South Africa only, and our results may not necessarily be representative of the general South African population. However, this study includes prospective data, which are limited in African research settings, especially with regard to the relatively new inflammatory marker suPAR.

CONCLUSION

We showed for the first time in a black population that suPAR is a strong predictor of both all-cause and cardiovascular mortality, independent of CRP and IL-6. Future research is needed to clarify the mechanism behind the association of suPAR with cardiovascular mortality and to explore the possibility of a suPAR cut-off value for early detection of cardiovascular morbidity and mortality risk in this population.

Acknowledgements

We are grateful towards the participants of this study, the PURE-SA research team, the field workers and supporting staff in the Africa Unit for Transdisciplinary Health Research (AUTHeR), North-West University, South Africa, as well as Dr S Yusuf (PURE-International) and the PURE project staff at the PHRI, Hamilton Health Sciences and McMaster University, ON, Canada.

We acknowledge the support of the Population Health Research Institute (PHRI), the North-West University and Roche Diagnostics, as well as the financial support of the South Africa-Netherlands Research Program on Alternatives in Development (SANPAD) (GUN number 08/15), the South African National Research Foundation (NRF) (GUN number FA2006040700010 and 2069139) and the German Academic Exchange Service (DAAD)-NRF (UID: 80516). Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the DAAD-NRF.

Conflict of interest

Jesper Eugen-Olsen is a founder, shareholder and board member of ViroGates A/S, Denmark, the company that produces the suPARnostic® assay. Jesper Eugen-Olsen is an inventor on a patent on suPAR and risk. Copenhagen University Hospital Hvidovre, Denmark, owns the patent, which is licensed to ViroGates A/S.

REFERENCES

[1] O Hartaigh B, Bosch JA, Carroll D, et al. Evidence of a synergistic association between heart rate, inflammation, and cardiovascular mortality in patients undergoing coronary angiography. Eur Heart J 2013; 34:932-41.

[2] Tripepi G, Mallamaci F, Zoccali C. Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. J Am Soc Nephrol 2005; 16:S83-S8.

[3] Harrison DG, Guzik TJ, Lob HE, et al. Inflammation, immunity, and hypertension. Hypertension 2011; 57:132-40.

[4] Wollert KC, Drexler H. The role of interleukin-6 in the failing heart. Heart Fail Rev 2001; 6:95-103.

[5] Eugen-Olsen J, Haugaard SB, Andersen O. Soluble urokinase plasminogen activator receptor (suPAR) as diagnostic marker for low-grade inflammation. Patent nr. EP2500730AI. Paris, FR: European Patent Office; 2012.

[6] Persson M, Engström G, Björkbacka H, Hedblad B. Soluble urokinase plasminogen activator receptor in plasma is associated with incidence of CVD. Results from the Malmö Diet and Cancer Study. Atherosclerosis 2012; 220:502-5.

[7] Eugen-Olsen J, Andersen O, Linneberg A, et al. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. J Intern Med 2010; 268:296-308.

[8] Harskamp RE, Roe MT. Soluble Urokinase-Type Plasminogen Activator Receptor: A Useful Biomarker for Coronary Artery Disease and Clinical Outcomes? J Am Heart Assoc 2014; 3:e001431.

[9] Eapen DJ, Manocha P, Ghasemzedah N, et al. Soluble Urokinase Plasminogen Activator Receptor Level Is an Independent Predictor of the Presence and Severity of Coronary Artery Disease and of Future Adverse Events. J Am Heart Assoc 2014; 3:e001118.

[10] Theilade S, Lyngbæk S, Hansen TW, et al. Soluble urokinase plasminogen activator receptor levels are elevated and associated with complications in patients with type 1 diabetes. J Intern Med 2015; 277(3): 362-371.

[11] Begum FD, HØGdall CK, Kjaer SK, et al. The prognostic value of plasma soluble urokinase plasminogen activator receptor (suPAR) levels in stage III ovarian cancer patients. Anticancer Res 2004; 24:1981-6.

[12] Dass K, Ahmad A, Azmi AS, Sarkar SH, Sarkar FH. Evolving role of uPA/uPAR system in human cancers. Cancer Treat Rev 2008; 34:122-36.

[13] Sidenius N, Sier CFM, Ullum H, et al. Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. Blood 2000; 96:4091-5.

[14] Lyngbæk S, Marott JL, Sehestedt T, et al. Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. Int J Cardiol 2013; 167:2904-11.

[15] Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care 2011; 15:R63.

[16] Jalkanen V, Vaahersalo J, Pettilä V, et al. The predictive value of soluble urokinase plasminogen activator receptor (SuPAR) regarding 90-day mortality and 12-month neurological outcome in critically ill patients after out-of-hospital cardiac arrest. Data from the prospective FINNRESUSCI study. Resuscitation 2014; 85:1562-7.

[17] Mölkänen T, Ruotsalainen E, Thorball C, Järvinen A. Elevated soluble urokinase plasminogen activator receptor (suPAR) predicts mortality in Staphylococcus aureus bacteremia. Eur J Clin Microbiol Infect Dis 2011; 30:1417-24.

[18] Lyngbæk S, Sehestedt T, Marott JL, et al. CRP and suPAR are differently related to anthropometry and subclinical organ damage. Int J Cardiol 2013; 167:781–5.

[19] Panichi V, Maggiore U, Taccola D, et al. Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-reactive protein in haemodialysis patients. Nephrol Dial Transplant 2004; 19:1154-60.

[20] Rao M, Guo D, Perianayagam MC, et al. Plasma interleukin-6 predicts cardiovascular mortality in hemodialysis patients. Am J Kidney Dis 2005; 45:324-33.

[21] Volpato S, Guralnik JM, Ferrucci L, et al. Cardiovascular disease, interleukin-6, and risk of mortality in older women the women's health and aging study. Circulation 2001; 103:947-53.

[22] Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999; 106:506-12.

[23] Bradshaw D, Pillay-Van Wyk V, Laubscher R, et al. Cause of death statistics for South Africa: Challenges and possibilities for improvement. South African MRC Burden of Disease Research Unit; 2010.

[24] Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S. The Prospective Urban Rural Epidemiology (PURE) study: Examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. Am Heart J 2009; 158:1-7.e1.

[25] Schutte AE, Schutte R, Huisman HW, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. Int J Epidemiol 2012; 41:1114-23.

[26] Marfell-Jones M, Olds T, Stewart A, Carter L. International standards for anthropometric assessment. Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry (ISAK); 2006.

[27] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16:31-41.

[28] UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2010. Geneva, Switzerland: Joint United Nations Programme on HIV/AIDS; 2010.

[29] Lloyd-Sherlock P, Beard J, Minicuci N, Ebrahim S, Chatterji S. Hypertension among older adults in low-and middle-income countries: prevalence, awareness and control. Int J Epidemiol 2014; 43:116-28.

[30] Lawn S, Myer L, Bangani N, Vogt M, Wood R. Plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR) and early mortality risk among patients enrolling for antiretroviral treatment in South Africa. BMC Infect Dis 2007; 7:41.

[31] Wittenhagen P, Kronborg G, Weis N, et al. The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality. Clin Microbiol Infect 2004; 10:409-15.

[32] Haupt TH, Petersen J, Ellekilde G, et al. Plasma suPAR levels are associated with mortality, admission time, and Charlson Comorbidity Index in the acutely admitted medical patient: a prospective observational study. Crit Care 2012; 16:R130.

[33] Jalkanen V, Yang R, Linko R, et al. SuPAR and PAI-1 in critically ill, mechanically ventilated patients. Intensive Care Med 2013; 39:489-96.

[34] Kruger R, Schutte R, Huisman HW, et al. NT-proBNP, C-Reactive Protein and Soluble uPAR in a Bi-Ethnic Male Population: The SAfrEIC Study. PLoS One 2013; 8:e58506.

[35] Du Plooy CS, Kruger R, Huisman HW, Rasmussen LM, Eugen-Olsen J, Schutte AE. Extracellular Matrix Biomarker, Fibulin-1 and its Association with Soluble uPAR in a Bi-ethnic South African Population: The SAfrEIC study. Heart Lung Circ 2015; 24(3): 298-305.

[36] Schutte AE, Myburgh A, Olsen MH, Eugen-Olsen J, Schutte R. Exploring soluble urokinase plasminogen activator receptor and its relationship with arterial stiffness in a bi-ethnic population: the SAfrEIC-study. Thromb Res 2012; 130:273-7.

[37] Sehestedt T, Lyngbæk S, Eugen-Olsen J, et al. Soluble urokinase plasminogen activator receptor is associated with subclinical organ damage and cardiovascular events. Atherosclerosis 2011; 216:237-43.

[38] Fan Z-X, Hua Q, Li Y-P, Liu R-K, Yang Z. Interleukin-6, but not soluble adhesion molecules, predicts a subsequent mortality from cardiovascular disease in patients with acute ST-segment elevation myocardial infarction. Cell Biochem Biophys 2011; 61:443-8.

[39] Mendall M, Strachan D, Butland B, et al. C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. Eur Heart J 2000; 21:1584-90.

[40] Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010; 375:132-40.

[41] Gorrasi A, Santi AL, Amodio G, et al. The Urokinase Receptor Takes Control of Cell Migration by Recruiting Integrins and FPR1 on the Cell Surface. PLoS One 2014; 9:e86352.

[42] Blasi F, Sidenius N. The urokinase receptor: Focused cell surface proteolysis, cell adhesion and signaling. FEBS Lett 2010; 584:1923-30.

[43] Donadello K, Scolletta S, Taccone FS, et al. Soluble urokinase–type plasminogen activator receptor as a prognostic biomarker in critically ill patients. J Crit Care 2014; 29:144-9.

[44] Chen W, Jin W-Q, Chen L-F, Williams T, Zhu W-l, Fang Q. Urokinase receptor surface expression regulates monocyte migration and is associated with accelerated atherosclerosis. Int J Cardiol 2012; 161:103-10.

[45] Svensson P-A, Olson FJ, Hägg DA, et al. Urokinase-type plasminogen activator receptor is associated with macrophages and plaque rupture in symptomatic carotid atherosclerosis. Int J Mol Med 2008; 22:459-64.

[46] Fuhrman B. The urokinase system in the pathogenesis of atherosclerosis. Atherosclerosis 2012; 222:8-14.

[47] Tsutamoto T, Hisanaga T, Wada A, et al. Interleukin-6 Spillover in the Peripheral Circulation Increases With the Severity of Heart Failure, and the High Plasma Level of Interleukin-6 Is an Important Prognostic Predictor in Patients With Congestive Heart Failure. J Am Coll Cardiol 1998; 31:391-8.

[48] Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. Thromb Haemost 2009; 102:215-22.

[49] Tamakoshi K, Yatsuya H, Kondo T, et al. The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. Int J Obes 2003; 27:443-9.

[50] Fourie C, van Rooyen J, Pieters M, Conradie K, Hoekstra T, Schutte A. Is HIV-1 infection associated with endothelial dysfunction in a population of African ancestry in South Africa? Cardiovasc J Afr 2011; 22:134-40.

[51] Schneider UV, Nielsen RL, Eugen-Olsen J. The prognostic value of the suPARnostic® ELISA in HIV-1 infected individuals is not affected by uPAR promoter polymorphisms. BMC Infect Dis 2007; 7:134-40.

SUPPLEMENTARY INFORMATION

Table S1 Cox proportional hazard ratios of suPAR, CRP and IL-6 with all-cause and cardiovascular mortality in HIV uninfected participants.

	All-cause mortality (*N* = 136)			Cardiovascular mortality (*N* = 48)		
	HR (95% CI)	R^2	*P*	HR (95% CI)	R^2	*P*
suPAR	1.43 (1.18-1.73)	0.60	<0.001	1.49 (1.08-2.07)	0.70	0.017
CRP	1.25 (1.02-1.54)	0.57	0.033	1.16 (0.81-1.67)	0.66	0.42
IL-6	1.40 (1.13-1.74)	0.59	0.003	1.69 (1.17-2.54)	0.77	0.013
suPAR*	1.41 (1.14-1.74)	0.63	0.002	1.49 (1.02-2.18)	0.80	0.040

Other covariates included in models: age, gender, body mass index, systolic blood pressure, heart rate, gamma-glutamyltransferase, tobacco use, glycated haemoglobin, triglyceride-to-high-density lipoprotein cholesterol ratio, creatinine clearance, anti-hypertensive and anti-inflammatory medication use. suPAR=soluble urokinase plasminogen activator receptor; CRP=C-reactive protein; IL-6=Interleukin-6; HR=hazard ratio; R^2=goodness of fit. *IL-6 included additionally as covariate in the model.

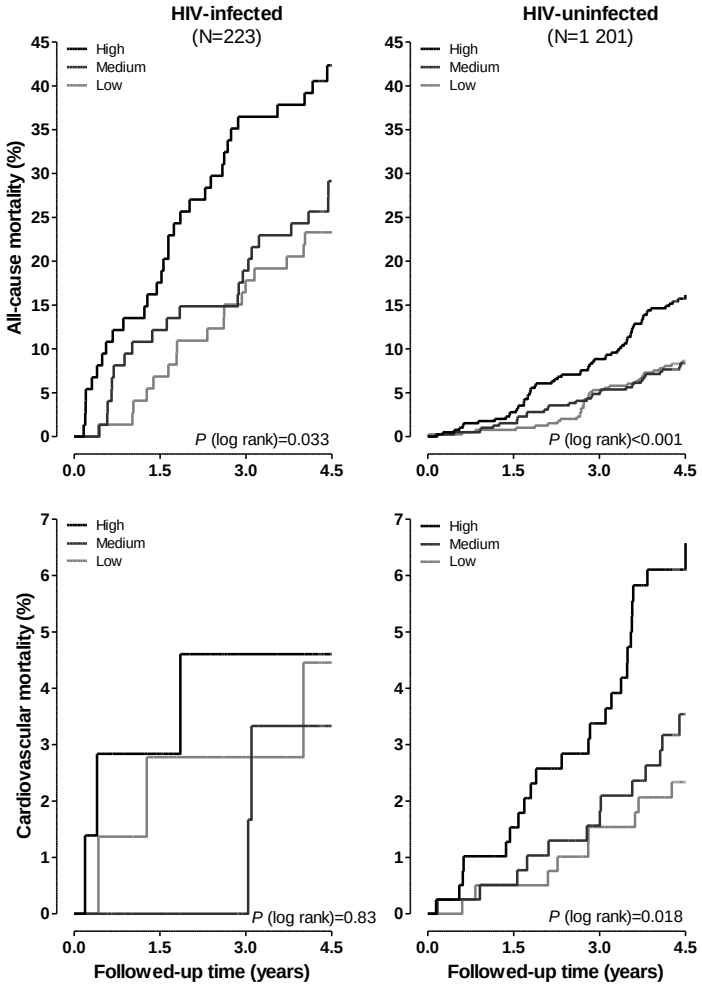


Figure S1 *Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of soluble urokinase plasminogen activator receptor (suPAR) in human immunodeficiency virus (HIV) infected and -uninfected groups, respectively.*

SUMMARY OF INSTRUCTIONS TO AUTHORS

JOURNAL DETAILS				
Title:	*International Journal of Cardiology* (Int J Cardiol)			
Impact factor:	6.175			
Publisher:	Elsevier			
Aim & Scope: Articles reporting clinical observations and interventions, experimental studies and theoretical concepts are all welcome provided they are of major scientific importance and clinical relevance. The journal covers all aspects of cardiology from genes to populations. The journal commissions high quality review articles from distinguished authors; unsolicited reviews will also be considered and will be subject to peer review.				
JOURNAL GUIDELINES				
Author guidelines:	http://www.internationaljournalofcardiology.com/content/authorinfo			
Title:	max 25 words		**Language:**	American English
Keywords:	3-6		**Spacing:**	double
Manuscript (words):	No limitation		**Font:**	---
Abstract (words):	max 250		**Margins:**	---
Conflict of interest:	Yes		**Page nr:**	---
Acknowledgements:	Yes		**Alignment:**	---
Tables & Figures:	No limit mentioned			
	Tables: Double spacing; only horisontal lines Figures: high-resolution TIF format			
References:	Any style (consistent)			
	[1] De Soyza N, Thenabadu PN, Murphy ML, Kane JJ, Doherty JE. Ventricular arrhythmia before and after aortocoronary bypass surgery. Int J Cardiol 1981; 1:123-30.			
Sections:	Title page, Structured Abstract, Key words, Introduction, Methods, Results, Discussion, Acknowledgments, References.			
Ethical considerations:	Manuscripts reporting data obtained from research conducted in human subjects must include a statement of assurance in the Methods section of the manuscript that (1) informed consent was obtained from each patient and (2) the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.			
Other:	▪ Include “Graphical abstract” and “Highlights” upon submission			

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

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 each manuscript will be discussed, interpreted, explained and compared to the relevant literature. Conclusions will be drawn and recommendations made to the reader with regards to suPAR as a potential marker of 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. Associations of suPAR with lifestyle and cardiometabolic risk factors

Transition in South Africa over the last decade has brought about an increase in prevalence of unhealthy behaviours,[1-4] which are known to negatively influence inflammatory status[5] and contribute to the development of CVD.[6,7] Africans have higher suPAR levels compared to Caucasians.[8] In this manuscript, we therefore aimed to explore whether there was a relationship between suPAR and health behaviours and cardiometabolic risk factors. We hypothesised that such relationships would indeed exist in the black South African population (Chapter 1, page 25).

In this cross-sectional analysis our results showed that suPAR positively and independently related to CRP and the health behaviours tobacco use, alcohol consumption, GGT levels and unemployment. However, no relationship was found between suPAR and indices of physical activity, dietary and psychological status, or with the cardiometabolic factors glucose, lipids, blood pressure and of measures of adiposity. The hypothesis was thus partly accepted, as suPAR independently associated with certain unhealthy lifestyle behaviours, such as tobacco use, alcohol consumption and unemployment, but not physical inactivity, dietary and psychological status. Furthermore, suPAR did not associate with cardiometabolic risk factors as was expected.

2.2. SuPAR and hypertension among black South Africans after five years

SuPAR has been shown to be a biomarker that links inflammation with cardiovascular disease,[7,9-13] but its role in the development of hypertension *per se*, among the hypertensive-prone, understudied black South African population,[14] is unknown. We aimed to determine whether suPAR was associated with the development of hypertension and hypothesised that, because of the known association of suPAR with CVD, black South Africans would have higher baseline suPAR levels if they were to develop hypertension compared to those that remained normotensive. It was further hypothesised that suPAR would associate with the five-year development of hypertension (Chapter 1, page 25).

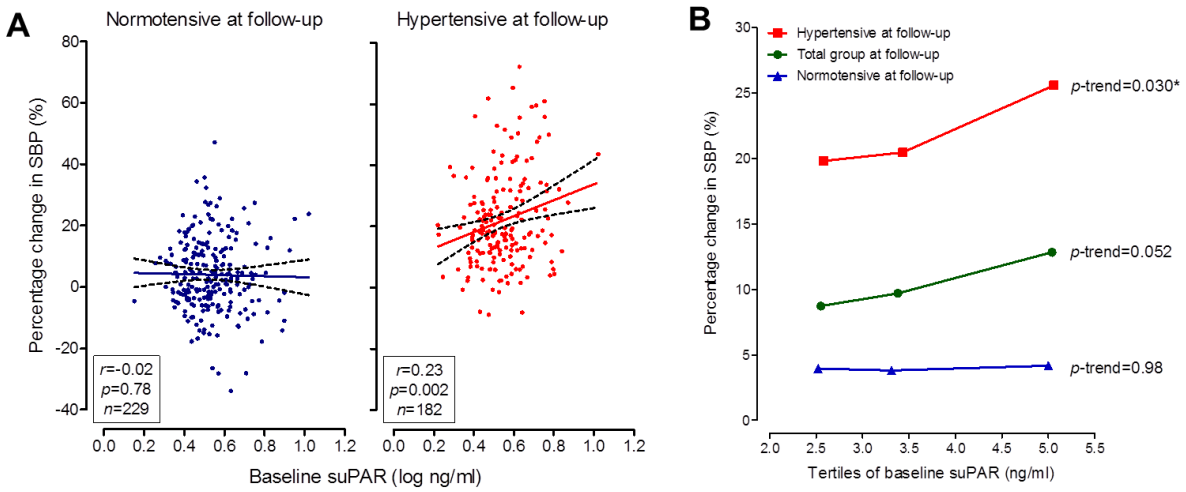


Figure 1 *Single linear regression analyses of the percentage change in SBP and baseline suPAR (A) and the percentage change in SBP, stratified by tertiles of baseline suPAR (adjusted for age, gender and baseline SBP) (B), in participants who remained normotensive and developed hypertension over five years. suPAR, soluble urokinase plasminogen activator receptor; SBP, systolic blood pressure.*

The results indicated that suPAR increased more in participants who developed hypertension, compared to those that remained normotensive over five years, even though suPAR levels were similar in these groups at baseline. In addition, change in systolic blood pressure (%SBP) correlated positively and associated independently with baseline suPAR, but only in those that developed hypertension. Further, a person was 1.41 times more likely to become hypertensive with each standard deviation increase in five-year percentage change in suPAR. The hypothesis for this manuscript can therefore be accepted in part. Although participants who developed hypertension did not have higher baseline suPAR levels, %SBP did associate with baseline suPAR in those

that became hypertensive, while change in suPAR associated with hypertensive status in black South Africans.

2.3. SuPAR as a prognostic marker of all-cause and cardiovascular mortality in a black population

Elevated inflammatory markers such as CRP and IL-6 are well-known risk factors for cardiovascular mortality.[15-18] SuPAR, which is less familiar, is known to predict cancer,[10,19,20] infections[21-24] and all-cause mortality.[25-27] However, the predictive value of suPAR in all-cause and cardiovascular mortality in black South Africans, who are highly burdened by cardiovascular disease and HIV infection, remains to be established. We therefore determined whether suPAR could independently predict all-cause and cardiovascular mortality in a black South African population, as in the case of other inflammatory markers such as CRP and IL-6. We further hypothesised that suPAR would independently predict all-cause and cardiovascular mortality among this population (Chapter 1, page 26).

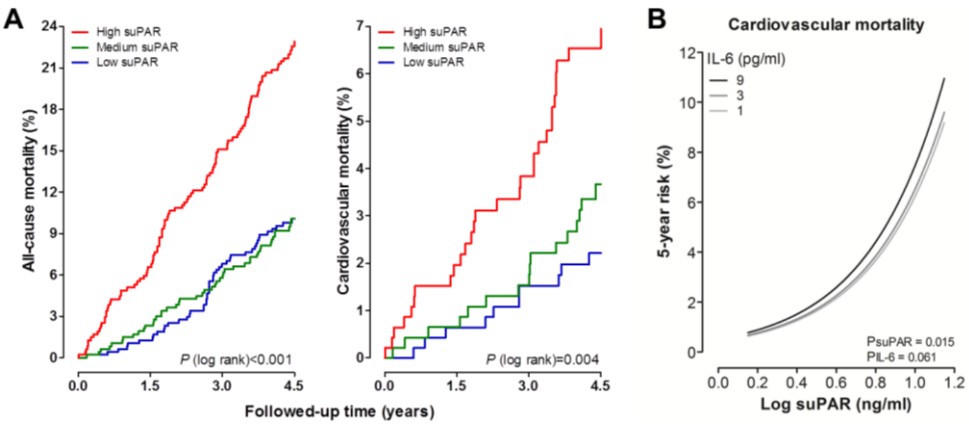


Figure 2 *A: Kaplan-Meier survival plots showing incidence of either all-cause or cardiovascular mortality by tertiles of suPAR. B: Absolute 5-year risk of cardiovascular mortality in relation to suPAR at different levels of IL-6. suPAR, soluble urokinase plasminogen activator receptor; IL-6, interleukin-6.*

We found that non-survivors had higher baseline suPAR, CRP and IL-6 levels than survivors. All three markers independently predicted all-cause mortality, but only suPAR and IL-6, both separately and combined, were predictive of cardiovascular mortality. In addition, suPAR predicted cardiovascular mortality independent of IL-6. The set hypothesis is therefore accepted, as suPAR predicted all-cause and cardiovascular mortality, independent of traditional risk factors, HIV and other inflammatory markers.

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. Most of our findings confirmed previous findings or contributed to the existing body of knowledge, while some were contradictory.

SuPAR and modifiable risk factors

The South African National Health and Nutrition Examination survey[28] recently found that 45.6% of households reported alcohol consumption, while 20.8% of the population reported to have never smoked before. In this study we found that 36.4% of participants consumed alcohol and 57.8% had either used or were using tobacco products. Even though the survey data were obtained for the South African population in general, it still supported the high prevalence of unhealthy behaviours that we found among the black South African community.

An unhealthy lifestyle is associated with low-grade inflammation[5] and in agreement, suPAR associated with unhealthy lifestyle behaviours in this study. To our knowledge, our study is however the first to report these findings in black South Africans. We firstly found a strong association between suPAR and GGT levels, a marker which is indicative of high alcohol consumption. To our knowledge, no studies have focussed directly on the link between suPAR and GGT levels, however, there are evidence that suPAR associates with heavy alcohol consumption and relates to alcoholic liver disease, liver function, cirrhosis and fibrosis.[25,29-31] Our results thus contribute to the current literature in this regard. In addition, suPAR associated with tobacco use, which corresponds with the findings of other studies where suPAR was higher in smokers from a Swedish population,[32] in 2 038 Danish women that smoked,[33] as well as in 1 933 Danish past or current smokers with impaired glucose regulation.[34] As of yet, to the best of our knowledge no studies have focussed on the association between suPAR and demographic factors such as unemployment. It is however known that unemployment often coincides with self-destructive behaviours such as alcohol abuse[3] and that the unemployed has an increased risk of CVD,[35] which underlines our findings.

We were surprised that suPAR related to heart rate only and not to other measures of cardiometabolic disorders. Nanchen *et al.* reported that, in 4 084 adults of ages 70-82 years, an increased heart rate did indeed associate with increased systemic

inflammation and endothelial dysfunction.[36] On the other hand, it is not clear from the literature whether a direct association between suPAR and blood pressure exists. It is however known that an inflammatory state alters high-density lipoprotein composition and function,[37] increases low-density lipoprotein and triglyceride levels[38] and that dyslipidaemia, oxidative stress and inflammation interact in the pathogenesis of coronary artery disease.[38] In HIV infected individuals receiving antiretroviral therapy, suPAR was indicated as marker of dysmetabolism[39] and was associated with metabolic changes such as glucose aberrations[40] and lipodystrophy.[41] These results contradict our findings, as we found no relationship between suPAR and the cardiometabolic factors dyslipidaemia, hyperglycaemia, adiposity and blood pressure. A possible explanation may be that black South Africans seem to have a more favourable lipid profile, which may assist in coronary protection.[42] It was further suggested that suPAR may play a role rather later than sooner in the atherosclerotic process where disease progression has already taken place,[8,43,44] as for instance in established coronary artery disease.[45] It should be kept in mind that 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.

SuPAR and hypertension

Following on from our first analyses on health behaviours, we found that baseline suPAR levels did not differ between those that would become hypertensive and those that would remain normotensive. However, we found a positive relationship between suPAR and change in blood pressure over five years, as well as an association between suPAR and hypertensive status, which highlight the complex interaction between inflammation and cardiovascular disease progression. Here our findings contributed to the literature, as data on the relationship between suPAR and hypertension, which may precede hard outcomes, are limited. Earlier studies showed that suPAR related to incidence of cardiovascular disease,[10,32] including ischaemic heart disease and stroke endpoints.[10] SuPAR further associated with subclinical organ damage and cardiovascular events,[33] including heart failure,[46] and with coronary artery calcifications in healthy middle-aged subjects.[7] In addition, suPAR is regarded as a biomarker of carotid plaque, stroke, and coronary artery disease[45] and of cardiovascular disease in chronic kidney disease patients.[13]

The possibility of reverse causality should be considered. As blood pressure measurements were taken five years apart, we were not able to determine the exact time point at which each participant became hypertensive after baseline and could therefore not calculate the duration that the participants were hypertensive. Also, five-year change in suPAR directly associated with hypertensive status only and not with change in blood pressure, while baseline suPAR did. The controversy in our findings mirrors that found in the literature where the interplay between inflammation and hypertension is complex and seem to occur in both directions.[47] In fact, some studies suggested that a vascular inflammatory response could initiate the development of hypertension[47-51] as inflammatory markers such as CRP and TNF-α were already increased in pre-hypertensive persons.[52,53] On the contrary, other researchers reported that hypertension exerts a number of pro-inflammatory effects[48,51,54,55] and that, through such inflammatory mechanisms, hypertension may accelerate the atherosclerotic process.[54] A plausible scenario is therefore that inflammation could initiate the development of pre-hypertension into a more severe hypertensive state; however, more extensive research is needed in order to confirm this hypothesis.

SuPAR and mortality

It can be inferred from the above evidence that a proven link between suPAR, modifiable risk factors and hypertension indeed exists in this population, which justifies the rest of our findings where suPAR predicted all-cause and cardiovascular mortality. Our findings both confirmed and added to the current literature. In parallel to our findings, studies have shown that suPAR was higher in non-survivors than in survivors who suffered from bacterial infections,[27,56] who were HIV infected,[57] or who were admitted to an acute medical unit.[58] Moreover, suPAR has a known value in the prognosis of cardiovascular disease and events,[7,10,12,45,46,59,60] critical illnesses[19,22,23,27,56,57,61,62] and all-cause mortality.[24-26,62-65] However, whether suPAR has predictive value for cardiovascular mortality *per se* is unclear from the literature, highlighting the appropriate contribution of our findings to the field.

Eapen and colleagues[12] estimated that a suPAR cut-off value of ≥3.5 ng/mℓ may predict future myocardial infarction and cardiac death risk. In this study, black South Africans who have died during the five years after baseline data collection, displayed a mean

baseline suPAR level of 4.29 ng/mℓ, which further adds to current knowledge of the high cardiovascular risk in this population.

SuPAR and other inflammatory markers

In line with our findings, where suPAR positively correlated with CRP and IL-6, others found a positive relationship between suPAR and other inflammatory markers such as interleukin-1 and TNF-α.[24,66,67] Lyngbæk *et al.*[44] found that the predictive value of suPAR in cardiovascular risk was strengthened when combined with CRP.

We found that, while suPAR associated with GGT and tobacco use and not with measures of adiposity such as body mass index, CRP did not associate with such lifestyle factors but did in fact relate to body mass index, regardless of the relatively low body mass indices of this population. Our findings are partly in agreement with the literature. Although earlier studies showed an association between CRP and both alcohol consumption and tobacco use,[68-70] CRP seems to be mostly related to obesity measures.[67,68,71,72] While hypertension was associated with suPAR, no such association was seen for CRP. There is much controversy on this topic in the literature. Sesso *et al*.[73] showed an association of CRP with future development of hypertension in 5 365 women who developed incident hypertension. In concert, Niskanen *et al*.[74] reported that 379 middle-aged men with CRP levels ≥3.0 mg/ℓ were 2.8 times more likely to develop hypertension than those with CRP <1.0 mg/ℓ. In contrast, the association between CRP and hypertension risk was strongly confounded by body mass index,[75] while Bautista *et al*.[76] found no association between CRP and high blood pressure after they accounted for other risk factors, IL-6 and TNF-α. Similarly, another study found that after adjustments for other blood pressure determinants, higher CRP levels associated with higher diastolic and not with systolic blood pressure.[50] In this regard, our findings therefore seem to both contradict and confirm the literature.

Finally, although all three inflammatory markers showed to be predictive of all-cause mortality, the predictive value for cardiovascular mortality was evident in the case of suPAR and IL-6 only, also independent of one another. Previous research showed that CRP and IL-6 predicted all-cause mortality in the elderly,[18] while IL-6 was predictive of cardiovascular mortality in 620 CVD female patients older than 65 years,[17] in haemodialysis patients[16] and in 263 admitted hospital patients with first acute ST-segment elevation myocardial infarction.[77] However, a meta-analysis of 160 309 people

without a history of vascular disease from 54 long-term prospective studies suggested that CRP has continuous associations with the risk of coronary heart disease, ischaemic stroke and vascular mortality[78] and, in another study, CRP predicted cardiovascular mortality among the elderly.[18] To the contrary, Mendall and colleagues[79] found no association between CRP and ischaemic heart disease mortality in a Welsh study involving 1 395 men, regardless of an association obtained with all-cause mortality.

The controversy in our findings therefore parallels that found in the literature and underlines the earlier suggestions that suPAR and CRP may represent different aspects of inflammation and different pathophysiological mechanisms which may lead to atherosclerosis and CVD.[10,44] Yet again, this flags the contribution of our findings and provides another strong motive for further research on the matter.

4. CHANCE AND CONFOUNDING

It is of importance 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 black participants from one of the nine provinces of South Africa only and this study is therefore not representative of the entire black South African population. Most of the participants were from the lower socio-economic grouping and 85.7% were unemployed, which may have biased our results. As blood pressure was measured five years apart, we could not calculate the exact duration that each participant was hypertensive, and reverse causality should therefore be considered.

We did not determine body temperature and leukocyte count or test for any opportunistic infections (this was only reported in questionnaires) and can therefore not exclude underlying infections which could have attenuated inflammatory marker concentrations. Only clinical, and not ambulatory blood pressure, was measured and participants with either masked or ‘white coat’ hypertension may not have been identified. We further did not measure genetic factors, which may have confounded some of the findings. One may further argue that, because some of the variables were self-reported (such as the alcohol consumption, tobacco use, physical activity and diet); a lack of sensitivity may exist and may have diluted the result. We did not determine cotinine levels, which could have confirmed tobacco product exposure. However, GGT

levels were measured, which is known to correlate with self-reported alcohol consumption in this population[80] and also in our study, indicating a degree of statistical reliability. Although these non-measured factors may have weakened the study, this study is of a prospective nature and scheduled follow-up studies will be able to incorporate these factors.

Statistical results were investigated from a physiological perspective and statistical significance does not necessarily indicate physiological significance. Nonetheless, we included a relatively large sample of the understudied black South African population which provided adequate statistical power.

5. RECOMMENDATIONS

In light of improving cardiovascular health and longevity of especially black South Africans, the following is recommended for future studies:

Other biochemical variables such as white blood cell count, oxidised low-density lipoprotein cholesterol, markers of oxidative stress and extracellular matrix proteins could be analysed. Further, other measures of cardiovascular structure and function, including intima-media thickness, plaque scores, pulse wave velocity and flow-mediated dilation, as well as 24 hour blood pressure could be performed. These indices may contribute to illuminate the mechanisms behind the association of suPAR, CVD development and mortality.

Given the prognostic value of suPAR, the possibility of a suPAR cut-off value for early detection of cardiovascular morbidity and mortality risk, which could be applied specifically to this population, should be explored. It is further recommended that suPAR should be investigated in experimental studies as an early diagnostic and treatment option for CVD in the black South African population where access and adherence to medication are limited.

On 30 July 2014, a news bulletin was released in Denmark that included phrases such as “Danish doctors are thrilled about breakthrough - Look into your future” and “Simple Blood Test Can Save Your Life”. These statements referred to the use of the suPARnostic® test as a relatively easy, rapid and inexpensive commercial assay to

identify high risk patients in this more developed country. In this regard, future research on the feasibility of this test in poor resource settings of South Africa is required, as suPAR have potential to identify high-risk individuals early on for preventative strategies and/or treatment intervention.

Given the high prevalence of unhealthy behaviours and the effect thereof on the already threatened cardiovascular health among black South Africans, there is an important need to address lifestyle awareness in order to limit the detrimental effect of modifiable risk factors on the health and mortality rate of this population.

Environmental and genetic risk factors of CVD interact in a highly complex manner with the inflammatory process. Additional studies should be considered where black consortia are formed in order to investigate and compare larger prospective population samples. Furthermore, such risk factors should be considered mutually inclusive, not exclusive when intervention strategies are developed, as risk management that includes multiple factors is the cornerstone of CVD management.

The longitudinal nature of this study has provided much valuable information. It is however clear that suPAR is able to provide even more information in cases where disease progression has already taken place. In addition to the five-year follow-up data which was used in this study, data from the 10-year follow-up study (which will be conducted in 2015) may be able to shed some light on some of the controversies and questions that have risen from this and other studies.

Therefore, given the complex interaction between inflammation, cardiovascular disease and mortality, this field remains open to further study of the black South Africans in the years to come.

6. FINAL CONCLUSIONS AND PERSPECTIVES

We showed for the first time that suPAR has potential as a marker of CVD development in black South Africans. In addition to the independent association of suPAR with unhealthy modifiable risk factors such as alcohol consumption and tobacco use, suPAR also associates with hypertension and independently predicts all-cause and cardiovascular mortality in the black South African population. These findings provide concerning evidence of the effect of unhealthy lifestyle factors on inflammatory status, and therefore on the CVD and mortality burden. This study permits the use of suPAR to provide a possible novel approach for early preventative and therapeutic strategies in order to combat the high prevalence of destructive behaviours and limit the already high CVD burden of this population.

7. REFERENCES

1. World Health Organization. Global status report on alcohol and health. Geneva, Switzerland: World Health Organization; 2011.
2. Ando K, Kawarazaki H, Miura K*, et al.* Report of the Salt Reduction Committee of the Japanese Society of Hypertension. (1) Role of salt in hypertension and cardiovascular diseases. *Hypertens Res.* 2013;36(12):1009-1019.
3. Bradshaw D. Determinants of health and their trends. In: Barron P, Roma-Reardon J, eds. Durban, SA: Health Systems Trust; 2008.
4. Mayosi BM, Lawn JE, van Niekerk A*, et al.* Health in South Africa: changes and challenges since 2009. *Lancet.* 2012;380(9858):2029-2043.
5. Egger G, Dixon J. Non-nutrient causes of low-grade, systemic inflammation: support for a 'canary in the mineshaft' view of obesity in chronic disease. *Obes Rev.* 2011;12(5):339-345.
6. Eugen-Olsen J, Haugaard SB, Andersen O. Soluble urokinase plasminogen activator receptor (suPAR) as diagnostic marker for low-grade inflammation. Patent nr. EP2500730AI. Paris, FR: European Patent Office; 2012.
7. Sørensen MH, Gerke O, Eugen-Olsen J*, et al.* Soluble urokinase plasminogen activator receptor is in contrast to high-sensitive C-reactive-protein associated with coronary artery calcifications in healthy middle-aged subjects. *Atherosclerosis.* 2014;237:60-66.
8. Schutte AE, Myburgh A, Olsen MH, Eugen-Olsen J, Schutte R. Exploring soluble urokinase plasminogen activator receptor and its relationship with arterial stiffness in a bi-ethnic population: the SAfrEIC-study. *Thromb Res.* 2012;130(2):273-277.
9. Theilade S, Lyngbæk S, Hansen TW, et al. Soluble urokinase plasminogen activator receptor levels are elevated and associated with complications in patients with type 1 diabetes. *J Intern Med* 2015; 277(3): 362-371.
10. Eugen-Olsen J, Andersen O, Linneberg A*, et al.* Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. *J Intern Med.* 2010;268(3):296-308.
11. Harskamp RE, Roe MT. Soluble Urokinase-Type Plasminogen Activator Receptor: A Useful Biomarker for Coronary Artery Disease and Clinical Outcomes? *J Am Heart Assoc.* 2014;3(5):e001431.

12. Eapen DJ, Manocha P, Ghasemzedah N*, et al.* Soluble Urokinase Plasminogen Activator Receptor Level Is an Independent Predictor of the Presence and Severity of Coronary Artery Disease and of Future Adverse Events. *J Am Heart Assoc.* 2014;3(5):e001118.
13. Meijers B, Poesen R, Claes K*, et al.* Soluble urokinase receptor is a biomarker of cardiovascular disease in chronic kidney disease. *Kidney Int.* 2015;87(1):210-216.
14. Lloyd-Sherlock P, Beard J, Minicuci N, Ebrahim S, Chatterji S. Hypertension among older adults in low-and middle-income countries: prevalence, awareness and control. *Int J Epidemiol.* 2014;43(1):116-128.
15. Panichi V, Maggiore U, Taccola D*, et al.* Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-reactive protein in haemodialysis patients. *Nephrol Dial Transplant.* 2004;19(5):1154-1160.
16. Rao M, Guo D, Perianayagam MC*, et al.* Plasma interleukin-6 predicts cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis.* 2005;45(2):324-333.
17. Volpato S, Guralnik JM, Ferrucci L*, et al.* Cardiovascular disease, interleukin-6, and risk of mortality in older women the women's health and aging study. *Circulation.* 2001;103(7):947-953.
18. Harris TB, Ferrucci L, Tracy RP*, et al.* Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med.* 1999;106(5):506-512.
19. Begum FD, HØGdall CK, Kjaer SK*, et al.* The prognostic value of plasma soluble urokinase plasminogen activator receptor (suPAR) levels in stage III ovarian cancer patients. *Anticancer Res.* 2004;24(3B):1981-1986.
20. Dass K, Ahmad A, Azmi AS, Sarkar SH, Sarkar FH. Evolving role of uPA/uPAR system in human cancers. *Cancer Treat Rev.* 2008;34(2):122-136.
21. Sidenius N, Sier CFM, Ullum H*, et al.* Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. *Blood.* 2000;96(13):4091-4095.
22. Donadello K, Scolletta S, Taccone FS*, et al.* Soluble urokinase–type plasminogen activator receptor as a prognostic biomarker in critically ill patients. *J Crit Care.* 2014;29(1):144-149.

23. Wrotek A, Jackowska T. The role of the soluble urokinase plasminogen activator (suPAR) in children with pneumonia. *Respir Physiol Neurobiol* 2015: Article in Press. DOI: 10.1016/j.resp.2014.12.018
24. Timmermans K, Vaneker M, Scheffer GJ, et al. SuPAR levels are related to plasma cytokine levels but have low predictive value for mortality in trauma patients. *J Crit Care* 2015: Article in Press. DOI: 10.1016/j.jcrc.2015.01.006
25. Koch A, Voigt S, Kruschinski C*, et al.* Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. *Crit Care.* 2011;15(1):R63.
26. Jalkanen V, Vaahersalo J, Pettilä V*, et al.* The predictive value of soluble urokinase plasminogen activator receptor (SuPAR) regarding 90-day mortality and 12-month neurological outcome in critically ill patients after out-of-hospital cardiac arrest. Data from the prospective FINNRESUSCI study. *Resuscitation.* 2014;85(11):1562-1567.
27. Mölkänen T, Ruotsalainen E, Thorball C, Järvinen A. Elevated soluble urokinase plasminogen activator receptor (suPAR) predicts mortality in Staphylococcus aureus bacteremia. *Eur J Clin Microbiol Infect Dis.* 2011;30(11):1417-1424.
28. Shisana O, Labadarios D, Rehle T, et al. South African National Health and Nutrition Examination Survey (SANHANES-1). Cape Town, SA: HSRC Press; 2013.
29. Zimmermann HW, Koch A, Seidler S, Trautwein C, Tacke F. Circulating soluble urokinase plasminogen activator is elevated in patients with chronic liver disease, discriminates stage and aetiology of cirrhosis and predicts prognosis. *Liver Int.* 2012;32(3):500-509.
30. Tuomi H, Kultti J, Danielsson J*, et al.* Serum soluble urokinase plasminogen activator receptor in alcoholics: relation to liver disease severity, fibrogenesis and alcohol use. *J Gastroenterol Hepatol.* 2014;29:1991-1995.
31. Salgado S, Garcia J, Vera J*, et al.* Liver cirrhosis is reverted by urokinase-type plasminogen activator gene therapy. *Mol Ther.* 2000;2(6):545-551.
32. Persson M, Engström G, Björkbacka H, Hedblad B. Soluble urokinase plasminogen activator receptor in plasma is associated with incidence of CVD. Results from the Malmö Diet and Cancer Study. *Atherosclerosis.* 2012;220(2):502-505.

33. Sehestedt T, Lyngbæk S, Eugen-Olsen J*, et al.* Soluble urokinase plasminogen activator receptor is associated with subclinical organ damage and cardiovascular events. *Atherosclerosis.* 2011;216(1):237-243.
34. Heraclides A, Jensen T, Rasmussen S*, et al.* The pro-inflammatory biomarker soluble urokinase plasminogen activator receptor (suPAR) is associated with incident type 2 diabetes among overweight but not obese individuals with impaired glucose regulation: effect modification by smoking and body weight status. *Diabetologia.* 2013;56(7):1542-1546.
35. Pisa P, Behanan R, Vorster H, Kruger A. Social drift of cardiovascular disease risk factors in Africans from the North West Province of South Africa: the PURE study. *Cardiovasc J Afr.* 2012;23(7):371-378.
36. Nanchen D, Stott DJ, Gussekloo J*, et al.* Resting heart rate and incident heart failure and cardiovascular mortality in older adults: role of inflammation and endothelial dysfunction: the PROSPER study. *Eur J Heart Fail.* 2012;15:581-588.
37. Marsche G, Saemann MD, Heinemann A, Holzer M. Inflammation alters HDL composition and function: Implications for HDL-raising therapies. *Pharmacol Ther.* 2013;137(3):341-351.
38. Tayal D, Goswami B, Tyagi S, Chaudhary M, Mallika V. Interaction between dyslipidaemia, oxidative stress and inflammatory response in patients with angiographically proven coronary artery disease: cardiovascular topics. *Cardiovasc J Afr.* 2012;23(1):23-27.
39. Andersen O, Eugen-Olsen J, Kofoed K, Iversen J, Haugaard SB. Soluble urokinase plasminogen activator receptor is a marker of dysmetabolism in HIV-infected patients receiving highly active antiretroviral therapy. *J Med Virol.* 2008;80(2):209-216.
40. Andersen O, Eugen-Olsen J, Kofoed K, Iversen J, Haugaard SB. suPAR associates to glucose metabolic aberration during glucose stimulation in HIV-infected patients on HAART. *J Infect.* 2008;57(1):55-63.
41. Fourie C, Rooyen J, Kruger A*, et al.* Soluble Urokinase Plasminogen Activator Receptor (suPAR) is Associated with Metabolic Changes in HIV-1-Infected Africans: A Prospective Study. *Inflammation.* 2012;35(1):221-229.
42. Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation.* 2005;112(23):3562-3568.

43. Lyngbæk S, Andersson C, Marott JL*, et al.* Soluble Urokinase Plasminogen Activator Receptor for Risk Prediction in Patients Admitted with Acute Chest Pain. *Clin Chem.* 2013;59(11):1621-1629.
44. Lyngbæk S, Marott JL, Sehestedt T*, et al.* Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *Int J Cardiol.* 2013;167:2904-2911.
45. Persson M, Östling G, Smith G*, et al.* Soluble Urokinase Plasminogen Activator Receptor A Risk Factor for Carotid Plaque, Stroke, and Coronary Artery Disease. *Stroke.* 2014;45(1):18-23.
46. Borné Y, Persson M, Melander O, Smith JG, Engström G. Increased plasma level of soluble urokinase plasminogen activator receptor is associated with incidence of heart failure but not atrial fibrillation. *Eur J Heart Fail.* 2014;16(4):377-383.
47. Pruijm M, Vollenweider P, Mooser V*, et al.* Inflammatory markers and blood pressure: sex differences and the effect of fat mass in the CoLaus Study. *J Hum Hypertens.* 2013;27:169-175.
48. Hilgers KF. Monocytes/macrophages in hypertension. *J Hypertens.* 2002;20(4):593-596.
49. Boos CJ, Lip GYH. Elevated high-sensitive C-reactive protein, large arterial stiffness and atherosclerosis: a relationship between inflammation and hypertension? *J Hum Hypertens.* 2005;19(7):511-513.
50. Li JJ, Fang CH, Hui RT. Is hypertension an inflammatory disease? *Med Hypotheses.* 2005;64(2):236-240.
51. Harrison DG, Guzik TJ, Lob HE*, et al.* Inflammation, immunity, and hypertension. *Hypertension.* 2011;57(2):132-140.
52. Chrysohoou C, Pitsavos C, Panagiotakos DB, Skoumas J, Stefanadis C. Association between prehypertension status and inflammatory markers related to atherosclerotic disease: The ATTICA Study. *Am J Hypertens.* 2004;17(7):568-573.
53. Stumpf C, John S, Jukic J*, et al.* Enhanced levels of platelet P-selectin and circulating cytokines in young patients with mild arterial hypertension. *J Hypertens.* 2005;23(5):995-1000.
54. Schillaci G, Pirro M. C-reactive protein in hypertension: clinical significance and predictive value. *Nutr Metab Cardiovasc Dis.* 2006;16(7):500-508.
55. Ohki R, Yamamoto K, Mano H*, et al.* Identification of mechanically induced genes in human monocytic cells by DNA microarrays. *J Hypertens.* 2002;20(4):685-691.

56. Wittenhagen P, Kronborg G, Weis N*, et al.* The plasma level of soluble urokinase receptor is elevated in patients with Streptococcus pneumoniae bacteraemia and predicts mortality. *Clin Microbiol Infect.* 2004;10(5):409-415.
57. Lawn S, Myer L, Bangani N, Vogt M, Wood R. Plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR) and early mortality risk among patients enrolling for antiretroviral treatment in South Africa. *BMC Infect Dis.* 2007;7(1):41.
58. Haupt TH, Petersen J, Ellekilde G*, et al.* Plasma suPAR levels are associated with mortality, admission time, and Charlson Comorbidity Index in the acutely admitted medical patient: a prospective observational study. *Crit Care.* 2012;16:R130.
59. Mekonnen G, Corban MT, Hung OY*, et al.* Plasma Soluble Urokinase-Type Plasminogen Activator Receptor Level is Independently Associated with Coronary Microvascular Function in Patients with Non-Obstructive Coronary Artery Disease. *Atherosclerosis.* 2015;239:55-60.
60. Lyngbæk S, Marott JL, Møller DV*, et al.* Usefulness of Soluble Urokinase Plasminogen Activator Receptor to Predict Repeat Myocardial Infarction and Mortality in Patients with ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Intervention. *Am J Cardiol.* 2012;110:1756-1763.
61. Thurison T, Christensen IJ, Lund IK, Nielsen HJ, Høyer-Hansen G. Circulating intact and cleaved forms of the urokinase-type plasminogen activator receptor: Biological variation, reference intervals and clinical useful cut-points. *Clin Chim Acta.* 2015;439:84-90.
62. Jalkanen V, Yang R, Linko R*, et al.* SuPAR and PAI-1 in critically ill, mechanically ventilated patients. *Intensive Care Med.* 2013;39:489-496.
63. Rabna P, Andersen A, Wejse C*, et al.* Urine suPAR Levels Compared with Plasma suPAR Levels as Predictors of Post-consultation Mortality Risk Among Individuals Assumed to be TB-negative: A Prospective Cohort Study. *Inflammation.* 2010;33(6):374-380.
64. Rabna P, Andersen A, Wejse C*, et al.* Utility of the Plasma Level of suPAR in Monitoring Risk of Mortality during TB Treatment. *PLoS One.* 2012;7(8):e43933.
65. Raggam R, Wagner J, Prüller F*, et al.* Soluble urokinase plasminogen activator receptor predicts mortality in patients with systemic inflammatory response syndrome. *J Intern Med.* 2014;276:651-658.

66. Galliera E, Drago L, Marazzi MG*, et al.* Soluble urokinase-type plasminogen activator receptor (suPAR) as new biomarker of the prosthetic joint infection: Correlation with inflammatory cytokines. *Clin Chim Acta.* 2015;441:23-28.
67. Lyngbæk S, Sehestedt T, Marott JL*, et al.* CRP and suPAR are differently related to anthropometry and subclinical organ damage. *Int J Cardiol.* 2013;167:781–785.
68. Villegas R, Xiang YB, Cai H*, et al.* Lifestyle determinants of c-reactive protein in middle-aged, urban Chinese men. *Nutr Metab Cardiovasc Dis.* 2012;22(3):223-230.
69. Imhof A, Froehlich M, Brenner H*, et al.* Effect of alcohol consumption on systemic markers of inflammation. *Lancet.* 2001;357(9258):763-767.
70. Bau PFD, Bau CHD, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. *Alcohol.* 2007;41(7):479-488.
71. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes.* 2007;56(4):1010-1013.
72. Fronczyk A, Molęda P, Safranow K, Piechota W, Majkowska L. Increased concentration of C-reactive protein in obese patients with type 2 diabetes is associated with obesity and presence of diabetes but not with macrovascular and microvascular complications or glycemic control. *Inflammation.* 2014;37(2):349-357.
73. Sesso HD, Buring JE, Rifai N*, et al.* C-reactive protein and the risk of developing hypertension. *JAMA.* 2003;290(22):2945-2951.
74. Niskanen L, Laaksonen DE, Nyyssönen K*, et al.* Inflammation, abdominal obesity, and smoking as predictors of hypertension. *Hypertension.* 2004;44(6):859-865.
75. Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension.* 2007;49(2):304-310.
76. Bautista L, Vera L, Arenas I, Gamarra G. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-α) and essential hypertension. *J Hum Hypertens.* 2005;19(2):149-154.
77. Fan Z-X, Hua Q, Li Y-P, Liu R-K, Yang Z. Interleukin-6, but not soluble adhesion molecules, predicts a subsequent mortality from cardiovascular disease in patients

with acute ST-segment elevation myocardial infarction. *Cell Biochem Biophys.* 2011;61(2):443-448.

78. Kaptoge S, Di Angelantonio E, Lowe G*, et al.* C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet.* 2010;375(9709):132-140.
79. Mendall M, Strachan D, Butland B*, et al.* C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *Eur Heart J.* 2000;21(19):1584-1590.
80. Zatu MC, van Rooyen JM, Wentzel-Viljoen E, Greeff M, Schutte AE. Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low resource settings: the PURE study. *J Hypertens.* 2014;32(4):749-755.

“The view is always worth the climb, live!”