

**Soluble urokinase plasminogen activator receptor and
cardiovascular function in African and Caucasian
populations: The SAfrEIC study**

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DECLARATION BY AUTHORS

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

Ms A Smith

Was involved in collecting and processing of cardiovascular data, namely pulse wave velocity and blood pressures as well as performing quality control regarding the correctness and completeness of each participant's questionnaires. Responsible for conducting the literature searches, statistical analyses, design and planning of manuscript, interpretation of results and writing of the manuscript.

Prof AE Schutte

Supervisor. Project leader of the SAfrEIC study. Responsible for design of study, collection of cardiovascular data, supervising the writing of the manuscript, making recommendations and giving professional input regarding the interpretation of data, and statistical analyses.

Dr R Schutte

Co-supervisor. Gave guidance and supervised the writing of the manuscript, collection and interpretation of data, and specific input regarding statistical analyses.

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Dr J Eugen-Olsen

International collaborator, responsible for the biochemical analyses of suPAR, as well as the interpretation of the results.

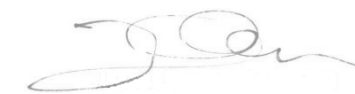
I declare that I have approved the above-mentioned manuscript, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that it may be form part of the MSc dissertation of Ms A Smith.

Prof AE Schutte



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SUMMARY

TITLE: Soluble urokinase plasminogen activator receptor and cardiovascular function in African and Caucasian populations: The SAfrEIC study

Motivation

Soluble urokinase plasminogen activator receptor (suPAR) is a known inflammatory marker, which is found in various body fluids. SuPAR reflects the immune and pro-inflammatory status of patients caused by HIV and tuberculosis, amongst others. However, recent studies have shown that suPAR is related to cardiovascular function. The cardiovascular health of the black South African population is a major health concern as this group suffers mostly from hypertension and stroke, leading to an alarming increase in cardiovascular morbidity and mortality. SuPAR may be able to contribute to early detection and prevention of cardiovascular diseases. No studies regarding the associations of suPAR with cardiovascular function have been investigated on black South Africans.

Objectives

To investigate suPAR as a possible marker of cardiovascular function in African and Caucasian men and women, by determining possible gender and ethnic-specific associations of suPAR with cardiovascular function.

Methodology

There were 207 African and 314 Caucasian men and women (aged 20-79 yrs.) included in this study. High-sensitivity C-reactive protein, glucose, lipids and creatinine were determined in fasting serum and suPAR was analyzed in plasma samples. Blood pressure was measured using the OMRON apparatus (HEM-747), with a 5-min rest interval between measurements. The Finometer device was used to determine the Windkessel compliance and the carotid dorsalis-pedis pulse wave velocity (PWV) was measured with the Complior (SP acquisition system) on the left side of each subject in the supine position. The means, adjusted means and proportions were compared between the groups by using independent t-tests, analysis of co-variance and the chi-square test, respectively. Associations were investigated between cardiovascular variables and suPAR using single and multiple regression analyses with either pulse wave velocity, systolic blood pressure, diastolic blood pressure or Windkessel compliance as dependent variable. Covariates included were age, body mass index, smoking, alcohol use, physical activity, glucose and high-density lipoprotein cholesterol.

Results and conclusion

SuPAR levels were significantly higher in Africans ($P < 0.001$) compared to Caucasians. After adjusting for body mass index, suPAR increased significantly with age in all groups, except for African women.

Moreover, the suPAR levels of African men and women were significantly higher than the Caucasians within each age quartile. While adjusting for age and body mass index, the cardiovascular profiles of the African and Caucasian men were less favourable compared to women, but suPAR levels were significantly higher in Caucasian women compared to men. In single regression, various measures of cardiovascular function correlated with suPAR in African men and Caucasian men and women. After adjusting for confounders the associations disappeared in Caucasian women, and remained non-significant in the African women. However, the association between PWV and suPAR remained significant in African men ($\beta=0.19$; $P=0.030$), while the association of systolic blood pressure ($\beta=0.20$; $P=0.017$), diastolic blood pressure ($\beta=0.17$; $P=0.020$) and Windkessel compliance ($\beta=-0.14$; $P=0.004$) with suPAR remained significant in Caucasian men. In conclusion, Africans presented higher suPAR levels compared to Caucasians, even when stratified by age. Gender specific associations indicated that suPAR was associated with arterial stiffness in African and Caucasian men only, therefore, indicating that suPAR could be a possible biomarker for predicting cardiovascular dysfunction.

Keywords: suPAR, cardiovascular function, ethnicity, gender, age

OPSOMMING

AFRIKAANSE TITEL: Oplosbare urokinase plasminogeen geaktiveerde reseptor en kardiovaskulêre funksie in swart en wit populasies: Die SAfrEIC studie

Motivering

Oplosbare urokinase plasminogeen geaktiveerde reseptor (suPAR) is a bekende inflammatoriese merker wat in verskeie liggaamsvloei-stowwe voorkom. SuPAR weerspieël die immuun en pro-inflammatoriese status van pasiënte wat onder andere deur MIV en tuberkulose geaffekteer is. Onlangse studies het egter gewys dat suPAR geassosieer word met kardiovaskulêre funksie. Die kardiovaskulêre gesondheid van die swart Suid-Afrikaanse populasie lei tot groot kommer aangesien hierdie groep hoofsaaklik aan hipertensie en beroerte onderwerp is en lei tot 'n drastiese toename in kardiovaskulêre morbiditeit en mortaliteit. SuPAR kan moontlik bydra tot vroeë opsporing en voorkoming van kardiovaskulêre siektes, maar geen studies aangaande die assosiasies van suPAR met kardiovaskulêre funksies is vantevore in die swart Suid-Afrikaanse populasie ondersoek nie.

Doelstelling

Die doel van die studie is om suPAR as 'n moontlike merker van kardiovaskulêre funksie in swart en wit mans en vrouens te ondersoek, om sodoende moontlike geslags- en ras-spesifieke assosiasies van suPAR met kardiovaskulêre funksie te ondersoek.

Metodologie

Die studie het uit 207 swart en 314 wit mans en vrouens (20-79 jaar oud) bestaan. Hoë-sensitiewe C-reaktiewe protein, glukose, lipiedes en kreatinien is in vastende serum bepaal en suPAR in die plasma monsters. Die OMRON apparaat (HEM-747) is gebruik om die bloeddruk te meet, met 'n 5-minuut rusinterval tussen metings. Die Finometer is gebruik om die Windkessel kompliansie te bepaal en die karotis dorsalis-pedis polsgolfsnelheid (PWV) is aan die linkerkant van elke proefpersoon gemeet met die Complior SP apparaat terwyl die proefpersoon op sy/haar rug lê. Die gemiddelde, aangepaste gemiddelde en verhoudings is tussen die groepe vergelyk deur gebruik te maak van 'n onafhanklike t-toets, analise van kovariansie en ook 'n chi-kwadraat toets, onderskeidelik. Assosiasies tussen suPAR en kardiovaskulêre veranderlikes is ondersoek deur die gebruik van enkel en meervoudige regressie analises met hetsy polsgolfsnelheid, sistoliese bloeddruk, diastoliese bloeddruk of Windkessel meegewendheid as die afhanklike veranderlike te gebruik. Ingeslote ko-veranderlikes was ouderdom, liggaamsmassa-indeks, rook, alkohol gebruik, fisieke aktiwiteit, glukose en hoë-digtheid lipoprotein cholesterol.

Resultate en gevolgtrekking

SuPAR vlakke is betekenisvol hoër onder die swart proefpersone ($P < 0.001$), in vergelyking met die wit proefpersone. Behalwe vir die wit vroue, het suPAR betekenisvol met ouderdom in al die groepe toegeneem, nadat vir liggaamsmassa-indeks aangepas is. Verder is die suPAR vlakke van swart mans en vrouens betekenisvol hoër as dié van die wit proefgroep in elke ouderdom kwartiel. Terwyl vir ouderdom en liggaamsmassa-indeks aangepas is, is die kardiovaskulêre profiele van die swart en wit mans swakker as dié van die vrouens, maar die suPAR vlakke is betekenisvol hoër onder die wit vrouens in vergelyking met die mans. In 'n enkel regressie het verskeie metings van kardiovaskulêre funksie met suPAR in die swart mans en wit mans en vrouens gekorreleer. Die assosiasie het onder die wit vrouens verdwyn en het nie-betekennisvol by die swart vrouens gebly nadat daar vir verskeie faktore aangepas is. Terwyl die assosiasie tussen PWV en suPAR betekenisvol gebly het in die swart mans ($\beta = 0.19$; $P = 0.030$). Die assosiasie van suPAR het ook in die wit mans betekenisvol gebly, met onder andere sistoliese bloeddruk ($\beta = 0.20$; $P = 0.017$), diastoliese bloeddruk ($\beta = 0.17$; $P = 0.020$) en Windkessel meegewendheid ($\beta = -0.14$; $P = 0.004$). Om saam te vat, swart proefpersone het hoër suPAR vlakke as die wit proefpersone getoon, selfs wanneer dit volgens ouderdom gestratifiseer is. Geslagspesifieke assosiasies toon dat suPAR geassosieer is met arteriële styfheid slegs in swart en wit mans. Dit dui daarop dat suPAR as 'n moonlike biomerker kan help in die voorspelling van kardiovaskulêre disfunksies.

Sleutelwoorde: suPAR, kardiovaskulêre funksie, etnisiteit, geslag, ouderdom

PREFACE

For the structure of this study it was decided to use the article format, as approved by the North-West University. This format basically consists of a manuscript ready for submission to a peer-reviewed journal. The manuscript is accompanied by an in-depth literature review as well as an interpretation of the results. The structured format of the information is as follows: Chapter 1 provides an introduction regarding the study, as well as the motivation, aims and hypotheses. A detailed literature overview pertaining to the topic is discussed in Chapter 2. Chapter 3 consists of the manuscript containing the abstract, introduction, methodology, results and interpretation of the study which will be submitted for publication to a peer-reviewed journal, namely *Thrombosis Research*. Chapter 4 is a critical summary of the results, providing final conclusions as well as recommendations. The appropriate references are provided at the end of each chapter according to the style of the journal.

OUTLINE OF THE DISSERTATION

Chapter 1 – Introduction, motivation, aims and hypothesis

Chapter 2 – Literature overview

Chapter 3 – *Manuscript*: Soluble urokinase plasminogen activator receptor and cardiovascular function in African and Caucasian populations: The SAfrEIC study

Chapter 4 – Summary of main findings

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LIST OF ABBREVIATIONS

β :	Beta
BMI:	Body mass index
CD4:	Cluster of differentiation 4
CRP:	C-reactive protein
Cwk:	Windkessel compliance
DBP:	Diastolic blood pressure
GPI:	Glycophosphatidylinositol
hs-CRP:	High-Sensitivity C-Reactive Protein
HAART:	Highly active antiretroviral therapy
HIV:	Human immunodeficiency virus
IL-6:	Interleukin-6
kg:	Kilogram
kg/m ² :	Kilogram per square meter
m:	Meter
mg/L:	Milligram per liter
mL/mmHg:	Milliliter per millimeter mercury
mmHg:	Millimeter mercury
mmol/L:	Millimolar per liter
m/s:	Meter per second
n:	Number of subjects
ng/ml:	Nanogram per milliliter
PWV:	Pulse wave velocity
SAfrEIC:	South African study on the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function
SD:	Standard deviation
suPAR:	Soluble urokinase plasminogen activator receptor
SBP:	Systolic blood pressure
uPA:	Urokinase plasminogen activator
uPAR:	Urokinase plasminogen activator receptor
WC:	Waist circumference
WHO:	World Health Organization

CHAPTER 1

Introduction

Introduction

Globally, cardiovascular disease is known to be a major health concern [1], however the prevalence of cardiovascular disease risk factors and related morbidity and mortality vary significantly amongst ethnic groups [2]. With a life expectancy of 44 years, black South Africans' life expectancy is amongst the lowest in the world [3]. This is mostly because of the high prevalence of Human Immunodeficiency Virus (HIV) infection, tuberculosis [4] and smoking amongst the African population [5]. Nevertheless, the majority of urban Africans also present multiple risk factors for cardiovascular disease [6].

Soluble urokinase plasminogen activator receptor (suPAR) is a soluble form of the urokinase plasminogen activator receptor (uPAR) and is found in various body fluids, such as urine, cerebrospinal fluid and plasma [7]. An elevated suPAR level reflects an inflammatory and immune system activation, which have been associated with poor clinical outcomes in patients suffering from infectious diseases [8], such as HIV-infection [9,10], tuberculosis [11,12] as well as several cancers [13]. High suPAR concentrations in the plasma independently predict high mortality in both tuberculosis patients and healthy individuals [12].

Recent studies point to suPAR's association with atherosclerosis, probably due to its inflammatory function. Low-grade inflammation is a sub-clinical chronic inflammatory state, which may contribute to the development of cardiovascular disease [14], through its association with atherosclerosis [14]. Although anti-inflammatory properties are present in the normal vascular endothelium, endothelial function is impaired in the presence of inflammatory conditions and increased oxidative stress [15]. Increased production of oxidative metabolic products is responsible for the activation of low-grade inflammatory mechanisms in the vascular wall [16], which impairs arterial function acutely and chronically [17-20].

Eugen-Olsen et al. [21] found that plasma suPAR is associated with the development of cancer, type 2 diabetes and cardiovascular disease in the general population. Their results also show that suPAR reflects different aspects of inflammation compared to C-reactive protein, for suPAR is not as well related to anthropometric parameters characterizing a dysmetabolic phenotype. The effect of suPAR was also age related, being associated more prominently with cardiovascular variables in the younger age groups. This indicates that young individuals are more susceptible to the detrimental effects of inflammation [21]. However, the study population consisted only of Caucasians. Not much is known regarding the relationship of suPAR with cardiovascular function in Africans.

Data generated from the present study would provide additional information on the relationship between suPAR and cardiovascular function, from groups of different ethnicity and gender.

Aim and objectives

The general aim of this study was to investigate suPAR as a possible marker of cardiovascular function in African and Caucasian men and women.

The detailed objectives are:

- to compare plasma suPAR levels of African and Caucasian groups stratified by gender and age; and
- to compare the associations of suPAR with measures of cardiovascular function in African and Caucasian men and women.

Hypotheses

Based on the available literature, the following hypotheses were formulated:

- Africans have higher suPAR levels than Caucasian men and women;
- in Africans, plasma suPAR is more strongly associated with measures of cardiovascular function when compared to Caucasians.

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CHAPTER 2

Literature overview

1. Africans and disease prevalence

South Africa is known as the country with the highest prevalence of HIV (Human Immunodeficiency Virus) [1]. The worldwide concern regarding Africa's HIV pandemic is justified, but it should not overshadow the need for treatment of other diseases. The incidence of tuberculosis remains high in Sub-Saharan Africa as a consequence of the immune suppression caused by HIV-infection [2]. Cancer prevalence is also increasing amongst African people of whom 36% is infection-related, namely hepatitis B and C as well as HIV [3]. Cardiovascular diseases are increasing and threatening the health of Sub-Sahara Africans and are contributing to the mortality and morbidity rates of the region [4]. Together, all of these diseases contribute to the low life expectancy of 44 years of black South Africans [5].

With urbanization, cardiovascular disease develops as observed in Africans and has become an increasing health burden that requires skilful, cost-effective management [6]. As shown in the INTERHEART study, hypertension is a strong contributor to the development of cardiovascular disease in black Africans [7]. Hypertension is eminently treatable and to some extent preventable [8]. In South Africa, hypertension is a major public health concern [9]. This disease is the most common risk factor for cardiovascular morbidity and mortality, and even moderately elevated blood pressure is associated with an increased risk of myocardial infarction, heart failure, stroke, and chronic renal failure [10]. In an epidemiological study performed in 2005 it has been established that 22.9% of men and 23.4% of women in the South African population are hypertensive [11]. The onset of hypertension is caused by many contributing factors including salt-sensitivity, socio-economic conditions, the low awareness and/or weak control of hypertension, and also urbanisation which is believed to be an important factor in the onset of hypertension among black South Africans [12]. Environmental stressors, the onset of obesity and lifestyle changes (including dietary changes) – associated with urbanisation, are likely to enhance mechanisms involving sympathetic activity and contribute to the early development and severe progression of hypertension in Africans [13].

Black Africans with higher-income are more susceptible to myocardial infarction than high-income white or other nonblack Africans [7]. Besides hypertension, the prevalence of diabetes mellitus is another major contributor to cardiovascular disease in Africa [14], such as myocardial infarction [7].

The increasing incidence of diabetes in Africa will also augment the severity of renal and cardiac damage caused by any given blood pressure level [8]. Even though the medication for hypertension is effective, there are some concerns regarding the high prevalence, the frequent underdiagnosis of hypertension and the adherence to medication, not only in developing countries but also in developed countries [8]. Unfortunately, follow-up and control of blood pressure in South Africa are

inadequate due to poor education and inability to understand the severity of the illness. The required facilities may also be lacking [8]. The total number of hypertensive subjects in the developing world is high, and a cost-analysis of possible antihypertensive drug treatment indicates that developing countries cannot afford the same treatment as developed countries [15].

As mentioned, with the increase in urbanisation in South Africa, many black people have been subjected to a process of rapid urbanization which may lead to social and cultural disruption causing increased levels of stress [16]. This is related to the fact that the traditional diet is exchanged with a more Western diet typified by decreases in carbohydrate and fiber and increases in fat, which leads to obesity. The traditional diet is associated with a low prevalence of degenerative diseases, whereas the Western diet is associated with increased prevalence [17].

Whatever the mechanism, urbanisation in South Africa has led to a significant increase in diseases of lifestyle such as hypertension and diabetes, resulting in coronary heart disease and cerebrovascular disease [16]. It is therefore necessary to identify biomarkers that may add in the identification of cardiovascular dysfunction as early as possible, which is not only cost-effective but also a good clinical marker.

2. Soluble urokinase plasminogen activator receptor (suPAR)

2.1 The origin of suPAR

SuPAR is the soluble form of the urokinase-type plasminogen activator receptor (uPAR), to which the urokinase plasminogen activator (uPA) system binds.

The uPA-system regulates cell movement through a mechanism that involves adhesion, proteolysis and chemotaxis which relies on the binding to plasminogen activator inhibitor-1 and to its receptor uPAR [18-21]. UPAR is, through its interaction with vitronectin, involved in cell adhesion and migration [22,23], as well as other immune functions such as fibrinolysis, angiogenesis and cell proliferation, which are expressed by immune cells, in particular neutrophils, monocytes and activated T-cells [22-24].

UPAR has three homologous domains (I, II and III) which are anchored within the plasma membrane by a glycoposphatidylinositol (GPI)-anchor at the uPAR(III) domain [25,26]. The amino-terminal domain is primarily involved in the molecular contact with uPA, where plasminogen activation is primary focused on the cell surface [27,28]. Through this interaction, cell migration and extracellular matrix remodelling are regulated by the uPA-uPAR system

[18,29]. Cleavage of the GPI anchor can shed both the intact and cleaved uPAR off the cell surface, which leads to soluble forms of uPAR (suPAR) in the circulation [26].

2.2 Functions of suPAR

SuPAR is present in plasma, red blood cells, urine, cerebrospinal fluid and serum [30-32] in various concentrations depending on the “activation” level of the immune system, since higher activation increases serum suPAR levels. SuPAR can be released into the circulation, peritoneal or ascetic fluid and is also found in high concentrations in the cystic fluid from ovarian cancer patients [33,34]. Moreover, a number of tumor cell lines [34,35] and monocytic cells release suPAR in the range of 0.8-3 ng/ml [36]. SuPAR could also originate from smooth muscle and vascular endothelial cells, which are potential sources of suPAR in the extracellular fluids and plasma [37].

SuPAR exists in plasma at concentrations of 1-10 ng/ml and, together with uPA, are elevated in cancer patients [38] as well as in patients with paroxysmal nocturnal haemoglobinuria due to a defect in enzymes responsible for synthesis of the GPI-anchor [39]. SuPAR is also found at elevated concentrations due to overexpression especially in diseases associated with hyperinflammation or in clinical sepsis syndrome [40].

In summary, suPAR has a variety of functions and is involved in numerous physiological pathways, including the plasminogen activating pathway, inflammation and modulation of cell adhesion, migration and proliferation [41-43].

2.3 SuPAR as a biomarker for diseases

SuPAR reflects the state of an individual’s immune activation which was substantiated by findings of increased suPAR levels in individuals suffering from parasitical, viral or bacterial infections as well as cancer and autoimmune diseases. In all of these conditions the prognosis of the disease worsened when the concentration of suPAR started to elevate [41-43,32]. SuPAR also shows significant positive correlations with increasing age [44] and gender [45], where women have slightly higher concentrations compared to men.

2.3.1 Cancer

The urokinase plasminogen system is particularly associated with the process of metastasis, namely to spread primary tumors to distant organs which is associated with poor prognosis and high mortality [46].

The urokinase plasminogen activator (uPA) system provides the most substantial amount of activated plasminogen when tissues are being degraded [47]. However, different components of the system are located in different areas dependent on the cancer type. SuPAR forms are prognostic markers in several cancers [48-51]. Variations of levels in suPAR are associated with poorer prognosis in malignant tumors, suPAR (II - III) in ovarian cancer [50] and suPAR (I) in prostate cancer [51].

2.3.2 Human Immunodeficiency Virus (HIV)

The immune and pro-inflammatory status of HIV-infected patients are reflected by the circulating suPAR, which is suppressed by highly active antiretroviral therapy (HAART) [44].

SuPAR is a strong predictor of mortality and immunologic failure in HIV-1 infected patients on HAART and has a prognostic strength similar to that of CD4+ T-cell count [52,53]. In patients receiving HAART, suPAR also demonstrated its potential as a treatment efficacy marker when its levels decreased with effective therapy [54] and, therefore, has potential clinical benefits.

The implications of suPAR as a clinical HIV management tool were recently extended to prognostic information of the metabolic status of patients undergoing HAART [44], as HIV-infected patients receiving HAART have an increased risk of various metabolic disorders, which may involve low-grade inflammation and other immunological perturbations. Anderson et al. found that suPAR remained elevated in some HIV-infected patients independently of the HAART's effects on it, which reflected a possible low-grade pro-inflammatory state [44]. They concluded that suPAR may reflect the metabolic status of the HIV-infected patients on HAART and linked dysmetabolism with low-grade inflammation [44], which was similar to the findings of Knob et al. and suggested that suPAR is a potential marker of dysmetabolism in HIV-infected patients on stable HAART [55].

2.3.3 Tuberculosis

As a consequence of the immune suppression caused by the HIV epidemic, the incident rate of tuberculosis still remains high in Sub-Saharan Africa [2]. Tuberculosis elevates suPAR levels, thereby increasing the patients' risk of mortality [44].

A great challenge is represented by tuberculosis patients regarding the diagnosis of active or latent infection and the monitoring of treatment efficacy. Eugen-Olsen et al. [44] investigated suPAR regarding its ability to predict tuberculosis treatment efficiency and found that suPAR levels at the time of tuberculosis treatment initiation are prognostic for survival during the 8-

month treatment period. In addition, in those who completed their treatment successfully, the suPAR levels decreased to the level of non-infected individuals. This makes suPAR a very promising biomarker in tuberculosis as well as for tuberculosis treatment efficacy, which was also confirmed by the investigation performed by Siawaya et al. [56].

2.3.4 Atherosclerosis

Atherosclerosis is currently recognised as an inflammatory disorder. Low grade inflammation contributes to all stages of atherosclerosis, from the initial phase of increased endothelial permeability up to the formation of the mature atherosclerotic plaque and plaque rupture [57].

Steins et al. [58] found that the uPA and its receptor uPAR are involved in atherosclerosis, with overexpression of uPA and uPAR in atherosclerotic lesions. Overexpression may be caused by activation of macrophages, endothelial cells and smooth muscle cells that synthesise and secrete these molecules [58].

Atherosclerotic lesions are common in the carotid artery of the elderly, but only a minority of plaque cause cerebrovascular ischemic events. These symptomatic carotid atherosclerotic plaques are characterised by degradation of the extracellular matrix, high macrophage density, and rupture of the fibrous cap which leads to hemorrhage and thrombosis [59]. UPAR is locally enriched within symptomatic plaques especially in regions with a ruptured fibrous cap and high macrophage density [60].

As mentioned, different forms of suPAR are prognostic markers in several cancers and can help identify the particular cancer at hand [48-51]. This explains the fact that circulating suPAR levels could be elevated to a measurable level when uPAR is over expressed in a relatively small tissue volume, which means that it is plausible that over expressed uPAR in atherosclerotic plaques could be detected systematically. Pawlak et al. performed some studies in uremic patients determining the relationship between circulating suPAR concentrations and atherosclerosis, and found that the collective suPAR concentrations were independently associated with carotid intima-media thickness [61,62], a marker of atherosclerotic disease and progression.

The progression of atherosclerotic vascular damage is inherent in coronary heart disease, stroke and peripheral artery disease, which are closely associated with arterial stiffness [63]. Systemic inflammation [64] and oxidative stress [65] are essential pathogenetic features of atherosclerosis, which initiates, progresses and develops disease complications. Inflammation and oxidative stress are responsible for endothelial dysfunction [66] and arterial stiffness [67],

which are essential characteristics of vascular deterioration. The endothelium is responsible for regulating vascular tone and structure [68], but when endothelial function is impaired it contributes to the progression of arterial stiffness [69,70]. Kals et al. [71] found that impairment of endothelial vasomotor capacity is related to degree of inflammation in the subclinical condition, whereas arterial stiffening is determined by the level of oxidative modifications in atherosclerosis. It is clear that endothelial dysfunction and premature arterial stiffening are important parameters in the stratification of cardiovascular risk [72].

Arterial stiffness can be determined by measuring the Windkessel compliance [73] or the pulse wave velocity, which is a useful predictor for future cardiovascular events in high-risk subjects such as those with hypertension [74,75]. Aortic augmentation index is also related to arterial properties via changes in pulse wave velocity [76]. Arterial stiffness increases pulse wave velocity and elevates systolic blood pressure and pulse pressure [77], an effect which increases arterial wall stress and potentiates the development of atherosclerosis [76].

2.3.5 Inflammation

Inflammation is a complex biological reaction to damaging stimuli and is a necessary response of the immune system to infection or trauma. Inflammation is the result of major increases in circulating levels of inflammatory mediators [78,79].

Low-grade inflammation is a sub-clinical chronic inflammatory state, which may contribute to the development of cancer [80], type 2 diabetes mellitus [81] and CVD [57]. Anti-inflammatory properties are present in the normal vascular endothelium; however, endothelial function is impaired in the presence of inflammatory conditions and increased oxidative stress [82]. Increased production of oxidative metabolic products is responsible for the activation of low-grade inflammatory mechanisms in the vascular wall [83]. An inflammatory stimulus impairs arterial function acutely and chronically [84-87]. Furthermore, chronic low-grade inflammation and oxidative stress are closely related to atherosclerosis by contributing to all its stages, from the initial phase of increased endothelial permeability up to the formation of the mature atherosclerotic plaque and plaque rupture [88-90].

C-reactive protein (CRP) is an acute phase inflammatory protein and is a well-known marker of inflammation and tissue damage [91]. CRP is known to be produced in the liver, and synthesised by hepatocytes in response to inflammatory cytokines, in particular IL-6 [92-94]. CRP is commonly used as a biomarker for low-grade inflammation and is measured by using a high sensitivity assay (hsCRP). The plasma concentration of CRP is associated with cancer mortality and total mortality [95]. Furthermore, CRP levels are also associated with arterial

function and has been related to pulse pressure, stiffness of elastic and muscular arteries and central wave reflections [87,96], which explains the association with increased risk of CVD [97]. Unlike suPAR, a significant amount of research has been done on CRP and its association with cardiovascular diseases. Various other inflammatory markers have also been investigated with regards to cardiovascular disease, which is listed in table 1.

Table 1: Other inflammatory markers associated with cardiovascular disease

Inflammation Marker	
Interleukin-6	Cesari et al. (98); Rodondi et al.(99) ; Ridker et al.(100)
Tumor necrosis factor-α	Cesari et al. (98); Ridker et al.(101)
MMP-9	Yasmin et al. (102); Noji et al. (103)
NT-proBNP	Olsen et al. (104); Bibbins-Domingo et al. (105)
Serum Amyloid A	Ridker et al. (100); Johnson et al.(106)
Fibrinogen	Stec et al.(107) ; Ernst et al.(108)
sICAM-1	Albert et al. (109); Ridker et al.(100)
Pentraxin 3	Suzuki et al. (110); Kotooka et al.(111)

Matrix metalloproteinase-9 (MMP-9); N-terminal pro-brain natriuretic paptide (NT-proBNP); soluble intercellular adhesion molecule (sICAM-1)

3. A good clinical marker

SuPAR seems to be a good potential clinical marker because of its high stability in plasma samples. For example, suPAR levels of healthy individuals are known to be very stable throughout the day [112]. In HIV-infected patients on stable HAART, the circadian variation in plasma suPAR levels are shown to be very limited [44]. In addition, suPAR remains stable after repeated freeze-thaw cycles of plasma samples [113]. Thus, suPAR measurements based on biological fluid derived from human subjects will be valid, independent of the sampling schedule or whether the subject was fasting or not [30].

4. References

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CHAPTER 3

Soluble urokinase plasminogen activator receptor and cardiovascular function in African and Caucasian populations: The SAfrEIC study

**Anélda Smith, Rudolph Schutte, Jesper Eugen-Olsen, Micheal H Olsen,
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ORIGINAL ARTICLE

Soluble urokinase plasminogen activator receptor and cardiovascular function in African and Caucasian populations: The SAfrEIC study

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Instructions for Authors: Thrombosis Research

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Abstract

Introduction: A high concentration of soluble urokinase-type plasminogen activator receptor (suPAR) is indicative of an inflammatory state. This is caused mainly by HIV-infection, tuberculosis and cancer. Recent studies also identified suPAR as an indicator of cardiovascular disease development, where suPAR was related to atherosclerosis amongst uremic patients. The black South African population is known to have a high prevalence of cardiovascular diseases, but the potential role of suPAR is unknown. We aimed to investigate suPAR as a possible marker of cardiovascular dysfunction in African and Caucasian men and women.

Materials and Methods: This study involved 207 African and 314 Caucasian men and women (aged 20-70 yrs). High-sensitivity C-reactive protein, glucose and lipids were determined in fasting serum and suPAR in plasma samples. Blood pressure, pulse wave velocity and Windkessel arterial compliance were measured.

Results: In single regression, various measures of cardiovascular function correlated with suPAR in African men and Caucasian men and women. However, after adjusting for confounders, associations between pulse wave velocity and suPAR remained significant in African men ($\beta=0.19$; $P=0.030$), while the association of systolic blood pressure ($\beta=0.20$; $P=0.017$), diastolic blood pressure ($\beta=0.17$; $P=0.020$) and Windkessel compliance ($\beta=-0.14$; $P=0.004$) with suPAR remained significant in Caucasian men. Independent associations were absent in African and Caucasian women.

Conclusion: Africans presented higher suPAR levels compared to Caucasians, even when stratified by age. Gender specific associations indicated that suPAR was associated with arterial stiffness only in African and Caucasian men.

KEYWORDS: suPAR; cardiovascular; African; Caucasian; men; women

ABBREVIATIONS:

suPAR: Soluble urokinase plasminogen activator receptor

uPAR: Urokinase plasminogen activator receptor

HIV: Human Immunodeficiency Virus

CRP: C-reactive protein

PWV: Pulse wave velocity

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Cwk: Windkessel compliance

Introduction

Cardiovascular disease is globally recognized as a major health concern [1]. It is known that African people have a higher risk of developing hypertension than Caucasians [2] and combined with various factors associated with urbanization, such as obesity and diabetes, the risk of cardiovascular morbidity and mortality increases dramatically [3].

Soluble urokinase plasminogen activator receptor (suPAR) is a soluble form of the urokinase plasminogen activator receptor (uPAR) and is found in various body fluids [4]. SuPAR reflects the immune and pro-inflammatory status of patients [5], mainly caused by human immunodeficiency virus (HIV)-infection [6,7], tuberculosis [8,9], as well as several cancers [10]. Apart from the clear link between suPAR and infectious diseases, recent studies point to its association with atherosclerosis [11] and the development of cardiovascular disease [12], especially in young individuals. In uremic patients, circulating suPAR concentrations were independently associated with carotid intima-media thickness [13,14], a marker of atherosclerotic disease. SuPAR reflects different aspects of inflammation as opposed to C-reactive protein (CRP). SuPAR is less related to anthropometric indices, and therefore characterizes a dysmetabolic phenotype [12].

Most black urban South Africans present multiple risk factors for cardiovascular disease [15]. It is, therefore, important to identify markers that may explain their increased risk and may contribute to the early detection and prevention of cardio-metabolic abnormalities. To date no evidence is available whether suPAR relates to cardiovascular function in black South Africans and whether ethnic differences exist regarding suPAR levels. The aims of this study were, therefore, to compare suPAR levels of Africans and Caucasians stratified by gender and age, as well as to evaluate the associations of suPAR with cardiovascular function in African and Caucasian people.

Subjects and Methods

Study design

The SAfrEIC (South African study on the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) study was a cross-sectional study, which included a total of 750 African and Caucasian men and women from the urban areas of the Potchefstroom district in the North West Province in South Africa.

Inclusion criteria were men and women aged between 20 and 70 years, while pregnant and lactating women were excluded. For the present study a subsample of 207 African and 314 Caucasian participants were included. A total of 229 participants were excluded due to the following reasons:

nonfasting (n=46); use of oral contraception (n=48); missing data (n=29); hyperglycemic (n=6); or previous diagnosis of any serious chronic illness, e.g. HIV infection (n=98) or diabetes (n=2).

All procedures were explained to the subjects in their home language prior to their participation in the study and they had the opportunity to ask questions. Thereafter, each subject signed an informed consent form and participated voluntarily. The study complied with the Declaration of Helsinki 1975 (as revised in 2008) for investigation of human subjects. The Ethics Committee of the North-West University, Potchefstroom Campus, approved the study (06M01 on 13 April 2007).

Questionnaires

Demographic, lifestyle and physical activity questionnaires were used to assess alcohol use, smoking habit and physical activity level.

Anthropometric measurements

Anthropometric measurements were done according to standard methods described by Marfell-Jones et al. [16]. Height measurements were taken using a stadiometer (Invicta, IP 1465, UK), and body weight measurements using a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). Waist circumference was measured at midway level between the inferior rib margin and superior margin of the iliac crest. All the measures were taken in triplicate.

Cardiovascular measurements

Each participant rested for 10 minutes prior to blood pressure measurements. Blood pressure was measured using the OMRON HEM-757 (Omron, Kyoto, Japan) apparatus, with the blood pressure cuff on the left upper arm. Two measurements were taken, with a 5-min rest interval between measurements.

Thereafter, the Finometer device (FMS; Finapres Measurement Systems, Amsterdam, The Netherlands) [17,18] was connected and a 5-min continuous measurement of cardiovascular variables was taken. During the recording, after 2 minutes, a return-to-flow systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial artery pressure [18]. The highest precision in cardiovascular measurements is obtainable only after this calibration [18]. The Finometer computed the Windkessel arterial compliance online and stored the data in result files [19]. The cardiovascular data were processed using Beatscope 1.1 (FMS, Finapres Medical Systems, Amsterdam, The Netherlands).

The carotid dorsalis-pedis pulse wave velocity (PWV) was measured by the same two observers, using the Complior SP acquisition system (Artech, Medical, Pantin, France) on the left side of each subject in the supine position and made use of the subtraction method when calculating the distances.

Biochemical analyses

A qualified nurse performed a finger prick to determine the fasting glucose level which was directly measured with an enzymatic method to screen for diabetes mellitus (LifeScan SureStep® Blood Glucose Monitoring System, LifeScan Inc., Melputas Ca 9535, 1995, USA). Afterwards a fasting blood sample was taken from the antebrachial vein branches using a sterile winged infusion set. Plasma and serum samples were prepared according to standard methods and stored at -80 °C until analyses were performed. Serum C-reactive protein, lipids and creatinine were determined with the Konelab 20i™ auto-analyser (Thermo Fisher Scientific, Oy, Vantaa, Finland). Creatinine clearance was estimated using the Cockcroft-Gault formula [13]. Plasma (EDTA) suPAR levels were determined using the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark).

Statistical analysis

For database management and statistical analyses, we used Statistica version 9.0 (Statsoft, Inc., Tulsa, OK, 2009). Variables with a non-Gaussian distribution were logarithmically transformed and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. We compared means, adjusted means and proportions by using independent t-tests, analysis of co-variance and the chi-square test, respectively. Associations were investigated between either suPAR or CRP and cardiovascular variables using single and multiple regression analyses with either pulse wave velocity, systolic blood pressure, diastolic blood pressure or Windkessel compliance as dependent variable. Covariates included were age, body mass index, glucose, high-density lipoprotein cholesterol, smoking, alcohol use and physical activity.

Results

Table 1 summarizes the participants' characteristics, stratified by ethnicity. The Africans and Caucasians were of similar age, however all anthropometric measures were higher in the Caucasians. Despite this, the Africans had higher blood pressure, pulse wave velocity and lower Windkessel compliance. In addition to C-reactive protein levels, suPAR levels were also significantly higher in Africans (P<0.001). Genders were compared and shown in Appendix A.

Table 1: Physical and metabolic characteristics of the African and Caucasian participants

	Africans	Caucasians	P-value
Number of participants	207	314	
Gender (men/women)	116/91	158/156	0.20
Age (years)	42.5 ± 13.1	41.2 ± 13.0	0.24
Anthropometric measurements			
Height (m)	1.64 ± 0.09	1.73 ± 0.09	< 0.001
Weight (kg)	61.8 ± 15.7	84.2 ± 19.5	< 0.001
Body mass index (kg/m ²)	23.2 ± 6.33	28.0 ± 5.92	< 0.001
Waist circumference (cm)	77.8 ± 12.5	88.9 ± 14.9	< 0.001
Cardiovascular measurements			
Systolic blood pressure (mmHg)	128 ± 21.8	120 ± 16.3	< 0.001
Diastolic blood pressure (mmHg)	85.3 ± 13.5	78.5 ± 9.98	< 0.001
Pulse pressure (mmHg)	42.6 ± 13.1	41.7 ± 10.7	0.37
Windkessel compliance (mL/mmHg)	1.57 ± 0.50	2.14 ± 0.59	< 0.001
Pulse wave velocity (m/s)	8.32 ± 1.60	7.88 ± 1.17	< 0.001
Lipid profile			
High-density lipoprotein cholesterol (mmol/L)	1.60 ± 0.59	1.36 ± 0.42	< 0.001
Low-density lipoprotein cholesterol (mmol/L)	2.35 ± 0.88	3.75 ± 1.12	< 0.001
Triglycerides (mmol/L)	1.02 [0.95;1.09]	1.31 [1.23;1.39]	< 0.001
Estimated creatinine clearance rate (mL/min)	108 ± 31.9	134 ± 33.0	< 0.001
Biochemical variables			
Glucose (mmol/L)	5.06 ± 0.76	5.53 ± 0.86	< 0.001
C-reactive protein (mg/L)	2.21 [1.74;2.81]	1.23 [1.00;1.50]	< 0.001
suPAR (ng/mL)	3.01 [2.86;3.17]	2.27 [2.20;2.34]	< 0.001
Lifestyle			
Physical activity	7.90 ± 1.35	7.57 ± 1.40	0.009
Smoking n, (%)	137 (66.5)	50 (16)	< 0.001
Alcohol use n, (%)	152 (73.4)	206 (65.6)	0.059
Hypertensive n, (%)	77 (37.2)	45 (14.3)	< 0.001
Medication			
Anti-hypertensive n, (%)	0 (0)	67 (21.4)	< 0.001
Anti-inflammatory n, (%)	1 (0.5)	22 (7.03)	< 0.001

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SuPAR, soluble urokinase plasminogen activator receptor.

When viewing suPAR according to quartiles of age (Figure 1), adjusted for body mass index, the suPAR levels of both African men (P<0.001) and Caucasian men (P<0.001) and women (P=0.020) increased significantly with age (P<0.001). Moreover, the suPAR levels of African men and women were significantly higher than the Caucasians within each age quartile.

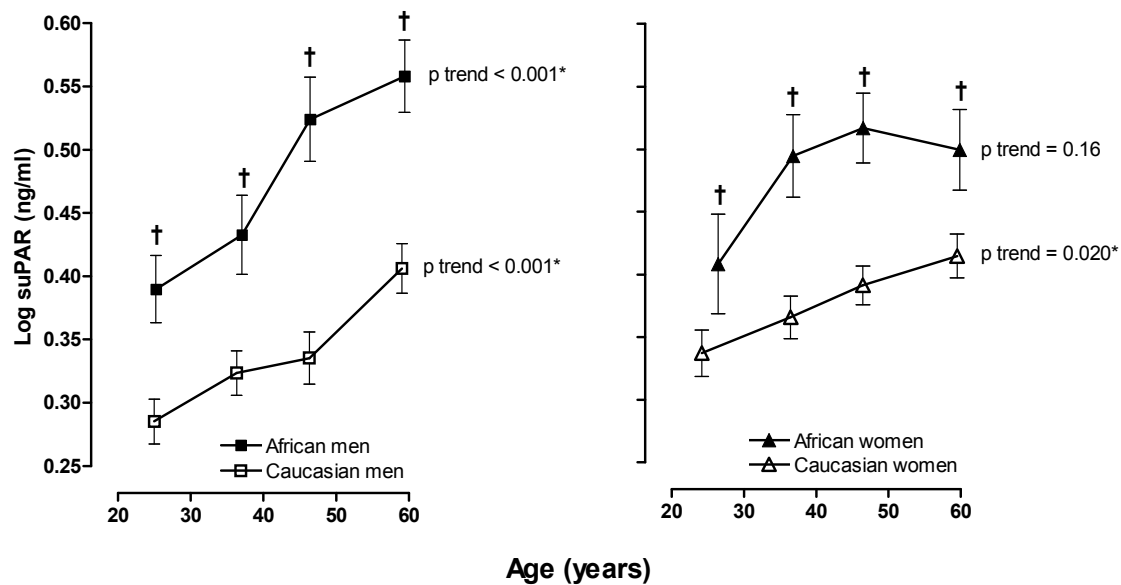


Figure 1: Soluble urokinase plasminogen activator receptor (suPAR) according to age quartiles of African and Caucasian men and women adjusted for body mass index

* Significant differences between quartile 1 and 4 ($P < 0.05$); † Significant ethnic differences between suPAR levels within a specific quartile ($P < 0.05$). Bars indicate standard error of mean.

In Table 2 the men and women were compared within each ethnic group while adjusting for age and body mass index. The cardiovascular profiles of the African and Caucasian men were less favourable compared to women. The inflammatory markers were similar in the African and Caucasian gender groups, except for suPAR levels that were significantly higher in Caucasian women compared to men.

Table 2: Gender differences of cardiovascular measurements and inflammatory markers of Africans and Caucasians.

	African men	African women	P	Caucasian men	Caucasian women	P
Number of participants	116	91		158	156	
Cardiovascular measurements						
SBP (mmHg)	133 ± 20.5	121 ± 20.2	<0.001	125 ± 13.7	115 ± 13.7	<0.001
DBP (mmHg)	86.8 ± 13.2	84.0 ± 12.9	0.16	79.8 ± 8.35	77.2 ± 8.35	0.006
Pulse pressure (mmHg)	46.7 ± 12.5	37.5 ± 12.3	<0.001	45.6 ± 9.60	37.7 ± 9.60	<0.001
Cwk (mL/mmHg)	1.71 ± 0.29	1.40 ± 0.29	<0.001	2.35 ± 0.30	1.92 ± 0.30	<0.001
PWV (m/s)	8.64 ± 1.37	7.92 ± 1.34	<0.001	8.11 ± 1.01	7.65 ± 1.01	<0.001
Inflammatory markers						
hs-CRP (mg/L)	2.07 [1.50;2.85]	2.57 [1.80;3.66]	0.40	1.18 [0.93;1.51]	1.27 [0.99;1.62]	0.70
suPAR (ng/mL)	2.99 [2.78;3.21]	3.06 [2.82;3.31]	0.69	2.16 [2.08;2.25]	2.39 [2.29;2.48]	<0.001

Data are arithmetic mean ± SD or geometric mean (fifth and 95th percentile intervals) for logarithmically transformed variables. All of the above data are adjusted for age and body mass index. SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Cwk, Windkessel compliance; PWV, Pulse wave velocity; CRP, C-reactive protein; suPAR, soluble urokinase plasminogen activator receptor.

In single regression analyses (Table 3), all groups, except African women, indicated significant correlations between suPAR and age, which is also indicated in Figure 1. Amongst the Africans, only the men showed significant associations of SBP ($r=0.23$; $P=0.013$), DBP ($r=0.25$; $P=0.006$), Cwk ($r=-0.33$; $P<0.001$) and PWV ($r=0.34$; $P<0.001$) with suPAR. In the Caucasian group, both genders had significant correlations of blood pressure and measures of arterial stiffness with suPAR.

Table 4: Single regression analyses of suPAR with obesity, cardiovascular measurements and CRP

	African men (N = 116)		African women (N = 91)		Caucasian men (N = 158)		Caucasian women (N = 156)	
	r	P	r	P	r	P	r	P
Age (years)	0.38	< 0.001	0.16	0.13	0.35	< 0.001	0.36	< 0.001
Anthropometric measurements								
BMI (kg/m ²)	-0.04	0.68	-0.02	0.83	0.19	0.018	0.32	< 0.001
WC (cm)	0.13	0.17	0.04	0.73	0.24	0.002	0.39	< 0.001
Cardiovascular measurements								
SBP (mmHg)	0.23	0.013	-0.004	0.97	0.23	0.004	0.22	0.006
DBP (mmHg)	0.25	0.006	0.01	0.89	0.28	< 0.001	0.27	0.001
Cwk (ml/mmHg)	-0.33	< 0.001	-0.11	0.32	-0.33	< 0.001	-0.09	0.26
PWV (m/s)	0.34	< 0.001	0.12	0.26	0.22	0.005	0.24	0.002
Inflammatory marker								
CRP (mg/L)	0.17	0.066	0.19	0.070	0.31	< 0.001	0.26	0.001

BMI, Body mass index; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Cwk, Windkessel compliance; PWV, Pulse wave velocity; CRP, C-reactive protein.

Forward stepwise multiple regression analyses (Table 4) confirmed the associations of suPAR with cardiovascular measurements in both African and Caucasian men, whereas the associations in African women remained absent and disappeared in Caucasian women. SuPAR remained significantly positively associated with pulse wave velocity only in African men, whereas in Caucasian men, suPAR correlated positively with systolic and diastolic blood pressure and negatively with Windkessel compliance. Another forward stepwise multiple regression analyses between CRP and cardiovascular measurements was performed (Appendix A) and showed no association in any group.

Table 4: Forward stepwise multiple regression analyses with either systolic blood pressure, diastolic blood pressure, pulse wave velocity, or Windkessel compliance as dependent variable.

suPAR	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	R ²	Adj R ²	β	P	R ²	Adj R ²	β	P
African men	0.287	0.253	0.095	0.30	0.319	0.279	0.139	0.12
Caucasian men	0.163	0.151	0.197	0.017	0.408	0.391	0.169	0.020
African women	0.406	0.347	-0.018	0.85	0.280	0.209	0.019	0.86
Caucasian women	0.362	0.323	-0.016	0.83	0.317	0.275	0.012	0.88

suPAR	Pulse wave velocity (m/s)				Windkessel compliance (mL/mmHg)			
	R ²	Adj R ²	β	P	R ²	Adj R ²	β	P
African men	0.370	0.352	0.185	0.030	0.721	0.698	-0.006	0.92
Caucasian men	0.247	0.226	0.079	0.34	0.727	0.721	-0.140	0.004
African women	0.531	0.504	0.091	0.27	0.717	0.693	-0.025	0.70
Caucasian women	0.391	0.359	0.023	0.76	0.662	0.641	0.007	0.90

Independent variables included suPAR, age, body mass index, smoking, alcohol use, physical activity, glucose and high-density lipoprotein cholesterol. SuPAR, soluble urokinase plasminogen activator receptor.

Discussion

This study investigated suPAR as a marker of cardiovascular dysfunction in African and Caucasian people. We found that African people had significant higher suPAR concentrations compared to Caucasians. SuPAR levels of African men and Caucasian men and women increased significantly with increasing age. In addition, suPAR was independently related to blood pressure and arterial stiffness in African and Caucasian men, but not in women.

The higher suPAR levels observed along with elevated CRP levels in Africans is not surprising since previous studies have shown that Africans have higher levels of inflammatory markers compared to Caucasians [20,21]. This study therefore confirms this but also adds to current knowledge by showing that suPAR levels were also higher in different age categories, independent of body mass index. Also additive to the literature is that we found no gender difference amongst the Africans. This is surprising for in the literature suPAR levels are indeed higher in Caucasian women compared to Caucasian men [12]. However, no previous research has been done on Africans to confirm the absence of this gender difference, and therefore needs confirmation. Another finding is that suPAR was associated with arterial stiffness, only amongst the men, suggesting that gender differences do exist in suPAR's involvement in atherosclerosis. However more research is needed to confirm this finding.

Data from this study correspond well with previous findings that suPAR is associated with increasing age [22]. Eugen-Olsen et al. [12] found that both suPAR concentrations and cardiovascular disease incidence increased with age. In our study, this was confirmed in all groups, except in African women.

In hemodialysis patients, Pawlak et al. [14] indicated that suPAR was significantly related to the prevalence of cardiovascular disease, due to observed uPAR upregulation at sites of vascular pathologies such as atherosclerotic plaque. This is also partly supported by Eugen-Olsen et al. [12] who found an association between elevated suPAR levels and cardiovascular disease in a population study consisting of Caucasian subjects. Not Eugen-Olsen et al. [12], nor any previous studies have made any specific reference to gender differences regarding the association with cardiovascular disease. Our results show that only men from both ethnic groups showed an independent relationship between suPAR and arterial stiffness.

The question could be asked why the association of suPAR with arterial stiffness is absent in women. Endothelial dysfunction is caused by diminished availability or production of nitric oxide, which is a vasodilator with anti-inflammatory and anti-atherosclerotic functions [23]. Estrogen is responsible for modulating and regulating important biological functions of different cellular components of the vascular wall. In addition, estrogen participates in the transcriptional regulation of endothelial nitric oxide synthases and is responsible for stimulating nitric oxide release directly [24]. This could indeed contribute to the absence of these associations. On the other hand, the observed associations in African and Caucasian men could be due to the fact that they smoked more, which could also have confounded our associations with arterial stiffness. Although we adjusted for smoking as a binomial variable, the confounding effects of smoking could perhaps not have been adequately adjusted for. Smoking reduces the bioavailability of endothelium-derived nitric oxide [23], augmenting inflammation of the arterial wall [25] and related arterial stiffness [26]. More evidence from other populations is thus necessary to confirm this gender specific association.

Plasma suPAR samples are known to be very stable throughout the day [27] and few variations are present in the suPAR concentrations of HIV-infected patients using antiretroviral therapy [28]. There is evidence that suPAR levels of plasma samples are also not even affected by repeated freeze-thaw procedures [29]. Our results, especially in men, suggest that together with the stability of the suPAR measurement, it may potentially enable clinicians to target early primary prevention more effectively in the future.

This study has to be interpreted within the context of its limitations and strengths. This was a cross-sectional study design with relatively young and apparently healthy participants. Older participants are needed to investigate suPAR levels with regard to disease development. Although our results are consistent, after multiple adjustments, we cannot exclude residual confounding. Participants without having tuberculosis or cancer could not be guaranteed due to ethical considerations and, therefore, could have influenced the suPAR levels. Also, unfortunately ambulatory blood pressure measurements were not performed, which could have given a better indication of suPAR's relationship with cardiovascular function. The difference in socioeconomic status between the African and Caucasian individuals is another limitation, where Africans had a much lower income than the Caucasians. Overall, this was a well-designed study implemented under controlled conditions. According to the best of our knowledge, this was the first study to investigate suPAR levels and associations with cardiovascular function in Africans and Caucasians.

To conclude, Africans from different age-groups presented higher suPAR levels than their Caucasian counterparts. In addition, suPAR was associated with arterial stiffness in men only, irrespective of race. This suggests that suPAR may be an important biomarker of cardiovascular dysfunction in men.

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CHAPTER 4

General conclusions and recommendations

INTRODUCTION

In this chapter, the main findings from this study will be summarized. The results will be discussed, interpreted, explained and compared to the relevant literature of Chapter 2. Conclusions will be drawn and recommendations will be made to researchers investigating soluble urokinase plasminogen activator receptor (suPAR) and cardiovascular function in African and Caucasian populations, or in general.

SUMMARY OF MAIN FINDINGS

This study aimed to compare suPAR levels as well as the associations between suPAR and cardiovascular function in African and Caucasian people. It was hypothesized that due to the high prevalence of inflammation and cardiovascular disease amongst African people, the Africans in this study will have higher suPAR levels than Caucasian men and women. The second hypothesis was that in Africans, plasma suPAR is more strongly associated with measures of cardiovascular dysfunction when compared to Caucasians.

We showed that suPAR levels were higher in the total groups of African people compared to the Caucasians, independent of gender and age. Therefore the first hypothesis is accepted. After adjusting for age and body mass index, the African and Caucasian men showed a less favorable cardiovascular profile compared to the women. A forward stepwise multiple regression analysis confirmed the association of suPAR with measures of arterial stiffness and blood pressure in African and Caucasian men. In African men, suPAR remained positively associated with pulse wave velocity and in Caucasian men with systolic blood pressure, diastolic blood pressure and negatively with Windkessel compliance. The second hypothesis is therefore rejected, showing a gender-specific association between suPAR and cardiovascular function, rather than an ethnic-specific association.

COMPARISON TO RELEVANT LITERATURE

The gender differences observed in Caucasians were confirming [1], however the lack of this difference in Africans is indeed contradictory.

Eugen-Olsen et al. [1] proposed that suPAR is related to cardiovascular diseases, which was supported by this study with the associations obtained between suPAR, blood pressure and arterial stiffness in African and Caucasian men, and could contribute to cardiovascular diseases.

Olson et al. [2] suggested that suPAR is a biomarker of cardiovascular disease risk, which is confirmed by our study. On the other hand, Olson et al. [2] proposed that suPAR is not a clinically useful biomarker of atherosclerosis based on plaque vulnerability. This is contradictory to our

findings with suPAR's association with arterial stiffness in men, and could indeed add to the literature that suPAR could be a useful biomarker for predicting cardiovascular dysfunction. However, this is pure speculation since the cross-sectional design of our study does not have predictive power.

STUDY LIMITATIONS

Before the main finding of this study is discussed, it is imperative to reflect on some of the factors that might have affected the results of this study. There are some methodological issues that could have influenced the outcomes of this study. The number of subjects included in this study could be questioned. An availability sample of the African (N=207) and Caucasian (N=314) population was used and may therefore introduce a selection bias. The whole South African population cannot be represented by this study group since this group was recruited from the Potchefstroom district in the North West Province of South Africa.

The differences in socio-economic classes and cultural background between the African and Caucasian people could also have influenced the findings of this study, for Africans had a lower income than their Caucasian counterparts, therefore limiting the Africans' medical care, as well as prejudicing their health.

Another factor that might have had an effect is the presence of cancer and tuberculosis amongst the participants. HIV-infected participants were excluded from the study, but there may have been participants suffering from cancer and/or tuberculosis, which may have increased suPAR levels. Importantly, participants perceived themselves as healthy, and since HIV-infected participants were excluded, those with accompanying tuberculosis did not partake in the study.

Confounding factors such as alcohol intake, diet, smoking and physical activity level could have influence the results, even though these confounders were accounted for in statistical analyses. More accurate or sensitive measures of alcohol intake, smoking or physical activity level may yield slightly different results.

Concerning the results, the possibility of chance should be taken into account. By using partial correlations and forward stepwise regression analyses, statistics indicated that one out of twenty significant correlations may be due to chance.

DISCUSSION OF THE MAIN FINDINGS

In the black urban South African population, incidence of cardiovascular diseases is on the increase due to the contribution of multiple risk factors [3]. SuPAR is mainly known to reflect an

inflammatory status, especially in HIV-infected, cancer and tuberculosis patients [4-7], but not much is known about the relationship between suPAR and cardiovascular function, especially in black South African people.

The focus of this study was, therefore, to investigate suPAR levels and the associations with cardiovascular function amongst Africans and Caucasians in order to explore potential novel mechanisms that might contribute to the increased cardiovascular mortality and morbidity in the African population.

Elevated levels of suPAR were observed in the African participants, however gender differences existed only in Caucasians with levels being higher in women [1]. The suPAR levels also increased with age in all groups [1], except African women. Recent studies from South Africa refute the “healthy obesity” concept [8], but our findings support this concept amongst African women [9]. In addition the suPAR levels were also higher amongst the African men and women compared to Caucasian men and women within each age quartile. This could be the result of the known higher inflammatory state amongst the African population [10].

The independent relationship of suPAR with arterial stiffness amongst the African and Caucasian men, could be due to the beneficial effects of estrogen in women [11] and because of the higher percentage smoking amongst the men [12]. However we adjusted for smoking in multiple regression analysis. This indicates that suPAR could be related to cardiovascular dysfunction and could help with early detection of cardiovascular dysfunction. Although the findings cannot be extrapolated to the whole South African population, it could provide a reference for future studies.

CONCLUSION

SuPAR levels were higher in the Africans compared to the Caucasians, which was also observed within each age quartile. Gender specific associations indicated that suPAR was associated with cardiovascular dysfunction amongst the African and Caucasian men only.

FUTURE RELEVANCE

Because of suPAR’s high stability in plasma samples, it could be a useful marker in the clinical setting. SuPAR may also enable physicians to target early disease development. SuPAR should not replace CRP, but it can be used as an additional measurement to determine the cardiovascular status, for each marker is responsible for different sets of information.

The following recommendations are proposed for future studies:

- Prospective studies are essential in order to investigate a cause-effect relationship instead of investigating tendencies in a cross-sectional design.
- SuPAR should be investigated amongst population groups from other ethnic origins to determine whether ethnic differences exist in suPAR levels, as well as with its association with cardiovascular function.
- When studying the relationship of suPAR with arterial stiffness, it is recommended to measure carotid intima-media thickness in addition to functional measures of arterial stiffness, to clarify whether suPAR is related to structural and/or functional changes.

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Appendix A

Table I: Physical and metabolic characteristics of the African men and women

	African men	African women	P-value
Number of participants	116	91	
Age (years)	41.1 ± 13.8	44.2 ± 11.9	0.097
Anthropometric measurements			
Height (m)	1.69 ± 0.07	1.57 ± 0.06	< 0.001
Weight (kg)	58.5 ± 13.1	66.1 ± 17.6	< 0.001
Body mass index (kg/m ²)	20.5 ± 4.20	26.7 ± 6.87	< 0.001
Waist circumference (cm)	74.3 ± 10.4	82.1 ± 13.5	< 0.001
Cardiovascular measurements			
Systolic blood pressure (mmHg)	131 ± 20.4	124 ± 23.0	0.030
Diastolic blood pressure (mmHg)	84.5 ± 13.6	86.2 ± 13.2	0.366
Pulse pressure (mmHg)	46.3 ± 11.4	38.0 ± 13.6	< 0.001
Windkessel compliance (mL/mmHg)	1.67 ± 0.52	1.45 ± 0.45	< 0.001
Pulse wave velocity (m/s)	46.3 ± 1.52	7.53 ± 1.64	0.007
Lipid profile			
High-density lipoprotein cholesterol (mmol/L)	1.66 ± 0.67	1.53 ± 0.47	0.098
Low-density lipoprotein cholesterol (mmol/L)	2.21 ± 0.89	2.51 ± 0.86	0.017
Triglycerides (mmol/L)	0.97 [1.46;164]	1.09 [1.60;1.88]	0.074
Estimated creatinine clearance (mL/min)	114 ± 31.1	102 ± 31.8	0.007
Biochemical variables			
Glucose (mmol/L)	5.02 ± 0.75	5.10 ± 0.77	0.450
C-reactive protein (mg/L)	1.61 [5.82;9.88]	3.29 [2.90;4.17]	0.003
suPAR (ng/mL)	2.96 [1.42;158]	3.08 [1.37;1.52]	0.433
Lifestyle			
Physical activity	8.21 ± 1.40	7.53 ± 1.21	< 0.001
Smoking n, (%)	85 (73.9)	52 (57.1)	0.011
Alcohol use n, (%)	96 (82.8)	56 (61.5)	< 0.001
Hypertensive n, (%)	44 (37.9)	33 (36.3)	0.805
Medication			
Anti-hypertensive n, (%)	0 (0)	0 (0)	-----
Anti-inflammatory n, (%)	0 (0)	1 (1.10)	-----

Data are arithmetic mean ± SD or geometric mean (fifth and 95th percentile intervals) for logarithmically transformed variables. SuPAR, soluble urokinase plasminogen activator receptor.

Table II: Physical and metabolic characteristics of the Caucasian men and women

	Caucasian men	Caucasian women	P-value
Number of participants	158	156	
Age (years)	40.1 ± 13.0	42.3 ± 12.9	0.136
Anthropometric measurements			
Height (m)	1.80 ± 0.07	1.66 ± 0.06	< 0.001
Weight (kg)	91.9 ± 17.5	76.3 ± 18.4	< 0.001
Body mass index (kg/m ²)	28.4 ± 4.98	27.7 ± 6.74	0.354
Waist circumference (cm)	94.6 ± 13.5	83.2 ± 14.1	< 0.001
Cardiovascular measurements			
Systolic blood pressure (mmHg)	125 ± 13.1	115 ± 17.7	< 0.001
Diastolic blood pressure (mmHg)	19.7 ± 9.00	77.3 ± 10.8	0.032
Pulse pressure (mmHg)	45.3 ± 8.81	38.0 ± 11.1	< 0.001
Windkessel compliance (mL/mmHg)	2.40 ± 0.59	1.87 ± 0.46	< 0.001
Pulse wave velocity (m/s)	8.06 ± 1.14	7.69 ± 1.18	0.005
Lipid profile			
High-density lipoprotein cholesterol (mmol/L)	1.19 ± 0.36	1.53 ± 0.40	< 0.001
Low-density lipoprotein cholesterol (mmol/L)	3.77 ± 1.15	3.74 ± 1.10	0.858
Triglycerides (mmol/L)	1.45 [1.69;1.92]	1.18 [1.60;1.80]	< 0.001
Estimated creatinine clearance rate (mL/min)	159 ± 40.3	110 ± 32.3	< 0.001
Biochemical variables			
Glucose (mmol/L)	5.70 ± 0.79	5.36 ± 0.89	< 0.001
C-reactive protein (mg/L)	1.23 [4.59;6.71]	1.23 [5.59;8.59]	0.993
suPAR (ng/mL)	2.15 [1.30;1.38]	2.40 [1.26;1.34]	< 0.001
Lifestyle			
Physical activity	7.88 ± 1.42	7.25 ± 1.32	< 0.001
Smoking n, (%)	32 (20.3)	18 (11.6)	0.370
Alcohol use n, (%)	120 (76)	86 (55.1)	< 0.001
Hypertensive n, (%)	23 (14.6)	22 (14.1)	0.909
Medication			
Anti-hypertensive n, (%)	36 (22.9)	31 (19.9)	0.510
Anti-inflammatory n, (%)	11 (7.01)	11 (7.05)	0.988

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SuPAR, soluble urokinase plasminogen activator receptor.

Table III: Forward stepwise multiple regression analyses for CRP, with either systolic blood pressure, diastolic blood pressure, pulse wave velocity or Windkessel compliance as dependent variable.

CRP	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	R ²	Adj R ²	β	P	R ²	Adj R ²	β	P
African men	0.288	0.231	0.075	0.41	0.311	0.256	0.094	0.30
Caucasian men	0.178	0.130	0.086	0.33	0.400	0.365	0.039	0.61
African women	0.406	0.348	0.032	0.74	0.281	0.210	-0.048	0.66
Caucasian women	0.363	0.324	0.046	0.60	0.317	0.275	0.023	0.80

CRP	Pulse wave velocity (m/s)				Windkessel compliance (mL/mmHg)			
	R ²	Adj R ²	β	P	R ²	Adj R ²	β	P
African men	0.372	0.321	0.045	0.60	0.724	0.701	-0.056	0.34
Caucasian men	0.249	0.205	-0.050	0.55	0.720	0.703	-0.011	0.84
African women	0.532	0.486	-0.024	0.78	0.721	0.693	-0.071	0.29
Caucasian women	0.403	0.367	0.139	0.11	0.665	0.644	-0.069	0.28

Independent variables included CRP, age, body mass index, smoking, alcohol use, physical activity, glucose and high-density lipoprotein cholesterol. CRP, C-reactive protein.