

Cardiovascular dysfunction in black South Africans: an investigation from various perspectives

IM Palmer M.Sc.

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PROMOTER: Prof. AE Schutte

CO-PROMOTER: Prof. HW Huisman

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• Getting it right • Re dira sentle • Ons doen dit reg

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~Psalm 28:7~

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ABBREVIATIONS: All abbreviations are indicated and explained where they first appear in the text, where after only the abbreviation is used.

AdipoR1	Adiponectin receptor-1
AdipoR2	Adiponectin receptor-2
AIDS	Acquired immunodeficiency syndrome
AMP	Adenosine monophosphate
AdipoR1 gene	Adiponectin gene
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARIC Study	Atherosclerosis Risk in Communities study
BMI	Body mass index
CARDIA Study	Coronary Artery Risk Development in Young Adults
COX-2	Cyclooxygenase-2
CVD	Cardiovascular disease
C _w	Arterial compliance/Windkessel compliance
DBP	Diastolic blood pressure
eCcr	Estimated creatinine clearance
ESC	European Society of Cardiology
ESH	European Society of Hypertension
ET-1	Endothelin-1
GFR	Glomerular filtration rate
GLUT-4	Glucose transporter type 4
HDL	High density lipoproteins
HIV	Human Immunodeficiency Virus
HMW	High molecular weight
HOMA-IR	Homeostasis model assessment for insulin resistance
hsCRP	High sensitivity C-reactive protein
ICAM	Intercellular adhesion molecule
IL-1RA	Interleukin-1 receptor antagonist
IL 6	Interleukin-6
IL-10	Interleukin-10
ISH	International Society of Hypertension
LDL	Low density lipoproteins

LMW	Low molecular weight
MANOVA	Multiple analysis of variance
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic (chemoattractant) protein-1
MetS	Metabolic syndrome
NO	Nitric oxide
PAI-1	Plasminogen activator inhibitor-1
PDGF	Platelet derived growth factor
PP	Pulse pressure
SAfrEIC	South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function
SBP	Systolic blood pressure
SOD	Superoxide dismutase
TC	Total cholesterol
TNF- α	Tumour necrosis factor- α
TPR	Total peripheral resistance
UA	Uric acid
VCAM	Vascular cell adhesion molecules
VSMC	Vascular smooth muscle cells
WC	Waist circumference
WHO	World Health Organization
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase

**TITLE: CARDIOVASCULAR DYSFUNCTION IN BLACK SOUTH AFRICANS: AN
INVESTIGATION FROM VARIOUS PERSPECTIVES**

SUMMARY

Motivation: The prevalence of cardiovascular dysfunction, especially hypertension, in Africans has increased dramatically over the past few decades. Despite considerable in-depth studies, cardiovascular diseases remain the leading cause of morbidity and mortality. Further escalations are predicted, especially in developing countries such as South Africa, if measures are not taken to combat the trend. Numerous cardiovascular risk factors have been investigated within African-Americans as well as Caucasians. However, it is not known to what extent African-Americans and Africans from South Africa are comparable. Therefore, it is essential to investigate risk factors and their possible contributory role in the high susceptibility of cardiovascular dysfunction in the black South African population.

Aim: To investigate potential risk factors and their possible involvement and association with the high prevalence of cardiovascular dysfunction within the black South African population.

Methodology: Manuscripts presented in Chapters 2, 3 and 4 made use of the data obtained from the cross-sectional SAfrEIC (The South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) study. The study group included 756 asymptomatic, apparently healthy African men and women as well as Caucasian men and women, recruited from the North West Province, South Africa. Anthropometric and cardiovascular measurements were taken as well as their lipid profiles, fasting insulin levels, and uric acid and adiponectin levels. Independent t-tests, analyses of variance (ANOVA) and analyses of covariance (ANCOVA) were used for comparison of variables between groups to determine significant differences. Partial correlations coefficients were used to show association between variables while adjusting for confounders. Multiple analyses of covariance (MANCOVA) were performed to compare variables between the groups, whilst adjusting for relevant confounders. Stepwise multiple and single regression analyses were also used to determine and confirm the most significant associations between variables.

All subjects gave informed consent in writing and the Ethics Committee of the North-West University approved the study. The reader is referred to the “Materials and Methods” section of Chapters 2, 3 and 4 for a more elaborate description of the subjects, study design and analytical methods used in each paper.

Results and conclusions of the individual manuscripts

- Results from Chapter 2 revealed significantly lower uric acid levels for African men compared to Caucasian men. Despite these lower levels, the association between uric acid and blood pressure is more pronounced within the African men. The strong positive relationship between uric acid and blood pressure might be explained by uric acid's independent relationship with vascular resistance. Uric acid also revealed a positive association with triglycerides in both the African and Caucasian men. These results suggest that uric acid *per se* can act as a risk factor in the development of cardiovascular dysfunction in African men.
- Results from Chapter 3 showed opposing changes in insulin secretion for African men and Caucasian men with increasing age. Whereas insulin levels increased in Caucasian men with progressive age, insulin levels in African men tended to decrease with ageing. Additionally, the insulin-blood pressure relationship within African men revealed opposite results as to what was expected. While the Caucasian men revealed a more positive association between insulin and blood pressure within the younger individuals, older individuals revealed a negative association between insulin and blood pressure. This implies that the vasoconstrictory actions of insulin seem to dominate in young individuals while the vasodilatory actions of insulin take over in older individuals. The turnaround probably acts as a counter protective mechanism against age-related cardiovascular dysfunction. On the contrary, despite decreased insulin secretion in older African men, they exhibit a more positive association between insulin and blood pressure, whereas younger subjects showed a more negative association. These results might suggest dissociation between insulin and blood pressure. Insulin *per se* might, therefore, not act as a risk factor, but rather the lack of insulin-mediated vasodilatory effects as observed within younger Africans.

- Results from Chapter 4 contradicted the notion found in the literature that age-related increase in adiponectin levels are due to impaired renal function. Although the results from this chapter confirmed a significant association between renal function (estimated creatinine clearance) and adiponectin levels – a multiple regression model revealed insulin resistance (HOMA-IR) as the major contributor to adiponectin levels. Adiponectin levels increased with progressive ageing only in the Africans. No such change was observed for the Caucasians. This might be due to development of functional adiponectin resistance or perhaps due to a decline in pancreatic cell mass with ageing.

In conclusion, the cardiovascular profile of Africans seems to be more detrimentally affected compared to Caucasians. Results from this study have elucidated on the associations and potential involvement of possible risk factors including, uric acid, insulin, C-peptide, as well as adiponectin, with regards to the high prevalence of cardiovascular dysfunction within the black South African population.

Keywords: Africans, cardiovascular dysfunction, ageing, hypertension, uric acid, insulin, C-peptide, adiponectin.

**ARIKAANSE TITEL: KARDIOVASKULÊRE DISFUNKSIE IN SWART SUID
AFRIKANERS: 'N ONDERSOEK VANUIT VERSKEIE
PERSPEKTIEWE**

OPSOMMING

Motivering: Die voorkoms van kardiovaskulêre disfunksie, veral hipertensie in swart Afrikane het dramaties toegeneem oor die afgelope paar dekades. Ten spyte van aansienlike in-diepte navorsing, bly kardiovaskulêre siektes een van die belangrikste oorsake van morbiditeit en mortaliteit. Verdere toenames word verwag, veral in ontwikkelende lande soos Suid-Afrika, indien daar nie stappe geneem word om hierdie tendens te verhoed nie. Verskeie kardiovaskulêre risikofaktore is reeds ondersoek in swart Afro-Amerikaners sowel as in Kaukasiërs, maar, dit is nie bekend tot watter mate swart Afro-Amerikaners en swart Afrikane van Suid-Afrika met mekaar vergelyk kan word nie. Dit is dus noodsaaklik om risikofaktore en hul moontlike bydraende rolle vir die hoë vatbaarheid van kardiovaskulêre disfunksies in die swart Afrikane populasie te ondersoek.

Doelstelling: Om potensiële risikofaktore en hul moontlike betrokkenheid en verwantskappe met die hoë voorkoms van kardiovaskulêre disfunksie in swart Afrikane te ondersoek.

Metodologie: Die manuskripte wat in Hoofstukke 2, 3 en 4 vervat is, het gebruik gemaak van die dwarsdeursnee S AfrEIC (*The South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function*) projek. Die studie groep het 756 asimptomatiese, ooglopend gesonde swart mans en vrouens sowel as Kaukasiër mans en vrouens vanuit die Noordwes Provinsie van Suid-Afrika ingesluit. Antropometriese en kardiovaskulêre metings is geneem, sowel as die lipiedprofiel, vastende insulienvlakke, asook uriensuur- en adiponektienvlakke. Onafhanklike t-toetse, analise van variansie (ANOVA) asook analise van kovariansie (ANKOVA) is gebruik om betekenisvolle verskille tussen groepe te bepaal. Meervoudige analise van kovariansie (MANKOVA) is gebruik om betekenisvolle verskille tussen groepe te bepaal terwyl daar gekorrigeer is vir relevante veranderlikes. Parsiële korrelasie koëffisiënte is gebruik om assosiasies tussen veranderlikes te bepaal terwyl daar vir sekere veranderlikes gekorrigeer is. Stapsgewyse meervoudige regressie analises

asook normale regressie analises is verder uitgevoer om die mees betekenisvolle assosiasies tussen veranderlikes te bepaal en bevestig.

Alle proefpersone het skriftelike ingeligte toestemming gegee. Die studie is goedgekeur deur die Etiekkomitee van die Noordwes-Universiteit. Die leser word verder verwys na die "Materials and Methods" afdeling van Hoofstukke 2, 3 en 4 vir 'n meer uitgebreide beskrywing van die proefpersone, studie-ontwerp en analitiese metodes wat gebruik is.

Resultate en gevolgtrekkings van die individuele manuskripte

- „ Resultate van Hoofstuk 2 toon aan dat swart mans betekenisvolle laer uriensuurvlakke het in vergelyking met Kaukasiër mans. Ten spyte van hul laer vlakke, is die assosiasie tussen uriensuur en bloeddruk veel meer prominent in die swart mans. Die sterk positiewe verwantskap tussen uriensuur en bloeddruk kan moontlik verduidelik word aan die hand van die onafhanklike assosiasie van uriensuur met vaskulêre weerstand. Uriensuur toon ook 'n positiewe assosiasie met trigliseriedes in beide die swart en Kaukasiër mans. Hierdie resultate toon aan dat uriensuur *per se* kan optree as 'n risikofaktor in die ontwikkeling van kardiovaskulêre disfunksie in swart mans.

- „ Resultate van Hoofstuk 3 toon teenoorgestelde veranderinge aan in insuliensekresie in swart en Kaukasiër mans met toenemende ouderdom. Insulienvlakke neem toe in Kaukasiër mans met toenemende ouderdom, waar in die swart mans dit 'n afnemende tendens toon. Die verwantskap tussen insulien en bloeddruk in swart mans het ook 'n teenoorgestelde resultaat getoon as wat verwag was. Terwyl jong Kaukasiër mans 'n meer positiewe verhouding tussen insulien en bloeddruk getoon het, het die ouer Kaukasiër mans 'n meer negatiewe verwantskap aangedui tussen insulien en bloeddruk. Dit wil meebring asof die vasokonstriktoriese funksies van insulien meer dominant is in jonger individue terwyl die vasodilatoriese funksies oorneem in ouer individue. Hierdie ommekeer dien moontlik as beskerming teen ouderdom verwante kardiovaskulêre disfunksie. Intendeel, ten spyte van 'n afname in insuliensekresie toon die ouer swart mans 'n meer positiewe assosiasie tussen bloeddruk en insulien, terwyl die jonger mans 'n meer negatiewe assosiasie aandui. Hierdie resultate dui op 'n moontlike dissosiasie tussen bloeddruk en insulien.

Insulien *per se* tree dus nie op as 'n risikofaktor nie, maar eerder die gebrek aan insulien-afhanklike vasodilatasie soos waargeneem in die jonger swart mans.

- 4i Resultate van Hoofstuk 4 het die teenstelling bewys rakende die stelling wat gevind word in die literatuur dat ouderdom-verwante toename in adiponektienvlakke te wyte is aan verswakte nierfunksie. Alhoewel die resultate van hierdie hoofstuk 'n sterk verwantskap bevestig tussen adiponektien en nierfunksie (benaderde kreatinien opruiming), het 'n veelvuldige regressie model getoon dat insulien weerstandbiedendheid (HOMA-IR) 'n groter bydrae maak tot adiponektien vlakke. Adiponektienvlakke het toegeneem met ouderdom in die swart mans maar nie in die Kaukasiër mans nie. Dit mag die gevolg wees van die ontwikkeling van funksionele adiponektien weerstandbiedendheid of moontlike afname in pankreasselmassa met toenemende ouderdom.

Ten slotte, die kardiovaskulêre profiel van die swart populasie is meer nadelig geaffekteerd in vergelyking met die van Kaukasiërs. Resultate van hierdie studie het meer duidelikheid gewerp op die assosiasies en potensiële betrokkenheid van moontlike risikofaktore insluitende uriensuur, insulien, C-peptide, asook adiponektien met betrekking tot die hoë voorkoms van kardiovaskulêre disfunksie in die swart Suid-Afrikaanse populasie.

Sleuterwoorde: Swart Afrikane, kardiovaskulêre disfunksie, veroudering, hipertensie, uriensuur, insulien, C-peptied, adiponektien.

PREFACE AND OUTLINE OF THE STUDY

This thesis consists of three manuscripts submitted for publication, of which two were accepted and published. Following Chapter 1 (Introductory chapter), Chapter 2 determines differences in uric acid levels as well as differences in correlates between uric acid levels and cardio-metabolic parameters within African and Caucasian men. Chapter 3 investigates the possibility of an insulin-blood pressure relationship within the African and Caucasian population. Chapter 4 assesses the relationship between ageing and adiponectin levels from the perspective of renal function in African and Caucasian people. Chapter 5 contains a summary and discussion of all the results provided, conclusions are drawn and recommendations are made. The relevant references are provided at the end of each chapter according to the authors' instructions of the specific journal in which the articles were published or submitted for publication. In order to keep the references in this thesis uniform, the technical style for the references used in the manuscripts submitted for publication are according to the relevant Author Instructions. For Chapters 1 and 5 references are according to the Vancouver style.

A brief description of the content of the different chapters is given below:

Outline of the study

CHAPTER	CONTENT
Chapter 1	General introduction, literature review, motivation, aims, objectives, and hypotheses
Chapter 2	Serum uric acid and the cardiovascular profile of African and Caucasian men
Chapter 3	Ethnic and gender differences regarding the insulin-blood pressure relationship
Chapter 4	Ageing and adiponectin levels in an African population: an investigation from a renal perspective
Chapter 5	General findings and conclusions

- ❖ Manuscript 1 (Chapter 2): Accepted and published in *Journal of Human Hypertension* (2009)
- ❖ Manuscript 2 (Chapter 3) Accepted and published in *Diabetes Research and Clinical Practice* (2007)
- ❖ Manuscript 3 (Chapter 4) Submitted to *Regulatory Peptides* (2010)

AUTHORS' CONTRIBUTIONS

The contribution of each of the researchers involved in this study is given in the following table:

<u>Name</u>	<u>Role in the study</u>
Miss. IM Palmer (M.Sc.) (Physiologist)	Responsible for literature searches, statistical analyses, collection of data, design and planning of manuscripts, interpretation of results and writing of all manuscripts
Prof. AE Schutte (Ph.D.) (Physiologist)	Promoter. Supervised the writing of the manuscripts, study design, collection of data, as well as initial planning and design of manuscripts
Prof. HW Huisman (Ph.D.) (Physiologist)	Co-promoter. Supervised the writing of the manuscripts, collection of data, as well as initial planning and design of manuscripts

The following is a statement from the co-authors confirming their individual role in each study and giving their permission that the three manuscripts may form part of this thesis.

I declare that I have approved the above-mentioned manuscripts, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that they may be published as part of the Ph.D. thesis of Lanthe Palmer.



Prof. AE Schutte

Prof. HW Huisman

CHAPTER 1

INTRODUCTION

1. GENERAL INTRODUCTION

1.1 Hypertension in Africa

The prevalence of hypertension within the African population has made a dramatic twist within the last 80 years. According to an article published by Donnison in 1929 (1), the prevalence of hypertension was unheard of. To the contrary, hypotension was much more common within a native Kenyan population. This observation was also confirmed by another study conducted by Williams in Uganda (2). A study performed by Kaminer and Lutz in 1960 (3) also revealed lower blood pressure levels in Bushmen from Sub-Saharan Africa.

Today, things look vastly different. The prevalence of cardiovascular disease (CVD) especially hypertension, evolved from a relative rarity to a major public health concern within the black population (4-7). The widely held perception that the burden of disease in Sub-Saharan Africa is composed mainly of communicable diseases is being set aside. This switch from communicable diseases to more chronic non-communicable diseases is known as “epidemiological transition” (8,9). Despite considerable in-depth studies regarding CVD, it still remains one of the leading causes of morbidity and mortality, and it is predicted to increase substantially in South Africa over the next few decades if measures are not taken to combat the trend (10).

Several epidemiological studies revealed that the African population has a much higher prevalence of hypertension and more severe target-organ damage compared to their Caucasian counterparts (4-7). Numerous contributing factors involved in CVD have been placed under extensive investigation over the past few years to establish possible associations and perhaps even cause-and-effect. However, most of these risk factors have been investigated within African-American or other African populations. Therefore, it is paramount to investigate risk factors linked to the high susceptibility of CVD within the black South African population. In Figure 1.1 a few possible risk factors are illustrated.



Figure 1.1: Modifiable, non-modifiable and other risk factors for cardiovascular disease. (Collected from various sources: (11,12))

It is not feasible to investigate all possible risk factors, thus, for the scope of this study only a few potential risk factors were selected to scrutinise and elucidate more on their possible involvement and contribution to the high prevalence of cardiovascular dysfunction within the black South African population.

2. MOTIVATION, AIMS, OBJECTIVES AND HYPOTHESES FOR EACH MANUSCRIPT

This thesis consists of three manuscripts submitted for publication. Since the relevant literature background for each manuscript is discussed in the papers and in the literature section, only a brief motivation for each chosen topic will be provided here.

2.1 Uric acid and the cardiovascular profile of African and Caucasian men (Chapter 2)

Motivation

The importance of uric acid as a risk factor for cardiovascular disease has been debated and investigated over the past few years (13-17). In a previous study we revealed that African women from South Africa have significantly lower uric acid levels compared to their Caucasian counterparts (18).

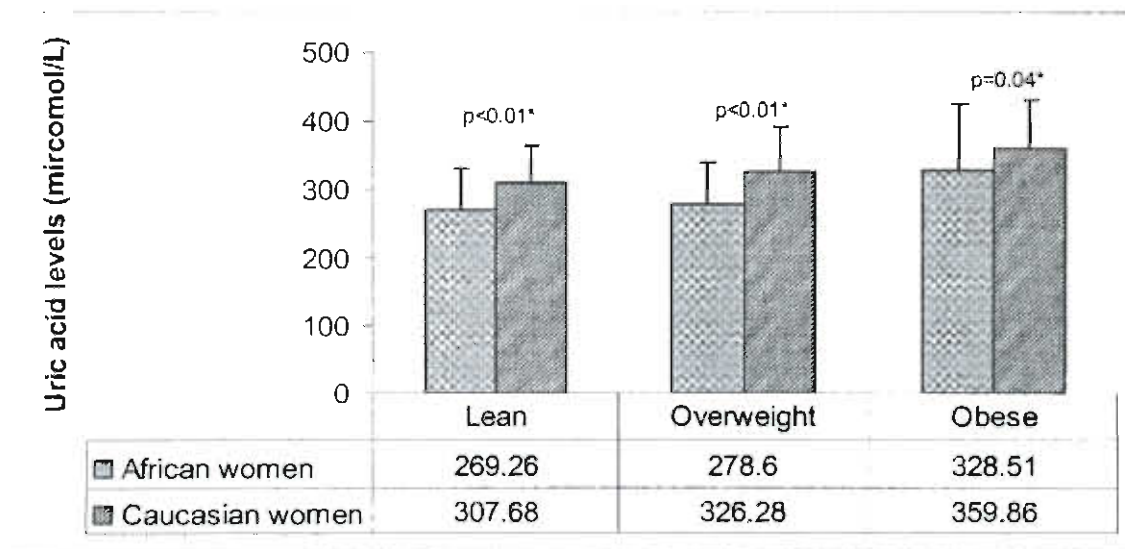


Figure 1.2: Uric acid levels of African and Caucasian women for the different obesity levels after adjusting for age and waist circumference (values are mean \pm standard deviation) (adopted from ref (18)).

These results contradict the literature which identifies the black population as a high-risk group regarding elevated serum uric acid levels (19-21). Our previous study only

included women and, therefore, no conclusion regarding uric acid levels in African and Caucasian men could be drawn. In their study Fang and Alderman (21) reported higher uric acid levels in African men compared to African women. Due to uric acid's strong link with cardiovascular disease (13,15,21-23) and the fact that the African men are extremely vulnerable to the development of hypertension (5,6), it is of utmost importance to investigate uric acid as a possible risk factor related to the high prevalence of cardiovascular disease amongst this group.

Aim

To investigate uric acid levels in African and Caucasian men.

Objectives

1. To determine whether differences in uric acid levels exist between African and Caucasian men.
2. To establish possible ethnic differences in correlates between uric acid and cardio-metabolic variables.

Hypotheses

Based on our previous work and the literature, the following hypotheses were formulated:

1. African men have lower uric acid levels compared to Caucasian men.
2. African men show stronger correlations between uric acid and cardio-metabolic variables compared to their Caucasian counterparts.

**2.2 Ethnic and gender differences regarding the insulin-blood pressure relationship
(Chapter 3)****Motivation**

Ageing is associated with several physiological changes in the body. Such changes include endocrinological alterations in the synthesis, secretion, circulating levels, metabolism, and biological activity of hormones (24). Age-related insulin secretory dysfunction is associated with increased insulin and C-peptide levels, which is frequently

characterised by insulin resistance and subsequently type 2 diabetes (25,26). Several epidemiological studies have shown that insulin secretory dysfunction associated with increasing age is linked to cardiovascular dysfunction (27-29), and might be the underlying cause of age-associated cardiovascular diseases such as hypertension. However, most of these studies only included African-Americans (30) and Caucasians (25), and data within a South African context are lacking. Furthermore, Schutte *et al.* (31) proposed a possible turnaround in the relationship between insulin and blood pressure, suggesting a more positive association within younger individuals, and a more negative association within elderly individuals.

It is, therefore, essential to have a better understanding of age-related changes in insulin secretion as well as changes in the insulin-blood pressure relationship, and how they relate to the high propensity of hypertension within the African population (5,6). Data gathered can also be applied to the development of preventive measures and therapeutic interventions within the African group.

Aim

To establish possible ethnic and gender differences regarding the insulin-blood pressure relationship within a South African context.

Objectives

1. To compare African and Caucasian groups with regards to the changes in the concentrations of fasting plasma insulin and C-peptide levels with increasing age.
2. To determine the relationship between fasting insulin/C-peptide with blood pressure of Africans and Caucasians using stratified age groups.

Hypotheses

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

1. African and Caucasian people indicate an increase in insulin and C-peptide with increasing age.
2. African and Caucasian people show a positive association between insulin and blood pressure within younger individuals, whereas the older individuals tend to have a more negative association between insulin and blood pressure.

2.3 Ageing and adiponectin levels in an African population: an investigation from a renal perspective (Chapter 4)

Motivation

Adiponectin is known as a cardiovascular-protective adipokine (32,33) due to its anti-atherogenic (34,35), anti-diabetic (35) and anti-inflammatory (36) properties. Since cardiovascular disease is allied with morbidity and mortality in the elderly population (37-39), it is expected that this important adipokine will show an inverse relationship with ageing. Conversely, previous studies have indicated that with an increase in age, there is a concomitant rise in adiponectin levels (40-42). In their paper, Isobe *et al.* concluded that this age-related incline in adiponectin levels can be explained in terms of impaired renal function (41).

Yet this observation is challenged by an article published by Guebre-Egziabher *et al.* (43) where it was reported that elevated adiponectin was more related to metabolic disturbances, especially obesity, than to a decline in renal function *per se*.

Aim

To assess the relationship between ageing and adiponectin levels from the perspective of renal function in apparently healthy African and Caucasian people.

Objectives

1. To establish whether differences exist regarding adiponectin levels between Africans and Caucasians and how these levels change with an increase in age.
2. To determine renal function by means of estimated creatinine renal clearance within each of the ethnic groups, and how this correlates with adiponectin and obesity levels with ageing.

Hypothesis

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

1. Africans have lower adiponectin levels compared to Caucasians, and both Africans and Caucasians will show a rise in adiponectin levels with increasing age.
2. Renal function (estimated renal clearance) has a strong positive correlation with adiponectin levels, but is dependent on metabolic disturbances.

3. LITERATURE REVIEW

3.1 Essential hypertension and its prevalence in South Africa

Essential hypertension is a common cardiovascular disorder and is multi-factorial in nature. The etiology differs immensely amongst different populations of the world, and by definition, essential hypertension refers to high blood pressure with no identifiable cause, and accounts for 95 to 99% of the cases reported (44). A few decades ago, Arthur Guyton proposed that every type of hypertension starts with volume overload due to the impaired ability of the kidney to excrete salt. However, researchers later on discovered that in essential hypertension, the kidney is the victim rather than the culprit, and it is now seen as a progressive cardiovascular disorder arising from complex and interrelated variables (45).

According to the World Health Organization (WHO)/International Society of Hypertension (ISH) blood pressure can be classified as follows (46):

Table 1.1: Classification of hypertension according to the World Health Organization (WHO)/International Society of Hypertension (ISH)

JNC 7 Blood pressure category	SBP (mmHg)	DBP (mmHg)
Optimal	<120	<80
Normal	120-129	80-84
High normal	130-139	85-89
<u>Hypertension:</u>		
Grade 1 (Mild)	140-149	90-99
Grade 2 (Moderate)	150-179	100-109
Grade 3 (Severe)	≥180	≥110
Isolated systolic hypertension	≥140	<90

The guidelines from the World Health Organization (WHO)/International Society of Hypertension (ISH) are similar to the guidelines stipulated by the European Society of Hypertension (ESH) and European Society of Cardiology (ESC). However, Europe is a

more homogenous community and the ESH/ESC guidelines are more appropriate for Europeans, whereas the WHO/ISH guidelines were endorsed more globally.

Hypertension is becoming a public health emergency worldwide, and despite intensive research, the global prevalence of CVD for the year 2000 was 26.4%, with a projected prevalence increase of 60% for the year 2025 (47). The current situation in South Africa is not better, and reflects the global trend with concerning high figures. According to recent studies conducted (7,48), it was revealed that the prevalence of hypertension is distressingly high within the black population. Adding to the burden of high epidemiological prevalence is the fact that hypertension occurs at a much younger age in the black population, being more severe and leading to earlier subsequent organ damage (48-50).

Despite the increasing incidence of hypertension in black South Africans, the underlying mechanisms related to high blood pressure are ill-defined and more research should be done to address this issue.

3.2 Basic principles and hemodynamics of the cardiovascular system

3.2.1 Arterial blood pressure

Blood pressure consists of two basic components namely the steady component (mean arterial pressure [MAP]) as well as the pulsatile component (systolic [SBP], diastolic [DBP] and pulse pressure [PP]) (51). Mean arterial pressure gives an indication of the average pressure during the cardiac cycle, and is determined by two hemodynamic measurements: cardiac output and vascular resistance (52,53) and is usually estimated by the following equation:

$$\text{MAP} \approx \text{DBP} + 1/3 [\text{SBP} - \text{DBP}] \quad 1.1$$

However, this rule has recently been challenged (54), and a new calculation method has been proposed using a reliable numerical integral of the calibrated pressure wave. According to this study, the mean pressure is underestimated using the traditional formula, however, this can be corrected by adding 40% of the pulse pressure to the diastolic pressure i.e.:

$$\text{MAP} = \text{DBP} + 0.4 \times \text{PP} \quad 1.2$$

The pulsatile component on the other hand is influenced by: ventricular ejection, large artery compliance and timing of wave reflection (52,53), and is estimated by pulse pressure (PP):

$$\text{PP} = \text{SBP} - \text{DBP} \quad 1.3$$

In contrast to mean arterial pressure, the pulse pressure does not remain constant throughout the circulatory system (53) with the central pulse pressure being lower than the brachial pulse pressure (55). This is the consequence of a combination of two hemodynamic phenomena: 1) as the blood propagates through the vascular conduits toward the peripheral vasculature, there is a progressive reduction in blood vessel diameter and increase in vascular resistance (56) and 2) changes in timing and amplitude of wave reflections (56).

Ventricular ejection generates a pressure wave that propagates in a forward direction, away from the heart (57) at a speed known as pulse wave velocity (51,57). This wave is reflected back toward the heart from any point in the arterial tree where a discontinuity is found (51,57) for instance at an arterial branch. Thus, the pulse wave velocity provides an overall, accessible estimate of the elastic properties of the central and peripheral arteries (58,59).

In healthy individuals, the reflected wave reaches the heart during the diastolic phase (58) promoting coronary perfusion. However, in the event of arterial stiffness the reflected wave reaches the aorta during the systolic phase augmenting the central blood pressure (51,58). Since the heart only perceives central pressure and not brachial pressure (60), the left ventricular afterload increases (60), consequently promoting left ventricular hypertrophy (61), and concomitant congestive heart failure (62).

3.2.2 Arterial stiffness and vascular resistance

The arterial system has two essential functions in the cardiovascular system. The first is a buffering function against the pressure oscillations (63,64). The second is to act as a conduit system – delivering blood at high pressure to peripheral tissues (64).

During systole, approximately 40% of the stroke volume is moved forward into the peripheral vasculature; however, the remaining 60% of the blood remains within the larger elastic arteries (51). During diastole, the arteries recoil back to their original shape and the accumulated blood gradually moves forward towards the peripheral tissues (51), providing constant perfusion to the tissues, especially to the coronary arteries.

In order to counteract the sudden rise in pressure, the walls of large central arteries are rich in elastin, a resilient protein, which allows the vessels to distend (65). It also contains a collagen fibre network which is efficient at maximum pressures (65,66). This ability of larger arteries (central arteries) to capacitise increased volumes of blood during systole is known as the Windkessel compliance effect (67). Furthermore, to ensure a steady and constant blood flow in the arteriolar and capillary system, a certain degree of resistance must be applied to the blood flow. The necessary resistance is determined by the cross-sectional diameter of the peripheral vascular system, and is known as vascular resistance (53).

Arterial stiffness, vascular resistance and associated cardiovascular risks

Arterial stiffness has long been recognized as a risk factor for cardiovascular diseases (68-70). This decrease in elasticity (increased stiffness) holds great potential risk for cardiovascular diseases (71), including myocardial infarction, left ventricular hypertrophy and hypertension (72,73). There are a number of factors and pathophysiological states that will significantly decrease arterial distensibility and compliance of the larger/conduit arteries. Atherosclerosis is considered one of the most important risk factors for arterial stiffness and is a progressive disease instigated from the amalgamation of endothelial dysfunction and inflammation (74). Continuous augmented central blood pressure leads to increased wall stress, responsible for plaque formation via indirect stimulation of vascular cell adhesion molecules (VCAM) and intercellular adhesion molecule – 1 (ICAM-1) (74).

A previous study reported that African-Americans are considered to be at high risk regarding arterial stiffness and that it occurs earlier in life or that it is more accelerated compared to Caucasians (75), possibly due to earlier exposure to risk factors. Ethnic differences in arterial stiffness are, therefore, of great importance, especially in the black

South African population due to their high susceptibility to cardiovascular diseases (5,6,7).

Increased vascular resistance is a hallmark of essential hypertension (76), and results primarily from a reduced luminal diameter (77,78). These changes in resistance might contribute to the pathogenesis of hypertension (79). A reduction in the luminal diameter of the vessels will result in an increase in the resistance, and a concomitant elevation of blood pressure (80).

A key role has been assigned to the vascular endothelium in the participation of cardiovascular dysfunction. Previously viewed as an inert vascular lining, the endothelium is now considered proactive, releasing vasoactive substances to regulate vasomotor function, and affect hemostasis (81,82). A trademark of endothelial dysfunction is impaired nitric oxide release (83), a potent vasodilator opposing the effects of endothelium-derived constrictors such as endothelin-1 and angiotensin-II (83). When the endothelial wall is damaged or injured, it loses its protective characteristics and converts to one that is vasoconstrictive, procoagulant and antifibrinolytic (81,84). Thus, the interaction of increased arterial stiffness and vascular resistance as well as vascular dysfunction is a detrimental combination, and can exacerbate the development of high blood pressure.

3.3 Risk factors for hypertension investigated in this thesis

As illustrated by Figure 1.1, hypertension is a multi-factorial condition and the extent of its etiology stretches far beyond the scope of this study. However, an attempt was made to look at a few possible contributing culprits. These include age, insulin and C-peptide, adiponectin as well as uric acid – all of which will be discussed in more detail below.

3.3.1 Age

“Senectus ipsa est morbu – senescence is a disease in itself” (85)

Human ageing is a complex process and is one of the most harmful, non-modifiable risk factors for cardiovascular disease. It plays a pivotal role in the underlying cause of hypertension and atherosclerotic disease (66,86), and with the world population of

people over 70 yrs estimated at approximately 610 million (87), it is becoming a rising public concern.

Ageing manifests as structural and functional changes within the cardiovascular system and might predispose the ageing heart to develop pathological changes (88). These changes are often the platform and hallmark of cardiovascular dysfunction observed in the elderly population (89,90).

Ageing and the heart

Cardiac structural alterations associated with ageing include cardiomyocyte cell loss, with subsequent hypertrophy of the remaining myocytes (88), and left ventricular hypertrophy (91). Hypertrophy of the left ventricle leads to decreased ventricle chamber diameter, collagen deposition (92) as well as a diminished responsiveness to β -adrenergic stimulation (85).

At a functional level, a decline in cardiac function may result from a diminution of the intrinsic contractile properties of the cardiac muscle. Prolonged contraction duration (93) results in a diminished heart rate, with each heartbeat exhibiting an increased end-diastolic end-filling (85).

Ageing and the vasculature

The vascular system is markedly altered by the ageing process, and is the main factor responsible for structural and functional changes in the arterial wall (94). Progressive ageing is accompanied by elastic fibres that are more disorganised, thinner and more fragmented compared to those of younger individuals (94). This might at least be partially explained by the up-regulation of elastase activity (95), a proteolytic enzyme responsible for fragmentation of elastin fibres found in the large elastic vessels as well as the skin (96). Elastin fibres play an essential role in the arteries, and any alterations of elastin will result in concomitant changes in arterial distensibility (86). Arterial stiffness is another sequel of arterial ageing due to the deposition of extracellular matrix (collagen), fibronectin as well as smooth muscle cells within the arterial compartments (97,98).

It is clear that vascular remodelling contributes in part to the high prevalence of cardiovascular dysfunction observed in the elderly population. The most fundamental of

these are impaired arterial distensibility. As a consequence of arterial stiffness, the pulse wave reflection returns during the late systolic phase, causing an increase in central systolic blood pressure (99) and ventricular afterload, and consequently left ventricular hypertrophy. Increased vascular stiffness also induces a raised pulse pressure (69) resulting in thickening of the intima media layer (100), strongly associated with cardiovascular diseases (101,102).

3.3.2 Insulin, C-peptide and associated vascular function

Insulin is a small peptide hormone, synthesised in significant quantities in the β -cells of the pancreas (103,104). It comprises of two polypeptide chains: A with 21 amino acid residues and B with 30 amino acid residues, connected to each other via disulphide bridges (103). Insulin is synthesized as pre-proinsulin which is the precursor for pro-insulin (103).

Pro-insulin consists of the two chains (A and B chains) with a connecting peptide, the so-called C-peptide connecting the two chains (103). The conversion of pro-insulin into mature insulin results from the cleavage of the C-peptide from the A and B chains (104). This is accomplished via the action of pro-hormone convertase 2 and 3 as well as carboxy peptidase H (103). The two peptides are then stored in secretory granules (105) from where they are later co-released in equimolar amounts into the circulation (106).

For many years, a general belief existed amongst researchers that C-peptide possesses little if any physiological function (107,108), that it was a mere by-product of insulin synthesis (109) and at most, facilitates the folding of the pro-insulin molecule and the generation of the disulfide bridges between the A-chain and B-chain (110).

Scientists argued that for peptides to be biologically active and exert their functions, they need a so-called "active site" which plays an important role in the binding of the ligand to the receptor (111). The lack of a known active site has long hampered the recognition of C-peptide as a biologically active hormone (111).

Another observation kept researchers believing that C-peptide was biologically inactive, and that was that C-peptide varies significantly between different species with regards to its amino acid sequence and peptide length (105,109).

However, over the last few years, new evidence emerged to contradict this view. Observations from several studies provide a basis for the hypothesis that C-peptide is biologically active with several physiological effects. These possible effects of C-peptide will be discussed in the following paragraphs.

Cardiovascular and metabolic function

Insulin is well known for its metabolic importance at cellular level, which encompasses carbohydrate metabolism, lipid metabolism and protein synthesis (112). Insulin is in many ways involved in carbohydrate metabolism. Its effects extend from facilitated diffusion of glucose into cells (113), increasing glycogen synthesis (114), stimulating glycolysis (115) and inhibiting gluconeogenesis (116). Lipid metabolism effects include fatty acid synthesis in adipocytes and the liver, as well as formation and storage of triglycerides (112).

In the cardiovascular system, insulin stimulates the production and release of several endothelial mediators, responsible for the dynamic control of vascular function (117). These include nitric oxide, endothelin-1 and reactive oxygen species (117,118). Through an array of signaling pathways, insulin mediated production of endothelial nitric oxide, starts with the phosphorylation of the insulin receptor substrate 1 (IRS-1) (118), leading up to increased endothelial nitric oxide synthase activity and nitric oxide production. In addition to its vasodilatory actions, insulin also exerts opposing vasoconstrictive effects resulting from sympathetic outflow (28). In healthy humans hyperinsulinemia increases sympathetic nerve activity (28,119), however, in the presence of insulin resistance, insulin-mediated vasodilation is blunted (120) and the vasoconstrictive pressor effects of insulin become dominant. This mechanism might elevate vascular resistance, contributing to elevated blood pressure (28).

During the condition of insulin resistance the tissues have a diminished ability to respond to the action of insulin – and usually the precursor for type 2 diabetes (121,122). To compensate for the resistance, the pancreas secretes even more insulin (hyperinsulinemia), and over time, the excess insulin secretion leads to a decline in insulin production as a result of exhaustion of the β -cells (121). Insulin resistance is a prominent component of cardiovascular diseases, including hypertension, coronary artery disease, and atherosclerosis (28,118), which are all characterised by endothelial

dysfunction (118). Several mechanisms linking endothelial dysfunction and diabetes or insulin resistance have been proposed. One possible mechanism linking insulin resistance and endothelial dysfunction includes stimulation of plasminogen activator inhibitor-1 (PAI-1) (124), a major inhibitor of fibrinolysis (125).

Additionally it is hypothesised that the elevated C-peptide levels might deposit in the vessel walls in early atherogenesis, due to the increased endothelial permeability (126). Through chemotactic effects C-peptide induces the migration of monocytes and CD4⁺ lymphocytes into the sub-endothelial space and the intima (Figure 1.3). The proposed mechanism might be a possible explanation why patients suffering from Type 2 diabetes and insulin resistance are so vastly susceptible to the development of atherosclerotic lesions.

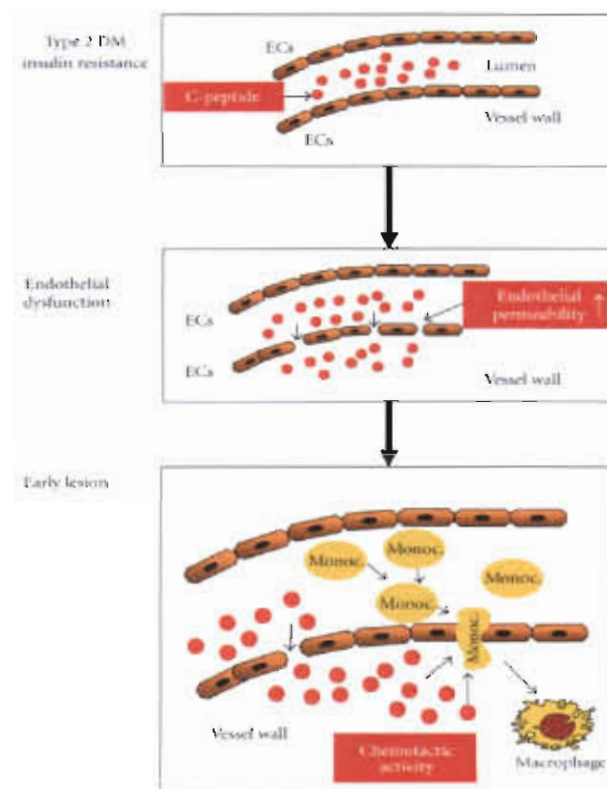


Figure 1.3. Potential role of C-peptide in early pathogenesis with insulin resistance and early Type 2 diabetes mellitus. (Type 2 DM: Type 2 diabetes mellitus; ECs: endothelial cells; Monoc: monocytes) (Reprinted from (126))

Another mechanism proposed for the development of plaque formation in diabetic patients, is that insulin (28,127) and C-peptide (128) promote smooth muscle cell proliferation, a hallmark of the atherosclerotic process (126). However, contradictory results were found by Kobayashi *et al.* (128) who demonstrated an inhibition of rat smooth muscle cells proliferation after treatment with human C-peptide.

According to Nakamoto *et al.* (129), coronary blood flow is increased during the early stages of diabetes mellitus. In their research they found that the administration of C-peptide to diabetic rats revealed no change in coronary blood flow, and an increase in nitric oxide production. However, a concomitant administration with insulin decreased both the coronary flow and nitric oxide production. It could be that in the presence of hyperinsulinemia the vasodilatory effects of elevated C-peptide are blunted. The hallmark of endothelial dysfunction is impaired nitric oxide release (130).

Nitric oxide is a potent vasodilator, opposing the effects of endothelium-derived constrictors such as angiotensin II (Ang II) and endothelin-1 (131,132). A reduction in the bioavailability of nitric oxide is an important step in the development of endothelial dysfunction and atherosclerosis, yet there are several studies performed that clearly showed that C-peptide has a nitric oxide-mediated vasodilatory effect (133).

Previous studies revealed that African women have a higher prevalence of Type 2 diabetes compared to Caucasian women (7% vs. 3.6%) (134-136). Overall, Africans tend to be more insulin resistant compared to Caucasians (137,138). Now more than a decade later, data concerning the prevalence of insulin resistance/Type 2 diabetes in the black South African population are still limited, and with urbanisation and obesity levels on the rise (139), it is so much more important to investigate this matter.

Renal function

Renal microvascular responses to insulin include renal vasodilation (140) mediated predominantly by the endothelial release of nitric oxide (141,142). Insulin also exerts a vasoconstrictor influence on renal vasculature via activation of the renin-angiotensin-system (143), however, in insulin-sensitive individuals the vasodilator effect is much greater than the vasoconstrictor effects (143). A possible mechanism proposed for the vasoconstrictor effect of insulin is that insulin stimulates proximal sodium reabsorption

(144,145) resulting in a decreased delivery of sodium to the macula densa, which in return will stimulate renin secretion (146). A study conducted by DeFronzo *et al.* (147) revealed that insulin also enhances phosphate reabsorption within the proximal tubular as well as sites distal to the proximal tube. Insulin resistance is also often accompanied by hyperuricemia (148). It is proposed that hyperinsulinemia enhances uric acid reabsorption via the proximal tubules leading to elevated levels of uric acid (149), which are also associated with cardiovascular disorders.

Patients suffering from Type 1 diabetes usually show glomerular hyperfiltration during the first years after the onset of the disease (150,151). In contrast, patients that suffer from Type 2 diabetes, who have normal levels of insulin and C-peptide (152) usually do not show any signs of the development of glomerular hyperfiltration or hypertrophy. Several experimental studies on rats delivered conclusive results that short-term infusion of C-peptide in Type 1 diabetic rats showed a marked decrease in glomerular filtration rate (105,151,153) – therefore relieving the condition of glomerular hyperfiltration. It can be speculated that C-peptide has a possible reno-protective function.

Insulin, C-peptide and obesity

Obesity, especially central obesity, represents one of the primary factors for the development of insulin resistance/hyperinsulinemia (154,155). A possible mechanism for the strong association between obesity and insulin resistance is that adipose tissue seems to alter the action of insulin through the release of several metabolically active adipokines (154). Increased adipose mass in obesity could lead to alterations in adipocytes hormones (adipokines) that regulate insulin sensitivity.

One such an adipokine is adiponectin, which in addition to its anti-inflammatory (36) and anti-atherogenic (34) properties, also exhibits insulin-sensitising characteristics (154). The primary mechanism by which adiponectin enhances insulin sensitivity seems to be mediated through the oxidation of fatty acids and the inhibition of hepatic gluconeogenesis by inhibiting key enzyme activities (155,156). Whereas obesity is usually associated with increased secretion of adipokines, adiponectin shows an inverse relation with increased adipose mass (154), in part associated with the development of insulin resistance.

Leptin is another adipocyte-derived hormone that plays a pivotal role in the regulation of food intake and energy expenditure (157). It is also responsible for lipid and glucose metabolism (158) as well as inhibiting insulin action by acting directly on pancreatic β -cells (159). Leptin resistance often accompanies obesity (160), resulting in defective leptin action on the β -cells, with consequential hyperinsulinemia.

Inflammatory markers produced by adipocytes include tumour necrosis factor- α (161,162) as well as interleukin-6 (163,164), both known to alter insulin sensitivity at different pathway levels (162,165,166).

The effect of age on insulin and C-peptide

Insulin resistance and impaired glucose tolerance are inevitable of the ageing process and known to incline progressively with increasing age (167). This can partially be explained by insulin secretory defects and action (25,168). Although the exact mechanisms responsible are not fully elucidated, a few possible mechanisms are hypothesized.

GLUT-4 protein is the primary transporter in skeletal muscle responsible for insulin-stimulated glucose uptake (169). Levels of muscle GLUT-4 decline with increasing age, and depletion of this transporter results in insulin resistance and diabetes (170). Another possible explanation is a diminished sensitivity of the β -cell to incretin hormones due to impairments in secretion and action of glucagon-like-peptide 1 and glucose-dependent insulinotropic peptide (168,171).

Studies in both animals (172,173) as well as humans (174) revealed significant age-related decline in insulin secretion with increasing age. This might possibly be contributed to progressive loss of β -cell function (175) with increasing age.

3.3.3 Adiponectin

Adiponectin (also referred to as AdipoQ, Acrp30 or apM1) is an expression of the apM1 gene (176), consists of 244-amino-acids and accounts for ~ 0.01 % of the total plasma proteins (43). It is composed of an N-terminal collagen-like sequence and a C-terminal globular domain (177), and in circulation can either exist as a full-length or a smaller proteolytic cleavage fragment (176), however, in plasma it exists mainly as a full-length

formation (177). In serum it is found as multiple oligomeric forms namely lower molecular weight (LMW) or as high molecular weight (HMW) (176).

Adiponectin is an adipocyte-derived hormone (178) with circulating concentration levels of about 5 - 30µg/ml (34). It is secreted in response to metabolic stimuli in order to sensitise the liver and muscles to the actions of insulin (179). The plasma half life of adiponectin is about 2.5 hours (180) compared to most other polypeptide hormones with a half-life time between 15 and 30 min.

Cardiovascular and metabolic function

Adiponectin received growing attention as a therapeutic treatment for cardiovascular disease due to its broad range of cardiovascular protective properties. Several studies indicated a link between low levels of adiponectin and the prevalence of cardiovascular risk events (181-185). Adiponectin has been shown to play an essential role in the suppression of inflammation (36,186) often associated with metabolic disorders that may result in Type 2 diabetes. According to researchers, it was found that adiponectin induced the expression of anti-inflammatory cytokines such as interleukin-10 (IL-10) (188,188) and interleukin-1 receptor antagonist (IL-1RA) in a variety of cells including monocytes and monocyte-derived macrophages (187). Interleukin-10 can inhibit the production of other pro-inflammatory cytokines such as tumour necrosis factor- α (TNF- α) as well as induce further production of IL-1RA, another anti-inflammatory product (187).

Adding to the abovementioned protective action is another beneficial feature of adiponectin, namely its anti-atherogenic properties (35). Adiponectin reduces monocyte attachment to aortic endothelial cells. This action is mediated by inhibiting the expression of vascular cell adhesion molecules (VCAM), intercellular adhesion molecule (ICAM), and E-selectin (189). Furthermore, adiponectin also suppresses smooth muscle cell proliferation and migration, induced by platelet-derived growth factor (PDGF) (190). Other vascular functions exerted by adiponectin include direct production of endothelial nitric oxide via the phosphatidylinositol 3-kinase pathways involving phosphorylation of endothelial nitric oxide synthase by means of activation of AMP protein kinase (34). The ability of adiponectin to stimulate nitric oxide production directly might lead to vasodilation and increased blood flow, enhancing glucose uptake (191), suggesting the notion that adiponectin possesses insulin-mimetic and insulin-sensitising actions (192).

The possibility that adiponectin directly stimulates GLUT-4 receptors cannot be ruled out (193,194). Other metabolic actions include regulating energy balance by stimulating free fatty acid oxidation (195).

Disparities exist concerning ethnic differences in adiponeclin levels. While some studies found that Africans have a lower adiponectin level compared to Caucasians (196,197), others have found no differences (198). Due to its beneficial properties within the cardiovascular system, it is important to investigate adiponectin and its associations with cardio-metabolic variables for possible treatment of cardiovascular dysfunction, especially within the black population.

Renal function

Decreased glomerular filtration rate (GFR) as observed in kidney disease, is often accompanied by elevated adiponectin levels (41) (Discussed in more detail under topic "*Ageing and adiponectin*"). It has been suggested that adiponectin is elevated compensatorily as a counter protective mechanism to improve endothelial dysfunction present in kidney disease (199).

Adiponectin and obesity

Adiponectin is an adipocyte-derived hormone (200), but unlike most other adipokines such as leptin (201), TNF- α (202) and resistin (203), circulating concentration levels of adiponectin tend to decrease with increasing adipocyte tissue mass (40). Hypoadiponectinemia is associated in particular with increased visceral fat (204), which might act as the causal link between obesity and associated cardiovascular diseases.

Ageing and adiponectin

Adiponectin levels have been reported to be elevated within the elderly population (42,206), but the exact underlying mechanism has not been clarified. However, a few possible mechanisms have been postulated.

The potential role of the kidney has been investigated as a possible culprit in the age-related hyperadiponectinemia (41,206). Ageing is associated with a number of physiological and pathophysiological changes, including renal impairment (207). Zocalli

et al. found significantly elevated levels of adiponectin in individuals with end-stage renal disease (208) entailing a central role of the kidneys in adiponectin clearance.

Another possible role can be attributed to sarcopenia, and is defined as the age-related loss of lean muscle mass (209,210). Adiponectin receptors, especially AdipoR1 are located within muscle cells (211), and with a loss in lean muscle, there might be a concomitant decline in these receptors, with compensatory hyperadiponectinemia and subsequently adiponectin resistance.

3.3.4 Uric acid

Uric acid is the final oxidation product of purine nucleotides catabolism (212) catalysed by the enzyme xanthine dehydrogenase/xanthine oxidase (XDH/XO) (213). It is mainly produced in the liver and the gut (214) and then secreted into the blood stream. However, according to a study by Matsuura (215), it was found that uric acid is also associated with visceral obesity (discussed in-depth later).

In most mammals uric acid is degraded by the hepatic enzyme, urate oxidase (uricase), to allantoin and clearance is reliant largely upon renal excretion (215). Approximately two thirds of the daily turnover of uric acid is cleared by means of renal excretion, whereas the remaining one third is excreted via the gut as faeces (216).

Cardiovascular function

There is an ongoing debate about the role of uric acid and its relation to cardiovascular disease. While there is no controversy about serum uric acid being a risk marker (13,22,23), the topic of uric acid as a risk factor is still disputed amongst researchers (17,217-220). Despite the disparities regarding elevated uric acid levels (hyperuricemia) as a cardiovascular risk marker or a risk factor, it is still important to understand the mechanism by which uric acid relates to cardiovascular function.

Hyperuricemia levels are commonly associated with hypertension (23,221), and present in approximately 25% of hypertensive subjects and 75% of people suffering from malignant hypertension (215). Although exact mechanisms are not fully understood, it is speculated that hypertension can develop due to uric acid-mediated renal vasoconstriction resulting from impaired endothelial nitric oxide availability (215,222,223)

especially at the macula densa (224), with a key role of the renin-angiotensin system (223,224).

Additional vascular effects include vascular smooth muscle cell proliferation (225,226). There is also evidence that uric acid possesses the ability to exacerbate inflammation (215). Uric acid induces monocyte chemoattractant protein-1 (MCP-1) synthesis by activating mitogen activated protein kinase (p38 MAP) (226). Uric acid also stimulates human mononuclear cells to produce interleukin-1, interleukin-6 and TNF- α (227,228). Another study reported that uric acid increases the expression of endothelin-1 (ET-1) (229).

Uric acid and its detrimental effects on the vascular system have been discussed in the previous paragraphs but there is, however, one beneficial feature. Uric acid possesses powerful antioxidant properties (230) and constitutes as much as two thirds of the antioxidant capacity in the human plasma (231-233). It is particularly effective in scavenging hydroxyl, superoxide, and peroxynitrate radicals (233). By binding with peroxynitrate, it forms a stable nitric oxide donor, thereby reducing the risk for potential peroxynitrate-induced oxidative damage, protecting the vascular system against oxidative stress (234). Superoxide dismutase (SOD3) is a critical enzyme in maintaining endothelial vascular function, and Hink *et al.* reported that uric acid can prevent the extracellular degradation of this important enzyme (235).

Perhaps somewhat paradoxical is the fact that uric acid can be converted to a pro-oxidant under certain circumstances producing reactive oxygen species instead of scavenging (236). In the early stages of atherosclerosis uric acid can act as an antioxidant, however, in the later, more developed stages of atherosclerosis, the antioxidant characteristics shift to take on those of a pro-oxidant.

According to several studies conducted, the black population is at higher risk for the development of hyperuricemia compared to Caucasian groups (19-21), conversely Alderman reports no ethnic difference (237) between African-Americans and Caucasians from the United States of America. Our own data have also shown lower uric acid levels in African women (18).

Renal function

Hyperuricemia is often a feature of insulin resistance (21), and this might be explained by the fact that hyperinsulinemia increase tubular reabsorption of sodium, resulting in a blunted ability of the kidney to excrete uric acid (238). Since uric acid clearance is mediated by the kidney, a reduction in glomerular filtration might lead to hyperuricemia (239).

Other effects include interstitial inflammation, microvascular rarefaction, afferent arteriopathy and interstitial fibrosis (19).

Effect of obesity on uric acid

For many years, the general notion existed that adipose tissue acts only as a storage depot, however, this view is no longer valid, and adipose tissue is now considered a complex and highly active endocrine organ (240). As previously mentioned, adipose tissue secretes an extensive variety of adipokines including, leptin (201), TNF- α (202), resistin (203), and adiponectin (240) to mention but a few.

To add to the complexity of obesity, one must take into account an even more relevant part of obesity: namely fat distribution. Two types of fat deposition have been categorised – one is subcutaneous adipose tissue and is located mainly underneath the skin (241), and the second and perhaps the most important one, is visceral adipose tissue, and is characterized mostly by depots of adipose tissue around the abdominal area and the gastrointestinal organs (242). The difference between these two is of great importance since metabolic and cardiovascular complications are directly related to visceral tissue depots rather than subcutaneous depots (242-244). This also holds true for hyperuricemia. Studies revealed that high uric acid levels are to a greater extent associated with visceral obesity, than subcutaneous obesity (214,245). Even though the exact mechanism is not clear, Matsuura *et al* suggest that free fatty acids (FFAs) which are often associated with visceral adipose tissue, might stimulate triglyceride synthesis in the liver (215). They further found a significant positive association between uric acid and triglycerides, suggesting that an overproduction of uric acid might be linked to increased triglyceride synthesis.

Ageing and uric acid

There is an age-related increase in serum uric acid levels noticeable especially in women (246). This might possibly be explained by commencement of menopause. Estrogen seems to have an enhancing effect on renal clearance of uric acid (247), however, with the onset of menopause, estrogen levels decline and the uricosuric effect is blunted (248), resulting in elevated uric acid levels.

To summarise, there is a broad extent of aspects identified as cardiovascular risk markers and factors. Those especially highlighted will be investigated further in the subsequent chapters.

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CHAPTER 2

URIC ACID AND THE CARDIOVASCULAR PROFILE OF AFRICAN AND CAUCASIAN MEN

IM Palmer, HW Huisman, AE Schutte

School for Physiology, Nutrition and Consumer Sciences, North-West University,
Potchefstroom Campus, South Africa

INSTRUCTIONS FOR AUTHORS: *Journal of Human Hypertension*

- ❖ Organise the manuscript into: 1) Title page, 2) Abstract and keywords, 3) Introduction, 4) Materials and Methods, 5) Results, 6) Discussion, 7) Acknowledgements, 8) Conflict of Interest, 9) References
- ❖ Abstract should not be more than 200 words with 3 to 6 keywords.
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Gasowski J, Fagard RH, Staessen JA, Grodzicki T, Pocock S, Boutitie F et al. Pulsatile blood pressure component as predictor of mortality in hypertension: a meta-analysis of clinical trial control groups. J Hypertens 2002; 20: 145–151.

- ❖ Abbreviations for titles of medical periodicals should conform to those used in the *Index Medicus*.
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Abstract

The African population is considered a high risk group for the development of hypertension, and identifying risk factors are therefore essential in preventive actions against cardiovascular disease (CVD). Elevated levels of uric acid (UA) are often associated with CVD. Our first aim was to establish possible ethnic differences in UA levels between African and Caucasian men. Our second aim was to determine any associations between UA levels and cardio-metabolic variables, and also how these correlates differ between the two groups. African (N=87) and Caucasian (N=121) men participated in this cross-sectional study. Our results showed that African men had significantly lower (353 ± 87.7 vs. 401 ± 98.2 $\mu\text{mol/L}$; $p < 0.01$) UA levels compared to Caucasian men. Waist circumference (WC) and triglycerides correlated strongly with UA in both ethnic groups. This was confirmed with a forward stepwise multiple regression analysis. After adjustment for confounders, the correlation between UA and triglycerides remained significant only in the Caucasians ($r = 0.29$; $p = 0.02$), whereas only the African men showed an independent correlation between UA and total peripheral resistance (TPR) ($r = 0.23$; $p = 0.04$). TPR increased significantly across UA tertiles only in the African men ($p = 0.01$ vs. $p = 0.96$). In conclusion, despite their lower UA levels, Africans showed an independent relationship between UA and vascular resistance, indicating a possible explanation for their high hypertension prevalence.

Keywords: Uric acid, cardiovascular disease, metabolic syndrome, African population, Caucasian population.

Introduction

Cardiovascular disease (CVD) remains one of the leading causes of mortality in the Westernized society (1,2) despite substantial research to address modifiable risk factors contributing to CVD related morbidity and mortality. One marker that is strongly linked to CVD is elevated uric acid (UA) levels (3-7). However, this association is difficult to interpret. Many studies argue that the association between UA and cardiovascular dysfunction is coincidental, and based on the presence of the individual components of the metabolic syndrome (MetS) (8). These components include hypertension, obesity, dyslipidemia, and hyperinsulinemia/insulin resistance.

Exact mechanisms linking elevated UA with the individual components of the metabolic syndrome is not entirely clear, but a few possible explanations are proposed. Although the contribution of obesity is not apparent, it is speculated that adipose tissue plays an important part in the production as well as inhibition of UA excretion (9). Elevated UA levels are not always the result of overproduction; it can result due to a decrease in excretion as well. Insulin, for example, is known to elevate UA levels by decreasing the excretion of UA via the proximal tubules (10). Hyperinsulinemia is therefore often associated with high levels of UA. UA is considered a natural anti-oxidant (11), and elevated UA levels found in the aforementioned conditions, could act as a possible counter mechanism to protect against endothelial damage. However, anti-oxidants can become pro-oxidants in certain conditions (urate redox shuttle) (11), resulting in damage to the endothelium and arterial vessel walls.

We have previously reported that African women from South Africa have significantly lower UA levels compared to their Caucasian counterparts (12). This is in contrast to the literature which classify the black population as a high risk group regarding hyperuricemia (4,8,13) or that the African population and Caucasian population have similar UA levels (14). The role of UA in the development of CVD and its concomitant associations with components of the MetS is receiving growing attention. A better understanding of the underlying mechanisms is essential in preventive actions against CVD especially in developing countries such as South Africa which suffers from a high prevalence of CVD (15,16).

African men are considered to be a high risk group for the development of hypertension in South Africa (15,16), and it is essential to identify risk factors contributing to their high susceptibility of cardiovascular disease. In a study by Fang & Alderman (4) it was found that men had significantly higher UA levels than women. This further strengthens the importance to investigate this matter in Africans. Our first aim was therefore to determine whether differences in UA exist between African and Caucasian men from South Africa. Our second

aim was to determine whether any associations between UA and cardio-metabolic variables exist, and whether these correlates differ between African and Caucasian men.

Materials and Methods

African (n=87) and Caucasian (n=121) men were recruited as volunteers from the Potchefstroom district in the North West Province, South Africa, to participate in a cross-sectional study. These men were apparently healthy, i.e. asymptomatic not previously diagnosed with any serious, chronic illnesses (except hypertension).

Exclusion criteria were subjects with diagnosed diabetes (type 1 and 2), HIV infected, and insulin levels above 27 $\mu\text{U/mL}$. Since insulin levels do not have clear defined cut-off values we used the proposed reference intervals according to a study done by Mack *et al.* (17). In total 181 African men and 163 Caucasian men were recruited for the study, however 136 were excluded according to the exclusion criteria.

The participants reported early in the morning at the Metabolic Clinic of the university, and were required to be in a fasting state. All subjects were informed about the outcome and procedures of the study beforehand, where after they signed informed consent forms. Field workers relayed all relevant information in the subject's home language. The study was approved by the Ethics Committee of the North-West University and complies with the Declaration of Helsinki as revised in 2004.

Blood sampling

A qualified nurse performed a finger prick to determine the fasting glucose level which was directly measured in the Metabolic Clinic 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 using a sterile winged infusion set and syringes. Plasma and serum samples were prepared according to standard methods and stored at -82°C until analyses were performed. Serum blood lipids, uric acid and high-sensitivity C-reactive protein (hsCRP) were determined later in the laboratory with the Konelab™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Insulin (ST AIA-PACK IRI, Cat. No 025260) was analysed by a two-site immunoassay on the TOSOH AIA System analyzers (San Francisco, CA, USA). Serum leptin levels were measured using a enzyme-linked immunosorbant (ELISA) kit (Diagnostic Systems laboratories, Inc., Cat. No. DSL-10-23100i). Human immunodeficiency virus (HIV) status was determined immediately after blood sampling with a rapid test

according to the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response Test and was repeated with Pareeshak test for confirmation.

Anthropometric measurements

Anthropometric measurements were performed according to standard methods described by Norton and Olds (18). Height measurements were taken using a stadiometer (Invicta, IP 1465, UK), and body weight measurements were measured up to the nearest kilogram using a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). The waist circumference was measured at midway level between the inferior rib margin and superior margin of the iliac crest. All the measurements were taken in triplicate to obtain a reliable median value.

Cardiovascular measurements

Each participant was allocated to a room where he rested for 10 min. before the blood pressure measurements. Participants were required to remain in the semi-Fowler's position while blood pressure was measured using the Finometer® device (FMS, Finapres Medical Systems, Amsterdam, the Netherlands). The vascular unloading technique of Peñáz together with the Physiocal criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are usable especially in comparative studies. The Finometer® device was connected and cuffs were attached to the left arm and the left middle finger of the participant. Resting blood pressure was recorded continuously for five minutes. After a recording of two minutes, a systolic return-to-flow calibration was performed. This calibration was performed to adjust the finger pressure of each specific participant with the brachial arterial pressure. Highest precision readings were obtained after this calibration. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), Windkessel compliance (C_w), and total peripheral resistance (TPR) were measured and stored.

General lifestyle and demographic questionnaires

Questionnaires were used to collect information regarding the subjects' smoking and alcohol habits.

Statistical analyses

Statistical analyses were performed using Statistica version 8.0 (Statsoft, Inc., Tulsa; OK, 2008). Statistical results are presented as means \pm standard deviation. Data that were not normally distributed (body mass index (BMI), leptin, glucose, total cholesterol (TC),

triglycerides, high density lipids (HDL), low density lipids (LDL), high-sensitivity C-reactive protein (hsCRP), insulin, MAP, and TPR) were log transformed and presented as means [5%; 95% percentile boundaries]. An independent t-test was used for comparison of variables between groups to determine significant differences. An analysis of covariance (ANCOVA) was performed to compare variables between the groups, whilst adjusting for age, body mass index, waist circumference, fasting insulin levels, smoking and alcohol consumption. Analysis of variance (ANOVA) were used to determine trend analysis.

To evaluate associations of UA with cardio-metabolic variables we used single regression analysis. To confirm the results from the single regression analysis a forward stepwise multiple regression analysis were performed. The model included only the significant associations found from the single regression analysis. These included UA as the dependent variable and age, log BMI, WC, log triglycerides, log insulin, log leptin, smoking and alcohol consumption, and log TPR as independent variables.

Results

The main clinical and anthropometrical characteristics of our study population are presented in Table 1. The two groups differed significantly with regards to all variables, except for age ($p = 0.93$) and hsCRP ($p = 0.56$). The Caucasian men had significantly higher obesity measures (BMI, WC, and HC), whereas the cardiovascular profile of the African men was more detrimental. This is reflected by their significantly higher SBP, DBP, MAP, and TPR and lower C_w .

On the other hand the lipid profile of the African men seemed to be more favourable with significantly higher HDL levels and lower triglycerides and LDL levels. They also had significantly lower leptin, glucose, and insulin levels compared to the Caucasian men. The African men had significantly lower UA levels compared to the Caucasian men.

Table 1. Physical and metabolic characteristics of the African and Caucasian men

Variable	African men (N=87)	Caucasian men (N=121)	P-value
Age (yrs)	40.3±13.2	40.4±13.9	0.93
<u>Anthropometrics</u>			
Weight (kg)	61.6±13.6	90.0±16.4	< 0.01
Height (m)	1.69±0.06	1.80±0.06	< 0.01
BMI (kg/m ²)	21.1 [16.5;28.9]	27.5 [21.7;35.2]	< 0.01
WC (cm)	76.8±10.8	93.4±12.5	< 0.01
HC (cm)	90.3±9.62	104±8.16	< 0.01
<u>Cardiovascular measures</u>			
SBP (mmHg)	138±23.2	132±15.4	0.01
DBP (mmHg)	85.9±11.4	79.6±7.95	< 0.01
MAP (mmHg)	106 [89.0;132]	99.2 [86.3;116]	< 0.01
CW (ml/mmHg)	1.69±0.51	2.36±0.58	< 0.01
TPR (mmHg.s/ml)	1.33 [0.92;2.18]	0.97 [0.63;1.54]	< 0.01
<u>Lipid profile</u>			
TC (mmol/L)	4.34 [2.68;6.34]	5.69 [3.72;8.19]	< 0.01
HDL (mmol/L)	1.54 [0.80;2.98]	1.18 [0.75;1.78]	< 0.01
LDL (mmol/L)	2.12 [0.94;3.82]	3.70 [2.17;6.06]	< 0.01
Trig (mmol/L)	1.02 [0.52;2.18]	1.39 [0.57;3.31]	< 0.01
<u>Biochemical variables</u>			
Fasting glucose (mmol/L)	4.97 [3.70;6.40]	5.50 [4.80;6.50]	< 0.01
Fasting insulin (uU/ml)	4.05 [0.80;18.1]	7.26 [2.60;18.0]	< 0.01
Fasting leptin (ng/ml)	1.10 [0.10;17.9]	5.57 [0.50;28.8]	< 0.01
hsCRP (mg/L)	1.34 [0.01;18.6]	1.15 [0.04;8.38]	0.56
Uric acid (umol/L)	353±87.7	401±98.2	< 0.01

Data expressed as mean±SD for normal distributed data and mean [5%;95% percentiles] for log-transformed data

BMI: body mass index, WC: waist circumference, HC: hip circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, Cw: Windkessel compliance, TPR: total peripheral resistance, HDL: high-density cholesterol, Trig: triglycerides, hsCRP: high sensitivity C-reactive protein

Table 2 reports the correlations between UA and cardio-metabolic variables for both African and Caucasian men.

Correlations between UA and cardio-metabolic variables in African and Caucasian men

UA showed significant correlations with obesity measures (BMI, WC, and HC), biochemical variables such as leptin, hsCRP, and triglycerides in both ethnic groups. SBP, DBP and MAP correlated significantly with UA, in the African men only. Noticeable only in the Caucasian men were significant correlations between both TC and LDL levels and UA. UA also correlated significantly with fasting glucose and insulin in the Caucasian men.

Table 2. Single regression analyses between uric acid and cardio-metabolic variables for African and Caucasian men

Variable	African men		Caucasian men	
Age (years)	$r = 0.17$	$p = 0.11$	$r = 0.16$	$p = 0.08$
Weight (kg)	$r = 0.41$	$p < 0.01$	$r = 0.26$	$p < 0.01$
Log BMI (kg/m^2)	$r = 0.40$	$p < 0.01$	$r = 0.27$	$p < 0.01$
WC (cm)	$r = 0.44$	$p < 0.01$	$r = 0.33$	$p < 0.01$
HC (cm)	$r = 0.41$	$p < 0.01$	$r = 0.27$	$p < 0.01$
SBP (mmHg)	$r = 0.26$	$p = 0.02$	$r = 0.12$	$p = 0.19$
DBP (mmHg)	$r = 0.33$	$p < 0.01$	$r = 0.12$	$p = 0.18$
MAP (mmHg)	$r = 0.33$	$p < 0.01$	$r = 0.11$	$p = 0.24$
TPR (mmHg.s/ml)	$r = 0.17$	$p = 0.11$	$r = -0.07$	$p = 0.47$
CW (ml/mmHg)	$r = -0.14$	$p = 0.21$	$r = -0.04$	$p = 0.63$
Log TC (mmol/L)	$r = 0.17$	$p = 0.11$	$r = 0.34$	$p < 0.01$
Log HDL (mmol/L)	$r = -0.07$	$p = 0.54$	$r = -0.09$	$p = 0.33$
Log LDL (mmol/L)	$r = 0.14$	$p = 0.20$	$r = 0.29$	$p < 0.01$
Log Trig(mmol/L)	$r = 0.27$	$p = 0.01$	$r = 0.37$	$p < 0.01$
Log Glucose (mmol/L)	$r = 0.17$	$p = 0.11$	$r = 0.26$	$p < 0.01$
Log-Insulin (uU/mL)	$r = 0.13$	$p = 0.23$	$r = 0.25$	$p < 0.01$
Leptin	$r = 0.40$	$p < 0.01$	$r = 0.26$	$p < 0.01$
Log hsCRP (mg/L)	$r = 0.23$	$p = 0.03$	$r = 0.27$	$p < 0.01$
Smoking	$r = 0.11$	$p = 0.30$	$r = -0.06$	$p = 0.54$
Alcohol	$r = -0.01$	$p = 0.88$	$r = 0.06$	$p = 0.50$

Data in bold have a p-value of ≤ 0.05

BMI: body mass index, WC: waist circumference, HC: hip circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, C_w: Windkessel compliance, TPR: total peripheral resistance, HDL: high-density cholesterol, Trig: triglycerides, hsCRP: high sensitivity C-reactive protein

Thereafter partial correlations were performed (Table 3) while adjusting for age, BMI, WC, fasting insulin, smoking and alcohol consumption. Almost all significant correlations mentioned in Table 2 disappeared leaving a significant correlation between UA and TPR noticeable only in the African men. The correlations between UA and lipids (TC, triglycerides and LDL) in the Caucasian men remained significant.

Table 3. Partial correlations between uric acid and cardio-metabolic variables (while adjusting for age, body mass index, waist circumference, fasting insulin, smoke, and alcohol) in African and Caucasian men

Variable	African men		Caucasian men	
Weight (kg)	r = 0.12	p = 0.24	r = 0.01	p = 0.91
HC (cm)	r = 0.14	p = 0.20	r = 0.06	p = 0.59
SBP (mmHg)	r = 0.10	p = 0.39	r = 0.02	p = 0.80
DBP (mmHg)	r = 0.17	p = 0.14	r = 0.02	p = 0.83
MAP (mmHg)	r = 0.17	p = 0.14	r = 0.00	p = 0.99
TPR (mmHg.s/ml)	r = 0.23	p = 0.04	r = -0.05	p = 0.59
CW (ml/mmHg)	r = -0.12	p = 0.30	r = -0.04	p = 0.69
Log TC (mmol/L)	r = 0.17	p = 0.13	r = 0.29	p < 0.01
Log HDL (mmol/L)	r = 0.13	p = 0.25	r = -0.02	p = 0.80
Log LDL (mmol/L)	r = 0.05	p = 0.66	r = 0.22	p = 0.02
Log Trig (mmol/L)	r = 0.10	p = 0.36	r = 0.29	p < 0.01
Log Glucose (mmol/L)	r = 0.02	p = 0.83	r = 0.18	p = 0.06
Leptin	r = 0.11	p = 0.34	r = 0.02	p = 0.81
Log hsCRP (mg/L)	r = 0.10	p = 0.37	r = 0.13	p = 0.17

BMI: body mass index, WC: waist circumference, HC: hip circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, CW: Windkessel compliance, TPR: total peripheral resistance, HDL: high-density cholesterol, Trig: triglycerides, hsCRP: high sensitivity C-reactive protein

A forward stepwise multiple regression analysis confirmed the association between UA and TPR in African men, and UA and triglycerides in the African and Caucasian men (Table 4). In both ethnic groups, WC showed a strong association with UA levels.

Table 4. Multiple regression analyses with uric acid as dependent variable for African and Caucasian men

Independent variables	African men $R^2 = 0.25$			Caucasian men $R^2 = 0.18$		
	β -value	Std. error	p-value	β -value	Std. error	p-value
Age	-	-	-	-	-	-
Log BMI	-	-	-	-0.26	0.19	0.18
WC	0.32	0.15	0.04	0.46	0.19	0.02
Log Trig	0.21	0.10	0.05	0.31	0.09	<0.01
Fasting insulin	-0.13	0.11	0.26	-	-	-
Leptin	0.18	0.14	0.22	-	-	-
Log TPR	0.21	0.10	0.03	-	-	-
Smoking	-	-	-	-	-	-
Alcohol intake	-	-	-	-	-	-

BMI: body mass index, WC: waist circumference, Trig: triglycerides, TPR: total peripheral resistance

We also plotted UA tertiles against WC, triglycerides and TPR to determine if there is a trend towards increasing WC, higher triglyceride and TPR levels with an increase in UA levels

(while adjusting for confounders) (Figure 1). There was a significant increase in WC in both ethnic groups with increasing UA levels. The Caucasian men showed a significant increase in triglyceride levels with increasing UA levels. Noticeable only in the African men is a significant increase in TPR with concomitant increase in UA levels.

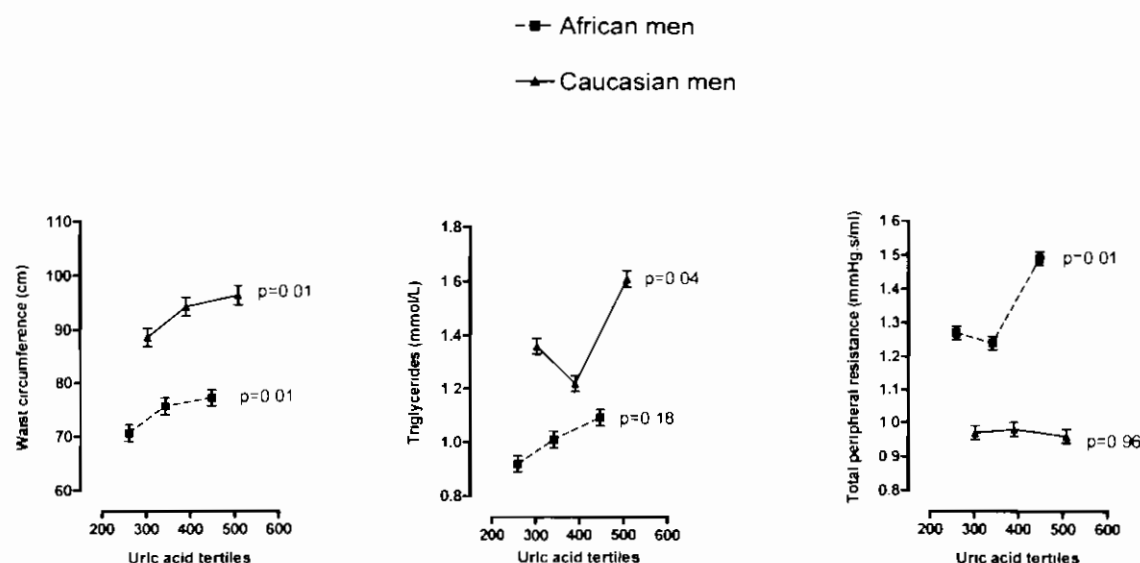


Figure 1. Waist circumference, triglyceride levels and total peripheral resistance with increasing serum uric acid tertiles in African and Caucasian men (adjusted for age, BMI, WC, fasting insulin levels, smoking and alcohol consumption)

Discussion

Epidemiological studies have shown that the African population is considered a high risk group for the development of hypertension (15,19) as well as hyperuricemia (8,13). Despite having significantly higher SBP, DBP and MAP compared to the Caucasian men, the African men in our study group had significantly lower UA levels. This data corresponds well with a previous study we performed on ethnic differences in UA levels between African and Caucasian women from South Africa (12), where the African women also had significantly lower UA levels. This discrepancy between our and previous epidemiological studies may be attributed to the different origin of the study populations, because it is not known to what extent Africans from South Africa and African-Americans are comparable (16,20,21).

WC is a reliable indicator of abdominal obesity (22,23) and UA levels are commonly associated with visceral fat (9). The higher UA levels in the Caucasian men might be partially explained by significant differences in abdominal obesity. Our multiple regression analysis also showed that WC is the strongest contributor to UA levels in both ethnic groups even though the African men had substantially smaller WC. This was confirmed by the significant increasing trends in WC according to UA tertiles of both ethnic groups.

Another possible explanation for the higher UA levels in the Caucasian men might be the consumption of protein (purine) rich food which is an exogenous source of uric acid (24). However this was not determined in our study, results from previous South African studies showed that Africans tend to have a lower protein intake compared to Caucasians (12, 25).

The presence of lower UA levels in Africans is indeed informative, but perhaps of greater interest, is how this marker relates to cardio-metabolic variables in apparently healthy or asymptomatic subjects. Despite their significantly lower UA levels, the positive association between UA and blood pressure was stronger and more pronounced in the African men. This observation is significant considering the fact that the black population is more susceptible to the development of hypertension (6). Our results are consistent with findings from the Coronary Artery Risk Development in Young Adults (CARDIA) study as well as the Atherosclerosis Risk in Communities (ARIC) study (6,26) signifying a stronger UA-blood pressure relationship in the black population. These findings are imperative for future health consequences regarding African men, especially for developing countries such as South Africa. A few years ago, the prevalence of hyperuricemia and the metabolic syndrome in the black population, was not a common finding. However, the adoption of a more westernised diet, due to industrialisation, had a great impact on the latter mentioned. Due to the high content of sugars (particularly fructose) in a westernised diet, the prevalence of obesity and diabetes increased (27,28) resulting in a concomitant increase in UA levels. Another study performed by Schwarzmeier *et al.* revealed that fructose *per se* causes an acute increase in UA levels (29) which will further increase the vulnerability for the development of UA dependent hypertension, especially in the African men. In addition, after adjusting for potential confounding factors such as obesity, age, fasting insulin, smoking and alcohol, the significance of the UA-blood pressure relationship diminished, leaving an independent relationship between UA and TPR. After dividing the group into UA tertiles, this significant independent increase in TPR was noticeable with an increase in UA levels only for our African participants.

This observation raises the possibility that UA may act as a risk factor, contributing to the development of cardiovascular disease in African men. Resistance arteries are essential in controlling blood pressure (30), and any structural changes (vascular remodelling) of these arteries will greatly influence vascular resistance (31). There are multiple ways in which UA can most likely cause vascular remodelling. UA can alter the proliferation and migration of vascular smooth muscle cells (32,33) by activating a series of pathways including mitogen-activated protein kinases (33,34), platelet-derived growth factors, chemokines (monocyte chemoattractant protein-1), and inflammatory enzymes (COX-2) (33,34).

Besides its possible direct effect on VSMC proliferation and migration, UA may also exert other detrimental effects on the vascular system. It is speculated that UA alters the release of endothelial NO (35,36), allowing the endothelium to convert from a vasoprotective environment to one that is vasoconstrictive, procoagulant and antifibrinolytic (37). Although this cross-sectional study limits us to draw any conclusions on causality, the latter mentioned relationship might suggest a possible mechanistic link between hyperuricemia and hypertension.

Our data also indicate that an independent relationship exist between UA and triglycerides. This is in agreement with previous studies (7,20,38) which have constantly found an independent correlation between triglycerides and UA levels. Confounding factors such as BMI or WC could explain this strong association. It is clear from our multiple regression model that WC was the main determinant for the variability in UA levels in both the African and Caucasian men. However, even after correcting for obesity risk factors, the strong correlation remained relatively strong and highly significant in the Caucasian men. According to Tavit *et al.* (39), higher UA levels are associated with atherogenesis. This suggests that a rise in UA levels with a concomitant rise in triglyceride levels may play some role in the aetiology of cardiovascular dysfunction.

Even though this study has contributed a part to the body of knowledge regarding UA levels and its correlates in African and Caucasian men, there are some limitations. Firstly, the cross-sectional design does not allow us to infer causality; therefore, our results need to be confirmed with future follow-up studies. Other limitations included the inability to account for additional factors that might also have confounded the association between UA and cardio-metabolic variables, including the use of diuretics (7) and the consumption of a protein (purine) rich diet (40). The study group was relatively young and healthy with a low degree of cardiovascular dysfunction. It is therefore recommended that in future a follow-up study be performed.

In conclusion, our findings indicate that African men have significantly lower UA levels compared to Caucasian men. However, the association between UA levels and vascular resistance is significant only in the African men. Despite the higher UA levels in the Caucasian men, their cardiovascular profile seems not to be affected detrimentally. UA showed strong correlations with triglycerides and abdominal obesity in both ethnic groups.

Table 5. Summary table

What is known about this topic:	What our study adds to this topic:
1. Uric acid is elevated in high risk groups for instance African-Americans (8)	1. Sub-Saharan African men have lower uric acid levels compared to Caucasian men from South Africa
2. Uric acid has a strong relationship with triglycerides (7,20) and specifically central obesity (9)	2. Triglycerides correlated strongly with uric acid in both ethnic groups, but remained significant only in Caucasians after adjustments
3. Uric acid correlates significantly with cardiovascular risk factors (7,32,37)	3. Only the African men showed correlations between uric acid and cardiovascular variables, in particular vascular resistance.

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Conflict of interest

None declared

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CHAPTER 3

ETHNIC DIFFERENCES REGARDING THE INSULIN-BLOOD PRESSURE RELATIONSHIP

IM Palmer, HW Huisman, AE Schutte

School for Physiology, Nutrition and Consumer Sciences, North-West University, Potchefstroom
Campus, South Africa

INSTRUCTIONS FOR AUTHORS: *Diabetes Research and Clinical Practice*

- ❖ Organise the manuscript into: 1) Title page, 2) Abstract and keywords, 3) Introduction, 4) Materials and Methods, 5) Results, 6) Discussion, 7) Acknowledgements, 8) References
- ❖ Abstract should not be more than 200 words with 3 to 5 keywords.
- ❖ References in the text should be numbered by Arabic numerals within square brackets in the order of first citation, and listed in numerical order at the end of the text.
- ❖ The first six authors should be quoted followed by *et al.*
- ❖ Abbreviations for titles of medical periodicals should conform to those used in the *Index Medicus*.
- ❖ Tables should be numbered consecutively with Arabic numerals, and contain only horizontal lines. A short descriptive heading and explanation above each table with footnotes underneath should be provided
- ❖ Use SI units throughout.
- ❖ Abbreviations and symbols should be standard.

Abstract

Ageing is associated with increasing insulin and C-peptide levels. Due to a lack of data, our first aim was to establish whether this also holds true for Africans from South Africa. Our second aim was to determine whether an association between insulin/C-peptide levels and blood pressure exist within an African and Caucasian population with increasing age, as well as to establish gender differences. African men and women (N=260) and Caucasian men and women (N=369) were recruited and stratified into age groups (18-35 yrs; >35-45 yrs and >45 yrs). ANCOVA's and partial correlations were performed. Results showed opposing changes in insulin/C-peptide levels of African and Caucasian men with increasing age. Insulin/C-peptide tended to decrease in African men, whereas insulin tended to increase and C-peptide increased significantly ($p=0.03$) in Caucasian men. Despite similar obesity levels, the oldest African women had significantly lower insulin ($p<0.01$) and C-peptide ($p<0.01$) levels compared to their Caucasian counterparts. In conclusion, insulin/C-peptide levels tended to decrease in the African population with increasing age. Despite significantly lower levels of insulin, blood pressure levels of African men seems to be affected more detrimentally compared to their Caucasian counterparts, leaving them more vulnerable for the development of cardiovascular diseases.

Keywords: insulin/C-peptide, ethnicity, gender, Africans, Caucasians

Introduction

Increasing age is associated with several physiological changes including insulin resistance [1;2] which is frequently characterised by compensatory and sustained hyperinsulinemia and subsequently Type 2 diabetes [3]. However, great controversy still exists in the literature investigating insulin levels in older people, with some studies suggesting normal or decreased insulin levels [4].

Since most of the studies addressing higher insulin levels in the elderly included mostly African-Americans [5] and Caucasians [1], our first aim was to establish whether this also holds true for African men and women and how their levels of insulin compare to those of their Caucasian counterparts. It is not known to what extent Africans from South Africa and African-Americans are comparable [3;6].

Several epidemiological studies have shown that the African population is a high risk group regarding the prevalence of hypertension [6-8] and it is therefore crucial to identify possible risk factors contributing to their high propensity for hypertension. Age-related insulin secretory dysfunction is associated with cardiovascular dysfunction [9-11], and might be a possible underlying cause in age-associated cardiovascular diseases such as hypertension, which is also known to occur more commonly with an increase in age [12].

A possible relationship between fasting insulin levels and blood pressure has been the grounds of many research debates over the past few years. While some researchers found positive correlations between insulin and blood pressure [13-18], other researchers [19-21] challenged these results on the grounds that 1) the relationship is often weak, 2) it depends on the characteristics of the study population (such as the ethnic group or inclusion criteria of a specific study), and 3) it is strongly confounded by age and obesity which is easily overcome by statistically adjusting for them.

However, Schutte *et al.* [22] suggested that statistical adjustment for age could mask the association between insulin and blood pressure since there is a possible turnaround in the correlation with increasing age. Instead, they propose age stratification instead of statistical adjustment for age. Their results indicate that the younger subjects tend to show a more positive insulin-blood pressure correlation, while the older subjects showed a more negative correlation. However, they concluded themselves that the study design was not ideal to directly address this issue.

Some researchers [23;24] suggest that C-peptide should be used when assessing insulin secretion. According to Brandenburg [25] and Kayali *et al.* [26] C-peptide can be used as an independent marker of insulin biosynthesis and secretion due to its unique properties. This suggestion has been made because insulin and C-peptide are released in equimolar amounts to the circulation [25;27]. C-peptide does not undergo hepatic extraction like insulin, therefore, the plasma half life of C-peptide in humans is approximately 30 minutes [28] as compared to no more than 6 minutes for insulin [29].

Due to the high susceptibility of Africans for the development of hypertension [6-8], it is important to identify possible risk factors contributing to their cardiovascular dysfunction, especially insulin since ethnic differences regarding insulin resistance and associated hyperinsulinemia was previously reported (30). Our second aim was, therefore, to determine the relationship between insulin or C-peptide with blood pressure of Africans and Caucasians using stratified age groups opposed to the whole subject group.

Gender-related cardiovascular dysfunction differ markedly between men and women [31;32] whereas men seem to develop hypertension at an earlier age compared to women [33]. We therefore also aimed to identify possible gender disparities between the insulin/C-peptide-blood pressure relationships.

Materials and Methods

The SAfrEIC (The South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) study took on the structure of a cross-sectional research design.

Apparently healthy, i.e. not previously diagnosed with any serious, chronic illnesses (except hypertension), men and women of African (n=260) and Caucasian (n=369) descent were recruited as volunteers from the Potchefstroom district in the North West Province, South Africa.

Exclusion criteria were subjects with diagnosed diabetes (type 1 and 2), HIV positive participants, pregnant and lactating women. Participants were in a fasting state.

Field workers relayed all relevant information in the subjects' home language. All subjects were informed about the outcome and procedures of the study beforehand, whereafter they signed informed consent forms. The study was approved by the Ethics Committee of the North-West University (Potchefstroom Campus) and complies with the Declaration of Helsinki as revised in 2004.

Blood sampling

A qualified nurse performed a finger prick to determine the fasting glucose level which was directly measured in the Metabolic Unit 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 using a sterile winged infusion set. Plasma and serum samples were prepared according to standard methods and stored at -82°C until analyses were performed. Insulin (ST AIA-PACK IRI, Cat. No 025260) and C-peptide (ST AIA-PACK C-PEP, Cat. No 025260) was analysed by a two-site immunoenzymometric assay on the TOSOH AIA System analyzers (San Francisco, CA, USA).

Anthropometrical measurements

Anthropometric measurements were performed according to standard methods described by Norton and Olds [34]. Height measurements were taken using a stadiometer (Invicta, IP 1465, UK), and body weight measurements were measured up to the nearest kilogram using a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). The waist circumference was measured at midway level between the inferior rib margin and superior margin of the iliac crest. All the measurements were taken in triplicate to obtain a reliable median value.

Blood pressure measurement

Each participant was allocated to a room where he/she rested for 10 min. before the blood pressure measurements. Participants were required to remain in the Fowler's position while blood pressure was measured using the Finometer® device (FMS, Finapres Medical Systems, Amsterdam, the Netherlands). The vascular unloading technique of Pe az together with the Physiological criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are usable especially in comparative studies [35;36]. The Finometer® device was connected and cuffs were attached to the left arm and the left middle finger of the participant. Resting blood pressure was recorded continuously for five minutes. After a recording of two minutes, a systolic return-to-flow calibration was performed. This calibration was performed to adjust the brachial arterial pressure of each specific participant with the finger pressure. Highest precision readings were obtained after this calibration. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) were measured and stored [37].

Questionnaires

Demographic information and menstrual status of the women were derived from results using a Demographic and Lifestyle questionnaire. Smoking and alcohol consumption was derived from a General Lifestyle questionnaire, indicated by "yes/no" answers.

Statistical analysis

Statistical analyses were performed using Statistica version 8.0 (Statsoft, Inc., Tulsa; OK, 2008). Statistical results are presented as means and 95 % confidence intervals. An independent t-test, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used for comparison of variables between groups to determine significant differences. A multiple analysis of covariance (MANCOVA) was performed to compare variables between the groups, whilst adjusting for age, body mass index, waist circumference, smoking and alcohol consumption.

Partial correlations (adjusting for age, body mass index, waist circumference, smoking and alcohol consumption) were performed within each group to determine associations between fasting insulin and mean arterial pressure as well as between fasting C-peptide and mean arterial pressure. Thereafter the experimental groups were stratified into age groups and the partial correlations were repeated while adjusting for only body mass index and waist circumference. Fisher's Z-transformation was used to indicate significant differences between correlation coefficients ($P < 0.05$).

Results

The two ethnic groups were stratified into three age groups: 18 - 35 years (Group 1); 36 - 45 years (Group 2), and older than 45 years (Group 3). The clinical and anthropometrical characteristics of each age group are summarised in Table 1.

The African and Caucasian women were comparable in all three age groups, except for Groups 2 and 3 where the African women had significantly higher mean arterial pressure compared to the Caucasian women. In Group 3 the Caucasian women had significantly higher fasting insulin ($p < 0.01$) and fasting C-peptide levels ($p < 0.01$). The smoking prevalence of the African women were significantly higher in Groups 2 and 3 compared to their Caucasian counterparts ($p < 0.01$ for both groups).

In all three male age groups, the Caucasian men had significantly higher body mass indexes, larger waist circumferences as well as higher fasting insulin- and C-peptide levels compared to the African men. The young African men (Group 1) had significantly higher blood pressure compared to the Caucasian men, with the same tendency in the two older age groups ($p = 0.06$ and $p = 0.08$, respectively). In both ethnic groups, there was a significant increase in blood pressure with aging, noticeable for men and women (data not shown). In all three age groups, the African men had a significantly higher smoking prevalence compared to the Caucasian men ($p < 0.01$ in all instances). Alcohol consumption were significantly higher in Groups 2 and 3 of the African men compared to their Caucasian counter parts ($p = 0.01$ and $p = 0.04$, respectively).

In all of the age groups, except for Group 1 of the women, the prevalence of smoking was significantly higher in the Africans compared to the Caucasians.

Table 1. Clinical and anthropometrical characteristics of the respective age groups

Variable	African females	Caucasian females	African men	Caucasian men
Group 1 (18-35yrs)	N=39	N=76	N=52	N=66
Age (yrs)	27.9 [26.3;29.4]	27.0 [25.8;28.1]	27.7 [26.3;29.2]	27.5 [26.2;28.7]
BMI (kg/m ²)	27.5 [25.2;29.9]	25.2 [23.9;26.6]	21.0 [19.8;22.2] ^a	27.2 [26.0;28.4] ^a
WC (cm)	79.4 [75.2;83.7]	76.1 [73.4;78.8]	72.6 [69.8;75.3] ^a	88.9 [85.7;92.1] ^a
Fasting insulin (uU/ml)	9.79 [7.92;11.7]	8.70 [7.30;10.1]	6.32 [4.99;7.56] ^a	8.85 [6.81;10.9] ^a
Fasting C-peptide (nmol/L)	0.61 [0.52;0.71]	0.62 [0.53;0.71]	0.46 [0.40;0.52] ^a	0.62 [0.52;0.72] ^a
MAP (mmHg)	96.4 [93.5;99.2]	95.1 [93.6;96.5]	101 [97.8;104] ^a	95.1 [93.6;96.8] ^a
Smoking, N (%)	9.00 (23.0)	11.0 (14.5)	33.0 (63.5) ^a	19.0 (28.8) ^a
Alcohol, N (%)	17.0 (43.6)	47.0 (61.8)	39.0 (75.0)	57.0 (86.4)
Group 2 (36-45yrs)	N=45	N=59	N=29	N=44
Age (yrs)	40.3 [39.4;41.1]	40.8 [40.0;42.0]	40.6 [39.6;41.7]	40.0 [39.0;40.9]
BMI (kg/m ²)	28.2 [25.8;30.6]	27.9 [26.3;29.5]	20.1 [18.4;21.7] ^a	30.4 [28.8;32.1] ^a
WC (cm)	83.2 [78.8;87.6]	82.6 [79.4;85.8]	73.8 [69.9;77.8] ^a	99.7 [95.2;104] ^a
Fasting insulin (uU/ml)	8.88 [6.93;10.8]	8.81 [7.10;10.5]	4.72 [2.49;6.94] ^a	9.06 [6.87;11.3] ^a
Fasting C-peptide (nmol/L)	0.68 [0.55;0.81]	0.65 [0.57;0.73]	0.43 [0.30;0.56] ^a	0.69 [0.51;0.86] ^a
MAP (mmHg)	104 [99.7;107] ^a	97.4 [94.9;100] ^a	105 [100;109]	100 [97.7;103]
Smoking, N (%)	24.0 (53.3) ^a	6.00 (10.2) ^a	25.0 (86.2) ^b	9.00 (20.5) ^b
Alcohol, N (%)	24.0 (53.3)	47.0 (61.8)	26.0 (89.7) ^a	27.0 (61.4) ^a
Group 3 (>45 yrs)	N=47	N=72	N=48	N=52
Age (yrs)	55.5 [53.5;57.5]	54.8 [53.4;56.2]	56.2 [54.4;58.0]	55.8 [54.2;57.4]
BMI (kg/m ²)	26.6 [24.4;28.8]	28.6 [27.1;30.2]	19.9 [18.8;21.0] ^a	28.1 [27.0;29.3] ^a
WC (cm)	84.7 [80.4;89.0]	86.5 [83.2;89.8]	76.1 [73.2;79.1] ^a	97.6 [94.8;100] ^a
Fasting insulin (uU/ml)	7.73 [6.08;9.39] ^a	10.3 [8.86;11.7] ^a	3.93 [2.44;5.41] ^b	11.2 [8.98;13.4] ^b
Fasting C-peptide (nmol/L)	0.61 [0.52;0.70] ^a	0.78 [0.70;0.86] ^a	0.38 [0.30;0.46] ^b	0.83 [0.71;0.95] ^b
MAP (mmHg)	110 [106;113] ^a	103 [100;105] ^a	110 [105;115]	105 [102;108]
Smoking, N (%)	33.0 (70.2) ^a	4.00 (5.55) ^a	38.0 (79.2) ^b	5.00 (9.62) ^b
Alcohol, N (%)	29.0 (61.7)	33.0 (45.8)	42.0 (87.5) ^a	38.0 (73.1) ^a

Values are expressed as the mean [± 95 % confidence intervals];

BMI, body mass index; WC, waist circumference; MAP, mean arterial pressure.

Mean values with the same superscript letter: statistically significant $p < 0.05$

Figures 1 and 2 shows the difference in fasting insulin and C-peptide levels between the African and Caucasian participants of the different age groups with and without adjustments for obesity, smoking and alcohol consumption.

Figures 1A and 1B show no significant changes in fasting insulin levels with an increase in age for either the African and Caucasian women or men. Significant differences were found between the insulin values of especially the oldest groups, with the oldest Caucasian groups showing the highest levels of insulin. The fasting insulin levels of all the African men age groups were significantly lower compared to the Caucasian men.

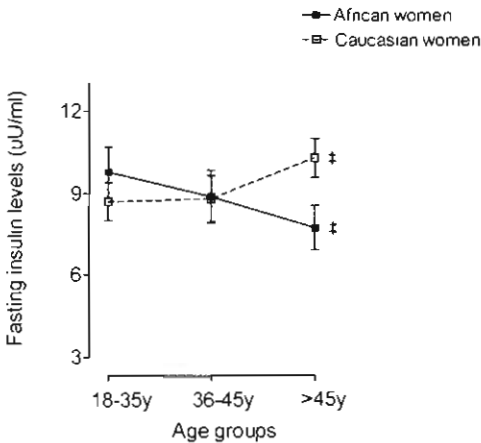


Figure 1A

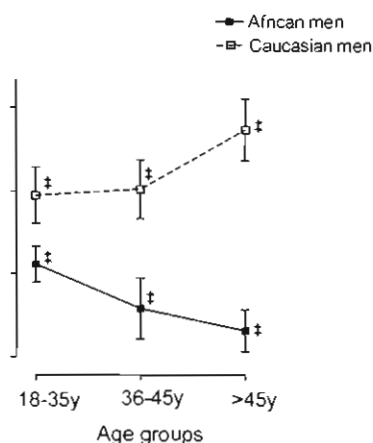


Figure 1B

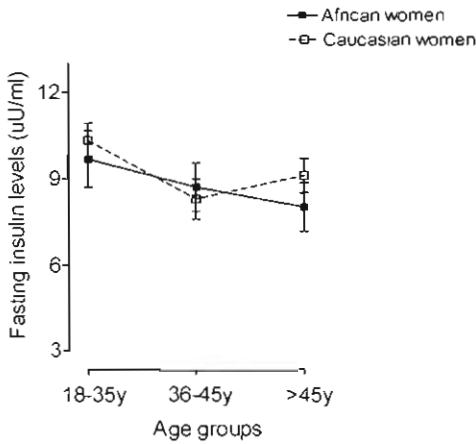


Figure 1C

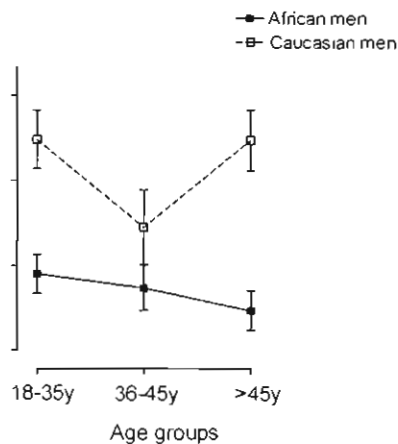


Figure 1D

Figure 1. Fasting insulin levels of the different age groups A) Fasting insulin levels of African and Caucasian women, B) Fasting insulin levels of African and Caucasian men, C) Fasting insulin levels of African and Caucasian women after adjusting for obesity, smoking and alcohol consumption, D) Fasting insulin levels of African and Caucasian men after adjusting for obesity, smoking and alcohol consumption. Results are plotted as mean±SEM. ‡ p<0.05 for differences between ethnic groups within the same age-group.

However, after adjusting for obesity measures (body mass index and waist circumference), smoking and alcohol consumption, the above-mentioned significant differences disappeared as seen in Figures 1C and 1D.

In Figures 2A and 2B a significant increase in fasting C-peptide levels with an increase in age for both the Caucasian women ($p=0.01$) and men ($p=0.03$) were shown. This was not evident for the African women or men. The significant increase observed within the Caucasian men, remained after adjusting for obesity (body mass index and waist circumference), smoking and alcohol consumption ($p=0.04$).

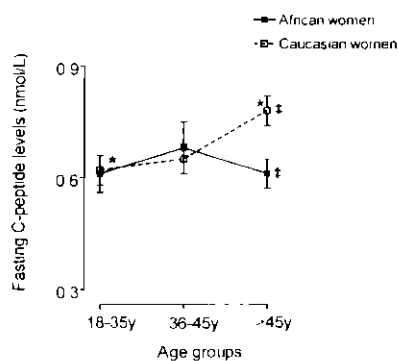


Figure 2A

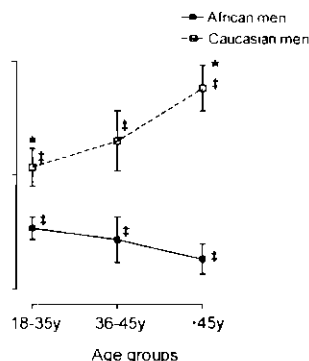


Figure 2B

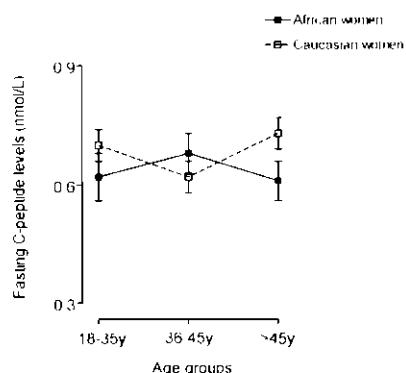


Figure 2C

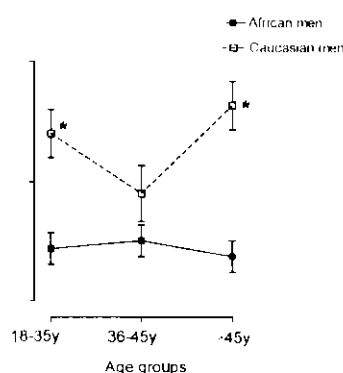


Figure 2D

Figure 2. Fasting C-peptide levels of the different age groups A) Fasting C-peptide levels of African and Caucasian women, B) Fasting C-peptide levels of African and Caucasian men, C) Fasting C-peptide levels of African and Caucasian women after adjusting for obesity, smoking and alcohol consumption D) Fasting C-peptide levels of African and Caucasian men after adjusting for obesity, smoking and alcohol consumption. Results are plotted as mean \pm SEM. * $P < 0.05$ for differences between age-groups within the same ethnic group, ‡ $p < 0.05$ for differences between ethnic groups within the same age-group.

As with the insulin levels, the African men had significantly lower C-peptide levels throughout the age spectrum compared to the Caucasian men. However, this significant difference disappeared after adjusting for obesity (body mass index and waist circumference), smoking and alcohol consumption. The only significant difference observed between the women was within the oldest

group, which also disappeared after adjusting for obesity (body mass index and waist circumference), smoking and alcohol consumption.

The correlations of fasting insulin- or C-peptide levels with mean arterial pressure (whilst adjusting for age, body mass index, waist circumference, smoking and alcohol consumption) for the whole group are shown in Table 2. The correlations were all omissible weak and insignificant.

Table 2. Partial correlations between fasting insulin- or C-peptide levels and mean arterial pressure (adjusted for age, body mass index, waist circumference, smoking and alcohol)

	Fasting insulin	Fasting C-peptide
African women	$r=-0.17$; $p=0.07$; $N=123$	$r=-0.11$; $p=0.24$; $N=128$
African men	$r=-0.03$; $p=0.73$; $N=124$	$r=-0.08$; $p=0.38$; $N=124$
Caucasian women	$r=-0.06$; $p=0.41$; $N=187$	$r=-0.08$; $p=0.30$; $N=188$
Caucasian men	$r=0.02$; $p=0.85$; $N=134$	$r=0.01$; $p=0.64$; $N=140$

After age stratification, partial correlations (adjusting for body mass index, waist circumference, smoking, and alcohol consumption) were performed between fasting insulin or fasting C-peptide and mean arterial pressure within each age group. In Figure 3A and 3B the correlation coefficients between fasting insulin and mean arterial pressure are shown for the African women and Caucasian gender groups.

It is clear from that the Caucasian women and men show similar correlations between insulin and blood pressure with increasing age, whereas the correlations between insulin and mean arterial pressure of the African women tended to become more positive with increasing age while those of the African men tended to become more negative with increasing age.

In general, all the groups showed very weak correlations for the insulin-blood pressure relationship, except for Group 2 of the African women who showed a significantly strong negative correlation. The African men revealed significant differences in the correlations between Groups 1 and 2 ($p<0.05$) as well as between Groups 1 and 3 ($p<0.05$). A significant difference also existed in the correlations between the African men and women of Group 1 ($p<0.05$).

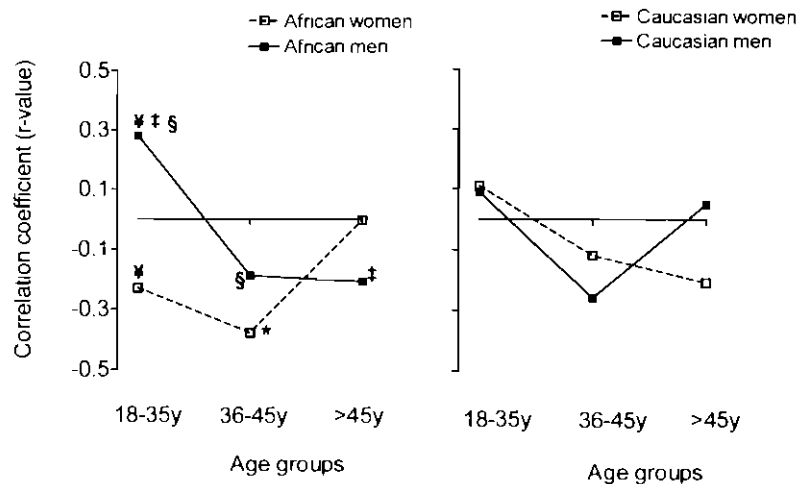


Figure 3A

Figure 3B

	Group 1	Group 2	Group 3
African women	$r=-0.23$; $p=0.23$	$r=-0.38$; $p=0.02^{* \dagger}$	$r=-0.00$; $p=0.98^{\ddagger}$
African men	$r=0.28$; $p=0.07$	$r=-0.19$; $p=0.38$	$r=-0.21$; $p=0.19$
Caucasian women	$r=0.11$; $p=0.40$	$r=-0.12$; $p=0.44$	$r=-0.21$; $p=0.10$
Caucasian men	$r=0.09$; $p=0.54$	$r=-0.26$; $p=0.20$	$r=0.05$; $p=0.77$

Figure 3. Partial correlations between fasting insulin and mean arterial pressure (adjusted for body mass index, waist circumference, smoking and alcohol consumption) in A) African women and men, B) Caucasian women and men. (*; significant correlation, $p=0.01$; † and §, significant difference in correlation between age-groups within the same ethnicity, $p < 0.05$, ¥, significant difference in correlation between ethnic-groups within the same age-group).

After adjusting for menstrual status, all of the results stayed the same, with the exception that the correlation in Group 2 of the African women became much stronger ($r=-0.58$, $p<0.01$).

Correlations between C-peptide and mean arterial pressure were very weak and insignificant, except for the African women in Group 2 who showed a relatively strong, borderline significant, negative correlation ($r=-0.33$, $p=0.06$) (data not shown). After adjusting for menstrual status in the women, all the results remained the same, except that the African women of Group 2 revealing a significant correlation ($r=-0.33$, $p=0.04$) (data not shown).

Discussion

In general, data from this study corresponds well with previous findings that the African population have higher blood pressure [6;38;39] compared to the Caucasian population. Since it is known from the literature that fasting insulin levels normally increase with age [1-3], we firstly aimed to

establish whether this also holds true for African men and women.

This study yielded interesting results namely opposite changes in both insulin and C-peptide levels of African and Caucasian men with an increase in age. Fasting insulin levels tended to increase with age in the Caucasian men opposed to a decrease in African men. The African men maintained significantly lower insulin levels compared to the Caucasian men throughout the age spectrum. These results were mirrored in the C-peptide data, since our results also showed a significant increase in fasting C-peptide secretion of the Caucasian men with ageing, that differs from the African men whose fasting C-peptide tended to decrease.

The higher fasting insulin and C-peptide levels observed in the Caucasian men might be explained by their significantly higher body mass index and larger waist circumference. Waist circumference is a reliable indicator of abdominal obesity [40; 41] which is often associated with an increase in insulin resistance [42;43] with concomitant compensatory hypersecretion of insulin, hence higher fasting insulin and C-peptide levels. The Caucasian men in our study showed a significant increase in their waist circumference ($p<0.01$) as well as body mass index ($p<0.01$) with increasing age. After adjusting for obesity (body mass index and waist circumference) the significant increase in insulin disappeared (data not shown). However, the Caucasian men still exhibited (after adjusting for obesity) a significant increase in fasting C-peptide levels with increasing age. Since C-peptide can be considered a reliable surrogate marker for insulin secretion, it can be speculated that more significant results for insulin will be revealed with a larger subject group.

The most striking finding from this study was the fact that the insulin and C-peptide levels of the African men showed a tendency to decrease with increasing age. In contrast to the Caucasian men, the insulin levels in the African men seem not to be dependent on obesity levels. In fact, body mass index and waist circumference of all three age-groups showed no significant change with increasing age ($p=0.35$ and $p=0.21$ respectively). The mechanism contributing to the age-related decline in insulin secretion cannot be determined from our study, and can only provide inferences on the effects of aging. Several possibilities exist that might explain this phenomenon. The decline in circulating insulin and C-peptide levels might be linked to progressive β -cell failure [44]. Joffe *et al.* [45] proposed that the African population either inherit or acquire decreased pancreatic β -cell mass [45] and that they are more prone to the development of insulinopenic non-insulin dependent diabetes mellitus. Another possible explanation is a diminished sensitivity to incretin hormones due to impairments in secretion and action of glucagon-like-peptide 1 and glucose-dependent insulintropic peptide [46].

The Caucasian women exhibited similar results as the Caucasian men – with the exception that there was no significant increase in fasting insulin levels with increasing age. However, their fasting C-peptide levels also showed a significant increase.

The only ethnic difference in insulin and C-peptide levels of the women were observed within the oldest group (Group 3). Despite similar obesity levels, the Caucasian women had significantly higher insulin and C-peptide levels compared to the African women. Other factors might therefore contribute to these differences, which cannot be concluded from this study.

Our second aim was to determine the relationship between insulin or C-peptide with blood pressure for Africans and Caucasians using stratified age groups versus the whole subject group.

Our data corresponds well with previous findings [19-21], namely that by statistically adjusting for age, the association between fasting insulin and mean arterial pressure is indeed very weak. This also holds true for the association between fasting C-peptide and mean arterial pressure. This was observed in both the African and Caucasian subjects. Although age stratification confirmed the observation made by Schutte *et al.* [22;47] (more positive correlation between fasting insulin and mean arterial pressure in the younger people and more negative correlations within the older group), it is imperative to emphasise the weakness and insignificance of these correlations. Despite the weakness of these correlations, a significant difference existed between the correlations of the youngest and oldest African men, revealing a turnaround in the insulin-blood pressure relationship, and thereby strengthening the observation made by Schutte *et al.* [22;47]

It seems as if insulin-mediated vasoconstrictive effects are more dominant within younger people. These vasoconstrictive effects might result from increased renal sodium reabsorption [48] and stimulation of the renin-angiotensin system, which in turn increase sympathetic nervous system outflow [49]. However, some researchers have also reported vasodilatory effects of insulin on the vascular system [50-52] and it is this insulin-mediated vasodilatation that could be more prominent in older people. Perhaps due to the fact that the latter mentioned group is considered such a high risk group for the development of cardiovascular diseases, this shift of insulin from a positive correlation towards a more negative correlation might act as a protective mechanism against further possible cardiovascular damage [47].

The African men showed an extrication of insulin with mean arterial blood pressure with progressive age. These results are purely associations, and we can only speculate regarding the implications. A possible indication is that in African men insulin *per se* is not considered a risk factor, but rather a possible lack of insulin-mediated vasodilatation giving secondary factors the

opportunity to instigate cardiovascular dysfunction. The African women on the other hand, showed a significant coupling between insulin and mean arterial pressure with progressive age, despite their lower insulin levels.

Although this study yielded important results, there are some potential limitations. Firstly, the mean age of the older group was approximately 55 years which is still relatively young and perhaps with a relatively low degree of cardiovascular dysfunction. It is therefore recommended that in future either a follow-up study be performed or older age groups should be included to determine cardiovascular dysfunction and its association with insulin for older ages. Secondly, our study is limited by its cross-sectional design, which prevents us from drawing any conclusion on cause and effects.

In conclusion, it seems that insulin and C-peptide levels differ between Africans and Caucasians with increasing age. The Caucasian population tended to exhibit an increase in insulin/c-peptide levels with increasing age whereas the African population tended to exhibit a decrease in circulating levels with increasing age. Obesity seems to play an essential role in the observed ethnic differences. Caucasian men and women showed an increase in obesity measures with increasing age resulting in increased insulin and C-peptide levels. However, obesity alone cannot be held responsible for latter mentioned differences. Despite similar obesity levels, the oldest Caucasian women had significantly higher insulin and C-peptide levels compared to the African women.

Even though the Caucasian men had significantly higher levels of insulin, it seems as if their blood pressure was not affected detrimentally. On the other hand, the African men with lower levels of insulin had higher blood pressure. It is therefore concluded that the lower insulin levels *per se* are not associated with cardiovascular dysfunction in African men with an increase in age and that other factors, such as sodium sensitivity [6] might be held accountable for the very high prevalence of hypertension observed in African men.

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CHAPTER 4

AGEING AND ADIPONECTIN LEVELS IN AN AFRICAN POPULATION: AN INVESTIGATION FROM A RENAL PERSPECTIVE

IM Palmer, HW Huisman, AE Schutte

School for Physiology, Nutrition and Consumer Sciences, North-West University,
Potchefstroom Campus, South Africa

INSTRUCTIONS FOR AUTHORS: *Regulatory Peptides*

- ❖ Divide the manuscript into the following sections into: 1) Introduction, 2) Materials and Methods, 3) Results, 3) Discussion, 4) Acknowledgements.
- ❖ Single spaced abstract should be included, with not more than 200 words.
- ❖ No more than 6 keywords should be provided.
- ❖ Citations in the text should be given as numbers within square brackets in the order of first citation, and listed at the end of the text.
- ❖ All the authors should be listed.
- ❖ Abbreviations for titles of medical periodicals should conform to those used in the *"List of Serial Title Word Abbreviations"*.
- ❖ Use SI units throughout.
- ❖ Abbreviations and symbols should be standard.

ABSTRACT

Ageing is associated with elevated adiponectin levels. Our aim was to assess whether age-related increase in adiponectin is associated with a decrease in renal function. The study comprised African (N=277) and Caucasian (N=326) men and women. Adiponectin levels, estimated creatinine clearance rate and obesity indices were determined. African men revealed significantly higher adiponectin levels compared to Caucasian men ($p<0.01$), reflecting the lower adiposity levels of the African men. No difference in obesity measures ($p=0.92$) and adiponectin levels ($p=0.27$) were observed between African and Caucasian women. A significant increase in adiponectin levels with ageing was observed in both African men and women ($p<0.01$). To the contrary, progressive ageing seems not to be significantly related to elevated adiponectin levels within Caucasians. Renal impairment decreased significantly within all of the groups ($p<0.01$). Single regression analyses performed in all specified groups revealed significant associations between adiponectin and estimated creatinine clearance. However a multiple regression model revealed that insulin resistance was the strongest contributor to adiponectin within all the groups. In conclusion, age-related rise in adiponectin levels observed in Africans is not due to renal impairment.

KEYWORDS

Adiponectin, renal function, ageing, Africans

INTRODUCTION

In addition to its major function as a storage depot for lipids, human adipose tissue is now also recognised as a secretory organ [1, 2]. A variety of active metabolic compounds called adipokines are released from white adipose tissue, such as tumour necrosis factor (TNF)- α , plasminogen activator inhibitor (PAI)-1, leptin, resistin and adiponectin, to mention a few [2-5]. As the adipose mass expands, the secretion of most of these compounds increases [6-8]. Adiponectin, however, is an exception and shows an inverse relation with adipose mass [9-11].

Adiponectin, an expression of the apM1 gene [12], consists of 244 amino acids and accounts for ~0.01% of the total plasma proteins [4]. Adiponectin possesses certain cardio-protective characteristics, including anti-atherogenic [13, 14], anti-diabetic [14] and anti-inflammatory properties [15-17]. Low adiponectin levels are unequivocally associated with obesity [11], Type 2 diabetes [1, 18] as well as higher cardiovascular risk [19-21]. Previous studies suggest that adiponectin deficiency might be a modulator of cardiovascular dysfunction [16, 22], and one possible mechanism is by impairing nitric oxide-mediated vasodilation [20, 23, 24].

Increased cardiovascular disease is common within the elderly population [25, 26]. It is, therefore, expected that increasing age is associated with adiponectin deficiency. To the contrary, various studies have reported the seemingly contradictory finding that despite higher cardiovascular risk in the elderly, ageing is associated with elevated levels of adiponectin [9, 27, 28]. Another study on the other hand found no age-related change in adiponectin levels [29].

Isobe *et al.* [9] concluded that a decrease in adiponectin clearance in the kidney might be the cause of high levels of adiponectin noticeable in the elderly. Apart from the increased adiponectin levels in the elderly, others also reported a significant negative correlation between adiponectin and renal function [30-32]. These studies included a Japanese study group, Type 1 diabetic subjects or patients with end stage renal disease. Our aim was to assess whether age-related increase in adiponectin is associated with a decrease in renal function.

MATERIALS AND METHODS

Participants

A total of 750 participants were recruited as volunteers from the Potchefstroom district in the North West Province, South Africa, to participate in the cross-sectional SAfrEIC study (South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function). The participants included in this investigation were apparently healthy, i.e. asymptomatic and not previously diagnosed with any serious or chronic illnesses (except hypertension).

Participants previously diagnosed with diabetes (Type 1 and 2), human immunodeficiency virus (HIV) infection, and those who were non-fasting were excluded. According to these criteria, 156 participants were excluded from the study group.

The participants reported early in the morning at the Metabolic Clinic of the university, and were required to be in a fasting state. All subjects were informed about the outcome and procedures of the study beforehand, where after they signed informed consent forms. Field workers relayed all relevant information in their home language. The study was approved by the Ethics Committee of the North-West University and complied with the Declaration of Helsinki as revised in 2008.

Blood sampling

A qualified nurse performed a finger prick to determine the fasting glucose level which was directly measured in the Metabolic Clinic with an enzymatic method (LifeScan SureStep® Blood Glucose Monitoring System. LifeScan Inc., Milpitas CA 9535, 1995, USA). Afterwards a fasting blood sample was taken from the antebachial vein using a sterile winged infusion set and syringes. Plasma and serum samples were prepared according to standard methods and stored at -82°C until analyses were performed. Serum blood lipids, creatinine and high-sensitivity C-reactive protein (hsCRP) were determined later in the laboratory with the Konelab™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Insulin (ST AIA-PACK IRI, Cat. No 025260) was analyzed by a two-site immunoassay on the TOSOH AIA System analyzers (San Francisco, CA, USA). Serum leptin levels were measured using an enzyme-linked immunosorbent (ELISA) kit (Diagnostic Systems Laboratories, Inc., Cat.

No. DSL-10-23100i). We measured adiponectin levels by making use of a quantitative sandwich enzyme immunoassay technique (Quantikine®, R&D Systems, Inc., Cat. No. DRP300).

Glomerular filtration rate (GFR) is an accurate quantification of renal function [33], and since GFR was not measured directly, a filtration marker namely estimated creatinine clearance was used [34-36]. Estimated creatinine clearance (eCcr) was calculated using the Cockcroft-Gault formula [37].

Insulin resistance was calculated using the homeostasis model assessment: $\text{HOMA-IR} = \text{fasting glucose} \times \text{fasting insulin} / 22.5$ [38].

HIV status was determined immediately after blood sampling with a rapid test. Serum was used for testing with the First Response Test (PMC Medical, Daman, India) and was repeated with the Pareeshak test (BHAT Bio-tech, Bangalore, India) for confirmation.

Anthropometric measurements

Anthropometric measurements were performed according to standard methods described by the International Society for the Advancement of Kinanthropometry (ISAK) [39]. Height measurements were taken using a stadiometer (Invicta, IP 1465, UK), and body weight measurements were measured up to the nearest kilogram using a calibrated electronic scale (Precision Health Scale, A&D Company, Japan). The waist circumference was measured at midway level between the inferior rib margin and superior margin of the iliac crest. All the measurements were taken in triplicate to obtain a reliable median value.

Cardiovascular measurements

Each participant was allocated to a room where he/she rested for at least 10 min. before the blood pressure measurements. Participants were required to remain in the semi-Fowler's position while blood pressure was measured using the Finometer® device (FMS, Finapres Medical Systems, Amsterdam, the Netherlands). The vascular unloading technique of Peñáz together with the Physiological criteria of Wesseling provided reliable, non-invasive and continuous estimates of blood pressure which are usable especially in comparative studies [40, 41]. The Finometer® device was connected and cuffs were

attached to the left arm and the left middle finger of the participant. Resting blood pressure was recorded continuously for five minutes. After a recording of two minutes, a systolic return-to-flow calibration was performed. This calibration was performed to adjust the finger pressure of each participant with the brachial arterial pressure. Highest precision readings were obtained after this calibration. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), Windkessel compliance (C_w), and total peripheral resistance (TPR) were measured and stored.

Statistical analyses

Statistical analyses were performed using Statistica version 8.0 (Statsoft, Inc., Tulsa; OK, 2008). Statistical results are presented as means \pm standard deviation. Data that were not normally distributed (adiponectin, leptin, glucose, insulin, homeostasis model assessment (HOMA), triglycerides, high-sensitivity C-reactive protein (hsCRP), eCcr, total peripheral resistance) were logarithmically transformed and presented as geometric means [5%; 95% percentile boundaries]. Independent t-tests were performed to determine significant differences between groups.

To evaluate the associations of adiponectin with age, eCcr, waist circumference, HOMA-IR and hsCRP, single regression analyses were performed within each ethnic/gender group. This was repeated while adjusting respectively for age, eCcr, waist circumference, HOMA-IR and hsCRP. Standard multiple regression analyses were performed within each specified group with adiponectin-log as dependent variable and age, eCcr-log, waist circumference, HOMA-IR-log and hsCRP-log as independent variables.

RESULTS

The main physical and metabolic characteristics of the study population are summarised in Table 1. When compared to their Caucasian counterparts, the African men and women weighed significantly less and were shorter. Overall, the Caucasians revealed a more favourable cardiovascular profile. This is reflected by their lower SBP, DBP, MAP, TPR and higher Windkessel compliance. In contrast, the lipid profile of the Africans seemed to be more favourable with significantly higher levels of high-density lipoproteins (observed only in the men), and lower triglycerides and low-density lipoproteins.

The African men had significantly higher adiponectin levels ($p<0.01$) compared to the Caucasian men corresponding well with their lower body mass ($p<0.01$) and waist circumference ($p<0.01$). No significant differences were observed between the African and Caucasian women with regards to adiponectin ($p=0.27$), body mass index ($p=0.66$) and waist circumference ($p=0.92$). Estimated creatinine clearance (eCcr) was significantly lower in the Africans compared to the Caucasians - observed in both men and women.

Table 1. Physical and metabolic characteristics of the study population

	African men	Caucasian men	<i>P</i> -value	African women	Caucasian women	<i>P</i> -value	Total group
	(N=117)	(N=121)		(N=160)	(N=205)		(N=603)
Age (yrs)	41.4±13.8	39.9±13.0	0.87	41.9±12.4	40.7±12.8	0.31	40.9±13.0
Anthropometric measures							
Weight (kg)	58.4±13.1	92.0±17.5	<0.01	67.6±19.0	74.5±17.5	<0.01	74.6±20.7
Height (m)	1.69±0.07	1.80±0.07	<0.01	1.57±0.06	1.66±0.06	<0.01	1.68±0.10
BMI (kg/m ²)	20.4±4.20	28.4±4.98	<0.01	27.3±7.49	27.2±6.41	0.66	26.2±6.58
WC (cm)	74.3±10.4	94.6±13.5	<0.01	82.5±14.2	81.6±13.5	0.92	83.8±14.9
Cardiovascular measures							
SBP (mmHg)	136±21.9	132±14.6	0.06	134±19.7	130±15.0	0.04	132±17.7
DBP (mmHg)	84.4±11.3	79.8±7.54	<0.01	81.7±9.83	76.8±7.45	<0.01	80.0±9.35
MAP (mmHg)	105±14.1	99.8±9.54	<0.01	104±12.5	98.3±9.35	<0.01	101±11.5
C _w (ml/mmHg)	1.67±0.51	2.41±0.58	<0.01	1.54±0.48	1.87±0.43	<0.01	1.91±0.59
TPR (mmHg.s/ml)	1.34 [0.89;2.20]	0.95 [0.59;1.54]	<0.01	1.28 [0.83;2.21]	1.06 [0.67;1.75]	<0.01	1.12 [0.68;1.97]
Lipid profile							
TC (mmol/L)	4.38±1.19	5.76±1.41	<0.01	4.46±1.04	5.89±1.43	<0.01	5.28±1.48
HDL-C (mmol/L)	1.72±0.78	1.20±0.35	<0.01	1.44±0.47	1.55±0.38	0.03	1.47±0.53
LDL-C (mmol/L)	2.20±0.89	3.81±1.25	<0.01	2.49±0.84	3.74±1.23	<0.01	3.22±1.30
Trig (mmol/L)	0.97 [0.52;2.01]	1.43 [0.57;3.60]	<0.01	1.04 [0.45;2.72]	1.19 [0.57;2.61]	0.01	1.17 [0.52;2.75]
Biochemical measures							
Adiponectin (µg/ml)	7.16 [2.92;19.8]	3.88 [1.24;10.2]	<0.01	6.27 [2.02;17.8]	6.59 [2.45;13.9]	0.27	5.76 [1.82;14.0]
Creatinine (µmol/L)	69.0±14.6	72.5±13.1	0.05	70.5±13.9	69.5±11.6	0.43	70.4±13.1
eGFR (ml/min)	102 [62.6;154]	155 [94.0;247]	<0.01	94.1 [57.9;168]	109 [70.9;174]	<0.01	115 [66.0;203]
Fasting glucose (mmol/L)	4.96 [4.03;6.37]	5.64 [4.61;7.27]	<0.01	5.13 [3.86;6.61]	5.25 [4.36;7.11]	0.09	5.27 [4.22;6.98]
Fasting insulin (µU/L)	3.14 [0.80;15.2]	8.26 [2.60;37.1]	<0.01	6.91 [1.60;20.7]	7.79 [2.70;20.6]	0.11	6.38 [1.10;21.7]
HOMA	0.70 [0.15;3.74]	2.07 [0.62;10.2]	<0.01	1.58 [0.27;6.04]	1.83 [0.56;5.91]	0.06	1.50 [0.24;6.05]
hsCRP (mg/L)	1.65 [0.01;21.4]	1.20 [0.02;9.53]	0.15	3.43 [0.40;31.5]	1.49 [0.06;15.7]	<0.01	1.71 [0.06;18.9]

Data expressed as mean±SD for normal distributed data and geometric mean [5%;95% percentile intervals] for log-transformed data; values in **bold** are statistical significant $p \leq 0.05$

BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, C_w: Windkessel compliance, TPR: total peripheral resistance, TC: total cholesterol, LDL-C: low-density cholesterol, HDL-C: high-density cholesterol, Trig: triglycerides, eGFR: estimated creatinine clearance, HOMA: Homeostasis model assessment, hsCRP: high sensitivity C-reactive protein

In order to determine whether increasing age is associated with elevated adiponectin levels as well as kidney impairment we plotted adiponectin and estimated creatinine clearance levels of each of the respective ethnic and gender groups (Fig 1).

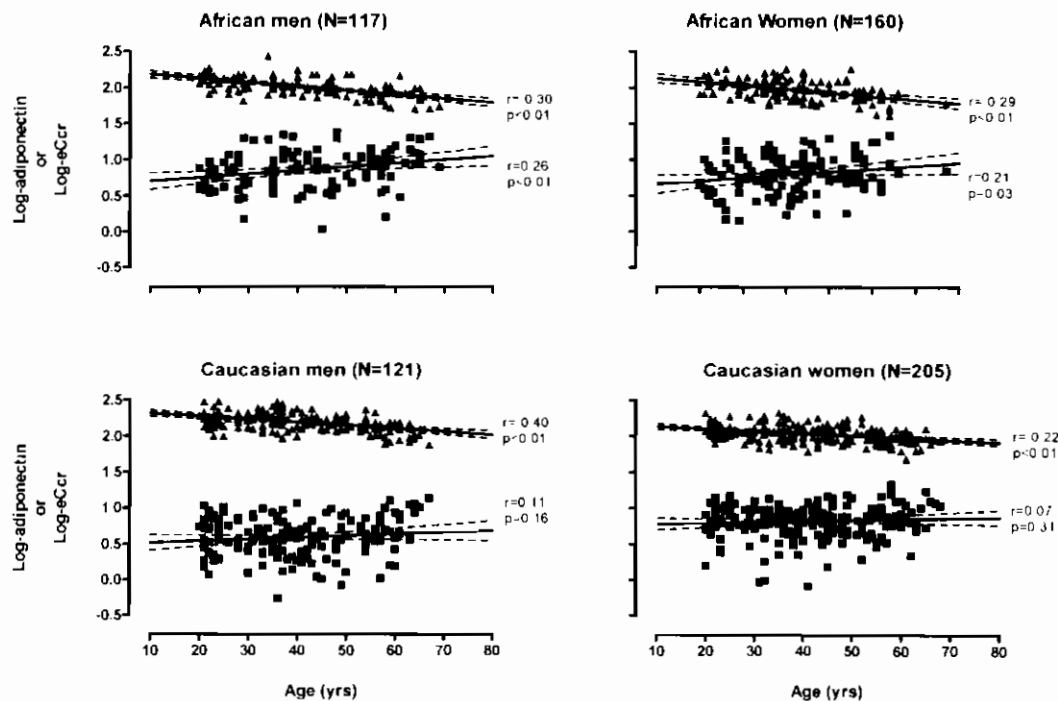


Figure 1. Single regression analyses between age and adiponectin (■) as well as age and estimated creatinine clearance (▲) within the respective age/ethnic groups.

Figure 1 illustrates a significant age-related decrease in kidney function (expressed as estimated creatinine clearance) observed in all of the groups ($r=-0.34$ to $r=-0.58$; $p<0.01$). Yet, a significant increase in adiponectin levels with progressive ageing was only noticeable in the African men ($r=0.26$; $p<0.01$) and women ($r=0.21$; $p=0.03$).

Table 2. Single and partial regression analyses between adiponectin and age

	Adiponectin									
	African men N=117		Caucasian men N=121		African women N=160		Caucasian women N=205		Whole group N=603	
	<i>r</i> -value	<i>p</i> -value	<i>r</i> -value	<i>p</i> -value	<i>r</i> -value	<i>p</i> -value	<i>r</i> -value	<i>p</i> -value	<i>r</i> -value	<i>p</i> -value
Age	0.26	< 0.01	0.11	0.16	0.21	0.03	0.07	0.31	0.15	< 0.01
eCcr	-0.30	< 0.01	-0.36	< 0.01	-0.29	< 0.01	-0.22	< 0.01	-0.42	< 0.01
WC	-0.27	< 0.01	-0.35	< 0.01	-0.36	< 0.01	-0.25	< 0.01	-0.42	< 0.01
HOMA-IR	-0.42	< 0.01	-0.39	< 0.01	-0.54	< 0.01	-0.39	< 0.01	-0.45	< 0.01
hs-CRP	-0.07	0.46	-0.12	0.15	-0.25	0.01	-0.06	0.38	-0.06	0.13
<i>Adjusted for age</i>										
eCcr	-0.18	0.06	-0.35	< 0.01	-0.23	0.01	-0.20	< 0.01	-0.40	< 0.01
<i>Adjusted for eCcr</i>										
Age	0.12	0.23	-0.06	0.46	0.09	0.33	0.00	0.98	-0.01	0.73
<i>Adjusted for WC</i>										
Age	0.34	< 0.01	0.25	< 0.01	0.31	< 0.01	0.17	0.02	0.28	< 0.01
<i>Adjusted for HOMA-IR</i>										
Age	0.15	0.12	0.18	0.04	0.15	0.10	0.17	0.03	0.16	< 0.01
<i>Adjusted for hsCRP</i>										
Age	0.27	0.01	0.14	0.09	0.26	0.01	0.10	0.15	0.18	< 0.01

WC: waist circumference; eCcr: estimated creatinine clearance; HOMA-IR: Homeostasis model assessment of insulin resistance; hsCRP: high sensitivity C-reactive protein

Furthermore, significant inverse correlations between adiponectin and the following variables were found within all the specified groups: eCcr ($r=-0.22$ to $r=-0.36$; $p<0.01$), waist circumference ($r=-0.25$ to $r=-0.36$; $p<0.01$), as well as HOMA-IR ($r=-0.39$ to $r=-0.54$; $p<0.01$). Adiponectin seems to be negatively related with hs-CRP in African women ($r=-0.25$; $p=0.01$) only.

Even after adjusting for either waist circumference or hsCRP the correlations between adiponectin and age remained significant for both the African men and women (Table 2). However, after adjusting for either eCcr or HOMA-IR, the significant positive associations disappeared.

To the contrary, the Caucasian men and women revealed weak yet significant correlations coefficients between adiponectin and age only after adjusting for waist circumference ($r=0.25$; $p<0.01$ and $r=0.17$; $p=0.02$ respectively) and HOMA-IR ($r=0.18$; $p=0.04$ and $r=0.17$; $p=0.03$ respectively).

When standard multiple regression models (Table 3) were performed within each ethnic/gender group, it was evident that insulin resistance (HOMA-IR) was the major contributor to adiponectin levels within each of the groups. Adiponectin levels were further explained by age and waist circumference in the African women. Adiponectin revealed a very weak and non-significant association with eCcr when included into a model with age, waist circumference, HOMA-IR and hsCRP as part of the independent variables. This weak correlations coefficient was observed in all the groups.

Table 3. Standard multiple regression models for the specified ethnic/gender groups with adiponectin as dependent variable.

Independent variables

African men

Adjusted R² = 0.18

	$\beta \pm SE$	p-value
HOMA-IR log	-0.26\pm0.11	0.02
Age	0.24 \pm 0.13	0.08
WC	-0.19 \pm 0.12	0.12
hsCRP log	-0.04 \pm 0.09	0.66
eCcr log	0.01 \pm 0.13	0.96

Caucasian men

Adjusted R² = 0.21

	$\beta \pm SE$	p-value
HOMA-IR log	-0.26\pm0.09	0.01
eCcr log	-0.17 \pm 0.12	0.15
WC	-0.16 \pm 0.13	0.22
Age	0.13 \pm 0.11	0.25
hsCRP log	-0.06 \pm 0.09	0.51

African women

Adjusted R² = 0.33

	$\beta \pm SE$	p-value
HOMA-IR log	-0.42\pm0.09	<0.01
Age	0.28\pm0.10	0.01
WC	-0.26\pm0.12	0.03
eCcr log	0.16 \pm 0.11	0.17
hsCRP log	-0.10 \pm 0.09	0.26

Caucasian women

Adjusted R² = 0.16

	$\beta \pm SE$	p-value
HOMA-IR log	-0.38\pm0.08	<0.01
Age	0.15 \pm 0.10	0.12
hsCRP log	-0.06 \pm 0.08	0.46
eCcr log	-0.04 \pm 0.11	0.68
WC	-0.02 \pm 0.12	0.85

WC: waist circumference; eCcr: estimated creatinine clearance; HOMA-IR: Homeostasis model assessment of insulin resistance; hsCRP: high sensitivity C-reactive protein
Variables indicated in bold were statistical significant: $p < 0.05$

DISCUSSION

The main findings from this study were that African men and women revealed significant increases in adiponectin levels with progressive ageing. These results comply with previous studies that also found that with an increase in age, there is a concomitant rise in adiponectin levels [2, 9, 42, 43]. Since cardiovascular disease is allied with morbidity and mortality in the elderly population [25], it is expected that this important adipokine will show an inverse relationship with ageing. We also found that there is a significant decline in renal function with ageing observed in African men and women. Renal function and its morphology are known to deteriorate with increasing age [44-46]. Therefore, one possible explanation that emerges from the literature is that increased adiponectin levels might be linked to impaired renal function [5, 32] especially observed in the elderly [9]. This can be partly explained due to a lower clearance rate [5, 47].

Further more, our results comply with previous studies [9, 30, 31] confirming the strong inverse association between adiponectin and renal function. To determine whether the age-related increase in adiponectin is dependent on renal function we performed partial regression analyses while adjusting for eCcr. The significant correlation between adiponectin and age observed within the African men and women became much weaker and non-significant. This might indicate that a decline in renal function could play a role in the elevated adiponectin levels observed with progressive ageing. However, when a standard multiple regression analysis was performed within each of the groups, it became apparent that renal function does not play a significant role and that the association between adiponectin and age is more strongly influenced by insulin resistance (HOMA-IR).

Adiponectin levels are inversely associated with insulin levels [48], therefore, changes in insulin concentrations could also have a possible influence on adiponectin levels. Although the exact mechanism is not entirely clear, Blumer *et al.* [49] proposed a suppressing action of insulin on adiponectin levels via possible down regulation. In a previous study we revealed that insulin levels of African men tended to decrease with progressive age [50], which might explain the elevated adiponectin levels with progressive ageing.

Besides insulin resistance, waist circumference also seems to play a significant part in age-related increase in adiponectin levels in the African women. Interesting though, despite the strong inverse correlation found between adiponectin and waist circumference, as well as a significant increase in waist circumference (data not shown), the African women still revealed a significant increase in adiponectin levels with progressive ageing, even after adjusting for waist circumference.

This leaves us with the question as to what is most likely responsible for the noticeable age-related increase in adiponectin levels within the African men and women. One possible explanation could be functional adiponectin resistance [51-53], possibly due to down-regulation of adiponectin receptors (AdipoR1 and AdipoR2) during old age. AdipoR1 is abundantly expressed in skeletal muscle while AdipoR2 is expressed predominantly in the liver [54;55]. However, the natural phenomenon, sarcopenia, is a gradual process resulting in reduced lean muscle mass and increased adipose deposition as a person grows older [56,57]. Since sarcopenia has been well documented, especially in skeletal muscle [57,59], it can be speculated that with loss of skeletal muscle there is a concomitant decrease in AdipoR1 expression. However, due to the design of this study we are unable to infer causality and a more sophisticated study design is necessary to elucidate on this hypothesis.

Adiponectin holds much potential as a therapeutic treatment for cardiovascular and metabolic dysfunction due to its beneficial properties. However, if the possibility of age-related functional adiponectin resistance exists, adiponectin treatment should be reconsidered in Africans. The implication of possible adiponectin resistance essential to consider in combating cardiovascular dysfunction within the African population, since adiponectin resistance could further exacerbate insulin resistance [51], which might augment the risk for the development of Type 2 diabetes, thereby adding to the burden of high susceptibility for cardiovascular dysfunction [60-63].

There are some limitations to this study. These include the use of serum creatinine levels and estimated creatinine clearance to define renal impairment. Although insulin clearance [64] would provide more accurate estimates of renal impairment, the Cockcroft-Gault formula is widely used in cross-sectional studies, it is also convenient, reproducible and inexpensive [65]. Direct measurement of glomerular filtration rate is

also difficult and expensive. Creatinine is derived from the metabolism of creatine within muscles as well as dietary protein intake, therefore, loss of muscle within the elderly (sarcopenia) as well as protein intake (both factors that were not measured) will have strong confounding effects on creatinine concentrations [35]. Another limitation includes the fact that we did not measure microalbuminuria (or albumin-creatinine ratio) which is an indication of chronic diseases such as diabetes, hypertension and kidney disease [66-68].

In conclusion the age-related increase in adiponectin levels observed within African men and women is not attributable to impaired renal function. This increase might be partially explained by a decrease in insulin levels with progressive ageing seen in African men or the possibility of adiponectin resistance in African women. Future studies should address this possibility when considering adiponectin treatment in the elderly.

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CHAPTER 5

GENERAL FINDINGS AND CONCLUSIONS

1. INTRODUCTION

A summary of the main findings from the three manuscripts in this thesis will be given. Firstly, the results from each manuscript will be discussed, interpreted, elucidated and compared to the relevant literature. The general discussion in this chapter will focus on the main findings following from each manuscript in the context of cardiovascular dysfunction within the African population from South Africa. As the title of this thesis suggests, an attempt was made to address various aspects related to cardiovascular function specifically in the black population from South Africa.

The development of cardiovascular dysfunction amongst black South Africans is a major health concern and should enjoy precedence in health research. The epidemiology and mechanisms of cardiovascular dysfunction will remain unknown unless more deliberate research is done to increase knowledge on the prevalence, incidents and pathogenesis of cardiovascular dysfunction in Africans. Risk factors and patterns related to cardiovascular dysfunction differ across ethnic groups and the impact might even be greater in Africa compared to Europe and America. It is against this background that this study was conducted.

A schematic summary of the main findings and conclusions from the three manuscripts are illustrated in Figure 5.1. Furthermore, as the title of this thesis suggests various known risk factors were investigated with specific focus on uric acid, ageing, insulin, C-peptide, adiponectin, renal function as well as ethnic and gender differences.

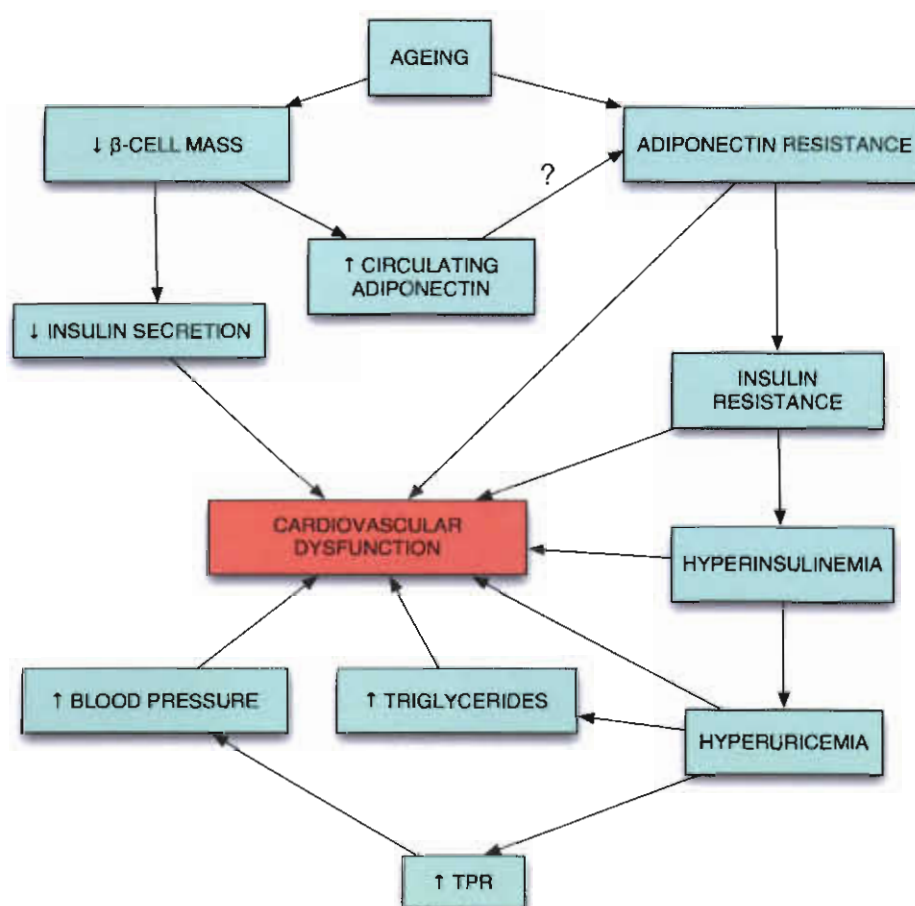


Figure 5.1. Schematic summary of main findings and conclusions

2. SUMMARY OF THE MAIN FINDINGS

The salient findings of the three manuscripts reported in this thesis were:

2.1 Uric acid and the cardiovascular profile of African and Caucasian men (Chapter 2)

The contradictory findings from our previous study conducted within African women from South Africa with regards to uric acid levels necessitated the follow-up manuscript. The aims of the first aspect of this study were: 1) to determine whether uric acid levels differ between African and Caucasian men from South Africa and 2) to determine possible associations between uric acid and cardio-metabolic variables and how these associations differ between the ethnic groups.

The results indicated that African men had significantly lower uric acid levels compared to the Caucasian men, confirming our observation in African women (1). In both the African and Caucasian men, waist circumference revealed the most significant contribution towards uric acid levels. Despite their significantly lower uric acid levels, the positive association between uric acid and blood pressure was more pronounced within the African men. A parallel increase in vascular resistance was observed only in the Africans with an increase in uric acid levels. A positive association was observed between uric acid and triglycerides in both ethnic groups. Yet, after adjusting for confounding factors (body mass index and waist circumference), a positive association between triglycerides and uric acid was observed only within the Caucasian men.

2.2 Ethnic and gender differences regarding the insulin-blood pressure relationship (Chapter 3)

The aims of the second aspect were: 1) to establish whether African men and women from South Africa show elevated levels of insulin with increasing age, and how their levels compare to their Caucasian counterparts; and 2) to determine the relationship between insulin or C-peptide and blood pressure of Africans and Caucasians using stratified age groups.

Opposed to the Caucasian men, whose insulin levels tended to show an age-related increase, the African men's insulin levels tended to decrease with increasing age. Compared to the Caucasian men, the African men maintained significantly lower insulin levels throughout the age spectrum, whereas the African women revealed significant lower insulin levels only within the oldest group, when compared to the Caucasian women. These significant differences disappeared in both gender groups after adjusting for confounding factors such as obesity, smoking and alcohol consumption.

Both the Caucasian men and women showed a significant age-related increase in C-peptide levels. This was not evident within either the African men or women. Yet again, the African men revealed significantly lower C-peptide levels throughout the age spectrum compared to the Caucasian men. The only significant difference observed between the African and Caucasian women was within the oldest age group. Even after

adjusting for obesity, smoking and alcohol consumption, the Caucasian men upheld their significant increase in C-peptide levels with increasing age.

Further investigation into the association between insulin or C-peptide and mean arterial blood pressure revealed that within the African women the association tends to be more positive within the younger people and later on becoming more negative within the older group. Opposite results were obtained within the African men where the younger group tended to show a more negative association between insulin and blood pressure, changing to a more positive association within the elderly.

It seems that insulin *per se* is not a risk factor within African men, but rather a lack of the insulin-mediated vasodilatory effects as observed within the younger population.

2.3 Ageing and adiponectin levels in an African population: an investigation from a renal perspective (Chapter 4)

Still on the topic of ageing, the aim of the third manuscript was to assess the relationship between ageing and adiponectin levels from the perspective of renal function in apparently healthy African and Caucasian people.

By comparing the Africans with their Caucasian counterparts it was observed that the African men had significantly higher adiponectin levels, whereas the African and Caucasian women revealed no differences. These differences seem to be a true reflection of the significant differences in obesity levels observed in the men, whereas the women of the two ethnic groups had similar levels of adiposity.

Results further indicated that there is a significant increase in adiponectin levels with increasing age – observed only within the African men and women. Unexpectedly the Caucasian men and women displayed no significant age-related rise in adiponectin levels as suggested in the literature. Adiponectin increased significantly in both African men and women with progressive age despite a concomitant increase in waist circumference. Insulin resistance seems to be a major contributor towards age-related rise in adiponectin levels.

Impaired renal function *per se* seems not to be responsible for the observed multiple regression result of age-related increase in adiponectin level within the African population. Changes in insulin levels and possibly adiponectin resistance in the elderly is proposed as a possible explanation for this result.

3. COMPARISON OF FINDINGS TO RELEVANT LITERATURE

When results from this study are compared to existing literature (as presented in Chapters 1, 2, 3, and 4) it is evident that certain findings confirmed and others contradicted those found in the literature, but the results from this study also added to the available literature.

The investigation regarding serum uric acid concentrations in African men confirmed findings from our previous study (1) regarding the differences between African and Caucasian women from South Africa. We have previously shown that African women also revealed lower uric acid levels. Although in compliance with our previous study regarding ethnic differences (1), the fact that the African men had lower uric acid levels than the Caucasian men is also a contradictory result. Several studies found that the black population (African-Americans) is considered a high-risk group for the development of hyperuricemia (2-4), some even found no ethnic differences at all (5).

Furthermore, the results are also in accordance with previous studies that indicated a stronger association between uric acid and blood pressure within the African population (6,7). Also, uric acid correlated strongly with abdominal obesity (8) and triglycerides which confirms the literature (9,10).

Adding to the available literature is the independent association between vascular resistance and uric acid observed in African men, which was not published before in Africans. This novel finding might indicate a possible mechanism regarding the resultant elevation in blood pressure found with increased uric acid levels.

Results from the second topic of investigation, namely changing relationship between insulin and C-peptide with increasing age, corresponds well with earlier studies (11-13). It was found that by statically adjusting for age, the association between insulin and blood pressure is indeed very weak. Using age stratification confirmed the observation

made by Schutte *et al.* (14) that a more positive association between insulin and blood pressure is observed within the younger population, whereas a more negative association exists within the elderly population. This is specifically true for the population of interest, namely the black South Africans.

Adding to the body of knowledge is the fact that African men showed opposing insulin and C-peptide levels with progressive ageing compared to Caucasian men. Whereas the Caucasian men showed a tendency towards higher insulin levels in the elderly, insulin levels in the African men tend to decrease. The African men also maintained significantly lower levels of both insulin and C-peptide throughout the age spectrum. These results could not be confirmed within the literature, since this was the first result regarding C-peptide levels in black South Africans and to the knowledge of the author, no other studies have been done on this topic to date.

The investigation regarding an explanation for the elevated plasma adiponectin levels with increasing age revealed confirmatory findings, namely that adiponectin levels increase with increasing age (15-17), only within the African men and women

The suggestion by Isobe *et al.* (15) that the elevated plasma adiponectin levels observed in elderly people could be explained by impaired renal function was tested. We found a strong inverse correlation between adiponectin and renal function (estimated creatinine clearance) which complies well with previous studies (15,18,19).

Multiple regression models indicated that renal function does not play a role in age-related increase in adiponectin levels, and that waist circumference is the major contributor to adiponectin levels with progressive ageing. However, despite significant increase in waist circumference, there is a contradictory rise in adiponectin levels observed in African men and women. Additional, contradictory results also indicate that the Caucasian men and women showed no significant change in adiponectin levels with progressive age (15-17). These results are supported by a previous study conducted by Ryan *et al.* (20) who concluded that age does not influence plasma adiponectin levels. The notion that renal function might be liable for the age-related increase in adiponectin is, therefore, contradicted. The possibility of the development of adiponectin resistance with ageing is proposed.

4. CHANCE AND CONFOUNDING

Before the main findings of this study are discussed, it is important to reflect critically on some important factors that may have affected the results. There are some methodological issues that could have caused weaknesses in the study and, therefore, might have influenced the different outcomes.

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. It is, therefore, important that the results found in this study, with specific reference to the correlations, should be confirmed in future studies.

Although an inclusion criterion for the study was that the subjects should be “apparently healthy”, their health is not a certainty. Attempts were made during the data-collection phase to obtain data that would provide information such as socio-economic status and physical activity level, but these are factors that have proved very difficult to measure in African populations – especially when making use of questionnaires. However, even without the direct data regarding socio-economic status, it was quite clear during data-collection that there was a discrepancy regarding the socio-economic status of the African and Caucasian participants in the SAfrEIC study. It is, therefore, possible that this factor might have influenced the results of the paper presented in this thesis. Obesity as a possible confounder was addressed for by statistical adjustments.

In the interpretation of the results in this thesis, it was attempted to interpret statistical results from a physiological standpoint at all times, while keeping in mind that a statistical significance does not necessarily mean physiological significance, and *vice versa*.

5. WEAKNESSES AND STRENGTHS OF THIS STUDY

Weaknesses of this study:

- Cross-sectional design of the study does not allow inferring causality.
- Socio-economic status differed between the two ethnic groups, with the possibility of differences in dietary intakes. Therefore, a westernised diet might be more common within the Caucasian population, and is often associated with

be more common within the Caucasian population, and is often associated with higher dietary intakes of protein and fructose - both known to elevate uric acid levels.

- Factors such as the use of diuretics, physical activity and direct measure of insulin secretion were not accounted for, since these are measures that are very difficult to obtain reliably in large studies.
- Markers such as insulin and iothalamate clearance would provide a better indication of renal function.
- An availability sample rather than a randomised sample was used, and results could, therefore, not be representative of the entire black population of South Africa.
- A large proportion of the African men were underweight. Their cardiovascular and metabolic profile could have differed compared to normal weight Africans, and, therefore, could have influenced the results of this study.
- The measure of high molecular adiponectin may be more accurate than total adiponectin levels.

Strengths of this study:

- Results from this study are the first to report on the lower uric acid levels in African men as well as insulin, C-peptide and adiponectin changes with increasing age in black South Africans.
- Limited data are available for relatively large groups of African people. These results, therefore, contribute significantly to existing knowledge.
- HIV as a cardiovascular confounder was tested for, and HIV positive participants were excluded.

6. RECOMMENDATIONS

Results from the three manuscripts reported in this thesis led to the formulation of the following recommendations that can be used by policy makers, health professionals and researchers:

- Elevated uric acid levels are considered a risk factor for the development of cardiovascular dysfunction and should, therefore, routinely be checked.

- More research is needed regarding hypertension in African men and the role of uric acid with specific reference to their underweight status.
- Intake of fructose sugar causes a direct increase in uric acid (21). Since fructose sugar is a common aspect of a westernised diet, it is strongly recommended that appropriate education regarding healthy eating habits should be communicated to the African population.
- Fasting insulin levels strongly correlate with blood pressure in African women, and should be taken into account when studying this population. This is particularly important since African women present the highest levels of obesity amongst all ethnic groups in South Africa (22).
- Africans might be susceptible to the development of adiponectin resistance with progressing age. This concept of adiponectin resistance is quite new, and requires further investigation. This should be taken into account when considering therapeutic measures for treatment of hypertension and other cardiovascular diseases.

7. DISCUSSION OF MAIN FINDINGS

The prevalence of cardiovascular diseases within the African population has evolved from almost non-existent (23-25) to concerning profusion (26-29). As previously illustrated in Figure 1.1 (Chapter 1), there are numerous etiological factors that might contribute to cardiovascular diseases. Since it is known that ethnic differences exist regarding the prevalence of cardiovascular diseases (30), the main focus of this study was to investigate various factors that are known to be directly or indirectly related to cardiovascular function in this ethnically distinct African population group. Although the findings cannot be generalised to the whole African population of South Africa, they serve as a foundation for future in-depth studies.

As expected, it was observed that the cardiovascular system of the Africans seems to be much more detrimentally affected. This was reflected by their significantly higher blood pressure levels, higher vascular resistance and lower Windkessel compliance. The clear vascular dysfunction observed in the African people seems to be partially instigated by direct adverse effects on the vascular system, induced by uric acid amongst various others, and on the other hand, due to possible lack of protective and beneficial properties of insulin and adiponectin, which are aggravated with progressive ageing.

Being considered a high risk group for elevated uric acid levels (2-4), the African men from this study revealed contradictory significantly lower uric acid levels compared to their Caucasian counterparts. Despite having significantly lower uric acid levels, the African men revealed a much stronger and more pronounced uric acid-blood pressure relationship. This result was also confirmed in previous studies (6,7). An independent relationship existed between uric acid and vascular resistance in African men, and this might serve as a possible underlying pathophysiological mechanism linking hyperuricemia with associated hypertension. Thus, uric acid seems to act as an independent risk factor, contributing to the development of cardiovascular diseases in an already highly susceptible population. Furthermore, uric acid correlated with triglycerides in both the African and Caucasian men. According to Tavit *et al.* (31), uric acid correlates strongly with atherogenesis, leading to the notion that hyperuricemia with a concomitant rise in triglycerides might have some etiological role in cardiovascular dysfunction. The implication of these results is essential in the combat against cardiovascular dysfunction especially hypertension. The adoption of a more westernised diet (high in fructose sugar) due to industrialisation might have a great impact on the prevalence of hyperuricemia. According to Schwarzmeier *et al.* (21), uric acid *per se* can cause an acute increase in uric acid levels. This might further increase the vulnerability for the development of uric acid dependent hypertension, especially in the African men.

Apart from uric acid, the role of insulin was also investigated. Progressive age seems to have opposite effects on insulin secretion in Africans and Caucasians. The Caucasians showed a tendency towards increased insulin levels with increasing age, resembling characteristics of insulin resistance and subsequent Type 2 diabetes. To the contrary insulin levels tended to decrease with ageing in Africans, taking on the characteristics of Type 1 diabetes. The cardio-metabolic complications resulting from either Type 1 diabetes or Type 2 diabetes differ in their manifestations (32). This might possibly explain why cardiovascular risk patterns differ between the two ethnic groups.

Insulin is known to have both pressor (33) as well as depressor effects (34) on the vascular system, and it seems that the insulin-mediated vasodilatory actions are more dominant within the elderly population. Thus, perhaps serves as a counter protective mechanism against age-related cardiovascular diseases (35). It became apparent from a previous study conducted by Schutte *et al.* (14) as well as the present study that

statistical adjustment for age could mask the relationship between insulin and blood pressure and that age stratification is recommended. This study confirmed that within younger African people there exists a more positive correlation, where in later years this correlation shifts to one that is more negative. However, African men seem to respond differently with regards to the insulin-blood pressure relationship. Within the younger population, a more negative correlation exists, whereas with progressive ageing, this correlation becomes more positive. The results further indicated opposing insulin and C-peptide secretions for the African and Caucasian men. The possible influence of the different levels in socio-economic status of the ethnic groups when interpreting these results can also not be ruled out. A substantial number of the African men were underweight and lean, whereas the Caucasian men tended to be on the other end of the scale, namely overweight or obese. The close association between obesity and insulin resistance in this regard is, therefore, quite important.

Yet, despite their lower insulin levels, the blood pressure of African men seems to be affected more detrimentally compared to their Caucasian counterparts, with higher insulin levels. Although purely speculative this might implicate that insulin *per se* is not responsible for the high incidence of hypertension, especially within the older African males, but perhaps the lack of insulin-mediated vasodilation giving secondary factors the opportunity to instigate cardiovascular dysfunction. The older African women also revealed significantly lower insulin levels compared to their Caucasian counterparts. Their lower insulin levels correlated more strongly with blood pressure, which might further increase the propensity for cardiovascular dysfunction observed within the elderly African women.

As seen in the preceding paragraphs, the effects of progressive ageing seem to affect the cardiovascular system of the African population detrimentally with regards to the protective actions of insulin. Another major finding of the study was the age-related rise in adiponectin levels observed in Africans. While Isobe *et al.* (15) proposed that a rise in adiponectin levels with concomitant ageing is due to impaired renal function, it became clear from this study that this association is strongly dependent on insulin resistance. No such change with regards to adiponectin was observed for the Caucasians. This result contradicts previous studies although a study done by Ryan *et al.* (20) found no age-associated change in adiponectin levels. Even though an exact mechanism for the

observed ethnic differences in adiponectin levels is not entirely clear at present, it is postulated by Furuta *et al.* (36) that pancreatic β -cell function is a significant regulator for circulating adiponectin levels. They further hypothesised that it is not insulin *per se* but rather the lack of β -cell products that affects the regulation of adiponectin concentrations. As previously mentioned, Africans from this study seem to resemble characteristics of Type 1 diabetes (decreasing insulin levels) with ageing, possibly due to acquired loss of pancreatic cell mass (37). If these results indicate the presence of an axis between adipocytes and β -cells this might explain the higher levels of adiponectin observed within the Africans. In addition, Gu (2009) reported elevated levels of adiponectin within type 1 diabetic patients (38), strengthening this hypothesis. It might also explain why no changes were observed within the Caucasians. Results from this study could indicate that the prevalence of insulin resistance and concomitant Type 2 diabetes might be more commonly associated with Caucasians, rather than Type 1 diabetes. Even though no significant changes were observed within the Caucasians, more significant results (increasing adiponectin levels with increasing age) may be revealed with a bigger subject group.

Regardless of their higher adiponectin levels, the cardiovascular profile of the Africans is much more detrimentally affected. It might, therefore, be speculated that the observed rise in adiponectin levels is a possible result of impaired adiponectin utilisation (functional adiponectin resistance) (39-41), perhaps due to down regulation of adiponectin receptors (AdipoR1 and AdipoR2), or possibly due to a decrease in insulin levels with ageing. Without the cardiovascular protective actions of adiponectin, cardiovascular diseases that often accompany old age might be exacerbated in the elderly Africans from South Africa.

8. GENERAL CONCLUSION

Cardiovascular dysfunction in the African population is a complex mixture of various risk factors. This study has irrefutably demonstrated direct and indirect results regarding various markers that are closely associated with the development of cardiovascular dysfunction, including uric acid, insulin and C-peptide, as well as the adipokine, adiponectin. The well-known cardiovascular deterioration with progressive ageing was more closely investigated with regards to insulin and C-peptide, as well as adiponectin.

Uric acid revealed an independent association with vascular resistance, and a direct association with triglycerides, a combination that might detrimentally affect vascular function. With progressive ageing the African men revealed decreased insulin secretion with a concomitant extrication of insulin and blood pressure, suggesting that insulin *per se* is not considered a cardiovascular risk factor, but rather the lack of beneficial actions. Adiponectin is also affected by age in Africans, revealing an increase in circulating levels, which may be due to the development of adiponectin resistance, and thus a loss of the cardio-protective functions of adiponectin.

The implications of this study's results are of great importance. It has elucidated some potential pathological mechanisms that might further promote cardiovascular risk in an already high risk population group. It was clear from this study that the cardiovascular health status of Africans seems to compare poorly to that of Caucasians, especially the African men. The complexity of cardiovascular dysfunction is further broadened through various other exposures such as urbanisation (42). Even though South Africa is striving towards a more urbanised lifestyle and better health care, it also still lingers under a burden of famine, poverty and high prevalence rates of infectious diseases such as the human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) (42,43). Concomitant with urbanisation is increased levels of stress (44), and according to Malan et al (45) persistent psychosocial stress or urbanisation has been associated with increases in blood pressure, which might explain why despite low body fat and a favourable lipid profile, the African population, especially the men, suffer from elevated blood pressure. These exposures might in future further increase the risk of cardiovascular dysfunction within black Africans from South Africa.

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