Insulin-like growth factor-1 and cardiometabolic function: a bi-ethnic population study

ASE Koegelenberg

Student number: 20568894

Thesis submitted for the degree

Doctor Philosophiae in Physiology at the Potchefstroom Campus of the North-West University.

Promoter:	Prof. AE Schutte
Co-Promoters:	Dr. W Smith
	Prof. R Schutte

July 2016

ACKNOWLEDGEMENTS

I would like to extend my gratitude to the following people for their input and support over the course of this project:

- To my loving and wonderful Heavenly Farther. Without you I can accomplish nothing. But you gave me the ability and the strength to fulfil my dreams. You comfort me during difficult times and for that I am forever grateful;
- To my promoters, thank you for your kindness, professional input, time, honesty, guidance and support throughout the duration of my studies. Prof Alta Schutte, you are such a great inspiration and I will admire you forever;
- The SABPA and SAfrEIC study participants for their willingness to participate and allowing me to use the data;
- The National Research Foundation (NRF-SARChI) for providing me with a scholarship for the duration of my studies;
- Yolandi Breet for your support, love and motivation. You are a true friend that is always willing to help;
- My beautiful husband Pieter, thank you for your love, support, encouragement and for always believing in me;
- To my adorable son Deaglan, without you knowing it, you were my greatest inspiration to fulfil this dream. You made me laugh during difficult times and taught me how to embrace every day and make the best out of it;
- To my family, thank you for your encouragement, love and support throughout my academic career.

Where God plants you, is where He will bless you and provide for you!

TABLE OF CONTENTS

Acknowledgements..... i

Table of contents..... ii

Preface..... v

Summary..... vii

Affirmation by the authors..... xi

List of tables..... xiii

List of figures..... xv

List of abbreviations..... xvii

CHAPTER 1: BACKGROUND, MOTIVATION AND LITERATURE OVERVIEW..... 1

1. Background..... 2
2. Literature overview..... 3
3. Summary..... 24
4. Aims, objectives and hypotheses..... 25
5. References..... 27

CHAPTER 2: STUDY DESIGN AND METHODOLOGY..... 49

1. The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study.... 50
    - 1.1 Study design..... 50
    - 1.2 Organisational procedures..... 53
    - 1.3 Questionnaires..... 54
    - 1.4 Anthropometric measurements..... 54
    - 1.5 Cardiovascular measurements..... 54
    - 1.6 Blood sampling and biochemical analyses..... 55

2. The South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC)..... 57
2.1 Study design..... 57
2.2 Organisational procedures..... 59
2.3 Questionnaires..... 59
2.4 Anthropometric measurements..... 60
2.5 Cardiovascular measurements..... 60
2.6 Blood sampling and biochemical analyses..... 60
3. References..... 62

CHAPTER 3: Bioavailable IGF-1 and its relation to the metabolic syndrome in a bi-ethnic population of men and women..... 64
CHAPTER 4: Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study..... 90
CHAPTER 5: IGF-1 and NT-proBNP in a black and white population: The SABPA study..... 111

CHAPTER 6: FINAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS..... 137
1. Introduction..... 138
2. Summary of main findings, discussion and comparison to the literature..... 138
3. Chance and confounding..... 145
4. Conclusion..... 147
5. Recommendations for future studies..... 148
6. References..... 149

ANNEXURES..... 154

ANNEXURE A: Declaration of language editing..... 154

ANNEXURE B: Turn-it-in report..... 156

ANNEXURE C: Published manuscript of research article 1..... 159

ANNEXURE C: Published manuscript of research article 2..... 160

PREFACE

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

- **Chapter 1**, the introductory chapter, provides an overall background and motivation and offers a detailed overview of the literature which supports the focused literature backgrounds presented in each of the manuscripts. The aim and hypotheses are also included in this chapter.
- **Chapter 2** elaborates in detail on the design of the SABPA and the SAfrEIC study and the methods of data collection.
- **Chapter 3**, the first manuscript investigated the relationship between bioavailable IGF-1 and the number of metabolic syndrome components and determined whether this relationship is modulated by inflammation, oxidative stress and liver dysfunction. These results were published in the journal, *Hormone and Metabolic Research*, 2015.
- **Chapter 4** explores the contribution of reduced IGF-1 to the development of vascular endothelial damage. This manuscript has been published in the journal, *Thrombosis Research*, 2015.
- **Chapter 5**, the third manuscript investigated the relationship between NT-proBNP, as a marker of systolic dysfunction and cardiac overload, and IGF-1. This article is under revision at the *European Journal of Clinical Investigation*.
- In the final chapter, **Chapter 6**, a summary of the main findings are provided – all the presented results are critically discussed, conclusions are drawn and applicable recommendations are made.

The promoter and co-promoters were included as co-authors in each manuscript. The first author, namely the PhD candidate, was responsible for the initiation and all parts of this thesis, including literature searches, data mining, statistical analyses, the interpretation of results, as well as writing of the manuscripts. All co-authors gave their consent that the manuscripts may form part of this thesis.

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

SUMMARY

Motivation

Due to rapid urbanisation, black South Africans have a high prevalence of cardiovascular disease and are prone to develop hypertension and ultimately hypertensive heart diseases. Studies have demonstrated that black populations exhibit lower concentrations of the vasculo- and cardioprotective peptide, insulin-like growth factor-1 (IGF-1), possibly increasing their susceptibility to cardiovascular abnormalities.

According to the literature, IGF-1 is reduced in individuals with cardiovascular and metabolic diseases. IGF-1 associates negatively with the metabolic syndrome and is suppressed by factors such as inflammation, oxidative stress and liver dysfunction. At a vascular level, IGF-1 has several vasculoprotective properties and reduced IGF-1 is linked to endothelial dysfunction, which may lead to endothelial damage. IGF-1 also confers various cardioprotective effects and reduced IGF-1 is associated with an increased risk for coronary artery disease, ischemic stroke and heart failure.

However, whether reduced IGF-1 among black South Africans is associated with their vulnerable metabolic and cardiovascular profile remain to be established.

Aim

The general aim of this thesis is to increase our understanding on the potential role of IGF-1 in the cardiovascular system by investigating associations of IGF-1 with the metabolic syndrome, and markers of vascular endothelial damage, cardiac overload and systolic dysfunction in black and white South Africans.

Methodology

Data from the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study was used and presented in the original research articles described in Chapter 4 and 5. Data from both the SABPA and the SAfrEIC (South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) studies was used and presented in the original article described in Chapter 3. The SABPA study included 409 black and white school teachers from the North West province, South Africa. The SAfrEIC study involved 750 black and white volunteers from urban areas in the North West province of South Africa. Standardised methods were used to capture all data and included general health questionnaires (lifestyle factors and medication use), anthropometric and cardiovascular measurements, as well as biochemical analyses. As part of the biochemical analyses, total IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3) were determined. Since approximately 80% of total IGF-1 is bound to IGFBP-3, the calculation of the molar ratio of IGF-1/IGFBP-3 allows us to use the ratio as an estimate of bioavailable IGF-1. In preparation for statistical analyses, non-Gaussian variables were logarithmically transformed and the central tendency and spread were represented by the geometric mean and the 5th and 95th percentile intervals. Analyses of variance (ANOVA) and analyses of covariance (ANCOVA) were used to explore IGF-1 distribution according to the number of metabolic syndrome components. ANCOVA was also used to explore the distribution of NT-proBNP by quartiles of IGF-1 and IGF-1/IGFBP-3 while adjusting for covariates.

We used single and multiple regression analyses to determine the associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components. Single regression analyses and forward stepwise multiple regression analyses were used to determine the associations of vWF and NT-proBNP with IGF-1 and IGF-1/IGFBP-3, respectively. The multivariate-adjusted analyses were performed upon both the inclusion and exclusion of individuals with diabetes. In

each case, covariates that were selected for inclusion in the models were selected based on the literature and on exploratory single regression analyses. All p-values refer to 2-sided hypothesis.

Results and conclusions

Three manuscripts were written in order to achieve the three main objectives of this thesis. In the first manuscript we explored the relationship between bioavailable IGF-1 and the number of metabolic syndrome components in men (n=457) and women (n=450). We also determined whether this relationship was independent of inflammation, oxidative stress and gamma-glutamyl transferase (γ-GT; a marker of liver dysfunction). We found similar total IGF-1 in men and women, however bioavailable IGF-1 was lower in women. In multi-variable adjusted analyses, bioavailable IGF-1 was inversely associated with the number of metabolic syndrome components in both sexes (men: β=-0.11; p=0.013 and women: β=-0.17; p=0.003). But the relationship was dependent on oxidative stress, liver dysfunction and inflammation, suggesting underlying processes by which the metabolic syndrome attenuates IGF-1.

Secondly, in order to determine the potential contribution of reduced IGF-1 to the development of vascular endothelial damage, we investigated the link between bioavailable IGF-1 and von Willebrand factor (vWF) as a marker of endothelial damage in black and white South Africans (42.6% black). We found that black individuals presented higher blood pressure and vWF_{ag} and lower IGF-1 than the white group. vWF_{ag} was inversely associated with IGF-1 (R^2=0.18; β=-0.17; p=0.044) and IGF-1/IGFBP-3 (R^2=0.18; β=-0.17; p=0.030) in blacks, with no associations in whites. These results suggest that low IGF-1 levels may contribute to vascular endothelial damage in blacks.

The third manuscript investigated whether lower IGF-1 levels in black individuals (n=160) were related to a marker of cardiac overload and systolic dysfunction, namely N-terminal prohormone

B-type natriuretic peptide (NT-proBNP), when compared to whites (n=195). We found similar NT-proBNP concentrations (p=0.72) in blacks and whites. In multi-variable adjusted forward stepwise regression analyses, we found a link between NT-proBNP and systolic blood pressure (SBP) in blacks (R^2=0.37; β=-0.28; p<0.001), but not with IGF-1. But in the white group NT-proBNP was inversely associated with both IGF-1 (R^2=0.39; β=-0.22; p<0.001) and SBP (R^2=0.39; β=-0.21; p=0.004). The link found in whites may support the direct cardioprotective role of IGF-1, however in the black group the absence of an association may be due to other factors, such as SBP, that has a greater contributory role in cardiac pathology in blacks.

General conclusion

In this thesis the cardiovascular protective role of IGF-1 was confirmed. Attenuated IGF-1 among black South Africans are linked to the metabolic syndrome and a marker of vascular endothelial damage. Furthermore, on a cardiac level, other factors such as systolic blood pressure may have a greater contributory role in cardiac pathology than IGF-1 among blacks.

Keywords: insulin-like growth factor-1; insulin-like growth factor binding protein-3; metabolic syndrome; von Willebrand factor, N-terminal prohormone B-type natriuretic peptide.

AFFIRMATION BY THE AUTHORS

The contribution of each researcher to this thesis is listed below:

Ms. ASE Koegelenberg

Responsible for initial proposal of this study along with all extensive literature searches and critical evaluation of study protocol and methodology (expertise in recording a 12-lead electrocardiograph (ECG) and using the Finometer device). Furthermore, responsible for data cleaning, statistical analyses, design and planning of research articles and the thesis, interpretation of results and writing of all sections of this thesis.

Prof. AE Schutte (promoter)

Supervised the design, planning and writing of the thesis, principal investigator of the SAfrEIC study, and for both the SAfrEIC and SABPA studies contributed to data collection, provided intellectual input on statistical analyses and writing of the manuscripts presented in Chapters 3, 4 and 5.

Dr. W Smith (co-promoter)

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

Prof. R Schutte (co-promoter)

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

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

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

Prof. AE Schutte

Dr. W Smith

Prof. R Schutte

LIST OF TABLES

CHAPTER 1

Table 1 - Criteria for clinical diagnosis of the metabolic syndrome by the International Diabetes Federation.

CHAPTER 3

Table 1 - Comparison of cardiovascular, biochemical and anthropometric measurements of men and women.

Table 2 - Mean values of IGF-1, IGF-1/IGFBP-3 ratio and metabolic syndrome components in categories stratified according to the number of metabolic syndrome components.

Table 3 - Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and potential confounders.

Table 4 - Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and potential confounders in participants without diabetes.

Table 1S - Comparison of assays used to determine IGF-1 levels in the SABPA and SAfrEIC studies.

Table 2S - Comparison of cardiovascular, biochemical and anthropometric measurements of men and women in the SABPA and SAfrEIC study, respectively.

Table 3S - Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and the individual contribution of additional confounders.

Table 4S - Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and additional covariates.

CHAPTER 4

Table 1 - Comparison of cardiovascular, biochemical and anthropometric measurements of black and white participants.

Table 2 - Independent associations of vWF_{ag} with IGF-1/IGFBP-3 and total IGF-1.

Table1S - Independent associations of vWF_{ag} with IGF-1/IGFBP-3 and total IGF-1 with inclusion of patients with diabetes.

CHAPTER 5

Table 1 - The cardiovascular, biochemical and anthropometric profiles of black and white participants.

Table 2 - Independent associations of NT-proBNP with total IGF-1 and IGF-1/IGFBP-3.

Table 1S - Independent associations of NT-proBNP with IGF-1.

Table 2S - Independent associations of NT-proBNP with total IGF-1 and IGF-1/IGFBP-3 with inclusion of patients with diabetes (n=38).

LIST OF FIGURES

CHAPTER 1

Figure 1 - IGF-1 signal transduction pathway.

Figure 2 - IGF-1 by quartiles of age of black and white men and women.

Figure 3 - The regulation of vWF secretion.

Figure 4 - Simplified scheme for IGF-1 induced signalling regulating survival of cardiomyocytes.

Figure 5 - Physiological pathway of NT-proBNP secretion.

Figure 6 - IGF-1 axis and the cardiovascular system investigated.

CHAPTER 2

Figure 1 - Geographical location of Klerksdorp and Potchefstroom in the North West province, South Africa.

Figure 2 - The Sympathetic activity and Ambulatory Blood pressure in Africans (SABPA) prospective cohort study population.

Figure 3 - The SAfrEIC (South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) study population.

Figure 4 - Data collection at the Metabolic Research Unit facility, North West University, South Africa.

CHAPTER 3

Figure 1 - The IGF-1 and IGF-1/IGFBP-3 ratio distribution according to the number of metabolic syndrome components, adjusted for age, ethnicity and study type.

CHAPTER 4

Figure 1 - von Willebrand factor (vWF) as a function of insulin-like growth factor-1 (IGF-1) and IGF-1/insulin-like growth factor binding protein-3 (IGFBP-3) in black and white individuals in single regression analyses.

CHAPTER 5

Figure 1 - Unadjusted correlations between NT-proBNP and IGF-1.

Figure 2 - NT-proBNP levels by quartiles of IGF-1, while adjusting for age, sex and BMI.

Figure 1S - Unadjusted correlations between NT-proBNP and IGF-1/IGFBP-3.

Figure 2S - NT-proBNP levels by quartiles of IGF-1/IGFBP-3, while adjusting for age, sex and BMI.

CHAPTER 6

Figure 1 - Forest plot indicating forward stepwise analyses of von Willebrand factor antigen with IGF-1/IGFBP-3 as main independent variable in blacks

Figure 2 - Forest plot indicating multiple regression analyses of NT-proBNP with IGF-1 as main independent variable in blacks.

Figure 3 - Forest plot indicating multiple regression analyses of NT-proBNP with IGF-1 as main independent variable in whites.

LIST OF ABBREVIATIONS

ABPM - Ambulatory blood pressure monitoring

AST - Aspartate aminotransferase

ALT - Alanine transaminase

ALT - Acid-liable subunit

ANOVA - Analysis of variance

ANCOVA - Analysis of covariance

BMI - Body mass index

BP - Blood pressure

CIMT - Carotid intima-media thickness

CRP - C-reactive protein

CSWA - Cross-sectional wall area

CVD - Cardiovascular disease

DBP - Diastolic blood pressure

ECG - Electrocardiogram

ECM - Extracellular matrix

eGFR - Estimated glomerular filtration rate

ELISA - Enzyme linked immunosorbent assay

GHD - Growth hormone deficiency

GHR - Growth hormone replacement

HbA1c - Glycated haemoglobin

HDL-C - High-density lipoprotein cholesterol

HIV - Human immunodeficiency virus

IGF-1 - Insulin-like growth factor-1

IGFBP-3 - Insulin-like growth factor binding protein-3

IL-6 - Interleukin-6

IRMA - Immunoradiometric assay

LDL - Low-density lipoprotein cholesterol

MMP - Matrix metalloproteinase

NAFLD - Non-alcoholic fatty liver disease

NCD - Non-communicable disease

NO - Nitric oxide

NOS - Nitric oxide synthase

NRF - National Research Foundation

NT-proBNP - N-terminal prohormone B-type natriuretic peptide

PGI_2 - Prostaglandin I_2

PURE - Prospective Urban and Rural Epidemiology

ROS - Reactive oxygen species

SABPA - Sympathetic activity and Ambulatory Blood Pressure in Africans

SAfrEIC - South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function

SBP - Systolic blood pressure

SMC - Smooth muscle cell

suPAR - Soluble urokinase plasminogen activator receptor

TC - Total cholesterol

TNF-α - Tumour necrosis factor-α

uPA - Urokinase plasminogen activator

uPAR - Urokinase plasminogen activator receptor

vWF - von Willebrand factor

WC - Waist circumference

γ-GT - Gamma-glutamyl transferase

Chapter 1

- Background and Motivation
- Literature overview
- Aim, Objectives and Hypotheses

1. BACKGROUND

During 2012, a total of 56 million deaths occurred worldwide, of which 38 million were due to non-communicable diseases (NCDs).[1] According to the World Health Organisation (WHO), NCDs are estimated to account for 43% of total deaths in South Africa, of which 18% are caused by cardiovascular diseases.[2] This seems due to Westernisation, which is characterised by lifestyle, nutritional and psychological well-being and health transitions.[3-4] Besides traditional risk factors, cardiovascular diseases are independently related to low levels of circulating insulin-like growth factor-1 (IGF-1).[5,6] Increasing evidence indicates that IGF-1 has several vasculo- and cardioprotective properties.[7] IGF-1 induces nitric oxide (NO) release,[8] enhances insulin sensitivity[9] and glucose uptake,[10] prevents postprandial dyslipidemia[11] and reduces gluconeogenesis.[10] IGF-1 also has anti-apoptotic[12] and anti-inflammatory properties[13] and scavenges free oxygen radicals.[10] Furthermore, IGF-1 has an impact on maintaining normal cardiac structure and function.[14,15]

Black South Africans present with attenuated IGF-1 levels, which is also associated with various cardiometabolic markers.[16,17] The loss in cardioprotection by IGF-1 together with cardiovascular risk factors found in blacks[18] could possibly increase the susceptibility of these individuals to vascular and metabolic abnormalities. Thus, the high prevalence of cardiovascular and metabolic diseases among blacks[18,19] may partly be due to their tendency for low IGF-1 concentrations. The relationship between the IGF-1 axis and various aspects of the cardiovascular system has been extensively investigated. However, it is still uncertain to what extent attenuated IGF-1 levels relate to the vascular and cardiac function of the hypertension prone black population of South Africa.

In the subsequent chapter, a broad overview of the literature is provided, mainly focussing on the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, and its role in the metabolic syndrome, vascular endothelium and cardiac dysfunction.

2. LITERATURE OVERVIEW

2.1. The growth hormone/insulin-like growth factor-1 axis

IGF-1, a 70 amino acid single chain protein, structurally homologous to IGF-2 and pro-insulin,[20] is mainly synthesised by the liver and transported to other tissue, acting as an endocrine hormone.[21,22] Besides the liver, other tissues including the vascular endothelium, exercising skeletal muscle and bone also contribute to a total secretion of approximately 3-10 mg/day.[23] IGF-1 secreted by extrahepatic tissue acts locally in a paracrine manner.[21,22] When GH binds to its hepatic receptors, it stimulates the expression and release of IGF-1 into the circulation.[21,22] Circulating IGF-1 is bound to a family of six different insulin like growth factor binding proteins (IGFBPs), also synthesised mainly by the liver. These proteins not only transport IGF-1, but also serve to prolong its half-life, modulate its tissue specificity by altering its affinity for receptors and modify its concentrations in the interstitial fluid.[24,25] IGF-1 synthesised and released from the liver has a high affinity for IGFBPs, whereas IGF-1 produced by other tissues has a lower affinity for IGFBPs.[21,22] Approximately 80% of total IGF-1 is bound by IGFBP-3 in serum and complexed with acid-labile subunit (ALS) into a ternary complex.[25] The remaining 20% is bound by the other IGFBPs.[25]

IGF-1 in its free form has a half-life of 15 minutes which accounts for its immediate availability.[26] The binary complex of IGF-1 with IGFBP-2, -4, -5, and -6 has a half-life of 90 minutes, thus regulating medium-term availability of IGF-1.[27] The ternary complex of IGF-1, ALS and IGFBP-3 has a half-life of 16 hours and is responsible for long-term availability,[27] while IGFBP-1 regulates the short-term availability of IGF-1 with a half-life of approximately 30 minutes.[28] Total IGF-1 is

the most reproducible measure of IGF-1 and is routinely measured in clinical studies,[29] however the free form of IGF-1 reflects IGF-1 bioactivity better than circulating total IGF-1.[30] Due to the short half-life of free IGF-1, a surrogate measure for free or bioavailable IGF-1 may be derived from the molar ratio of total IGF-1 and IGFBP-3.[30]

IGF-1 exerts all of its known physiological effects by binding to two different cell-surface receptors: the IGF-1 receptor (IGF-1R) and the IGF-2 receptor (IGF-2R). The IGF-1R has a high affinity for IGF-1 and IGF-2, but a lower affinity for insulin.[31] Due to the structural homology between IGF-1 and insulin, and between the insulin receptor and the IGF-1R, IGF-1 can also bind to the insulin receptor but with a lower affinity than that of insulin.[31] IGF-2R binds IGF-2 with a higher affinity than IGF-1, but does not bind with insulin.[31]

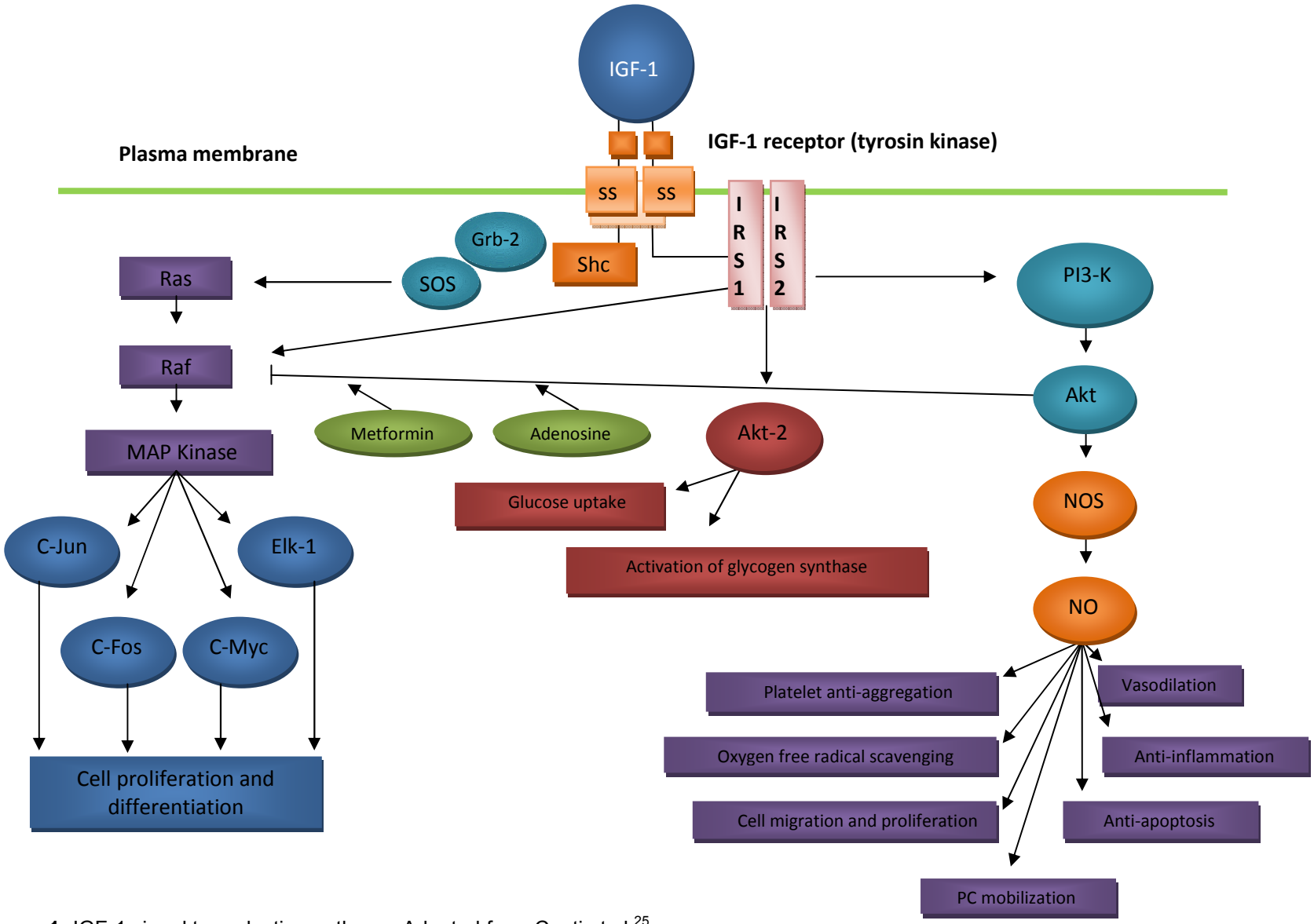


Figure 1: IGF-1 signal transduction pathway. Adapted from Conti et al.[25]
Ras, Renin-angiotensin system; MAP kinase, mitogen-activated protein kinase; SOS, Sons of sevenless; IRS-1; insulin receptor substrate-1; IRS-2, insulin receptor substrate-2; PI3-K, phosphoinositide 3-kinase; NOS, nitric oxide synthase; NO, nitric oxide.

IGF-1R signalling involves the autophosphorylation and tyrosine phosphorylation of cellular proteins, including the adaptor protein Shc and members of the insulin receptor substrate (IRS) family (Figure 1). IRS activates multiple signaling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, the main action of which is to synthesise nitric oxide (NO).[32] NO has multiple metabolic and vasculoprotective effects including vasodilation,[33] anti-inflammatory,[13] anti-apoptosis,[12] anti-platelet aggregation and scavenging oxygen free radicals,[10] while promoting cell migration and proliferation.[34] In addition, IGF-1 enhances insulin sensitivity,[9] suppresses plasma non-esterified free fatty acids,[35] increases glucose metabolism (oxidative and non-oxidative)[10] and reduces triglyceride concentrations.[36] IGF-1 therefore counteracts endothelial dysfunction, atherosclerosis, plaque development and ischemic myocardial damage.[37,38]

By activating Akt-2, IGF-1 promotes glucose transporter 4 translocation to the plasma membrane and activates glycogen synthase.[25] Receptor interaction with Shc activates the rennin-angiotensin system (Ras), Raf and mitogen-activated protein kinase (MAPK) pathway, the actions of which are related to the carcinogenic activity of IGF-1.[25] In addition, IGF-1 exerts mitogenic, migratory and proliferatory effects on the smooth muscle cells via the MAPK pathway.[25] The combination of the mitogenic and anti-apoptotic effects of IGF-1 has a profound impact on tumor growth.[25] Therefore, cross-talk between the various signaling pathways needs to be investigated thoroughly when considering the potential therapeutic impact of IGF-1.[25,39]

Owing to its vasculo- and cardioprotective properties, evidence has accrued showing that decreased IGF-1 levels contribute to endothelial dysfunction, and associate with cardiometabolic risk factors, endothelial damage and cardiac pathology.

2.2. IGF-1, cardiovascular risk factors and endothelial dysfunction

Cardiovascular risk factors cause endothelial dysfunction, endothelial apoptosis and impaired endothelial-dependent vascular reactivity and are therefore considered as promoters of vascular disease.[40] Reduced levels of IGF-1 may at least in part contribute to these effects, since various risk factors such as aging,[17,41,42] obesity,[43] diabetes,[43,44] hypertension,[43,45,46] reduced high-density lipoprotein cholesterol (HDL-C),[43] hypertriglyceridaemia,[43] smoking,[47] and alcohol abuse[48] have been associated with low levels of circulating IGF-1.

Aging

Various cross-sectional studies reported lower IGF-1 levels with increasing age.[17,41,42] Since IGF-1 exerts vascular protective effects,[49] this decline in IGF-1 levels may add to age-related diseases such as cardiovascular and metabolic diseases.[50] In a study by Schutte et al.[17] IGF-1 declined significantly by age quartiles in both blacks and whites (Figure 2). However, black South Africans showed a steeper decline in IGF-1 levels at much younger ages when compared to whites,[17] or from what has previously been described in other populations.[51,52]

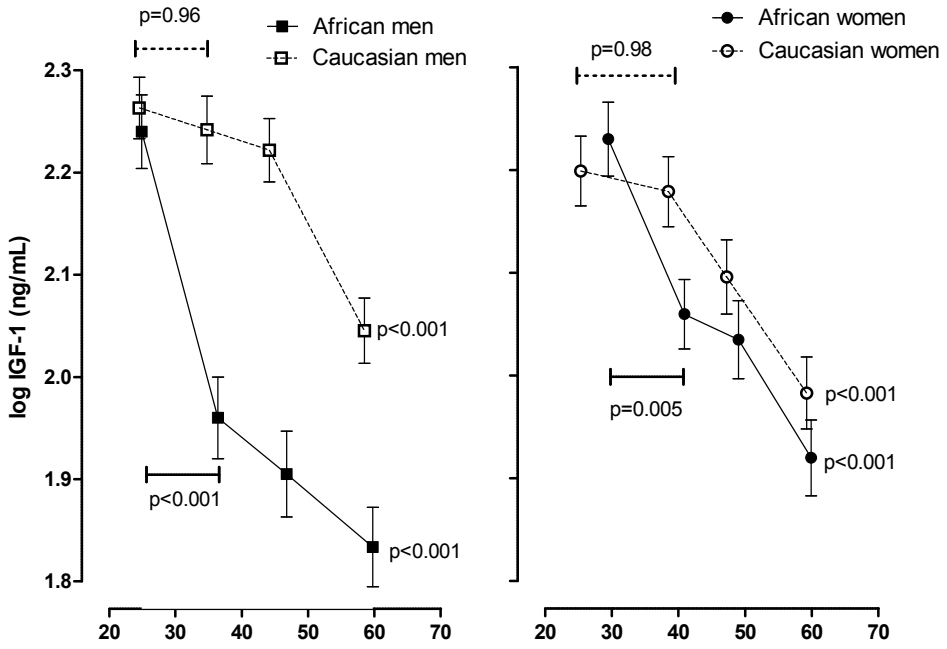


Figure 2: IGF-1 by quartiles of age of black and white men and women.[17]

They also found an association between low IGF-1 and various cardiometabolic risk factors in blacks and therefore suggested that the decline in IGF-1, at much younger ages, may at least in part contribute to the high prevalence of cardiovascular diseases among blacks.[17]

Smoking and alcohol abuse

As a result of the chemicals contained in tobacco products, long-term smoking can result in an inflammatory reaction due to oxidative stress.[53] Both inflammation and oxidative stress reduce IGF-1 levels,[54] and unsurprisingly various studies found that IGF-1 is inversely associated with smoking.[47,55] Also the ingestion of moderate amounts of alcohol significantly reduces total IGF-1 levels in healthy individuals.[56] In a longitudinal study, increased alcohol intake was associated with a decrease in IGF-1 in black men.[48] Schutte et al.[17] also found low IGF-1 to be associated with high alcohol use in blacks.

Obesity

A strong relationship exists between obesity and co-morbidities such as type 2 diabetes, hypertension, dyslipidemia and coronary heart disease.[57,58] Various studies suggest that reduced IGF-1 levels play a key role in this relationship.[59,60] In a study by Rasmussen et al.,[61] IGF-1 was inversely associated with abdominal fat and the percentage body fat of obese subjects. Also, obese subjects presented lower levels of IGF-1 when compared to normal subjects, but after weight loss reduced levels returned to normal.[61] To the contrary, some studies have also shown an increase in IGF-1 in obesity.[62] Utz et al.[63] demonstrated that overweight women had increased IGF-1, whereas obese women showed reduced IGF-1 levels. Imrie et al.[64] suggested an initial compensatory increase in IGF-1 in the presence of IGF-1 resistance associated with obesity, and that the duration of obesity might have an impact on IGF-1 levels.

Reduced HDL-C

The structurally complex HDL-C interacts with highly specific receptors in peripheral tissues as well as the liver and exerts a variety of anti-atherogenic effects.[65] It promotes reverse cholesterol transport by which excess cholesterol is delivered back to the liver,[65] where it is disposed of as bile salts.[65] Apart from that, HDL-C stimulates nitric oxide and prostacyclin production (which is also anti-thrombotic), inhibits thrombosis and endothelial cell apoptosis, decreases platelet aggregation and inhibits low density lipoprotein (LDL) oxidation.[66] Low levels of HDL-C are associated with an increased risk for coronary artery disease (CAD),[67] myocardial infarction (MI),[68] and stroke.[69] Diseases associated with low IGF-1 such as obesity and diabetes mellitus are also associated with low HDL-C.[70] A positive association between IGF-1 and HDL-C has been reported in 132 elderly patients,[71] in 139 offspring of mothers with type 1 diabetes[70] and in non-diabetic individuals between the ages of 20 and 69 years.[72] In addition, patients with GH deficiency have lower HDL-C than age-matched controls, and with GH replacement therapy

these patients' HDL-C levels increased significantly.[73] Succurro et al.[72] suggested that IGF-1 may be an independent modulator of HDL-C concentrations.

Contrary to these findings, studies performed in healthy individuals,[35] in men who survived MI,[74] and in primary care patients with various cardiometabolic risk factors,[75] found a negative association between IGF-1 and HDL-C. It is possible that differences in clinical characteristics and associated treatments for cardiometabolic risk factors may explain the contradictory findings. Also, circulating concentrations of small and large HDL-C particles may also explain the inconsistent relationship found between HDL-C and IGF-1 in different studies, since concentrations of large HDL particles display an inverse relationship with cardiovascular risk whereas small particles reveal a positive correlation with cardiovascular risk.[76]

Hypertriglyceridaemia

GH secretion results in the release of free fatty acids following triglyceride breakdown in fat tissue and in increased free fatty acid oxidation in the liver.[77] Therefore, in patients with extreme insulin resistance, co-treatment with IGF-1 improved their glucose tolerance and decreased hypertriglyceridaemia.[36]

Hypertension

The stimulating effect of IGF-1 on NO production may involve IGF-1 in blood pressure (BP) and regional blood flow regulation.[78] Sowers et al.[79] suggest a decreased ability of IGF-1 to modulate the NO system in some patients with essential hypertension. Because of its blood pressure lowering function, recombinant IGF-1 treatment has been considered as a potential therapeutic target to treat hypertension.[25] Various cross-sectional studies, including studies done on black South Africans, show a significant inverse association between BP and IGF-1.[43,45,80,81] Conversely, other cross-sectional studies found no relationship between BP and IGF-

1,[82,83] while others found that IGF-1 concentrations were significantly higher among hypertensive patients compared to normotensives.[84-88] The relationship between BP and IGF-1 however seems dependent on the concentration of IGF-1.[89] Low IGF-1 levels (associated with vascular deterioration), as well as very high IGF-1 levels (possibly due to IGF-1 resistance that develops over time), could attenuate the vasculoprotective functions of IGF-1.[89]

Gamma-glutamyl transferase

Recent studies indicate that elevated gamma-glutamyl tranferase (γ-GT) is an independent predictor of diabetes, hypertension and the metabolic syndrome,[90,91] and is also inversely associated with circulating IGF-1.[17] Traditionally, serum γ-GT has been used as a biomarker of alcoholic fatty liver disease[91] and various studies have demonstrated an inverse association between IGF-1 levels and alcohol intake.[17,48] In addition, increased γ-GT is frequently observed in liver damage and non-alcoholic fatty liver disease (NAFLD).[92] Furthermore, Ichikawa et al.[93] found that low IGF-1 serves as a predictor for the development of liver fibrosis and steatosis in NAFLD patients. IGF-1 has anti-fibrotic and cell protective actions but inflammatory cytokines, which are increased in NAFLD, inhibit IGF-1 secretion from hepatocytes and causes hepatic fibrosis.[93]

Inflammation and oxidative stress

Cardiometabolic risk factors such as hypertension,[94] hypercholesterolemia[94] and insulin resistance[95] contribute to endothelial dysfunction accompanied by inflammation, the activation of platelets, thrombus formation and oxidative stress. Direct injury to the vessel wall will result in the synthesis of pro-inflammatory proteins, such as cytokines, that initiate the inflammatory response.[96] Endothelial dysfunction characterised by the secretion of endothelin-1 or reduced NO production will also increase the synthesis of pro-inflammatory cytokines.[97] Thus,

inflammation contributes to endothelial dysfunction, while endothelial dysfunction advances inflammation.

IGF-1 and the inflammatory system have a close biological link since IGF-1 decreases the expression of pro-inflammatory cytokines such as interleukin-6 (IL-6).[98] In a study consisting of children with severe burn injuries, the administration of IGF-1 reduced pro-inflammatory cytokines.[99] Consistent with these findings, Sukhanov et al.[98] demonstrated that IGF-1 modulates macrophage function and suggested that this could represent a key mechanism mediating the anti-inflammatory properties of IGF-1. IGF-1 suppresses macrophage infiltration into atherosclerotic lesions and consequently down-regulates the expression of a pro-inflammatory cytokine called tumor necrosis factor-alpha (TNF-α).[98] In addition, IGF-1 also reduces lipoprotein lipase mRNA in cultured macrophages.[100]

To the contrary, studies also reported that cytokines decrease circulating IGF-1 by decreasing GH or increasing the body's resistance to GH.[54] Low circulating levels of IGF-1 have also been associated with high levels of C-reactive protein (CRP),[83] a systemic marker of inflammation and also an independent predictor of cardiovascular disease,[97] diabetes[101] and the metabolic syndrome.[102,103] CRP exerts direct pro-inflammatory effects on endothelial cells.[104] Its synthesis is stimulated by pro-inflammatory cytokines such as IL-6, IL-1 and TNF-α.[105,106]

Both high CRP and low IGF-1 are closely related to oxidative stress. In monocytes and macrophages, reactive oxygen species increase pro-inflammatory cytokine production[107] that will consequently reduce bioavailable IGF-1.[54] Additionally, activated inflammatory cells at the tissue level will increase the expression of oxidant-generating enzymes that may result in oxidative damage, and therefore further induce the inflammatory response.[107] Oxidative damage will attenuate IGF-1, since oxidative stress ultimately causes hepatocyte necrosis and

apoptosis, where IGF-1 is mainly synthesised.[108-110] However, high IGF-1 reduces oxidative stress and is responsible for free oxygen radical scavenging through the activation of endothelial nitric oxide synthase (eNOS).[111]

2.3. IGF-1 and the metabolic syndrome

Cardiovascular risk factors such as hypertension, hyperglycaemia, hypertriglyceridaemia, abdominal obesity and decreased HDL-C, cluster together as part of the metabolic syndrome (Table 1).[112]

Table 1: Criteria for clinical diagnosis of the metabolic syndrome by the International Diabetes Federation.[112]

Measure	Categorical Cut Points
Elevated waist circumference	Male ≥94 cm; Female ≥80 cm (Sub-Saharan Africa)
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator)	≥150 mg/dL (1.7 mmol/L)
Reduced HDL-C (drug treatment for elevated HDL-C is an alternate indicator)	Male <40 mg/dL (1.0 mmol/L); Female <50 mg/dL (1.3 mmol/L)
Elevated blood pressure (anti-hypertensive medication is an alternate indicator)	Systolic ≥130mmHg and/or diastolic ≥85 mmHg
Elevated fasting glucose (drug treatment for elevated glucose is an alternate indicator)	≥100mg/dL (5.6 mmolL)
The presence of any 3 of 5 risk factors constitutes a diagnosis of the metabolic syndrome.	

HDL-C, high-density lipoprotein cholesterol

According to the International Diabetes Federation, insulin resistance plays a major role in the development of the metabolic syndrome and is widely believed to be a central feature of the metabolic syndrome.[113]

Similarities between insulin and IGF-1 suggest a possible role of IGF-1 in the development of the metabolic syndrome.[114] Large longitudinal studies found a higher risk of the metabolic

syndrome and insulin resistance in participants with low IGF-1.[28,115] In a study on 3281 healthy adults, Sierra-Johnson et al.[115] investigated the number of metabolic syndrome components present and found that the mean values of bioavailable IGF-1 decreased significantly as the number of metabolic syndrome components increased. Sesti et al.[43] established that IGF-1 concentrations were significantly lower in subjects with the metabolic syndrome compared with subjects without metabolic syndrome. In addition, in other cohorts with young and middle-aged subjects inverse associations between IGF-1 and the metabolic syndrome were consistently found.[116-118]

On balance, in cohort studies with older subjects the relationship between IGF-1 and the metabolic syndrome was more inconsistent. Van Bunderen et al.[119] examined 1258 elderly Dutch people and found that high-normal IGF-1 levels were associated with prevalent metabolic syndrome. Yeap et al.[120] studied a cohort of men, 70 years and older, and found a U-shape relationship, where both low and high IGF-1 levels were associated with an increased risk for the metabolic syndrome. The authors suggested that the association of IGF-1 with individual cardiovascular risk factors in these individuals could contribute to the discrepancy found in different age groups on the relationship between IGF-1 and the metabolic syndrome.[120] Schneider et al.[121] also showed a U-shaped relationship between IGF-1 and the risk of developing type 2 diabetes and suggested that a lack of protective effects against diabetes seems to play a role in a low IGF-1 state. In states of high IGF-1, potentially increased GH secretion may result in reduced insulin sensitivity and consequently increase the risk of developing type 2 diabetes.[121]

2.4. IGF-1 and vascular endothelial damage

The endothelial-protective activities of IGF-1 are vital to ensure normal vascular functioning.[37] However, reduced IGF-1 levels are linked to endothelial dysfunction,[25] which is considered the initial step in the development of endothelial damage. With endothelial damage, substances responsible for haemostasis, fibrinolysis, the synthesis of growth factors and the regulation of vessel tone under normal physiological conditions, are increased.[122] When endothelial damage occurs, collagen and tissue factor (TF) are exposed at the site of injury. While collagen triggers the initiation of primary haemostasis, TF is responsible for the initiation of secondary haemostasis.[123] During primary haemostasis, a substance known as von Willebrand factor (vWF) is released which is responsible for platelet adhesion and aggregation at the site of injury.[124] During secondary haemostasis an unstable plug is formed and stabilised by fibrin.[125]

For the purpose of this thesis, vWF will be discussed as a marker of endothelial damage.

The regulation of vWF secretion and function

vWF is a glycoprotein that plays an essential role in haemostasis.[126] vWf release is increased during endothelial cell damage and has therefore been proposed as a marker of endothelial damage.[127] Also high plasma levels have been found to predict the subsequent occurrence of fatal and non-fatal cardiovascular events.[128]

vWF is non-covalently complexed with Factor VIII (FVIII) in plasma to maintain adequate FVII levels which are involved in thrombin generation.[129,130] In addition, it is also responsible for the formation of a molecular bridge between platelets and the sub-endothelium of an injured vessel, as part of the platelet adhesion process.[131,129] vWF has a role in platelet aggregation and binds to collagen and heparin in the sub-endothelial matrix.[132] It does not interact with platelets in the absence of injury, but damage to the endothelium enables vWF to bind constituents of sub-

endothelial connective tissue.[133] This consequently allows vWF to bind platelets with sufficient affinity and keep them at the site of injury.[133] vWF-platelet adhesion is dependent on fluid shear stress, therefore in veins and healthy arteries with low shear rate, platelet adhesion is not stimulated.[133] But in small arterioles with small diameters and in partially occluded arteries high shear rate occur and platelet adhesion is stimulated.[133]

vWF is expressed by endothelial cells and megakaryocytes and stored in Weibel-Palade (WP) bodies and α-granules, respectively.[134] It is synthesised as a precursor called pro-vWF.[135] Pro-vWF in endothelial cells undergoes several maturation steps and is cleaved into 2 products namely mature vWF and a pro-peptide.[135] Mature vWF synthesised in endothelial cells is either released constitutively into plasma or the sub-endothelium or stored in WP bodies.[134] Apart from endothelial cells, vWF synthesised by megakaryocytes is contained in platelets. The platelet factor is contained in organelles called α-granules.[136] From endothelial cells vWF is released at a steady state, whereas α-granules only release vWF upon platelet activation at the site of injury.[124] vWF, released from WP bodies, consists of large multimers and is the main determinant of plasma vWF levels.[124]

A large number of secretion agonists for vWF have been identified (Figure 3).[124] One group includes agonists that mediate a rise in cytosolic free calcium which induced acute vWF release.[124] Another group of agonists include vasoactive hormones, such as epinephrine, adenosine, vasopressin and desmopressin, are known to raise vWF plasma levels.[124,137] Factors such as hypoxia, shear stress and cytokines, including TNF-α, IL-8 and IL-6, can also increase vWF secretion.[138-141]

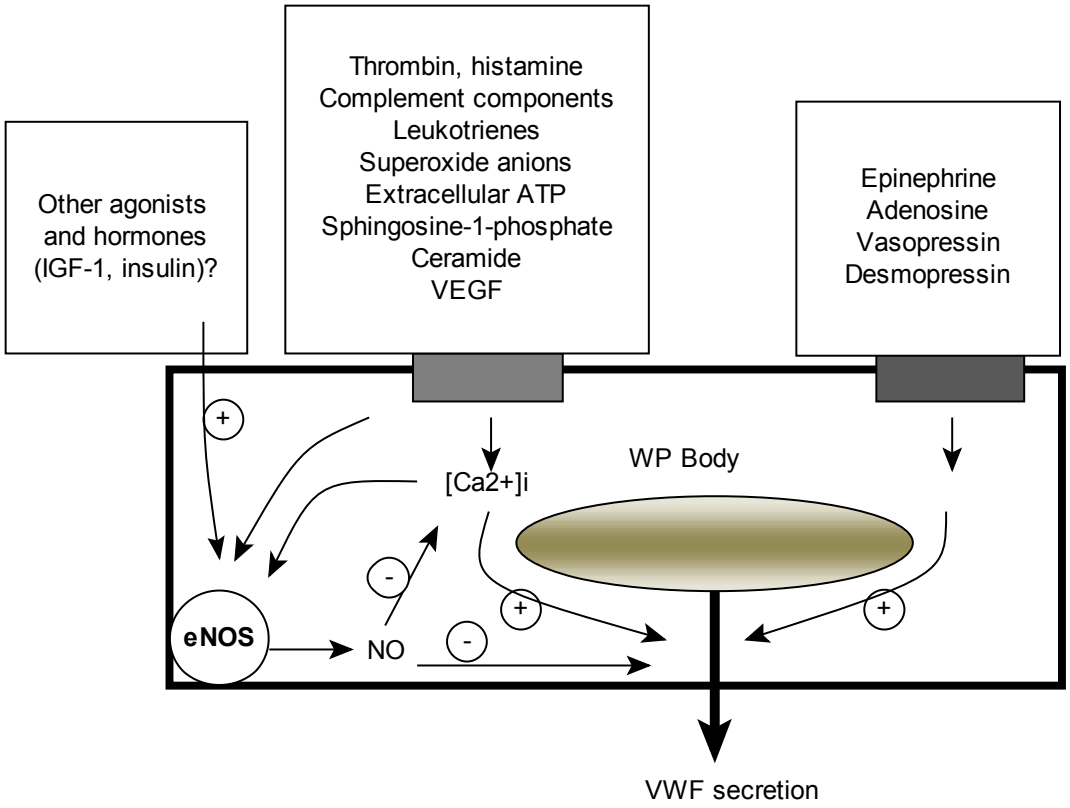


Figure 3: The regulation of vWF secretion. Adapted from Vischer et al.[124]
IGF-1, insulin-like growth factor-1; VEGF, vascular endothelial growth factor; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; WP body, Weibel-Palade body; cAMP, cyclic adenosine monophosphate; vWF, von Willebrand factor; $[Ca^{2+}]_I$, intracellular calcium concentration.

Apart from the multiple agonists, there are also inhibitors of vWF secretion such as NO and prostacyclin.[142,143] NO mediates a negative feedback inhibition on vWF secretion possibly by inhibiting calcium mobilisation form intracellular stores or by blocking the granule-membrane fusion process.[124] An inverse association between vWF and flow-mediated dilation exists suggesting that vWF increases due to impaired endothelial NO synthesis.[144,145] eNOS expression and NO production are stimulated when IGF-1 interacts with high-affinity endothelial binding sites.[8] In addition to IGF-1, insulin also activates eNOS expression and may therefore also be an inhibitor of vWF secretion.[124] Furthermore, high vWF levels are also associated with other factors, also known to be associated with reduced NO and IGF-1, including inflammatory markers such as CRP,[146,147] and components of the metabolic syndrome.[148,149]

IGF-1 is also responsible for the activation of phospholipase A_2 which induces prostacyclin (prostaglandin I_2 (PGI_2)) synthesis.[150] Prostacyclin inhibits platelet aggregation, induces disaggregation of aggregated platelets[151] and is also responsible for inducing vasodilation.[150] When adults and children with primary pulmonary hypertension were treated with long-term prostacyclin, vWF levels were significantly decreased.[152]

It is possible that intervention with agents that reduces circulating vWF, as mentioned above, may lead to a reduction in adverse cardiovascular events associated with high levels of vWF.[127]

2.5. IGF-1 and the heart

IGF-1 plays a crucial role in the preservation of cardiac structure and function[14,15] and has beneficial effects in maintaining the structure and function of a damaged or failing heart.[15] In a study by Donath et al.[153] the infusion of IGF-1 in patients with heart failure improved left ventricular performance. In addition, low IGF-1 levels is associated with an increased risk for heart failure.[50]

IGF-1 receptor signaling in cardiomyocytes

Cardiac effects of IGF-1 are mediated by the activation of the plasma membrane IGF-1R.[154] When the ligand is bound to the receptor, autophosphorylation of the tyrosine residue in the IGF-1R will initiate signaling pathways including the PI3K/Akt mechanistic target of rapamycin (mTOR) signalling pathway and the Ras/Raf/Mitogen-activated protein kinase (MEK)/ERK pathway.[154] Activation of a third pathway (the phospholipase C (PLC)/inositol 1,4,5-triphosphate ($InsP_3$) pathway) leads to an increase in cytosolic Ca^{2+}.[155] Activation of these pathways will ultimately link IGF-1 to the regulation of cardiomyocyte contractility, protein synthesis and hypertrophy, autophagy, apoptosis and metabolism since the viability of cardiomyocytes is of fundamental importance.[7]

Contractility

There are several in vitro studies demonstrating the direct effects of IGF-1 on intrinsic cardiac contractility.[156,157] Also, when animals are treated with GH, in vitro assessments of cardiomyocytes show improved contractility.[158,159] The GH/IGF-1 axis induce increased contractility by (1) altering the intracellular Ca^{2+} transient through an increase in L-type calcium channel activity,[160] (2) by increasing the sensitivity of myofilaments to Ca^{2+}[161] and (3) by upregulating sarcoplasmic reticulum ATPase levels.[162]

Growth

IGF-1 is a positive regulator of cardiac growth by increasing protein synthesis, cardiomyocyte size, amino acid uptake and muscle specific gene expression.[163,164] Systemic and local IGF-1 are essential during embryonic development for appropriate organ growth.[165] Besides physiological growth, IGF-1 also promotes hypertrophy of tissues with high energy demands. In a study by Duerr et al.,[166] IGF-1 administration to a severely dysfunctional rat heart in evolving MI underwent additional hypertrophy and improved cardiac function. Cardiac hypertrophy requires concomitant remodelling of the heart and it has been shown that IGF-1 promotes collagen synthesis by fibroblasts[167] and that GH increases collagen deposition in the heart.[168]

Apoptosis

The heart is susceptible to numerous stressors that will result in cell death which is a hallmark for diseases such as heart failure and MI.[169] Therefore, the anti-apoptotic and pro-survival properties of IGF-1 are fundamentally important in order to prevent cardiomyocyte loss.[164] At cellular level, the anti-apoptotic effects of IGF-1 are mediated by the activation of the Ras/Raf/MEK/ERK and the PI3K/Akt/mTOR signalling pathways (Figure 4).[164,170,171]

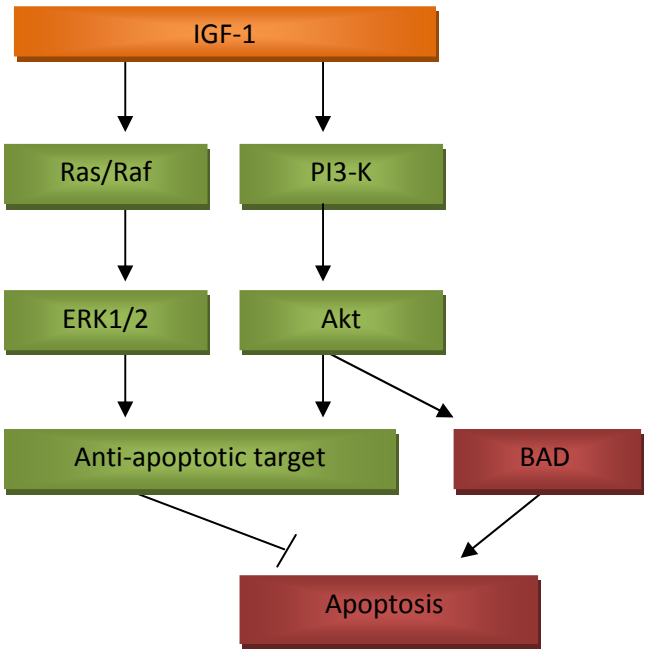


Figure 4: Simplified scheme for IGF-1 induced signalling regulating survival of cardiomyocytes. Adapted from Mehrhof et al.[170,171]

IGF-1, insulin-like growth factor-1; PI3-K, phosphoinositide 3-kinase; RAS, Renin-angiotensin system.

The Ras/Raf/MEK phosphorylation of ERK1 and ERK2 will activate pro-survival activities and protection form apoptosis.[164,172] The activation of Akt initiated by PI3K is also crucial for the anti-apoptotic actions of IGF-1. Akt exerts its anti-apoptotic properties by inactivating pro-apoptotic factors and by activating anti-apoptotic targets. Bcl-2-associated death promoter (BAD) protein, procaspase-9, cyclic adenosine monophosphate (cAMP) response-element-binding protein, nuclear factor-κB, Forkhead family transcription factor and glycogen synthasekinase-3β are some of the downstream targets of Akt.[171] Nonphosphorylated BAD exerts apoptotic effects and the activation of IGF-1R will result in Akt-dependent phosphorylation of BAD.[164]

Autophagy

Autophagy is a catabolic process that occurs virtually in all cells to maintain homeostatic functions such as protein and organelle turnover.[173] Despite this vital role, autophagy

contributes to cell death when it is extensively or inefficiently executed. Autophagy can be strongly induced by stress conditions including nutrient starvation, oxidative stress, infection, hypoxia and other stressors.[174] Various studies indicate that IGF-1 exerts an inhibitory effect upon cellular autophagy. Short term treatment with IGF-1 during nutrient-deprivation stress prevents cardiac autophagy by activating the PI3K/Akt/mTOR pathway since mTOR is a negative regulator of autophagy.[175,176] In addition, IGF-1 increases intracellular adenosine triphosphate (ATP) levels, mitochondrial Ca^{2+} levels and oxygen consumption, which is reduced during nutrient-deprivation stress.[175] As a consequence, IGF-1 inhibits adenosine monophosphate (AMP)-activated protein kinase in cardiomyocytes, which also triggers cardiac autophagy.[175]

During conditions such as heart failure where there is added cardiomyocyte stress, an upregulation of cardiac natriuretic peptide production occurs, which acts in a counteractive manner in order to limit overload.[177-179] As part of this thesis, to assess the relationship between IGF-1 and a marker of cardiac dysfunction, the natriuretic peptide called the N-terminal prohormone B-type natriuretic peptide (NT-proBNP) will be investigated.

N-terminal prohormone B-type natriuretic peptide

The heart secretes, amongst others, two cardiac natriuretic peptides with a homologous structure, namely arterial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).[180-182] The main stimulus for BNP peptide synthesis and secretion is cardiac wall stress.[183] Under normal conditions, BNP related peptides are predominantly produced by the atria, with notable contributions derived from cardiac fibroblasts.[184,185] However, in conditions associated with volume overload where significant myocyte stretch occurs, such as in hypertension, left ventricular hypertrophy (LVH), MI and heart failure, ventricular cardiomyocytes become the main producers of BNP peptides.[177,178,186,187] Human BNP is produced as a 108 amino acid

prohormone (proBNP-108).[188] ProBNP-108 is an inactive proform which is split into two parts through cleavage by proteolytic enzymes.[188] The first end-product is the biologically active peptide BNP32, and the second is an inactive N-terminal (NT-proBNP).[184]

After secretion, BNP binds to the natriuretic peptide receptor type 1 (NPR-1) or 2 (NPR-2) which is linked to guanylyl-cyclase which up-regulates cyclic guanosine monophosphate (cGMP) levels after ligand binding (Figure 6).[182,189] cGMP exerts its biological effects by the activation of cGMP-dependent protein kinase G type I.[189]

Biological effects of BNP include diuresis, natriuresis, vasodilation and the inhibition of renin and aldosterone production.[190] Through inhibition of the calcineurin-nuclear factor of activated T cells signaling pathway, BNP can also inhibit cardiac and vascular myocyte hyperthrophy.[190]

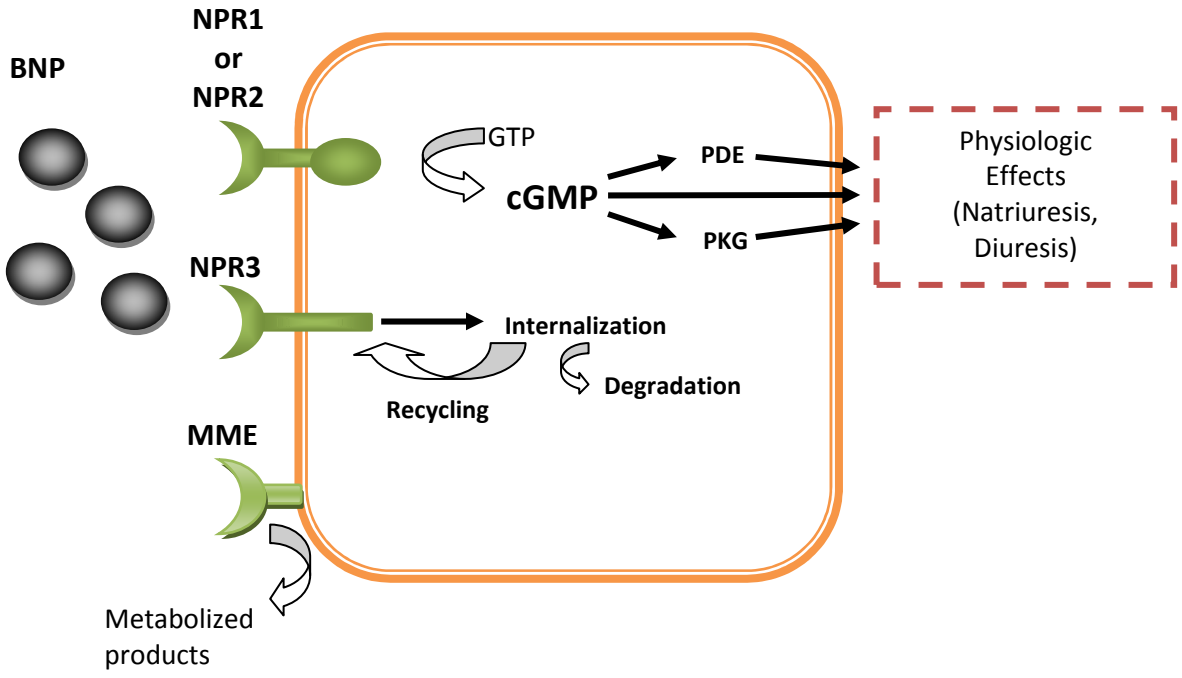


Figure 5: Physiological pathway of NT-proBNP secretion.[189]
BNP, brain natriuretic peptide; NPR, natriuretic peptide receptor; GTP, guanosin triphosphate; cGMP, cyclic guanosine monophaphate; PDE, phosphodiesterase inhibitors; PKG, protein kinase G.

NT-proBNP and cardiovascular disease

NT-proBNP is used as a reliable marker of cardiovascular risk.[191] It has a half-life of 5.45 times longer than that of BNP[192] and rises 2-10 times higher than BNP in patients with left ventricular dysfunction.[193] Thus, the greater rise during or prior to heart failure and the longer half-life make it a better marker than BNP. Plasma NT-proBNP levels have been reported to be higher in hypertensive patients when compared to normotensive patients, and is most pronounced when LVH is present.[190,190,194] Even in patients with hypertension and LVH without diabetes or clinically overt cardiovascular disease, NT-proBNP strongly predicts cardiovascular events.[190] In a study by Choi et al.[195] higher NT-proBNP was significantly associated with incident heart failure in an asymptomatic multi-ethnic population, independent of traditional risk factors and LV mass index. Hence, NT-proBNP levels are used to assess cardiac overload, left ventricular dysfunction and heart failure.[196]

Conflicting findings are consistently reported on the relationship between NT-proBNP and IGF-1. In acromegaly patients, the overtly elevated IGF-1 levels were associated with the development of chronic heart failure,[197] of which elevated NT-proBNP is a well-established biomarker. On the other hand, IGF-1 exerts beneficial cardiovascular effects by playing a role in the development, growth and function of the cardiovascular system.[7] In a study by Duerr et al.[166] the administration of IGF-1 to cardiac failure patients resulted in improved cardiac function. Petretta et al.[198] found that high NT-proBNP levels accompanied by a low IGF-1 /GH ratio was useful to stratify chronic heart failure (CHF) patients at higher risk of cardiac death. Alternatively, adult patients with GH deficiency had increased NT-proBNP levels which were significantly reduced after 12 months of GH replacement therapy.[199] However, in a study by Gruson et al.[200] the decrease observed in NT-proBNP levels following GH treatment occurred independently of changes in cardiac structure or function. They suggest that the effects of GH on NT-proBNP levels may be independent of cardiovascular changes.[200] Collectively, these

studies point to a protective effect of the GH/IGF-1 axis against cardiac dysfunction and failure, with an inverse association between circulating IGF-1 and NT-proBNP.

Although some findings indicated lower NT-proBNP in African-Americans than a white population,[201,202] evidence is sparse on whether ethnic differences exist within a South African population. NT-proBNP levels were reported to be elevated in black South Africans when compared to whites.[203] Supporting these findings, Sliwa et al.[18] recorded data for 4162 hospitalised patients in South Africa, and found that blacks were more likely to be diagnosed with heart failure than the rest of the cohort.

2.6. IGF-1 and ethnicity

Platz et al.[204] investigated IGF-1 levels in American men and found that blacks had lower IGF-1 concentrations than whites and Asians. With regards to South African data, Schutte et al.[17] found that black populations display lower levels of both total and bioavailable IGF-1, when compared to whites. Between the ages of 20 and 30 years, IGF-1 levels were comparable. However in black men and women, a sudden decline in IGF-1 levels at approximately 40 years of age was observed.[17] When compared to other populations,[51,52] the rapid decline takes place at a much younger age in blacks. Low IGF-1 levels are also accompanied by various cardiometabolic risk factors in young black Africans and may therefore result in earlier disease onset.[18]

3. SUMMARY

Black South Africans are prone to hypertension development,[205] have a high prevalence of cardiovascular disease and a high cardiovascular event and mortality rate.[18,206] To better understand the pathophysiological underpinnings of cardiovascular disease development in this particular black population, several mechanisms have been proposed.[207,208] Recently, studies

demonstrated a significant decline in IGF-1 levels among blacks which were associated with various cardiometabolic risk factors.[16,17] Low IGF-1 concentrations may attenuate its protective effects on the vasculature and heart among these individuals. To better understand the high prevalence of CVD in black South Africans, it is necessary to understand the potential involvement of IGF-1 in this particular population.

4. AIM, OBJECTIVES AND HYPOTHESES

Aim

The central aim of this thesis is to increase our understanding on the potential roles of IGF-1 in the cardiovascular system of black and white South Africans. To achieve this, the relationship of IGF-1 with increasing cardiometabolic risk, and with markers of vascular endothelial damage cardiac overload and systolic dysfunction will be investigated.

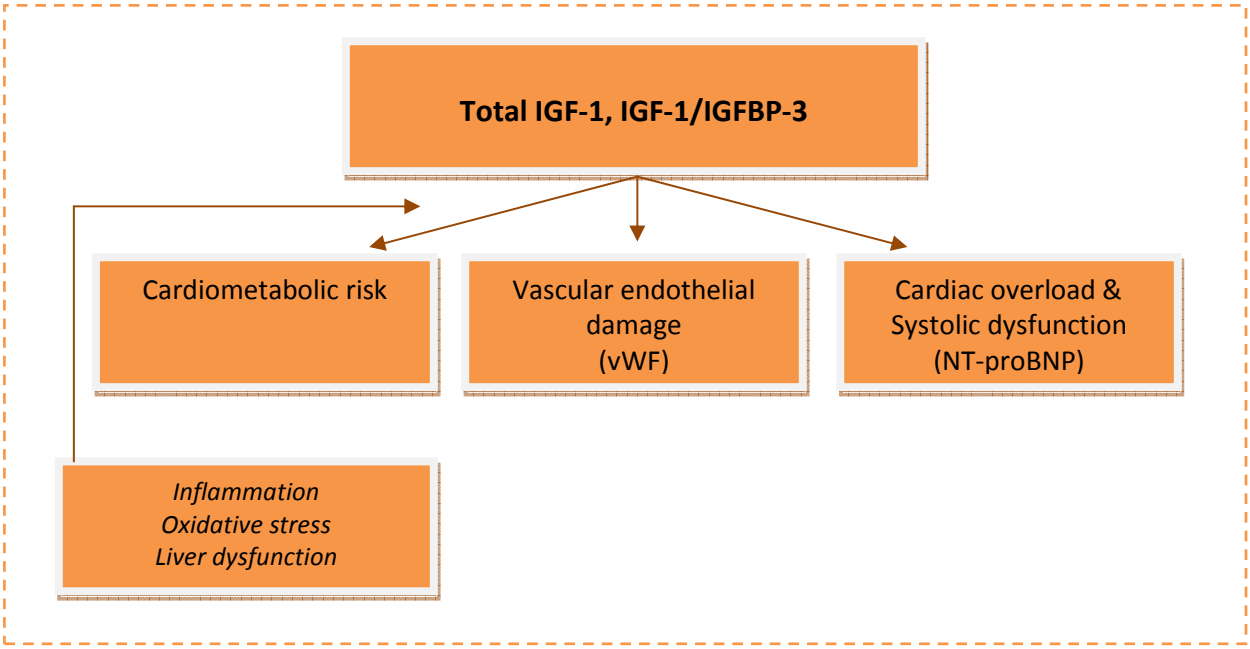


Figure 6: IGF-1 axis and the cardiovascular system investigated.
vWF, von Willebrand factor; NT-proBNP, N-terminal prohormone B-type natriuretic peptide

Objectives

1. To investigate the relationship between bioavailable IGF-1 and the number of components of the metabolic syndrome in black and white men and women.
2. To establish whether bioavailable IGF-1 relates to von Willebrand factor (vWF), as a marker of endothelial damage, in black and white South Africans.
3. To explore the association between NT-proBNP, a marker of cardiac overload and systolic dysfunction, and IGF-1 in a black and white South African population.

Hypotheses

1. Bioavailable IGF-1 is inversely associated with the number of metabolic syndrome components in both sexes and ethnicities.
2. Bioavailable IGF-1 is inversely associated with vWF in both ethnicities.
3. NT-proBNP is inversely associated with IGF-1 in black and white individuals.

5. REFERENCES

1. World Health Organisation. Global status report on noncommunicable diseases 2014. 2014; Available at: http://www.who.int/nmh/publications/ncd-status-report-2014/en/. Accessed 4/14, 2016.
2. World Health Organisation. Noncommunicable diseases profiles by country. 2014; Available at: http://www.who.int/nmh/publications/ncd-profiles-2014/en/. Accessed 4/5, 2016.
3. Mbanya JCN, Motala AA, Sobngwi E, et al. Diabetes in sub-Saharan Africa. Lancet 2010;375(9733):2254-66.
4. Pieters M, Vorster HH. Nutrition and hemostasis: a focus on urbanization in South Africa. Mol Nutr Food Res 2008;52(1):164-72.
5. Juul A, Scheike T, Davidsen M, et al. Low Serum Insulin-Like Growth Factor I Is Associated With Increased Risk of Ischemic Heart Disease A Population-Based Case-Control Study. Circulation 2002;106(8):939-44.
6. Laughlin GA, Barrett-Connor E, Criqui MH, et al. The prospective association of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-1 levels with all cause and cardiovascular disease mortality in older adults: the Rancho Bernardo Study. J Clin Endocrin Metab 2004;89(1):114-20.
7. Troncoso R, Ibarra C, Vicencio JM, et al. New insights into IGF-1 signaling in the heart. Trends Endocrinol Metab 2014;25(3):128-37.
8. Schini-Kerth V. Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells. J Endocrinol Invest 1999;22(5):82-8.

9. Conti E, Andreotti F, Sestito A, et al. Reduced levels of insulin-like growth factor-1 in patients with angina pectoris, positive exercise stress test, and angiographically normal epicardial coronary arteries. Am J Cardiol 2002;89(8):973-5.

10. Dimmeler S, Zeiher AM. Exercise and Cardiovascular Health Get Active to “AKTivate” Your Endothelial Nitric Oxide Synthase. Circulation 2003;107(25):3118-20.

11. Twickler MT, Cramer MM, Koppeschaar HP. Unraveling Reaven’s syndrome X: serum insulin-like growth factor-I and cardiovascular disease. Circulation 2003;107(20):e190-2.

12. Hutter R, Sauter BV, Reis ED, et al. Decreased reendothelialization and increased neointima formation with endostatin overexpression in a mouse model of arterial injury. Circulation 2003;107(12):1658-63.

13. Spies M, Nesic O, Barrow R, et al. Liposomal IGF-1 gene transfer modulates pro-and anti-inflammatory cytokine mRNA expression in the burn wound. Gene Ther 2001;8(18):1409-15.

14. Täng MS, Redfors B, Lindbom M, et al. Importance of circulating IGF-1 for normal cardiac morphology, function and post infarction remodeling. Growth Horm IGF Res 2012;22(6):206-11.

15. Duerr RL, McKirnan MD, Gim RD, et al. Cardiovascular effects of insulin-like growth factor-1 and growth hormone in chronic left ventricular failure in the rat. Circulation 1996;93(12):2188-96.

16. Schutte A, Schutte R, Smith W, et al. Compromised bioavailable IGF-1 of black men relates favourably to ambulatory blood pressure: The SABPA study. Atherosclerosis 2014;233(1):139-44.

17. Schutte AE, Huisman HW, van Rooyen JM, et al. A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. J Clin Endocrin Metab 2010;95(5):2503-7.

18. Sliwa K, Wilkinson D, Hansen C, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. Lancet 2008;371(9616):915-22.

19. Bradshaw D, Norman R, Pieterse D, et al. Estimating the burden of disease attributable to diabetes in South Africa in 2000. S Afr Med J 2007;97(8):700-6.

20. Rinderknecht E, Humbel RE. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem 1978;253(8):2769-76.

21. Jones JI, Clemmons DR. Insulin-Like Growth Factors and Their Binding Proteins: Biological Actions*. Endocr Rev 1995;16(1):3-34.

22. Delafontaine P. Insulin-like growth factor I and its binding proteins in the cardiovascular system. Cardiovasc Res 1995;30(6):825-34.

23. Baxter RC. Circulating Levels and Molecular Distribution of the Acid-Labile (α) Subunit of the High Molecular Weight Insulin-Like Growth Factor-Binding Protein Complex*. J Clin Endocrin Metab 1990;70(5):1347-53.

24. Hwa V, Oh Y, Rosenfeld RG. The Insulin-Like Growth Factor-Binding Protein (IGFBP) Superfamily 1. Endocr Rev 1999;20(6):761-87.

25. Conti E, Musumeci M, De Giusti M, et al. IGF-1 and atherothrombosis: relevance to pathophysiology and therapy. Clin Sci 2011;120:377-402.

26. Paye JM, Forsten-Williams K. Regulation of insulin-like growth factor-I (IGF-I) delivery by IGF binding proteins and receptors. Ann Biomed Eng 2006;34(4):618-32.

27. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res 2003;13(4):113-70.

28. Sandhu MS, Heald AH, Gibson JM, et al. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. Lancet 2002;359(9319):1740-5.

29. Frystyk J. Utility of free IGF-I measurements. Pituitary 2007;10(2):181-7.

30. Frystyk J. Free insulin-like growth factors–measurements and relationships to growth hormone secretion and glucose homeostasis. Growth Horm IGF Res 2004;14(5):337-75.

31. Steele-Perkins G, Turner J, Edman JC, et al. Expression and characterization of a functional human insulin-like growth factor I receptor. J Biol Chem 1988;263(23):11486-92.

32. Kooijman R. Regulation of apoptosis by insulin-like growth factor (IGF)-I. Cytokine Growth Factor Rev 2006;17(4):305-23.

33. Izhar U, Hasdai D, Richardson DM, et al. Insulin and insulin-like growth factor-I cause vasorelaxation in human vessels in vitro. Coron Artery Dis 2000;11(1):69-76.

34. Shizukuda Y, Tang S, Yokota R, et al. Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinase Cdelta activity. Circ Res 1999;85(3):247-56.

35. Colao A, Spiezia S, Di Somma C, et al. Circulating insulin-like growth factor-I levels are correlated with the atherosclerotic profile in healthy subjects independently of age. J Endocrinol Invest 2005;28(7):440-8.

36. Kolaczynski JW, Caro JF. Insulin-like growth factor-1 therapy in diabetes: physiologic basis, clinical benefits, and risks. Ann Intern Med 1994;120(1):47-55.

37. Conti E, Carrozza C, Capoluongo E, et al. Insulin-like growth factor-1 as a vascular protective factor. Circulation 2004;110(15):2260-5.

38. Weroha SJ, Haluska P. IGF-1 receptor inhibitors in clinical trials—early lessons. J Mammary Gland Biol Neoplasia 2008;13(4):471-83.

39. LeRoith D, Werner H, Beitner-Johnson D, et al. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 1995;16(2):143-63.

40. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003;348(7):593-600.

41. Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am J Epidemiol 1997;145(11):970-6.

42. Juul A, Bang P, Hertel NT, et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 1994;78(3):744-52.

43. Sesti G, Sciacqua A, Cardellini M, et al. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care 2005;28(1):120-5.

44. Gomez JM, Maravall FJ, Gomez N, et al. Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. Clin Endocrinol (Oxf) 2003;58(2):213-9.

45. Colao A, Di Somma C, Cascella T, et al. Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: an observational, exploratory study in 404 subjects. Eur J Endocrinol 2008;159(4):389-97.

46. Hu Y, Pete G, Walsh MF, et al. Central IGF-1 decreases systemic blood pressure and increases blood flow in selective vascular beds. Horm Metab Res 1996;28(5):211-4.

47. Teramukai S, Rohan T, Eguchi H, et al. Anthropometric and behavioral correlates of insulin-like growth factor I and insulin-like growth factor binding protein 3 in middle-aged Japanese men. Am J Epidemiol 2002;156(4):344-8.

48. Gapstur SM, Kopp P, Chiu BC, et al. Longitudinal associations of age, anthropometric and lifestyle factors with serum total insulin-like growth factor-I and IGF binding protein-3 levels in Black and White men: the CARDIA Male Hormone Study. Cancer Epidemiol Biomarkers Prev 2004;13(12):2208-16.

49. Perticone F, Sciacqua A, Perticone M, et al. Low-plasma insulin-like growth factor-I levels are associated with impaired endothelium-dependent vasodilatation in a cohort of untreated, hypertensive Caucasian subjects. J Clin Endocrin Metab 2008;93(7):2806-10.

50. Vasan RS, Sullivan LM, D'Agostino RB, et al. Serum insulin-like growth factor I and risk for heart failure in elderly individuals without a previous myocardial infarction: the Framingham Heart Study. Ann Intern Med 2003;139(8):642-8.

51. Yamamoto H, Sohmiya M, Oka N, et al. Effects of aging and sex on plasma insulin-like growth factor I (IGF-I) levels in normal adults. Acta Endocrinol (Copenh) 1991;124(5):497-500.

52. Hilding A, Hall K, Wivall-Helleryd I, et al. Serum Levels of Insulin-Like Growth Factor I in 152 Patients with Growth Hormone Deficiency, Aged 19–82 Years, in Relation to Those in Healthy Subjects 1. J Clin Endocrin Metab 1999;84(6):2013-9.

53. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010;34(3):258-65.

54. De Benedetti F, Alonzi T, Moretta A, et al. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997;99(4):643-50.

55. Landin-Wllhelmsen K, Wllhelmsen L, Lappast G, et al. Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. Clin Endocrinol (Oxf) 1994;41(3):351-7.

56. Rojdmark S, Rydvald Y, Aquilonius A, et al. Insulin-like growth factor (IGF)-1 and IGF-binding protein-1 concentrations in serum of normal subjects after alcohol ingestion: evidence for decreased IGF-1 bioavailability. Clin Endocrinol (Oxf) 2000;52(3):313-8

57. Ren J, Kelley RO. Cardiac health in women with metabolic syndrome: clinical aspects and pathophysiology. Obesity 2009;17(6):1114-23.

58. Tam C, Clement K, Baur L, et al. Obesity and low-grade inflammation: a paediatric perspective. Obes Rev 2010;11(2):118-26.

59. Franco C, Bengtsson B, Johannsson G. The GH/IGF-1 axis in obesity: physiological and pathological aspects. Metab Syndr Relat Disord 2006;4(1):51-6.

60. Kreitschmann-Andermahr I, Suarez P, Jennings R, et al. GH/IGF-I regulation in obesity--mechanisms and practical consequences in children and adults. Horm Res Paediatr 2010;73(3):153-60.

61. Rasmussen MH, Hvidberg A, Juul A, et al. Massive weight loss restores 24-hour growth hormone release profiles and serum insulin-like growth factor-I levels in obese subjects. J Clin Endocrinol Metab 1995;80(4):1407-15.

62. Hochberg Z, Hertz P, Colin V, et al. The distal axis of growth hormone (GH) in nutritional disorders: GH-binding protein, insulin-like growth factor-I (IGF-I), and IGF-I receptors in obesity and anorexia nervosa. Metab Clin Exp 1992;41(1):106-12.

63. Utz A, Yamamoto A, Sluss P, et al. Androgens may mediate a relative preservation of IGF-I levels in overweight and obese women despite reduced growth hormone secretion. J Clin Endocrinol Metab 2008;93(10):4033-40.

64. Imrie H, Abbas A, Viswambharan H, et al. Vascular insulin-like growth factor-I resistance and diet-induced obesity. Endocrinology 2009;150(10):4575-82.

65. Toth PP. Reverse cholesterol transport: high-density lipoprotein's magnificent mile. Curr Atheroscler Rep 2003;5(5):386-93.

66. Nofer J, Kehrel B, Fobker M, et al. HDL and arteriosclerosis: beyond reverse cholesterol transport. Atherosclerosis 2002;161(1):1-16.

67. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease--the Framingham Heart Study. Can J Cardiol 1988;4 Suppl A:5A-10A.

68. Stubbe I, Gustafson A, Nilsson-Ehle P. Alterations in plasma proteins and lipoproteins in acute myocardial infarction: effects on activation of lipoprotein lipase. Scand J Clin Lab Invest 1982;42(5):437-44.

69. Ninomiya JK, L'Italien G, Criqui MH, et al. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. Circulation 2004;109(1):42-6.

70. Nelson SM, Freeman DJ, Sattar N, et al. IGF-1 and leptin associate with fetal HDL cholesterol at birth: examination in offspring of mothers with type 1 diabetes. Diabetes 2007;56(11):2705-9.

71. Ceda GP, Dall'Aglio E, Magnacavallo A, et al. The Insulin-Like Growth Factor Axis and Plasma Lipid Levels in the Elderly 1. J Clin Endocrin Metab 1998;83(2):499-502.

72. Succurro E, Arturi F, Grembiale A, et al. Positive association between plasma IGF1 and high-density lipoprotein cholesterol levels in adult nondiabetic subjects. Eur J Endocrinol 2010;163(1):75-80.

73. Gotherstrom G, Bengtsson B, Bosaeus I, et al. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 2007;92(4):1442-5.

74. Ruotolo G, Båvenholm P, Brismar K, et al. Serum insulin-like growth factor-I level is independently associated with coronary artery disease progression in young male survivors of myocardial infarction: beneficial effects of bezafibrate treatment. J Am Coll Cardiol 2000;35(3):647-54.

75. Schneider HJ, Klotsche J, Saller B, et al. Associations of age-dependent IGF-I SDS with cardiovascular diseases and risk conditions: cross-sectional study in 6773 primary care patients. Eur J Endocrinol 2008;158(2):153-61.

76. Kontush A. HDL particle number and size as predictors of cardiovascular disease. Frontiers in pharmacology 2015;6:218.

77. DiGirolamo M, Edén S, Enberg G, et al. Specific binding of human growth hormone but not insulin-like growth factors by human adipocytes. FEBS Lett 1986;205(1):15-9.

78. Diez J. Insulin-like growth factor I in essential hypertension. Kidney Int 1999:55(2):744-59.

79. Sowers JR. Insulin and insulin-like growth factor in normal and pathological cardiovascular physiology. Hypertension 1997;29(3):691-9.

80. Succurro E, Andreozzi F, Sciacqua A, et al. Reciprocal association of plasma IGF-1 and interleukin-6 levels with cardiometabolic risk factors in nondiabetic subjects. Diabetes Care 2008;31(9):1886-8.

81. Dall'Aglio E, Maggio M, Benedetti N, et al. IGF-I levels in obesity: their relationship to blood pressure levels. J Endocrinol Invest 1999;22(10):49-50.

82. Kawachi S, Takeda N, Sasaki A, et al. Circulating insulin-like growth factor-1 and insulin-like growth factor binding protein-3 are associated with early carotid atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25(3):617-21.

83. Colangelo LA, Liu K, Gapstur SM, et al. Insulin-like growth factor-1, insulin-like growth factor binding protein-3, and cardiovascular disease risk factors in young black men and white men: the CARDIA Male Hormone Study. Am J Epidemiol 2004;160(8):750-7.

84. Diez J, Laviades C. Insulin-like growth factor I and collagen type III synthesis in patients with essential hypertension and left ventricular hypertrophy. J Hum Hypertens 1994;8(1):S21-5.

85. Laviades C, Gil MJ, Monreal I, et al. Is the tissue availability of circulating insulin-like growth factor I involved in organ damage and glucose regulation in hypertension? J Hypertens 1997;15(10):1159-65.

86. Laviades C, Mayor G, Diez J. Elevated circulating levels of insulin-like growth factor I in essential hypertensive patients with left ventricular hypertrophy. Arch Mal Coeur Vaiss 1991;84(8):1039-41.

87. Andronico G, Mangano M, Nardi E, et al. Insulin-like growth factor 1 and sodium-lithium countertransport in essential hypertension and in hypertensive left ventricular hypertrophy. J Hypertens 1993;11(10):1097-101.

88. Diez J, Ruilope LM, Rodicio JL. Insulin response to oral glucose in essential hypertensives with increased circulating levels of insulin growth factor I. J Hypertens 1991;9(6):S174-75.

89. Schutte AE, Volpe M, Tocci G, et al. Revisiting the relationship between blood pressure and insulin-like growth factor-1. Hypertension 2014;63(5):1070-7.

90. Onat A, Can G, Çiçek G, et al. Serum γ-Glutamyltransferase: Independent Predictor of Risk of Diabetes, Hypertension, Metabolic Syndrome, and Coronary Disease. Obesity 2012;20(4):842-8.

91. Nemesanszky E, Lott JA. Gamma-glutamyltransferase and its isoenzymes: progress and problems. Clin Chem 1985;31(6):797-803.

92. Tahan V, Canbakan B, Balci H, et al. Serum gamma-glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk. Hepatogastroenterology 2008;55(85):1433-8.

93. Ichikawa T, Nakao K, Hamasaki K, et al. Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. Hepatology international 2007;1(2):287-94.

94. Drexler H. Endothelial dysfunction: clinical implications. Prog Cardiovasc Dis 1997;39(4):287-324.

95. Vincent MA, Montagnani M, Quon MJ. Molecular and physiologic actions of insulin related to production of nitric oxide in vascular endothelium. Curr Diab Rep 2003;3(4):279-88.

96. Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91(11):2844-50.

97. Epstein FH, Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340(2):115-26.

98. Sukhanov S, Higashi Y, Shai SY, et al. IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 2007;27(12):2684-90.

99. Jeschke MG, Barrow RE, Suzuki F, et al. IGF-I/IGFBP-3 equilibrates ratios of pro- to anti-inflammatory cytokines, which are predictors for organ function in severely burned pediatric patients. Mol Med 2002;8(5):238-46.

100. Sukhanov S, Higashi Y, Shai S, et al. Differential requirement for nitric oxide in IGF-1-induced anti-apoptotic, anti-oxidant and anti-atherosclerotic effects. FEBS Lett 2011;585(19):3065-72.

101. Han TS, Sattar N, Williams K, et al. Prospective study of C-reactive protein in relation to the development of diabetes and metabolic syndrome in the Mexico City Diabetes Study. Diabetes Care 2002;25(11):2016-21.

102. Frohlich M, Imhof A, Berg G, et al. Association between C-reactive protein and features of the metabolic syndrome: a population-based study. Diabetes Care 2000;23(12):1835-9.

103. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes Care 1999;22(12):1971-7.

104. Kaushal K, Heald AH, Siddals KW, et al. The impact of abnormalities in IGF and inflammatory systems on the metabolic syndrome. Diabetes Care 2004;27(11):2682-8.

105. Kushner I. Regulation of the acute phase response by cytokines. Perspect Biol Med 1993;36(4):611-22.

106. Castell JV, Gómez-Lechón MJ, David M, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 1989;242(2):237-9.

107. Matata BM, Galinanes M. Peroxynitrite is an essential component of cytokines production mechanism in human monocytes through modulation of nuclear factor-kappa B DNA binding activity. J Biol Chem 2002;277(3):2330-5.

108. Scheidegger KJ, James RW, Delafontaine P. Differential effects of low density lipoproteins on insulin-like growth factor-1 (IGF-1) and IGF-1 receptor expression in vascular smooth muscle cells. J Biol Chem 2000;275(35):26864-9.

109. Higashi Y, Peng T, Du J, et al. A redox-sensitive pathway mediates oxidized LDL-induced downregulation of insulin-like growth factor-1 receptor. J Lipid Res 2005;46(6):1266-77.

110. Guicciardi ME, Malhi H, Mott JL, et al. Apoptosis and necrosis in the liver. Compr Physiol 2013;3(2):977-1010.

111. Li Y, Higashi Y, Itabe H, et al. Insulin-like growth factor-1 receptor activation inhibits oxidized LDL-induced cytochrome C release and apoptosis via the phosphatidylinositol 3 kinase/Akt signaling pathway. Arterioscler Thromb Vasc Biol 2003;23(12):2178-84.

112. Alberti K, Eckel RH, Grundy SM, et al. Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120(16):1640-5.

113. Alberti, Kurt George Matthew Mayer, Zimmet P, et al. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. Diabetic Med 2006;23(5):469-80.

114. Aguirre G, De Ita JR, de la Garza R, et al. Insulin-like growth factor-1 deficiency and metabolic syndrome. J Transl Med 2016;14(1):3.

115. Sierra-Johnson J, Romero-Corral A, Somers V, et al. IGF-I/IGFBP-3 ratio: a mechanistic insight into the metabolic syndrome. Clin Sci 2009;116(6):507-12.

116. Saydah S, Ballard-Barbash R, Potischman N. Association of metabolic syndrome with insulin-like growth factors among adults in the US. Cancer Causes Control 2009;20(8):1309-16.

117. Hypponen E, Boucher BJ, Berry DJ, et al. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. Diabetes 2008;57(2):298-305.

118. Tong PC, Ho C, Yeung VT, et al. Association of testosterone, insulin-like growth factor-I, and C-reactive protein with metabolic syndrome in Chinese middle-aged men with a family history of type 2 diabetes. J Clin Endocrin Metab 2005;90(12):6418-23.

119. van Bunderen CC, Oosterwerff MM, van Schoor NM, et al. Serum IGF1, metabolic syndrome, and incident cardiovascular disease in older people: a population-based study. Eur J Endocrinol 2013;168(3):393-401.

120. Yeap BB, Chubb SA, Ho KK, et al. IGF1 and its binding proteins 3 and 1 are differentially associated with metabolic syndrome in older men. Eur J Endocrinol 2010;162(2):249-57.

121. Schneider HJ, Friedrich N, Klotsche J, et al. Prediction of incident diabetes mellitus by baseline IGF1 levels. Eur J Endocrinol 2011;164(2):223-9.

122. Blann A. von Willebrand factor and the endothelium in vascular disease. Br J Biomed Sci 1993;50(2):125-34.

123. Furie B, Furie BC. Mechanisms of thrombus formation. N Engl J Med 2008;359(9):938-49.

124. Vischer U. von Willebrand factor, endothelial dysfunction, and cardiovascular disease. J Thromb Haemost 2006;4(6):1186-93.

125. Lisman T, Weeterings C, de Groot PG. Platelet aggregation: involvement of thrombin and fibrin (ogen). Front Biosci 2005;10:2504-17.

126. Horvath B, Hegedus D, Szapary L, et al. Measurement of von Willebrand factor as the marker of endothelial dysfunction in vascular diseases. Exp Clin Cardiol 2004;9(1):31-4.

127. Blann AD, McCollum CN. Von Willebrand factor, endothelial cell damage and atherosclerosis. Eur J Vasc Surg 1994;8(1):10-5.

128. Jansson JH, Nilsson TK, Johnson O. von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. Br Heart J 1991;66(5):351-5.

129. Ruggeri ZM. von Willebrand factor. J Clin Invest 1997;99(4):559-64.

130. Cruz MA, Yuan H, Lee JR, et al. Interaction of the von Willebrand factor (vWF) with collagen Localization of the primary collagen-binding site by analysis of recombinant vWF a domain polypeptides. J Biol Chem 1995;270(18):10822-7.

131. Sadler JE. von Willebrand factor. J Biol Chem 1991;266(34):22777-80.

132. Blann AD, McCollum CN. Von Willebrand factor, endothelial cell damage and atherosclerosis. Eur J Vasc Surg 1994;8(1):10-5.

133. Sadler JE. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem 1998;67(1):395-424.

134. Lip GY, Blann A. von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? Cardiovasc Res 1997;34(2):255-65.

135. Vischer UM, Wagner DD. von Willebrand factor proteolytic processing and multimerization precede the formation of Weibel-Palade bodies. Blood 1994;83(12):3536-44.

136. Mannucci PM. Platelet von Willebrand factor in inherited and acquired bleeding disorders. Proc Natl Acad Sci U S A 1995;92(7):2428-32.

137. Vischer UM, de Moerloose P. von Willebrand factor: from cell biology to the clinical management of von Willebrand's disease. Crit Rev Oncol 1999;30(2):93-109.

138. Bhatia R, Matsushita K, Yamakuchi M, et al. Ceramide triggers Weibel-Palade body exocytosis. Circ Res 2004;95(3):319-24.

139. Pinsky DJ, Naka Y, Liao H, et al. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies. A mechanism for rapid neutrophil recruitment after cardiac preservation. J Clin Invest 1996;97(2):493-500.

140. Galbusera M, Zoja C, Donadelli R, et al. Fluid shear stress modulates von Willebrand factor release from human vascular endothelium. Blood 1997;90(4):1558-64.

141. Bernardo A, Ball C, Nolasco L, et al. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. Blood 2004;104(1):100-6.

142. Veyradier A, Nishikubo T, Humbert M, et al. Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension. Circulation 2000;102(20):2460-2.

143. Zarei S, Frieden M, Kaufmann J, et al. The regulation of endothelial VWF secretion by nitric oxide: is it physiological? J Thromb Haemost 2006;4(1):263-5.

144. Lee KW, Blann AD, Lip GY. High pulse pressure and nondipping circadian blood pressure in patients with coronary artery disease: relationship to thrombogenesis and endothelial damage/dysfunction. Am J Hypertens 2005;18(1):104-15.

145. Felmeden D, Blann A, Spencer CG, et al. A comparison of flow-mediated dilatation and von Willebrand factor as markers of endothelial cell function in health and in hypertension: relationship to cardiovascular risk and effects of treatment A substudy of

the Anglo-Scandinavian Cardiac Outcomes Trial. Blood Coagulation Fibrinol 2003;14(5):425-31.

146. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350(14):1387-97.

147. Haverkate F, Thompson SG, Duckert F. Haemostasis factors in angina pectoris; relation to gender, age and acute-phase reaction. Results of the ECAT Angina Pectoris Study Group. Thromb Haemost 1995;73(4):561-7.

148. Meigs JB, Mittleman MA, Nathan DM, et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: the Framingham Offspring Study. JAMA 2000;283(2):221-8.

149. Juhan-Vague I, Thompson SG, Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome. A study of 1500 patients with angina pectoris. The ECAT Angina Pectoris Study Group. Arterioscler Thromb 1993;13(12):1865-73.

150. Webb JG, Tan Y, Jaffa MA, et al. Evidence for prostacyclin and cAMP upregulation by bradykinin and insulin-like growth factor 1 in vascular smooth muscle cells. J Recept Signal Transduct Res 2010;30(2):61-71.

151. Moncada S, Vane JR. The role of prostacyclin in vascular tissue. Fed Proc 1979;38(1):66-71.

152. Friedman R, Mears JG, Barst RJ. Continuous infusion of prostacyclin normalizes plasma markers of endothelial cell injury and platelet aggregation in primary pulmonary hypertension. Circulation 1997;96(9):2782-4.

153. Donath MY, Sütsch G, Yan X, et al. Acute Cardiovascular Effects of Insulin-Like Growth Factor I in Patients with Chronic Heart Failure 1. J Clin Endocrinol Metab 1998;83(9):3177-83.

154. Becker AB, Roth RA. Insulin receptor structure and function in normal and pathological conditions. Annu Rev Med 1990;41(1):99-115.

155. Ibarra C, Estrada M, Carrasco L, et al. Insulin-like growth factor-1 induces an inositol 1,4,5-trisphosphate-dependent increase in nuclear and cytosolic calcium in cultured rat cardiac myocytes. J Biol Chem 2004;279(9):7554-65.

156. Vetter U, Kupferschmid C, Lang D, et al. Insulin-like growth factors and insulin increase the contractility of neonatal rat cardiocytes in vitro. Basic Res Cardiol 1988;83(6):647-54.

157. Freestone NS, Ribaric S, Mason WT. The effect of insulin-like growth factor-1 on adult rat cardiac contractility. Mol Cell Biochem 1996;163(1):223-9.

158. Stromer H, Cittadini A, Douglas PS, et al. Exogenously administered growth hormone and insulin-like growth factor-I alter intracellular Ca2+ handling and enhance cardiac performance. In vitro evaluation in the isolated isovolumic buffer-perfused rat heart. Circ Res 1996;79(2):227-36.

159. Tajima M, Weinberg EO, Bartunek J, et al. Treatment with growth hormone enhances contractile reserve and intracellular calcium transients in myocytes from rats with postinfarction heart failure. Circulation 1999;99(1):127-34.

160. Solem ML, Thomas AP. Modulation of cardiac Ca 2 channels by IGF1. Biochem Biophys Res Commun 1998;252(1):151-5.

161. Buerke M, Murohara T, Skurk C, et al. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. Proc Natl Acad Sci U S A 1995;92(17):8031-5.

162. Houck WV, Pan LC, Kribbs SB, et al. Effects of growth hormone supplementation on left ventricular morphology and myocyte function with the development of congestive heart failure. Circulation 1999;100(19):2003-9.

163. Castellano G, Affuso F, Conza PD, et al. The GH/IGF-1 Axis and Heart Failure. Curr Cardiol Rev 2009;5(3):203-15.

164. Opgaard OS, Wang PH. IGF-I is a matter of heart. Growth Horm IGF Res 2005;15(2):89-94.

165. Wang J, Zhou J, Powell-Braxton L, et al. Effects of Igf1 gene deletion on postnatal growth patterns. Endocrinology 1999;140(7):3391-4.

166. Duerr RL, Huang S, Miraliakbar HR, et al. Insulin-like growth factor-1 enhances ventricular hypertrophy and function during the onset of experimental cardiac failure. J Clin Invest 1995;95(2):619-27.

167. Butt RP, Laurent GJ, Bishop JE. Collagen production and replication by cardiac fibroblasts is enhanced in response to diverse classes of growth factors. Eur J Cell Biol 1995;68(3):330-5.

168. Bruel A, Oxlund H. Biosynthetic growth hormone increases the collagen deposition rate in rat aorta and heart. Eur J Endocrinol 1995;132(2):195-9.

169. Chiong M, Wang Z, Pedrozo Z, et al. Cardiomyocyte death: mechanisms and translational implications. Cell Death Dis 2011;2(12):e244.

170. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev 1999;13(22):2905-27.

171. Mehrhof FB, Muller FU, Bergmann MW, et al. In cardiomyocyte hypoxia, insulin-like growth factor-I-induced antiapoptotic signaling requires phosphatidylinositol-3-OH-kinase-dependent and mitogen-activated protein kinase-dependent activation of the transcription factor cAMP response element-binding protein. Circulation 2001;104(17):2088-94.

172. Foncea R, Gálvez A, Pérez V, et al. Extracellular regulated kinase, but not protein kinase C, is an antiapoptotic signal of insulin-like growth factor-1 on cultured cardiac myocytes. Biochem Biophys Res Commun 2000;273(2):736-44.

173. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132(1):27-42.

174. Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. N Engl J Med 2013;368(7):651-62.

175. Troncoso R, Vicencio JM, Parra V, et al. Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Cardiovasc Res 2012;93(2):320-9.

176. Murrow L, Debnath J. Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. Annu Rev Pathol 2013;8:105-37.

177. Hystad M, Geiran O, Attramadal H, et al. Regional cardiac expression and concentration of natriuretic peptides in patients with severe chronic heart failure. Acta Physiol Scand 2001;171(4):395-403.

178. Luchner A, Stevens TL, Borgeson DD, et al. Differential atrial and ventricular expression of myocardial BNP during evolution of heart failure. Am J Physiol 1998;274(5):H1684-9.

179. Mukoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J Clin Invest 1991;87(4):1402-12.

180. Flynn TG, Mercedes L, Adolfo J. The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochem Biophys Res Commun 1983;117(3):859-65.

181. Sudoh T, Kangawa K, Minamino N, et al. A new natriuretic peptide in porcine brain. Nature 1988;332(6159):78-81.

182. Suga S, Nakao K, Hosoda K, et al. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. Endocrinology 1992;130(1):229-39.

183. Magga J, Marttila M, Mantymaa P, et al. Brain natriuretic peptide in plasma, atria, and ventricles of vasopressin- and phenylephrine-infused conscious rats. Endocrinology 1994;134(6):2505-15.

184. Hall C. Essential biochemistry and physiology of (NT-pro) BNP. European Journal of Heart Failure 2004;6(3):257-60.

185. Tsuruda T, Boerrigter G, Huntley BK, et al. Brain natriuretic Peptide is produced in cardiac fibroblasts and induces matrix metalloproteinases. Circ Res 2002;91(12):1127-34.

186. Kohno M, Horio T, Yokokawa K, et al. Brain natriuretic peptide as a cardiac hormone in essential hypertension. Am J Med 1992;92(1):29-34.

187. Sumida H, Yasue H, Yoshimura M, et al. Comparison of secretion pattern between A-type and B-type natriuretic peptides in patients with old myocardial infarction. J Am Coll Cardiol 1995;25(5):1105-10.

188. Sawada Y, Suda M, Yokoyama H, et al. Stretch-induced hypertrophic growth of cardiocytes and processing of brain-type natriuretic peptide are controlled by proprotein-processing endoprotease furin. J Biol Chem 1997;272(33):20545-54.

189. Lanfear DE, Stolker JM, Marsh S, et al. Genetic variation in the B-type natiuretic peptide pathway affects BNP levels. Cardiovasc Drugs Ther 2007;21(1):55-62.

190. Hall C. NT-ProBNP: the mechanism behind the marker. J Card Fail 2005;11(5):S81-3.

191. Martinez-Rumayor A, Richards AM, Burnett JC, et al. Biology of the natriuretic peptides. Am J Cardiol 2008;101(3):S3-8.

192. Kemperman H, van den Berg M, Kirkels H, et al. B-type natriuretic peptide (BNP) and N-terminal proBNP in patients with end-stage heart failure supported by a left ventricular assist device. Clin Chem 2004;50(9):1670-2.

193. Hunt P, Richards A, Nicholls M, et al. Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment. Clin Endocrinol (Oxf) 1997;47(3):287-96.

194. Takeda T, Kohno M. Brain natriuretic peptide in hypertension. Hypertens Res 1995;18(4):259-66.

195. Choi EY, Bahrami H, Wu CO, et al. N-terminal pro-B-type natriuretic peptide, left ventricular mass, and incident heart failure: Multi-Ethnic Study of Atherosclerosis. Circ Heart Fail 2012;5(6):727-34.

196. Weber M, Mitrovic V, Hamm C. B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide - Diagnostic role in stable coronary artery disease. Exp Clin Cardiol 2006;11(2):99-101.

197. Clayton R. Cardiovascular function in acromegaly. Endocr Rev 2003;24(3):272-7.

198. Petretta M, Colao A, Sardu C, et al. NT-proBNP, IGF-I and survival in patients with chronic heart failure. Growth Horm IGF Res 2007;17(4):288-96.

199. Wallaschofski H, Saller B, Spilcke-Liss E, et al. Effects of growth hormone treatment on B-type natriuretic peptide as a marker of heart failure in adults with growth hormone deficiency. Horm Metab Res 2006;38(10):656-61.

200. Gruson D, Alexopoulou O, Pasquet A, et al. Impact of growth hormone (GH) treatment on circulating Nt-proBNP concentrations and on cardiac function in adult GH-deficient patients. Scand J Clin Lab Invest 2012;72(5):387-94.

201. Das SR, Drazner MH, Dries DL, et al. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the Dallas Heart Study. Circulation 2005;112(14):2163-8.

202. Maisel AS, Clopton P, Krishnaswamy P, et al. Impact of age, race, and sex on the ability of B-type natriuretic peptide to aid in the emergency diagnosis of heart failure: results from the Breathing Not Properly (BNP) multinational study. Am Heart J 2004;147(6):1078-84.

203. Kruger R, Schutte R, Huisman HW, et al. NT-proBNP and potential vascular calcification in Black and Caucasian African men: the SAfrEIC study. Ethn Dis 2012;22(4):398.

204. Platz EA, Pollak MN, Rimm EB, et al. Racial variation in insulin-like growth factor-1 and binding protein-3 concentrations in middle-aged men. Cancer Epidemiol Biomarkers Prev 1999;8(12):1107-10.

205. Schutte AE, Schutte R, Huisman HW, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. Int J Epidemiol 2012;41(4):1114-23.

206. Connor MD, Thorogood M, Casserly B, et al. Prevalence of stroke survivors in rural South Africa: results from the Southern Africa Stroke Prevention Initiative (SASPI) Agincourt field site. Stroke 2004;35(3):627-32.

207. Vorster H. The emergence of cardiovascular disease during urbanisation of Africans. Public Health Nutr 2002;5(1a):239-43.

208. Alberts M, Urdal P, Steyn K, et al. Prevalence of cardiovascular diseases and associated risk factors in a rural black population of South Africa. Eur J Cardiovasc Prev Rehabil 2005;12(4):347-54.

Chapter 2

Study Design and Methodology

Within this thesis data is used form two cross-sectional studies conducted in South Africa, namely the SABPA and the SAfrEIC studies. The methodology of each of these studies is discussed below:

1. The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study:

1.1. STUDY DESIGN

The SABPA I study is a cross-sectional study conducted in 2008 and 2009 with the original aim to determine neural mechanistic pathways involved in emotional distress and vascular remodelling in black and white South Africans.[1] However, data on a detailed range of health measures were collected, and were explored in secondary data analyses in the present study.

The target population for this study included urban-dwelling black and white male and female school teachers enrolled in the 43 schools of the Dr. Kenneth Kaunda Education District (Klerksdorp and Potchefstroom), North West province, South Africa (Figure 1). The reason for this selection was an attempt to attain a homogenous sample from a similar socio-economic class.

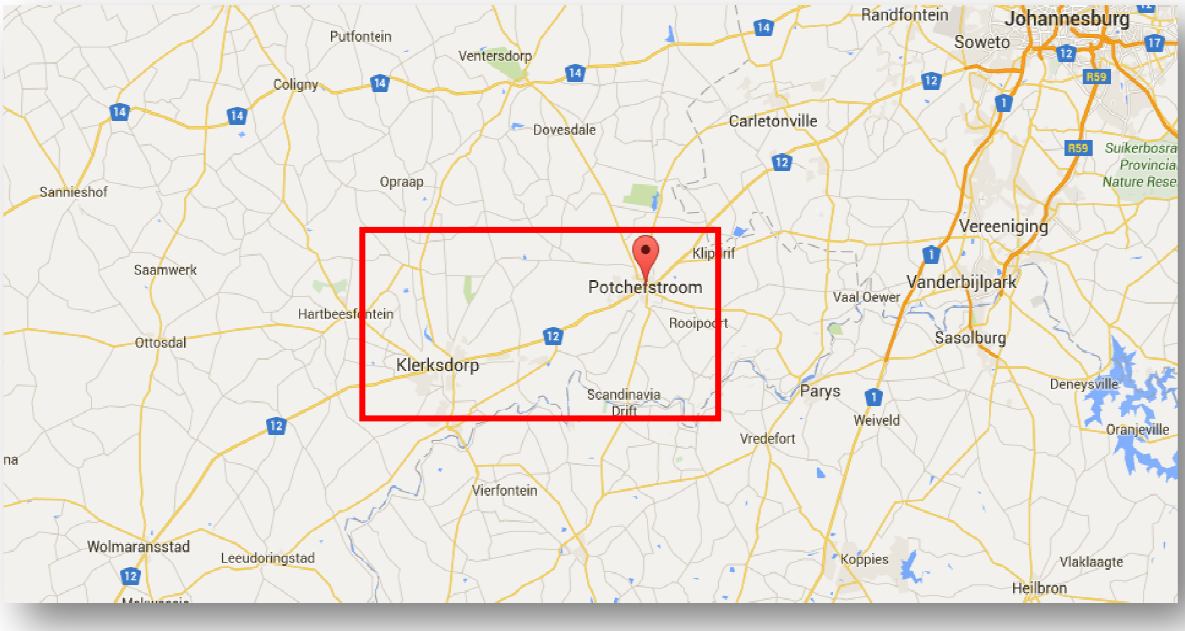


Figure 1: Geographical location of Klerksdorp and Potchefstroom in the North West province, South Africa.

During the recruitment phase all volunteering teachers were screened to determine if they met the study eligibility criteria and 409 teachers were selected (Figure 2). Power calculations based on the largest standard deviation of 24h systolic blood pressure showed that 50 participants per group in this type of study were more than sufficient to show significant differences in biological profiles. Participants were informed in detail in English and native languages about the nature, benefits, risks, objectives and procedures of the study, prior to their inclusion. All participants signed an informed consent form.

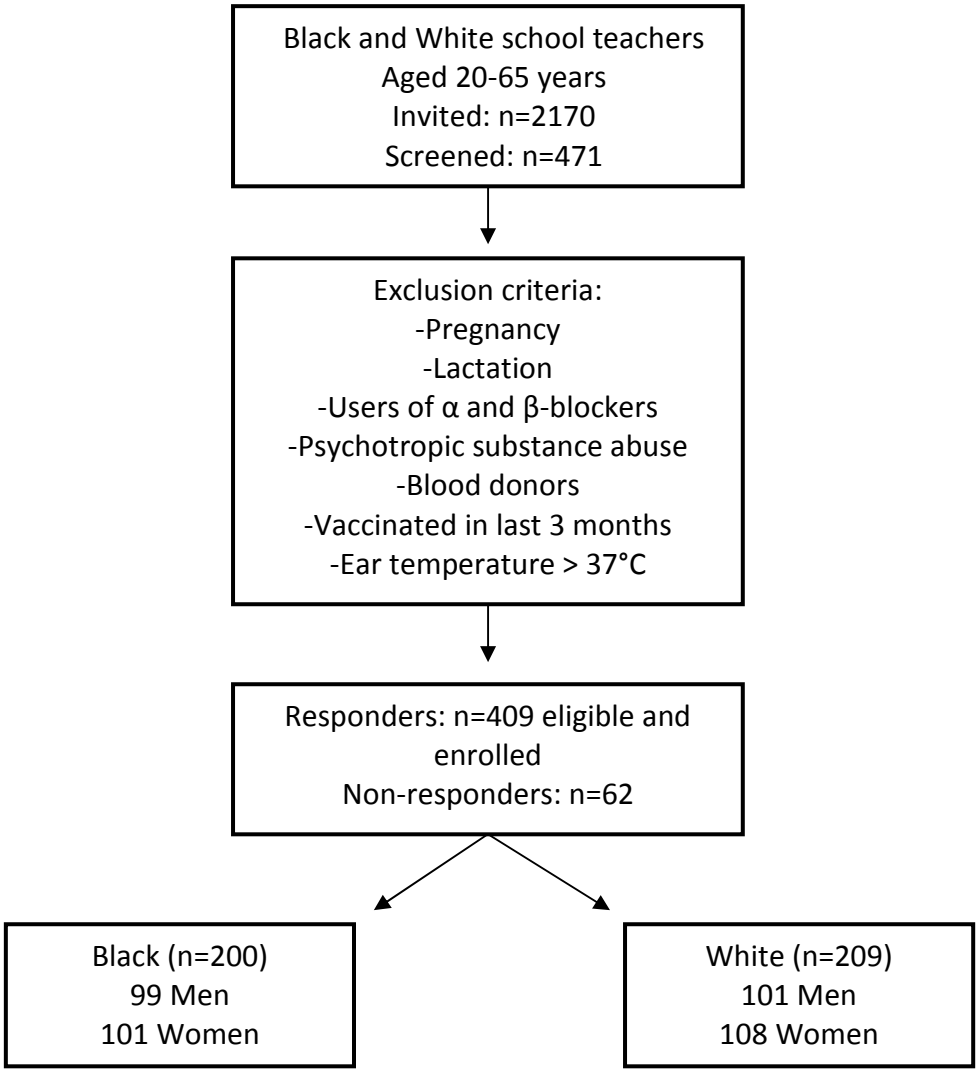


Figure 2: The Sympathetic activity and Ambulatory Blood pressure in Africans (SABPA) study population.

The SABPA study complied with all the stipulations of the Declaration of Helsinki (1975, revised 2004) for investigation of human participants and was approved by the Ethics Committee of the North-West University (NWU-00036-07-S6). A consent and cooperation agreement were obtained from the Department of Education, North-West Province, South African Democratic Teacher Unions and Headmasters of Schools.

A detailed layout of the experimental protocol and data collection procedures was previously described.[1] The methodology appropriate to this sub-study is discussed in more detail below.

1.2. ORGANISATIONAL PROCEDURES

Each morning between 07h00 and 08h00 an ambulatory blood pressure (ABPM) and electrocardiogram apparatus (MeditechCE120® Cardiotens, Budapest, Hungary) was attached to the participants' non-dominant arm at their workplace. Participants reported to the Metabolic Research Unit of the North-West University (consisting of 10 bedrooms, two bathrooms, a living room and kitchen) at 16h30 where they were informed of the procedures of the following day. Testing for the Human Immunodeficiency Virus (HIV) was performed, and each participant received pre-counselling and post-counselling during testing from a registered nurse. Participants received a standardised dinner at 18h00, and had their last beverages (tea/coffee) and two biscuits at 20h30. Thereafter they relaxed by reading, watching television, or social interaction and refrained from consuming alcohol, caffeine, smoking and doing exercise. They were encouraged to go to bed at around 22h00. An overnight spot urine sample was collected in the morning. At 06h00 the ABPM apparatus was removed and anthropometric measurements were taken. After a five minute resting period whilst in a sitting position, blood pressure measurements were taken. Following these measurements, a resting 12-lead electrocardiograph activity of six cardiac cycles (Norav NHH1200® Kiryat Bialik, Israel) was recorded after being in a semirecumbent position for two hours. Hereafter, a five minute continuous measurement of cardiovascular variables was recorded (Finometer, Finapres Medical Systems, Amsterdam, Netherlands). Blood samples were then collected from the participant's right arm brachial vein branches with a sterile winged infusion set.

After completion of all procedures, participants were thanked for their participation and enjoyed a breakfast. After freshening up, participants were transported back to their workplace and received feedback on their health profile within one week.

1.3. QUESTIONNAIRES

Participants completed a general health and sociodemographic questionnaires to assess lifestyle habits (such as smoking) as well as medication use. These included among others the use of hypertension, diabetes, lipid-lowering and anti-inflammatory medication.

1.4. ANTHROPOMETRIC MEASUREMENTS

Registered anthropometrists obtained anthropometric measurements in triplicate using standard methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Leicester, UK).[2,3] The stature of each participant was measured to the nearest 0.1cm, and the weight to the nearest 0.1kg. Waist circumference (WC) was measured over the abdomen between the costal margin and the iliac crest. Measurements were taken to the nearest 0.1 cm using a non-stretchable standard tape (Holtain Instruments Ltd. Wales). Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2).

1.5. CARDIOVASCULAR MEASUREMENTS

A validated ABPM apparatus (Meditech CE120® Cardiotens; Meditech, Budapest, Hungary) was programmed to measure blood pressure at 30 minute intervals during the day (08h00-22h00) and every hour during night-time (22h00-6h00). We downloaded ambulatory blood pressure data onto a database using the CardioVisions 1.9.0 Personal Edition software (Meditech, Budapest, Hungary). Office blood pressure (BP) was also measured with a mercury sphygmomanometer, using the Riva-Rocci/Korotkoff method,[4] on the non-dominant arm, whilst being in a sitting position. Two duplicate measures were taken with a five minute interval

between the two measurements. A resting 12- lead electrocardiograph activity of six cardiac cycles (Norav NHH1200® Kiryat Bialik, Israel) was recorded. Data from the 12-lead electrocardiography (ECG) was used to determine the gender specific Cornell product as a marker of left ventricular hypertrophy ((RaVL + SV3) × QRS ≥ 244.0 mV.ms for men and (RaVL + SV3 + 0.8 mV) × QRS ≥ 244.0 mV.ms for women).[5-7] Continuous BP was monitored by making use of a validated Finometer device (FMS, Finapres Medical System, Amsterdam, Netherlands).[5,8] This involved a five minute recording of each participant's BP under resting, yet awake, conditions. After the first two minutes, the finger pressure was calibrated with the upper arm (brachial) pressure (that is, return-to-flow systolic calibration). This optimised the accuracy of the readings taken. Finometer measurements were processed with Beatscope 1.1 software to obtain the stoke volume, cardiac output and total peripheral resistance during resting conditions. A high resolution ultrasound was applied to determine the carotid intima-media thickness (CIMT).[9] Standardised images[10] of the left and right common carotid arteries were obtained from at least two optimum angles using the Sonosite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) and 6 - 13 MHz linear array transducer. The images were digitised and imported into the Artery Measurement Systems automated software (AMS) II v1.139 (Gothenburg, Sweden). We calculated the cross-sectional wall area (CSWA) using the following equation: $CSWA = \pi (d/2 + CIMT)^2 – \pi (d/2)^2$, where d denotes luminal diameter.

1.6. BLOOD SAMPLING AND BIOCHEMICAL ANALYSES

A registered nurse obtained a fasting blood sample with a sterile winged infusion set from the antebrachial vein branches. Blood samples were stored at -80ºC until analyses were performed.

Serum samples for total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (γ-GT), creatinine and high-sensitivity C-reactive protein (CRP) were analysed using

two sequential multiple analysers (Konelab 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800 - Beckman and Coulter®, Germany). Low density lipoprotein cholesterol (LDL) was determined with the Friedewald formula: LDL = TC – HDL – TG/2.17.[11] The Chronic Kidney Disease Epidemiology Formulas (CKD-EPI) was used to calculate estimate glomerular filtration rate (eGFR) from serum creatinine levels, age, sex and ethnicity.[12]

Plasma samples for blood glucose determinations were collected in tubes containing sodium fluoride and centrifuged soon after blood collection. Blood glucose was determined using the Beckman and Coulter time-end method (Unicel DXC 800 - Beckman and Coulter®, Germany). The intra- and interassay coefficients of variation for standard biochemical measures performed with auto-analysers (Konelab 20i, Unicel DXC 800 - Beckman and Coulter) were <10%. The percentage of glycated haemoglobin (HbA1c) was determined by means of the turbidimetric inhibition immunoassay on whole blood using the Roche Integra 400 (Roche, Basel, Switzerland). Participants were considered as having diabetes if they had fasting glucose levels of ≥7.0 mmol/L or HbA1c levels of ≥6.5 % and/or made use of diabetes medication.[13]

Total serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) were determined with immunoradiometric assays (IRMA) from Immunotech (Beckman and Coulter®, Germany; IGF-1 – A15729; IGFBP-3 – DSL-6600). The intra- and interassay coefficients of variation for IGF-1 were 2.92%, and 4.49%, respectively. The intra-assay variability for IGFBP-3 ranged between 2.71% and 7.95%, and the inter-assay variability ranged between 3.20% and 9.30%.

Serum peroxides were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognised as an efficient method for evaluating reactive oxygen species (ROS) in the body.[14] The Bio-Tek FL600 Microplate

Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure ROS levels, where 1.0 mg/L H_2O_2 represents one unit of ROS. The intra- and interassay coefficients of variation for ROS were <10%.

Plasma Interleukin-6 (IL-6) was analysed using the IL-6 Quantikine high sensitivity enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN USA). The intra- and interassay coefficients of variation for IL-6 were 7.4%, and 17.0%, respectively. Citrated samples were used for the analysis of von Willebrand factor antigen (vWF_{ag}). vWF_{ag} levels were determined with a sandwich ELISA. Polyclonal rabbit anti-vWF antibody and rabbit anti-vWF-horseradish peroxidase antibody (DAKO, Glostrup, Denmark) were used to perform the assay. The 6th International Standard for vWF/FVIII was used to create the standard curve against which the samples were measured. N-terminal prohormone B-type natriuretic peptide (NT-proBNP) levels were determined with the electrochemiluminesence method on the e411 (Roche, Basel, Switzerland). The intra- and interassay coefficients of variation for NT-proBNP were 4.2%, and 4.6%, respectively. HIV status was determined with an antibody test First Response Kit (Premier Medical Corporation LTD, Daman, India) and confirmatory Pareekshak test (Bhat Bio-tech India (P) LTD, Bangalore, India).

2. The South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) study:

2.1. STUDY DESIGN

The SAfrEIC study had a cross-sectional design and was conducted in 2007. The general aim of this study was to investigate the influence of sex, ethnicity and ageing on the relationship between insulin sensitivity and blood pressure. For the purposes of this thesis the existing larger dataset was also applied to address some of the specific aims.

Trained field workers recruited volunteers from urban areas of the Potchefstroom district in the North West province of South Africa, which included black and white men and women. Prior to this study all the participants that were apparently ill, pregnant or lactating were excluded from the study.

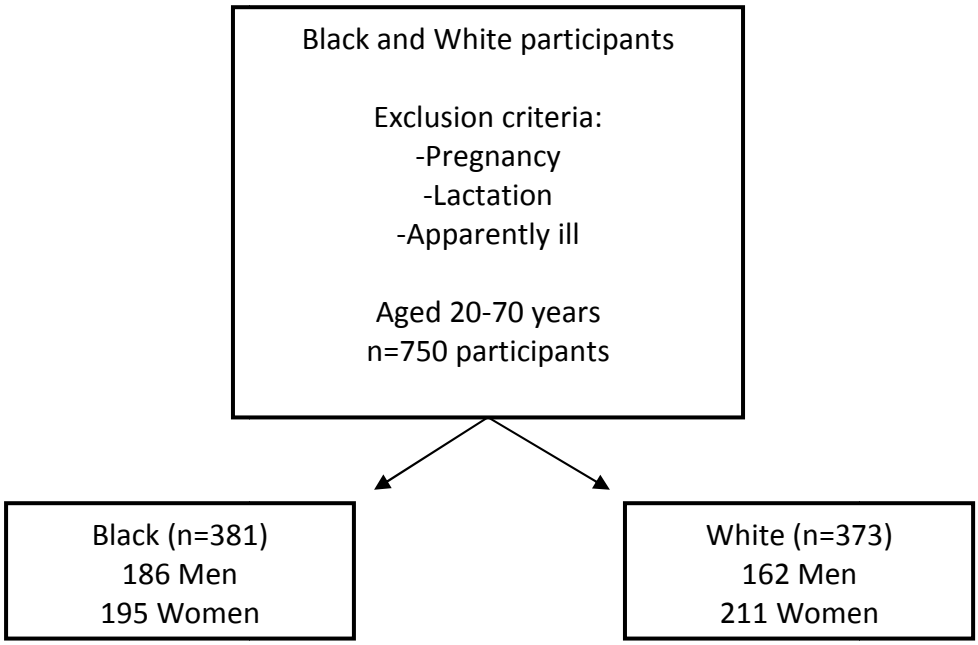


Figure 3: The SAfrEIC (South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) study population.

Figure 4: Data collection at the Metabolic Research Unit facility, North West University, South Africa.

The Ethics Committee of the North-West University (Potchefstroom campus) approved this study and the study complied with all the stipulations of the Declaration of Helsinki (1975,

revised 2004) for investigation of human participants. All participants gave written informed consent after the procedures were thoroughly explained to them. African field workers were available to relay information to the African participants in their home language.

A detailed layout of the experimental protocol and data collection procedures was previously described.[15,16] The specific methodology applicable to this sub-study is discussed below.

2.2. ORGANISATIONAL PROCEDURES

The participants were invited for a clinical examination at a Metabolic Unit facility on the campus of the North-West University. They arrived at 07h00 and were introduced to the research environment after which all the organisational procedures were explained to them and informed consent forms were signed. During the course of the morning, basic health and demographic questionnaires were completed. A fasting blood sample was collected from each participant by a registered nurse from the ante-brachial vein, using a sterile winged infusion set and syringe. Anthropometric measurements were subsequently taken in a private bedroom. Afterwards cardiovascular measurements were obtained. Each participant received a feedback report containing their basic health information, breakfast as well as financial compensation for travel expenses to the facility.

2.3. QUESTIONNAIRES

Each participant completed a demographic and lifestyle questionnaire, including questions on smoking and alcohol habits and medication use.

2.4. ANTHROPOMETRIC MEASUREMENTS

Anthropometric measurements were done by qualified anthropometrists according to standard procedures.[2,3] Height was measured to the nearest 0.1cm using the Invicta Stadiometer (Invicta Plastics Ltd, IP 1465, UK) and body weight was measured to the nearest 0.1kg using a calibrated digital scale (Precision Health Scale, A&D Company, Japan). WC was measured at midway level between the inferior rib margin and superior margin of the iliac crest. Measurements were taken to the nearest 0.1 cm using a non-stretchable standard tape (Holtain Instruments Ltd. Wales). All the measures were taken in triplicate. BMI was calculated by weight divided by height squared (kg/m^2).

2.5. CARDIOVASCULAR MEASUREMENTS

BP measurements were taken in duplicate after a 10 minute resting period and a five minute interval between the two measurements. The OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) apparatus was used to measure systolic and diastolic blood pressure with the cuff on the left upper arm in the sitting position. Appropriate cuff sizes were used for obese participants.

2.6. BLOOD SAMPLING AND BIOCHEMICAL ANALYSES

Fasting blood samples were obtained by a registered nurse. A plasma sample for fasting blood glucose was collected in tubes containing sodium fluoride, kept on ice and centrifuged. Glucose and serum samples for lipids (TC, HDL-C, triglycerides), AST, ALT, γ-GT and CRP were analysed using the Konelab 20i auto-analyser (Thermo Scientific, Vantaa, Finland). Participants were considered as having diabetes if they had fasting glucose levels of ≥7.0 mmol/L and/or made use of diabetes medication.[13] The intra- and interassay coefficients of variation for standard biochemical measures performed with the Konelab 20i auto-analyser were <10%. LDL was determined with the Friedewald formula: LDL = TC – HDL – TG/2.17.[11]

Total IGF-1 was determined using an ELISA by BiocodeHycel (Liége, Belgium, catalog no. EL2010). The intra- and interassay coefficients of variation for IGF-1 were 15.1% and 7.9%, respectively. IGFBP-3 was determined with an IRMA method from Immunotech (Beckman and Coulter®, Germany; IGFBP-3 – DSL-6600). The intra-assay variability for IGFBP-3 ranged between 2.7% and 7.9%, and the inter-assay variability ranged between 3.2% and 9.3%. Serum ROS were determined by a similar method as previously described for the SABPA study. The intra- and interassay coefficients of variation for ROS were <10%. The 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 the Pareeshak test for confirmation.

3. REFERENCES

1. Malan L, Hamer M, Frasure-Smith N, et al. Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study. Int J Epidemiol 2015;44(6):1814-1822.
2. Marfell-Jones MJ, Olds T, Stewart A, et al. International standards for anthropometric assessment: International Society for the Advancement of Kinanthropometry (ISAK); 2006.
3. Norton K, Olds T, Commossion AS. Anthropometrica: a textbook of body measurement for sports and health courses: University of New South Wales Press; 1996.
4. Verrij E, Van Montfrans G, Bos J. Reintroduction of Riva-Rocci measurements to determine systolic blood pressure. Neth J Med 2008;66(11):480-482.
5. Guelen I, Westerhof BE, van der Sar GL, et al. Validation of brachial artery pressure reconstruction from finger arterial pressure. J Hypertens 2008;26(7):1321-1327.
6. Julius S, Alderman MH, Beevers G, et al. Cardiovascular risk reduction in hypertensive black patients with left ventricular hypertrophy: the LIFE study. J Am Coll Cardiol 2004;43(6):1047-1055.
7. Okin PM, Roman MJ, Devereux RB, et al. Electrocardiographic identification of increased left ventricular mass by simple voltage-duration products. J Am Coll Cardiol 1995;25(2):417-423.
8. Imholz BP, Wieling W, van Montfrans GA, et al. Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. Cardiovasc Res 1998;38(3):605-616.
9. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force

endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21(2):93-111.

10. Liang YL. Non-invasive measurements of arterial structure and function: repeatability, interrelationships and trial sample size. Clin Sci 1998;95(6):669-679.
11. Esteban-Salan M, Aguilar-Doreste JA, Arranz-Pena ML, et al. Multicentric evaluation of the homogeneous LDL-cholesterol Plus assay: Comparison with beta-quantification and Friedewald formula. Clin Biochem 2008;41(16):1402-1409.
12. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150(9):604-12.
13. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2016;39:S13-22.
14. Hayashi I, Morishita Y, Imai K, et al. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat Res 2007;631(1):55-61.
15. Schutte AE, Huisman HW, van Rooyen JM, et al. A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. J Clin Endocrinol Metab 2010;95(5):2503-2507.
16. Palmer I, Schutte A, Huisman H. Ethnic and gender differences regarding the insulin–blood pressure relationship. Diabetes Res Clin Pract 2009;85(1):102-110.

Chapter 3

Bioavailable IGF-1 and its relation to the metabolic syndrome in a bi-ethnic population of men and women

Endocrine Research

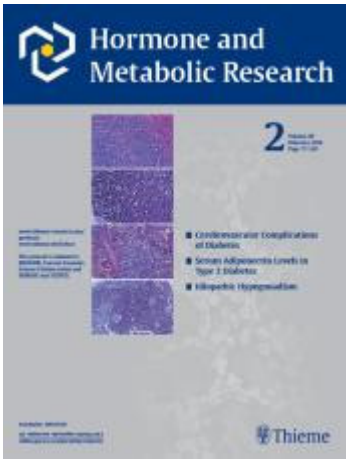
Bioavailable IGF-1 and its Relation to the Metabolic Syndrome in a Bi-Ethnic Population of Men and Women

Authors

A. S. E. Koegelenberg[1], R. Schutte[1,2], W. Smith[1], A. E. Schutte[1,2]

Affiliations

[1] Hypertension In Africa Research Team (HART), North-West University, Potchefstroom, South Africa
[2] MRC Research Unit for Hypertension and Cardiovascular disease, North-West University, Potchefstroom, South Africa



This manuscript was published in *Hormone and Metabolic Research*, Volume 2, Pages 130-136, 2016

(Annexure C, page 159)

ABSTRACT

Aims: Insulin-like growth factor-1 (IGF-1), an insulin sensitivity and vasculoprotective factor, associates negatively with the metabolic syndrome. However, IGF-1 is reduced by factors such as inflammation, oxidative stress and liver dysfunction. We investigated the relationship between bioavailable IGF-1 and the number of metabolic syndrome components and determined whether this relationship is independent of inflammation, oxidative stress and gamma-glutamyl transferase (γ-GT; a marker of liver dysfunction).

Methods: This study included 907 black and white participants stratified by sex (aged 43.0 ± 11.8 years). Among them 63 participants had fasting glucose levels of ≥7.0 mmol/L and/or used diabetes medication. Via standard methods we determined waist circumference, fasting glucose, triglycerides, high-density lipoprotein cholesterol and blood pressure. We also determined high-sensitivity C-reactive protein (CRP), reactive oxygen species (ROS), γ-GT, IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3). We used IGF-1/IGFBP-3 as an estimate of bioavailable IGF-1.

Results: Total IGF-1 was similar between men and women ($p=0.10$), however bioavailable IGF-1 was lower in women ($p<0.001$). In multivariate-adjusted analyses, IGF-1/IGFBP-3 was inversely associated with the number of metabolic syndrome components in both sexes (men: $\beta=-0.11$; $p=0.013$ and women: $\beta=-0.17$; $p=0.003$). Upon inclusion of ROS, γ-GT and CRP, significance was lost. In patients without diabetes, the results for men changed marginally, but were consistent for women.

Conclusion: We found an inverse association between bioavailable IGF-1 and the number of metabolic syndrome components. But the relationship was dependent on oxidative stress, liver dysfunction and inflammation, suggesting underlying processes by which the metabolic syndrome attenuates IGF-1.

Keywords: metabolic syndrome; insulin-like growth factor binding protein-3; inflammation; oxidative stress; γ-glutamyltransferase

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) has several metabolic and vasculoprotective effects. IGF-1 induces nitric oxide (NO) release [1]; it enhances insulin sensitivity [2] and glucose uptake [3]; prevents postprandial dyslipidemia [4]; reduces gluconeogenesis and scavenges free oxygen radicals [3]. IGF-1 also has anti-inflammatory properties [5]. The biological effects of IGF-1 are modulated by six different insulin-like growth factor binding proteins (IGFBPs) which bind to IGF-1 increasing its half-life, manages delivery to tissues and regulates movement between intravascular and extravascular compartments [6]. Approximately 80% of total IGF-1 is bound in serum by IGFBP-3, while the remaining 20% is bound by the other five IGFBPs [6]. Therefore, the molar ratio of IGF-1 and IGFBP-3 could be used as a surrogate measure for bioavailable IGF-1.

IGF-1 is reduced in individuals suffering from cardiovascular and metabolic diseases [7,8]. Cardiometabolic factors such as hypertension [8-10], hyperglycaemia [9], hypertriglyceridaemia [8], reduced high-density lipoprotein (HDL) cholesterol and abdominal obesity [8] are all inversely associated with IGF-1. These cardiometabolic factors aggregate together as part of the metabolic syndrome [11], which itself is negatively associated with IGF-1 [8,12]. A negative association also exists between the number of metabolic syndrome components present and IGF-1 [12,13].

In addition, circulating IGF-1 is adversely affected by various factors including inflammation [14,15], oxidative stress [16] and liver dysfunction [17] and it is undetermined whether the relationship between IGF-1 and the number of metabolic syndrome components are

independent of these factors. We therefore examined the relationship of bioavailable IGF-1 with the number of metabolic syndrome components in men and women; and how this relationship is modulated by inflammation, oxidative stress and liver dysfunction to identify the possible underlying pathophysiological mechanisms between IGF-1 and the metabolic syndrome.

SUBJECTS AND METHODS

Study design and subject selection

We included data from the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) and the SAfrEIC (South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) studies conducted by the same research team using similar methodology. The SABPA study was conducted in 2008 and 2009, and included 409 black and white teachers between the ages of 25 and 65 years in the North West province of South Africa. The SAfrEIC study conducted in 2007 involved 750 black and white volunteers from urban areas of the North West Province of South Africa (aged 20-70 years). Exclusion criteria for the SABPA study were participants with an elevated ear temperature, making use of α- and β-receptor blockers, psychotropic substance dependence or abuse, blood donors, individuals vaccinated in the past three months and pregnant women. The SAfrEIC study excluded participants that were apparently ill, pregnant or lactating. For the purpose of this study we excluded participants due to missing data (n=233) and those infected with the human immunodeficiency virus *(*HIV) (n=19*).* The overall sample of this sub-study consisted of 907 participants divided into men (n=457) and women (n=450) of whom 63 individuals were having fasting glucose levels of ≥7.0 mmol/L and/or made use of diabetes medication. Approval for both the SABPA and the SAfrEIC studies was obtained from the Ethics Committee of the North-West University adhering to the principles of the Declaration of Helsinki. All participants gave written informed consent after the procedures of the study were thoroughly

explained to them. The organisational procedures of both studies were described in detail elsewhere [17,18].

Anthropometric measurements

Height (stature) and weight of participants were measured while being in their underwear. Measurements were taken in triplicate using standard methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Leicester, UK) [19]. Waist circumference (WC) was measured over the abdomen between the costal margin and the iliac crest. Measurements were taken to the nearest 0.1 cm using a non-stretchable standard tape (Holtain Instruments Ltd. Wales). Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2).

Blood pressure measurements

In the SAfrEIC study blood pressure (BP) measurements were taken in duplicate after a 10 min resting period and a five min interval between the two measurements. The OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) apparatus was used to determine systolic and diastolic blood pressure with the cuff on the left upper arm in the sitting position. In the SABPA study participants rested for five minutes in a semi-recumbent position before the first measurement was taken. BP was measured with a mercury sphygmomanometer, using the Riva-Rocci/Korotkoff method [20], on the non-dominant arm. Two duplicate measures were taken with a five minute interval between the two measurements.

Biochemical analyses

Serum samples for lipids (total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides), aspartate aminotranferase (AST), alanine transaminase (ALT), and high-sensitivity C-reactive protein (CRP) were analysed using the Konelab 20i auto-analyser

(Thermo Scientific, Vantaa, Finland (2008)). Low density lipoprotein cholesterol (LDL) was determined with the Friedewald formula: LDL = TC – HDL – TG/2.17. In the SAfrEIC study a venous sample for fasting blood glucose was collected in tubes containing sodium fluoride, kept on ice and centrifuged. In the SABPA study, these sodium fluoride tubes were centrifuged soon after blood collection. During the SAfrEIC study glucose and gamma-glutamyl transferase (γ-GT) were determined using the Konelab 20i auto-analyser (Thermo Scientific, Vantaa, Finland), while during the SABPA study glucose and γ-GT were determined using Beckman and Coulter time-end method (Unicel DXC 800 - Beckman and Coulter®, Germany (2009)). Participants were considered as having diabetes if they had fasting glucose levels of ≥7.0 mmol/L and/or made use of diabetes medication [21]. The intra- and interassay coefficients of variation for standard biochemical measures performed with auto-analysers (Konelab 20i, Unicel DXC 800 - Beckman and Coulter) were <10%. In the SABPA study total IGF-1 was determined with an immunoradiometric assay (IRMA) from Immunotech (Beckman and Coulter®, Germany; IGF-1 – A15729). The intra- and interassay coefficients of variation for IGF-1 were 2.92%, and 4.49%, respectively. In the SAfrEIC study we determined total IGF-1 using an enzyme linked immunosorbert assay (ELISA) by BiocodeHycel (Liége, Belgium, catalog no. EL2010). The intra- and interassay coefficients of variation for IGF-1 were 15.08% and 7.89%, respectively. The properties of these two IGF-1 methods are comparable (Table 1S). IGFBP-3 was determined with an IRMA method from Immunotech (Beckman and Coulter®, Germany; IGFBP-3 – DSL-6600) in both the SABPA and SAfrEIC studies. The intra-assay variability for IGFBP-3 ranged between 2.71% and 7.95%, and the inter-assay variability ranged between 3.20% and 9.30%, for both studies. Serum reactive oxygen species (ROS) were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognised as an efficient method for evaluating oxidative stress in the body. The Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure serum peroxide levels, where 1.0 mg/L H_2O_2

represents one unit of ROS [22]. The intra- and interassay coefficients of variation for ROS were <10%. HIV testing was done according to standard procedures of the South African Department of Health.

Statistical Analyses

For database management and statistical analyses we used Statistica version 12.0 (StatSoft, Inc.,Tulsa, OK).The associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components were tested for interaction with sex, ethnicity or study type by introducing appropriate interaction terms in multiple regression analyses. Variables that were not normally distributed were logarithmically transformed (BMI, fasting glucose, triglycerides, CRP, γ-GT, AST and ALT). We subsequently compared means and proportions between men and women with independent T-tests and Chi-square tests, respectively. We explored the IGF-1 and IGF-1/IGFBP-3 distribution according to the number of metabolic syndrome components by using analyses of variance (ANOVA) and analyses of covariance (ANCOVA). The number of metabolic syndrome components was calculated using the criteria for the metabolic syndrome compiled by the International Diabetes Federation and other international bodies in 2009 [11]. We used single and multiple regression analyses to determine the associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components. The models included the following independent variables: age, ethnicity, study type and the number of metabolic syndrome components. We then further explored how this relationship is modulated by inflammation, oxidative stress and γ-GT. The models then included age, ethnicity, study type, the number of metabolic syndrome components, CRP, ROS and γ-GT as independent variables. The multi-variable adjusted analyses were performed in the total group and repeated upon exclusion of individuals with diabetes.

RESULTS

We stratified the 907 participants according to sex due to significant interactions with sex regarding the associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components ($p<0.001$ and $p=0.010$, respectively). No significant interactions were found for ethnicity or study type (all $p>0.05$).

Table 1 displays the characteristics of the men and women (Table 2S for each study, respectively). Despite a younger mean age for men compared to women (41.9 versus 44.1 years, $p=0.006$), men showed a higher mean waist circumference ($p<0.001$), triglycerides ($p<0.001$), glucose ($p<0.001$) and BP ($p<0.001$), accompanied by lower HDL-C ($p<0.001$). γ-GT were significantly higher in men ($p<0.001$) while women had higher levels of CRP and ROS (all $p<0.001$). IGF-1 did not differ between men and women ($p=0.101$), whereas women had higher IGFBP-3 ($p<0.001$) and a lower IGF-1/IGFBP-3 ($p<0.001$).

Table 1: Comparison of cardiovascular, biochemical and anthropometric measurements of men and women.

	Men (n=457)	**Women (n=450)**	***p* value**
Age (yrs)	41.9 ± 12.2	44.1 ± 11.4	**0.006**
Ethnicity, black, n (%)	200 (43.8)	184 (40.9)	0.38
Anthropometric measurements			
Body mass index (kg/m^2)	25.8 (17.7; 37.6)	27.5 (19.3; 41.7)	**<0.001**
Waist circumference (cm)	91.0 ± 16.7	85.9 ± 15.0	**<0.001**
Biochemical measures			
IGF-1 (ng/ml)	157 ± 73.1	150 ± 70.2	0.10
IGFBP-3 (nmol/l)	134 ± 33.9	144 ± 28.1	**<0.001**
IGF-1/IGFBP-3	1.17 ± 0.45	1.03 ± 0.44	**<0.001**
Fasting glucose (mmol/L)	5.56 (4.31; 7.32)	5.26 (4.19; 6.90)	**<0.001**
Total cholesterol (mmol/L)	5.22 ± 1.39	5.27 ± 1.47	0.64
High-density lipoprotein cholesterol (mmol/L)	1.26 ± 0.56	1.43 ± 0.42	**<0.001**
Low-density lipoprotein cholesterol (mmol/L)	3.27 ± 1.27	3.30 ± 1.26	0.70
Triglycerides (mmol/L)	1.27 (0.54; 3.17)	1.01 (0.46; 2.51)	**<0.001**
C-reactive protein (mg/L)	1.66 (0.11; 14.5)	2.52 (0.13; 25.6)	**<0.001**
Reactive oxygen species (mg/L)	78.8 ± 18.3	98.2 ± 27.9	**<0.001**
Gamma-glutamyl transferase (U/L)	46.3 (16.0; 237)	28.6 (8.00; 183)	**<0.001**
Aspartate aminotrasferase (U/L)	25.8 (14.3; 69.0)	19.9 (11.8; 44.0)	**<0.001**
Alanine transaminase (U/L)	23.7 (9.62; 66.3)	15.0 (5.63; 44.6)	**<0.001**
Cardiovascular measurements			
Systolic blood pressure (mmHg)	130 ± 17.6	122 ± 18.9	**<0.001**
Diastolic blood pressure (mmHg)	84.8 ± 12.6	81.1 ± 11.4	**<0.001**
Lifestyle measures			
Smoking self-reported, n (%)	155 (34.0)	86 (19.2)	**<0.001**
Intake of medication			
Anti-hypertensive medication, n (%)	81 (17.8)	76 (16.9)	0.73
Anti-diabetic medication, n (%)	7 (1.53)	3 (0.67)	0.21
Lipid-lowering medication, n (%)	18 (3.94)	17 (3.78)	0.90
Diabetes, n (%)	40 (8.75)	23 (5.11)	0.031
*Hypertensive, n (%)	146 (32.0)	110 (24.4)	**0.015**

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables.
n, number of participants; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3
*Classification as hypertensive: ≥140/90 mmHg.

In Table 2 we compared IGF-1, IGF-1/IGFBP-3 and the cardiometabolic profile according to the number of metabolic syndrome components. As expected, both sexes showed that waist circumference, glucose, triglycerides and BP increased significantly (all p<0.001), and HDL-C decreased (p<0.001) as the number of metabolic syndrome components increased. For men we

found a tendency of decreasing IGF-1/IGFBP-3 (p for trend=0.078), but IGF-1 decreased as the number of metabolic syndrome components increased (p for trend=0.032). In women, IGF-1 and IGF-1/IGFBP-3 decreased significantly with the increase in metabolic syndrome components (both p for trend<0.001, Table 2). As shown in Figure 1, this decrease persisted after adjusting for age, ethnicity and study type (p for trend<0.001). However, the decrease of IGF-1 in men was not significant after adjustments (p for trend=0.240).

Table 2: Mean values of IGF-1, IGF-1/IGFBP-3 ratio and metabolic syndrome components in categories stratified according to the number of metabolic syndrome components.

	Number of metabolic syndrome components			***p for trend***
	0	**1-2**	**>3**	
	Men			
n	73	196	186	
IGF-1 (ng/ml)	166 ± 79.9	147 ± 73.4	164 ± 69.3	0.032
IGF-1/IGFBP-3	1.28 ± 0.53	1.14 ± 0.24	1.15 ± 0.44	0.078
Waist circumference (cm)	74.8 ± 8.12	84.9 ± 14.1*	104 ±12.2*	<0.001
Glucose (mmol/L)	4.83 (4.03; 5.49)	5.32 (4.22; 6.42)*	6.12 (4.96; 8.91)*	<0.001
Triglycerides(mmol/L)	0.80 (0.42; 1.38)	1.03 (0.54; 2.02)*	1.90 (0.87; 4.44)*	<0.001
High density lipoprotein (mmol/L)	1.50 ± 0.60	1.44 ± 0.61	0.98 ± 0.31*	<0.001
Systolic blood pressure (mmHg)	116 ± 7.56	131 ± 17.9*	136 ± 17.2*	<0.001
Diastolic blood pressure (mmHg)	74.3 ± 5.88	84.4 ± 12.7*	89.2 ± 11.8*	<0.001
	Women			
n	80	211	157	
IGF-1 (ng/ml)	190 ± 79.3	148 ± 67.3*	129 ± 60.3*	<0.001
IGF-1/IGFBP-3	1.22 ± 0.47	1.06 ± 0.44*	0.90 ± 0.39*	<0.001
Waist circumference (cm)	71.0 ± 5.31	83.5 ± 12.8*	96.6 ± 13.1*	<0.001
Glucose (mmol/L)	4.76 (4.05; 5.50)	5.02 (4.22; 6.01)*	5.89 (4.21; 10.29)*	<0.001
Triglycerides (mmol/L)	0.74 (0.45; 1.30)	0.90 (0.43; 1.74)*	1.38 (0.61; 2.94)*	<0.001
High-density lipoprotein (mmol/L)	1.64 ± 0.25	1.48 ± 0.44*	1.26 ± 0.40*	<0.001
Systolic blood pressure (mmHg)	106 ± 9.10	121 ± 18.1*	132 ± 18.1*	<0.001
Diastolic blood pressure (mmHg)	71.0 ± 6.52	80.5 ± 11.1*	87.1 ± 9.81*	<0.001

n, number of participants; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3

*Significance of the difference from previous category (p<0.001).

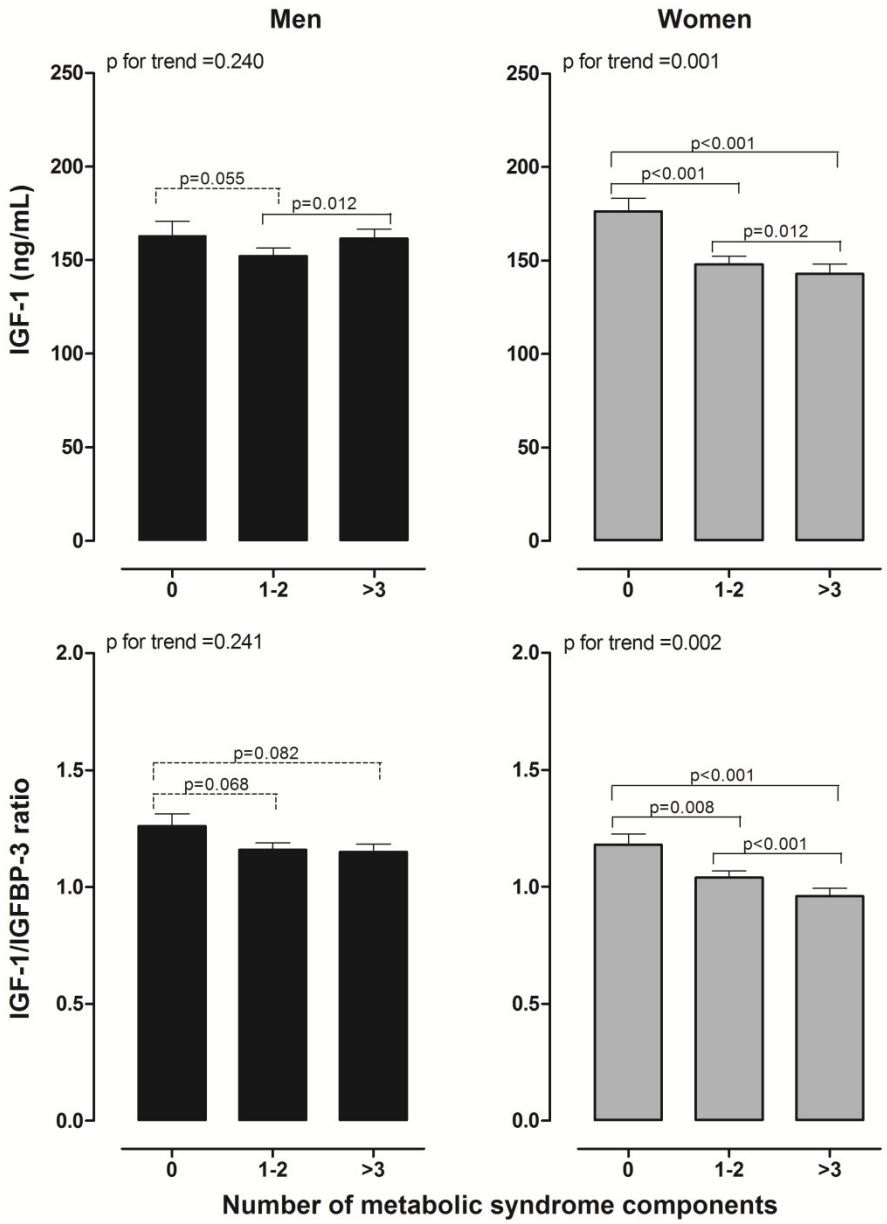


Figure 1: The IGF-1 and IGF-1/IGFBP-3 ratio distribution according to the number of metabolic syndrome components, adjusted for age, ethnicity and study type.

We performed multiple regression analyses with IGF-1 and IGF-1/IGFBP-3 as our dependent variables in men and women (Table 3). In Model 1 we included the number of metabolic syndrome components as well as age, ethnicity and study type as independent variables. We found in all instances a negative relationship between the number of metabolic syndrome components and IGF-1/IGFBP-3 in both sexes and with IGF-1 in women. Adjustments for CRP,

ROS and γ-GT nullified the relationships of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components (Model 2). CRP was not significantly associated with either IGF-1 or IGF-1/IGFBP-3, but ROS and γ-GT were prominent contributors to the variance of IGF-1 and IGFBP-3 in both sexes.

Table 3: Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and potential confounders.

	Men (n=457)		**Women (n=450)**	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2=0.30**	**R^2=0.14**	**R^2=0.21**	**R^2=0.20**
Age (yrs)	β=-0.41; p<0.001	β=-0.31 ; p<0.001	β=-0.36 ; p<0.001	β=-0.32 ; p<0.001
Ethnicity (White/African)	β=-0.38 ; p<0.001	β=-0.12 ; p=0.012	β=-0.12 ; p=0.004	β=0.10 ; p=0.016
Study (SAfrEIC/SABPA)	β=0.17 ; p<0.001	β=0.24 ; p<0.001	β=0.16 ; p<0.001	β=0.24 ; p<0.001
Number of MetS components (0-5)	-	β=-0.11 ; p=0.013	β=-0.11 ; p=0.014	β=-0.17 ; p=0.003
Model 2 (Model 1 + CRP, ROS, γ-GT)	**R^2=0.34**	**R^2=0.20**	**R^2=0.26**	**R^2=0.26**
Number of MetS components (0-5)	-	-	-	-
C-reactive protein log (mg/L)	-	-	-	-
ROS (mg/L)	β=-0.10 ; p=0.016	β=-0.13 ; p=0.008	β=-0.19 ; p<0.001	β=-0.23 ; p<0.001
γ-GT log (U/L)	β=-0.20 ; p<0.001	β=-0.26 ; p<0.001	-	β=-0.12 ; p=0.023

IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; ROS, reactive oxygen species; γ-GT, gamma-glutamyl transferase; MetS, metabolic syndrome.

A separate summary of the individual contribution of CRP, ROS and γ-GT to Model 1 is given in Table 3S. In sensitivity analyses we repeated Model 2 with smoking and the usage of anti-hypertensive medication as additional covariates. This did not affect the outcome of our results (Table 4S).

When repeating these analyses after excluding participants with fasting glucose levels of ≥7.0 mmol/L and/or using diabetes medication (Table 4), we found in Model 1 that the negative relationship between the number of metabolic syndrome components and IGF-1/IGFBP-3 remained significant in women, but marginally changed in men (β=-0.09 ; p=0.078). After including CRP, ROS and γ-GT as covariates, the relationship between IGF-1/IGFBP-3 and the number of metabolic syndrome components disappeared. Apart from ROS and γ-GT being

prominent contributors to the variance of IGF-1 and IGFBP-3 in men and women, CRP also related to IGF-1/IGFBP-3 in men (β=0.10 ; p=0.045), and IGF-1 in women (β=-0.11 ; p=0.043). To explore whether the aforementioned association between CRP and IGF-1/IGFBP-3 in men are possibly driven by obesity, we included waist circumference to the model and found that the association between CRP and IGF-1/IGFBP-3 became non-significant (β=0.085 ; p=0.089).

Table 4: Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and potential confounders in participants without diabetes.

	Men (n=417)		**Women (n=427)**	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2=0.31**	**R^2=0.14**	**R^2=0.20**	**R^2=0.19**
Age (yrs)	β=-0.42; p<0.001	β=-0.33 ; p<0.001	β=-0.37 ; p<0.001	β=-0.34 ; p<0.001
Ethnicity (White/African)	β=-0.38 ; p<0.001	β=-0.11 ; p=0.027	β=-0.12 ; p=0.008	β=0.11 ; p=0.015
Study (SAfrEIC/SABPA)	β=0.15 ; p<0.001	β=0.22 ; p<0.001	β=0.15 ; p<0.001	β=0.22 ; p<0.001
Number of MetS components (0-5)	-	β=-0.09 ; p=0.078	β=-0.12 ; p=0.015	β=-0.14 ; p=0.002
Model 2 (Model 1 + CRP, ROS, γ-GT)	**R^2=0.35**	**R^2=0.20**	**R^2=0.26**	**R^2=0.26**
Number of MetS components (0-5)	-	-	-	-
C-reactive protein log (mg/L)	-	β=0.10 ; p=0.050	β=-0.11 ; p=0.046	-
ROS (mg/L)	β=-0.09 ; p=0.035	β=-0.11 ; p=0.024	β=-0.18 ; p<0.001	β=-0.23 ; p<0.001
γ-GT log (U/L)	β=-0.20 ; p<0.001	β=-0.26 ; p<0.001	-	β=-0.12 ; p=0.025

IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; ROS, reactive oxygen species; γ-GT, gamma-glutamyl transferase; MetS, metabolic syndrome.

DISCUSSION

We examined the relationship of bioavailable IGF-1 with increasing cardiometabolic risk, and how this relationship is affected by inflammation, oxidative stress and liver dysfunction. We focused specifically on the whole population sample but repeated our analyses upon exclusion of patients with diabetes. We found an independent inverse association between bioavailable IGF-1 and the number of metabolic syndrome components in the whole population of men and women. However, significance of this relationship was lost after adjusting for CRP, ROS and γ-GT (as a marker of liver dysfunction), where both ROS and γ-GT were negatively associated with bioavailable IGF-1 in both sexes. When individuals with diabetes were excluded, the

negative association between bioavailable IGF-1 and the number of metabolic syndrome components remained significant in women, but a borderline association were found in men. Again in women the significance of the relationship was lost upon inclusion of CRP, ROS and γ-GT to the model.

In agreement with our findings, several studies reported a negative association between bioavailable IGF-1 and the number of metabolic syndrome components. Sierra-Johnson et al. examined 3281 subjects from the Third National Health and Nutrition Examination Survey (NHANES III), and found that bioavailable IGF-1 decreased significantly as the number of metabolic syndrome components increased [13]. In addition, Sesti et al. examined 509 European subjects of whom 20% were classified as having the metabolic syndrome. IGF-1 was also lower in subjects with the metabolic syndrome compared to those without [8]. However, none of these studies accounted for markers of inflammation, oxidative stress or γ-GT in their analyses.

When CRP, ROS and γ-GT were added to the regression model the link between bioavailable IGF-1 and the number of metabolic syndrome components became non-significant. Indeed both ROS and γ-GT were negatively associated with bioavailable IGF-1 in men and women. IGF-1 reduces oxidative stress and is responsible for free oxygen radical scavenging [3]. But oxidative stress also attenuates IGF-1 levels, given that oxidative stress particularly affects hepatocytes where IGF-1 is mainly synthesised [16,23]. Furthermore γ-GT, as a surrogate marker of liver damage and liver diseases such as alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD), are associated with reduced IGF-1 production [24-27]. However it is beyond the scope of this article to differentiate whether the increase in γ-GT is due to alcohol or NAFLD.

We did not find an independent association between CRP and bioavailable IGF-1 in the whole population. This was unexpected since CRP synthesis is stimulated by pro-inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor alpha (TNF-α) and IL-1 that are also known to decrease both circulatory and tissue concentrations of IGF-1 [28,29]. However, CRP has been shown to result in increased superoxide production [30], and therefore we might speculate that CRP attenuated IGF-1 via the process of oxidative stress. This was possibly represented by ROS that was independently related to bioavailable IGF-1 in our regression model. In the population without diabetes we found a positive association between CRP and bioavailable IGF-1 in men. However, this relationship disappeared upon inclusion of waist circumference to the model. Therefore we might speculate that the relationship between CRP and IGF-1 is driven by obesity since it is known that obesity and CRP are strongly associated [31].

Our finding that both ROS and γ-GT modulate the association between bioavailable IGF-1 and the number of metabolic syndrome components in men and women emphasise the fact that these components should be taken into account when investigating the link between IGF-1 and the metabolic syndrome. These results may reflect potential mechanistic pathways (namely via oxidative stress and liver dysfunction) by which the metabolic syndrome attenuates IGF-1 levels, given that the metabolic syndrome is associated with the overproduction of reactive oxygen species [32] and increased levels of γ-GT [33].

This study should be viewed in context of its strengths and limitations. A limitation of our study is that it consists of two different studies, but both studies used validated techniques. Thus, we pooled IGF-1 data that was measured with different assays (ELISA and IRMA) in the two studies. The γ-GT test is a non-specific measure that can be elevated with many types of liver diseases; therefore we did not specify a liver disease associated with the increase in γ-GT in

our study. The results of our study were consistent after multiple adjustments however, we cannot exclude residual confounding. In addition, due to the cross-sectional nature of this study, causality cannot be inferred. The strengths of this study include the availability of bioavailable IGF-1 (IGF-1/IGFBP-3) in a relatively large group of participants.

To conclude, cardiometabolic risk is inversely associated with bioavailable IGF-1, but this association is potentially mediated by oxidative stress, inflammation and liver dysfunction. These factors should be taken into account when investigating the link between IGF-1 and the metabolic syndrome. Therefore, strategies aimed at improving low-grade inflammation, oxidative stress and liver function in individuals that are metabolically compromised should be investigated due to their potentially attenuating effects on IGF-1.

Acknowledgements

The SABPA and SAfrEIC studies would not have been possible without the voluntary collaboration of the participants. We gratefully acknowledge the contribution of the research teams, and the Department of Education, South Africa (SABPA study), for their involvement in these studies. Research included in the present study was partially funded by the Medical Research Council, South Africa; Roche Diagnostics; the North-West University; and the Metabolic Syndrome Institute, France. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Schini-Kerth V.* Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells. J Endocrinol Invest 1999; 22: 82-88
2. *Conti E, Andreotti F, Sestito A, Riccardi P, Menini E, Crea F, Maseri A, Lanza GA.* Reduced levels of insulin-like growth factor-1 in patients with angina pectoris, positive exercise stress test, and angiographically normal epicardial coronary arteries. Am J Cardiol 2002; 89: 973-975
3. *Dimmeler S, Zeiher AM.* Exercise and Cardiovascular Health Get Active to "AKTivate" Your Endothelial Nitric Oxide Synthase. Circulation 2003; 107: 3118-3120
4. *Twickler MT, Cramer MM, Koppeschaar HP.* Unraveling Reaven's syndrome X: serum insulin-like growth factor-I and cardiovascular disease. Circulation 2003; 107: e190-e192
5. *Spies M, Nesic O, Barrow R, Perez-Polo J, Herndon D.* Liposomal IGF-1 gene transfer modulates pro-and anti-inflammatory cytokine mRNA expression in the burn wound. Gene Ther. 2001; 8: 1409-1415
6. *Conti E, Musumeci M, De Giusti M, Dito E, Mastromarino V, Volpe M.* IGF-1 and atherothrombosis: relevance to pathophysiology and therapy. Clin Sci 2011; 120: 377-402
7. *Vasan RS, Sullivan LM, D'Agostino RB, Roubenoff R, Harris T, Sawyer DB, Levy D, Wilson PW.* Serum insulin-like growth factor I and risk for heart failure in elderly individuals without a previous myocardial infarction: the Framingham Heart Study. Ann Intern Med 2003; 139: 642-648
8. *Sesti G, Sciacqua A, Cardellini M, Marini MA, Maio R, Vatrano M, Succurro E, Lauro R, Federici M, Perticone F.* Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care 2005; 28: 120-125

9. *Colao A, Di Somma C, Cascella T, Pivonello R, Vitale G, Grasso LF, Lombardi G, Savastano S.* Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: an observational, exploratory study in 404 subjects. Eur J Endocrinol 2008; 159: 389-397

10. *Hu Y, Pete G, Walsh MF, Sowers J, Dunbar JC.* Central IGF-1 decreases systemic blood pressure and increases blood flow in selective vascular beds. Horm Metab Res 1996; 28: 211-214

11. *Alberti K, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart J, James WPT, Loria CM, Smith SC.* Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120: 1640-1645

12. *Parekh N, Roberts CB, Vadiveloo M, Puvananayagam T, Albu JB, Lu-Yao GL.* Lifestyle, anthropometric, and obesity-related physiologic determinants of insulin-like growth factor-1 in the Third National Health and Nutrition Examination Survey (1988–1994). Ann Epidemiol 2010; 20: 182-193

13. *Sierra-Johnson J, Romero-Corral A, Somers V, Lopez-Jimenez F, Malarstig A, Brismar K, Hamsten A, Fisher R, Hellenius M.* IGF-I/IGFBP-3 ratio: a mechanistic insight into the metabolic syndrome. Clin Sci 2009; 116: 507-512

14. *De Benedetti F, Alonzi T, Moretta A, Lazzaro D, Costa P, Poli V, Martini A, Ciliberto G, Fattori E.* Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997; 99: 643-650

15. *Efstratiadis G, Tsiaousis G, Athyros VG, Karagianni D, Pavlitou-Tsiontsi A, Giannakou-Darda A, Manes C.* Total serum insulin-like growth factor-1 and C-reactive protein in metabolic syndrome with or without diabetes. Angiology 2006; 57: 303-311

16. *Scheidegger KJ, James RW, Delafontaine P.* Differential effects of low density lipoproteins on insulin-like growth factor-1 (IGF-1) and IGF-1 receptor expression in vascular smooth muscle cells. J Biol Chem 2000; 275: 26864-26869

17. *Schutte AE, Huisman HW, van Rooyen JM, Malan L, Malan NT, Fourie CMT, Louw R, van der Westhuizen FH, Schutte R.* A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. J Clin Endocrinol Metab 2010; 95: 2503-2507

18. *Hamer M, Malan L, Schutte A, Huisman H, Van Rooyen J, Schutte R, Fourie C, Malan N, Seedat Y.* Conventional and behavioral risk factors explain differences in sub-clinical vascular disease between black and Caucasian South Africans: The SABPA study. Atherosclerosis 2011; 215: 237-242

19. *Norton K, Olds T. Anthropometrica: a textbook of body measurement for sports and health courses*, University of New South Wales Press; 1996.

20. *Verrij E, Van Montfrans G, Bos J.* Reintroduction of Riva-Rocci measurements to determine systolic blood pressure. Neth J Med 2008; 66: 480-482

21. *American Diabetes Association.* Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37: 81-90

22. *Hayashi I, Morishita Y, Imai K, Nakamura M, Nakachi K, Hayashi T.* High-throughput spectrophotometric assay of reactive oxygen species in serum Mutat Res 2007; 631: 55-61

23. *Higashi Y, Peng T, Du J, Sukhanov S, Li Y, Itabe H, Parthasarathy S, Delafontaine P.* A redox-sensitive pathway mediates oxidized LDL-induced downregulation of insulin-like growth factor-1 receptor. J Lipid Res 2005; 46: 1266-1277

24. *Ichikawa T, Nakao K, Hamasaki K, Furukawa R, Tsuruta S, Ueda Y, Taura N, Shibata H, Fujimoto M, Toriyama K.* Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. Hepatology International 2007; 1: 287-294

25. *Wu D, Cederbaum AI.* Alcohol, oxidative stress, and free radical damage. Alcohol Res Health 2003; 27: 277-284

26. *Møller S, Juul A, Becker U, Henriksen JH.* The acid-labile subunit of the ternary insulin-like growth factor complex in cirrhosis: relation to liver dysfunction. J Hepatol 2000; 32: 441-446

27. *Bassanello M, De Palo EF, Lancerin F, Vitale A, Gatti R, Montin U, Ciarleglio FA, Senzolo M, Burra P, Brolese A.* Growth hormone/insulin-like growth factor 1 axis recovery after liver transplantation: A preliminary prospective study. Liver transplantation 2004; 10: 692-698

28. *Succurro E, Andreozzi F, Sciacqua A, Hribal ML, Perticone F, Sesti G.* Reciprocal association of plasma IGF-1 and interleukin-6 levels with cardiometabolic risk factors in nondiabetic subjects. Diabetes Care 2008; 31: 1886-1888

29. *Willerson JT, Ridker PM.* Inflammation as a cardiovascular risk factor. Circulation 2004; 109: II2-10

30. *Singh U, Devaraj S, Vasquez-Vivar J, Jialal I.* C-reactive protein decreases endothelial nitric oxide synthase activity via uncoupling. J Mol Cell Cardiol 2007; 43: 780-791

31. *Kim C, Park H, Kawada T, Kim J, Lim D, Hubbard N, Kwon B, Erickson K, Yu R.* Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes 2006; 30: 1347-1355

32. *Yubero-Serrano EM, Delgado-Lista J, Peña-Orihuela P, Perez-Martinez P, Fuentes F, Marin C, Tunez I, Tinahones FJ, Perez-Jimenez F, Roche HM.* Oxidative stress is

associated with the number of components of metabolic syndrome: LIPGENE study. Exp Mol Med 2013; 45: e28

33. *Grundy SM.* Gamma-glutamyl transferase: another biomarker for metabolic syndrome and cardiovascular risk. Arterioscler Thromb Vasc Biol 2007; 27: 4-7

SUPPLEMENTARY MATERIAL

Table 1S: Comparison of assays used to determine total IGF-1 levels in the SABPA and SAfrEIC studies.

	IRMA (Immunotech A15729, Beckman & Coulter) (SABPA)	**ELISA (BiocodeHycel EL2010) (SAfrEIC)**
Unit of measurement for IGF-1	ng/ml	ng/ml
Intra-assay CV (%)	2.92	15.08
Inter-assay CV (%)	4.49	7.89
Sensitivity (ng/ml)	2	4.9
Dilution test (%)	88 - 111	73 - 108
Recovery test (%)	91 - 103	101.5 – 109.8
Kit calibrators		
CAL 0	0	0
CAL 1	30	45
CAL 2	100	77
CAL 3	300	133
CAL 4	600	270
CAL 5	1200	570
CAL 6	-	1330

IGF-1, insulin-like growth factor-1; CV, coefficient of variation.

Table 2S: Comparison of cardiovascular, biochemical and anthropometric measurements of men and women in the SABPA and SAfrEIC study, respectively.

	SABPA study			**SAfrEIC study**		
	Men (n=189)	**Women (n=198)**	***p* value**	**Men (n=268)**	**Women (n=252)**	**p value**
Age (yrs)	44.1 ± 9.92	45.2 ± 9.60	0.26	40.4 ± 13.3	43.1 ± 12.5	**0.016**
Ethnicity, black, n (%)	88 (46.6)	90(45.5)	0.83	112 (41.8)	94 (37.3)	0.30
Anthropometric measurements						
Body mass index (kg/m^2)	27.9 (20.7; 38.6)	28.5 (20.0; 42.2)	0.16	25.1 (16.9; 35.7)	26.8 (18.7; 40.9)	**<0.001**
Waist circumference (cm)	97.9 ± 15.6	88.9 ± 15.0	**<0.001**	86.1 ± 15.8	83.5 ± 14.5	**0.049**
Biochemical measures						
IGF-1 (ng/ml)	165 ± 59.8	158 ± 63.2	0.26	152 ± 80.9	143 ± 74.8	0.19
IGFBP-3 (nmol/l)	133 ± 27.0	138 ± 26.7	0.077	134 ± 38.0	149 ± 28.4	**<0.001**
IGF-1/IGFBP-3	1.24 ± 0.40	1.13 ± 0.41	**0.009**	1.11 ± 0.48	0.96 ± 0.45	**<0.001**
Fasting glucose (mmol/L)	5.87 (4.60; 9.50)	5.18 (4.19; 6.55)	**<0.001**	5.35 (4.22; 7.13)	5.32 (4.19; 7.60)	0.72
Total cholesterol (mmol/L)	5.23 ± 1.24	5.02 ± 1.37	0.11	5.21 ± 1.48	5.46 ± 1.53	0.066
HDL cholesterol (mmol/L)	1.03 ± 0.33	1.32 ± 0.38	**<0.001**	1.42 ± 0.63	1.52 ± 0.43	**0.027**
LDL cholesterol (mmol/L)	3.45 ± 1.22	3.26 ± 1.18	0.12	3.15 ± 1.36	3.34 ± 1.33	0.11
Triglycerides (mmol/L)	1.35 (0.58; 3.23)	0.84 (0.42; 2.13)	**<0.001**	1.22 (0.53; 3.11)	1.16 (0.51; 2.80)	0.38
C-reactive protein (mg/L)	2.19 (0.65; 9.60)	3.87 (0.99; 28.7)	**<0.001**	1.36 (0.01; 15.9)	1.80 (0.07; 24.0)	**0.019**
Reactive oxygen species (mg/L)	79.5 ± 17.3	105 ± 31.0	**<0.001**	78.2 ± 19.0	92.6 ± 23.9	**<0.001**
Gamma-glutamyltransferase (U/L)	40.1 (13.0; 156)	21.2 (7.00; 89.8)	**<0.001**	51.2 (18.6; 334)	36.2 (14.0; 242)	**<0.001**
Aspartate aminotrasferase (U/L)	22.3 (13.7; 40.0)	17.0 (11.2; 30.0)	**<0.001**	28.6 (15.4; 89.9)	22.6 (13.3; 67.6)	**<0.001**
Alanine transaminase (U/L)	19.9 (7.80; 55.6)	11.0 (4.53; 30.8)	**<0.001**	26.8 (11.4; 74.3)	19.1 (9.82; 47.9)	**<0.001**
Cardiovascular measurements						
Systolic blood pressure (mmHg)	134 ± 17.9	126 ± 16.4	**<0.001**	103 ± 16.9	118 ± 20.2	**<0.001**
Diastolic blood pressure (mmHg)	89.1 ± 12.8	81.7 ± 10.0	**<0.001**	65 ± 11.5	80.7 ± 12.3	0.29
Lifestyle measures						
Smoking self-reported, n (%)	42 (22.2)	16 (8.08)	**<0.001**	113 (42.2)	70 (27.8)	**<0.001**
Intake of medication						
Anti-hypertensive medication, n (%)	45 (23.8)	45 (22.7)	0.80	36 (13.4)	31 (12.3)	0.68
Anti-diabetic medication, n (%)	7 (3.70)	3 (1.51)	0.18	0 (0)	0 (0)	-
Lipid-lowering medication, n (%)	7 (3.70)	4 (2.02)	0.32	11 (4.10)	13 (5.16)	0.57
Diabetes, n (%)	14 (5.22)	18 (7.14)	0.36	26 (13.8)	5 (2.53)	**<0.001**
*Hypertensive, n (%)	80 (42.3)	62 (31.3)	**0.025**	66 (24.6)	48 (19.1)	0.12

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables.
n, number of participants; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; HDL cholesterol, High-density lipoprotein; LDL cholesterol, Low-density lipoprotein.
*Classification as hypertensive: ≥140 and/or 90 mmHg.

Table 3S: Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and the individual contribution of additional confounders.

	Men (n=457)		Women (n=450)	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2=0.30**	**R^2=0.14**	**R^2=0.21**	**R^2=0.20**
Age (yrs)	β=-0.41; p<0.001	β=-0.31 ; p<0.001	β=-0.36 ; p<0.001	β=-0.32 ; p<0.001
Ethnicity (White/African)	β=-0.38 ; p<0.001	β=-0.12 ; p=0.012	β=-0.12 ; p=0.004	β=0.10 ; p=0.016
Study (SAfrEIC/SABPA)	β=0.17 ; p<0.001	β=0.24 ; p<0.001	β=0.16 ; p<0.001	β=0.24 ; p<0.001
Number of MetS components (0-5)	-	β=-0.11 ; p=0.013	β=-0.11 ; p=0.014	β=-0.17 ; p=0.003
Model 2 (Model 1 + CRP)	**R^2=0.30**	**R^2=0.14**	**R^2=0.23**	**R^2=0.22**
Number of MetS components (0-5)	-	β=-0.14 ; p=0.010	-	-
C-reactive protein log (mg/L)	-	-	β=-0.21 ; p<0.001	β=-0.18 ; p<0.001
Model 3 (Model 1 + ROS)	**R^2=0.32**	**R^2=0.16**	**R^2=0.25**	**R^2=0.26**
Number of MetS components (0-5)	-	β=-0.11 ; p=0.030	-	β=-0.10 ; p=0.033
ROS (mg/L)	β=-0.13 ; p=0.002	β=-0.13 ; p=0.005	β=-0.23 ; p<0.001	β=-0.26 ; p<0.001
Model 4 (Model 1 + γ-GT)	**R^2=0.33**	**R^2=0.19**	**R^2=0.21**	**R^2=0.21**
Number of MetS components (0-5)	-	-	β=-0.10 ; p=0.044	β=-0.12 ; p=0.013
γ-GT log (U/L)	β=-0.22 ; p<0.001	β=-0.27 ; p<0.001	β=-0.12 ; p=0.028	β=-0.15 ; p=0.007

IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; ROS, reactive oxygen species; γ-GT, gamma-glutamyl transferase; MetS, metabolic syndrome.

Table 4S: Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and additional covariates.

	Men (n=457)		Women (n=450)	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2=0.34**	**R^2=0.20**	**R^2=0.26**	**R^2=0.26**
Age (yrs)	β=-0.37; p<0.001	β=-0.26 ; p<0.001	β=-0.35 ; p<0.001	β=-0.32 ; p<0.001
Ethnicity (White/African)	β=-0.22 ; p<0.001	-	-	β=0.19 ; p<0.001
Study (SAfrEIC/SABPA)	β=0.91 ; p=0.044	β=0.14 ; p=0.005	β=0.19 ; p<0.001	β=0.25 ; p<0.001
C-reactive protein (mg/L)	-	-	-	-
ROS (mg/L)	β=-0.09 ; p=0.035	β=-0.12 ; p=0.012	β=-0.19 ; p<0.001	β=-0.23 ; p<0.001
γ-GT log (U/L)	β=-0.19 ; p<0.001	β=-0.26 ; p<0.001	-	β=-0.12 ; p=0.020
Smoking (yes/no)	-	-	-	-
Antihypertensive medication (yes/no)	-	-	-	-
Number of MetS components (0-5)	-	-	-	-

IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; ROS, reactive oxygen species; γ-GT, gamma-glutamyl transferase; MetS, metabolic syndrome.

JOURNAL DETAILS	
Title:	*Hormone and Metabolic Research*
Impact Factor:	2.121
Publisher:	Thieme
Aim & Scope: The aim of the journal is to disseminate new and reliable experimental data from across the field of endocrinology and metabolism to researchers, scientists and doctors worldwide. Hormone and Metabolic Research accepts manuscripts in English in the fields of endocrinology and metabolism from clinical and laboratory research, with a preference for experimental over observational studies. Reports dealing with effects of plant extracts and other chemicals on diabetes and other endocrine functions are not subjects covered by this journal. There is a special focus on manuscripts dealing with pituitary, adrenal, thyroid and bone disorders as well as on diseases of the endocrine pancreas. The following types of manuscripts will be accepted: **Original Articles** (Endocrine Care/Endocrine Research), Reviews and Mini Reviews, Immediate Interest, Hypotheses, Commentaries, Letters to the Editor, Symposium Proceedings and Editorials.	

JOURNAL GUIDELINES				
Author guidelines:				
Short running title:	Max: 40 characters		**Conflict of interest:**	Yes
Abstract:	Max: 250 words		**Acknowledgements:**	Yes
Keywords:	3-6		**Language:**	English
Manuscript:	Max: 6 printed pages		**Spacing:**	Double-spaced
Tables & Figures:	3 Tables, 4 Figures			
References in text:	References should be numbered consecutively using square brackets in the order in which they are cited in text.			
List of references:	The references should be listed in numbered order according to the sequence they appear in the text. All authors or groups of authors of each publication should be mentioned. The name of the author(s) should be followed by the full title of the paper, name of the journal in which it has been published, year of publication, volume, first and last page.			
Sections:	Page 1: Title page Page 2: Abstract and keywords Page 3: Introduction, subjects and methods, results, discussion, acknowledgements, conflict of interest, list of references.			

Chapter 4

Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study

A.S.E. Koegelenberg [a], R. Schutte [a,b], W. Smith [a], A.E. Schutte [a,b,*]

This manuscript was published in Thrombosis Research, Volume 5, Pages 1007-1012, 2015

(Annexure C, page 160)

ABSTRACT

Introduction: Insulin-like growth factor-1 (IGF-1) has vasculoprotective effects and can directly oppose endothelial dysfunction in several ways. To improve our understanding on the potential contribution of reduced IGF-1 to the development of vascular endothelial damage, we investigated the link between bioavailable IGF-1 and von Willebrand factor (vWF) as a marker of endothelial damage. We performed this study in black South African school teachers, known to be prone to hypertension.

Materials and methods: From the larger Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study we included 179 black and 207 white non-diabetic men and women (aged 44.5 ± 9.96 years). We measured ambulatory blood pressure and determined IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3) and vWF antigen from blood samples. We used the molar IGF-1/IGFBP-3 ratio as an estimate of bioavailable IGF-1.

Results: Black individuals presented higher blood pressure and vWF_{ag} and lower IGF-1 than the white group (all $p<0.001$). In multi-variable adjusted analyses, vWF_{ag} was inversely associated with IGF-1 ($R^2=0.18$; $\beta=-0.17$; $p=0.044$) and IGF-1/IGFBP-3 ($R^2=0.18$; $\beta=-0.17$; $p=0.030$) in blacks, with no associations in whites. Since IGF-1 is attenuated and vWF_{ag} elevated in diabetes, we included patients with diabetes (n=38) and the aforementioned associations found in blacks remained robust.

Conclusion: The inverse association between bioavailable IGF-1 and vWF in black South Africans suggests that suppressed IGF-1 may result in endothelial damage independent of traditional risk factors.

Keywords: insulin-like growth factor-1; insulin-like growth factor binding protein-3; von Willebrand factor.

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) has vasculoprotective effects by activating nitric oxide synthase (NOS) and consequently increasing nitric oxide (NO) production through the PI3-K signalling pathway [1]. These protective effects include, among others, anti-apoptosis [2], endothelial-dependent vasodilation [3], oxygen free radical scavenging [4], anti-inflammatory [5] and anti-platelet aggregation properties [4]. IGF-1 is synthesised upon stimulation by growth hormone (GH) and is bound to insulin-like growth factor binding proteins (IGFBPs) that function as carrier proteins to regulate circulating IGF-1 transport, turnover and distribution [6]. IGF-1 is only biologically active in its free form, therefore total circulating IGF-1 provides only a crude estimate of biologically active IGF-1 [7]. Free IGF-1, which only account for 1% of total IGF-1, may therefore have greater physiological and clinical relevance than total IGF-1 [8]. Since approximately 80% of total IGF-1 is bound to IGFBP-3 [6], the calculation of the molar ratio of IGF-1/IGFBP-3 allows us to use the ratio as an estimate of bioavailable IGF-1, which may be a more beneficial marker to use than total IGF-1.

The endothelial-protective activities of IGF-1 are vital, with reduced IGF-1 levels linked to endothelial dysfunction [6], which may ultimately lead to endothelial damage. Also, attenuated IGF-1 levels associate with various cardiovascular risk factors such as hypertension [9, 10] and diabetes mellitus [11], which may fully exert their detrimental effects through the pathway of endothelial dysfunction, endothelial apoptosis and the development of unstable plaque [2, 12, 13]. Endothelial damage results in the elevation of von Willebrand factor (vWF) levels [14] – a blood glycoprotein synthesised by and stored in endothelial cells [15]. Cell injury associated with cardiovascular risk factors such as diabetes will also result in high levels of vWF [14].

Due to rapid urbanisation, black South Africans are prone to hypertension development [16] and have a high prevalence of cardiovascular disease [17]. The evidence that an urban dwelling

lifestyle may be detrimental to the cardiometabolic health of Africans motivated us to develop the SABPA study that included black and white school teachers [18]. We have demonstrated in the SABPA and another South African study that black individuals have lower levels of IGF-1 than white South Africans making them more vulnerable to vascular abnormalities [9, 19]. We therefore investigated whether endothelial damage, measured by vWF, is related to IGF-1 in black and white South Africans.

MATERIALS AND METHODS

Study population

This study forms part of the SABPA study conducted between February and May, 2008 and 2009. We recruited a total of 409 urbanised black and white teachers from the North West province, South Africa. The reason for this selection was an attempt to attain a homogenous sample from a similar socio-economic class. We invited all eligible participants between the ages of 25 and 65 years to participate. Exclusion criteria were participants with an elevated ear temperature, making use of α- and β-receptor blockers, psychotropic substance dependence or abuse, blood donors, and individuals vaccinated in the past three months. For this sub-study, participants were excluded due to missing data on IGF-1 availability (n=4). Human immunodeficiency virus *(*HIV) infected participants (n=19) and participants with glycated haemoglobin (HbA1c) levels ≥6.5% and/or using diabetes medication (n=38) were also excluded. The overall sample of this sub-study consisted of 348 participants divided into a black (n=148) and white (n=200) group. Participants were fully informed about the objectives and procedures of the study prior to their inclusion. All participants signed an informed consent form. The study complied with the Helsinki declaration of 1975 (as revised in 2008) for investigation of human participants. The Health Research Ethics Committee of the North-West University (Potchefstroom Campus) approved the study.

Organisational Procedures

Each morning at approximately 08h00, an ambulatory blood pressure monitor (ABPM) device was attached to the participants' non-dominant arm at their workplace. At 16h30 participants were transported to the Metabolic Unit Research Facility of the North-West University. This facility consists of 10 bedrooms, two bathrooms, a living room and kitchen. Participants received a standardised dinner and had their last beverages (tea/coffee) and two biscuits at 20h30. Thereafter they relaxed by reading, watching television, or social interaction and refrained from consuming alcohol, caffeine, smoking and doing exercise. They were encouraged to go to bed at around 22h00. Urine was collected overnight. At 06h00, subjects were woken at which time the ABPM apparatus was removed and subsequent measurements commenced. After the collection of a urine sample and anthropometric data, nurses obtained a fasting venous blood sample. Actical® accelerometers (Montréal, Québec) were attached around the hip of each participant before they leave for work, in order to assess physical activity during a normal working day.

Questionnaires

We used a general health questionnaire to determine lifestyle habits and medication use.

Anthropometric measurements

Height (stature) and weight of participants were measured while being in their underwear. Measurements were taken in triplicate using standard methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK) [20]. Waist circumference (WC) was measured with measurements taken to the nearest 0.1 cm using a non-stretchable standard tape (Lufkin, Cooper Tools, Apex, North Carolina, US). Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2).

Blood Pressure Measurements

An ABPM apparatus (Meditech CE120® Cardiotens; Meditech, Budapest, Hungary) was attached on the participant at their workplace, programmed to measure blood pressure at 30 minute intervals during the day (08h00-22h00) and every hour during night time (22h00-6h00). We downloaded blood pressure data onto a database using the CardioVisions 1.9.0 Personal Edition software (Meditech, Budapest, Hungary).

Blood Sampling and Biochemical Analyses

A registered nurse obtained a blood sample with a sterile winged infusion set from the antebrachial vein branches. Serum samples for total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglycerides, gamma-glutamyl transferase (γ-GT), and high-sensitivity C-reactive protein (CRP) were analysed using two sequential multiple analysers (Konelab 20i; Thermo Scientific, Vantaa, Finland (2008); Unicel DXC 800 - Beckman and Coulter®, Germany (2009)). Interleukin-6 (IL-6) was analysed using the IL-6 Quantikine high sensitivity enzyme linked immunosorbent assay (R&D Systems, Minneapolis, MN USA). Total IGF-1 and IGFBP-3 were determined with immunoradiometric assays (IRMA) from Immunotech (Beckman and Coulter®, Germany; IGF-1 – A15729; IGFBP-3 – DSL-6600). Serum peroxides were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognised as an efficient method for evaluating reactive oxygen species (ROS) in the body. The Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure ROS levels, where 1.0 mg/L H_2O_2 represents one unit of ROS [21]. Citrated samples were used for the analysis of von Willebrand factor antigen (vWF_{ag}). vWF_{ag} levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA). Polyclonal rabbit anti-vWF antibody and rabbit anti-vWF-horseradish peroxidase antibody (DAKO, Glostrup, Denmark) were used to perform the assay. The 6th International Standard for vWF/FVIII was used to create the standard curve against

which the samples were measured. The percentage of HbA1c was determined by means of the turbidimetric inhibition immunoassay on whole blood using the Roche Integra 400 (Roche, Basel, Switzerland). In the present sub-study, participants were considered as having diabetes if they had HbA1c levels of ≥6.5 % or made use of diabetes medication [22]. HIV testing was done according to standard procedures of the South African Department of Health.

Statistical Analyses

For database management and statistical analyses we used Statistica version 12.0 (StatSoft, Inc.,Tulsa, OK). We tested the interaction of sex and ethnicity on the association between vWF_{ag} and IGF-1/IGFBP-3 by introducing appropriate interaction terms in multiple regression analyses. Variables that were not normally distributed were logarithmically transformed (vWF_{ag}, IL-6, CRP, HbA1c, triglycerides, and γ-GT). We subsequently compared means and proportions between black and white groups with independent T-tests and Chi-square tests, respectively. We used single regression analyses and forward stepwise multiple regression analyses to determine the associations of vWF_{ag} with total IGF-1 and IGF-1/IGFBP-3. The models included the following covariates: age, sex, BMI, HbA1c, total cholesterol:HDL, triglycerides, CRP, ROS, γ-GT, smoking, pulse pressure, and antihypertensive medication. The multivariate-adjusted analyses were also repeated upon inclusion of individuals with diabetes. All p-values refer to 2-sided hypothesis.

RESULTS

We found no interaction of ethnicity (p=0.36) and sex (p=0.65 and p=0.48 for blacks and whites, respectively) on the association between vWF_{ag} and IGF-1/IGFBP-3. We therefore pooled the men and women, but stratified the groups according to ethnicity in line with our aim and the literature [9, 23].

Characteristics of the study population

Table 1 displays the characteristics of the black and white participants. Age did not differ between blacks and whites, however black participants showed higher BMI (p<0.001), 24hr systolic and diastolic blood pressure (all p<0.001), vWF_{ag} (p<0.001), CRP (p<0.001) and IL-6 (p=0.001). IGF-1 (p<0.001) and IGFBP-3 (p<0.001) were lower in the black group, but IGF-1/IGFBP-3 was similar between the two groups (p=0.26).

Table 1: Comparison of cardiovascular, biochemical and anthropometric measurements of black and white participants.

	Black (n=148)	**White (n=200)**	***p* value**
Age (yrs)	43.8 ± 8.54	45.0 ± 10.9	0.28
Sex (women, men)	79/69	105/95	0.87
Anthropometric measurements			
Body mass index (kg/m^2)	29.9 ± 6.91	27.4 ± 5.75	<0.001
Waist circumference (cm)	92.4 ± 14.7	92.4 ± 15.7	0.96
Biochemical measures			
HbA1c (%)	5.70 (5.10; 3.60)	5.46 (5.00; 6.10)	<0.001
Total cholesterol:HDL ratio (mmol/L)	4.25 ± 1.61	4.96 ± 1.60	<0.001
Triglycerides (mmol/L)	1.07 (0.49; 2.77)	0.99 (0.43; 2.74)	0.20
IGF-1 (ng/ml)	142 ± 54.4	178 ± 62.7	<0.001
IGFBP-3 (nmol/l)	122 ± 24.6	148 ± 22.9	<0.001
IGF-1/IGFBP-3	0.15 ± 0.05	0.16 ± 0.05	0.26
vWF_{ag} (%)	90.5 (57.9; 148)	62.1 (41.0; 95.4)	<0.001
C-reactive protein (mg/L)	4.49 (0.65; 33.0)	2.00 (0.99; 9.00)	<0.001
IL-6 (pg/mL)	1.17 (0.33; 3.14)	0.91 (0.31; 3.03)	0.001
Reactive oxygen species (mg/L)	90.7 (57.1; 145)	87.0 (57.2; 138)	0.17
Cardiovascular measurements			
Ambulatory systolic blood pressure (mmHg)	132 ± 16.4	124 ± 11.5	<0.001
Ambulatory diastolic blood pressure (mmHg)	82.4 ± 10.8	76.3 ± 7.87	<0.001
Ambulatory pulse pressure (mmHg)	49.7 ± 9.19	47.2 ± 7.22	0.004
Lifestyle measures			
Gamma-glutamyl transferase (U/L)	44.6 (20.1; 177)	18.9 (7.00; 75.0)	<0.001
Smoking self-reported, n (%)	26 (17.6)	29 (14.5)	0.45
Medication use			
Anti-hypertensive medication, n (%)	49 (33.1)	25 (12.5)	<0.001
Lipid-lowering medication, n (%)	2 (1.35)	8 (4.00)	0.14
Anti-inflammatory medication, n (%)	10 (7.76)	12 (6.00)	0.77
Hypertensive, n (%)	103 (69.6)	87 (43.5)	<0.001

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables.
n, number of participants; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; HbA1c, glycated haemoglobin; vWF_{ag}, *von Willebrand factor antigen*.*Classification as hypertensive: ≥140/90 mmHg.

Regression analyses

In unadjusted analyses (Figure 1) we observed an inverse association between vWF_{ag} and both IGF-1 and IGF-1/IGFBP-3 in the black participants, with no associations found in the white group. After adjusting for age, sex and BMI the association between vWF_{ag} and IGF-1 in blacks remained significant (r=-0.17; p=0.041) while the inverse association between vWF_{ag} and IGF-1/IGFBP-3 became borderline (r=-0.14; p=0.096). These additional adjustments did not change the non-significant results in the white group.

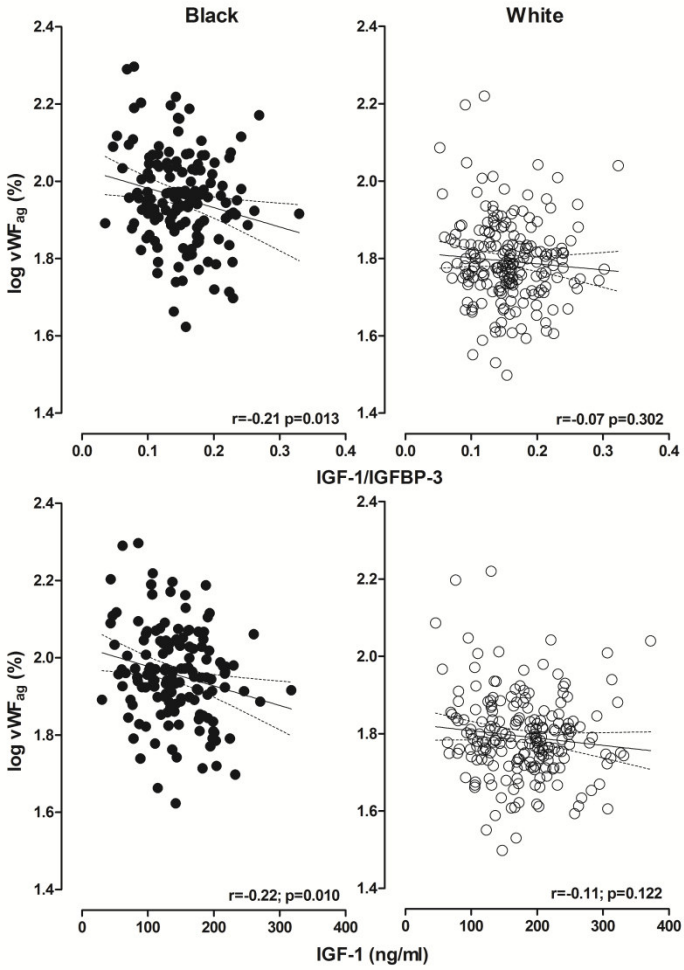


Figure 1: von Willebrand factor (vWF) as a function of insulin-like growth factor-1 (IGF-1) and IGF-1/insulin-like growth factor binding protein-3 (IGFBP-3) in black and white individuals in single regression analyses. Solid and dashed lines represent the regression line and the 95% CI boundaries

Table 2: Independent associations of vWF_{ag} with IGF-1/IGFBP-3 and total IGF-1.

	Black vWF_{ag}		**White** vWF_{ag}	
	β (95% CI)	***p value***	**β (95% CI)**	***p value***
n	148		200	
Model 1				
Adjusted R^2	0.18		0.04	
Main independent variable: IGF-1/IGFBP-3	-0.17 (-0.33;-0.01)	0.044	-	
Age (yrs)	-		0.11 (-0.03;0.25)	0.13
Sex (women, men)	-		0.12 (-0.07;0.31)	0.098
BMI (kg/m^2)	0.21 (0.03;0.39)	0.025	0.11 (-0.04;0.26)	0.16
HbA1c (%)	-0.11 (-0.27;0.05)	0.18	-	
TC/HDL ratio	-0.08 (-0.24;0.08)	0.31	-	
C-reactive protein (mg/L)	0.23 (0.04;0.40)	0.016	-	
Reactive oxygen species (mg/L)	-		0.19 (0.02;0.36)	0.032
γ-GT (U/L)	-		-0.12 (-0.31;0.01)	0.066
Smoking (no/yes)	-		-0.07 (-0.21;0.07)	0.32
Anti-hypertensive medication (no/yes)	-0.19 (-0.35;-0.04)	0.016	-	
Model 2				
Adjusted R^2	0.18		0.04	
Main independent variable: IGF-1 (ng/ml)	-0.17 (-0.37;-0.02)	0.030	-	
Age (yrs)	-		0.11 (-0.03;0.25)	0.13
Sex (women, men)			0.12 (-0.07;0.31)	0.22
BMI (kg/m^2)	0.21 (0.03;0.39)	0.025	0.11 (-0.04;0.26)	0.16
HbA1c (%)	-0.11 (-0.27;0.04)	0.15	-	
C-reactive protein (mg/L)	0.22 (0.04;0.39)	0.019	-	
Reactive oxygen species (mg/L)	-		0.19 (0.02;0.36)	0.032
γ-GT (U/L)	-		-0.15 (-0.31;0.01)	0.066
Smoking (no/yes)	-		-0.07 (-0.21;0.07)	0.32
Anti-hypertensive medication (no/yes)	-0.21 (-0.37;-0.06)	0.006	-	

β, partial regression coefficient; 95% CI, 95% confidence intervals of β; vWF_{ag}, *von Willebrand factor antigen;* IGF-1, insulin-like growth factor-1; IGF-1/IGFBP-3 ratio, insulin-like growth factor-1/insulin-like growth factor binding protein-3; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL ratio, total cholesterol:high-density lipoprotein cholesterol; γ-GT, gamma-glutamyl transferase.
Covariates included IGF-1/IGFBP-3 or IGF-1, age, sex, BMI, HbA1c, TC:HDL, triglycerides, C-reactive protein; pulse pressure, reactive oxygen species, γ-GT, smoking and antihypertensive medication.
$p < 0.05$ regarded as statistically significant.

We further performed multi-variable adjusted forward stepwise regression analyses with vWF_{ag} as dependent variable (Table 2), and assessed the association with IGF-1/IGFBP-3 (Model 1) and total IGF-1 (Model 2) as our main independent variables. We found an inverse association between vWF_{ag} and IGF-1/IGFBP-3 (R^2=0.18; β=-0.17; p=0.044) and between vWF_{ag} and IGF-1 (R^2=0.18; β=-0.17; p=0.030) in blacks only. Also when including participants with diabetes

(n=38) (Table 1S), the inverse associations of vWF_{ag} with IGF-1/IGFBP-3 (R^2=0.14; β=-0.17; p=0.020) and IGF-1 (R^2=0.15; β=-0.21; p=0.005) were confirmed in the blacks only.

DISCUSSION

We found an independent inverse association between vWF, as a marker of endothelial damage, and bioavailable IGF-1 in black individuals who also presented with significantly higher blood pressure and vWF, and lower IGF-1 than white individuals. This finding was robust in a population irrespective of including or excluding those with diabetes. No significant associations were found in the white group.

We confirm our previous results in a different population sample indicating that IGF-1 levels are significantly lower in blacks compared to whites [9]. Higher levels of vWF among blacks also support previous findings [24, 25], and taken together with the low IGF-1 profile, these results may indicate and partly explain why black populations have an increased risk for hypertension development and cardiovascular events [17]. Although our black population had higher ambulatory blood pressure [9], we found the association between endothelial damage and IGF-1 to be independent of pulse pressure and other risk factors. This association was found not only with total IGF-1 but also with the IGF-1/IGFBP-3 ratio, which may indicate that both total and bioavailable IGF-1 associate independently with endothelial damage.

Empen et al. analysed the association between IGF-1 and endothelial function, as measured by flow-mediated dilation, in a study population including 1482 participants (49.7% women) aged 25-85 years [26]. Although we used vWF and not flow mediated dilation as a marker of endothelial function, our results support the findings of Empen that low serum levels of IGF-1 are associated with impaired endothelial function, after adjustments for major cardiovascular confounders [26]. The role of IGF-1 in vasodilation is supported by Abdu et al. who showed that

patients (nonsmokers, without diabetes, hypertension or vascular disease) with low levels of IGF-1 due to growth hormone deficiency have reduced vasodilation. However, the administration of growth hormone led to the normalisation of previously reduced vasodilation [27].

von Willebrand factor is increased following endothelial cell injury [28], therefore vWF is an indicator of endothelial damage [14]. vWF has various functions in hemostasis: it acts as a bridging molecule for platelet adhesion and aggregation at the site of cell injury [29]. vWF-mediated platelet adhesion is dependent on a high shear rate, which occurs in arterioles and in partially occluded arteries [30]. Therefore damage to the endothelium enables vWF to bind to platelets at the site of injury, which does not occur in the absence of injury [30]. vWF also acts as a carrier for coagulation factor VIII and is responsible for maintaining normal levels of factor VIII [31]. In addition to the important functions of vWF in hemostasis, vWF is also involved in arterial thrombus formation and atherosclerosis [32].

Since IGF-1 has vasculoprotective properties [1] we may speculate on some mechanisms to be involved in the link found between vWF and IGF-1. IGF-1 directly opposes endothelial dysfunction, mainly by increasing NO production [1]. NO has multiple endothelial-protective activities, therefore a reduction in IGF-1 will consequently decrease NO production with a loss in its vasculoprotective effects, resulting in endothelial dysfunction [33] and may ultimately lead to endothelial damage. NO further plays a major role in the regulation of vWF secretion [34]. NO is responsible for the inhibition of platelet activation, and may thus inhibit endothelial exocytosis such as vWF release [34, 35]. On the other hand, a reduction in NO levels may potentiate vWF secretion [34]. Another valuable protective activity of IGF-1 is the activation of phospholipase A_2 which induces prostacyclin (prostaglandin I_2 (PGI_2)) synthesis [36]. PGI_2 has important anti-platelet effects and is also responsible for inducing vasodilation [36]. In a study by Veyradier et

al. vWF levels were significantly decreased when patients with pulmonary arterial hypertension were treated with prostacyclin [37]. Given that IGF-1 is responsible for NO and prostacyclin production [1, 36] and that both NO and prostacyclin exert an autocrine negative feedback on vWF secretion [34, 37], one might expect that a reduction in IGF-1 would potentiate vWF secretion with possible implications for endothelial damage and hemostasis. This was confirmed by the significant inverse association found between vWF and IGF-1 in the black population. Therefore endothelial damage in blacks, who also presented a more unfavourable cardiometabolic profile compared to whites, may be due to a decline in bioavailable IGF-1 levels.

This study should be viewed in context of its strengths and limitations. Participants consisted of urban black and white teachers, and the results can therefore not be extrapolated to the rest of South Africa. Due to the cross-sectional nature of this study, causality cannot be inferred. In addition, information on platelets and white blood cell counts, as well as flow mediated dilation were not available and may have provided more insight on the mechanisms involved regarding the link between vWF and IGF-1. The results of our study were consistent after multiple adjustments however, we cannot exclude residual confounding. Strengths of this study include the availability of bioavailable IGF-1 (molar IGF-1/IGFBP-3), detailed cardiovascular assessments and socio-economic compatibility in a relatively large group of bi-ethnic participants.

To conclude, the independent inverse relationship between vWF and IGF-1 in black individuals suggests that endothelial damage may be a consequence of the reduction in endothelial-protective activities of IGF-1. Our findings support the notion that reduced IGF-1 could be considered as an independent risk factor for cardiovascular disease.

Acknowledgements

The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study would not have been possible without the voluntary collaboration of the participants and the Department of Education, North West province, South Africa. The authors gratefully acknowledge the technical assistance of Mrs. Tina Scholtz, Sr. Chrissie Lessing and Dr. Szabolcs Péter. Research included in the present study was partially funded by the Medical Research Council, South Africa; Roche Diagnostics; the North-West University; and the Metabolic Syndrome Institute, France. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

[1] V. Schini-Kerth, Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells, J. Endocrinol. Investig. 22 (1999) 82–88.

[2] R.Hutter, B.V. Sauter, E.D.Reis, M. Roque, D.Vorchheimer, F.E. Carrick, et al., Decreased reendothelialization and increased neointima formation with endostatin overexpression in a mouse model of arterial injury, Circulation 107 (2003) 1658–1663.

[3] U. Izhar, D. Hasdai, D.M. Richardson, P. Cohen, A. Lerman, Insulin and insulin-like growth factor-I cause vasorelaxation in human vessels in vitro, Coron. Artery Dis. 11 (2000) 69–76.

[4] S. Dimmeler, A.M. Zeiher, Exercise and cardiovascular health get active to “AKTivate” your endothelial nitric oxide synthase, Circulation 107 (2003) 3118–3120.

[5] M. Spies, O. Nesic, R. Barrow, J. Perez-Polo, D. Herndon, Liposomal IGF-1 gene transfer modulates pro-and anti-inflammatory cytokine mRNA expression in the burn wound, Gene Ther. 8 (2001) 1409–1415.

[6] E. Conti, M. Musumeci, M. De Giusti, E. Dito, V. Mastromarino, M. Volpe, IGF-1 and atherothrombosis: relevance to pathophysiology and therapy, Clin. Sci. 120 (2011) 377–402.

[7] H Ørskov, JWeeke, J Frystyk, A Kaal, S Nielsen, C Skjaerbaek, et al., Growth hormone determination in serum from patients with growth disorders, Growth Hormone in Adults. Cambridge University Press, Cambridge, 1996 109–121.

[8] J. Frystyk, C. Skjærbæk, B. Dinesen, H. Ørskov, Free insulin-like growth factors (IGF-I and IGF-II) in human serum, FEBS Lett. 348 (1994) 185–191.

[9] A.E. Schutte, H.W. Huisman, J.M. van Rooyen, L. Malan, N.T. Malan, C.M.T. Fourie, et al., A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability, J. Clin. Endocrinol. Metab. 95 (2010) 2503–2507.

[10] A.E. Schutte, M. Volpe, G. Tocci, E. Conti, Revisiting the relationship between blood pressure and insulin-like growth factor-1, Hypertension 63 (2014) 1070–1077.

[11] M.S. Sandhu, A.H. Heald, J.M. Gibson, J.K. Cruickshank, D.B. Dunger, N.J. Wareham, Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study, Lancet 359 (2002) 1740–1745.

[12] Y. Okura, M. Brink, A.A. Zahid, A. Anwar, P. Delafontaine, Decreased expression of insulin-like growth factor-1 and apoptosis of vascular smooth muscle cells in human atherosclerotic plaque, J. Mol. Cell. Cardiol. 33 (2001) 1777–1789.

[13] V.A. Patel, Q.J. Zhang, K. Siddle,M.A. Soos, M. Goddard, P.L.Weissberg, et al., Defect in insulin-like growth factor-1 survival mechanism in atherosclerotic plaque-derived vascular smooth muscle cells ismediated by reduced surface binding and signaling, Circ. Res. 88 (2001) 895–902.

[14] G.Y. Lip, A. Blann, von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? Cardiovasc. Res. 34 (1997) 255–265.

[15] B.Horvath,D.Hegedus, L. Szapary, Z. Marton, T.Alexy, K. Koltai, et al., Measurement of von Willebrand factor as the marker of endothelial dysfunction in vascular diseases, Exp. Clin. Cardiol. 9 (2004) 31–34.

[16] A.E. Schutte, R. Schutte, H.W. Huisman, J.M. van Rooyen, C.M. Fourie, N.T. Malan, et al., Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study, Int. J. Epidemiol. 41 (2012) 1114–1123.

[17] K. Sliwa, D. Wilkinson, C. Hansen, L. Ntyintyane, K. Tibazarwa, A. Becker, et al., Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): A cohort study, Lancet 371 (2008) 915–922.

[18] L. Malan,M. Hamer, N. Frasure-Smith,H.S. Steyn, N.T.Malan, Cohort profile: sympathetic activity and ambulatory blood pressure in Africans (SABPA) prospective cohort study, Int. J. Epidemiol. (2014)http://dx.doi.org/10.1093/ije/dyu199.

[19] A. Schutte, R. Schutte, W. Smith, H. Huisman, C. Mels, L. Malan, et al., Compromised bioavailable IGF-1 of black men relates favourably to ambulatory blood pressure: the SABPA study, Atherosclerosis 233 (2014) 139–144.

[20] K. Norton, T. Olds, Anthropometrica: A Textbook of Body Measurement for Sports and Health Courses, University of New South Wales Press, 1996.

[21] I. Hayashi, Y. Morishita, K. Imai,M. Nakamura, K. Nakachi, T. Hayashi, High-throughput spectrophotometric assay of reactive oxygen species in serum, Mutat. Res. 631 (2007) 55–61.

[22] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 37 (2014) S81–S90.

[23] L. Lammertyn, A.E. Schutte, M. Pieters, R. Schutte, D-dimer relates positively with increased blood pressure in black South Africans: the SABPA study, Thromb. Res. 133 (2014) 1152–1157.

[24] C. Miller, E. Haff, S. Platt, P. Rawlins, C. Drews, A. Dilley, et al., Measurement of von Willebrand factor activity: relative effects of ABO blood type and race, J. Thromb. Haemost. 1 (2003) 2191–2197.

[25] P. Lutsey, M. Cushman, L. Steffen, D. Green, R. Barr, D. Herrington, et al., Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study, J. Thromb. Haemost. 4 (2006) 2629–2635.

[26] K. Empen, R. Lorbeer, H. Volzke,D.M. Robinson, N. Friedrich, A. Krebs, et l.,Association of serum IGF1 with endothelial function: results from the population-based study of health in Pomerania, Eur. J. Endocrinol. 163 (2010) 617–623.

[27] T. Abdu, T. Elhadd, H. Buch, D. Barton, R. Neary, R. Clayton, Recombinant GH replacement in hypopituitary adults improves endothelial cell function and reduces calculated absolute and relative coronary risk, Clin. Endocrinol. (Oxf) 61 (2004) 387–393.

[28] B. Boneu,M. Abbal, J. Plante, R. Bierme, Factor-VIII complex and endothelial damage, Lancet 305 (1975) 1430.

[29] A.D. Blann, C.N. McCollum, von Willebrand factor, endothelial cell damage and atherosclerosis, Eur. J. Vasc. Surg. 8 (1994) 10–15.

[30] J.E. Sadler, Biochemistry and genetics of vonWillebrand factor, Annu. Rev. Biochem. 67 (1998) 395–424.

[31] S.F. De Meyer, H. Deckmyn, K. Vanhoorelbeke, von Willebrand factor to the rescue, Blood 113 (2009) 5049–5057. [32] A. Hamsten, The hemostatic system and coronary heart disease, Thromb. Res. 70 (1993) 1–38.

[33] E. Conti, C. Carrozza, E. Capoluongo, M. Volpe, F. Crea, C. Zuppi, et al., Insulin-like growth factor-1 as a vascular protective factor, Circulation 110 (2004) 2260–2265.

[34] S. Zarei, M. Frieden, J. Kaufmann, U. Vischer, The regulation of endothelial vWF secretion by nitric oxide: is it physiological? J. Thromb. Haemost. 4 (2006) 263–265.

[35] S. Massberg, M. Sausbier, P. Klatt, M. Bauer, A. Pfeifer, W. Siess, et al., Increased adhesion and aggregation of platelets lacking cyclic guanosine 3′,5′-monophosphate kinase I, J. Exp. Med. 189 (1999) 1255–1264.

[36] J.G.Webb, Y. Tan, M.A. Jaffa, A.A. Jaffa, Evidence for prostacyclin and cAMP upregulation by bradykinin and insulin-like growth factor 1 in vascular smooth muscle cells, J. Recept. Signal Transduct. 30 (2010) 61–71.

[37] A. Veyradier, T. Nishikubo, M. Humbert, M. Wolf, O. Sitbon, G. Simonneau, et al., Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension, Circulation 102 (2000) 2460–2462.

SUPPLEMENTARY MATERIAL

Table 1S: Independent associations of vWF_{ag} with IGF-1/IGFBP-3 and total IGF-1 with inclusion of patients with diabetes.

	Black		**White**	
	vWF_{ag}		**vWF_{ag}**	
	β (95% CI)	***p value***	**β (95% CI)**	***p value***
N	178		208	
Model 1				
Adjusted R^2	0.14		0.05	
Main independent variable: IGF-1/IGFBP-3	-0.17 (-0.32;-0.06)	0.020	-	-
Age (yrs)	-	-	0.15 (0.01;0.28)	0.034
Sex (women, men)	-0.10 (-0.26;0.05)	0.19	0.16 (-0.03;0.35)	0.098
BMI (kg/m^2)	-	-	0.13 (-0.02;0.28)	0.10
Triglycerides (mmol/L)	-	-	-0.09 (-0.27;0.09)	0.30
C-reactive protein (mg/L)	0.25 (0.09;0.41)	0.002	-	-
Reactive oxygen species (mg/L)	-	-	0.21 (0.04;0.38)	0.0.14
γ-GT (U/L)	-	-	-0.12 (-0.28;0.05)	0.18
Anti-hypertensive medication (no/yes)	-0.21 (-0.35;-0.06)	0.020	-	-
Model 2				
Adjusted R^2	0.15		0.05	
Main independent variable: IGF-1 (ng/ml)	-0.21 (-0.35;-0.07)	0.005	-	-
Age (yrs)	-	-	0.15 (0.01;0.28)	0.034
Sex (women, men)	-0.12 (-0.28;0.03)	0.11	0.16 (-0.03;0.35)	0.098
BMI (kg/m^2)	-	-	0.13 (-0.02;0.28)	0.10
Triglycerides (mmol/L)	-	-	-0.09 (-0.27;0.09)	0.304
C-reactive protein (mg/L)	0.24 (0.07;0.38)	0.004	-	-
Reactive oxygen species (mg/L)	-	-	0.21 (0.04;0.38)	0.014
γ-GT (U/L)	-	-	-0.12 (-0.28;0.05)	0.178
Anti-hypertensive medication (no/yes)	-0.22 (-0.36;-0.07)	0.003	-	-

β, partial regression coefficient; 95% CI, 95% confidence intervals of β; vWF_{ag}, *von Willebrand factor antigen;* IGF-1/IGFBP-3 ratio, insulin-like growth factor-1/insulin-like growth factor binding protein-3; IGF-1, insulin-like growth factor-1; BMI, body mass index; TC:HDL ratio, total cholesterol:high-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; SBP, systolic blood pressure; γ-GT, gamma-glutamyl transferase.
Potential confounders included IGF-1/IGFBP-3 or IGF-1,age, sex, BMI, HbA1c, total cholesterol:HDL, triglycerides, C-reactive protein, ROS, γ-GT, smoking, pulse pressure and antihypertensive medication.
$p \leq 0.05$ regarded as statistically significant.

JOURNAL DETAILES	
Title:	*Thrombosis Research*
Impact Factor:	2.427
Publisher:	ELSEVIER
Aim & Scope: *Thrombosis Research* is an international journal with a goal of rapid dissemination of new information on thrombosis, haemostasis, and vascular biology to advance science and clinical care. The journal publishes **peer-reviewed original research**, along with reviews, editorials, and opinions and critics. Both basic and clinical studies are published. Publication of research which will lead to novel approaches in diagnosis, therapy, prognosis and prevention of thrombotic and hemorrhagic diseases is given high priority.	

JOURNAL GUIDELINES				
Author guidelines:				
Title:	Concise & Informative		**Conflict of interest:**	Yes
Abstract:	Max: 250 words		**Acknowledgements:**	Yes
Keywords:	Max: 6		**Language:**	English
Manuscript:	5000-6000 words			
References in text:	Indicate references by number(s) in square brackets in line with the text.			
List of references:	References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Number the references (in square brackets) in the list in the order in which they appear in the text.			
Sections:	Page 1: Title page Page 2: Abstract and keywords Page 3: Introduction, materials and methods, results, discussion and conclusion, acknowledgements, list of references.			
Ethical approval:	Studies on patients or volunteers require ethics committee approval and informed consent, which should be documented in the paper.			

Chapter 5

IGF-1 and NT-proBNP in a black and white population: The SABPA study

This manuscript is under revision at the European Journal of Clinical Investigation

ABSTRACT

Background: Black populations exhibit lower concentrations of the cardioprotective peptide, insulin-like growth factor-1 (IGF-1), and are more prone to develop hypertensive heart disease than whites. We therefore determined whether lower IGF-1 in black individuals relates to a marker of cardiac overload and systolic dysfunction, namely N-terminal prohormone B-type natriuretic peptide (NT-proBNP).

Materials and methods: We included 160 black and 195 white non-diabetic South African men and women (aged 44.4 ± 9.81 years), and measured ambulatory blood pressure, NT-proBNP, IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3).

Results: Although the black group presented elevated ambulatory blood pressure accompanied by lower IGF-1 compared to the white group (all $p<0.001$), we found similar NT-proBNP concentrations ($p=0.72$). Furthermore, in blacks we found a link between NT-proBNP and systolic blood pressure (SBP) ($R^2=0.37$; $\beta=0.28$; $p<0.001$), but not with IGF-1. In the white group, NT-proBNP was inversely associated with IGF-1 ($R^2=0.39$; $\beta=-0.22$; $p<0.001$) after adjusting for covariates and potential confounders. Since IGF-1 is attenuated in diabetes, we added the initially excluded patients with diabetes (n=38) and the aforementioned associations remained robust.

Conclusion: Contrary to the white group, we found no association between NT-proBNP and IGF-1 in black adults. Our findings suggest that SBP and other factors may play a greater contributory role in cardiac pathology in blacks.

Keywords: insulin-like growth factor-1; insulin-like growth factor binding protein-3; N-terminal prohormone B-type natriuretic peptide, ethnicity, race, African

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) signalling has vascular and cardioprotective effects [1], and reduced levels of circulating IGF-1 associates independently with the risk of developing cardiovascular diseases [2]. IGF-1 is mainly synthesised in the liver, however other tissues such as the myocardium and the endothelium not only express receptors for IGF-1 but also produce IGF-1 locally, and functions in an autocrine and paracrine manner [3]. IGF-1 exerts multiple endothelial protective effects in the vasculature mainly via the phosphatidylinositol 3-kinase (PI3-K) pathway [3]. These include vasodilation by stimulating nitric oxide (NO) production [4], plaque stabilizing [5], anti-apoptotic [6], anti-platelet [7] and anti-inflammatory properties [8].

Besides vasculoprotective effects, in cardiomyocytes, IGF-1 increases contractility [9, 10], regulates metabolism [11], increases protein synthesis [12], promotes hypertrophy in tissues with high energy demands [13], prevents cardiac autophagy [14] and inhibits apoptosis [15]. In doing so, IGF-1 may consequently reduce wall stress and improve cardiac performance in patients with cardiac overload [16].

We have shown that low IGF-1 associates with a marker of endothelial damage [17] and higher ambulatory blood pressure [18] in a hypertension-prone black population. Black populations from South Africa have lower IGF-1 and higher blood pressure than whites [17, 19, 20] and are also more prone to develop hypertensive heart disease [21]. In a study on hospitalised South Africans with newly diagnosed cardiovascular diseases, blacks were more likely to be diagnosed with heart failure than other ethnic groups [21]. Supporting these findings, Kruger et al. found in the general population that N-terminal prohormone B-type natriuretic peptide (NT-proBNP) levels, which is a marker of cardiac overload, left ventricular dysfunction and heart failure [22], were significantly higher among blacks when compared to whites [23].

It is therefore warranted to determine if IGF-1 levels which are typically reduced in blacks, associate in a protective manner with cardiac overload and systolic dysfunction. The purpose of this study was therefore to explore the association between NT-proBNP and IGF-1 in a black and white South African population.

MATERIALS AND METHODS

Study population

This study forms part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study conducted between February and May, 2008 and 2009 [24]. We recruited a total of 409 urbanised black and white teachers from the North West Province, South Africa. The reason for this selection was an attempt to attain a homogenous sample from a similar socio-economic class. We invited all eligible participants between the ages of 25 and 65 years to participate. Exclusion criteria were participants with an elevated ear temperature, making use of α- and β-receptor blockers, psychotropic substance dependence or abuse, blood donors, and individuals vaccinated in the past three months. For this sub-study, participants were excluded due to missing data on IGF-1 (n=5) and NT-proBNP (n=11). Participants with glycated haemoglobin (HbA1c) levels ≥6.5% and/or using diabetes medication (n=38) were also excluded. The overall sample of this sub-study consisted of 355 participants stratified by ethnicity (45.1% black). Participants were fully informed about the objectives and procedures of the study prior to their inclusion. All participants signed an informed consent form. The study complied with the Helsinki Declaration for investigation of human participants. The Health Research Ethics Committee of the North-West University (Potchefstroom Campus) approved the study.

Organisational procedures

Each morning at approximately 08h00, an ambulatory blood pressure monitor (ABPM) was attached to the participants' non-dominant arm at their workplace. At 16h30 participants were transported to the Metabolic Unit Research Facility of the North-West University. This facility consists of 10 bedrooms, two bathrooms, a living room and kitchen. Participants received a standardised dinner and had their last beverages (tea/coffee) and two biscuits at 20h30. Thereafter they relaxed by reading, watching television, or social interaction and refrained from consuming alcohol, caffeine, smoking and doing exercise. They were encouraged to go to bed at around 22h00. At 06h00, subjects were woken at which time the ABPM apparatus was removed and subsequent measurements commenced. After anthropometric measurements, nurses obtained a fasting venous blood sample.

Questionnaires

We used a general health questionnaire to evaluate lifestyle such as smoking, alcohol consumption and medication use.

Anthropometric measurements

Height (stature) and weight of participants were measured while being in their underwear. Measurements were taken in triplicate using standard methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK) [25]. Waist circumference (WC) was measured with measurements taken to the nearest 0.1 cm using a non-stretchable standard tape (Lufkin, Cooper Tools, Apex, North Carolina, US). Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2).

Cardiovascular assessment procedures

The ABPM apparatus (Meditech CE120® Cardiotens; Meditech, Budapest, Hungary) was attached to the participant at their workplace, programmed to measure blood pressure (BP) at 30 minute intervals during the day (08h00-22h00) and every hour during night-time (22h00-6h00). We downloaded blood pressure data into a database using the CardioVisions 1.9.0 Personal Edition software (Meditech, Budapest, Hungary). High resolution ultrasound was applied to determine carotid intima-media thickness in the common carotid artery (CIMT) [26]. Standardized images [27] of the left and the right common carotid artery were obtained, from at least two optimum angles using a Sonosite MicroMaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) and 6 -13 MHz linear array transducer. The images were digitised and imported into the Artery Measurement Systems automated software (AMS) II v1.139 (Gothenburg, Sweden). We calculated the cross-sectional wall area (CSWA) using the following equation: $CSWA = \pi (d/2 + CIMT)^2 – \pi (d/2)^2$, where d denotes luminal diameter. Beat-to-beat blood pressure was continuously assessed using the Finometer device (Finapres Medical Systems, Amsterdam). The BeatScope version 1.1a software further calculates an integrated age-dependent aortic flow curve from the surface area beneath the pressure/volume curve determining cardiac output and stroke volume. A standard 12-lead ECG was recorded during resting conditions (PC 1200, v5.030, Norav Medical, Yokneam, Israel). ECG derived left ventricular mass was calculated using the Cornell product [28, 29].

Blood sampling and biochemical analyses

A registered nurse obtained a blood sample with a sterile winged infusion set from the antebrachial vein branches. Serum samples for total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides, gamma-glutamyl transferase (γ-GT), creatinine and high-sensitivity C-reactive protein (CRP) were analysed using two sequential multiple analysers

(Konelab 20i; Thermo Scientific, Vantaa, Finland (2008); Unicel DXC 800 - Beckman and Coulter®, Germany (2009)). Total IGF-1 and IGFBP-3 were determined with immunoradiometric assays (IRMA) from Immunotech (Beckman and Coulter®, Germany; IGF-1 – A15729; IGFBP-3 – DSL-6600). Since approximately 80% of total IGF-1 is bound to IGFBP-3 [3], the calculation of the molar ratio of IGF-1/IGFBP-3 allows us to use the ratio as an estimate of bioavailable IGF-1. Serum peroxides were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognised as an efficient method for evaluating reactive oxygen species (ROS) in the body. The Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure serum peroxide levels, where 1.0 mg/L H_2O_2 represents one unit of ROS [30]. NT-proBNP levels were determined with the electrochemiluminesence method on the e411 (Roche, Basel, Switzerland). We determined the percentage of glycated haemoglobin (HbA1c) by means of the turbidimetric inhibition immunoassay on whole blood using the Roche Integra 400 (Roche, Basel, Switzerland). In the present sub-study, participants were considered as having diabetes if they had HbA1c levels of ≥6.5 % or made use of diabetes medication [31]. We used the Chronic Kidney Disease Epidemiology Formulas (CKD-EPI) to calculate estimate glomerular filtration rate (eGFR) from serum creatinine levels, age, sex and ethnicity [32]. HIV testing was done according to standard procedures of the South African Department of Health.

Statistical Analyses

For database management and statistical analyses we used Statistica version 12.0 (StatSoft, Inc.,Tulsa, OK). We tested the interaction of sex and ethnicity on the association between NT-proBNP and IGF-1 by introducing appropriate interaction terms in multiple regression analyses. Variables that were not normally distributed were logarithmically transformed (NT-proBNP, HbA1c, CRP and γ-GT). We subsequently compared means and proportions between black and

white groups using independent T-tests and Chi-square tests, respectively. We explored associations with NT-proBNP by quartiles of IGF-1 and IGF-1/IGFBP-3 while adjusting for age, sex and BMI with ANCOVA analyses. Multi-variable adjusted forward stepwise regression analyses were performed to investigate independent associations of NT-proBNP with IGF-1 and IGF-1/IGFBP-3. We considered all variables in Table 1 as potential covariates and included in final multiple regression models only those independent variables that correlated with IGF-1 and NT-proBNP in bi-variate analyses. The final model included age, sex, BMI, HbA1c, total cholesterol:HDL-C, CRP, ROS, γ-GT, systolic blood pressure (SBP), eGFR and self-reported smoking. These analyses were also repeated upon inclusion of individuals with diabetes. All p-values refer to 2-sided hypothesis.

RESULTS

We found no interaction with ethnicity ($p=0.24$) or sex (blacks, $p=0.076$; whites, $p=0.88$) on the association between NT-proBNP and IGF-1. We therefore pooled the men and women, but stratified the total group according to ethnicity in line with our aim and the literature [20, 33]. Table 1 lists the general characteristics of the black and white participants. Although the mean ages of the groups were similar, the black participants showed higher BMI ($p<0.001$) and 24hr systolic and diastolic blood pressure (both $p<0.001$) compared to the whites. NT-proBNP levels did not differ ($p=0.72$) between the groups, whereas IGF-1 ($p<0.001$) and IGFBP-3 ($p<0.001$) were lower the black group, but IGF-1/IGFBP-3 was similar ($p=0.17$).

Table 1: The cardiovascular, biochemical and anthropometric profiles of black and white participants.

	Black (n=160)	White (n=195)	p
Age (yrs)	43.8 ± 8.33	45.0 ± 10.8	0.25
Women, n (%)	83 (51.9)	102 (52.3)	0.94
Anthropometric measurements			
Body mass index (kg/m^2)	29.8 ± 6.93	27.4 ± 5.76	<0.001
Waist circumference (cm)	92.2 ± 14.7	92.5 ± 15.8	0.85
Biochemical measures			
NT-proBNP (pg/ml)	32.7 (7.08; 153)	33.8 (9.46; 120)	0.72
IGF-1 (ng/ml)	140 ± 53.0	179 ± 62.7	<0.001
IGFBP-3 (nmol/l)	122 ± 24.8	148 ± 23.3	<0.001
IGF-1/IGFBP-3	0.15 ± 0.05	0.16 ± 0.05	0.17
HbA1c (%)	5.69 (5.10; 6.30)	5.47 (5.00; 6.10)	<0.001
Total cholesterol:HDL-C ratio (mmol/L)	4.38 ± 2.06	4.95 ±1.61	0.004
Triglycerides (mmol/L)	1.30 ± 1.03	1.16 ± 0.70	0.12
C-reactive protein (mg/L)	4.19 (0.61; 32.3)	1.97 (0.99; 9.00)	<0.001
Reactive oxygen species (mg/L)	94.4 ±25.8	89.4 ± 27.0	0.078
eGFR (ml/min/1.73m^2)	110 ± 20.2	96.8 ± 14.9	<0.001
Cardiovascular measurements			
Ambulatory systolic blood pressure (mmHg)	132 ± 16.0	123 ± 11.5	<0.001
Ambulatory diastolic blood pressure (mmHg)	82.6 ± 10.5	76.2 ± 7.81	<0.001
Ambulatory pulse pressure (mmHg)	49.3 ± 9.00	47.2 ± 7.26	0.013
Ambulatory heart rate (b/min)	79.1 ± 11.2	73.2 ± 10.2	<0.001
Cornell Product (mV.ms)	68.8 ± 37.7	50.3 ± 28.7	<0.001
Stroke volume (ml)	100 ± 27.6	97.3 ± 24.4	0.29
Cardiac output (l/min)	6.70 ±1.89	6.34 ± 1.92	0.082
Total peripheral resistance (mmHg/ml/s)	1.02 ± 0.31	1.01 ± 0.29	0.80
CSWA (mm^2)	13.5 ± 4.23	13.0 ± 4.15	0.303
Lifestyle measures			
Gamma-glutamyl transferase (U/L)	45.1 (20.0; 166)	19.0 (7.00; 76.0)	<0.001
Smoking self-reported, n (%)	28 (17.5)	28 (14.4)	0.43
Medication use			
Anti-hypertensive medication, n (%)	50 (31.3)	24 (12.3)	<0.001
Hypertensive, n (%)	100 (62.5)	72 (36.9)	<0.001
HIV positive, n (%)	14 (8.75)	0 (0)	<0.001

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables.
n, number of participants;NTproBNP, N-terminal prohormone B-type natriuretic peptide; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; HbA1c, glycated haemoglobin; eGFR, estimated glomerular filtration rate; CSWA, cross-sectional wall area; HIV, human immunodeficiency virus.

Unadjusted correlations between NT-proBNP and IGF-1 are plotted in Figure 1. A negative association between NT-proBNP and IGF-1 (r=-0.36; p<0.001) was found in whites, but not in

blacks (r=-0.13; p=0.093). A negative correlation was obtained between NT-proBNP and IGF-1/IGFBP-3 in both the black (r=-0.16; p=0.049) and white (r=-0.34; p=0.001) groups (Figure 1S).

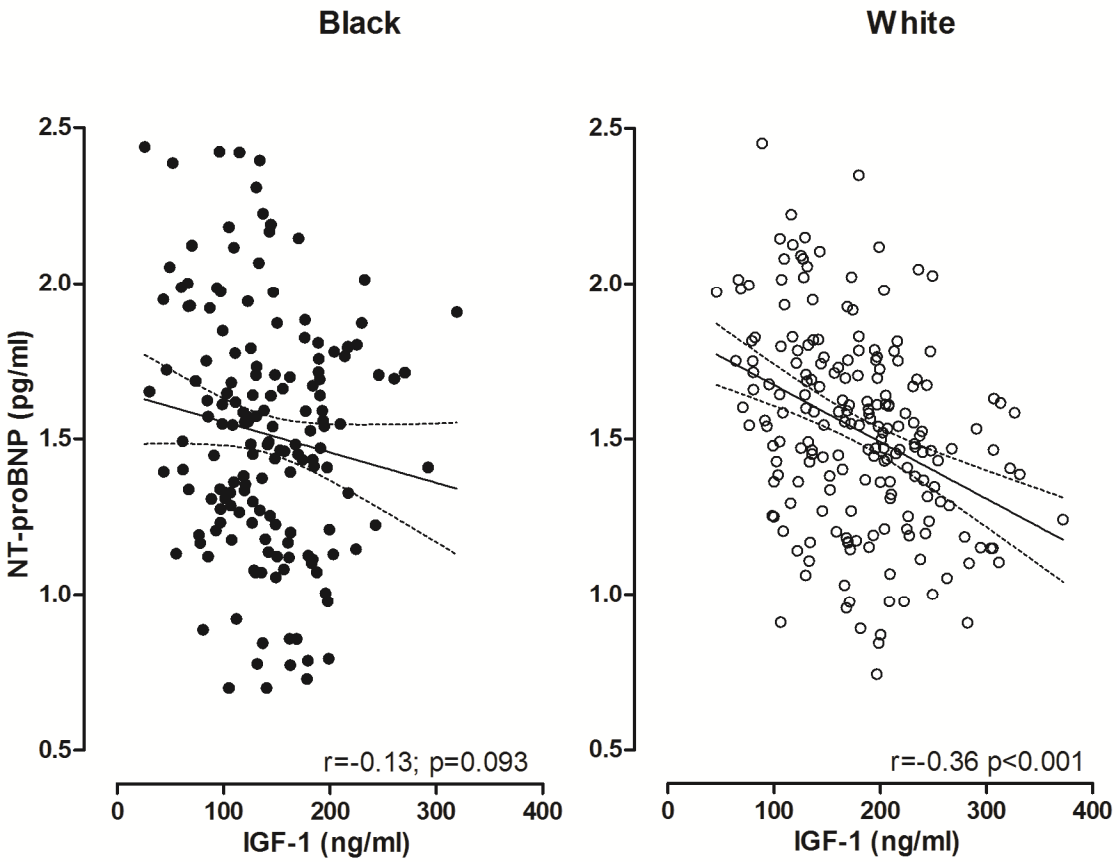


Figure 1: Unadjusted correlations between NT-proBNP and IGF-1.

We also plotted NT-proBNP by quartiles of IGF-1 (Figure 2), while adjusting for age, sex and BMI. The negative association between NT-proBNP and IGF-1 remained significant in the white group (p for trend = 0.047), with NT-proBNP in quartile 1 being significantly higher than quartile 2-4 (p≤0.006). No significant trend was found in the black group (p for trend = 0.90). In IGF-1/IGFBP-3 quartiles (Figure 2S), the aforementioned association with NT-proBNP remained

significant in the white group (p for trend = 0.049), with no associations in the black group (p for trend =0.30)

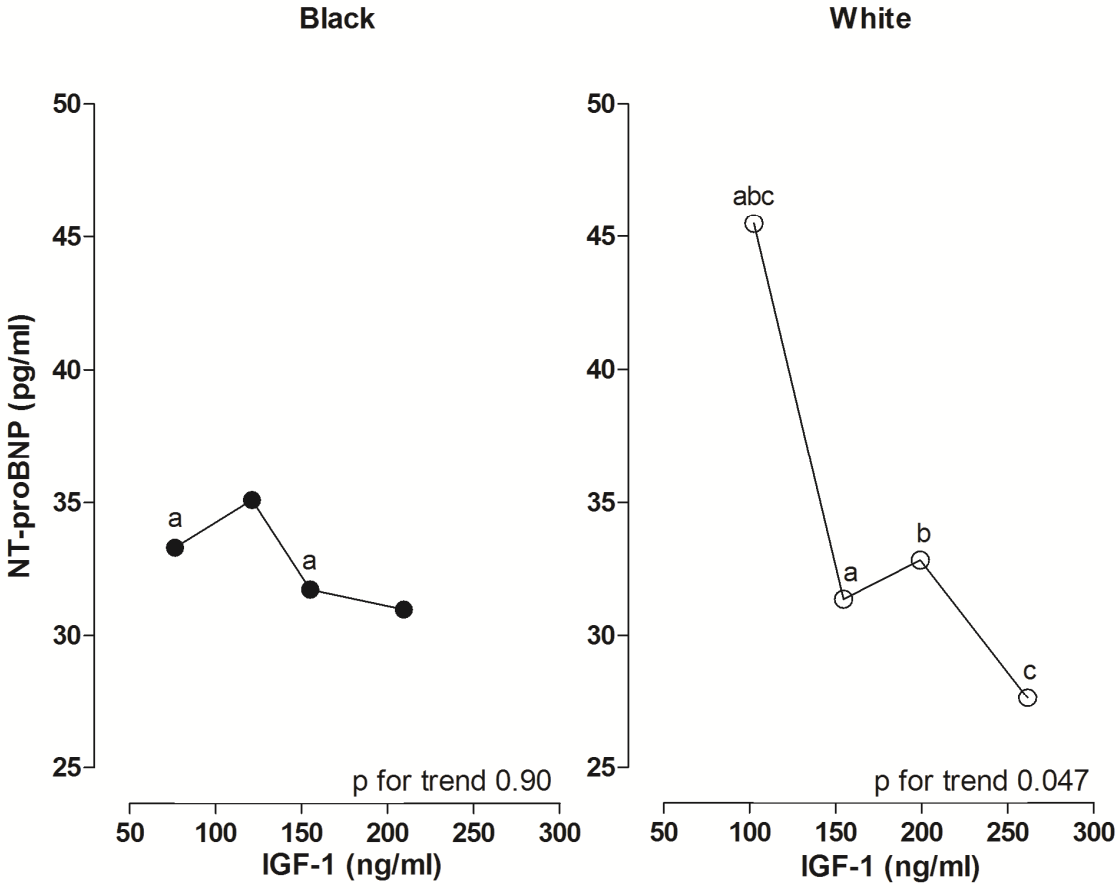


Figure 2: NT-proBNP levels by quartiles of IGF-1, while adjusting for age, sex and BMI.

We further performed multi-variable adjusted forward stepwise regression analyses with NT-proBNP as the dependent variable (Table 2) and assessed the association with IGF-1 (Model 1) and IGF-1/IGFBP-3 (Model 2) as our main independent variables. After full adjustments, the associations of NT-proBNP with IGF-1 (R^2=0.39; β=-0.22; p<0.001) and IGF-1/IGFBP-3 (R^2=0.38; β=-0.21; p=0.001) were confirmed in the white group. In the black group neither IGF-1 or IGF-1/IGFBP-3 entered the model.

Table 2: Independent associations of NT-proBNP with total IGF-1 and IGF-1/IGFBP-3.

NT-proBNP	Black (n=160)		White(n=195)	
	β (95% CI)	p	β (95% CI)	p
Model 1				
Adjusted R^2	0.37		0.39	
IGF-1 (ng/ml)	-		-0.22 (-0.35; -0.10)	<0.001
Age (yrs)	0.37 (0.23; 0.50)	<0.001	-	
Sex (women, men)	0.38 (0.22; 0.53)	<0.001	0.25 (0.11; 0.39)	<0.001
BMI (kg/m^2)	-0.16 (-0.31; -0.01)	0.032	-0.18 (-0.33; -0.04)	0.015
HbA1c (%)	-0.15 (-0.29; -0.01)	0.032	-0.14 (-0.26; -0.02)	0.029
TC/HDL ratio	-		-0.19 (-0.34; -0.05)	0.009
C-reactive protein (mg/L)	-		0.18 (0.05; 0.30)	0.007
SBP (mmHg)	0.28 (0.14; 0.42)	<0.001	0.21 (0.07; 0.35)	0.004
eGFR (ml/min/1.73m^2)	-		-0.26 (-0.38; -0.14)	<0.001
Self-reported smoking (yes/no)	-		-0.13 (-0.24; -0.02)	0.027
Model 2				
Adjusted R^2	0.37		0.38	
IGF-1/IGFBP-3	-		-0.21 (-0.33; -0.09)	0.001
Age (yrs)	0.37 (0.23; 0.50)	<0.001	-	
Sex (women, men)	0.38 (0.22; 0.53)	<0.001	0.18 (0.03; 0.33)	0.024
BMI (kg/m^2)	-0.16 (-0.31; -0.02)	0.032	-0.18 (-0.33; -0.03)	0.017
HbA1c (%)	-0.15 (-0.29; -0.01)	0.032	-0.13 (-0.26; -0.01)	0.040
TC/HDL ratio	-		-0.19 (-0.34; -0.04)	0.012
C-reactive protein (mg/L)	-		0.22 (0.09; 0.37)	0.002
γ-GT (U/L)	-		-0.14 (-0.28; -0.01)	0.056
SBP (mmHg)	0.28 (0.14; 0.42)	<0.001	0.23 (0.09; 0.37)	0.001
eGFR (ml/min/1.73m^2)	-		-0.28 (-0.40; -0.17)	<0.001
Self-reported smoking (yes/no)	-		-0.11 (-0.22; -0.01)	0.054

β, partial regression coefficient; 95% CI, 95% confidence intervals of β; NTproBNP, N-terminal prohormone B-type natriuretic peptide; IGF-1, insulin-like growth factor-1; IGF-1/IGFBP-3 ratio, insulin-like growth factor-1/insulin-like growth factor binding protein-3; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL ratio, total cholesterol:high-density lipoprotein cholesterol; γ-GT, gamma-glutamyl transferase; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate.
Covariates included IGF-1 or IGF-1/IGFBP-3, age, sex, BMI, HbA1c, TC:HDL, C-reactive protein, γ-GT, reactive oxygen species, systolic blood pressure, eGFR and self-reported smoking.
$p < 0.05$ regarded as statistically significant.

In sensitivity analyses we repeated Model 1 with Cornell product as additional covariate. In neither ethnic group did Cornell product enter the model and the results remained unchanged (Table 1S).

We further included participants with diabetes (n=38) into the analyses (Table 2S). The inverse associations of NT-proBNP with IGF-1 (R^2=0.38; β=-0.22; p<0.001) and IGF-1/IGFBP-3 (R^2=0.37; β=-0.20; p=0.002) were confirmed in the white participants only.

Lastly, we also repeated the analyses after excluding HIV infected participants (n=14), but the results remained unchanged for the inverse association between NT-proBNP and IGF-1 in whites (R^2=0.39; β=-0.22; p<0.001) and no association in blacks.

DISCUSSION

In a black population with elevated blood pressure, higher subclinical ECG-derived left ventricular mass and lower IGF-1 levels compared to whites, we found no link between NT-proBNP and IGF-1. In contrast an independent negative association was found between NT-proBNP and IGF-1 in whites. These findings remained robust after the inclusion of those with diabetes.

Conflicting findings are consistently reported on the association between NT-proBNP and IGF-1 [34-37]. Elevated NT-proBNP is a well established biomarker of chronic heart failure [34] and in acromegaly patients, the overtly elevated IGF-1 levels, were significantly associated with the development of chronic heart failure [35]. Contradictory to these findings, Duerr et al. found that the administration of IGF-1 in cardiac failure patients resulted in improved cardiac function, suggesting a beneficial effect of IGF-1 in cardiac failure patients [36]. Alternatively, adult patients with growth hormone deficiency had increased NT-proBNP levels which were significantly reduced after 12 months of growth hormone replacement therapy [37]. However changes in NT-proBNP may occur independently of changes in cardiac structure or function,

suggesting that the effects of growth hormones on NT-proBNP levels may be independent of cardiovascular changes [38] or reflect subtle cardiac changes.

Our results are consistent with our previous findings in a different population sample that have shown higher blood pressure and significantly lower IGF-1 in blacks compared to whites [20]. In the latter study lower IGF-1 was accompanied by multiple traditional cardiovascular risk factors including high blood pressure, arterial stiffness, γ-GT, and smoking [20]. We demonstrated further that lower IGF-1 in blacks related to high blood pressure and von Willebrand factor, reflecting endothelial damage [17, 19]. However, when we investigated IGF-1 on a cardiac level in the present study, we did not find any association between NT-proBNP and IGF-1 in the blacks. Also, blacks had similar NT-proBNP levels than whites which are in contrast with findings from Kruger et al. in another South African population that found higher NT-proBNP in blacks compared to whites [33]. It is important to note that the prevalence of smoking was higher and the socioeconomic status lower in the blacks than whites in the study of Kruger et al., which could contribute to elevated cardiovascular risk, while blacks and whites from the present study are from a similar socioeconomic class. Nevertheless, SBP correlated positively with NT-proBNP in the black group in both Kruger et al. and the present study [33]. Since NT-proBNP did not associate with IGF-1 in this group, but mainly with SBP it could suggest a disparate manner in which cardiovascular risk factors contribute to NT-proBNP concentrations, also confirmed by our multiple regression models. It is therefore plausible that IGF-1 could differentially influence cardiac function, in whites through direct effects, and in blacks indirectly through blood pressure. An additional explanation for the lack of an association between NT-proBNP and IGF-1 in blacks may be due to the significantly reduced total IGF-1 concentrations in blacks which may disturb the regulation of IGF-1 on cardiac function. Also, NT-proBNP concentrations do not seem to be elevated in conditions of low IGF-1 for blacks within the

lowest IGF-1 quartile (Figure 1). This again may confirm the disparate regulatory manner of IGF-1 in different ethnic groups.

The association between NT-proBNP and IGF-1 in whites may thus indicate the direct regulatory cardioprotective function of IGF-1 [1, 11, 15, 16]. Such direct effect of IGF-1 on cardiac function may possibly be explained by the following mechanisms. In the vasculature IGF-1 exerts beneficial effects mainly by increasing NO production [39]. IGF-1 in cardiomyocytes protects the heart by regulating cardiac growth and metabolism by increasing protein synthesis, cardiomyocyte size, amino acid uptake and muscle specific gene expression [11, 15]. IGF-1 is also an inhibitor of apoptosis to prevent cardiomyocyte loss [15]. At cellular level, the anti-apoptotic effects of IGF-1 are mediated by the activation of the Ras/Raf/Mitogen-activated protein kinase (MEK)/ERK and the PI3K/Akt/mechanistic target of rapamycin (mTOR) signalling pathways [15]. Furthermore, the IGF-1 axis influences cardiac contractility through various mechanisms, including altering the intracellular Ca^{2+} transient through an increase in L-type calcium channel activity [40], by enhancing myofilament sensitivity to Ca^{2+} [41] and by upregulating sarcoplasmic reticulum ATPase (SERCA) levels [42]. Thus, IGF-1 opposes endothelial dysfunction, while its cardiac effects may act to reduce wall stress and result in preserved cardiac performance [16].

Although NT-proBNP is a stable marker of cardiac function including systolic dysfunction and cardiac overlaod, additional echocardiography should be considered in future studies since it would give a better cardiac profile especially with regards to left ventricular hyperthrophy. This study needs to be interpreted within the context of its strengths and limitations. Since this is a cross-sectional study, causality cannot be inferred. It is possible that our findings were due to confounding variables or unknown factors that are associated with both NT-proBNP and IGF-1.

Nevertheless, this was a well-designed study implemented under controlled conditions, and a first to examine the link between NT-proBNP and IGF-1 in a bi-ethnic population of South Africa.

In conclusion, IGF-1 was higher in whites compared to blacks and associated inversely with NT-proBNP, supporting the cardioprotective role of IGF-1. We found no association between NT-proBNP and lower IGF-1 in a hypertension-prone black population which could possibly be attributed to other factors such as SBP, which may have a greater contributory role on cardiac pathology.

Acknowledgements

The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study would not have been possible without the voluntary collaboration of the participants and the Department of Education, North West province, South Africa. The authors gratefully acknowledge the technical assistance of Mrs. Tina Scholtz, Sr. Chrissie Lessing and Dr. Szabolcs Péter. This work was partially funded by the Medical Research Council, South Africa; Roche Diagnostics; the North-West University; and the Metabolic Syndrome Institute, France. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.

The Authors declare that there is no conflict of interest.

REFERENCES

1. Troncoso R, Ibarra C, Vicencio JM, Jaimovich E, Lavandero S. New insights into IGF-1 signaling in the heart. Trends Endocrinol Metab 2014;25:128-37.
2. Ungvari Z, Csiszar A. The emerging role of IGF-1 deficiency in cardiovascular aging: recent advances. J Gerontol A Biol Sci Med Sci 2012;67:599-610.
3. Conti E, Musumeci M, De Giusti M, Dito E, Mastromarino V, Volpe M. IGF-1 and atherothrombosis: relevance to pathophysiology and therapy. Clin Sci 2011;120:377-402.
4. Izhar U, Hasdai D, Richardson DM, Cohen P, Lerman A. Insulin and insulin-like growth factor-I cause vasorelaxation in human vessels in vitro. Coron Artery Dis 2000;11:69-76.
5. Jan H, Borensztajn KS, Moimas S, van Heiningen S, Teeling P, van Berkel TJ, et al. IGF-1 has plaque-stabilizing effects in atherosclerosis by altering vascular smooth muscle cell phenotype. Am J Pathol 2011;178:924-34.
6. Hutter R, Sauter BV, Reis ED, Roque M, Vorchheimer D, Carrick FE, et al. Decreased reendothelialization and increased neointima formation with endostatin overexpression in a mouse model of arterial injury. Circulation 2003;107:1658-63.
7. Dimmeler S, Zeiher AM. Exercise and Cardiovascular Health Get Active to “AKTivate” Your Endothelial Nitric Oxide Synthase. Circulation 2003;107:3118-20.
8. Spies M, Nesic O, Barrow R, Perez-Polo J, Herndon D. Liposomal IGF-1 gene transfer modulates pro-and anti-inflammatory cytokine mRNA expression in the burn wound. Gene Ther 2001;8:1409-15.
9. Vetter U, Kupferschmid C, Lang D, Pentz S. Insulin-like growth factors and insulin increase the contractility of neonatal rat cardiocytes in vitro. Basic Res Cardiol 1988;83:647-54.

10. Freestone NS, Ribaric S, Mason WT. The effect of insulin-like growth factor-1 on adult rat cardiac contractility. Mol Cell Biochem 1996;163:223-9.

11. Castellano G, Affuso F, Conza PD, Fazio S. The GH/IGF-1 Axis and Heart Failure. Curr Cardiol Rev 2009;5:203-15.

12. Nagoshi T, Yoshimura M, MC Rosano G, D Lopaschuk G, Mochizuki S. Optimization of cardiac metabolism in heart failure. Curr Pharm Des 2011;17:3846-53.

13. McMullen JR, Shioi T, Huang WY, Zhang L, Tarnavski O, Bisping E, et al. The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase (p110alpha) pathway. J Biol Chem 2004;279:4782-93.

14. Troncoso R, Vicencio JM, Parra V, Nemchenko A, Kawashima Y, Del Campo A, et al. Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Cardiovasc Res 2012;93:320-9.

15. Opgaard OS, Wang PH. IGF-I is a matter of heart. Growth Horm IGF Res 2005;15:89-94.

16. Isgaard J, Arcopinto M, Karason K, Cittadini A. GH and the cardiovascular system: an update on a topic at heart. Endocrine 2015;48:25-35.

17. Koegelenberg A, Schutte R, Smith W, Schutte A. Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study. Thromb Res 2015;136:1007-12.

18. Schutte AE, Volpe M, Tocci G, Conti E. Revisiting the relationship between blood pressure and insulin-like growth factor-1. Hypertension 2014;63:1070-7.

19. Schutte A, Schutte R, Smith W, Huisman H, Mels C, Malan L, et al. Compromised bioavailable IGF-1 of black men relates favourably to ambulatory blood pressure: The SABPA study. Atherosclerosis 2014;233:139-44.

20. Schutte AE, Huisman HW, van Rooyen JM, Malan L, Malan NT, Fourie CMT, et al. A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. J Clin Endocrinol Metab 2010;95:2503-7.

21. Sliwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. The Lancet 2008;371:915-22.

22. Weber M, Mitrovic V, Hamm C. B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide - Diagnostic role in stable coronary artery disease. Exp Clin Cardiol 2006;11:99-101.

23. Kruger R, Schutte R, Huisman HW, Olsen MH, Schutte AE. NT-proBNP and potential vascular calcification in Black and Caucasian African men: the SAfrEIC study. Ethnicity and Disease 2012;22:398-403.

24. Malan L, Hamer M, Frasure-Smith N, Steyn HS, Malan NT. Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study. Int J Epidemiol 2015:44:1814-22.

25. Norton K, Olds T. Anthropometrica: A textbook of body measurement for sports and health courses: University of New South Wales Press; 1996.

26. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21:93-111.

27. McGRATH B. Non-invasive measurements of arterial structure and function: repeatability, interrelationships and trial sample size. Clin Sci 1998;95:669-79.

28. Dahlof B, Devereux RB, Julius S, Kjeldsen SE, Beevers G, de Faire U, et al. Characteristics of 9194 patients with left ventricular hypertrophy: the LIFE study. Losartan Intervention For Endpoint Reduction in Hypertension. Hypertension 1998;32:989-97.

29. Okin PM, Roman MJ, Devereux RB, Kligfield P. Electrocardiographic identification of increased left ventricular mass by simple voltage-duration products. J Am Coll Cardiol 1995;25:417-23.

30. Hayashi I, Morishita Y, Imai K, Nakamura M, Nakachi K, Hayashi T. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat Res 2007;631:55-61.

31. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37 Suppl 1:S81-90.

32. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-12.

33. Kruger R, Schutte R, Huisman HW, Hindersson P, Olsen MH, Schutte AE. N-terminal prohormone B-type natriuretic peptide and cardiovascular function in Africans and Caucasians: the SAfrEIC study. Heart Lung Circ 2012;21:88-95.

34. Andreadis EA, Geladari C. Brain Natriuretic Peptide: Structure, Action and Role in the Diagnosis and Prognosis of Heart Failure. Hospital Chronicles 2015;10:202-9.

35. Clayton R. Cardiovascular function in acromegaly. Endocr Rev 2003;24:272-7.

36. Duerr RL, Huang S, Miraliakbar HR, Clark R, Chien KR, Ross J,Jr. Insulin-like growth factor-1 enhances ventricular hypertrophy and function during the onset of experimental cardiac failure. J Clin Invest 1995;95:619-27.

37. Wallaschofski H, Saller B, Spilcke-Liss E, Lerch MM, Lohmann T, Eigenthaler M. Effects of growth hormone treatment on B-type natriuretic peptide as a marker of heart failure in adults with growth hormone deficiency. Horm Metab Res 2006;38:656-61.

38. Gruson D, Alexopoulou O, Pasquet A, Cumps J, Ketelslegers J, Maiter D. Impact of growth hormone (GH) treatment on circulating Nt-proBNP concentrations and on cardiac function in adult GH-deficient patients. Scand J Clin Lab Invest 2012;72:387-94.

39. Conti E, Carrozza C, Capoluongo E, Volpe M, Crea F, Zuppi C, et al. Insulin-like growth factor-1 as a vascular protective factor. Circulation 2004;110:2260-5.

40. Solem ML, Thomas AP. Modulation of cardiac Ca 2 channels by IGF1. Biochem Biophys Res Commun 1998;252:151-5.

41. Buerke M, Murohara T, Skurk C, Nuss C, Tomaselli K, Lefer AM. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. Proc Natl Acad Sci U S A 1995;92:8031-5.

42. Houck WV, Pan LC, Kribbs SB, Clair MJ, McDaniel GM, Krombach RS, et al. Effects of growth hormone supplementation on left ventricular morphology and myocyte function with the development of congestive heart failure. Circulation 1999;100:2003-9.

SUPPLEMENTARY MATERIAL

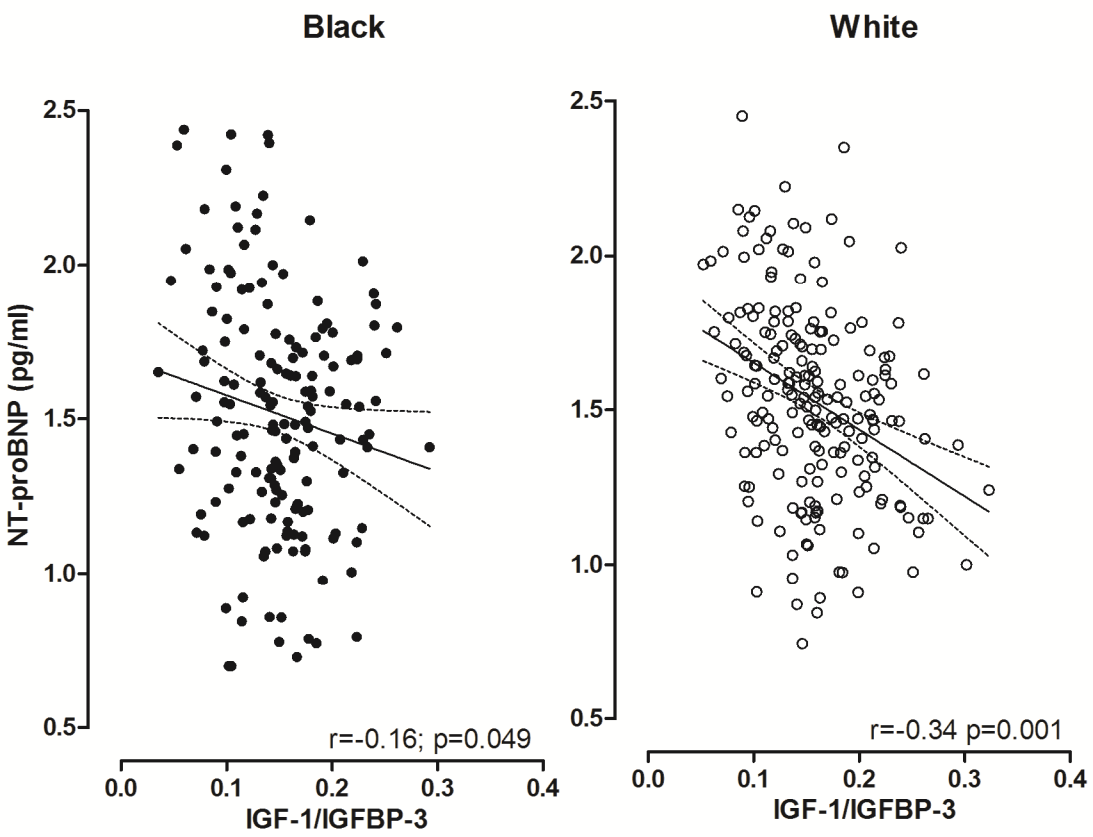


Figure 1S: Unadjusted correlations between NT-proBNP and IGF-1/IGFBP-3.

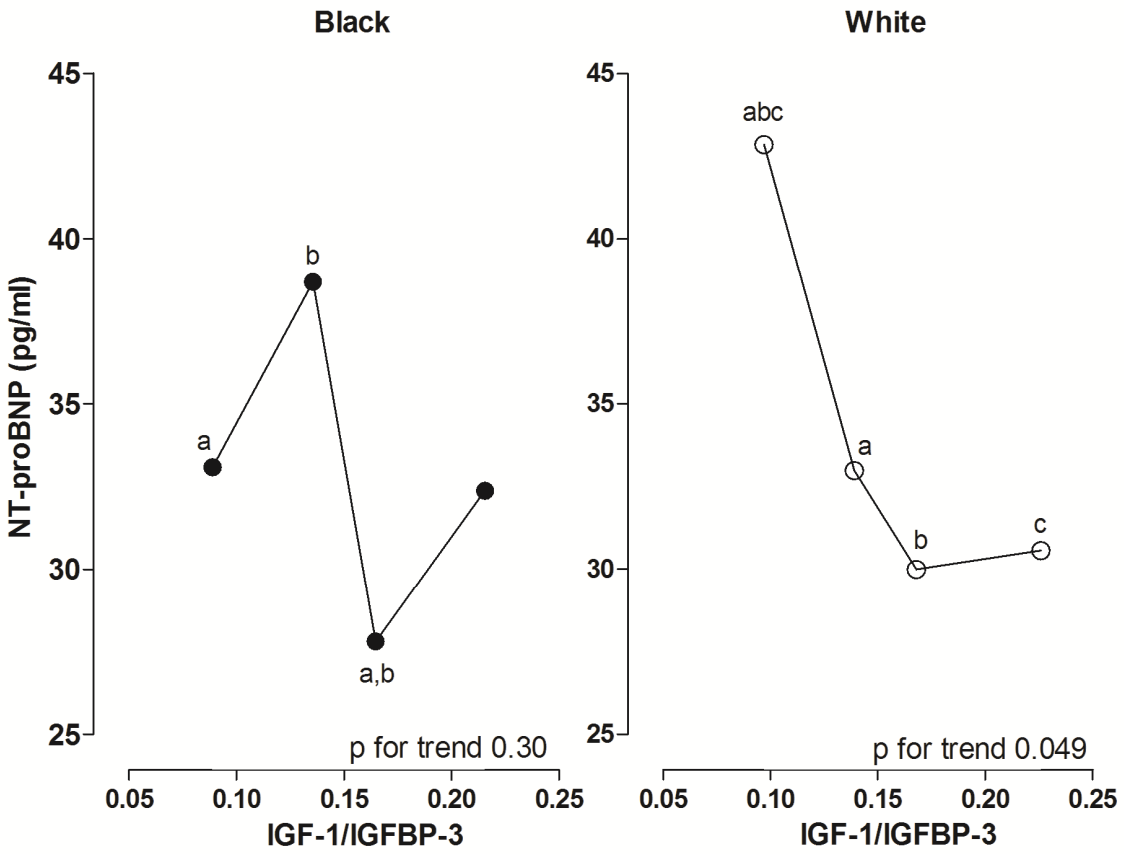


Figure 2S: NT-proBNP levels by quartiles of IGF-1/IGFBP-3, while adjusting for age, sex and BMI.

Table 1S: Independent associations of NT-proBNP with IGF-1.

NT-proBNP	Black (n=160)		White(n=195)	
	β (95% CI)	p	β (95% CI)	p
Adjusted R^2	0.37		0.38	
Main independent variable: IGF-1 (ng/ml)	-		-0.22 (-0.35; -0.09)	<0.001
Age (yrs)	0.37 (0.22; 0.52)	<0.001	-	
Sex (women, men)	0.38 (0.21; 0.55)	<0.001	0.25 (0.10; 0.40)	0.001
BMI (kg/m^2)	-0.16 (-0.33; -0.01)	0.047	0.18 (-0.34; -0.03)	0.023
HbA1c (%)	-0.15 (-0.30; -0.01)	0.047	-0.14 (-0.27; -0.01)	0.041
TC/HDL ratio	-		-0.19 (-0.35; -0.04)	0.014
C-reactive protein (mg/L)	-		0.18 (0.04; 0.31)	0.012
Systolic blood pressure (mmHg)	0.28 (0.13; 0.43)	<0.001	0.21 (0.06; 0.36)	0.007
eGFR ($ml/min/1.73m^2$)	-		-0.26 (-0.39; -0.13)	<0.001
Self-reported smoking	-		-0.13 (-0.25; -0.01)	0.038

β, partial regression coefficient; 95% CI, 95% confidence intervals of β; NTproBNP, N-terminal prohormone B-type natriuretic peptide; IGF-1, insulin-like growth factor-1; IGF-1/IGFBP-3 ratio, insulin-like growth factor-1/insulin-like growth factor binding protein-3; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL ratio, total cholesterol:high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.
Covariates included IGF-1 or IGF-1/IGFBP-3, age, sex, BMI, HbA1c, TC:HDL, C-reactive protein, gamma-glutamyl transferase, reactive oxygen species, systolic blood pressure, eGFR, self-reported smoking and Cornell product.
$p < 0.05$ regarded as statistically significant.

Table 2S: Independent associations of NT-proBNP with total IGF-1and IGF-1/IGFBP-3 with inclusion of patients with diabetes (n=38).

NT-proBNP	Black (n=191)		White(n=202)	
	β (95% CI)	p	β (95% CI)	p
Model 1				
Adjusted R^2	0.33		0.38	
Main independent variable: IGF-1 (ng/ml)	-		-0.22 (-0.35; -0.10)	<0.001
Age (yrs)	0.33 (0.23; 0.48)	<0.001	-	
Sex (women, men)	0.41 (0.32; 0.62)	<0.001	0.27 (0.13; 0.42)	<0.001
BMI (kg/m^2)	-0.20 (-0.31; -0.04)	0.009	-0.17 (-0.32; -0.02)	0.029
HbA1c (%)	-0.18 (-0.31; -0.06)	0.010	-0.16 (-0.30; -0.03)	0.014
TC/HDL ratio	-		-0.17 (-0.31; -0.02)	0.025
C-reactive protein (mg/L)	-		0.17 (0.04; 0.29)	0.013
Systolic blood pressure (mmHg)	0.30 (0.16; 0.42)	<0.001	0.20 (0.05; 0.34)	0.009
eGFR (ml/min/1.73m^2)	-		-0.26 (-0.38; -0.15)	<0.001
Self-reported smoking (yes,no)	-		-0.12 (-0.24; -0.01)	0.037
Model 2				
Adjusted R^2	0.33		0.37	
Main independent variable: IGF-1/IGFBP-3	-		-0.20 (-0.30; -0.03)	0.002
Age (yrs)	0.33 (0.20; 0.47)	<0.001	-	
Sex (women, men)	0.41 (0.25; 0.56)	<0.001	0.21 (0.05; 0.36)	0.010
BMI (kg/m^2)	-0.20 (-0.35; -0.05)	0.009	-0.17 (-0.32; -0.02)	0.032
HbA1c (%)	-0.18 (-0.32; -0.05)	0.010	-0.16 (-0.29; -0.03)	0.019
TC/HDL ratio	-		-0.16 (-0.31; -0.01)	0.033
C-reactive protein (mg/L)	-		0.21 (0.08; 0.35)	0.003
γ-GT (U/L)	-		-0.14 (-0.28; 0.01)	0.061
Systolic blood pressure (mmHg)	0.30 (0.15; 0.45)	<0.001	0.22 (0.07; 0.37)	0.004
eGFR (ml/min/1.73m^2)	-		-0.29 (-0.41; -0.17)	<0.001
Self-reported smoking (yes,no)	-		-0.10 (-0.22; 0.01)	0.076

β, partial regression coefficient; 95% CI, 95% confidence intervals of β; NTproBNP, N-terminal prohormone B-type natriuretic peptide; IGF-1, insulin-like growth factor-1; IGF-1/IGFBP-3 ratio, insulin-like growth factor-1/insulin-like growth factor binding protein-3; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL ratio, total cholesterol:high-density lipoprotein cholesterol; γ-GT, gamma-glutamyl transferase; CSWA, cross sectional wall area; eGFR, estimated glomerular filtration rate.
Covariates included IGF-1 or IGF-1/IGFBP-3, age, sex, BMI, HbA1c, TC:HDL, C-reactive protein;γ-GT, reactive oxygen species, systolic blood pressure, eGFR and self-reported smoking.
$p < 0.05$ regarded as statistically significant.

JOURNAL DETAILS	
Title:	*European Journal of Clinical Investigation*
Impact Factor:	2.734
Publisher:	Wiley Online Library

Aim & Scope: EJCI considers any original contribution from the most sophisticated basic molecular sciences to applied clinical and translational research and evidence-based medicine across a broad range of subspecialties. The EJCI publishes reports of highquality research that pertain to the genetic, molecular, cellular, or physiological basis of human biology and disease, as well as research that addresses prevalence, diagnosis, course, treatment, and prevention of disease. We are primarily interested in studies directly pertinent to humans, but submission of robust in vitro and animal work is also encouraged. Interdisciplinary work and research using innovative methods and combinations of laboratory, clinical, and epidemiological methodologies and techniques is of great interest to the journal.

JOURNAL GUIDELINES				
Author guidelines:				
Title:	Not exceed 70 characters. Avoid using declarative sentences or questions		**Conflict of interest:**	Yes
Abstract:	Max: 250 words		**Acknowledgements:**	Yes
Keywords:	Max: 6		**Language:**	English
Manuscript:	2700 words			
References in text:	Indicate references by number(s) in square brackets in line with the text.			
List of references:	List 6 authors, followed by 'et al' if the number exceeds 6. An example of a correct reference is given below: 1. Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.			
Sections:	Page 1: Title page Page 2: Abstract and keywords Page 3: Introduction, materials and methods, results, discussion and conclusion, acknowledgements, list of references.			

Chapter 6

Final Discussion, Conclusions and Recommendations

1. INTRODUCTION

In this concluding chapter, a summary of the main findings of the three manuscripts reported in this thesis will be presented. The results from each manuscript will be discussed, interpreted, explained and compared to the relevant literature. Conclusions will be drawn and recommendations made to the reader with regards to the relationship between IGF-1 and cardiometabolic risk as well as markers of vascular endothelial damage and cardiac overload.

2. SUMMARY OF MAIN FINDINGS, DISCUSSION AND COMPARISON TO THE LITERATURE

The relevant findings and a comparison with the literature for each hypothesis, as set in Chapter 1, are discussed below. The three main hypotheses were addressed in the manuscripts reported in this thesis (Chapter 3, 4 and 5).

Hypothesis 1: *Bioavailable IGF-1 is inversely associated with the number of metabolic syndrome components in both sexes and ethnicities (Chapter 3).*

Insulin-like growth factor 1, a vascular protective factor, is reduced in patients with cardiovascular and metabolic diseases,[1,2] and is inversely associated with the metabolic syndrome.[3] However whether this relationship holds true for the black population of South Africa, in whom a high metabolic syndrome prevalence[4] and attenuated IGF-1 levels[5,6] have been reported, required further investigation. In the manuscript, *Bioavailable IGF-1 and its relation to the metabolic syndrome in a bi-ethnic population of men and women*, we therefore aimed to explore this relationship in black and white men and women.

In this cross-sectional analyses our results showed that bioavailable IGF-1 was inversely associated with the number of metabolic syndrome components in both sexes of black and white participants. The set hypothesis is therefore accepted.

This study is the first to explore the relationship between IGF-1 and the metabolic syndrome in a large population sample from South Africa (n=907). Our findings therefore add to the literature in this regard. In line with our findings, others also found an inverse association between bioavailable IGF-1 and the metabolic syndrome in other populations.[7-9] Low IGF-1 levels have been found to associate with reduced insulin sensitivity, glucose intolerance and type 2 diabetes.[10-12] Sierra-Johnson et al.[3] examined 3281 participants from the NHANES III (Third National Health and Nutrition Examination Survey) study and found that bioavailable IGF-1 decreased notably as the number of metabolic syndrome components increased. Sesti et al.[2] found IGF-1 concentrations to be significantly lower in individuals with the metabolic syndrome compared to those without. However, in older subjects, the link between IGF-1 and the metabolic syndrome contradict our results. Van Bunderen et al.[13] found in individuals older than 64 years of age that high-normal IGF-1 levels were associated with prevalent metabolic syndrome. Schneider et al.[14] as well as Yeap et al.[15] found a U-shape relationship where both low and high IGF-1 levels were associated with the risk of developing the metabolic syndrome and type 2 diabetes. The relationship between IGF-1 and the metabolic syndrome thus appears to differ in different age groups. However, our study did not allow for the assessment of this relationship within different age groups as the range of our population was not broad enough, thus further exploration is needed on this matter.

Nevertheless, the underlying pathophysiological mechanisms between IGF-1 and the metabolic syndrome remain unclear. To add to the literature in this regard, we further investigated whether this relationship is independent of factors known to adversely affect IGF-1 levels, including markers of inflammation and oxidative stress that are also characteristics of non-alcoholic and alcoholic fatty liver disease (hence the inclusion of gamma-glutamyl tranferase as an indicator of liver dysfunction).[6,16-19] To the best of our knowledge, none of the previous studies investigating the association between IGF-1 and the metabolic syndrome accounted for these risk factors in

their analyses. We found the inverse association between IGF-1 and the number of metabolic syndrome components in both sexes of black and white South Africans to be dependent on these risk factors, alluding to the potential underlying processes by which the metabolic syndrome attenuates IGF-1. These findings not only add but also support the literature given that the metabolic syndrome is associated with the overproduction of inflammatory markers,[20,21] reactive oxygen species[22] and increased levels of gamma-glutamyl transferase,[23] which will in turn attenuate IGF-1 levels.[6,16-19]

Hypothesis 2: *Bioavailable IGF-1 is inversely associated with vWF in both ethnicities (Chapter 4).*

Black South Africans are susceptible to hypertension[24] and cardiovascular disease,[25] which seems to originate from vascular abnormalities.[24,25] Furthermore, black individuals display attenuated IGF-1 levels,[5,6] which could compromise IGF-1's endothelial protective properties.[26] As already found in Chapter 3, reduced IGF-1 levels are associated with various cardiometabolic risk factors that may exert their detrimental effects through the pathway of endothelial dysfunction.[27-29] To improve our understanding on the potential role of reduced IGF-1 in the development of endothelial dysfunction, we aimed to establish whether a relationship exists between bioavailable IGF-1 and von Willebrand factor (vWF), a marker which is indicative of vascular endothelial damage. This study was performed in black and white South African school teachers in the manuscript: *Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study.*

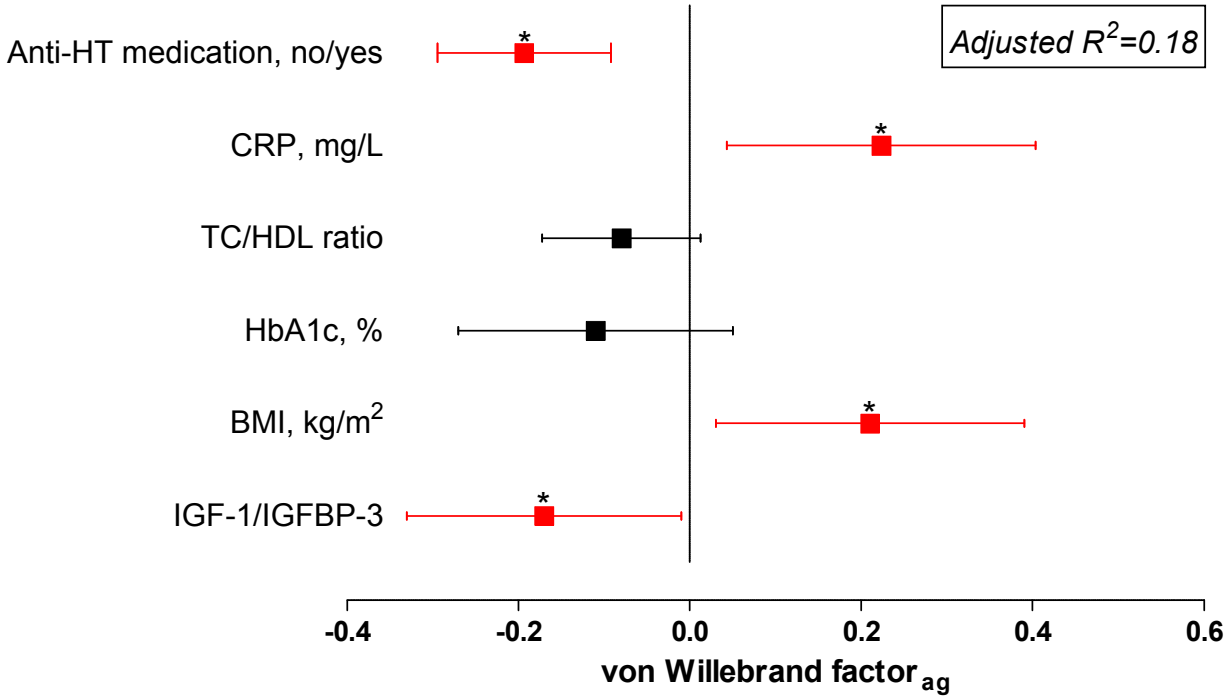


Figure 1: Forest plot indicating forward stepwise regression analyses of von Willebrand factor antigen with IGF-1/IGFBP-3 as main independent variable in blacks. Values are indicated as standardised β (± 95% confidence interval).
CRP, C-reactive protein; TC/HDL ratio, total cholesterol/high density lipoprotein ratio; HbA1c, glycated haemoglobin; BMI, body mass index; IGF-1/IGFBP-3, insulin-like growth factor-1/Insulin-like growth factor binding protein-3.
Covariates included IGF-1/IGFBP-3 or IGF-1, age, sex, BMI, HbA1c, TC:HDL, triglycerides, C-reactive protein; pulse pressure, reactive oxygen species, γ-GT, smoking and antihypertensive medication.
* p ≤.05

Our results indicated that vWF was inversely associated with bioavailable IGF-1 in black South Africans (Figure 1) only, who also displayed significantly higher vWF and lower IGF-1 than whites. The hypothesis for this manuscript can therefore only be partly accepted since a negative association was evident in blacks, but no association was found in whites.

Parallel to our findings, Lutsey et al.[30] found that black American individuals display higher levels of vWF than white, Hispanic or Chinese individuals. In addition, Platz et al.[31] indicated that black Americans had lower IGF-1 concentrations than whites and Asians. To our knowledge no previous studies focussed directly on the association between IGF-1 and vWF,

highlighting the contribution of our findings to the literature. However there is evidence that reduced IGF-1 is associated with impaired endothelial function as measured by flow-mediated dilation.[32] Abdu et al.[33] also indicated that individuals with low IGF-1 have reduced vasodilation. It is known that IGF-1 induces nitric oxide (NO) release and prostacyclin synthesis[34,35] which both exert an autocrine negative feedback on vWF secretion.[36,37] Therefore, we might expect that a reduction in IGF-1 among black South Africans, who also presented a more unfavourable cardiometabolic profile compared to whites, would possibly increase their susceptibility to endothelial damage as supported by the negative relationship found between vWF and IGF-1. The increased risk for hypertension development and cardiovascular events among black South Africans[24,25] may be partly due to these findings.

Hypothesis 3: *NT-proBNP is inversely associated with IGF-1 in black and white individuals (Chapter 5).*

The vascular and cardioprotective peptide, IGF-1, is inversely associated with blood pressure[38] and as indicated in this thesis, with a marker of endothelial damage[39] in black individuals who display low IGF-1 concentrations. Kruger et al.[40] found that NT-proBNP, which is a marker of cardiac overload, left ventricular dysfunction and heart failure,[41] were significantly higher among blacks when compared to whites in the SAfrEIC study. In order to investigate whether IGF-1 levels associate in a protective manner with a marker of cardiac overload and systolic dysfunction, the aim of the last manuscript, entitled *IGF-1 and NT-proBNP in a black and white population: The SABPA study,* was to explore the association between NT-proBNP and IGF-1 in black and white school teachers.

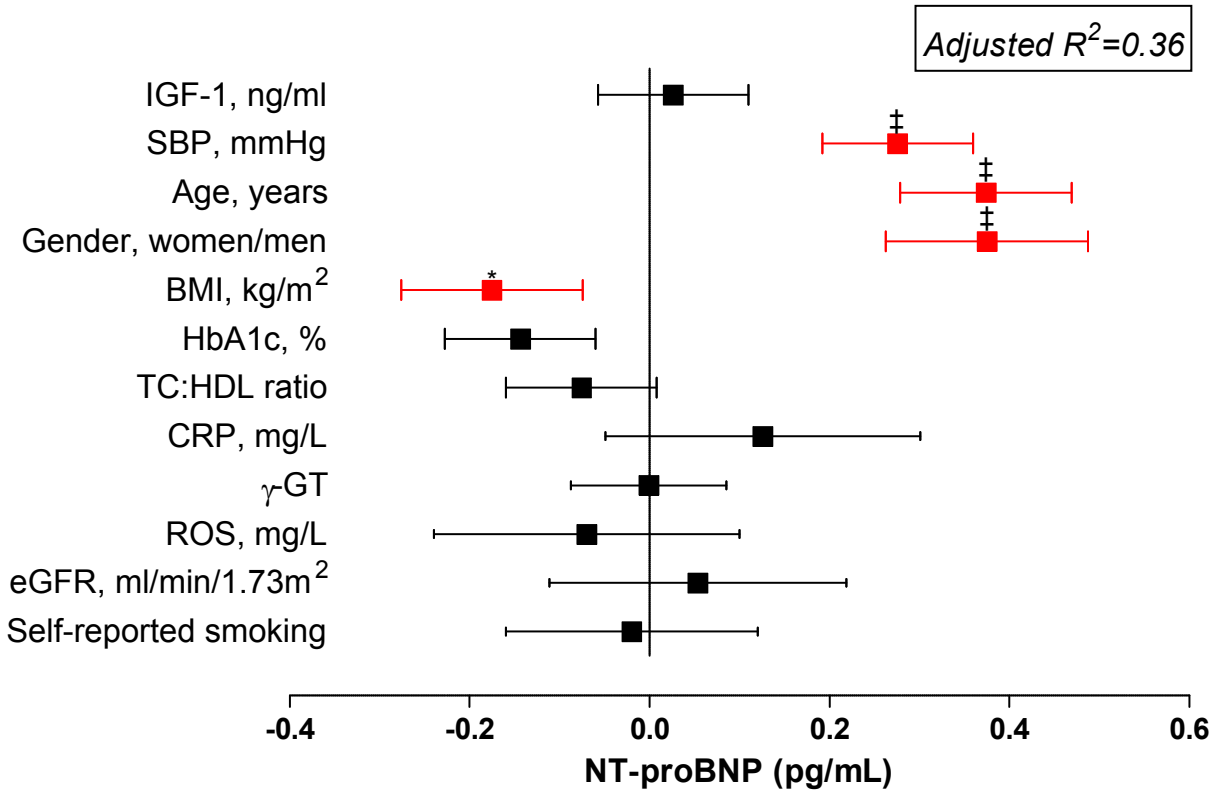


Figure 2: Forest plot indicating multiple regression analyses of NT-proBNP with IGF-1 as main independent variable in blacks. Values are indicated as standardised β (± 95% confidence interval). IGF-1, insulin-like growth factor-1; SBP, systolic blood pressure; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL, total cholesterol/high density lipoprotein ratio; CRP, C-reactive protein; γ-GT, gamma-glutamyl transferase; ROS, reactive oxygen species; eGFR, estimated glomerular filtration rate. * p ≤.05; † p ≤.01; ‡ p≤.001

Compared to the white group, the black group presented elevated blood pressure accompanied by lower IGF-1 levels. Circulating NT-proBNP concentrations did not differ between the groups. Furthermore, in blacks NT-proBNP was positively associated with systolic blood pressure, but not with IGF-1 (Figure 2). In whites, NT-proBNP was inversely associated with IGF-1, even after multiple adjustments (Figure 3). The hypothesis is thus partly accepted, as NT-proBNP was negatively associated with IGF-1 in whites, but with no association found in blacks.

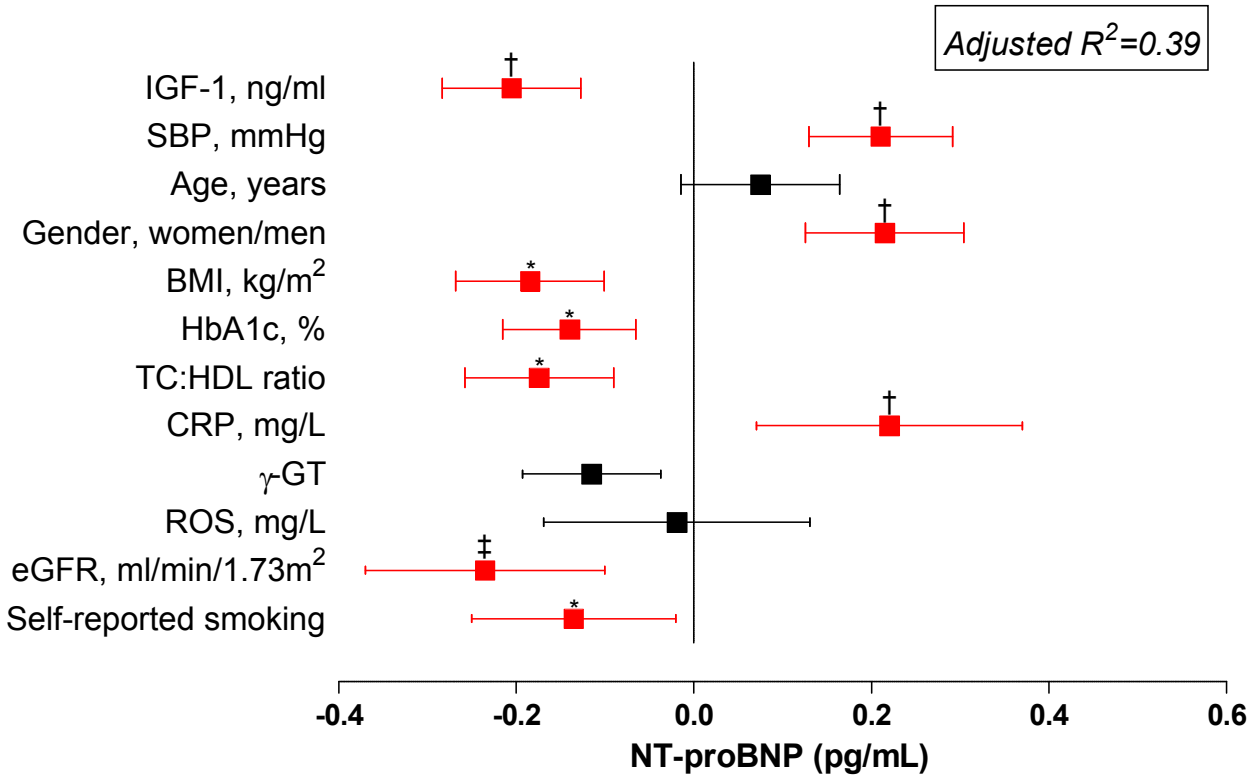


Figure 3: Forest plot indicating multiple regression analyses of NT-proBNP with IGF-1 as main independent variable in whites. Values are indicated as standardised β (± 95% confidence interval). IGF-1, insulin-like growth factor-1; SBP, systolic blood pressure; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL, total cholesterol/high density lipoprotein ratio; CRP, C-reactive protein; γ-GT, gamma-glutamyl transferase; ROS, reactive oxygen species; eGFR, estimated glomerular filtration rate. * p ≤.05; † p ≤.01; ‡ p≤.001

In our study, blacks had similar NT-proBNP levels than whites which contradict the findings of Kruger et al.[40] who found higher NT-proBNP levels in blacks compared to whites in another South African population. A possible explanation may be that the black and white school teachers from our study were from a similar socio-economic class, whereas the black population in the study of Kruger et al.[40] was from a lower socio-economic class than the whites, which may have increased their cardiovascular risk.[42]

In keeping with the inverse association between NT-proBNP and IGF-1 found in whites, Andreassen et al.[43] also found an inverse association when investigating 642 Danish whites. Duerr et al.[44] did not explore the direct link between NT-proBNP and IGF-1 but their results

showed improved cardiac function in cardiac failure patients after the administration of IGF-1. Also, Wallaschofski et al.[45] found increased NT-proBNP among patients with growth hormone (GH) deficiency which was significantly reduced after GH replacement therapy. Our results therefore support the cardioprotective role of IGF-1 among white South Africans.

Surprisingly, we found a lack of an association among the black population. This could suggest a disparate manner in which cardiovascular risk factors contribute to NT-proBNP concentrations, also confirmed by our multiple regression models. It is therefore plausible that IGF-1 could differentially influence cardiac function, in whites through direct effects, and in blacks indirectly through blood pressure. In addition, significantly reduced IGF-1 concentrations in blacks may disturb the cardio-protective function of IGF-1 and therefore no association was found between NT-proBNP and IGF-1. Although NT-proBNP is a stable and sensitive marker of cardiac function,[41] additional echocardiography should be considered in future research since it would give a better cardiac profile and provide a clearer understanding why no association were found among blacks.

3. CHANCE AND CONFOUNDING

It is important to reflect on possible factors that may have confounded the results obtained in this thesis. The results stemmed from a particular target population residing in one province of South Africa and for the larger part of the thesis school teachers were investigated. Therefore this particular population does not necessarily represent the entire black and white population. Although the results do not reveal the general health of the entire population of South Africa, the number of participants that were included in each of the studies provided sufficient statistical power to obtain reliable results.

The likelihood of chance findings should be taken into account when reviewing the results obtained. Within partial and forward stepwise regression analyses, one out of twenty significant correlations may be due to chance. The findings are likely to be robust, as results remained significant before and after full adjustments for covariates and potential confounders. Adjusting for covariates such as age, sex, ethnicity, study, BMI, SBP, pulse pressure, HbA1c, TC/HDL ratio, triglycerides, CRP, ROS, γ-GT, eGFR, smoking, and anti-hypertensive medication, could have influenced the results by over- or underestimation of the association between IGF-1 and the various variables investigated in each article. We therefore kept the number of covariates that could influence the relationship to a minimum of only one covariate for every ten participants. It is also possible that our findings were due to unknown factors we did not measure or did not account for in our analyses that associate with IGF-1.

In addition, since the data for all the articles was analysed cross-sectionally, cause and effect cannot be determined. One should also take into account that statistical significance does not necessarily indicate physiological significance. However, not only were the results from each article statistically significant, it also corresponded with the literature physiologically, therefore it is likely that the results indicate physiological significance.

4. CONCLUSION

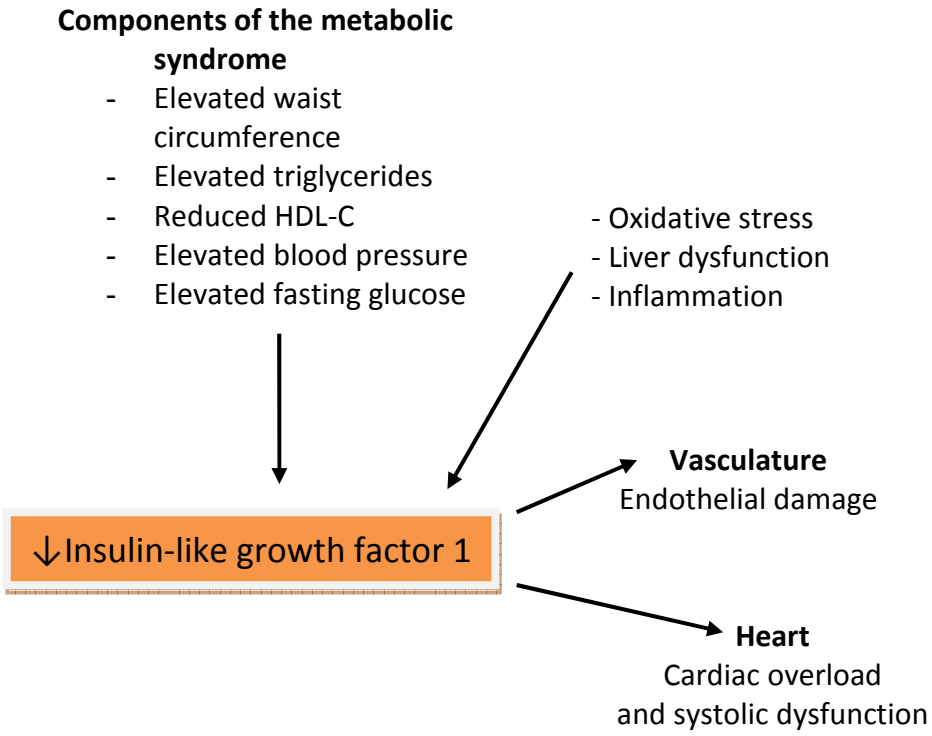


Figure 4: IGF-1 and the cardiometablic system investigated in this thesis.

In black and white South Africans IGF-1 is inversely associated with the number of metabolic syndrome components. But the association in both groups is dependent on oxidative stress, liver dysfunction and inflammation, suggesting possible underlying pathophysiological mechanisms by which the metabolic syndrome attenuates IGF-1 levels in these groups. Black participants however presented with a more unfavourable metabolic profile than whites which may possibly contribute to their lower IGF-1 levels. With low IGF-1 levels we may suggest a loss in cardiovascular protection in this group. Thus, the significant inverse relationship between IGF-1 and a marker of endothelial dysfunction among the black group was not surprising. It is however unexpected that no association between IGF-1 and a marker of cardiac overload and systolic dysfunction was evident among blacks. But since this marker was significantly

associated with systolic blood pressure in this group we may suggest that attenuated IGF-1 could contribute to cardiac pathology through blood pressure elevation. In whites however, IGF-1 associated negatively with a marker of cardiac overload and systolic dysfunction, supporting the cardioprotective role of IGF-1.

5. RECOMMENDATIONS FOR FUTURE STUDIES

The following recommendations are made to future researchers investigating IGF-1 and cardiovascular function:

- Taking into account the early age-related decline of IGF-1 in black South Africans,[6] together with the findings of this thesis, it is suggested that longitudinal studies in Africa should be performed to investigate the prognostic value of IGF-1 for incident hypertension, fatal and non-fatal cardiovascular events.

- Although the methodology used in this thesis provided clear findings, the inclusion of more direct measurements are encouraged for future studies. These include e.g. 24-hour ambulatory blood pressure (only performed in the SABPA, and not the SAfrEIC study), flow mediated dilation to determine endothelial dysfunction and echocardiography to determine cardiac structure and function.

- Based on the low levels of IGF-1 in blacks as found in our studies and that of others,[5,6] it is recommended that carefully designed intervention studies should investigate recombinant human IGF-1 treatment in order to possibly improve vascular function among blacks.

6. REFERENCES

1. Vasan RS, Sullivan LM, D'Agostino RB, et al. Serum insulin-like growth factor I and risk for heart failure in elderly individuals without a previous myocardial infarction: the Framingham Heart Study. Ann Intern Med 2003;139(8):642-8.
2. Sesti G, Sciacqua A, Cardellini M, et al. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care 2005;28(1):120-5.
3. Sierra-Johnson J, Romero-Corral A, Somers V, et al. IGF-I/IGFBP-3 ratio: a mechanistic insight into the metabolic syndrome. Clin Sci 2009;116:507-12.
4. Peer N, Lombard C, Steyn K, et al. High prevalence of metabolic syndrome in the Black population of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. Eur J Prev Cardiol 2015;22(8):1036-42.
5. Schutte A, Schutte R, Smith W, et al. Compromised bioavailable IGF-1 of black men relates favourably to ambulatory blood pressure: The SABPA study. Atherosclerosis 2014;233(1):139-44.
6. Schutte AE, Huisman HW, van Rooyen JM, et al. A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. J Clin Endocrinol Metab 2010;95(5):2503-7.
7. Saydah S, Ballard-Barbash R, Potischman N. Association of metabolic syndrome with insulin-like growth factors among adults in the US. Cancer Causes Control 2009;20(8):1309-16.
8. Hypponen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. Diabetes 2008;57(2):298-305.

9. Tong PC, Ho C, Yeung VT, et al. Association of testosterone, insulin-like growth factor-I, and C-reactive protein with metabolic syndrome in Chinese middle-aged men with a family history of type 2 diabetes. J Clin Endocrinol Metab 2005;90(12):6418-23.

10. Succurro E, Andreozzi F, Marini MA, et al. Low plasma insulin-like growth factor-1 levels are associated with reduced insulin sensitivity and increased insulin secretion in nondiabetic subjects. Nutr Metab Cardiovasc Dis 2009;19(10):713-9.

11. Sandhu MS, Heald AH, Gibson JM, et al. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. Lancet 2002;359(9319):1740-5.

12. Mannino GC, Greco A, De Lorenzo C, et al. A Fasting Insulin–Raising Allele at IGF1 Locus Is Associated with Circulating Levels of IGF-1 and Insulin Sensitivity. PloS One 2013;8(12):e85483.

13. van Bunderen CC, Oosterwerff MM, van Schoor NM, et al. Serum IGF1, metabolic syndrome, and incident cardiovascular disease in older people: a population-based study. Eur J Endocrinol 2013;168(3):393-401.

14. Schneider HJ, Friedrich N, Klotsche J, et al. Prediction of incident diabetes mellitus by baseline IGF1 levels. Eur J Endocrinol 2011;164(2):223-9.

15. Yeap BB, Chubb SA, Ho KK, et al. IGF1 and its binding proteins 3 and 1 are differentially associated with metabolic syndrome in older men. Eur J Endocrinol 2010;162(2):249-57.

16. De Benedetti F, Alonzi T, Moretta A, et al. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997;99(4):643-50.

17. Efstratiadis G, Tsiaousis G, Athyros VG, et al. Total serum insulin-like growth factor-1 and C-reactive protein in metabolic syndrome with or without diabetes. Angiology 2006;57(3):303-11.

18. Scheidegger KJ, James RW, Delafontaine P. Differential effects of low density lipoproteins on insulin-like growth factor-1 (IGF-1) and IGF-1 receptor expression in vascular smooth muscle cells. J Biol Chem 2000;275(35):26864-9.

19. Ichikawa T, Nakao K, Hamasaki K, et al. Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. Hepatol Int 2007;1(2):287-94.

20. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm 2010:2010. pii: 289645. Epub 2010 Jul 14.

21. Lee Y, Pratley RE. The evolving role of inflammation in obesity and the metabolic syndrome. Curr Diab Rep 2005;5(1):70-75.

22. Yubero-Serrano EM, Delgado-Lista J, Peña-Orihuela P, et al. Oxidative stress is associated with the number of components of metabolic syndrome: LIPGENE study. Exp Mol Med 2013;45(6):e28.

23. Grundy SM. Gamma-glutamyl transferase: another biomarker for metabolic syndrome and cardiovascular risk. Arterioscler Thromb Vasc Biol 2007;27(1):4-7.

24. Schutte AE, Schutte R, Huisman HW, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. Int J Epidemiol 2012;41(4):1114-23.

25. Sliwa K, Wilkinson D, Hansen C, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. Lancet 2008;371(9616):915-22.

26. Conti E, Carrozza C, Capoluongo E, et al. Insulin-like growth factor-1 as a vascular protective factor. Circulation 2004;110(15):2260-5.

27. Hutter R, Sauter BV, Reis ED, et al. Decreased reendothelialization and increased neointima formation with endostatin overexpression in a mouse model of arterial injury. Circulation 2003;107(12):1658-63.

28. Okura Y, Brink M, Zahid AA, et al. Decreased expression of insulin-like growth factor-1 and apoptosis of vascular smooth muscle cells in human atherosclerotic plaque. J Mol Cell Cardiol 2001;33(10):1777-89.

29. Patel VA, Zhang QJ, Siddle K, et al. Defect in insulin-like growth factor-1 survival mechanism in atherosclerotic plaque-derived vascular smooth muscle cells is mediated by reduced surface binding and signaling. Circ Res 2001;88(9):895-902.

30. Lutsey P, Cushman M, Steffen L, et al. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. J Thromb Haemost 2006;4(12):2629-35.

31. Platz EA, Pollak MN, Rimm EB, et al. Racial variation in insulin-like growth factor-1 and binding protein-3 concentrations in middle-aged men. Cancer Epidemiol Biomarkers Prev 1999;8(12):1107-10.

32. Empen K, Lorbeer R, Volzke H, et al. Association of serum IGF1 with endothelial function: results from the population-based study of health in Pomerania. Eur J Endocrinol 2010;163(4):617-23.

33. Abdu T, Elhadd T, Buch H, et al. Recombinant GH replacement in hypopituitary adults improves endothelial cell function and reduces calculated absolute and relative coronary risk. Clin Endocrinol (Oxf) 2004;61(3):387-93.

34. Schini-Kerth V. Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells. J Endocrinol Invest 1999;22(5):82-8.

35. Webb JG, Tan Y, Jaffa MA, et al. Evidence for prostacyclin and cAMP upregulation by bradykinin and insulin-like growth factor 1 in vascular smooth muscle cells. J Recept Signal Transduct Res 2010;30(2):61-71.

36. Veyradier A, Nishikubo T, Humbert M, et al. Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension. Circulation 2000;102(20):2460-2.

37. Zarei S, Frieden M, Kaufmann J, et al. The regulation of endothelial VWF secretion by nitric oxide: is it physiological? J Thrombo Haemost 2006;4(1):263-5.

38. Schutte AE, Volpe M, Tocci G, et al. Revisiting the relationship between blood pressure and insulin-like growth factor-1. Hypertension 2014;63(5):1070-7.

39. Koegelenberg A, Schutte R, Smith W, et al. Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study. Thromb Res 2015;136(5):1007-12.

40. Kruger R, Schutte R, Huisman HW, et al. N-terminal prohormone B-type natriuretic peptide and cardiovascular function in Africans and Caucasians: the SAfrEIC study. Heart Lung Circ 2012;21(2):88-95.

41. Weber M, Mitrovic V, Hamm C. B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide - Diagnostic role in stable coronary artery disease. Exp Clin Cardiol;11(2):99-101.

42. Clark AM, DesMeules M, Luo W, et al. Socioeconomic status and cardiovascular disease: risks and implications for care. Nat Rev Cardiol 2009;6(11):712-2.

43. Andreassen M, Raymond I, Kistorp C, et al. IGF1 as predictor of all cause mortality and cardiovascular disease in an elderly population. Eur J Endocrinol 2009;160(1):25-31.

44. Duerr RL, Huang S, Miraliakbar HR, et al. Insulin-like growth factor-1 enhances ventricular hypertrophy and function during the onset of experimental cardiac failure. J Clin Invest 1995;95(2):619-27.

45. Wallaschofski H, Saller B, Spilcke-Liss E, et al. Effects of growth hormone treatment on B-type natriuretic peptide as a marker of heart failure in adults with growth hormone deficiency. Horm Metab Res 2006;38(10):656-61.

ANNEXURE A

Declaration of language editing

DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator, and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a thesis written by ASE Koegelenberg (Student number: 20568894, North-west University).

Title of the thesis: Insulin-like growth factor-1 and cardiometabolic function: a bi-ethnic population study

C Vorster

12/7/2016

C Vorster

Date

9 Lanyon Street

Potchefstroom

2520

082 440 4102

ANNEXURE B

Turn-it-in report

Turnitin Originality Report

20568894:ASE_Koegelenberg.docx by SUZANNE KOEGELENBERG

From Postgraduate reports 3 (19c4d674-2dba-48a9-bac6-fac00abf8790)

Processed on 12-Jul-2016 14:36 SAST
ID: 689222024
Word Count: 17087

Similarity Index	Similarity by Source	
19%	Internet Sources:	22%
	Publications:	13%
	Student Papers:	10%

sources:

1 5% match (Internet from 25-Mar-2014)
http://dspace.nwu.ac.za/bitstream/handle/10394/8208/Kruger_R.pdf?sequence=2

2 4% match (Internet from 22-May-2016)
http://dspace.nwu.ac.za/bitstream/handle/10394/8708/Koegelenberg_S.pdf?sequence=1

3 2% match (Internet from 24-Jun-2016)
https://thieme-connect.de/products/ejournals/abstract/10.1055/s-0035-1559768?lang=de

4 2% match (Internet from 28-May-2016)
http://dspace.nwu.ac.za/bitstream/handle/10394/15353/Lammertyn_L_2015.pdf?isAllowed=y&sequence=1

5 1% match (Internet from 30-May-2016)
http://f1000.com/prime/ext/725937201

6 1% match (Internet from 12-Oct-2015)
https://www.thieme-connect.com/media/hmr/EFirst/supmat/10-1055-s-0035-1559768-2015-02-0056-0001.pdf

7 1% match (Internet from 22-Mar-2016)
http://www.clinsci.org/content/120/9/377

8 1% match (student papers from 02-Mar-2015)
Submitted to North West University on 2015-03-02

9 1% match (publications)
Aletta E Schutte. "Blood pressure variability is significantly associated with ECG left ventricular mass in normotensive Africans: The SABPA Study", Hypertension Research, 10/2011

10 1% match (publications)

Malan, L., M. Hamer, M.P. Schlaich, G. Lambert, T. Ziemssen, M. Reimann, N. Frasure-Smith, J.H. Amirkhan, R. Schutte, J.M. van Rooyen, C.M. Mels, C.M.T. Fourie, A.S. Uys, and N.T. Malan. "Defensive coping facilitates higher blood pressure and early sub-clinical structural vascular disease via alterations in heart rate variability: The SABPA study", Atherosclerosis, 2013.

---

11 1% match (publications)

Graziella Castellano. "The GH/IGF-1 Axis and Heart Failure", Current Cardiology Reviews, 08/01/2009

---

12 1% match (publications)

Handbook of Growth and Growth Monitoring in Health and Disease, 2012.

---

13 1% match (publications)

Malan, L., M. Hamer, N. Frasure-Smith, H. S. Steyn, and N. T. Malan. "Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study", International Journal of Epidemiology, 2014.

ANNEXURE C

Published manuscript of research article 1

Bioavailable IGF-1 and its Relation to the Metabolic Syndrome in a Bi-Ethnic Population of Men and Women

DOI 10.1055/s-0035-1559768
Horm Metab Res 2016; 48: 130–136

ISSN 0018-5043

Bioavailable IGF-1 and its Relation to the Metabolic Syndrome in a Bi-Ethnic Population of Men and Women

Authors
A. S. E. Koegelenberg[1], R. Schutte[1,2], W. Smith[1], A. E. Schutte[1,2]

Affiliations
[1] Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa
[2] MRC Research Unit for Hypertension and Cardiovascular disease, North-West University, Potchefstroom, South Africa

Key words
- metabolic syndrome
- insulin-like growth factor binding protein-3
- inflammation
- oxidative stress
- γ-glutamyltransferase

Abstract

Insulin-like growth factor 1 (IGF-1), an insulin sensitivity and vasculoprotective factor, associates negatively with the metabolic syndrome. However, IGF-1 is reduced by factors such as inflammation, oxidative stress and liver dysfunction. We investigated the relationship between bioavailable IGF-1 and the number of metabolic syndrome components and determined whether this relationship is independent of inflammation, oxidative stress and gamma glutamyl transferase (γ-GT; a marker of liver dysfunction). This study included 907 black and white participants stratified by sex (aged 43.0 ± 11.8 years). Among them 63 participants had fasting glucose levels of ≥ 7.0 mmol/l and/or used diabetes medication. Via standard methods we determined waist circumference, fasting glucose, triglycerides, high-density lipoprotein cholesterol and blood pressure. We also determined high-sensitivity C-reactive protein (CRP), reactive oxygen species (ROS), γ-GT, IGF-1 and insulin-like growth factor binding protein 3 (IGFBP-3). IGF-1/IGFBP-3 was used as an estimate of bioavailable IGF-1. Total IGF-1 was similar between men and women ($p = 0.10$), however, bioavailable IGF-1 was lower in women ($p < 0.001$). In multivariate-adjusted analyses, IGF-1/IGFBP-3 was inversely associated with the number of metabolic syndrome components in both sexes (men: $\beta = -0.11$; $p = 0.013$ and women: $\beta = -0.17$; $p = 0.003$). Upon inclusion of ROS, γ-GT and CRP, significance was lost. In patients without diabetes, the results for men changed marginally, but were consistent for women. We found an inverse association between bioavailable IGF-1 and the number of metabolic syndrome components. But the relationship was dependent on oxidative stress, liver dysfunction and inflammation, suggesting underlying processes by which the metabolic syndrome attenuates IGF-1.

Supporting Information for this article is available online at http://www.thieme-connect.de/products

received 19.02.2015
accepted 30.07.2015

Bibliography
DOI http://dx.doi.org/10.1055/s-0035-1559768
Published online: September 8, 2015
Horm Metab Res 2016; 48: 130–136

ISSN 0018-5043

Correspondence
A. E. Schutte, PhD
Hypertension in Africa Research Team (HART)
Private Bag X6001
North-West University
Potchefstroom 2520
South Africa
Tel.: +27/18/299 2444
Fax: +27/18/285 2432
alta.schutte@nwu.ac.za

Introduction

Insulin-like growth factor 1 (IGF-1) has several metabolic and vasculoprotective effects. IGF-1 induces nitric oxide (NO) release [1]; it enhances insulin sensitivity [2] and glucose uptake [3]; prevents postprandial dyslipidemia [4]; reduces gluconeogenesis and scavenges free oxygen radicals [3]. IGF-1 also has anti-inflammatory properties [5]. The biological effects of IGF-1 are modulated by 6 different insulin-like growth factor binding proteins (IGFBPs) which bind to IGF-1 increasing its half-life, manages delivery to tissues and regulates movement between intravascular and extravascular compartments [6]. Approximately 80% of total IGF-1 is bound in serum by IGFBP-3, while the remaining 20% is bound by the other 5 IGFBPs [6]. Therefore, the molar ratio of IGF-1 and IGFBP-3 could be used as a surrogate measure for bioavailable IGF-1.

IGF-1 is reduced in individuals suffering from cardiovascular and metabolic diseases [7,8]. Cardiometabolic factors such as hypertension [8–10], hyperglycaemia [9], hypertriglyceridaemia [8], reduced high-density lipoprotein (HDL) cholesterol and abdominal obesity [8] are all inversely associated with IGF-1. These cardiometabolic factors aggregate together as part of the metabolic syndrome [11], which itself is negatively associated with IGF-1 [8,12]. A negative association also exists between the number of metabolic syndrome components present and IGF-1 [12,13].

In addition, circulating IGF-1 is adversely affected by various factors including inflammation [14,15], oxidative stress [16] and liver dysfunc-

tion [17] and it is undetermined whether the relationship between IGF-1 and the number of metabolic syndrome components are independent of these factors. We therefore examined the relationship of bioavailable IGF-1 with the number of metabolic syndrome components in men and women; and how this relationship is modulated by inflammation, oxidative stress and liver dysfunction to identify the possible underlying pathophysiological mechanisms between IGF-1 and the metabolic syndrome.

Subjects and Methods

Study design and subject selection

We included data from the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) and the SAfrEIC (South African study regarding the influence of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function) studies conducted by the same research team using similar methodology. The SABPA study was conducted in 2008 and 2009, and included 409 black and white teachers between the ages of 25 and 65 years in the North West Province of South Africa. The SAfrEIC study conducted in 2007 involved 750 black and white volunteers from urban areas of the North West Province of South Africa (aged 20–70 years). Exclusion criteria for the SABPA study were participants with an elevated ear temperature, making use of α- and β-receptor blockers, psychotropic substance dependence or abuse, blood donors, individuals vaccinated in the past 3 months and pregnant women. The SAfrEIC study excluded participants that were apparently ill, pregnant or lactating. For the purpose of this study we excluded participants due to missing data (n = 233) and those infected with the human immunodeficiency virus (HIV) (n = 19). The overall sample of this sub-study consisted of 907 participants divided into men (n = 457) and women (n = 450) of whom 63 individuals were having fasting glucose levels of ≥ 7.0 mmol/l and/or made use of diabetes medication. Approval for both the SABPA and the SAfrEIC studies was obtained from the Ethics Committee of the North-West University adhering to the principles of the Declaration of Helsinki. All participants gave written informed consent after the procedures of the study were thoroughly explained to them. The organisational procedures of both studies were described in detail elsewhere [17, 18].

Anthropometric measurements

Height (stature) and weight of participants were measured while being in their underwear. Measurements were taken in triplicate using standard methods with calibrated instruments (Precision Health Scale, A&D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Leicester, UK) [19]. Waist circumference (WC) was measured over the abdomen between the costal margin and the iliac crest. Measurements were taken to the nearest 0.1 cm using a non-stretchable standard tape (Holtain Instruments Ltd. Wales). Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2).

Blood pressure measurements

In the SAfrEIC study blood pressure measurements were taken in duplicate after a 10 min resting period and a 5 min interval between the 2 measurements. The OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) apparatus was used to determine systolic and diastolic blood pressure with the cuff on the left upper arm in the sitting position. In the SABPA study, participants rested for 5 min in a semi-recumbent position before the first measurement was taken. Blood pressure was measured with a mercury sphygmomanometer, using the Riva-Rocci/Korotkoff method [20], on the non-dominant arm. Two duplicate measures were taken with a 5 min interval between the 2 measurements.

Biochemical analyses

Serum samples for lipids (total cholesterol, high density lipoprotein cholesterol (HDL), triglycerides), aspartate aminotranferase (AST), alanine transaminase (ALT), and high-sensitivity C-reactive protein (CRP) were analysed using the Konelab 20i autoanalyser (Thermo Scientific, Vantaa, Finland (2008)). Low density lipoprotein cholesterol (LDL) was determined with the Friedewald formula: LDL = TC – HDL – TG/2.17. In the SAfrEIC study, a venous sample for fasting blood glucose was collected in tubes containing sodium fluoride, kept on ice and centrifuged. In the SABPA study, these sodium fluoride tubes were centrifuged soon after blood collection. During the SAfrEIC study glucose and gamma-glutamyltransferase (γ-GT) were determined using the Konelab 20i auto-analyser (Thermo Scientific, Vantaa, Finland), while during the SABPA study glucose and γ-GT were determined using Beckman and Coulter time-end method (Unicel DXC 800 – Beckman and Coulter®, Germany (2009)). Participants were considered as having diabetes if they had fasting glucose levels of ≥ 7.0 mmol/l and/or made use of diabetes medication [21]. The intra- and interassay coefficients of variation for standard biochemical measures performed with auto-analysers (Konelab 20i, Unicel DXC 800 – Beckman and Coulter) were < 10 %. In the SABPA study, total IGF-1 was determined with an immunoradiometric assay (IRMA) from Immunotech (Beckman and Coulter®, Germany; IGF-1 – A15729). The intra- and interassay coefficients of variation for IGF-1 were 2.92, and 4.49 %, respectively. In the SAfrEIC study, we determined total IGF-1 using an enzyme linked immunosorbert assay (ELISA) by BiocodeHycel (Liége, Belgium, Catalogue No. EL2010). The intra- and interassay coefficients of variation for IGF-1 were 15.08 and 7.89 %, respectively. The properties of these 2 IGF-1 methods are comparable (**Table 1S**). IGFBP-3 was determined with an IRMA method from Immunotech (Beckman and Coulter®, Germany; IGFBP-3 – DSL-6600) in both the SABPA and SAfrEIC studies. The intra-assay variability for IGFBP-3 ranged between 2.71 and 7.95 %, and the inter-assay variability ranged between 3.20 and 9.30 %, for both studies. Serum reactive oxygen species (ROS) were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognised as an efficient method for evaluating oxidative stress in the body. The Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure serum peroxide levels, where 1.0 mg/l H_2O_2 represents one unit of ROS [22]. The intra- and interassay coefficients of variation for ROS were < 10 %. HIV testing was done according to standard procedures of the South African Department of Health.

Statistical analyses

For database management and statistical analyses we used Statistica version 12.0 (StatSoft, Inc.,Tulsa, OK). The associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components were tested for interaction with sex, ethnicity or study type by introducing appropriate interaction terms in

multiple regression analyses. Variables that were not normally distributed were logarithmically transformed (BMI, fasting glucose, triglycerides, CRP, γ-GT, AST and ALT). We subsequently compared means and proportions between men and women with independent T-tests and Chi-square tests, respectively. The IGF-1 and IGF-1/IGFBP-3 distribution was explored according to the number of metabolic syndrome components by using analyses of variance (ANOVA) and analyses of covariance (ANCOVA). The number of metabolic syndrome components was calculated using the criteria for the metabolic syndrome compiled by the International Diabetes Federation and other international bodies in 2009 [11]. We used single and multiple regression analyses to determine the associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components. The models included the following independent variables: age, ethnicity, study type and the number of metabolic syndrome components. We then further explored how this relationship is modulated by inflammation, oxidative stress and γ-GT. The models then included age, ethnicity, study type, the number of metabolic syndrome components, CRP, ROS and γ-GT as independent variables. The multivariate-adjusted analyses were performed in the total group and repeated upon exclusion of individuals with diabetes.

Results

We stratified the 907 participants according to sex due to significant interactions with sex regarding the associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components ($p < 0.001$ and $p = 0.010$, respectively). No significant interactions were found for ethnicity or study type (all $p > 0.05$). **Table 1** displays the characteristics of the men and women (**Table 2S** for each study, respectively). Despite a younger mean age for men compared to women (41.9 vs. 44.1 years, $p = 0.006$), men showed a higher mean waist circumference ($p < 0.001$), triglycerides ($p < 0.001$), glucose ($p < 0.001$) and blood pressure ($p < 0.001$), accompanied by lower HDL-C ($p < 0.001$). γ-GT were significantly higher in men ($p < 0.001$) while women had higher levels of CRP and ROS (all $p < 0.001$). IGF-1 did not differ between men and women ($p = 0.101$), whereas women had higher IGFBP-3 ($p < 0.001$) and a lower IGF-1/IGFBP-3 ($p < 0.001$).

In **Table 2**, we have compared IGF-1, IGF-1/IGFBP-3 and the cardiometabolic profile according to the number of metabolic syndrome components. As expected, both sexes showed that waist circumference, glucose, triglycerides and blood pressure increased significantly (all $p < 0.001$), and HDL-C decreased ($p < 0.001$) as the number of metabolic syndrome components increased. For men we found a tendency of decreasing IGF-1/IGFBP-3 (p for trend = 0.078), but IGF-1 decreased as the number of metabolic syndrome components increased (p for trend = 0.032). In women, IGF-1 and IGF-1/IGFBP-3 decreased significantly with the increase in metabolic syndrome components (both p for trend < 0.001, **Table 2**). As shown in **Fig. 1**, this decrease persisted after adjusting for age, ethnicity and study type (p for trend < 0.001). However, the decrease of IGF-1 in men was not significant after adjustments (p for trend = 0.240).

Table 1 Comparison of cardiovascular, biochemical, and anthropometric measurements of men and women.

	Men (n = 457)	Women (n = 450)	p-Value
Age (years)	41.9 ± 12.2	44.1 ± 11.4	**0.006**
Ethnicity, black, n (%)	200 (43.8)	184 (40.9)	0.38
Anthropometric measurements			
Body mass index (kg/m^2)	25.8 (17.7; 37.6)	27.5 (19.3; 41.7)	**<0.001**
Waist circumference (cm)	91.0 ± 16.7	85.9 ± 15.0	**<0.001**
Biochemical measures			
IGF-1 (ng/ml)	157 ± 73.1	150 ± 70.2	0.10
IGFBP-3 (nmol/l)	134 ± 33.9	144 ± 28.1	**<0.001**
IGF-1/IGFBP-3	1.17 ± 0.45	1.03 ± 0.44	**<0.001**
Fasting glucose (mmol/l)	5.56 (4.31; 7.32)	5.26 (4.19; 6.90)	**<0.001**
Total cholesterol (mmol/l)	5.22 ± 1.39	5.27 ± 1.47	0.64
High-density lipoprotein cholesterol (mmol/l)	1.26 ± 0.56	1.43 ± 0.42	**<0.001**
Low-density lipoprotein cholesterol (mmol/l)	3.27 ± 1.27	3.30 ± 1.26	0.70
Triglycerides (mmol/l)	1.27 (0.54; 3.17)	1.01 (0.46; 2.51)	**<0.001**
C-reactive protein (mg/l)	1.66 (0.11; 14.5)	2.52 (0.13; 25.6)	**<0.001**
Reactive oxygen species (mg/l)	78.8 ± 18.3	98.2 ± 27.9	**<0.001**
Gamma-glutamyltransferase (U/l)	46.3 (16.0; 237)	28.6 (8.00; 183)	**<0.001**
Aspartate aminotrasferase (U/l)	25.8 (14.3; 69.0)	19.9 (11.8; 44.0)	**<0.001**
Alanine transaminase (U/l)	23.7 (9.62; 66.3)	15.0 (5.63; 44.6)	**<0.001**
Cardiovascular measurements			
Systolic blood pressure (mm Hg)	130 ± 17.6	122 ± 18.9	**<0.001**
Diastolic blood pressure (mm Hg)	84.8 ± 12.6	81.1 ± 11.4	**<0.001**
Lifestyle measures			
Smoking self-reported, n (%)	155 (34.0)	86 (19.2)	**<0.001**
Intake of medication			
Anti-hypertensive medication, n (%)	81 (17.8)	76 (16.9)	0.73
Anti-diabetic medication, n (%)	7 (1.53)	3 (0.67)	0.21
Lipid-lowering medication, n (%)	18 (3.94)	17 (3.78)	0.90
Diabetes, n (%)	40 (8.75)	23 (5.11)	0.031
Hypertensive *, n (%)	146 (32.0)	110 (24.4)	**0.015**

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

n: Number of participants; IGF-1: Insulin-like growth factor 1; IGFBP-3: Insulin-like growth factor-binding protein 3

* Classification as hypertensive: ≥ 140/90 mm Hg

	Number of metabolic syndrome components			p for trend
	0	1–2	>3	
	Men			
n	73	196	186	
IGF-1 (ng/ml)	166 ± 79.9	147 ± 73.4	164 ± 69.3	0.032
IGF-1/IGFBP-3	1.28 ± 0.53	1.14 ± 0.24	1.15 ± 0.44	0.078
Waist circumference (cm)	74.8 ± 8.12	84.9 ± 14.1 *	104 ± 12.2 *	<0.001
Glucose (mmol/l)	4.83 (4.03; 5.49)	5.32 (4.22; 6.42) *	6.12 (4.96; 8.91) *	<0.001
Triglycerides (mmol/l)	0.80 (0.42; 1.38)	1.03 (0.54; 2.02) *	1.90 (0.87; 4.44) *	<0.001
High density lipoprotein (mmol/l)	1.50 ± 0.60	1.44 ± 0.61	0.98 ± 0.31 *	<0.001
Systolic blood pressure (mm Hg)	116 ± 7.56	131 ± 17.9 *	136 ± 17.2 *	<0.001
Diastolic blood pressure (mm Hg)	74.3 ± 5.88	84.4 ± 12.7 *	89.2 ± 11.8 *	<0.001
	Women			
n	80	211	157	
IGF-1 (ng/ml)	190 ± 79.3	148 ± 67.3 *	129 ± 60.3 *	<0.001
IGF-1/IGFBP-3	1.22 ± 0.47	1.06 ± 0.44 *	0.90 ± 0.39 *	<0.001
Waist circumference (cm)	71.0 ± 5.31	83.5 ± 12.8 *	96.6 ± 13.1 *	<0.001
Glucose (mmol/l)	4.76 (4.05; 5.50)	5.02 (4.22; 6.01) *	5.89 (4.21; 10.29) *	<0.001
Triglycerides (mmol/l)	0.74 (0.45; 1.30)	0.90 (0.43; 1.74) *	1.38 (0.61; 2.94) *	<0.001
High-density lipoprotein (mmol/l)	1.64 ± 0.25	1.48 ± 0.44 *	1.26 ± 0.40 *	<0.001
Systolic blood pressure (mm Hg)	106 ± 9.10	121 ± 18.1 *	132 ± 18.1 *	<0.001
Diastolic blood pressure (mm Hg)	71.0 ± 6.52	80.5 ± 11.1 *	87.1 ± 9.81 *	<0.001

n: Number of participants; IGF-1: Insulin-like growth factor 1; IGFBP-3: Insulin-like growth factor-binding protein 3

* Significance of the difference from previous category ($p < 0.001$)

Table 2 Mean values of IGF-1, IGF-1/IGFBP-3 ratio, and metabolic syndrome components in categories stratified according to the number of metabolic syndrome components.

We performed multiple regression analyses with IGF-1 and IGF-1/IGFBP-3 as our dependent variables in men and women (**Table 3**). In Model 1, the number of metabolic syndrome components as well as age, ethnicity and study type were included as independent variables. We found in all instances a negative relationship between the number of metabolic syndrome components and IGF-1/IGFBP-3 in both sexes and with IGF-1 in women. Adjustments for CRP, ROS and γ-GT nullified the relationships of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components (Model 2). CRP was not significantly associated with either IGF-1 or IGF-1/IGFBP-3, but ROS and γ-GT were prominent contributors to the variance of IGF-1 and IGFBP-3 in both sexes.

A separate summary of the individual contribution of CRP, ROS and γ-GT to Model 1 is given in **Table 3S**. In sensitivity analyses we repeated Model 2 with smoking and the usage of anti-hypertensive medication as additional covariates. This did not affect the outcome of our results (**Table 4S**).

When repeating these analyses after excluding participants with fasting glucose levels of ≥ 7.0 mmol/l and/or using diabetes medication (**Table 4**), we found in Model 1 that the negative relationship between the number of metabolic syndrome components and IGF-1/IGFBP-3 remained significant in women, but marginally changed in men ($\beta = -0.09$; $p = 0.078$). After including CRP, ROS and γ-GT as covariates, the relationship between IGF-1/IGFBP-3 and the number of metabolic syndrome components disappeared. Apart from ROS and γ-GT being prominent contributors to the variance of IGF-1 and IGFBP-3 in men and women, CRP also related to IGF-1/IGFBP-3 in men ($\beta = 0.10$; $p = 0.045$), and IGF-1 in women ($\beta = -0.11$; $p = 0.043$). To explore whether the aforementioned association between CRP and IGF-1/IGFBP-3 in men are possibly driven by obesity, we included waist circumference to the model and found that the association between CRP and IGF-1/IGFBP-3 became non-significant ($\beta = 0.085$; $p = 0.089$).

Discussion

We examined the relationship of bioavailable IGF-1 with increasing cardiometabolic risk, and how this relationship is affected by inflammation, oxidative stress and liver dysfunction. We focused specifically on the whole population sample but repeated analyses upon exclusion of patients with diabetes. An independent inverse association between bioavailable IGF-1 and the number of metabolic syndrome components was found in the whole population of men and women. However, significance of this relationship was lost after adjusting for CRP, ROS and γ-GT (as a marker of liver dysfunction), where both ROS and γ-GT were negatively associated with bioavailable IGF-1 in both sexes. When individuals with diabetes were excluded, the negative association between bioavailable IGF-1 and the number of metabolic syndrome components remained significant in women but a borderline association were found in men. Again in women the significance of the relationship was lost upon inclusion of CRP, ROS and γ-GT to the model.

In agreement with our findings, several studies reported a negative association between bioavailable IGF-1 and the number of metabolic syndrome components. Sierra-Johnson et al. examined 3 281 subjects from the Third National Health and Nutrition Examination Survey (NHANES III), and found that bioavailable IGF-1 decreased significantly as the number of metabolic syndrome components increased [13]. In addition, Sesti et al. examined 509 European subjects of whom 20% were classified as having the metabolic syndrome. IGF-1 was also lower in subjects with the metabolic syndrome compared to those without [8]. However, none of these studies accounted for markers of inflammation, oxidative stress or γ-GT in their analyses.

When CRP, ROS and γ-GT were added to the regression model the link between bioavailable IGF-1 and the number of metabolic syndrome components became non-significant. Indeed both ROS and γ-GT were negatively associated with bioavailable IGF-1 in men and women. IGF-1 reduces oxidative stress and is responsible for free oxygen radical scavenging [3]. But oxidative

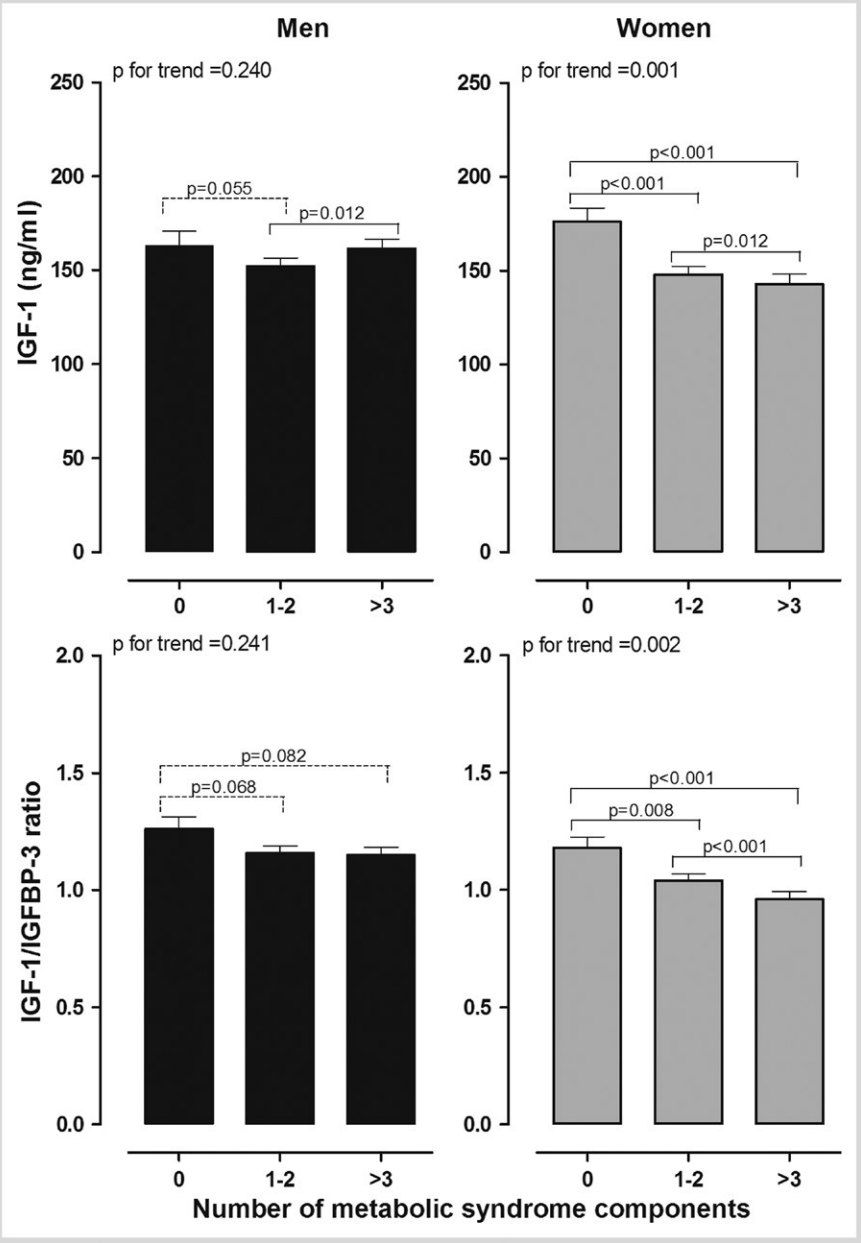


Fig. 1 The IGF-1 and IGF-1/IGFBP-3 ratio distribution according to the number of metabolic syndrome components, adjusted for age, ethnicity and study type.

	Men (n = 457)		Women (n = 450)	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2 = 0.30**	**R^2 = 0.14**	**R^2 = 0.21**	**R^2 = 0.20**
Age (years)	β = −0.41; p < 0.001	β = −0.31; p < 0.001	β = −0.36; p < 0.001	β = −0.32; p < 0.001
Ethnicity (White/African)	β = −0.38; p < 0.001	β = −0.12; p = 0.012	β = −0.12; p = 0.004	β = 0.10; p = 0.016
Study (SAfrEIC/SABPA)	β = 0.17; p < 0.001	β = 0.24; p < 0.001	β = 0.16; p < 0.001	β = 0.24; p < 0.001
Number of MetS components (0–5)	–	β = −0.11; p = 0.013	β = −0.11; p = 0.014	β = −0.17; p = 0.003
Model 2 (Model 1 + CRP, ROS, γ-GT)	**R^2 = 0.34**	**R^2 = 0.20**	**R^2 = 0.26**	**R^2 = 0.26**
Number of MetS components (0–5)	–	–	–	–
C-reactive protein log (mg/l)	–	–	–	–
ROS (mg/l)	β = −0.10; p = 0.016	β = −0.13; p = 0.008	β = −0.19; p < 0.001	β = −0.23; p < 0.001
γ-GT log (U/l)	β = −0.20; p < 0.001	β = −0.26; p < 0.001	–	β = −0.12; p = 0.023

Table 3 Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and potential confounders.

IGF-1: Insulin-like growth factor 1; IGFBP-3: Insulin-like growth factor-binding protein 3; ROS: Reactive oxygen species; γ-GT: Gamma-glutamyltransferase; MetS: Metabolic syndrome

stress also attenuates IGF-1 levels, given that oxidative stress particularly affects hepatocytes where IGF-1 is mainly synthesised [16,23]. Furthermore γ-GT, as a surrogate marker of liver damage and liver diseases such as alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD), are associated with reduced IGF-1 production [24–27]. However it is beyond

	Men (n = 417)		Women (n = 427)	
	IGF-1	IGF-1/IGFBP-3	IGF-1	IGF-1/IGFBP-3
Model 1	**R^2 = 0.31**	**R^2 = 0.14**	**R^2 = 0.20**	**R^2 = 0.19**
Age (years)	β = −0.42; p < 0.001	β = −0.33; p < 0.001	β = −0.37; p < 0.001	β = −0.34; p < 0.001
Ethnicity (White/African)	β = −0.38; p < 0.001	β = −0.11; p = 0.027	β = −0.12; p = 0.008	β = 0.11; p = 0.015
Study (SAfrEIC/SABPA)	β = 0.15; p < 0.001	β = 0.22; p < 0.001	β = 0.15; p < 0.001	β = 0.22; p < 0.001
Number of MetS components (0–5)	–	β = −0.09; p = 0.078	β = −0.12; p = 0.015	β = −0.14; p = 0.002
Model 2 (Model 1 + CRP, ROS, γ-GT)	**R^2 = 0.35**	**R^2 = 0.20**	**R^2 = 0.26**	**R^2 = 0.26**
Number of MetS components (0–5)	–	–	–	–
C-reactive protein log (mg/l)	–	β = 0.10; p = 0.050	β = −0.11; p = 0.046	–
ROS (mg/l)	β = −0.09; p = 0.035	β = −0.11; p = 0.024	β = −0.18; p < 0.001	β = −0.23; p < 0.001
γ-GT log (U/l)	β = −0.20; p < 0.001	β = −0.26; p < 0.001	–	β = −0.12; p = 0.025

For abbreviations, see Table 3

Table 4 Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and potential confounders in participants without diabetes.

the scope of this article to differentiate whether the increase in γ-GT is due to alcohol or NAFLD.

We did not find an independent association between CRP and bioavailable IGF-1 in the whole population. This was unexpected since CRP synthesis is stimulated by pro-inflammatory cytokines such as interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and IL-1 that are also known to decrease both circulatory and tissue concentrations of IGF-1 [28,29]. However, CRP has been shown to result in increased superoxide production [30], and therefore we might speculate that CRP attenuated IGF-1 via the process of oxidative stress. This was possibly represented by ROS that was independently related to bioavailable IGF-1 in our regression model. In the population without diabetes we found a positive association between CRP and bioavailable IGF-1 in men. However, this relationship disappeared upon inclusion of waist circumference to the model. Therefore, we might speculate that the relationship between CRP and IGF-1 is driven by obesity since it is known that obesity and CRP are strongly associated [31].

Our finding that both ROS and γ-GT modulate the association between bioavailable IGF-1 and the number of metabolic syndrome components in men and women emphasise the fact that these components should be taken into account when investigating the link between IGF-1 and the metabolic syndrome. These results may reflect potential mechanistic pathways (namely via oxidative stress and liver dysfunction) by which the metabolic syndrome attenuates IGF-1 levels, given that the metabolic syndrome is associated with the overproduction of reactive oxygen species [32] and increased levels of γ-GT [33].

This study should be viewed in context of its strengths and limitations. A limitation of our study is that it consists of 2 different studies, but both studies used validated techniques. Thus, we pooled IGF-1 data that were measured with different assays (ELISA and IRMA) in the 2 studies. The γ-GT test is a non-specific measure that can be elevated with many types of liver diseases; therefore we did not specify a liver disease associated with the increase in γ-GT in our study. The results of our study were consistent after multiple adjustments however, we cannot exclude residual confounding. In addition, due to the cross-sectional nature of this study, causality cannot be inferred. The strengths of this study include the availability of bioavailable IGF-1 (IGF-1/IGFBP-3) in a relatively large group of participants.

To conclude, cardiometabolic risk is inversely associated with bioavailable IGF-1, but this association is potentially mediated by oxidative stress, inflammation and liver dysfunction. These factors should be taken into account when investigating the link between IGF-1 and the metabolic syndrome. Therefore, strategies aimed at improving low-grade inflammation, oxidative stress and liver function in individuals that are metabolically compromised should be investigated due to their potentially attenuating effects on IGF-1.

Acknowledgements

The SABPA and SAfrEIC studies would not have been possible without the voluntary collaboration of the participants. We gratefully acknowledge the contribution of the research teams, and the Department of Education, South Africa (SABPA study), for their involvement in these studies. Research included in the present study was partially funded by the Medical Research Council, South Africa; Roche Diagnostics; the North-West University; and the Metabolic Syndrome Institute, France. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.

Conflict of Interest

The authors declare no conflict of interest.

References

1 *Schini-Kerth V*. Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells. J Endocrinol Invest 1999; 22: 82–88
2 *Conti E*, *Andreotti F*, *Sestito A*, *Riccardi P*, *Menini E*, *Crea F*, *Maseri A*, *Lanza GA*. Reduced levels of insulin-like growth factor-1 in patients with angina pectoris, positive exercise stress test, and angiographically normal epicardial coronary arteries. Am J Cardiol 2002; 89: 973–975
3 *Dimmeler S*, *Zeiher AM*. Exercise and Cardiovascular Health Get Active to "AKTivate" Your Endothelial Nitric Oxide Synthase. Circulation 2003; 107: 3118–3120
4 *Twickler MT*, *Cramer MM*, *Koppeschaar HP*. Unraveling Reaven's syndrome X: serum insulin-like growth factor-I and cardiovascular disease. Circulation 2003; 107: e190–e192
5 *Spies M*, *Nesic O*, *Barrow R*, *Perez-Polo J*, *Herndon D*. Liposomal IGF-1 gene transfer modulates pro- and anti-inflammatory cytokine mRNA expression in the burn wound. Gene Ther 2001; 8: 1409–1415
6 *Conti E*, *Musumeci M*, *De Giusti M*, *Dito E*, *Mastromarino V*, *Volpe M*. IGF-1 and atherothrombosis: relevance to pathophysiology and therapy. Clin Sci 2011; 120: 377–402
7 *Vasan RS*, *Sullivan LM*, *D'Agostino RB*, *Roubenoff R*, *Harris T*, *Sawyer DB*, *Levy D*, *Wilson PW*. Serum insulin-like growth factor I and risk for heart failure in elderly individuals without a previous myocardial infarction: the Framingham Heart Study. Ann Intern Med 2003; 139: 642–648

8 *Sesti G*, *Sciacqua A*, *Cardellini M*, *Marini MA*, *Maio R*, *Vatrano M*, *Succurro E*, *Lauro R*, *Federici M*, *Perticone F*. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care 2005; 28: 120–125

9 *Colao A*, *Di Somma C*, *Cascella T*, *Pivonello R*, *Vitale G*, *Grasso LF*, *Lombardi G*, *Savastano S*. Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: an observational, exploratory study in 404 subjects. Eur J Endocrinol 2008; 159: 389–397

10 *Hu Y*, *Pete G*, *Walsh MF*, *Sowers J*, *Dunbar JC*. Central IGF-1 decreases systemic blood pressure and increases blood flow in selective vascular beds. Horm Metab Res 1996; 28: 211–214

11 *Alberti K*, *Eckel RH*, *Grundy SM*, *Zimmet PZ*, *Cleeman JI*, *Donato KA*, *Fruchart J*, *James WPT*, *Loria CM*, *Smith SC*. Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120: 1640–1645

12 *Parekh N*, *Roberts CB*, *Vadiveloo M*, *Puvananayagam T*, *Albu JB*, *Lu-Yao GL*. Lifestyle, anthropometric, and obesity-related physiologic determinants of insulin-like growth factor-1 in the Third National Health and Nutrition Examination Survey (1988–1994). Ann Epidemiol 2010; 20: 182–193

13 *Sierra-Johnson J*, *Romero-Corral A*, *Somers V*, *Lopez-Jimenez F*, *Malarstig A*, *Brismar K*, *Hamsten A*, *Fisher R*, *Hellenius M*. IGF-I/IGFBP-3 ratio: a mechanistic insight into the metabolic syndrome. Clin Sci 2009; 116: 507–512

14 *De Benedetti F*, *Alonzi T*, *Moretta A*, *Lazzaro D*, *Costa P*, *Poli V*, *Martini A*, *Ciliberto G*, *Fattori E*. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997; 99: 643–650

15 *Efstratiadis G*, *Tsiaousis G*, *Athyros VG*, *Karagianni D*, *Pavlitou-Tsiontsi A*, *Giannakou-Darda A*, *Manes C*. Total serum insulin-like growth factor-1 and C-reactive protein in metabolic syndrome with or without diabetes. Angiology 2006; 57: 303–311

16 *Scheidegger KJ*, *James RW*, *Delafontaine P*. Differential effects of low density lipoproteins on insulin-like growth factor-1 (IGF-1) and IGF-1 receptor expression in vascular smooth muscle cells. J Biol Chem 2000; 275: 26864–26869

17 *Schutte AE*, *Huisman HW*, *van Rooyen JM*, *Malan L*, *Malan NT*, *Fourie CMT*, *Louw R*, *van der Westhuizen FH*, *Schutte R*. A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. J Clin Endocrinol Metab 2010; 95: 2503–2507

18 *Hamer M*, *Malan L*, *Schutte A*, *Huisman H*, *Van Rooyen J*, *Schutte R*, *Fourie C*, *Malan N*, *Seedat Y*. Conventional and behavioral risk factors explain differences in sub-clinical vascular disease between black and Caucasian South Africans: The SABPA study. Atherosclerosis 2011; 215: 237–242

19 *Norton K*, *Olds T*. Anthropometrica: a textbook of body measurement for sports and health courses. University of New South Wales Press; 1996

20 *Verrij E*, *Van Montfrans G*, *Bos J*. Reintroduction of Riva-Rocci measurements to determine systolic blood pressure. Neth J Med 2008; 66: 480–482

21 *American Diabetes Association*. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37: 81–90

22 *Hayashi I*, *Morishita Y*, *Imai K*, *Nakamura M*, *Nakachi K*, *Hayashi T*. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat Res 2007; 631: 55–61

23 *Higashi Y*, *Peng T*, *Du J*, *Sukhanov S*, *Li Y*, *Itabe H*, *Parthasarathy S*, *Delafontaine P*. A redox-sensitive pathway mediates oxidized LDL-induced downregulation of insulin-like growth factor-1 receptor. J Lipid Res 2005; 46: 1266–1277

24 *Ichikawa T*, *Nakao K*, *Hamasaki K*, *Furukawa R*, *Tsuruta S*, *Ueda Y*, *Taura N*, *Shibata H*, *Fujimoto M*, *Toriyama K*. Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. Hepatology International 2007; 1: 287–294

25 *Wu D*, *Cederbaum AI*. Alcohol, oxidative stress, and free radical damage. Alcohol Res Health 2003; 27: 277–284

26 *Møller S*, *Juul A*, *Becker U*, *Henriksen JH*. The acid-labile subunit of the ternary insulin-like growth factor complex in cirrhosis: relation to liver dysfunction. J Hepatol 2000; 32: 441–446

27 *Bassanello M*, *De Palo EF*, *Lancerin F*, *Vitale A*, *Gatti R*, *Montin U*, *Ciarleglio FA*, *Senzolo M*, *Burra P*, *Brolese A*. Growth hormone/insulin-like growth factor 1 axis recovery after liver transplantation: A preliminary prospective study. Liver transplantation 2004; 10: 692–698

28 *Succurro E*, *Andreozzi F*, *Sciacqua A*, *Hribal ML*, *Perticone F*, *Sesti G*. Reciprocal association of plasma IGF-1 and interleukin-6 levels with cardiometabolic risk factors in nondiabetic subjects. Diabetes Care 2008; 31: 1886–1888

29 *Willerson JT*, *Ridker PM*. Inflammation as a cardiovascular risk factor. Circulation 2004; 109: II2–10

30 *Singh U*, *Devaraj S*, *Vasquez-Vivar J*, *Jialal I*. C-reactive protein decreases endothelial nitric oxide synthase activity via uncoupling. J Mol Cell Cardiol 2007; 43: 780–791

31 *Kim C*, *Park H*, *Kawada T*, *Kim J*, *Lim D*, *Hubbard N*, *Kwon B*, *Erickson K*, *Yu R*. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes 2006; 30: 1347–1355

32 *Yubero-Serrano EM*, *Delgado-Lista J*, *Peña-Orihuela P*, *Perez-Martinez P*, *Fuentes F*, *Marin C*, *Tunez I*, *Tinahones FJ*, *Perez-Jimenez F*, *Roche HM*. Oxidative stress is associated with the number of components of metabolic syndrome: LIPGENE study. Exp Mol Med 2013; 45: e28

33 *Grundy SM*. Gamma-glutamyl transferase: another biomarker for metabolic syndrome and cardiovascular risk. Arterioscler Thromb Vasc Biol 2007; 27: 4–7

Supporting Information

Bioavailable IGF-1 and its Relation to the Metabolic Syndrome in a Bi-Ethnic Population of Men and Women

Authors

A. S. E. Koegelenberg[1], R. Schutte[1,2], W. Smith[1], A. E. Schutte[1,2]

Affiliations

[1]Hypertension in Africa Research Team (HART), North-West University, South Africa

[2]MRC Research Unit for Hypertension and Cardiovascular disease, North-West University, South Africa

Correspondence

A. E. Schutte, PhD

Hypertension in Africa Research Team (HART)

Private Bag X6001

North-West University

Potchefstroom

2520

South Africa

Tel.: +27/18/299 2444

Fax: +27/18/285 2432

alta.schutte@nwu.ac.za

© Georg Thieme Verlag KG · DOI: 10.1055/s-0035-1559768 · Horm Metab Res 2015; 47: e1–e5 · Koegelenberg ASE et al.

Table 1S Comparison of assays used to determine total IGF-1 levels in the SABPA and SAfrEIC studies.

	IRMA (Immunotech A15729, Beckman & Coulter)	**ELISA (BiocodeHycel EL2010)**
	(SABPA)	**(SAfrEIC)**
Unit of measurement for IGF-1	ng/ml	ng/ml
Intra-assay CV (%)	2.92	15.08
Inter-assay CV (%)	4.49	7.89
Sensitivity (ng/ml)	2	4.9
Dilution test (%)	88–111	73–108
Recovery test (%)	91–103	101.5–109.8
Kit calibrators		
CAL 0	0	0
CAL 1	30	45
CAL 2	100	77
CAL 3	300	133
CAL 4	600	270
CAL 5	1200	570
CAL 6	-	1330

IGF-1, insulin-like growth factor 1; CV, coefficient of variation

Table 2S Comparison of cardiovascular, biochemical and anthropometric measurements of men and women in the SABPA and SAfrEIC study, respectively.

	SABPA study			SAfrEIC study		
	Men (n=189)	**Women (n=198)**	***p* value**	**Men (n=268)**	**Women (n=252)**	**p value**
Age (yrs)	44.1±9.92	45.2±9.60	0.26	40.4±13.3	43.1±12.5	**0.016**
Ethnicity, black, n (%)	88 (46.6)	90 (45.5)	0.83	112 (41.8)	94 (37.3)	0.30
Anthropometric measurements						
Body mass index (kg/m^2)	27.9 (20.7; 38.6)	28.5 (20.0; 42.2)	0.16	25.1 (16.9; 35.7)	26.8 (18.7; 40.9)	**<0.001**
Waist circumference (cm)	97.9±15.6	88.9±15.0	**<0.001**	86.1±15.8	83.5±14.5	**0.049**
Biochemical measures						
IGF-1 (ng/ml)	165±59.8	158±63.2	0.26	152±80.9	143±74.8	0.19
IGFBP-3 (nmol/l)	133±27.0	138±26.7	0.077	134±38.0	149±28.4	**<0.001**
IGF-1/IGFBP-3	1.24±0.40	1.13±0.41	**0.009**	1.11±0.48	0.96±0.45	**<0.001**
Fasting glucose (mmol/L)	5.87 (4.60; 9.50)	5.18 (4.19; 6.55)	**<0.001**	5.35 (4.22; 7.13)	5.32 (4.19; 7.60)	0.72
Total cholesterol (mmol/L)	5.23±1.24	5.02±1.37	0.11	5.21±1.48	5.46±1.53	0.066
HDL cholesterol (mmol/L)	1.03±0.33	1.32±0.38	**<0.001**	1.42±0.63	1.52±0.43	**0.027**
LDL cholesterol (mmol/L)	3.45±1.22	3.26±1.18	0.12	3.15±1.36	3.34±1.33	0.11
Triglycerides (mmol/L)	1.35 (0.58; 3.23)	0.84 (0.42; 2.13)	**<0.001**	1.22 (0.53; 3.11)	1.16 (0.51; 2.80)	0.38
C-reactive protein (mg/L)	2.19 (0.65; 9.60)	3.87 (0.99; 28.7)	**<0.001**	1.36 (0.01; 15.9)	1.80 (0.07; 24.0)	**0.019**
Reactive oxygen species (mg/L)	79.5±17.3	105±31.0	**<0.001**	78.2±19.0	92.6±23.9	**<0.001**
Gamma-glutamyltransferase (U/L)	40.1 (13.0; 156)	21.2 (7.00; 89.8)	**<0.001**	51.2 (18.6; 334)	36.2 (14.0; 242)	**<0.001**
Aspartate aminotrasferase (U/L)	22.3 (13.7; 40.0)	17.0 (11.2; 30.0)	**<0.001**	28.6 (15.4; 89.9)	22.6 (13.3; 67.6)	**<0.001**
Alanine transaminase (U/L)	19.9 (7.80; 55.6)	11.0 (4.53; 30.8)	**<0.001**	26.8 (11.4; 74.3)	19.1 (9.82; 47.9)	**<0.001**
Cardiovascular measurements						
Systolic blood pressure (mmHg)	134±17.9	126±16.4	**<0.001**	103±16.9	118±20.2	**<0.001**
Diastolic blood pressure (mmHg)	89.1±12.8	81.7±10.0	**<0.001**	65±11.5	80.7±12.3	0.29
Lifestyle measures						
Smoking self-reported, n (%)	42 (22.2)	16 (8.08)	**<0.001**	113 (42.2)	70 (27.8)	**<0.001**
Intake of medication						
Anti-hypertensive medication, n (%)	45 (23.8)	45 (22.7)	0.80	36 (13.4)	31 (12.3)	0.68
Anti-diabetic medication, n (%)	7 (3.70)	3 (1.51)	0.18	0 (0)	0 (0)	-
Lipid-lowering medication, n (%)	7 (3.70)	4 (2.02)	0.32	11 (4.10)	13 (5.16)	0.57
Diabetes, n (%)	14 (5.22)	18 (7.14)	0.36	26 (13.8)	5 (2.53)	**<0.001**
*Hypertensive, n (%)	80 (42.3)	62 (31.3)	**0.025**	66 (24.6)	48 (19.1)	0.12

Data are arithmetic mean±SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables
n, number of participants; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3; HDL cholesterol, High-density lipoprotein; LDL cholesterol, Low-density lipoprotein
*Classification as hypertensive: ≥140 and/or 90 mmHg

Table 3S Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and the individual contribution of additional confounders.

	Men (n=457)		**Women (n=450)**	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2=0.30**	**R^2=0.14**	**R^2=0.21**	**R^2=0.20**
Age (yrs)	β=-0.41; p<0.001	β=-0.31; p<0.001	β=-0.36; p<0.001	β=-0.32; p<0.001
Ethnicity (White/African)	β=-0.38; p<0.001	β=-0.12; p=0.012	β=-0.12; p=0.004	β=0.10; p=0.016
Study (SAfrEIC/SABPA)	β=0.17; p<0.001	β=0.24; p<0.001	β=0.16; p<0.001	β=0.24; p<0.001
Number of MetS components (0-5)	-	β=-0.11; p=0.013	β=-0.11; p=0.014	β=-0.17; p=0.003
Model 2 (Model 1+CRP)	**R^2=0.30**	**R^2=0.14**	**R^2=0.23**	**R^2=0.22**
Number of MetS components (0-5)	-	β=-0.14; p=0.010	-	-
C-reactive protein log (mg/L)	-	-	β=-0.21; p<0.001	β=-0.18; p<0.001
Model 3 (Model 1+ROS)	**R^2=0.32**	**R^2=0.16**	**R^2=0.25**	**R^2=0.26**
Number of MetS components (0-5)	-	β=-0.11; p=0.030	-	β=-0.10; p=0.033
ROS (mg/L)	β=-0.13; p=0.002	β=-0.13 ; p=0.005	β=-0.23; p<0.001	β=-0.26; p<0.001
Model 4 (Model 1+γ-GT)	**R^2=0.33**	**R^2=0.19**	**R^2=0.21**	**R^2=0.21**
Number of MetS components (0-5)	-	-	β=-0.10; p=0.044	β=-0.12; p=0.013
γ-GT log (U/L)	β=-0.22; p<0.001	β=-0.27; p<0.001	β=-0.12; p=0.028	β=-0.15; p=0.007

IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3; ROS, reactive oxygen species; γ-GT, gamma-glutamyltransferase; MetS, metabolic syndrome

Table 4S Independent associations of IGF-1 and IGF-1/IGFBP-3 with the number of metabolic syndrome components and additional covariates.

	Men (n=457)		**Women (n=450)**	
	IGF-1	**IGF-1/IGFBP-3**	**IGF-1**	**IGF-1/IGFBP-3**
Model 1	**R^2=0.34**	**R^2=0.20**	**R^2=0.26**	**R^2=0.26**
Age (yrs)	β=-0.37; p<0.001	β=-0.26; p<0.001	β=-0.35; p<0.001	β=-0.32; p<0.001
Ethnicity (White/African)	β=-0.22; p<0.001	-	-	β=0.19; p<0.001
Study (SAfrEIC/SABPA)	β=0.91; p=0.044	β=0.14; p=0.005	β=0.19; p<0.001	β=0.25; p<0.001
C-reactive protein (mg/L)	-	-	-	-
ROS (mg/L)	β=-0.09; p=0.035	β=-0.12; p=0.012	β=-0.19; p<0.001	β=-0.23; p<0.001
γ-GT log (U/L)	β=-0.19; p<0.001	β=-0.26; p<0.001	-	β=-0.12; p=0.020
Smoking (yes/no)	-	-	-	-
Antihypertensive medication (yes/no)	-	-	-	-
Number of MetS components (0–5)	-	-	-	-

IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3; ROS, reactive oxygen species; γ-GT, gamma-glutamyltransferase; MetS, metabolic syndrome

ANNEXURE D

Published manuscript of research article 2

Thrombosis Research 136 (2015) 1007–1012

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

Bioavailable IGF-1 and its relationship with endothelial damage in a bi-ethnic population: The SABPA study

A.S.E. Koegelenberg [a], R. Schutte [a,b], W. Smith [a], A.E. Schutte [a,b,*]

[a] Hypertension in Africa Research Team (HART), North-West University, South Africa
[b] MRC Research Unit for Hypertension and Cardiovascular disease, North-West University, South Africa

ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 6 August 2015
Accepted 29 August 2015
Available online 3 September 2015

Keywords:
Insulin-like growth factor-1
Insulin-like growth factor binding protein-3
von Willebrand factor

ABSTRACT

Introduction: Insulin-like growth factor-1 (IGF-1) has vasculoprotective effects and can directly oppose endothelial dysfunction in several ways. To improve our understanding on the potential contribution of reduced IGF-1 to the development of vascular endothelial damage, we investigated the link between bioavailable IGF-1 and von Willebrand factor (vWF) as a marker of endothelial damage. We performed this study in black South African school teachers, known to be prone to hypertension.

Materials and methods: From the larger Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study we included 179 black and 207 white non-diabetic men and women (aged 44.5 ± 9.96 years). We measured ambulatory blood pressure and determined IGF-1, insulin-like growth factor binding protein 3 (IGFBP-3) and vWF antigen from blood samples. We used the molar IGF-1/IGFBP-3 ratio as an estimate of bioavailable IGF-1.

Results: Black individuals presented higher blood pressure and vWF_{ag} and lower IGF-1 than the white group (all $p < 0.001$). In multivariate-adjusted analyses, vWF_{ag} was inversely associated with IGF-1 ($R^2 = 0.18$; $\beta = -0.17$; $p = 0.044$) and IGF-1/IGFBP-3 ($R^2 = 0.18$; $\beta = -0.17$; $p = 0.030$) in blacks, with no associations in whites. Since IGF-1 is attenuated and vWF_{ag} elevated in diabetes, we included patients with diabetes ($n = 38$) and the aforementioned associations found in blacks remained robust.

Conclusion: The inverse association between bioavailable IGF-1 and vWF in black South Africans suggests that suppressed IGF-1 may result in endothelial damage independent of traditional risk factors.

1. Introduction

Insulin-like growth factor-1 (IGF-1) has vasculoprotective effects by activating nitric oxide synthase (NOS) and consequently increasing nitric oxide (NO) production through the PI3-K signalling pathway [1]. These protective effects include, among others, anti-apoptosis [2], endothelial-dependent vasodilation [3], oxygen free radical scavenging [4], anti-inflammatory [5] and anti-platelet aggregation properties [4]. IGF-1 is synthesised upon stimulation by growth hormone (GH) and is bound to insulin-like growth factor binding proteins (IGFBPs) that function as carrier proteins to regulate circulating IGF-1 transport, turnover and distribution [6]. IGF-1 is only biologically active in its free form, therefore total circulating IGF-1 provides only a crude estimate of biologically active IGF-1 [7]. Free IGF-1, which only account for 1% of total IGF-1, may therefore have greater physiological and clinical relevance than total IGF-1 [8]. Since approximately 80% of total IGF-1 is bound to IGFBP-3 [6], the calculation of the molar ratio of IGF-1/IGFBP-3 allows us to use the ratio as an estimate of bioavailable IGF-1, which may be a more beneficial marker to use than total IGF-1.

The endothelial-protective activities of IGF-1 are vital, with reduced IGF-1 levels linked to endothelial dysfunction [6], which may ultimately lead to endothelial damage. Also, attenuated IGF-1 levels associate with various cardiovascular risk factors such as hypertension [9,10] and diabetes mellitus [11], which may fully exert their detrimental effects through the pathway of endothelial dysfunction, endothelial apoptosis and the development of unstable plaque [2,12,13]. Endothelial damage results in the elevation of von Willebrand factor (vWF) levels [14] — a blood glycoprotein synthesised by and stored in endothelial cells [15]. Cell injury associated with cardiovascular risk factors such as diabetes will also result in high levels of vWF [14].

Due to rapid urbanization, black South Africans are prone to hypertension development [16] and have a high prevalence of cardiovascular disease [17]. The evidence that an urban dwelling lifestyle may be detrimental to the cardiometabolic health of Africans motivated us to develop the SABPA study that included black and white school teachers [18]. We have demonstrated in the SABPA and another South African study that black individuals have lower levels of IGF-1 than white South Africans making them more vulnerable to vascular abnormalities

* Corresponding author at: Hypertension in Africa Research Team (HART), Private Bag X6001, North-West University, Potchefstroom, 2520, South Africa.
E-mail address: alta.schutte@nwu.ac.za (A.E. Schutte).

http://dx.doi.org/10.1016/j.thromres.2015.08.022
0049-3848/

[9,19]. We therefore investigated whether endothelial damage, measured by vWF, is related to IGF-1 in black and white South Africans.

2. Materials and methods

2.1. Study population

This study forms part of the SABPA study conducted between February and May 2008 and 2009. We recruited a total of 409 urbanised black and white teachers from the North West Province, South Africa. The reason for this selection was an attempt to attain a homogenous sample from a similar socio-economic class. We invited all eligible participants between the ages of 25 and 65 years to participate. Exclusion criteria were participants with an elevated ear temperature, making use of α- and β-receptor blockers, psychotropic substance dependence or abuse, blood donors, and individuals vaccinated in the past three months. For this sub-study, participants were excluded due to missing data on insulin-like growth factor-1 availability (n = 4). Human immunodeficiency virus (HIV) infected participants (n = 19) and participants with glycated haemoglobin (HbA1c) levels ≥6.5% and/or using diabetes medication (n = 38) were also excluded. The overall sample of this sub-study consisted of 348 participants divided into a black (n = 148) and white (n = 200) group. Participants were fully informed about the objectives and procedures of the study prior to their inclusion. All participants signed an informed consent form. The study complied with the Helsinki declaration of 1975 (as revised in 2008) for investigation of human participants. The Health Research Ethics Committee of the North-West University (Potchefstroom Campus) approved the study.

2.2. Organisational procedures

Each morning at approximately 08:00, an ambulatory blood pressure monitor (ABPM) device was attached to the participants' non-dominant arm at their workplace. At 16:30 participants were transported to the Metabolic Unit Research Facility of the North-West University. This facility consists of 10 bedrooms, two bathrooms, a living room and kitchen. Participants received a standardised dinner and had their last beverages (tea/coffee) and two biscuits at 20:30. Thereafter they relaxed by reading, watching television, or social interaction and refrained from consuming alcohol, caffeine, smoking and doing exercise. They were encouraged to go to bed at around 22:00. Urine was collected overnight. At 06:00, subjects were woken at which time the ABPM apparatus was removed and subsequent measurements commenced. After the collection of a urine sample and anthropometric data, nurses obtained a fasting venous blood sample. Actical® accelerometers (Montréal, Québec) were attached around the hip of each participant before they leave for work, in order to assess physical activity during a normal working day.

2.3. Questionnaires

We used a general health questionnaire to determine lifestyle habits and medication use.

2.4. Anthropometric measurements

Height (stature) and weight of participants were measured while being in their underwear. Measurements were taken in triplicate using standard methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK) [20]. Waist circumference (WC) was measured with measurements taken to the nearest 0.1 cm using a non-stretchable standard tape (Lufkin, Cooper Tools, Apex, North Carolina, US). Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2).

2.5. Blood pressure measurements

An ABPM apparatus (Meditech CE120® Cardiotens; Meditech, Budapest, Hungary) was attached on the participant at their workplace, programmed to measure blood pressure at 30 min intervals during the day (08:00–22:00) and every hour during night time (22:00–06:00). We downloaded blood pressure data onto a database using the CardioVisions 1.9.0 Personal Edition software (Meditech, Budapest, Hungary).

2.6. Blood sampling and biochemical analyses

A registered nurse obtained a blood sample with a sterile winged infusion set from the antebrachial vein branches. Serum samples for total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglycerides, gamma-glutamyl transferase (γ-GT), and high-sensitivity C-reactive protein (CRP) were analysed using two sequential multiple analyzers (Konelab 20i; Thermo Scientific, Vantaa, Finland (2008); Unicel DXC 800 — Beckman and Coulter®, Germany (2009)). Interleukin-6 (IL-6) was analysed using the IL-6 Quantikine high sensitivity enzyme linked immunosorbent assay (R&D Systems, Minneapolis, MN USA). Total IGF-1 and IGFBP-3 were determined with immunoradiometric assays (IRMA) from Immunotech (Beckman and Coulter®, Germany; IGF-1 — A15729; IGFBP-3 — DSL-6600). Serum peroxides were determined by an improved assay system based on the principle of the derivatives of reactive oxygen metabolites test, which is recognised as an efficient method for evaluating reactive oxygen species (ROS) in the body. The Bio-Tek FL600 Microplate Fluorescence Reader (Bio-Tek, Instruments, Inc., Highland Park, Winooski, VT, USA) was used to measure ROS levels, where 1.0 mg/L H_2O_2 represents one unit of ROS [21]. Citrated samples were used for the analysis of von Willebrand factor antigen (vWF_{ag}). vWF_{ag} levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA). Polyclonal rabbit anti-vWF antibody and rabbit anti-vWF-horseradish peroxidase antibody (DAKO, Glostrup, Denmark) were used to perform the assay. The 6th International Standard for vWF/FVIII was used to create the standard curve against which the samples were measured. The percentage of glycated haemoglobin (HbA1c) was determined by means of the turbidimetric inhibition immunoassay on whole blood using the Roche Integra 400 (Roche, Basel, Switzerland). In the present sub-study, participants were considered as having diabetes if they had HbA1c levels of ≥6.5% or made use of diabetes medication [22]. HIV testing was done according to standard procedures of the South African Department of Health.

2.7. Statistical analyses

For database management and statistical analyses we used Statistica version 12.0 (StatSoft, Inc.,Tulsa, OK). We tested the interaction of sex and ethnicity on the association between vWF_{ag} and IGF-1/IGFBP-3 by introducing appropriate interaction terms in multiple regression analyses. Variables that were not normally distributed were logarithmically transformed (vWF_{ag}, IL-6, CRP, HbA1c, triglycerides, ROS and γ-GT). We subsequently compared means and proportions between black and white groups with independent T-tests and Chi-square tests, respectively. We used single regression analyses and forward stepwise multiple regression analyses to determine the associations of vWF_{ag} with total IGF-1 and IGF-1/IGFBP-3. The models included the following covariates: age, sex, BMI, HbA1c, total cholesterol:HDL, triglycerides, CRP, ROS, γ-GT, smoking, pulse pressure, and antihypertensive medication. The multivariate-adjusted analyses were also repeated upon inclusion of individuals with diabetes. All p-values refer to 2-sided hypothesis.

3. Results

We found no interaction of ethnicity ($p = 0.36$) and sex ($p = 0.65$ and $p = 0.48$ for blacks and whites, respectively) on the association

between vWF_{ag} and IGF-1/IGFBP-3. We therefore pooled the men and women, but stratified the groups according to ethnicity in line with our aim and the literature [9,23].

4. Characteristics of the study population

Table 1 displays the characteristics of the black and white participants. Age did not differ between blacks and whites, however black participants showed higher BMI ($p < 0.001$), 24 h systolic and diastolic blood pressure (all $p < 0.001$), vWF_{ag} ($p < 0.001$), CRP ($p < 0.001$) and IL-6 ($p = 0.001$). IGF-1 ($p < 0.001$) and IGFBP-3 ($p < 0.001$) were lower in the black group, but IGF-1/IGFBP-3 was similar between the two groups ($p = 0.26$).

5. Regression analyses

In unadjusted analyses (Fig. 1) we observed an inverse association between vWF_{ag} and both IGF-1 and IGF-1/IGFBP-3 in the black participants, with no associations found in the white group. After adjusting for age, sex and BMI the association between vWF_{ag} and IGF-1 in blacks remained significant ($r = -0.17$; $p = 0.041$) while the inverse association between vWF_{ag} and IGF-1/IGFBP-3 became borderline ($r = -0.14$; $p = 0.096$). These additional adjustments did not change the non-significant results in the white group.

We further performed multi-variable adjusted forward stepwise regression analyses with vWF_{ag} as dependent variable (Table 2), and assessed the association with IGF-1/IGFBP-3 (Model 1) and total IGF-1 (Model 2) as our main independent variables. We found an inverse association between vWF_{ag} and IGF-1/IGFBP-3 ($R^2 = 0.18$; $\beta = -0.17$; $p = 0.044$) and between vWF_{ag} and IGF-1 ($R^2 = 0.18$; $\beta = -0.17$; $p = 0.030$) in blacks only. Also when including participants with diabetes ($n = 38$) (Supplementary Table 1), the inverse associations of vWF_{ag} with IGF-1/IGFBP-3 ($R^2 = 0.14$; $\beta = -0.17$; $p = 0.020$) and IGF-1 ($R^2 = 0.15$; $\beta = -0.21$; $p = 0.005$) were confirmed in the blacks only.

6. Discussion

We found an independent inverse association between vWF, as a marker of endothelial damage, and bioavailable IGF-1, in black individuals who also presented with significantly higher blood pressure and vWF, and lower IGF-1 than white individuals. This finding was robust in a population irrespective of including or excluding those with diabetes. No significant associations were found in the white group.

We confirm our previous results in a different population sample indicating that IGF-1 levels are significantly lower in blacks compared to whites [9]. Higher levels of vWF among blacks also support previous findings [24,25], and taken together with the low IGF-1 profile, these results may indicate and partly explain why black populations have an increased risk for hypertension development and cardiovascular events [17]. Although our black population had higher ambulatory blood pressure [9], we found the association between endothelial damage and IGF-1 to be independent of pulse pressure and other risk factors. This association was found not only with total IGF-1 but also with the IGF-1/IGFBP-3 ratio, which may indicate that both total and bioavailable IGF-1 associate independently with endothelial damage.

Empen et al. analysed the association between IGF-1 and endothelial function, as measured by flow-mediated dilation, in a study population including 1482 participants (49.7% women) aged 25–85 years [26]. Although we used vWF and not flow mediated dilation as a marker of endothelial function, our results support the findings of Empen that

Table 1
Comparison of cardiovascular, biochemical and anthropometric measurements of black and white participants.

	Black (n = 148)	White (n = 200)	p value
Age (yrs)	43.8 ± 8.54	45.0 ± 10.9	0.28
Sex (women, men)	79/69	105/95	0.87
Anthropometric measurements			
Body mass index (kg/m^2)	29.9 ± 6.91	27.4 ± 5.75	<0.001
Waist circumference (cm)	92.4 ± 14.7	92.4 ± 15.7	0.96
Biochemical measures			
HbA1c (%)	5.70 (5.10; 3.60)	5.46 (5.00; 6.10)	<0.001
Total cholesterol:HDL ratio (mmol/L)	4.25 ± 1.61	4.96 ± 1.60	<0.001
Triglycerides (mmol/L)	1.07 (0.49; 2.77)	0.99 (0.43; 2.74)	0.20
IGF-1 (ng/ml)	142 ± 54.4	178 ± 62.7	<0.001
IGFBP-3 (nmol/l)	122 ± 24.6	148 ± 22.9	<0.001
IGF-1/IGFBP-3	0.15 ± 0.05	0.16 ± 0.05	0.26
vWF_{ag} (%)	90.5 (57.9; 148)	62.1 (41.0; 95.4)	<0.001
C-reactive protein (mg/L)	4.49 (0.65; 33.0)	2.00 (0.99; 9.00)	<0.001
IL-6 (pg/mL)	1.17 (0.33; 3.14)	0.91 (0.31; 3.03)	0.001
Reactive oxygen species (mg/L)	90.7 (57.1; 145)	87.0 (57.2; 138)	0.17
Cardiovascular measurements			
Ambulatory systolic blood pressure (mm Hg)	132 ± 16.4	124 ± 11.5	<0.001
Ambulatory diastolic blood pressure (mm Hg)	82.4 ± 10.8	76.3 ± 7.87	<0.001
Ambulatory pulse pressure (mm Hg)	49.7 ± 9.19	47.2 ± 7.22	0.004
Lifestyle measures			
Gamma-glutamyltransferase (U/L)	44.6 (20.1; 177)	18.9 (7.00; 75.0)	<0.001
Smoking self-reported, n (%)	26 (17.6)	29 (14.5)	0.45
Medication use			
Anti-hypertensive medication, n (%)	49 (33.1)	25 (12.5)	<0.001
Lipid-lowering medication, n (%)	2 (1.35)	8 (4.00)	0.14
Anti-inflammatory medication, n (%)	10 (7.76)	12 (6.00)	0.77
[a]Hypertensive, n (%)	103 (69.6)	87 (43.5)	<0.001

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables.
n, number of participants; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; HbA1c, glycated haemoglobin; vWF_{ag}, von Willebrand factor antigen.
[a]Classification as hypertensive: ≥140/90 mm Hg.

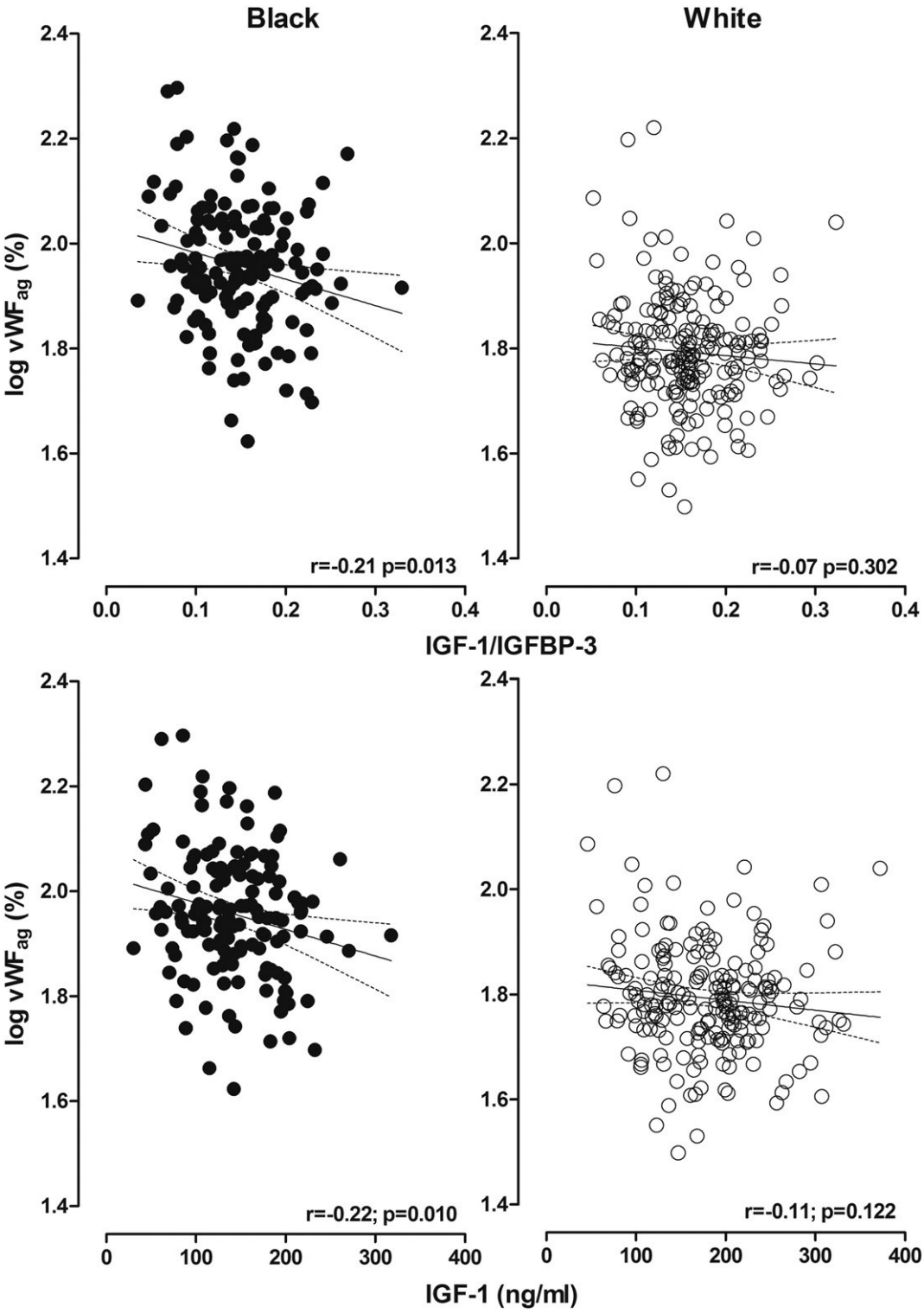


Fig. 1. von Willebrand factor (vWF) as a function of insulin-like growth factor-1 (IGF-1) and IGF-1/insulin-like growth factor binding protein-3 (IGFBP-3) in black and white individuals in single regression analyses. Solid and dashed lines represent the regression line and the 95% CI boundaries.

low serum levels of IGF-1 are associated with impaired endothelial function, after adjustments for major cardiovascular confounders [26]. The role of IGF-1 in vasodilation is supported by Abdu et al. who showed that patients (nonsmokers, without diabetes, hypertension or vascular disease) with low levels of IGF-1 due to growth hormone deficiency have reduced vasodilation. However, the administration of growth hormone led to the normalisation of previously reduced vasodilation [27].

von Willebrand factor is increased following endothelial cell injury [28], therefore vWF is an indicator of endothelial damage [14]. vWF has two functions in haemostasis: it acts as a bridging molecule for platelet adhesion and aggregation at the site of cell injury [29]. vWF-mediated platelet adhesion is dependent on a high shear rate, which occurs in arterioles and in partially occluded arteries [30]. Therefore damage to the endothelium enables vWF to bind to platelets at the

Table 2
Independent associations of vWF_{ag} with IGF-1/IGFBP-3 and total IGF-1.

	Black		White	
	vWF_{ag}		vWF_{ag}	
	β (95% CI)	p value	β (95% CI)	p value
n	148		200	
Model 1				
Adjusted R^2	0.18		0.04	
Main independent variable: IGF-1/IGFBP-3	−0.17 (−0.33; −0.01)	0.044	–	
Age (yrs)	–		0.11 (−0.03; 0.25)	0.13
Sex (women, men)	–		0.12 (−0.07; 0.31)	0.098
BMI (kg/m^2)	0.21 (0.03; 0.39)	0.025	0.11 (−0.04; 0.26)	0.16
HbA1c (%)	−0.11 (−0.27; 0.05)	0.18	–	
TC/HDL ratio	−0.08 (−0.24; 0.08)	0.31	–	
C-reactive protein (mg/L)	0.23 (0.04; 0.40)	0.016	–	
Reactive oxygen species (mg/L)	–		0.19 (0.02; 0.36)	0.032
γ-GT (U/L)	–		−0.12 (−0.31; 0.01)	0.066
Smoking (no/yes)	–		−0.07 (−0.21; 0.07)	0.32
Anti-hypertensive medication (no/yes)	−0.19 (−0.35; −0.04)	0.016	–	
Model 2				
Adjusted R^2	0.18		0.04	
Main independent variable: IGF-1 (ng/ml)	−0.17 (−0.37; −0.02)	0.030	–	
Age (yrs)	–		0.11 (−0.03; 0.25)	0.13
Sex (women, men)			0.12 (−0.07; 0.31)	0.22
BMI (kg/m^2)	0.21 (0.03; 0.39)	0.025	0.11 (−0.04; 0.26)	0.16
HbA1c (%)	−0.11 (−0.27; 0.04)	0.15	–	
C-reactive protein (mg/L)	0.22 (0.04; 0.39)	0.019	–	
Reactive oxygen species (mg/L)	–		0.19 (0.02; 0.36)	0.032
γ-GT (U/L)	–		−0.15 (−0.31; 0.01)	0.066
Smoking (no/yes)	–		−0.07 (−0.21; 0.07)	0.32
Anti-hypertensive medication (no/yes)	−0.21 (−0.37; −0.06)	0.006	–	

β, partial regression coefficient; 95% CI, 95% confidence intervals of β; vWF_{ag}, von Willebrand factor antigen; IGF-1, insulin-like growth factor-1; IGF-1/IGFBP-3 ratio, insulin-like growth factor-1/insulin-like growth factor binding protein-3; BMI, body mass index; HbA1c, glycated haemoglobin; TC:HDL ratio, total cholesterol:high-density lipoprotein cholesterol; γ-GT, gamma-glutamyl transferase.
Covariates included IGF-1/IGFBP-3 or IGF-1, age, sex, BMI, HbA1c, TC:HDL, triglycerides, C-reactive protein; pulse pressure, reactive oxygen species, γ-GT, smoking and antihypertensive medication.
$p < 0.05$ regarded as statistically significant.

site of injury, which does not occur in the absence of injury [30]. vWF also acts as a carrier for coagulation factor VIII and is responsible for maintaining normal levels of factor VIII [31]. In addition to the important functions of vWF in haemostasis, vWF is also involved in arterial thrombus formation and atherosclerosis [32].

Since IGF-1 has vasculoprotective properties [1] we may speculate on some mechanisms to be involved in the link found between vWF and IGF-1. IGF-1 directly opposes endothelial dysfunction, mainly by increasing NO production [1]. NO has multiple endothelial-protective activities, therefore a reduction in IGF-1 will consequently decrease NO production with a loss in its vasculoprotective effects, resulting in endothelial dysfunction [33] and may ultimately lead to endothelial damage. NO further plays a major role in the regulation of vWF secretion [34]. NO is responsible for the inhibition of platelet activation, and may thus inhibit endothelial exocytosis such as vWF release [34,35]. On the other hand, a reduction in NO levels may potentiate vWF secretion [34]. Another valuable protective activity of IGF-1 is the activation of phospholipase A_2 which induces prostacyclin (prostaglandin I_2 (PGI_2)) synthesis [36]. PGI_2 has important anti-platelet effects and is also responsible for inducing vasodilation [36]. In a study by Veyradier et al. vWF levels were significantly decreased when patients with pulmonary arterial hypertension were treated with prostacyclin [37]. Given that IGF-1 is responsible for NO and prostacyclin production [1, 36] and that both NO and prostacyclin exert an autocrine negative feedback on vWF secretion [34,37], one might expect that a reduction in IGF-1 would potentiate vWF secretion with possible implications for endothelial damage and haemostasis. This was confirmed by the significant inverse association found between vWF and IGF-1 in the black population. Therefore endothelial damage in blacks, who also presented a more unfavourable cardiometabolic profile compared to whites, may be due to a decline in bioavailable IGF-1 levels. Due to the cross-sectional nature of this study, causality cannot be inferred.

This study should be viewed in context of its strengths and limitations. A limitation of our study is that the participants consisted of urban black and white teachers, and the results can therefore not be extrapolated to the rest of South Africa. In addition, information on platelets and white blood cell counts, as well as flow mediated dilation were not available and may have provided more insight on the mechanisms involved regarding the link between vWF and IGF-1. The results of our study were consistent after multiple adjustments however, we cannot exclude residual confounding. A strength of this study includes the availability of bioavailable IGF-1 (molar IGF-1/IGFBP-3), detailed cardiovascular assessments and socio-economic compatibility in a relatively large group of bi-ethnic participants.

To conclude, the independent inverse relationship between vWF and IGF-1 in black individuals suggests that endothelial damage may be a consequence of the reduction in endothelial-protective activities of IGF-1. Our findings support the notion that reduced IGF-1 could be considered as an independent risk factor for cardiovascular disease.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2015.08.022.

Conflict of interests

None.

Acknowledgements

The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study would not have been possible without the voluntary

collaboration of the participants and the Department of Education, North West province, South Africa. The authors gratefully acknowledge the technical assistance of Mrs. Tina Scholtz, Sr. Chrissie Lessing and Dr. Szabolcs Péter. Research included in the present study was partially funded by the Medical Research Council, South Africa; Roche Diagnostics; the North-West University; and the Metabolic Syndrome Institute, France. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged.

Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.

References

[1] V. Schini-Kerth, Dual effects of insulin-like growth factor-I on the constitutive and inducible nitric oxide (NO) synthase-dependent formation of NO in vascular cells, J. Endocrinol. Investig. 22 (1999) 82–88.
[2] R. Hutter, B.V. Sauter, E.D. Reis, M. Roque, D. Vorchheimer, F.E. Carrick, et al., Decreased reendothelialization and increased neointima formation with endostatin overexpression in a mouse model of arterial injury, Circulation 107 (2003) 1658–1663.
[3] U. Izhar, D. Hasdai, D.M. Richardson, P. Cohen, A. Lerman, Insulin and insulin-like growth factor-I cause vasorelaxation in human vessels in vitro, Coron. Artery Dis. 11 (2000) 69–76.
[4] S. Dimmeler, A.M. Zeiher, Exercise and cardiovascular health get active to "AKTivate" your endothelial nitric oxide synthase, Circulation 107 (2003) 3118–3120.
[5] M. Spies, O. Nesic, R. Barrow, J. Perez-Polo, D. Herndon, Liposomal IGF-1 gene transfer modulates pro-and anti-inflammatory cytokine mRNA expression in the burn wound, Gene Ther. 8 (2001) 1409–1415.
[6] E. Conti, M. Musumeci, M. De Giusti, E. Dito, V. Mastromarino, M. Volpe, IGF-1 and atherothrombosis: relevance to pathophysiology and therapy, Clin. Sci. 120 (2011) 377–402.
[7] H Ørskov, J Weeke, J Frystyk, A Kaal, S Nielsen, C Skjaerbaek, et al., Growth hormone determination in serum from patients with growth disorders, Growth Hormone in Adults. Cambridge University Press, Cambridge, 1996 109–121.
[8] J. Frystyk, C. Skjærbæk, B. Dinesen, H. Ørskov, Free insulin-like growth factors (IGF-I and IGF-II) in human serum, FEBS Lett. 348 (1994) 185–191.
[9] A.E. Schutte, H.W. Huisman, J.M. van Rooyen, L. Malan, N.T. Malan, C.M.T. Fourie, et al., A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability, J. Clin. Endocrinol. Metab. 95 (2010) 2503–2507.
[10] A.E. Schutte, M. Volpe, G. Tocci, E. Conti, Revisiting the relationship between blood pressure and insulin-like growth factor-1, Hypertension 63 (2014) 1070–1077.
[11] M.S. Sandhu, A.H. Heald, J.M. Gibson, J.K. Cruickshank, D.B. Dunger, N.J. Wareham, Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study, Lancet 359 (2002) 1740–1745.
[12] Y. Okura, M. Brink, A.A. Zahid, A. Anwar, P. Delafontaine, Decreased expression of insulin-like growth factor-1 and apoptosis of vascular smooth muscle cells in human atherosclerotic plaque, J. Mol. Cell. Cardiol. 33 (2001) 1777–1789.
[13] V.A. Patel, Q.J. Zhang, K. Siddle, M.A. Soos, M. Goddard, P.L. Weissberg, et al., Defect in insulin-like growth factor-1 survival mechanism in atherosclerotic plaque-derived vascular smooth muscle cells is mediated by reduced surface binding and signaling, Circ. Res. 88 (2001) 895–902.
[14] G.Y. Lip, A. Blann, von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? Cardiovasc. Res. 34 (1997) 255–265.
[15] B. Horvath, D. Hegedus, L. Szapary, Z. Marton, T. Alexy, K. Koltai, et al., Measurement of von Willebrand factor as the marker of endothelial dysfunction in vascular diseases, Exp. Clin. Cardiol. 9 (2004) 31–34.
[16] A.E. Schutte, R. Schutte, H.W. Huisman, J.M. van Rooyen, C.M. Fourie, N.T. Malan, et al., Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study, Int. J. Epidemiol. 41 (2012) 1114–1123.
[17] K. Sliwa, D. Wilkinson, C. Hansen, L. Ntyintyane, K. Tibazarwa, A. Becker, et al., Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): A cohort study, Lancet 371 (2008) 915–922.
[18] L. Malan, M. Hamer, N. Frasure-Smith, H.S. Steyn, N.T. Malan, Cohort profile: sympathetic activity and ambulatory blood pressure in Africans (SABPA) prospective cohort study, Int. J. Epidemiol. (2014)http://dx.doi.org/10.1093/ije/dyu199.
[19] A. Schutte, R. Schutte, W. Smith, H. Huisman, C. Mels, L. Malan, et al., Compromised bioavailable IGF-1 of black men relates favourably to ambulatory blood pressure: the SABPA study, Atherosclerosis 233 (2014) 139–144.
[20] K. Norton, T. Olds, Anthropometrica: A Textbook of Body Measurement for Sports and Health Courses, University of New South Wales Press, 1996.
[21] I. Hayashi, Y. Morishita, K. Imai, M. Nakamura, K. Nakachi, T. Hayashi, High-throughput spectrophotometric assay of reactive oxygen species in serum, Mutat. Res. 631 (2007) 55–61.
[22] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 37 (2014) S81–S90.
[23] L. Lammertyn, A.E. Schutte, M. Pieters, R. Schutte, D-dimer relates positively with increased blood pressure in black South Africans: the SABPA study, Thromb. Res. 133 (2014) 1152–1157.
[24] C. Miller, E. Haff, S. Platt, P. Rawlins, C. Drews, A. Dilley, et al., Measurement of von Willebrand factor activity: relative effects of ABO blood type and race, J. Thromb. Haemost. 1 (2003) 2191–2197.
[25] P. Lutsey, M. Cushman, L. Steffen, D. Green, R. Barr, D. Herrington, et al., Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study, J. Thromb. Haemost. 4 (2006) 2629–2635.
[26] K. Empen, R. Lorbeer, H. Volzke, D.M. Robinson, N. Friedrich, A. Krebs, et al., Association of serum IGF1 with endothelial function: results from the population-based study of health in Pomerania, Eur. J. Endocrinol. 163 (2010) 617–623.
[27] T. Abdu, T. Elhadd, H. Buch, D. Barton, R. Neary, R. Clayton, Recombinant GH replacement in hypopituitary adults improves endothelial cell function and reduces calculated absolute and relative coronary risk, Clin. Endocrinol. (Oxf) 61 (2004) 387–393.
[28] B. Boneu, M. Abbal, J. Plante, R. Bierme, Factor-VIII complex and endothelial damage, Lancet 305 (1975) 1430.
[29] A.D. Blann, C.N. McCollum, von Willebrand factor, endothelial cell damage and atherosclerosis, Eur. J. Vasc. Surg. 8 (1994) 10–15.
[30] J.E. Sadler, Biochemistry and genetics of von Willebrand factor, Annu. Rev. Biochem. 67 (1998) 395–424.
[31] S.F. De Meyer, H. Deckmyn, K. Vanhoorelbeke, von Willebrand factor to the rescue, Blood 113 (2009) 5049–5057.
[32] A. Hamsten, The hemostatic system and coronary heart disease, Thromb. Res. 70 (1993) 1–38.
[33] E. Conti, C. Carrozza, E. Capoluongo, M. Volpe, F. Crea, C. Zuppi, et al., Insulin-like growth factor-1 as a vascular protective factor, Circulation 110 (2004) 2260–2265.
[34] S. Zarei, M. Frieden, J. Kaufmann, U. Vischer, The regulation of endothelial vWF secretion by nitric oxide: is it physiological? J. Thromb. Haemost. 4 (2006) 263–265.
[35] S. Massberg, M. Sausbier, P. Klatt, M. Bauer, A. Pfeifer, W. Siess, et al., Increased adhesion and aggregation of platelets lacking cyclic guanosine 3′,5′-monophosphate kinase I, J. Exp. Med. 189 (1999) 1255–1264.
[36] J.G. Webb, Y. Tan, M.A. Jaffa, A.A. Jaffa, Evidence for prostacyclin and cAMP upregulation by bradykinin and insulin-like growth factor 1 in vascular smooth muscle cells, J. Recept. Signal Transduct. 30 (2010) 61–71.
[37] A. Veyradier, T. Nishikubo, M. Humbert, M. Wolf, O. Sitbon, G. Simonneau, et al., Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension, Circulation 102 (2000) 2460–2462.