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Angiogenesis and Cardiovascular Dysfunction in Urbanised Africans:

The PURE study

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"... and I will take the stony heart out of their flesh, and will give them an heart of flesh..." Ez. 11:19.

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DECLARATION BY AUTHORS

The researchers who collaborated in the completion of this study were:

- The Author: PC Venter (Physiologist), responsible for literature survey, experimental acquisition of raw data, data processing and statistical analyses, as well as the interpretation of results;
- The Supervisor: Dr. L Malan (Physiologist and Registered Nurse), who co-ordinated the successful planning, design of the project and manuscript, statistical advice, as well as editing of the written dissertation;
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The following statement confirms the individual contribution of collaborators in the study and their written permission that this dissertation should be completed in article format.

I declare that I have approved the above-mentioned manuscript, that my role in the study is indicated above, is representative of my actual contribution and that I hereby give consent that it may be published as part of the M.Sc dissertation of PC Venter.

Dr. L Malan

Prof. AE Schutte

OPSOMMING

Probleemstelling: Hipertensie is 'n hoof bydraende risiko faktor tot vele kardiovaskulêre siektes en mag die oorsaak of gevolg van kardiovaskulêre disfunksie wees. Veral swart Afrikane ly aan hipertensie a.g.v. lewenstylveranderinge wat plaasvind tydens verstedeliking en wat kan lei tot simpato-adrenale hiperaktiwiteit. Vaskulêre endoteel groeifaktor-A (VEGF-A) and angiopoietien-2 (Ang-2) is beide reguleerders van angiogenese wat betekenisvol verhoog is in toestande wat geassosieer word met vaskulêre disfunksie. Vlakke van angiogenese faktore in Afrikane is onbekend en kan moontlik nie ooreenkom met vlakke wat reeds in Kaukasiërs gerapporteer is nie.

Doelstellings: Die doel van hierdie studie is eerstens, om te bepaal of verskille voorkom tussen die vlakke van VEGF-A en Ang-2 in verstedelikte in vergelyking met landelike swart Afrikane. Tweedens, om te bepaal of verhoogde VEGF-A en Ang-2 vlakke verband hou met hipertensie in swart Afrikane.

Metodiek: Hierdie is 'n substudie wat berus op die Prospective Urbanisation and Rural Epidemiologic (PURE) Studie. Oënskynlik gesonde vastende Afrikaan mans en vroue (N = 272; ouderdomme 35 - 50 jaar) vanuit die Noordwes Provinsie van Suid Afrika is deur 'n mediese geneesheer geselekteer vir deelname. Groepe is gestratifiseer volgens geslag en verstedelikingstatus op grond van inligting uit sosiodemografiese vraelyste. Kardiovaskulêre parameters (Omron HEM-757), polsgolfsnelheid (Complier SP), plasma angiogenese faktor vlakke (ELISA) en antropometriese mates is bepaal. 'n Onafhanklike t-toets en Pearson Chi-square toets is aangewend om verstedelikte en landelike data te vergelyk gevolg deur 'n ko-variansieanalise (ANCOVA) waar gekorrigeer is vir risikofaktore (ouderdom, liggaamsmassa-indeks, fisieke aktiwiteit en rookgewoontes). ANCOVAs (korrigeer vir risikofaktore) het gevolg waar hipertensiewe en normotensiewe groepe vergelyk binne die hele groep en verstedelikte groepe. Korrelasies, gekorrigeer vir risikofaktore, tussen kardiovaskulêre veranderlikes en angiogenese faktore is bepaal binne die hele groep en verstedelikte groepe.

Resultate en gevolgtrekking: Plasma VEGF-A waardes vir alle Afrikane is baie laag terwyl die ANG-2 vlakke meer verhoog is in vergelyking met kontrole vlakke verkry vir Kaukasiërs (hetsy normo- of hipertensief). Verstedelikte mans is meer oorgewig met 'n hoër voorkoms van hipertensie (42.47%) en VEGF-A maar met laer Ang-2 vlakke as landelike mans. Verstedelikte vrouens is meer oorgewig, fisies minder aktief en rook minder maar vertoon hoër diastoliese bloeddruk en VEGF-A met 'n laer polsgolfsnelheid in vergelyking met hul landelike eweknie. Ang-2 vlakke toon 'n negatiewe verband met diastoliese bloeddrukdata in landelike vroue. Geen verbande tussen hipertensiewe individue en verhoogde angiogenetiese faktor vlakke is verkry nie. Gevolglik blyk dit dat verstedeliking 'n groter effek op die angiogenese faktore het as op die hipertensiewe staat. As lae vlakke van VEGF-A voorkom kan dit die uitkoms van ANG-2 stimulering bepaal en die eienskappe van ANG-2 moontlik verander van anti-angiogeneties na pro-angiogeneties met gevolglike bloedvat-onstabiliteit en vaskulêre disfunksie soos waargeneemis in hipertensiewe verstedelikte mans. Dit kan wees dat hoër ANG-2 vlakke betrokke is by die afregulering van die Tie-2 reseptor met gevolglike laer vlakke van VEGF-A vlakke. Verdere studie is egter nodig aangesien Tie-2 reseptor aktiwiteit nie bepaal is in hierdie studie nie.

Sleutelwoorde: VEGF-A, Ang-2, angiogenese, verstedeliking, swart Afrikane.

SUMMARY

Argument: Hypertension is a main contributing risk factor to many cardiovascular diseases and may be the cause or the result of cardiovascular dysfunction. Black Africans, especially, suffer from hypertension because of lifestyle changes that occur during westernisation, which may lead to sympatho-adrenal hyperactivity. Vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2 (Ang-2) are regulators of angiogenesis and are significantly up regulated during states of vascular dysfunction. Levels of angiogenic factors are unknown for African people and may not be the same as levels thus far reported for Caucasians.

Aims: The aim of this study is firstly, to determine whether differences exist regarding the levels of VEGF-A and Ang-2 in urbanised compared to rural black Africans and secondly, to determine whether increased levels of VEGF-A and Ang-2 factors are related to hypertension in black Africans.

Methodology: This is a sub study that is based upon the Prospective Urban and Rural Epidemiological (PURE) study. Apparently healthy, fasting African men and women (N=272, aged 35 to 50 years) from the North-West province of South Africa were selected by a medical doctor to participate in this study. Groups were stratified according to gender and urbanisation status based upon information derived from sociodemographic questionnaires. Cardiovascular parameters (Omron HEM-757), pulse wave velocity (PWV) (Complier SP), plasma angiogenic factor levels (ELISA) and anthropometric measures were determined. An independent t-test and Pearson Chi-square test were used to compare urban and rural data, followed by an analysis of covariance (ANCOVA) while correcting for confounders (age, body mass index, physical activity and tobacco usage). ANCOVAs (corrected for confounders) were applied where hypertensive and normotensive groups were compared within the whole group and urbanised groups. Correlations, correcting for confounders, between cardiovascular variables and angiogenic factors were determined within the whole group and urbanised groups.

Results and conclusion: Plasma VEGF-A values for all black Africans were very low while the ANG-2 levels were elevated compared to control values for Caucasians (normotensive and hypertensive) in literature. Urbanised men were more overweight and indicated a higher incidence of hypertension (42.47%) and elevated VEGF-A levels, but lower Ang-2 levels compared to rural men. Urbanised women were generally overweight, physically less active and smoked less, but indicated higher diastolic blood pressure (BP), VEGF-A levels and lower PWV compared with their rural counterparts. Ang-2 levels indicate a negative relationship to diastolic BP data in rural women. No relationships between hypertensive individuals and high angiogenic factor levels were uncovered. Conclusive evidence suggested that angiogenic factor levels were affected more by urbanisation than by the state of hypertension. If low levels of VEGF-2 occur, ANG-2 stimulation and properties may be altered, thereby switching ANG-2 from an anti-angiogenic to a pro-angiogenic molecule, inferring blood vessel destabilisation and vascular dysfunction, such as is observed in hypertensive urbanised men. Higher ANG-2 levels may result in Tie-2 receptor down regulation, hence causing VEGF-A levels to be lower. Further study is needed to ascertain this mechanism since Tie-2 receptor activity was not determined in this study.

Keywords: VEGF, Ang-2, angiogenesis, urbanisation, black Africans.

PREFACE

To promote uniformity in this dissertation, the whole study was done according to the article format. Chapter 1 introduces the reader to the subject under question by supplying a general introduction with motivation, aims and hypotheses. A more detailed literature study in Chapter 2 provides background knowledge, also needed to interpret results. The actual article in Chapter 3 consists of all the subdivisions as mentioned in the instructions for author's page found at the onset of this chapter. Chapter 4 is a basic conclusive chapter on study results, their implications and recommendations for future research. Resources of chapters are provided in the reference section at the end of each chapter.

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ABBREVIATIONS

ANCOVA:	Analysis of covariance
Ang-1:	Angiopoietin-1
Ang-2:	Angiopoietin-2
BMI:	Body mass index
BP:	Blood pressure
CHF:	Congestive heart failure
CKD:	Chronic kidney disease
CO:	Cardiac output
CVD:	Cardiovascular disease
DBP:	Diastolic blood pressure
ELISA:	Enzyme linked immunosorbant assay
ESH:	European Society of Hypertension
HCM:	Hypertrophic cardiomyopathy
HIV/AIDS:	Human immune deficiency virus / acquired immune deficiency syndrome
HT:	Hypertensive
ISH:	International Society of Hypertension
JNC 7:	The seventh report of the Joint National Committee on the prevention, detection, evaluation, and treatment of high blood pressure
KEEP:	Kidney Early Evaluation Program
mmHg:	Millilitre mercury
mRNA:	Messenger ribonucleic acid
NADPH:	Nicotinamide adenosine dinucleotide phosphate protonated
ng/ml:	Nano gram per millilitre
NP-1:	Neuropilin receptor type 1
NP-2:	Neuropilin receptor type 2
NT:	Normotensive
PAI:	Physical activity index

pg/ml:	Pico gram per millilitre
PURE:	Prospective Urban and Rural Epidemiological study
PWV:	Pulse wave velocity
SBP:	Systolic blood pressure
THUSA:	Transition and Health during Urbanisation in South Africa
TIE-1:	Tyrosine kinase receptor-1
TIE-2:	Tyrosine kinase receptor-2
TPR:	Total peripheral resistance
USA:	United States of America
VEGF-A:	Vascular endothelial growth factor-A (first isoform of B, C, D, E.)
VEGFR-1:	Vascular endothelial growth factor receptor-1
vWF:	Von Willebrand factor
WHO:	World Health Organisation

CHAPTER 1

GENERAL INTRODUCTION

ANGIOGENESIS AND CARDIOVASCULAR DYSFUNCTION IN URBANISED AFRICANS: THE PURE STUDY

GENERAL INTRODUCTION

It is known that African-Americans suffer from hypertension[1] and that their blood pressure is poorly controlled.[1-2] African-American men are more likely than other studied ethnic groups from the United States of America (USA), to present with pre-hypertension and hypertension.[3]

This observation is no different in countries such as South Africa. Obesity and malnutrition are a well-known aftermath of "westernisation" or urbanisation in Africa[4-5] and causes an early increase in the systolic (37 mmHg) and diastolic (23 mmHg) blood pressure (BP) as seen by Longo-Mbenza *et al.*[6] in semi-urbanised black African boys. In adults (aged 16 – 64 years), CVD related events are the second highest cause of death in South Africans, and the incidence thereof is still rising.[7]

Urbanisation causes a lifestyle change and with it a reduction in physical activity. This combined change is a known risk factor for increasing hypertension, which according to Niakara *et al.*[8] may explain the 40.2 % prevalence of hypertension found in West-African urban subjects (35 years and older). Hypertension was also studied in Chicago communities where hypertension was associated with black Americans in disadvantaged neighbourhoods.[9] Opie & Mayosi[4] and Opie & Seedat[5] studied and reviewed data on sub-Saharan black Africans within this field and came across the same results. It is also known that black Africans pose higher vascular reactivity,[10-11] but whether this contributes to smooth muscle cell changes, and the underlying mechanism thereof, is largely unknown.

Angiogenesis, defined as the process during which new blood vessels are formed, should physiologically only take place during development, reproduction and wound repair.[12]

Primary angiogenic regulators like Angiopoietin-2 (Ang-2) and vascular endothelial growth factor-A (VEGF-A) (expressed in all vascularised tissues) maintain vascular functional homeostasis when present in low physiological concentrations.[13] Hypertension, on its own, is also known to cause mechanical signal transduction and activation of VEGF and Ang-2 within vascular smooth muscle cells.[14] This stress associated induction by chronically elevated blood pressure may activate angiogenic adaptive remodelling causing hypertrophy and/or intimal hyperplasia of blood vessel walls.[15] VEGF-A (interchangeably also known as VEGF by certain authors) and vascular remodelling, related to coronary heart disease, was studied in Caucasians, but whether the levels of VEGF is causative or a result of acute events remains controversial.[13]

VEGF-A and Ang-2 mediate angiogenic blood vessel remodelling when used therapeutically, according to Eaton *et al.*,[13] but cause pathologic neovascularisation when naturally present in patients at even more elevated levels.[16] A study by Nadar *et al.*[17] indicated elevated levels of plasma VEGF (ave = 1500 pg/mL) and Ang-2 (ave = 1.8 ng/mL) in hypertensive patients compared to normotensives (VEGF ave = 500 pg/mL; Ang-2 ave = 1.5 ng/mL). Investigating the levels of Ang-2 and VEGF-A in a subject's plasma may possibly be used as a new novel marker of vascular dysfunction, a factor present during angiogenesis[16+18] and hypertensive states.[15]

Angiogenic factors as predictors of the adverse outcomes of hypertension in African patients are largely still unknown[19] and the mediating risk factors of hypertension for blacks in urban environments are in the process of being researched.[2] According to the best knowledge of the author, VEGF and Ang-2 blood plasma level data on sub-Saharan black Africans are non-existent and may prove different from studies done elsewhere.

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CHAPTER 2

LITERATURE STUDY

ANGIOGENESIS AND CARDIOVASCULAR DYSFUNCTION IN URBANISED AFRICANS: THE PURE STUDY

LITERATURE STUDY

HYPERTENSION IN SOUTH AFRICANS

A survey on hypertension among adult individuals in the Durban area of KwaZulu-Natal, conducted by Seedat[1] in 1983, conclusively indicated that 25% of the urbanised black Zulu people suffered from hypertension compared to 9% in their rural counterparts, after adjusting for age. Hypertension was then classified by the World Health Organisation (WHO) as BP at or above 160/95 mmHg.[2] In 1984, a study of 4,993 rural Zulus in the Natal region of South Africa indicated that hypertension had become much more of a problem in the urban Zulu population.[3] In 2003, the WHO[4] and later the European Society of Hypertension[5] (2007) classified hypertension as BP measurements which exceeds 140/90 mmHg, which means that the 1983 survey results may even be worse if subjects were classified by the latest criteria.

Surveys undertaken from the 1970's to the 1990's on the incidence of hypertension indicated that 7 % of the rural Lesotho and 9.4 % of the rural Zulu tribes were classified as hypertensive. The criterion for hypertension classification, as set forth for this period, was a BP at or above 160/95 mmHg. These statistics already indicated a notable change in the occurrence of hypertension in Africans, since, according to The Lancet,[6] black Africans did not suffer from hypertension or an increase in BP with age in 1929.[2]

In 2003, the THUSA study[7] (Transition and Health during Urbanisation in South Africa) focused on the degree of urbanisation and how it relates to the health status in a black Tswana-speaking South African community. This study concluded that ethnic differences regarding BP may be explained by a genetic renal physiological and environmental socio-economic status difference. Accordingly, hypertensive patients experience above normal alpha-adrenergic peripheral vascular resistance when confronted with urbanisation, instead of normal increased cardiac output caused by sympatho-adrenal hyperactivity.[8,9]

Hypertension

The term hypertension, as well as the questionable pre-hypertension category introduced by the JNC-7,[10] may be misleading due to several factors mentioned in a report by the European Society of Hypertension and Cardiology.[5] BP classification should be considered in context of other individual risk factor criteria to estimate absolute CVD risk more correctly.[11] Although most data for guidelines concerning CVD risk assessment is currently based on western society, a predictive function for CVD such as was proposed by the Framingham study, may now be equally applicable on the South African subjects since the same guidelines have been adopted.[11]

The cause of hypertension is multifactorial[12] and includes factors such as age, gender, urbanisation, obesity and malnutrition. Many of these risk factors coincide with the same factors causative of coronary heart disease.[7] Hypertension significantly and specifically contributes to heart disease, stroke and end-organ failure (e.g. kidney failure). Currently one fourth of the United States of American (USA) population is affected and one third of the USA population still have uncontrolled BP.[13]

Blood pressure – A basic understanding

A complete evaluation of the cardiovascular system's hemodynamic status is possible when the mean arterial BP is known. This value is mathematically the product of the hemodynamic parameters, cardiac output and systemic vascular resistance. Elevations in any of or a combination of the two parameters may cause the cardiovascular pathological state of hypertension, hence it is also known as a hemodynamic disease.[14] Gu *et al.*[15] divided the BP data of test cases into three categories according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, the JNC 7 report.[10]

Table 1 Guidelines for BP classification (Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, the JNC 7 report (2003)).[10]

Classification	Systolic BP (mmHg)	Diastolic BP (mmHg)	
Optimal /	< 120 mmHg	< 80 mmHg	
Normotensive:			
Normal:	120 – 129	80 – 84	} Pre-hypertensive
High Normal:	130 – 139	85 – 89	
Hypertensive:	≥ 140	≥ 90	

Abbreviations: BP, blood pressure.

Where the mean systolic BP (SBP) ranges between and including 120 mmHg and 139 mmHg or the mean diastolic BP (DBP) ranges between and including 80 mmHg and 89 mmHg (Table 1) pre-hypertension was introduced as a new BP category.[15,16] If a subject under study reports that he or she is currently on high BP prescription medication, the subject is classified as being hypertensive. Such subjects usually are excluded from epidemiologic studies[7,15] regardless of what the BP may be at baseline measurement during initial examination.

BP classification formulation is important in CVD primary management care.[16] However, guidelines to diagnosis are not rigid and need to be adapted to relate to personal, medical and cultural situations. After critical examination of current literature,[5] the WHO/ISH guidelines was selected as the most appropriate resource to use in clinical studies and literature.[17]

Gu *et al.*[15] conducted a study to evaluate the relationship between BP and CVD outcome. Results revealed that pre-hypertensives (with mean BP of 125/77 mmHg) and hypertensives (with mean BP of 145/81 mmHg) were inclined to be older, male, overweight / obese, hypercholesterolemic, diabetic, chronic kidney disease patients, who had congestive heart failure, previous heart attack and were prior stroke patients. Black hypertensive patient data indicated a 60 % higher risk of CVD mortality. Within this same study, results indicated that non-Hispanic black USA subjects were more likely to

be hypertensive, as were subjects who were classified as sedentary individuals. As per 1000 persons, 95 % of all test subjects who died of CVD within 8.5 years of the first study were hypertensive (14.6, 95 %) and pre-hypertensive (3.7, 95 %).

Factors contributing to hypertension (ESH Guidelines 2007)[18]

Since angiogenic factors such as VEGF, a possible indicator of vascular damage, are believed to increase in states of hypertension and CVD,[19] it seems reasonable that contributing factors leading to hypertension and angiogenic factor levels during states of hypertension must be researched as a possible molecular diagnostic tool. It is possible that angiogenic activity may causally be linked to individual contributing factors of hypertension, but data on such relationships does not exist for all factors. Thus, only contributing factors to hypertension will be discussed here.

- *Ethnicity and gender*

Hypertension is already known to be poorly controlled in black Americans of African descent, especially men,[20,21] apart from black Africans.[2] Furthermore, the cost of treatment is not affordable for most black citizens of developing African countries.[2] In a cross sectional study done by Duru *et al.*[20] on 8,256 subjects screened for chronic kidney disease using the Kidney Early Evaluation Program (KEEP), it was found that hypertensive African American men with chronic kidney disease progressed 5 times more rapidly to the fatal condition of end stage renal disease compared to whites and 1.4 times faster than African American women. Hypertension is ethnic and gender related and is linked causally or consequently to chronic kidney disease which predisposes to CVD.[20]

According to Grady and Ramirez,[22] a growing body of researchers is looking at residential segregation as an ethnical disparity in the USA population that may be a contributing factor leading to related chronic medical conditions in African Americans or black Americans.

- *Social and residential disparities on the basis of ethnicity and socioeconomic status*

Social and residential disparities on the basis of ethnicity and socioeconomic status were studied in the USA population by Morenoff *et al.*,[21] who found that hypertension which had an earlier onset, was more aggressive and difficult to treat, thus more severe in causing end target organ damage in black African Americans. Conclusively, hypertension as a risk factor for heart-, kidney- and cerebrovascular disease was found to be unevenly distributed among the USA population.

- ***Obesity/Physical activity***

Urbanisation presents lifestyle changes that affect the contributing components of CVD, such as dyslipidemia, alcoholism and heightened peripheral vascular resistance,[7] as well as a reduction in physical activity. The relationship of urbanisation and sedentary lifestyle was shown by Niakara *et al.*[23] to increase hypertension in West-African urban subjects where the prevalence of hypertension was 40.2 % (Data generated was mainly for subjects of 35 years and older). Obesity and overweight positively correlates with CVD, coronary heart disease,[24] associated vascular dysfunction and angiogenesis[25] which is in turn associated with older men, being physically inactive and hypertensive.[26]

Increased adipose tissue in the obese and its role in cardiovascular and auto-immune diseases through leptin hormone secretion, have already been reviewed by Peelman *et al.*[27] Leptin levels may even explain changes in cardiovascular parameters: cardiac output (CO), total peripheral resistance (TRP) and BP, related to obesity in African women.[28] Therefore, obesity (like age) needs to be adjusted for since this factor may influence possible associations with cardiovascular dysfunction.[28]

Physical activity forms part of the initial onslaught to combat hypertension. As a matter of fact, it is reported that lifestyle counselling by a physician can improve hypertension outcome, even without using medication.[13] It is also known that this basic health service

is scarce in rural black communities and may contribute to the increased epidemiology of hypertension among black communities.[29] In a survey by Kruger *et al.*[24] on a black African population in the North-West province of South Africa, anthropometric data and demographic data concluded that physical activity index (PAI) showed a negative correlation association with the indicators of obesity, namely body mass index (BMI) and waist circumference (WC).

An independent association between obesity and angiogenesis exists. The endocrine, autocrine and paracrine function of adipose tissue and a possible mechanism to explain the relationship of obesity to angiogenesis was already explored.[25] Theoretically, adipose tissue, endothelial cells and associated cell types secrete increased amounts of angiogenic factors, such as vascular endothelial growth factor (VEGF), VEGF type C (VEGF-C), VEGF type D (VEGF-D) and soluble VEGF and angiopoietin-2 (Ang-2) (even after adjusting for age and gender), although experimental procedures indicate that only VEGF-C (0.44 – 0.46), VEGF-D (0.34 – 0.40), soluble VEGF-receptor 2 (0.44 – 0.48) and Ang-2 (0.34 – 0.34) correlate positively with BMI.[25] Angiogenic VEGF in increased plasma concentration has already been identified in hypertensives where such levels correlate positively with increased endothelial damage / dysfunction and cardiovascular risk.[19]

- ***Aging***

Cooper *et al.*[30] not only found that age posed an increased risk of coronary artery disease in hypertensive patients, but that the risk of hypertension also increases with age. The risk of hypertension generally increases above the age of 35 years, but incidentally the age at which individuals experience urbanisation is also found to have a synergistic effect in precipitating hypertension.[23]

Aging brings about changes in arterial wall properties, such as elasticity (distensibility) and thus it's buffering capacity (compliance). A decrease in arterial compliance causes elevated pulse pressure, leading to isolated systolic hypertension, detectible by non-

invasive echo-tracking techniques.[31,32] Aortal and large artery stiffness is largely influenced by physiological (e.g. age and gender), environmental (e.g. smoking and lifestyle) and disease parameters (e.g. hypertension and atherosclerosis). In this regard, pulse wave velocity (PWV) is a good indicator of arterial elastic properties and this measurement also correlatively increases with age.[32,33]

Breithaupt-Grögler & Belz[33] and Van Bortel *et al.*[34] indicated that arterial wall properties change with age, but a study by Wagatsuma[35] on young, adult and old mice proved that capillary supply or average blood capillary number per muscle fibre does not change with increasing age. His study also concluded that angiogenesis-related factors VEGF and Angiopoietin-1 (Ang-1) and Ang-2 levels remained unchanged in skeletal muscle tissue with advancing age.

In contrast to Wagatsuma's study[35] on rodents, an earlier study by Felmeden *et al.*[36] indicated correlative increases in blood plasma VEGF and the well-known cardiovascular risk factor, von Willebrand factor (vWf), with CVD risk factors such as age and BP.

Even though the capillary supply in animal models may remain unchanged,[35] the human chance of becoming hypertensive increases with age to about 65% at the age of 65 years and older, as was observed in Americans during an investigation by Gu *et al.*[15] According to them a definitive relationship exists between high BP and an increased risk of CVD events, as was studied in middle-aged men. Results indicated that the risk of CVD increased above BP values of 130-139/85-89 mmHg as compared to BP values below 120/80 mmHg, even though vascular disease mortality risk exists at BP values of round about 115/75 mmHg.[15] Hypertension may be the cause or result of endothelial dysfunction but is primarily related (as a major risk factor) to the pathogenesis of CVD.[12]

Age and gender work hand in hand at causing variation in BP among subjects.[29,36] Even though increasing age relates to higher BP and thus CVD,[7] it was found by Gu *et al,*[15] that test subjects within the age category of 65 – 74 years and the age group older than or

including 75 years who died of CVD indicated mortality rates indifferent throughout all BP categories.

Cardiovascular risk factors can potentially change with age,[15] as is particularly the case with women. Menopausal status is considered a cardiovascular risk factor by Tomg *et al.*,[37] because atherogenic risk may increase in post-menopausal woman, being indicated by so-called atherogenic lipids. Atherogenic lipids include plasma cholesterol levels, low-density lipoprotein cholesterol and triglycerides.

Post-menopause is considered during the age of 45 to 54 years of age. BP changes in natural menopausal women were not indicated in previous studies,[37] but may occur within 6 years of menopause if no change in menopausal status occurs. BP and body weight are both risk factors of CVD that change with age in women during menopause. During the menopausal transitional period, the SBP increases only slightly. Later, the lack of the oestrogen hormone causes both the SBP and DBP to increase. This change is attenuated by oestrogen administration.[37]

- *Smoking and Alcohol abuse*

Hypertension, cigarette smoking and hypercholesterolemia are the three major independent risk factors causative of coronary heart disease.[38] Amongst other factors, cigarette smoking adds synergistically to the pathological working of all other risk factors in causing vascular endothelial dysfunction and increased intima-media thickness.[39] Endothelial-dependant vasodilatation is thus obscured and worsens thrombotic, hemorrhagic and vasoconstrictive pathologies which precipitate occlusive and ischemic conditions in tissues. Chronic smoking causes dysfunctional blood flow auto regulation, while acute smoking leads to mean arterial pressure increases in rat models.[38] Current cigarette smoking, diabetic and dyslipidemic patients are traditional additive risk factors of coronary heart disease and associated vascular dysfunction.[26]

Smoking is a predisposing factor for CVD and atherosclerosis. These diseases are caused by endothelial dysfunction and conditionally initiate a phenotypic alteration of the endothelium towards a pro-inflammatory and pro-thrombotic modus.[38] Upon induction, atherosclerotic inflammatory mediators are released from activated monocytes, which cause smooth muscle cell and endothelial cell growth factor secretion, leading to atherogenesis, plaque instability[38] and ultimately angiogenic changes.

The effect of alcohol consumption on the vasculature is a subject of disagreement. The results of a study by Su *et al.*[39] indicated beneficial effects of alcohol consumption on the intima-medial thickness and also reports that the same results were indicated by other studies. Moderate drinking, especially red wine, have been proven by researchers to bring about beneficial cardiovascular effects (anti-oxidant, cardio protective and arterial damage protection). Research results indicated that these beneficial effects, compared to total alcohol related mortality, was graphically illustrated by a J-shaped relationship curve, such that abuse may lead to the opposite results.[40,41]

- *Lifestyle changes caused by Urbanisation*

Tribal African communities in the past lived in a peaceful, rural context, with little change, as documented by Donnison in 1926.[6] During this period, no incidence of hypertension was recorded. Historically, BP patterns between Europeans and black Africans were recorded to be dissimilar, but from the age of about 40 years and older, when Caucasians experienced an age related increase in BP, no such increase appeared in black Africans.

Migration of black Africans to urban areas is said to cause conflict in tribal people, with poverty and limited food resources. When stricken with the psychological stress that accompanies European lifestyle change, these same communities falter in adaptation[42] and these same tests reveal heightened BP with increasing age.[2] Today, it is known that the prevalence of hypertension is increased in urbanised black South Africans compared to other ethnic groups within the same context.[28]

The Sahara divides Africa into two main regions, namely the northern peri-Mediterranean and the sub-Saharan region, which includes South Africa. The effects of westernisation are especially evident when studying the BP patterns of sub-Saharan Khoisan nomadic tribes, which revealed no rise in BP with age, as with Europeans and North Americans of the Westernised world up to 1960. Since then, a western or civilised lifestyle has spread like a disease over the African continent and indirectly affected the BP of its native people,[43] leading to CVDs.[7,9] This "westernisation monster" is described by Opie & Mayosi[43] and is said to occur in three stages:

The first stage of westernisation "programs", or installs, is the risk factor to the foetus during the developmental stage by way of under nutrition in early life, which may, later in life, contribute to detrimental health.[43] Arterial hypertension development is introduced through rural-urban migration[7] or westernisation, and maternal malnutrition of the foetus, which leads to intrauterine growth retardation.[29] In the second stage, the already preconditioned human is weakened by disease and environmental related factors which inevitably leads to the third and final stage of CVD and death.[43]

The determinants of CVD in black Africans are still obscure and demand more research. CVD is, at this stage, causally related to hypertension since their risk factor patterns coincide. These major risk factors, which include age, gender, obesity and nutritional status, are strongly influenced when black Africans migrate from rural to urban areas.[7] Other environmental-related diseases and factors (apart from hypertension) that lead to CVD include HIV/AIDS related deaths, by way of tuberculoses pericarditis, rheumatic valvular disease, cardiomyopathy and diabetes mellitus, to name but a few.[43]

A mechanism proposed by Malan *et al.*[9] and Van Rooyen *et al.*[8] suggests that urbanisation causes abnormal sympatho-adrenal hyperactivity. This progresses to hypertension via the mechanism proposed by Schutte *et al.*[7] in which beta-adrenergic activity is down regulated, but alpha-adrenergic activity and thus peripheral vascular resistance is up regulated.

The Vascular endothelium and hypertension

The blood vessel intima or inner surface layer is a continuous lining or monolayer of cells called the vascular endothelium. The human body's approximately 10^{13} endothelial cells, structurally separates the blood vessel wall from the blood over a surface area of about 1-7 m^2 spread over the entire vascular system, altogether weighing roughly 1 kg in mass.[38] This layer of cells is functionally involved in homeostasis of the blood vessel concerning coagulation, vascular permeability, vascular tonus and vascular remodelling, to name but a few. Anatomically the endothelial cell is the target of many cytokines and growth factors because it is in direct contact with blood plasma and other blood cellular components.[44] Endothelial cells may be involved in outocrine, paracrine and endocrine actions by releasing vasoactive substances. Receptors on the endothelial cell membrane may sense hemodynamic changes and in return synthesize and/or activate the release of vasoactive substances.[38]

BP is regulated, among other mechanisms, by local blood flow or vascular tonus, which is mediated by vasoactive substances, such as vasodilator- and vasoconstrictor substances. Functional endothelial cells are vital for resistance arteries to work optimally and hemodynamically correctly.[38]

- *Endothelial dysfunction*

Endothelial cells may be impaired in disease states and become incapable of performing their normal function. Dysfunctional endothelial cells may form part of the initiation and progression of certain CVD processes, such as hypertension, atherosclerosis and hypertrophic cardiomyopathy (HCM), to name but a few.[39] Physiologically, certain haemostatic processes may also be activated, such as platelet aggregation after activation, inflammation in concert with immune modulation and increased vascular permeability, as well as angiogenic processes, such as smooth muscle cell proliferation.[38]

- *Angiogenesis*

An important consequence of many CVDs is the pathologic condition of angiogenesis or neovascularisation of blood vessels.[45] Angiogenesis may be defined as the process during which new blood vessels are formed from already existing vessels, a process that consists of proliferation, sprouting and tube formation by endothelial cells. Physiologically, this happens during development, reproductive processes and wound recovery.[38,46] Clinically, neovascularisation is marked by an increased density of new blood vessels in the intima, an increased occurrence of luminal stenosis, chronic inflammatory infiltrate extent, and granulation formation in tissues, as well as atheromateous changes.[26]

Induction of the process takes place when pro-angiogenic proteins, such as growth factors (e.g. VEGF), are released by either metastatic and tumour cells,[25] like melanoma or choriocarcinoma cells, or under tissue ischemic conditions, as in congestive heart failure (CHF).[47] Mediators augmenting proliferation and migration of endothelial cells are also induced by heparinase and plasmin derived from neovascularising endothelial cells.[38] Neovascularisation is thus regulated by a balance between pro-angiogenic and anti-angiogenic proteins that prevents excessive angiogenesis under physiological conditions.[25,46]

Vascular endothelial growth factor-A and angiopoietin-1 and angiopoietin-2

The diffusible factors that are released by the angiogenesis inducing vascular endothelial cells include VEGF, angiopoietins, transforming growth factor, platelet-derived growth factor, tumour necrosis factor-α, interleukins and fibroblast growth factors.[46] Some of these factors like Ang-1 and VEGF are primarily synthesised by the peri-endothelial cells, like vascular smooth muscle cells, underlying the endothelial cells. Receptors for Ang-1 and VEGF are mainly in the endothelial cells from embryonic development up to adulthood.[44]

Angiopoietins and VEGF promote proliferation or regression, depending on vascular functional status. The two factors function interdependently. VEGF may increase Ang-2 gene expression in endothelial cells, as well as interact with tyrosine kinase receptor-1 (Tie-1), an angiopoietin receptor.[45]

The angiopoietin growth factor family apparently has both angiogenic and metastatic properties exclusive to the endothelium and associates closely with VEGF.[47] The association is so interwoven that Ang-2 mRNA expression and release is potently stimulated by VEGF.[48] The putative mechanism for the pathophysiological angiogenic activity and vascular permeability induced by VEGF and Ang-2 may sprout from their relationship to anti-angiogenic factors. The pathophysiology in vascular disease may putatively be explained as an imbalance between the pro-angiogenic and anti-angiogenic factors.[49] Where VEGF increases vascular endothelial permeability and endothelial cell mitogenesis[25] at most points of the angiogenic cascade, Ang-1 promotes endothelial cell survival and possibly maturation, endothelial-support cell interaction stability and decreases vascular permeability. Pro-angiogenic Ang-2 may cause vessel regression in the absence of VEGF and cell migration and proliferation in the presence of VEGF. Increased plasma levels of VEGF, and therefore Ang-2, cause leaky, friable and proliferating vessels, thus leading to endothelial dysfunction.[48,49]

It is also possible that the two factors, VEGF and the angiopoietins, may act interchangeably and coordinate vessel generation and regression. In tests performed by Korff *et al.*,[50] using endothelial monolayer cell culture techniques, it was found that the application of Ang-2, in conjunction with VEGF, initiates endothelial cell sprouting at the end of capillaries and therefore promotes vessel elongation. VEGF, a cytokine factor, induces vascular mitogenesis,[19] angiogenesis and permeability[25] in contrast to Ang-1, which reduces permeability. Even in the presence of VEGF, Ang-1 aborts functional antagonistic effects of VEGF. The mechanism of antagonism of Ang-2 to Ang-1 lies in the fact that Ang-2 also binds to tyrosine kinase receptor-2 (Tie-2) but infers no activational signal in the endothelial cell. In so doing, Ang-2 induces endothelial cell function loss and therefore blood vessel regression, but may promote angiogenesis in the

presence of VEGF and so infer destabilization of the blood vessel. Thus the two cytokines, ANG-2 and VEGF, act synergistically, where Ang-2 acts to facilitate the function of VEGF.[50]

The sub factor VEGF-A, or commonly VEGF, causes modulation of the vessel wall by interacting predominantly with the receptors VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2)[45] on the vascular endothelial cells. VEGF inhibits intima media thickening, but promotes regeneration of vascular endothelial cells, thereby furthering endothelial dysfunction. The same factor is reported to promote chemotaxis of monocytes and plaque neovascularisation as a mechanism of atherosclerosis in collatero-genesis, thus vascular remodelling.[26] Therefore, VEGF-A is prone to cause pro-angiogenic activity by both receptors and to maintain mature vessels.

VEGF promotes tube formation, apoptosis inhibition, proliferation and migration of vascular endothelial cells.[26,44,45] VEGF, previously thought to be the main regulator of angiogenesis, mitogenesis and vascular permeability, increases the risk of coronary heart disease by the same mechanism when levels are raised. VEGF expression induces thrombosis via atherosclerotic lesion formation and vascular remodelling, causing increased plaque instability.[26]

Elevated plasma levels of VEGF along with the von Willebrandt factor (vWf) were tested for in humans[36] and may suggest hypertension related angiogenesis. vWf is an assessment marker for endothelial damage or dysfunction and predicts abnormal endothelial function involved in the pathogenesis of hypertension. In this study by Felmeden *et al.*,[36] general plasma VEGF level values, as determined by ELISA, may increase from 100 pg/mL in control subjects to as high as 300 – 350 pg/mL in the highest of hypertensive risk profile patients.

A population based study by Eaton *et al.*[26] revealed a linear association between baseline levels of VEGF and an increased independent relationship with coronary heart disease mortality. This relationship proved to be dose dependant and a predictor of poor

prognosis. Baseline levels of VEGF-A, that posed increased risk of coronary heart disease, were considered to be approximately 381 pg/ml, compared to the average level of 186 pg/ml in normal individuals. Post mortem results of patients who died of coronary heart disease, indicated elevated plasma VEGF-A levels of 400 pg/ml to 303 pg/ml ($p=0.004$), utilising the ELISA technique. The effect of VEGF is found in all vascularised tissues, including large blood vessels and the myocardium and is up regulated in ischemic skeletal muscles when angiogenesis occurs.[26]

The **Angiopoietin** growth factor family plays a major role in angiogenesis and metastasis according to oncology research[47] and is considered a novel member of the so-called endothelial cell specific factors. Where Ang-2 is physiologically expressed in high quantity at vascular remodelling sites in the ovary, uterus, placenta and endothelial cells in the postnatal phase,[51] Ang-1 expression extends to the brain, skeletal muscle, prostate and small intestine of adult tissues (excluding the endothelial cells). The tissue distribution of Ang-3 is very different from Ang-1 and Ang-2.[49] In total there are four angiopoietins: Ang-1, Ang-2, Ang-3 and Ang-4 and all four ligands bind to the Tie-2 receptor, postnatally restricted to the endothelial cells.[51]

For the angiopoitin binding receptor, Tie, there exist two (already mentioned) heterodimers Tie-1 and Tie-2.[45] The best studied ligands to bind to Tie-2 are the angiopoietins: Ang-1 and Ang-2.[51] Ang-2 binds to the receptor Tie-2 and competes with Ang-1 for opposite action.[45,49] Ang-1, which is a pro-angiogenic endothelial cell growth factor, is a survival factor, as well as a blood vessel stabiliser or maturation promoter.[50] Ang-1 and Ang-2 plasma levels and regulation differ because of the differences in local availability. Unlike Ang-2, Ang-1 is incorporated into the extra cellular matrix upon secretion, heightening the availability to target cells.[48] Upon binding to the Tie-2 receptor, angiopoietins form a pro-angiogenic receptor/ligand system that promotes blood vessel assembly and maturation.[50]

VEGF and Angiopoietin interact. VEGF also binds to Tie-1 and may induce the same second messenger pathway, as mentioned above,[45] although some researchers mention that no ligands for the Tie-1 have been identified.[49]

Ang-1 maintains endothelial cell survival via anti-apoptotic action and promotes blood vessel stability (integrity), in other words maturation of new vessels.[49] Ang-2, as the competitor, modulates the receptor function by promoting pro-angiogenic blood vessel destabilisation, rapidly expanding capillary diameter, basal lamina remodelling and sprouting of existing blood vessels in the presence of VEGF or stimulated by Ang-1. In the absence of VEGF, Ang-2 augments endothelial cell death and blood vessel regression.[47] Experimentation by researchers has revealed that transgenic over expression of Ang-2 disrupted mouse embryo blood vessel formation.[49] Both the angiogenic growth factors Ang-1 and Ang-2 bind to the endothelial cell Tie-2 receptor but infer neither mitogenic nor proliferative characteristics, in the absence VEGF.[47] This means that proliferation and migration of endothelial cells (sprouting of new blood vessels) will mostly take place during the event where both VEGF and Ang-2 are present. Ang-2 therefore exerts its actions mainly by antagonising Ang-1.[45]

AIMS

The aims of this study are:

1. To determine whether differences exist regarding the levels of VEGF-A and Ang-2 in urbanised compared to rural black Africans; and
2. To determine whether increased levels of VEGF-A and Ang-2 factors are related to hypertension states in black Africans when compared to normotensive Africans.

HYPOTHESES

1. Increased levels of angiogenic factors (VEGF-A and Ang-2) are prevalent in urbanised compared to rural black Africans.
2. Hypertensive African people present with higher levels of VEGF-A and Ang-2 than normotensive Africans.

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CHAPTER 3

ANGIOGENESIS AND CARDIOVASCULAR DYSFUNCTION IN URBANISED AFRICANS: THE PURE STUDY

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ABSTRACT

Increased plasma vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2 (Ang-2) has been associated with vascular dysfunction and hypertension. The aim was to assess angiogenic markers in black Africans during urbanisation and associated hypertensive states. African men and women (N = 272), matched for age, were recruited from rural and urban communities in the North-West province of South Africa. Sociodemographic questionnaires were completed and anthropometric measurements taken. Blood pressure (Omron HEM-757) and pulse wave velocity (PWV) (Complior SP) were obtained. Angiogenic factors were determined with ELISAs. Covariates included: age, body mass index, physical activity and smoking. Results indicated much lower VEGF-A values for black Africans, but high Ang-2 levels (for normo- and hypertensives). Urbanised men were overweight with a higher prevalence of hypertension (42.47%) and VEGF-A, but lower Ang-2 levels compared to rural men. Urbanised women were overweight, physically inactive and smoked less, but indicated higher diastolic blood pressure (DBP) and VEGF-A levels with a lower pulse wave velocity (PWV) than their rural counterparts. Ang-2 levels indicated negative relationships with DBP data in rural women. No other relationships between hypertensive individuals and increased angiogenic factor levels could be distinguished. Urbanisation conclusively had a greater effect on angiogenic factors than on the state of hypertension. The low VEGF-A levels may speculatively be the result of Ang-2 binding to Tie-2 receptors in men and so down regulating this receptor availability in urbanised hypertensive men.

INTRODUCTION

African-Americans,[1] and specifically men, are more likely to have a higher prevalence of hypertension[2] associated with disadvantaged neighbourhoods.[3] Opie & Mayosi[4] and Opie & Seedat[5] studied and reviewed data on sub-Saharan black Africans within this field and described it as an aftermath of westernisation causing an early increase in the systolic blood pressure (SBP) of 37 mmHg and diastolic blood pressure (DBP) of 23 mmHg, because of various reasons.[1,6,7,8,9] It is known that urbanised black Africans pose higher vascular reactivity,[10,11,12] but whether this contributes to smooth muscle cell changes remains largely unknown.

Angiogenesis, the process during which new blood vessels are formed, should physiologically only take place during development, reproduction and wound repair.[13] Primary angiogenic regulators, such as vascular endothelial growth factor-A (VEGF-A or simply VEGF) and Angiopoietin-2 (Ang-2) (expressed in all vascularised tissues), maintain vascular functional homeostasis when present in low physiological concentrations.[14] When naturally present in patients at even more elevated levels, angiogenic factors may cause pathologic neovascularisation.[15] A study by Nadar *et al.*[16] indicated elevated levels of plasma VEGF and Ang-2 in hypertensive patients compared to normotensives. Mechanical stress on the blood vessel wall, because of chronic high blood pressure (BP), activates large artery adaptation or remodelling. It could activate the VEGF and Ang-2 pathway within vascular smooth muscle cells by stretch-induced changes in membrane protein receptors,[17] activating angiogenic adaptive remodelling causing hypertrophy and/or intimal hyperplasia of blood vessel walls.[18]

Investigating the levels of VEGF and Ang-2 in a subject's plasma may possibly be used as a new, novel marker of vascular dysfunction[17,19] present in hypertensive states.[15] According to the best knowledge of the authors, VEGF and Ang-2 plasma level data on sub-Saharan black Africans are non-existant. We, therefore, firstly aimed to determine the differences in angiogenic factor (VEGF and Ang-2) levels by comparing rural to urbanised black Africans and hypothesised that increased levels of angiogenic factors are prevalent in urbanised compared to rural black Africans. Secondly, we hypothesised that hypertensive black Africans present with higher levels of VEGF and Ang-2 than normotensive Africans.

MATERIALS AND METHODS

Study design

The objectives of this cross-sectional study are addressed within a sub-study of the international Prospective Urban and Rural Epidemiological (PURE) study.[20] This sub-study consisted of two phases. In phase I the recruitment of black African participants took place through screening by a registered medical doctor in the North-West region of South Africa. Hereafter, black Africans will be referred to as Africans. Inclusion criteria for the sub-study were apparently healthy, fasting black African men and women aged 35 to 50 years. Exclusion criteria included pregnant and lactating women. During recruitment, the protocol was explained in the subjects' home language and they had the opportunity to ask questions. Afterwards, informed consent forms were signed. The study was approved by the Ethics Committee of the North-West University in accordance with the Declaration of Helsinki.[21] Phase II included completion of questionnaires and

measurements. On arrival of fasting participants at 07:00, measurements commenced with blood sampling. Fasting implied the abstinence of food or beverages for at least 8 hours (except water). Blood samples were drawn by a registered medical doctor from the ante-brachial vein in the dominant arm and handled according to standardised procedures. Centrifugation and separation were performed within 2 hours of collection and blood plasma was then stored at -70°C. The completion of questionnaires followed, and included the completion of general socio-demographic, health and physical activity questionnaires under the supervision of a clinical psychologist in the patient's home language. BP and arterial stiffness measurements were then conducted by cardiovascular physiologists or trained postgraduate students. Lastly, anthropometric measurements were made. Participants were immediately informed of their health status based on blood pressure measurements.

The sub-study sample consisted of 272 fasting men (N = 111) and women (N = 161) and participants were stratified into rural and urban gender groups. Rural status was allocated to communities having limited access to running water and electricity, whereas urban status was allocated to communities having access to both.[22] Hereafter, they were also subdivided into normotensive and hypertensive status groups (normotensive, SBP < 120 mmHg and DBP < 80 mmHg; hypertensive, SBP ≥ 140 mmHg and DBP ≥ 90 mmHg) (ESH guidelines[23] and JNC 7 classification).[24]

Questionnaires

Standardised interviewer-based questionnaires were used to collect general socio-demographic and health data. The physical activity index (PAI) was determined using the Baecke scale[22] for rural and urban populations. A PAI of 2.25 represents a subject performing domestic service in a standing and walking position, travelling by taxi, with no participation in sports activities. A 2.90 level PAI represents a subject walking to work at a moderate pace for 31 to 60 minutes, who spends leisure time in a standing or walking related activity or even indulges in sport activities when not at work. Current tobacco product use and current alcohol use was determined by a simple yes or no answer by participants.

Anthropometrical assessment

Anthropometric measurements were made using the guidelines adopted from the NIH sponsored Arlie Conference (1988) where the subject wore minimal or no clothing. 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 (Lufkin, Cooper Tools, Apex, NC). Weight was measured to the nearest 0.1 kg with the subject being barefoot using a portable digital scale (precision Health Scale, A&D Company, Tokyo, Japan). Height was measured to the nearest 0.1 cm in the upright standing position with a stadiometer (IP 1465, Invicta, London, UK). The subject was measured barefoot with the head held in the Frankfurt plane. Body mass index (BMI) was calculated by weight divided by height squared (kg/m^2), where overweight was indicated by a BMI of $\geq$ 25.0 to 29.9 kg/m2 and obesity by a BMI of $\geq$ 30.0 kg/m^2.[25]

Cardiovascular measurements

Participants should not have smoked or exercised in the past 15-30 minutes before BP recordings.[20] BP was recorded using the validated Omron HEM-757 automatic digital blood pressure monitor on the dominant arm whilst in a sitting position. Two measurements were taken with the arm at heart level at 5-minute intervals and the mean BP was computed. Cardiovascular parameters obtained were SBP, DBP and heart rate (HR). The distance between the carotid and radial arteries was measured while the participants were in the supine position. Firstly, between the radial point and the suprasternal notch and secondly, the distance between the carotid point and the suprasternal notch. The latter was subtracted from the first and notated. After 5 minutes, the pulse wave velocity (PWV) for arterial stiffness was determined non-invasively on the left hand side of each subject by means of the Complior SP device (Artech-Medical, France).[26,27,28]

Biochemical analyses

Human Ang-2 in EDTA blood plasma samples was measured using a Quantikine sandwich solid-phase enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Ltd., Abingdon, UK, Cat. No. DANG20). Human Ang-2 Immunoassay kits had a minimum detectable dose (MDD) in the range of 1.20 – 21.3 pg/mL and mean minimum sensitivity of 8.29 pg/mL. Human VEGF ELISA kits (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan, Code No. 27171) were used quantitatively to detect VEGF-A levels in human serum samples without any freeze-thaw occurring. The sensitivity for these kits was 1.01 pg/mL. All disposable equipment and potentially contaminated

materials utilised in the ELISA procedures were decontaminated by autoclaving for a minimum of 1 hour at 121.5°C. Glass-distilled and autoclaved water was used for reagent preparation.

Statistical analyses

Statistical analyses were performed using Statistica version 8 (StatSoft Inc., Tulsa, OK, USA, 2008) software. An independent *t*-test was carried out to compare subject characteristics of rural and urban African gender groups. The prevalence of current smoking, alcohol consumption and hypertension (normotensive and hypertensive) categorical data were computed using the Pearson Chi-square test. The analysis of covariance (ANCOVA) was firstly applied to compare rural and urban community group data within gender groups for SBP, DBP, PWV and angiogenic factors (VEGF-A and Ang-2) levels while adjusting for age, BMI, PAI and prevalence of smoking. Secondly, ANCOVAs tested for differences between normotensives and hypertensives within gender groups. Partial correlations for associations between cardiovascular measurements and angiogenic factors for rural and urban gender groups were performed, adjusting for age, BMI, PAI and prevalence of smoking.

RESULTS

Table I - subject characteristics data were compared between rural and urban African women and men. Urban women were generally older, more overweight, less active and smoked less but presented with lower PWV and marginally significant increased DBP and VEGF-A values than rural women. Urbanised men tended to be more overweight,

have increased DBP (p = 0.07), VEGF-A levels (p ≤ 0.01), lower Ang-2 levels and a higher prevalence of hypertension (Figure 1).

Table 1 Comparing subject characteristics (mean ± n SD) of African women and men between rural and urban communities without any adjustment for variables

	Women			*Men*		
	Rural N = 93	*Urban* N = 68	P-*value*	*Rural* N = 54	*Urban* N = 57	P-*value*
Age (years)	**42.20 ± 5.43**	**43.80 ± 4.67**	**0.05**	42.40 ± 5.36	41.90 ± 5.05	0.58
BMI (kg/m^2)	**24.90 ± 5.10**	**26.90 ± 5.16**	**0.01**	**19.40 ± 2.16**	**20.60 ± 3.77**	**0.05**
WC (cm)	77.50 ± 12.00	80.00 ± 10.70	0.17	72.80 ± 5.39	73.40 ± 9.47	0.72
PAI	**3.05 ± 0.53**	**2.86 ± 0.56**	**0.03**	3.03 ± 0.49	3.37 ± 2.11	0.27
Tobacco (%)	**63.40%**	**41.20%**	**0.02**	48.20%	61.40%	0.16
Alcohol (%)	23.70%	30.90%	0.57	48.20%	59.70%	0.22
SBP (mm Hg)	121 ± 19.80	126 ± 19.50	0.13	121 ± 17.40	127 ± 16.60	0.71
DBP (mm Hg)	83.30 ± 14.40	87.20 ± 12.50	0.07	79.10 ± 12.20	83.50 ± 14.40	0.08
PWV (m/s)	**10.80 ± 2.30**	**10.10 ± 1.70**	**0.04**	11.60 ± 3.04	11.40 ± 2.08	0.62
VEGF-A (pg/mL)	7.48 ± 9.07	10.20 ± 9.78	0.07	**5.84 ± 7.50**	**11.80 ± 10.30**	**≤0.01**
Ang-2 (ng/mL)	2.15 ± 0.83	2.15 ± 1.15	0.98	**2.53 ± 1.25**	**2.09 ± 1.07**	**0.05**

Abbreviations: Ang-2, angiopoietin-2; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; N, number of participants; PAI, physical activity index; PWV, pulse wave velocity; SD, standard deviation; SBP, systolic blood pressure; VEGF-A, vascular endothelial growth factor-A.

Our first aim was to compare angiogenic factor (VEGF-A and Ang-2) levels between rural and urban groups according to normotensive (NT) and hypertensive (HT) status.[23]

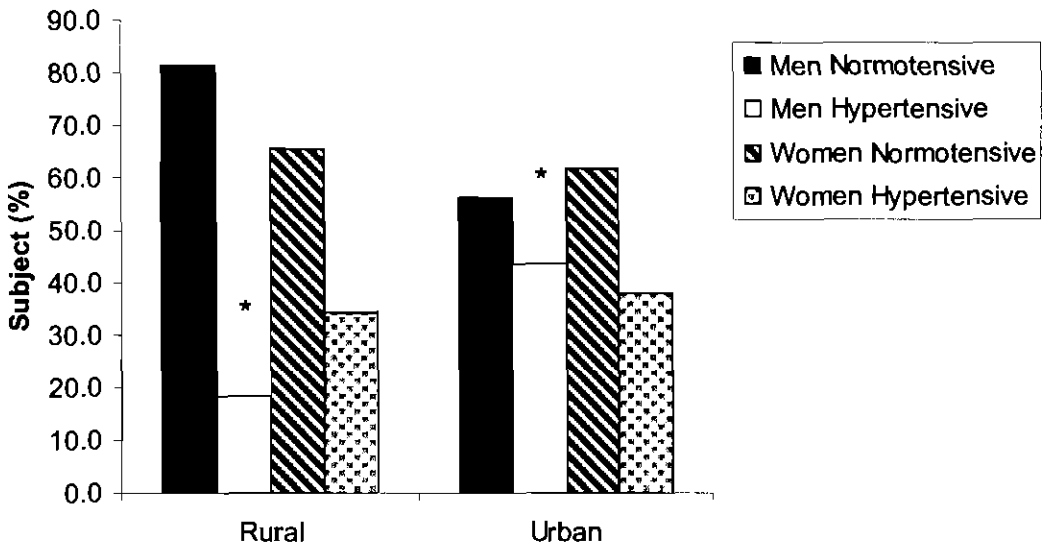

Figure 1 The prevalence of HT (%) within rural and urban gender groups. *$P \leq 0.01$ between rural (18.50%) and urban (43.90%) hypertensive men.

In Table 2, whole group data indicated increased levels of VEGF-A in urban women and men, but this increase is seen more specifically in normotensive urban women and men ($p = 0.02$). Within the whole group, urban men exhibited marginally lower Ang-2 levels.

Table 2 Comparing (mean ± CI) cardiovascular and angiogenic factors of rural versus urban groups (within the whole group as well as NT and HT groups) adjusting for confounders (age, BMI, PAI and smoking).

		Women			*Men*		
		Rural *N = 86*	*Urban* *N = 65*	P-*value*	*Rural* *N = 51*	*Urban* *N = 53*	P-*value*
Whole group *N = 255*	SBP (mmHg)	120 (116; 125)	125 (120; 130)	0.19	121 (116; 126)	126 (121; 130)	0.16
	DBP (mmHg)	83.4 (80.4; 86.4)	86.6 (83.1; 90.1)	0.18	79.0 (75.3; 82.7)	82.5 (78.8; 86.1)	0.20
	PWV (m/s)	10.8 (10.3; 11.3)	10.3 (9.73; 10.8)	0.14	11.6 (10.9; 12.3)	11.4 (10.8; 12.2)	0.77
	VEGF-A (pg/mL)	**6.65 (4.56; 8.75)**	**11.0 (8.61; 13.4)**	**≤0.01**	**6.30 (3.72; 8.89)**	**11.2 (8.72; 13.8)**	**≤0.01**
	Ang-2 (ng/mL)	2.12 (1.90; 2.34)	2.21 (1.96; 2.47)	0.59	2.50 (2.18; 2.83)	2.10 (1.78; 2.42)	0.09
Normotensive *N = 172*	SBP (mmHg)	110 (107; 113)	115 (111; 119)	0.07	117 (113; 120)	113 (109; 118)	0.28
	DBP (mmHg)	**75.3 (73.1; 77.4)**	**79.9 (77.2; 82.5)**	**0.01**	75.9 (72.8; 79.0)	73.6 (69.9; 77.2)	0.35
	PWV (m/s)	10.2 (9.77; 10.7)	10.0 (9.48; 10.6)	0.62	11.5 (10.8; 12.2)	10.8 (9.98; 11.6)	0.20
	VEGF-A (pg/mL)	**6.68 (4.07; 9.29)**	**11.7 (8.55; 14.8)**	**0.02**	**5.67 (3.00; 8.34)**	**10.1 (6.97; 13.3)**	**0.04**
	Ang-2 (ng/mL)	2.20 (1.91; 2.48)	2.25 (1.90; 2.60)	0.83	2.40 (2.06; 2.73)	2.09 (1.69; 2.49)	0.27
Hypertensive *N = 83*	SBP (mmHg)	141 (134; 148)	143 (135; 150)	0.80	144 (135; 152)	143 (138; 147)	0.79
	DBP (mmHg)	99.6 (96.0; 103)	98.7 (94.8; 103)	0.73	92.4 (83.6; 101)	95.5 (90.4; 101)	0.56
	PWV (m/s)	11.9 (10.9; 12.9	10.7 (9.64; 11.7)	0.11	11.6 (9.07; 14.2)	12.5 (11.0; 14.0)	0.56
	VEGF-A (pg/mL)	6.80 (2.87; 10.7)	9.69 (5.41; 14.0)	0.35	12.1 (3.87; 20.4)	11.7 (6.83; 16.5)	0.93
	Ang-2 (ng/mL)	1.96 (1.60; 2.32)	2.13 (1.75; 2.51)	0.53	2.79 (1.82; 3.75)	2.20 (1.63; 2.76)	0.33

Abbreviations: Ang-2, angiopoietin-2; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; PAI, physical activity index; PWV, pulse wave velocity; SBP, systolic blood pressure; VEGF-A, vascular endothelial growth factor-A.
Values are adjusted means and 95% confidence intervals (minimum and maximum value).

Table 3 Comparing (mean ± CI) cardiovascular and angiogenic factors of NT versus HT within gender groups (within whole, rural and urban groups) adjusting for confounders (age, BMI, PA1 and smoking).

		Women			*Men*		
		Normotensive N = 99	*Hypertensive N = 52*	P-*value*	*Normotensive N = 73*	*Hypertensive N = 31*	P-*value*
Whole group N = 272	SBP (mmHg)	**112 (110; 115)**	**142 (138; 146)**	**≤0.01**	**115 (113; 118)**	**143 (138; 147)**	**≤0.01**
	DBP (mmHg)	**77.1 (75.4; 78.8)**	**99.3 (96.9; 102)**	**≤0.01**	**75.0 (72.6; 77.4)**	**94.4 (90.7; 98.2)**	**≤0.01**
	PWV (m/s)	**10.2 (9.77; 10.6)**	**11.3 (10.8; 11.9)**	**≤0.01**	**11.2 (10.6; 11.8)**	**12.3 (11.4; 13.3)**	**≤0.05**
	VEGF-A (pg/mL)	8.74 (6.81; 10.7)	8.20 (5.55; 10.9)	0.75	7.90 (5.70; 10.1)	11.0 (7.53; 14.5)	0.15
	Ang-2 (ng/mL)	2.22 (2.02; 2.42)	2.03 (1.76; 2.31)	0.28	2.25 (1.98; 2.53)	2.41 (1.98; 2.83)	0.56
Rural N = 147	SBP (mmHg)	**109 (106; 113)**	**142 (137; 147)**	**≤0.01**	**116 (112; 120)**	**144 (135; 152)**	**≤0.01**
	DBP (mmHg)	**74.8 (72.4; 77.2)**	**99.7 (96.2; 103)**	**≤0.01**	**75.4 (72.4; 78.5)**	**94.1 (87.4; 101)**	**≤0.01**
	PWV (m/s)	**10.3 (9.72; 10.9)**	**12.1 (11.2; 12.9)**	**≤0.01**	11.6 (10.6; 12.6)	12.5 (10.3; 14.7)	0.46
	VEGF-A (pg/mL)	7.01 (4.62; 9.40)	6.98 (3.54; 10.4)	0.99	5.40 (3.06; 7.75)	7.83 (2.63; 13.0)	0.40
	Ang-2 (ng/mL)	2.24 (2.01; 2.46)	1.99 (1.65; 2.32)	0.23	2.48 (2.11; 2.84)	2.83 (2.02; 3.64)	0.44
Urban N = 125	SBP (mmHg)	**116 (111; 120)**	**143 (138; 149)**	**≤0.01**	**115 (111; 119)**	**142 (137; 147)**	**≤0.01**
	DBP (mmHg)	**80.2 (77.7; 82.6)**	**99.2 (95.9; 102)**	**≤0.01**	**74.8 (70.8; 78.8)**	**93.9 (89.0; 98.7)**	**≤0.01**
	PWV (m/s)	10.0 (9.47; 10.5)	10.4 (9.73; 11.1)	0.35	**10.6 (9.87; 11.2)**	**12.4 (11.5; 13.2)**	**≤0.01**
	VEGF-A (pg/mL)	11.6 (8.50; 14.7)	8.80 (4.68; 12.9)	0.29	12.6 (8.90; 16.2)	10.5 (6.02; 15.0)	0.50
	Ang-2 (ng/mL)	2.17 (1.80; 2.55)	2.13 (1.64; 2.62)	0.90	1.94 (1.53; 2.34)	2.24 (1.76; 2.73)	0.37

Abbreviations: Ang-2, angiopoietin-2; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; PAI, physical activity index; PWV, pulse wave velocity; SBP, systolic blood pressure; VEGF-A, vascular endothelial growth factor-A.
Values are adjusted means and 95% confidence intervals (minimum and maximum value).

Table 3 shows that BP and PWV values were significantly higher in the HT participants within the whole group, as well as in rural and urban strata in most cases.

To determine the associations between angiogenic factor levels (VEGF-A and Ang-2) and cardiovascular factors in the rural and urban groups, correlations were performed and then adjusted for age, BMI, PAI and smoking (Table 4).

Table 4 Correlations between angiogenic factors and cardiovascular variables (before and after adjustment for age, BMI, PAI and smoking)

		Women				*Men*			
		Rural *N = 91*		*Urban* *N = 67*		*Rural* *N = 53*		*Urban* *N = 56*	
		r-*value*	P-*value*	r-*value*	P-*value*	r-*value*	P-*value*	r-*value*	P-*value*
VEGF	SBP	-0.01	1.00	-0.14	0.24	0.11	0.43	0.13	0.36
	DBP	-0.08	0.45	-0.10	0.40	0.09	0.54	0.13	0.35
	PWV	-0.17	0.11	-0.10	0.41	-0.02	0.88	-0.03	0.85
Ang-2	SBP	**-0.21**	**0.05**	-0.02	0.87	-0.24	0.08	0.12	0.38
	DBP	**-0.24**	**0.02**	-0.05	0.71	-0.22	0.11	0.18	0.18
	PWV	-0.01	0.90	0.11	0.38	0.04	0.77	0.14	0.30
		Adjusted for age, BMI, PAI and smoking							
VEGF	SBP	-0.02	0.85	-0.13	0.32	0.10	0.52	-0.10	0.48
	DBP	-0.13	0.24	-0.14	0.30	0.04	0.77	-0.12	0.40
	PWV	-0.17	0.14	-0.09	0.47	0.01	0.96	-0.05	0.76
Ang-2	SBP	-0.19	0.09	-0.08	0.53	-0.11	0.48	0.14	0.34
	DBP	**-0.25**	**0.03**	-0.06	0.67	-0.19	0.20	0.22	0.13
	PWV	-0.06	0.63	0.04	0.74	0.01	0.93	-0.03	0.84

Abbreviations: Ang-2, angiopoietin-2; DBP, BP, blood pressure; diastolic blood pressure; SBP, systolic blood pressure; PWV, pulse wave velocity; VEGF-A, vascular endothelial growth factor-A.

Weak negative associations showed between Ang-2 and SBP in rural men (marginally) and women without any adjustments. The same trend was found between Ang-2 and DBP in rural women only, before and after adjustment for confounders.

DISCUSSION

Our first hypothesis stated that increased levels of angiogenic factors (VEGF and Ang-2) are prevalent in urbanised compared to rural black Africans. Previous research has indicated higher BP values in African-Americans[2] associated with disadvantaged communities[3] and that angiogenic factor levels are proven to be increased in states of raised BP.[16,17,18] The relationship between angiogenic factors and urbanisation may therefore relate via the mechanism of increased BP.

Urban women, despite being overweight, less active and using less tobacco, showed lower PWV but higher DBP and VEGF-A ($p = 0.07$) values. Urbanised men showed the same trend of being more overweight although within the normal range limits,[25] but with higher DBP and VEGF-A ($p \leq 0.01$) values supporting literature and our first hypothesis. Urbanised Africans have previously indicated higher vascular reactivity and hypertension prevalence,[10,11] thus supporting present data (Figure 1). The decreased PWV measurements and Ang-2 levels of urbanised Africans, however, indicated less possible arterial stiffening, vascular damage, and neovascularisation and associated risk for CVD.[27,29] Previous studies have indicated that higher BP values do not necessarily account for higher PWV, although black African hypertensive urban men have indicated both increased stiffness and SBP.[27,30] Hypertension has been associated with increased sympathetic activity, which influences both BP and PWV characteristics.[31] Although carotid-radial PWV is considered a measure of peripheral (radial artery) arterial stiffness, overall rural-urban comparative data for hypertension occurrence and age, which normally suggest increased stiffness, apparently did not cause increased vascular stiffness.[27,30]

Cardiovascular factors (SBP, DBP and PWV) and angiogenic factors were compared once again between rural and urban groups, adjusting for confounders. Of particular importance was the obesity marker (BMI), since WC (for central obesity) indicated no significant difference between groups. It is known that adipose tissue is heterogeneous in nature, and that increased perivascular fat deposits interact with endothelial cells via an autocrine/paracrine mechanism, thus promoting vascular dysfunction.[32] A physically active lifestyle (PAI) improves endothelial function, related to hypertension,[33] whereas cigarette smoking acts synergistically with hypertension and hypercholesterolemia, contributing towards coronary heart disease.[34] Since menopausal status and age does influence the cardiovascular risk factor profile of women, such as blood pressure,[35] this factor was corrected by using a narrowed down age group within 35 to 50 years.

Data indicated that for the whole group, both urban women and men experienced significant increases in VEGF-A levels, although VEGF-A levels in the whole group indicated lower detection values compared to previous studies done in literature (90 – 100 pg/mL).[14,29,36,37] The whole groups' values ranged between 0.00 and 53.03 pg/mL VEGF-A. The whole groups' Ang-2 levels ranged between 0.67 and 6.88 ng/mL and was considered to indicate angiogenic activity according to some literature.[29,36] In a study by Nadar *et al.*,[16] both angiogenic factors were expected to be increased in hypertensive subjects.

If the values and the mechanism for angiogenesis, as proposed by Nadar *et al.*,[16] are taken into account, the low levels of VEGF-A in Africans could speculatively determine the outcome of Ang-2 stimulation and possibly change the characteristics of Ang-2 from anti-angiogenic to pro-angiogenic. This will theoretically result in destabilisation of the

vessels and vascular dysfunction as is seen in urbanised hypertensive men. It may in turn suggest that higher Ang-2 levels are involved during down regulation of the Tie-2 receptor and so explain the lower VEGF-A levels in African subjects.

It was found that Ang-2 levels, even though mean values were above normal,[16] were lower in urbanised compared to rural men ($p = 0.05$). This comparative finding was altogether new to current literature since angiogenic factors were considered to be raised in states where vascular dysfunction was expected.[17,18]

Our first hypothesis was that angiogenic factors may be increased in urbanised compared to rural black Africans. Ang-2 did not conform to this hypothesis or the second, which stated that angiogenic factors may be increased in hypertensives. Such a disparity between VEGF-A and Ang-2 levels in African people is unknown to current literature, where the balance between pro-angiogenic factor levels may be an important contributor in their synergistic effect on blood vessels, although VEGF-A is considered to be more potent.[13,19]

When, upon deeper investigation, rural and urban data separating normotensive and hypertensive groups were compared, results indicated that the higher VEGF-A levels of urban participants occurred mainly within the normotensive group. It is also the normotensive urban women that experienced increased DBP levels. Urban men who presented with the highest incidence of hypertension and increased angiogenic factor (VEGF-A) levels in the whole group, evidently indicated that these increases do not necessarily occur within the hypertensive group. We, therefore, partially accept our first

hypothesis in that it only applies to whole group statistics comparing rural and urban communities.

Testing our second hypothesis, which stated that hypertensive African people present with higher levels of angiogenic factors, all data were compared between normotensive and hypertensive individuals. Apart from the strong corresponding nature of increased SBP, DBP and PWV values that occurred mainly in rural and urban hypertensive women and men, indicating increased arterial stiffness,[30] sympathetic activity and possible vascular damage,[31] no significant differences between angiogenic factor levels were detected after adjusting for confounders. This finding defied the proposed mechanism, that urban subjects were expected to develop vascular dysfunction through a state of increased BP and angiogenic factors.

When the strength of association between angiogenic factor levels and the environment (rural and urban areas) were evaluated, only weak negative associations existed for rural women and men considering SBP data. After adjustment for confounders, it was mainly the DBP that consistently indicated a statistically stronger negative correlation with Ang-2 levels in rural women, meaning that when BP increased in rural women, Ang-2 levels decreased. The result may be angiogenesis according to our proposed mechanism.

Results, therefore, did and also did not agree with currently held views that angiogenic factor levels are increased in states of chronically raised BP. Chong *et al.*[36] found that after comparison of angiogenic markers (Ang-1, Ang-2, tie-2 and VEGF) with hypertension and smoking, none of the markers were significantly influenced. The incidence of hypertension which was thought to be contextually (environmentally)

influenced was also previously disproved[3] even though BP is supposed to be related to angiogenic factor levels.[16] The second hypothesis is therefore rejected.

We conclude firstly, that urbanised Africans present with increased levels of the angiogenic factor VEGF-A in both urbanised men and women and the first hypothesis is partially accepted since Ang-2 did not conform to the same expectations. Concerning the second hypothesis which states that hypertensive African people present with higher levels of both angiogenic factors compared to rural Africans, is completely rejected since comparison after adjustments indicated no significant differences. We, therefore, propose that urbanisation of Africans plays a greater role in increasing angiogenic factor (VEGF-A) levels than a state of raised BP or hypertension. The mechanism whereby Ang-2 interrelates with VEGF-A in African people is questionable. We could speculate that the low levels of VEGF-A can change the characteristics of Ang-2 from anti-angiogenic to pro-angiogenic, resulting in vascular dysfunction as is seen in the urbanised hypertensive men. Higher levels of Ang-2 could involve down regulation of the Tie-2 receptor, explaining the lower levels of VEGF-A. Clearly more investigation is needed.

This study was subject to limitations. One fundamental limitation is that data for the angiogenic factor VEGF-A in black Africans were much lower compared to data generated elsewhere.[29] We would also recommend that the study group size be increased to improve statistical power, even though smaller groups have been used in literature.[36] Concerning true BP measurement accuracy for BP categorical designation, it is also recommended that ambulatory BP monitoring be performed for future community surveys.[38] The inflammatory as well as HIV/AIDS status, which may putatively influence BP results, could also be deemed necessary for future studies. Furthermore, our results

were based on a selection done by a medical doctor, and cannot therefore represent the general population.

Table 5 indicates the contributions of this study to current knowledge on this topic

Current knowledge on angiogenesis and cardiovascular dysfunction in urbanised Africans

1. Hypertension, as a coronary heart disease and cardiovascular disease risk factor,[2] is an established phenomenon during urbanisation of Africans;[8,12,39]
2. Coronary heart disease risk is linked to angiogenesis by the up regulation of angiogenic factors (e.g. VEGF-A)[14] (known to endothelial dysfunction) causing vascular structural remodelling just as hypertension does,[17] eventually leading to target organ damage.[40]

What this study adds

1. Angiogenic factor (VEGF-A and Ang-2) level data on Africans;
2. The angiogenic factor VEGF-A is elevated in urbanised men and women and plays a more prominent role than Ang-2;
3. Hypertension does, comparatively, indicate increased arterial stiffness,[31] but may not be a possible indicator of arterial dysfunction as indicated by angiogenic markers. No relation exists between angiogenic data and the state of hypertension in Africans.

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CHAPTER 4

CLOSING CHAPTER:

General findings and conclusions

INTRODUCTION

Chapter 4 is a closing chapter that unifies the findings as discussed in the previous chapter. A summary is provided with deductions from which conclusions are formulated and a new viewpoint established, whilst referring to previous work on the same subject.

SUMMARY OF MAIN FINDINGS

The title and main area of research of this study and article reported in this study is:

Angiogenesis and cardiovascular dysfunction in urbanised Africans: The PURE study

The aims of this study were firstly, to measure the differences in the levels of the angiogenic factors: vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2 (Ang-2) in urbanised and rural black Africans (hereafter referred to as Africans) and to compare the levels between the two communities. Our second aim was to determine whether increased levels of VEGF-A and Ang-2 are related to the state of hypertension in Africans. Therefore, the first hypothesis stated that increased levels of angiogenic factors are prevalent in urbanised compared to rural Africans and subsequently the second hypothesis stated that hypertensive African people present with higher levels of angiogenic factors than normotensive Africans.

Although VEGF-A levels were low in all Africans, a significantly increased level of VEGF-A was found in urbanised compared to rural Africans, even after adjusting for confounders (age, BMI, PAI and smoking). The first hypothesis was therefore partially accepted even though Ang-2 levels indicated a possible inverted relationship. When the strength of relationships was measured between the angiogenic factors and cardiovascular parameters, while adjusting for the same confounders, only a weak negative relationship existed between Ang-2 and blood pressure (BP) in rural African women. To establish if angiogenic factor levels were in fact raised in hypertensive gender and community groups, a comparison adjusting for covariates was performed, but no significant differences were indicated. No association could therefore be established within either the rural or urban groups, whereupon the second hypothesis was rejected.

COMPARISON OF FINDINGS WITH THE LITERATURE

Comparing the results of this study holistically with previous studies done in the same and other ethnic groups, regarding the same issues, produces findings that are / are not supported by the literature, whereas some may be altogether new contributions to literature. Findings that agree with the literature are that urban African men are more hypertensive[1] than rural African men (according to our study) and that the angiogenic factor VEGF-A, as a measure of vascular dysfunction, was increased in a state of increased peripheral vascular reactivity.[2,3]

Findings contradicting the literature include results that indicate that Ang-2 level results indicated altogether reversed relationships with BP and VEGF-A data.[4] This result alone contradicts most, but not all[5] of our understanding of the mechanism regarding BP and angiogenic factors thus far.[6] This finding could support the mechanism of down regulation of the Ang-2 – Tie-2 receptor complex, resulting in lower levels of VEGF-A.

Findings in the literature, not evident in the study, included findings by the study that increased BP in the state of hypertension is not responsible for angiogenic activity, although it indirectly supports previous studies on coronary heart failure (CHF) where hypertension was a causative component.

A finding in this study that is new to current literature is that both diastolic blood pressure (DBP) and VEGF-A simultaneously were significantly increased in normotensive urban women (Table 2). This result alone may indicate that within the BP categories (normotensive, hypertensive) a different mechanism applies to regulate angiogenic factor levels and that this may only be applicable to the DBP. The findings of higher vascular reactivity in urbanised Africans could imply sympathomedullary activity in association with angiogenesis.[7]

CHANCE AND CONFOUNDING

When reflecting on the acquisition of results, it is important to critically evaluate the methods of this study and point out possible shortcomings that may have influenced results. In this way, conclusions may be evaluated much more objectively.

It may be that research on the angiogenic factor levels of African rural and urban people has never been done before. The results, in fact, only represent subjects taken from the North-West district of South Africa and therefore are not representative of all the sub-Saharan African communities.

Apparently, healthy subjects were also carefully selected from a larger group within the greater PURE study according to inclusion criteria and as a sub-study may not even represent the whole of the PURE study group.

Cardiovascular parameters were measured according to the 'in office' method and therefore do not represent true BP values for subjects, as may be the case when ambulatory BP values are used.

CONFOUNDERS

One of the main confounding factors of this study was the inclusion criterion: "apparently healthy subjects", which is not a certainty, although participants were screened by a

certified medical doctor. Other confounders not adjusted for, such as age groups other than that selected (35 to 50 years), alcohol abuse, drug abuse, HIV/AIDS status, waste circumference (WC) and socio-economic status, could have influenced results.

DISCUSSION OF MAIN FINDINGS

According to sources[8,9] and our results, urbanised people and especially men[1] generally present with elevated BP levels that may be due to increased sympathetic activity in the peripheral vasculature.[2,3] Raised BP may, in fact, cause vascular dysfunction by several mechanisms,[6] although it is not always proven that these components of CHF (e.g. hypertension and smoking) affect angiogenic factor levels.[5] In this study, subject characteristics indicated that the general BP of urbanised men was significantly increased, which was also the case in African American men,[1] but because no comparison between rural and urban communities concerning angiogenic factor levels has been done previously, these results could not be compared.

It is known that BP disparities exist between ethnic groups.[1,10,11] The results of this study did, however, indicate dissimilar results from findings in the literature. It was expected that angiogenic factors would be elevated in states of hypertension, because BP elevations cause vascular dysfunction.

Elevated VEGF-A level results of the PURE study subjects indicated that urban Africans may present with higher vascular angiogenic activity,[12] which is functionally only the

case during a state of cardiovascular dysfunction. The VEGF-A levels, although elevated, were much lower than for control subject levels found in the literature (VEGF ave = 500 pg/mL).[12,13]

It is, however, known that VEGF-A and Ang-2 as angiogenic factors correlate with each other[4] and work synergistically in promoting angiogenesis in cardiovascular dysfunctional states. This was not the case according to results, since Ang-2 indicated a reversed relationship to BP in rural African women only and no increase in angiogenic markers could be established for hypertensives. Values for PWV substantiate that rural women did indeed indicate possible cardiovascular dysfunction, as measurements were elevated in hypertensive rural women, which may explain the negatively correlating Ang-2 factor levels.

This study may contribute to current literature by elucidating the fact that the two angiogenic factors (VEGF-A and Ang-2) do not correlate with each other in Africans as they have in other studies.[4] This finding contradicts previous studies[14] that suggested the synergistic role of angiogenic factors in states of angiogenesis. VEGF-A and Ang-2 angiogenic factor levels were also measured for the first time in Africans, which proved to be different from data for other ethnic groups.[4,5] Hypertension could not be established as a contributing factor for increasing plasma VEGF-A levels in Africans. Because Ang-2 increased inversely to increases in DBP, it is possible that another mechanism is at work, promoting angiogenesis in urban African communities.

If the values and the mechanism for angiogenesis, as proposed by Nadar *et al.*[13] are taken into account, the low levels of VEGF-A in Africans could speculatively determine the outcome of Ang-2 stimulation and possibly change the characteristics of Ang-2 from anti-angiogenic to pro-angiogenic. This will theoretically result in destabilisation of the vessels and vascular dysfunction as is seen in urbanised hypertensive men. It may, in turn, suggest that higher Ang-2 levels are involved during down regulation of the Tie-2 receptor and so explain the lower VEGF-A levels in African subjects.

CONCLUSION

Pathophysiologic angiogenesis, as indicated by elevated angiogenic plasma levels, has been shown to occur during states of increased BP and hypertension, where hypertension may also be caused by urbanisation. Although elevated VEGF-A levels (though sub-normal) occurred in urbanised compared to rural black Africans, levels were not significantly increased in hypertensive subjects, so that hypertension could be considered a mechanism for vascular dysfunction in urbanised Africans.

RECOMMENDATIONS FOR FUTURE STUDIES

Possible shortcomings of this study indicated possible recommendations for future studies:

- The first hypothesis was partly accepted on the finding that VEGF-A levels were increased in urban Africans. This group only included the age group of 35 to 50 years and cannot be generalised to all age groups.
- We would also recommend that the study group size be increased to improve statistical power, even though smaller groups have been used in literature.[5]
- Concerning true BP measurement accuracy for BP categorical designation, it is also recommended that ambulatory BP monitoring be performed for future community surveys.[15]
- Furthermore, our results were based on a selection done by a medical doctor of African participants in the North-West province of South Africa, and results cannot therefore be generalised to represent the entire African population.
- Environmental influence as opposed to blood pressure *per se* seems to influence angiogenesis to a greater extent. For this reason, adaptive behaviour or coping mechanisms during urbanisation (causative of higher sympatho vascular discharge) should rather be the focus of similar investigations in future.

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