Brain derived neurotrophic factor and structural vascular disease in black Africans: the SABPA study

A J Smith

20798881

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Supervisor: Prof L Malan

Co-supervisor: Prof NT Malan

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OPSOMMING

TITEL: Brein-afgeleide Neurotrofe Faktor (BANF) en Strukturele Vaskulêre Siekte in Swart Afrikaaners: Die SABPA studie.

Motivering

Die brein-afgeleide neurotrofe faktor (BANF) is ’n proteïene kompleks wat hoofsaaklik deur die sentrale senuweestelsel gesinteseer en afgeskei word. Die senuweestelsel is normaalweg betrokke by die onderhoud van neurone. Navorsing dui daarop dat BANF verband hou met verskeie neurologiese en psigologiese siektes, terwyl onlangse bewyse getoon het dat hierdie neurotrofe ook in die periferie van die liggaam aktief is. Trouens, die spesifieke rol en werking van BANF in die kardiovaskulêre sisteem, veral in die geval van die etniese gemeenskappe van Afrika, moet egter nog bepaal word. Die kardiovaskulêre gesondheidsprofiel van swart Suid-Afrikaners is veral ’n groot bekommernis aangesien navorsing getoon het dat hierdie groep aan verskeie kardiovaskulêre risikofaktore ly wat orgaanbeskadiging tot gevolg kan hê. Sub-kliniese aterosklerose of strukturele endoteel wanfunksie dra by tot die toeneemende morbiditeit asook sterftes in die wêreld. Tot dusver is daar egter geen studies aangaande die verband tussen BANF en strukturele endoteel wanfunksie uitgevoer op die etniese gemeenskappe van Afrika nie.

Doelstellings

Die doel van hierdie studie was om te bepaal of BANF geassosieer kan word met veranderinge in ambulatoriese bloeddruk, en of daar ’n verband bestaan tussen BANF en strukturele endoteel wanfunksie in die swart mans en vrouens, deur die die karotis se deursnee wandoppervlak bereken en die verhouding tussen albumien en kreatinien (ACR), te bepaal .

Metode

Die studie het uit 172 swart onderwysers (82 mans en 90 vrouens) wat in die Kenneth Kaunda distrik in die Noordwes Provinsie, Suid Afrika werksaam is, bestaan. ’n Meditech CE120 CardioTens® apparaat is gebruik om hulle ambulatoriese bloeddrukmetings te verkry. Bloeddruklesings is elke 30 min gedurende die dag en elke 60 min tydens die nag geneem. Antropometriese metings is in drievoud uitgevoer en volgens gestandardiseerde prosedures verskaf deur geregistreerde vlak II antropometriste. ’n Hoë-resolusie ultraklank skandering met karotis intima-media dikte (KIMD) beelde, bestaande uit ten minste twee opitimale hoeke van die linker en regter gemeenskaplike karotis slagare, is verkry met behulp van ’n SonoSite Micromaxx ultraklank stelsel. Die lumen deursnee tussen die naby- en verafgeleë wande van die lumen-intima koppelvlak sowel as die gemiddeldes van beide die linker en regter gemeenskaplike karotis slagaar is bereken. Gevolglik is die karotis se deursnee wandoppervlak bereken.

Deelnemers wat tydens hul nagrus gevas het, het bloed- en urinemonsters voorsien om die BDNF serumvlakke en ander metabolise merkers, soos byvoorbeeld, chroniese hiperglisemie (HbA1c) en gamma glutamiel transferase (GGT) te bepaal. Albumien- and kreatinienvlakke in urine is bepaal met behulp van ’n Unicel DXC 800 analiseerder van Beckman and Coulter (Duitsland) en is weergegee as ’n verhouding tussen albumien en kreatinien (ACR). BANF mediaan split x geslag interaksie se effekte is bepaal en het die stratifikase van mans en vrouens in lae en hoë ($\leq$ / > 1.37 ng/ml) BANF groepe regverdig.

Resultate en Gevolgtrekking

Oor die algemeen was manlike deelnemers oorgewig (BMI 25-30kg/m2) en het hulle 'n beduidende hoeveelheid alkoholverbruik getoon. Die mans het ‘n meer riskante kardiometaboliese profile getoon deurdat talle metabolise veranderlikes hoër was as aanvaarde afsnyvlakke (European Society of Hypertension). Die manlike populasie het verhoogde vlakke

van akute en chroniese glukose (HbA1c) getoon, wat aanduidend kan wees op ‘n pre-diabetiese toestand, asook ‘n verspreide lipiedprofiel met verlaagte vlakke HdL en verhoogte vlakke trigliseriede. Algehele BANF vlakke was laer as verwysings waardes (6.97 – 42.6 ng/ml). Mans het laer gemiddelde vlakke BANF getoon met ambulatoriese bloeddruk vlakke, wat die normaal waardes oorskry (ambulatoriese SBD > 130mmHg; DBD > 80mmHg). Mans was meer hipertensief wanneer hulle met die vroulike populasie vergelyk word.

Rakende die strukturele endoteel wanfunksie, was die gemiddelde ACR vlak van die manlike populasie hoër as normale waardes (<3.5mg/mmol).

Die vroulike populasie het ‘n algehele obese profile getoon met ‘n lae graadse inflammasie (CRP, 12.27 ± 11.67mg/l).

‘n ANCOVA interaksie op die hoof effekte (BANF median split x Geslag), het ‘n betekenisvolle interaksie getoon vir KIMDf [F (1.164); 3.99, p=0.05] en cholesterol [F (1.164); 4.12, p=0.05]. Gevolglik was die median split metode gebruik om die totale populasie te stratifiseer in geslagsgroepe met lae (≤ 1.37 ng/ml) en hoër BANF vlakke (> 1.37 ng/ml)

Die lae BANF mans het hoër vlakke cholesterol getoon in vergelyking met die hoë BANF mans, onafhanklik van ouderdom en BMI. Slegs die vroue in die lae BANF groep het betekinisvolle hoër vlakke vir strukturele endoteel wanfunksie getoon, (p< 0.05) in vergelyking met dieselfde geslag in die hoë BANF groep.

Ten slotte aanvaar ons die gestelde hipotese dat vaskulêre hermodulering van die karotis arterie geassosieer is met lae BANF vlakke. Hierdie verskynsel mag moontlik impliseer dat verswakte en moontlike af-regulerende BANF vlakke, dien as ‘n kompensatoriese meganisme, vir die hoë bloeddrukvlakke. Metaboliese risiko en hipertrofiese hermodulering was duidelik in die groep met die hoër BANF vlak, wat mag aandui dat daar moontlik, verskeie onderliggende meganismes bestaan, aangaande die versteurde neurotrofien gesondheid in mans en vroue.

Bevindinge dui op die impak van sentrale neurale regulering op die kardiovaskulêre sisteem, wat mag bydra tot die kardiometaboliese risiko in Afrikaners.

Sleutelwoorde: Swart Afrikaners, BANF, bloeddruk, karotis intima-media dikte, HbA1c

SUMMARY

TITLE: Brain-derived Neurotrophic Factor (BDNF) and Structural Vascular Disease in Black Africans: The SABPA Study

Motivation

Brain-derived neurotrophic factor (BDNF) is a protein complex, synthesised and secreted mainly by the central nervous system and is involved in neuronal maintenance. Research suggests that BDNF is implicated in various neurological and psychiatric diseases, while recent evidence suggests a role for the neurotrophin on the periphery as well. Indeed, the specific functional role of BDNF and its action mechanism in the cardiovascular system, especially in that of Africans, is yet to be determined. The cardiovascular health profile of black South Africans is a major concern as research has shown that this group suffers from an array of cardiovascular risk factors that may result in organ damage. Sub-clinical atherosclerosis or structural endothelial dysfunction contributes to ever-increasing morbidity and mortality in the world. However, no studies regarding the associations between BDNF and structural vascular disease have been undertaken relating to black African participants.

Objectives

The objective of this study was to determine whether BDNF is associated with changes in ambulatory blood pressure (BP) and whether a relationship between BDNF and structural endothelial dysfunction exists in black African male and female participants, determined by cross sectional wall area (CSWA) and albumin:creatinine ratio (ACR).

Methodology

The study included 172 black African teachers (82 males and 90 females) who were employed by the Kenneth Kaunda Education district of the North-West Province, South Africa. Ambulatory blood pressure recordings were obtained with the use of a Meditech CE120 CardioTens ® apparatus. Blood pressure readings were measured at 30 min intervals during the day and 60 min intervals during the night. Anthropometric measurements were performed in triplicate by registered level II anthropometrists according to standardised procedures. A high-resolution ultrasound scan with carotid intima-media thickness (CIMT) images from at least two optimal angles of the left and right common carotid artery were obtained using a SonoSite Micromaxx ultrasound system. The lumen diameter between the near and far wall of the lumen-intima interface and the averages of both the left and right common carotid arteries were calculated. Subsequently, the carotid cross-sectional wall area (CSWA) was calculated. Participants, who fasted overnight, provided eight-hour blood and urine samples to determine serum BDNF and metabolic markers, for example, hyperglycaemia (HbA1c) and gamma glutamyl transferase (GGT). Urinary albumin and creatinine levels were determined by means of a turbidimetric method with the use of a Unicel DXC 800 analyser from Beckman and Coulter (Germany) and expressed as a ratio between albumin and creatinine (ACR). BDNF median split x Gender interaction effects for structural ED justified stratification of BDNF into low and high ($\leq$ / > 1.37 ng/ml) gender groups.

Results and Conclusion

On average, male participants were overweight (BMI 25-30kg/m^2) and abused more alcohol.[21] African men revealed a vulnerable cardiometabolic profile with values exceeding cut–points (European Society of Hypertension). These men demonstrated increased acute and chronic glucose (HbA1c) levels indicating a pre-diabetic state; as well as a disturbed lipid profile with lower HdL and increased triglycerides. Overall BDNF levels were lower than reference ranges (6.97 – 42.6 ng/ml). The men revealed mean lower BDNF levels, ambulatory BP values exceeding guideline cut-points (ambulatory SBP > 130mmHg; DBP > 80mmHg) as well as a hypertensive state compared to their female counterparts. Pertaining to structural endothelial dysfunction, the mean ACR value in men exceeded normal laboratory values
(< 3.5mg/mmol). The African women displayed an obese state with low grade inflammation (CRP, 12.27 ± 11.67mg/l).

A single two-way ANCOVA interaction on main effects (BDNF median split x Gender) demonstrated significant interaction for CIMTf [F (1,164); 3.99, p=0.05] and cholesterol [F (1,164); 4.12, p=0.05]. Therefore, a median split approach was followed which stratified gender groups into lower (≤ 1.37 ng/ml) and higher BDNF levels (>1.37 ng/ml).

The low BDNF men revealed higher cholesterol than the high BDNF group, independent of BMI and age. Only the low BDNF women indicated significantly higher values for structural vascular markers ($p< 0.05$) than the high BDNF female group.

In conclusion, we accept our hypothesis, as hypertrophic remodelling of the carotid artery was associated with lower BDNF levels. This may imply attenuated or possibly down-regulated BDNF levels acting as a compensatory mechanism for the mean higher BP levels. In women, metabolic risk and hypertrophic remodelling were evident within higher circulating levels of BDNF, underpinning different underlying mechanisms for impaired neurotrophin health in men

and women. Novel findings of BDNF revealed the impact of central neural regulation on the circulatory system, which may contribute to cardiometabolic risk in Africans.

Keywords: African, BDNF, blood pressure, carotid intima-media thickness, HbA1c

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LIST OF ABBREVIATIONS

ABP	Ambulatory blood pressure
ACR	Albumin:Creatinine ratio
AD	Alzheimer's disease
ANCOVA	Analysis of covariance
ANS	Autonomic nervous system
Aβ	Amyloid Beta
BDNF	Brain derived neurotrophic factor
BMI	Body mass index
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CCA	Common carotid artery
CIMT	Carotid intima media thickness
CNS	Central nervous system
CREB	cAMP Response element-binding protein
CRP	C-reactive protein
CSWA	Cross sectional wall area
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ED	Endothelial dysfunction
eNOS	Endothelial nitric oxide synthase
ESH	European Society of Hypertension
FSL	Flinders sensitive line
GGT	Gamma-glutamyl transferase
GSH	Glutathione
GSSG	Oxidised glutathione

HbA1c	Glycated haemoglobin
HdL	High density lipoprotein
HIV	Human immunodeficiency virus
Hs-CRP	High sensitivity C-reactive protein
ICA	Internal carotid artery
kcal	Kilo-calories
kDa	Kilo-dalton
mmHg	Millimetre mercury
mmol/L	Millimolar per litre
NCD	Non-communicable diseases
NGF	Nerve growth factor
NO	Nitric oxide
NS	Non-significant
NT-3	Neurotrophin-3
p75-NTR	p75 neurotrophin receptor
pg/ml	Pictogram per litre
PI3K	phosphatidylinositol-3-kinase
PLC-γ	phospholipase C
SABPA	Sympathetic activity and ambulatory blood pressure in Africans
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SNS	Sympathetic nervous system
trk	Tyrosine kinase receptor
WHO	World Health Organisation
WT	Wild type

α	Alpha
β	Beta

PREFACE

The style of this dissertation was structured according to the rules of the North-West University. The article format was used for the completion of this dissertation. This dissertation is written in English, while an Afrikaans summary of the article has been included at the beginning of the dissertation, as required by the institution. This is a format approved and recommended by the North-West University, consisting basically of a manuscript, which has been submitted to a peer-reviewed journal. The manuscript is accompanied by an in-depth literature review and an interpretation of the results. Appropriate references are presented at the end of each chapter. The chosen journal for this project is the *Journal of Human Hypertension.*

OUTLINE OF THE STUDY

The layout of this dissertation is as follow:

Chapter 1	Comprises the introductory chapter containing an introduction and literature study.
Chapter 2	Consists of the article as submitted, according to journal guidelines, to the *Journal of Human Hypertension,* titled: *Attenuated Brain-derived Neurotrophic Factor and hypertrophic remodelling: The SABPA Study.*
Chapter 3	Contains the summary of the main findings.

DECLARATION BY THE AUTHORS

The following is a statement by the co-authors confirming their individual roles and responsibilities in this study. They hereby give permission for the manuscript to form part of the dissertation.

Mr AJ Smith

Responsible for the research of current literature, performing statistical analyses, and processing of the SABPA data, designing and planning of the manuscript, interpretation and writing of the manuscript.

Prof L Malan: *Supervisor*

Principal Investigator of the SABPA study, supervised the design of the study, collection and statistical analysis of data, planning of the manuscript, and the construction of tables and figures, as well as the recommendations regarding the writing and construction of the research article and the script.

Prof NT Malan (*Co-supervisor*), Prof BH Harvey and Dr AS Uys: *Assistant supervisors*

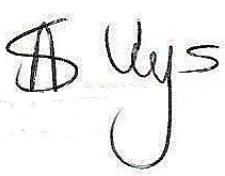
Provided recommendations regarding the statistical analysis of the data, the construction of tables, interpretation of the data, and the writing and construction of the research article and script.

Hereby, I declare that I approve of the above-mentioned manuscript, and that my role in this study, as indicated above represents my authentic contribution and that I give consent for this Mini-Dissertation to be submitted in partial fulfillment of the requirements for the degree *Magister* in Physiology, for Mr Alwyn Johannes Smith, at the Potchefstroom Campus of the North-West University

Prof L. Malan

Prof N.T. Malan

Prof B.H. Harvey



Dr. L. Uys

CHAPTER ONE

INTRODUCTION AND LITERATURE STUDY

LITERATURE OVERVIEW

Introduction

The prevalence of cardiovascular disease (CVD) in most developing countries is a major health concern, contributing to approximately 15 million deaths per year [1]. In 1999, Pearson [2] stated that "cardiovascular disease is already the leading cause of death, not only in developing countries but in developed countries as well", a statement not supported by the World Health Report in the year 2000 [3].

However, a decade later the World Health Report (2011) by the World Health Organisation (WHO) presented statistics which indicated that of the 57 million deaths recorded in 2008, 36 million (63 %) deaths were due to non-communicable diseases (NCD), principally consisting of cardiovascular diseases, diabetes and cancers [4]. Moreover, at least 48 % of the deaths caused by NCD were cardiovascular diseases. The WHO also predicted a 15-20 % increase in CVD globally between the years 2010 and 2020, with Africa and Asia experiencing the greatest impact.

According to Steyn [5], approximately 195 people in South Africa die per day due to CVD, between 1997 and 2004. Very recent research has projected that deaths relating to CVD will increase among young age groups, with deaths projected to increase by over 40 % by the year 2030 [4, 5]. It is thought that this rising number is indirectly influenced by several risk factors pertaining to CVD. According to the World Heart Federation, these factors include hypertension, abnormal blood lipid levels, diabetes, physical inactivity and the use of tobacco. Other factors include age, gender and ethnicity [6].

The prevalence of cardiovascular disease in sub-Saharan Africa is increasing rapidly, and the rate of increase is projected to be higher in urban than in rural populations [7, 8]. In 2005, Opie *et al.* [9] concluded that black urban Africans are at greater risk for developing cardiovascular

related symptoms such as hypertension. In a study conducted by Seedat *et al.* [10], hypertension was found to be the most common risk factor in black South Africans, however this statement was limited to the Durban population. Seedat *et al.* [10] also showed that the prevalence of hypertension is higher in urban Zulus when compared to the same rural group. In 2012, Malan *et al.* [11] demonstrated that in a cohort of African men, diastolic blood pressure (DBP) and chronic hyperglycaemia levels were higher when compared to Caucasian men. It is evident in present data and research that the rise in cardiovascular mortality and morbidity will have detrimental effects on the world population, and that certain races may be more susceptible to death caused by CVD. It is therefore important to continue research into the possible problems and solutions regarding cardiovascular health, especially in developing countries.

Rothman *et al.* [12] proposed that brain derived neurotrophic factor (BDNF) plays a major role in the mediation of adaptive responses of the cardiovascular-, nervous-, and energy-regulating systems of the body. This is supported by Ejiri *et al.* [13] who demonstrated in 2005 that BDNF is up-regulated in subjects suffering from unstable angina pectoris. BDNF was also classified as being an essential target derived survival factor and a subsequent signalling molecule involved in the survival of arterial baroreceptors during vascular innervation [14]. It is clear that BDNF might play an important role in cardiovascular physiology and may be a platform for future research and drug development. Therefore, the current study is essential as no data exists on BDNF and CVD in Sub-Sahara Africans. BDNF and its relationship with cardiovascular risk markers will now be discussed.

1.1.1. BDNF structure and cellular response

The BDNF is a member of the family of proteins called neurotrophins, which mainly function as growth factors and are able to promote the survival of neurons. BDNF was discovered in 1982 by Barde *et al.*, [15] using purified extracts of pig brains. Neurotrophins are polypeptides that regulate the plasticity and survival of developing neurons in the central and peripheral nervous system [16]. The BDNF protein consists of 247 amino acid residues, encoded from chromosome 11, and shares about 50 % of its amino acid identity with other neurotrophic factors such as nerve growth factor (NGF) and neurotrophin-3 (NT-3) [17]. The BDNF protein is synthesised as a 32 kDa pre-pro-BDNF molecule. This molecule undergoes splicing to yield pro-BDNF which in turn gets cleaved to mature 14kDa BDNF, either by intra- or extracellular enzymes [18].

The BDNF functions are mediated by two types of receptors: 1) a high affinity tyrosine receptor kinase (Trk) and 2) a low affinity pan-neurotrophin receptor called p75 [19]. There are various subtypes of the Trk receptor; however the specific receptor for BDNF is TrkB to which it binds with high affinity [20, 21]. The binding of BDNF to the TrkB receptor activates an auto-phosphorylation of multiple tyrosine residues that creates specific binding sites for intracellular proteins. These proteins include phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PLC-ɣ). Activation of these intracellular proteins leads to signalling cascades such as the phosphorylation of cAMP response element binding protein (CREB) [22, 23] which elicits distinct cellular responses. CREB is an intracellular transcriptional factor that regulates the transcription of various downstream genes responsible for enhancing the survival and differentiation of cells [24].

In addition to binding to TrkB, BDNF also binds to the p75 receptor designated p75NTR, the binding of which leads to the activation of various intracellular signalling pathways, which includes Jun-kinase and sphingo-myelin hydrolysis [25]. One action of p75NTR-BDNF

interaction is the ability to initiate programmed cell death or apoptosis [25, 26]. Although the proposed intracellular mechanism after BDNF-receptor interaction is very complex, a simplified pathway is depicted in Figure 1, adapted from Sossin *et al.* and Chunha *et al.* [27, 21].

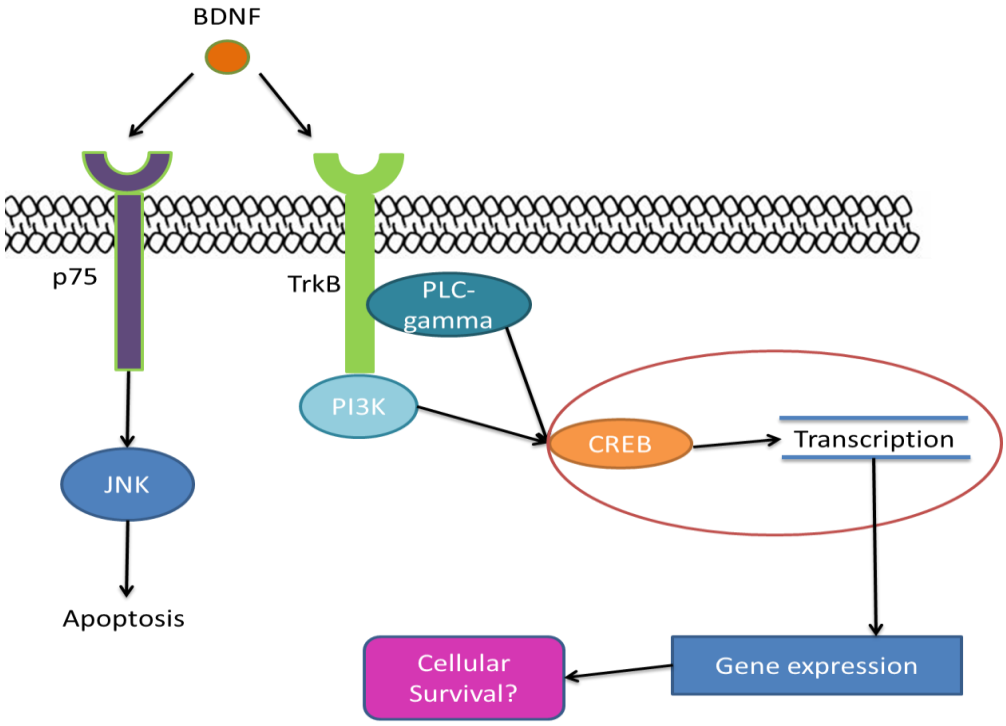


Figure 1.1 The proposed intracellular signalling mechanism of receptor-bound BDNF. Adapted from Sossin *et al.* and Chunha *et al.* [27, 21]

1.1.2. BDNF and possible determinants

BDNF, like many other biological variables, is susceptible to change brought on by many independent factors that might possess the ability to inhibit or enhance the secretion, availability and the functioning of the protein. BDNF and its receptors are not only synthesised or derived from the brain as the name implies, but are also found in other non-neuronal cell types and/or tissues. It is widely distributed in the brain in specific cell types including astrocytes [28], neurons [29], and microglia [28]. Other peripheral non-neuronal types of tissues/cells that mediate BDNF synthesis and express the trkB receptor, include the developing heart endothelial

cells [16], atherosclerotic vessels, vascular smooth muscle cells [13] and macrophages [30]. The latter observations has contributed to the belief that BDNF may be implicated in cardiovascular diseases.

It is important to note that the measurement of BDNF levels in blood are affected by various determinants such as sampling method, socio-demographics, lifestyle indicators and certain diseases [31], as discussed below.

1.1.2.1. Sampling method

In 1995, Rosenfeld *et al.* [32] produced the first evidence for the presence of BDNF in human plasma and serum. Interestingly, the average serum BDNF levels were more than 100 times higher than the levels recorded in human plasma [33]. A possible reason for this difference was proposed by Fujimara *et al.* [34] in 2002 who postulated that the degranulation of platelets during the clotting process is responsible for this difference. Indeed, human platelets contain a large amount of BDNF [34, 35, and 36]. Fujimara *et al.* [34] also demonstrated that the amount of BDNF in washed platelets is nearly identical to the amount of BDNF in serum. We may therefore hypothesise that the difference between plasma- and serum BDNF levels may reflect the amount of BDNF stored in circulating platelets.

BDNF sample handling has to comply with standardised procedures including the storage of whole blood samples at -20 °C which has not been associated with any significant changes over a period of 5 years [37]. However, long term storage (6-10months) of serum has been associated with a significant decrease in serum BDNF concentration [37].

1.1.2.2. BDNF and socio-demographics

Serum BDNF appears to be negatively correlated with age [38]. However, mixed results have been published in this regard. Age correlated significantly with BDNF (p=0.048) in a study conducted by Lang *et al.*, [39], although no gender differences were observed by the author in a study with 118 volunteers (64 Female and 54 Male) between the ages of 29 and 55. Ziegenhorn *et al.* [40] discovered that a negative correlation exists between age and BDNF in a group of 465 healthy subjects between the ages of 70 and103 years. Supporting this is the finding of Golden *et al*. [41] who showed that serum BDNF tends to decrease with age in both genders and that BDNF levels are higher in females when compared to male subjects of the same race. In a study conducted on old age individuals, women with a lower BDNF indicated a higher all-cause mortality risk [42]. Gender influences on BDNF levels have also been reported by Bus *et al.* [31] where an age-related increase in females was found. Another study carried out by Bus *et al.* a year later found that changes in serum BDNF were less pronounced in men [43].

1.1.2.3. Lifestyle

Pertaining to lifestyle, Bus *et al.* showed attenuated BDNF levels and associated changes in subjects classified as binge drinkers [31]. BDNF levels were lower in subjects who were alcohol dependent [44], although Elfving *et al.* [45] found no relationship between BDNF and alcohol consumption. Similarly Janak *et al.* [46] reported that decreases in BDNF are associated with an increase in alcohol consumption. Other lifestyle indicators such as smoking and living in an urban area, are associated with an increase in BDNF levels [31]. However, to the contrary, the results of Kim *et al.* [47] found that, when comparing smokers to non-smokers, the smoking group had a significantly lower level of BDNF and that the level of BDNF increased after the

cessation of smoking. Subjects living in a rural setting exhibited lower levels of BDNF when compared to urbanised subjects [31], which may be the result of chronic psychological stress. Urban living has fallen under the research spotlight for many years and is indeed associated with higher levels of chronic psychological stress [48, 49]. In 2012, Malan *et al.* [50] showed that black African subjects living in an urban environment and experiencing stress experienced an increase in vascular responsiveness and higher baseline blood pressure. Harvey *et al.* [51] found that the metabolic risk factor, waist circumference, accounts for 49 % of the variance in BDNF in depressed men, with the redox status accounting for 42 % of such variance in women, suggesting the possible contribution that chronic psychosocial stress and its effects on BDNF may adversely affect cardiometabolic health.

1.1.3. BDNF and cardiometabolic risk factors

Previous studies that have explored the possible relationship between BDNF and cardiovascular health variables found a positive correlation between BDNF and triglycerides, low density lipoprotein and total cholesterol [50]. Although certain researchers found that BDNF was positively correlated with lipid profiles, others found negative correlations between body mass index (BMI) [38], cholesterol [53] and BDNF. Studies conducted on animals in which the BDNF gene has been eliminated from the brain after birth supports this theory. Indeed BDNF gene knockout mice display elevated serum cholesterol levels [54], thus confirming the possible metabolic role of BDNF and high levels of BDNF which might lower the risk of hypercholesteremia. We therefore cautiously suggest that this action may afford a possible cardio-protective ability of BDNF.

Another cardiometabolic risk factor could be eating habits and the presence of chronically high levels of blood glucose levels. Several studies have been conducted regarding the possible role of

BDNF and energy-regulation. Some studies have suggested that BDNF is increased in several brain regions such as the hippocampus and cerebral cortex when subjects were kept on an intermittent fasting regime [55, 56].

Krabbe *et al*. [57] noted that low levels of circulating BDNF are evident in persons with obesity and type II diabetes. In humans, glycated haemoglobin (HbA1c), glucose and insulin resistance have been found to be positively associated with BDNF [58]. These discoveries might imply that altered BDNF levels have an impact on the development of obesity and perhaps the risk to develop metabolic syndrome [57, 58]. HbA1c is indeed implicated in cardiovascular disease, especially in the black Africans. In 2012 Malan *et al.* [59] demonstrated the relationship between HbA1c and endothelial dysfunction in black Africans. Adapting to an over-challenging environment impedes effective coping. Indeed, in black males utilising highly defensive coping, chronic hyperglycaemia facilitated endothelial dysfunction. A psychological "loss of control" and susceptibility to stroke risk was observed. This fact may have implications for circulating BDNF levels and possible down regulation of BDNF and/or neurodegeneration of the brain. The possible link of this process with psychosocial stress has also been demonstrated in Flinders Sensitive Line (FSL) rats, a genetic rodent model of depression [60, 61]

1.1.3.1. BDNF and Neuropathology

During recent years, BDNF has been found to be associated with a wide variety of diseases and specific insight has been sought to find the link between neurodegenerative diseases and BDNF. Phillips *et al.* [62] found that patients diagnosed with Alzheimer's disease (AD) had lower levels of BDNF-mRNA in the hippocampus, a result supported later by Lee *et al.* [63] who noted that AD sufferers had a marked decrease in BDNF in the temporal neocortex. Since amyloid beta (Aβ) protein is implicated in AD, Ciaramella *et al.* [64] found the presence of Aβ to significantly

decrease the expression of BDNF in dendritic cells. Current literature clearly indicates that BDNF plays a major role in the apparent maintenance of the cellular structure and functioning of the brain [62,63]. Thus a lack of BDNF seems to be catastrophic regarding the normal functioning of the brain, especially with regard to functions such as cognition, memory, and the development of depression. BDNF appears to play a pivotal role in the pathophysiology of Major depressive disorder (MDD) since research has found that the baseline BDNF level of patients suffering from MDD is lower than that of control subjects [65]. The same author also found that patients with MDD who were treated with antidepressants exhibited an increase in the level of BDNF, a notion supported by Zanardini *et al*. [66] who found increased levels of BDNF in depressed patients after repetitive transcranial magnetic stimulation. The question as to a possible neuroprotective role for BDNF in the central nervous system seems to be clear; however, whether BDNF plays the same role in the periphery and its possible impact on cardiovascular risk also needs to be considered. Since BDNF has the ability to cross the blood–brain barrier by a high capacity, saturable transport system [67], significant correlations between cerebral and serum BDNF levels were found and therefore, BDNF found in peripheral blood [68] has been regarded as a reliable marker of brain BDNF [69]. Significant associations between cerebral and serum BDNF levels, have been described in rats [70]; other animal studies indicate that peripheral BDNF not only represents a biomarker of depression, but also exerts profound central nervous system effects [71].

1.1.3.2. BDNF and the Autonomic Nervous System

The autonomic nervous system (ANS), especially the sympathetic nervous system (SNS), plays an important role in the maintenance of "normal" cardiac- and vascular functioning. Studies have proposed that BDNF might play a role in the regulation of heart rate by the ANS. Both exercise

and dietary energy restriction increase BDNF levels [72, 73], while studies of animals have implicated BDNF in the regulation of parasympathetic and/or sympathetic inputs to the heart. Thus Rothman *et al.* [12] demonstrated that BDNF knockout mice exhibit a 50 % reduction in BDNF mRNA, together with a significantly elevated heart rate compared to wild type (WT) mice. Further, when exposed to restraint stress, the heart rate of the BDNF knockout mice failed to increase compared to that of the WT mice, indicating an impaired cardiovascular stress response [12].

In similar studies of humans, participants with the BDNF polymorphism (Val66Met), which is known to decrease the activity of BDNF, showed decreased activity-dependent secretion of BDNF, with subjects presenting with an altered sympathovagal balance and sympathetic dominance [74]. In another study, carriers of this mutation also exhibited an altered heart rate in response to stress [75]. Clearly, the literature would suggest that BDNF has a distinct interaction with the ANS.

Further research regarding BDNF and the cardiovascular system showed that BDNF, when injected into the rostral ventrolateral medulla of anesthetised rats, induces an increase in blood pressure [76], and that both the blood pressure and the heart rate are increased when it is injected into the third ventricle [77]. Increases in blood pressure, heart rate, and lumbar sympathetic nerve activity have been observed when BDNF is injected into the brains of anesthetised rats [78]. Also when BDNF and its receptors are inhibited, blood pressure and heart rate decrease significantly, suggesting the ability of BDNF to regulate these all important cardiovascular variables [78]. These findings suggest a strong relationship between BDNF and the regulation of various cardiovascular variables through an action on the ANS. Indeed, Malan *et al.* [79] demonstrated ANS dysfunction that was associated with vascular disease and elevated blood

pressure in an African male cohort. Since depressed heart rate variability [79] as well as attenuated baroreceptor sensitivity [80] has been documented in Africans, the risk for down-regulated BDNF and associated cardiovascular risk seems likely.

1.1.3.3. BDNF and the Cardiovascular System

While the role of BDNF as a neurotrophin in the physiology and pathology of the Central Nervous System (CNS) is well-documented, much remains to be learned of its role in the cardiovascular system. In cardiac ischaemia, the levels of BDNF in the local circulation were up-regulated after reperfusion of the ventricles [81]. Other studies have found that BDNF has the ability to improve angiogenesis and the functioning of the ischaemic left ventricle [82], and that BDNF is important in maintaining vessel stability in the heart [16].

Contrary to the latter statement, serum levels of BDNF were found to be decreased in participants with acute coronary syndromes [83], while increased levels of BDNF were found in coronary sinus blood samples in subjects with unstable angina compared to controls or those with stable angina [13]. A possible hypothesis might be that BDNF is up regulated after an insult to the myocardium, such as ischaemia, representing a possible protective mechanism; however, this needs to be confirmed.

BDNF has several effects on the vasculature. Endothelial nitric oxide synthase (eNOS), which is important for angiogenesis via the synthesis of nitric oxide (NO), is able to up-regulate the production of BDNF soon after an ischaemic stroke [84, 85]. BDNF and the trkB receptor have been found in atherosclerotic lesions [86], while the presence of p75NTR is thought to be

important in the apoptosis of muscle cells and lesion development after vascular injury [87]. The central role for oxidative stress is further exemplified in a study carried out by Harvey *et al.* [51] who found a positive association between BDNF and the oxidative stress factors glutathione (GSH) and oxidised glutathione (GSSG) in depressed African men and women.

To summarise, most of the variables such as socio demographics and other lifestyle factors that adversely contribute to poor cardiovascular health in black Africans seem to play an important role in regulating BDNF. Black Africans have demonstrated significantly higher blood pressure levels and lipid profiles when compared to Caucasians; they also tend to indulge in smoking and alcohol abuse more often, all of which are factors that may contribute to increased intima media thickness (IMT), as documented by Hamer *et al.* [88, 89]. Urbanisation and an unhealthy diet are also known risk factors that increase the chance of cardiovascular disease [52, 88], particularly via effects on the structural composition of the vasculature. Moreover, functional endothelial damage and target organ damage have been found to be significantly higher in African men vs Caucasian men by measuring the albumin: creatinine ratios (ACR) [90]. Stehouwer *et al.* [91] supported the notion that endothelial damage was associated with the presence of micoalbuminuria. Moreover, Gerstein *et al.* [92] used data from a cohort study and found that any degree of albuminuria was seen as a risk factor for individuals who may or may not have been suffering from Diabetes Mellitus. Gerstein *et al.* [92] also suggested that the screening for albuminuria can identify persons at high risk for cardiovascular events. It is also believed that microalbuminuria confers a 4-fold increased risk of heart diseases upon hypertensive subjects [93]. Literature indicates that the albumin: creatinine ratios are strongly associated with the prevalence of cardiovascular diseases; hence the data presented by Okpechi *et al.* [94], showed significant correlations exist between ACR and systolic blood pressure, as well as diastolic blood pressure and fasting glucose levels, specifically in black Africans.

The literature study also clearly indicates that BDNF might directly influence the cardiovascular risk profile of humans, or indirectly do so via its effects on the structural and/or functional endothelium, thereby modulating endothelium dysfunction which could lead to a reduced risk for cardiovascular mortality.

It is therefore important that all facets of BDNF be investigated, including in the CNS and in the periphery of the human body, since this neurotrophin may have important therapeutic benefits. Indeed, Fumagalli *et al.* [95] and Allen *et al.* [96] have recently reviewed the therapeutic options of BDNF in neurodegenerative diseases.

QUESTIONS EMERGING FROM THE LITERATURE

The following questions emerged from the literature:

- How is BDNF implicated in the normal physiology and pathology of the cardiovascular system?
- Is there a link between BDNF and long term glucose metabolism?
- Does the link between BDNF and vascular disease exist in black South African men and women?
- Do increased levels of BDNF have an impact on blood pressure in black South African men and women?

MOTIVATION AND AIMS

No studies regarding the relationship between BDNF and structural vascular disease have been undertaken within the black South African population. Therefore, the main aim of the study was to investigate this relationship in African men and women in order to gain insight into potential novel interactions. Subsequent aims were to investigate the relationship between BDNF and blood pressure, markers of changes in the structural vasculature and markers of endothelial dysfunction.

HYPOTHESIS

Low Brain-derived Neurotrophic Factor (BDNF) will be associated with cardiometabolic risk in a black African cohort.

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CHAPTER 2

Attenuated Brain-derived Neurotrophic Factor and hypertrophic remodelling: The SABPA Study

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Word count: 3150
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Attenuated Brain-derived Neurotrophic Factor and hypertrophic remodelling: The SABPA Study

Running Title: Brain-derived Neurotrophic Factor and Endothelial dysfunction

Alwyn J. Smith[1], Leoné Malan[1], Aletta S. Uys[1], Nicolaas T. Malan[1], Brian H. Harvey[2], Tjalf Ziemssen[3]

[1]Hypertension in Africa Research Team (HART); School for Physiology, Nutrition and Consumer Sciences; North-West University; Potchefstroom; South Africa.

[2] Unit for Drug Research and Development, Division of Pharmacology, School for Pharmacy, North-West University, Potchefstroom, South Africa

[3] Department of Neurology, Medical Faculty Carl Gustav Carus, Technische Universität, Dresden, Germany.

Correspondence:

Prof Leoné Malan (RN, PhD)
HART, Hypertension in
Africa Research Team
Subject Group Physiology
Faculty of Health Sciences
Private Bag X6001
North-West University
Potchefstroom
2520
SOUTH AFRICA
Tel +27 18 2992438
Fax +27 18 2992433
E-mail: Leone.Malan@nwu.ac.za

Abstract

Brain-derived neurotrophic factor (BDNF) has been linked to neurological pathologies however the role of BDNF in cardiometabolic disturbances is limited. We aimed to assess the association between BDNF levels and structural endothelial dysfunction (ED) as determined by cross sectional wall area (CSWA) and albumin:creatinine ratio (ACR) in black Africans.

Ambulatory blood pressure (BP) and ultrasound CSWA values were obtained from 82 males and 90 females. Fasting blood and 8h overnight urine samples were collected to determine serum BDNF and cardiometabolic risk markers, i.e. glycated haemoglobin (HbA1c), lipids, inflammation and ACR. BDNF median split x Gender interaction effects for structural ED justified stratification of BDNF into low and high (≤ / > 1.37 ng/ml) gender groups.

BDNF values (0.86-1.98 ng/ml) were substantially lower than reference ranges (6.97 – 42.6 ng/ml) in the African gender cohort, independent of age and body mass index. No relationship was revealed between BDNF and renal function opposed by an inverse relationship between BDNF and CSWA ($r = -0.17$; $p = 0.03$) in the African cohort. Linear regression analyses revealed a positive relationship between systolic BP and structural remodelling in the total cohort and low BDNF gender groups. In the high BDNF females, HbA1C was associated with structural remodelling.

Attenuated or possible down-regulated BDNF levels were associated with hypertrophic remodelling maybe as a compensatory mechanism for the higher BP in Africans. Additionally, metabolic risk and hypertrophic remodelling in women with high BDNF, underpin different underlying mechanisms for impaired neurotrophin health in men and women.

Keywords: African, BDNF, blood pressure, carotid intima media thickness, HbA1c

Introduction

The prevalence of cardiovascular disease in sub-Saharan Africa is increasing rapidly, and the rate of increase is projected to be higher in urban than in rural populations.[1] Disturbed brain-heart responses have been shown in Africans who demonstrated coping disability when residing in an urban-dwelling environment.[2] Adapting to an over-demanding environment seems to increase the cardiovascular vulnerability in the African population.[1,2]

A fairly novel marker, i.e. serum brain-derived neurotrophic factor (BDNF), may expand knowledge on the brain-heart responses in assessing cardiovascular vulnerability. BDNF is a member of the neurotrophin family of growth factors and regulates specific aspects of neuronal survival and plasticity.[3] It has the ability to cross the blood-brain barrier via a high capacity saturation transport systems revealing significantly high correlations between cerebral- and peripheral serum BDNF levels in rats.[3,4] If this holds true for humans, is unclear as it has not been well-described.[3,4] Altered BDNF especially has been associated with several pathologies of the central nervous system (CNS), particularly psychiatric and neurodegenerative diseases.[5-8] In addition, BDNF has also been associated with cardiovascular disease, although the nature of the relationship between BDNF and heart disease is poorly understood. Lower[9] and higher[10] circulating levels of BDNF has been demonstrated in patients with acute coronary syndromes such as unstable angina pectoris and myocardial infarctions.

Lower BDNF secretion in human subjects, supported by the BDNF polymorphism (Val66Met), was associated with sympathovagal imbalance favouring sympathetic dominance.[11] The latter is a known risk factor in cardiovascular pathology and -disease and was demonstrated in the male cohort under study.[12] If BDNF is injected into the rostral ventrolateral medulla[13] and the third ventricle,[14] it acutely elevates blood pressure, indicating the profound influence it has on cardiovascular tone via a central neural mechanism. As ambulatory blood pressure responses

were associated with functional and structural endothelial changes in Africans[2] it is possible that BDNF via augmented blood pressure responses may increase shear stress and injury on the endothelial wall. Indeed, Donovan *et al*.[15] revealed that the BDNF receptor, tyrosine kinase B (TrkB), is up-regulated in rodent vascular endothelium following injury.

Therefore, the potential effect of BDNF on the vasculature motivated the hypothesis that low BDNF levels, independent of lifestyle risk factors, will demonstrate disturbed endothelial changes in an African cohort.

Method and Materials

Participants

The sub-study is nested in the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study, which is a target population study including 200 black African Teachers, aged 21-62 years. They are from similar socio-economic status working in the Dr Kenneth Kaunda Education district, North West Province, South Africa.[2] The study was conducted between February and May in 2008 avoiding seasonal changes. Exclusion criteria included ear temperature above 37.5 °C, use of psychotropic substances, use of α- and β- blockers, blood donors or individuals vaccinated during the past three months. The current sub-study further excluded participants on medication for diabetes mellitus (n=9) and with HIV positive status (n=19). Of the 200 participants, the final sample size comprised 82 male and 90 female black Africans (hereafter referred to as Africans).

Participants were fully informed about the objectives and procedures of the study prior to their recruitment. All participants signed an informed consent form. The study was approved by the Ethics Review Board of the North-West University, Potchefstroom Campus, South Africa (project number: NWU-00036-07-S6). The study complied with the declaration of Helsinki[16] for studies involving human participants.

General Procedure

Before 09:00, participants were fitted with an ambulatory blood pressure (ABP) monitor (Meditech CE120 CardioTens; Meditech, Budapest, Hungary) at their workplace. A cuff of appropriate size was fitted to the non-dominant arm of each participant. Measurements were conducted during the week on workdays. The apparatus was programmed to measure BP at 30 minute intervals during the day (08:00-22:00) and every hour during night (22:00-06:00). Participants were asked to continue with normal daily activities and to record any abnormalities such as headache, nausea, or feeling stressed on their ambulatory diary cards. Inflations were successful for 75 % of the males and 70 % in the female group. Participants were also fitted with an Actical® accelerometer (Montréal, Québec) to measure physical activity for 24 hours, taking their resting metabolic rate into account.

Data were derived for physical activity energy expenditure (kcal). The ABP data were analysed using the CardioVisions 1.15.2 Personal Edition software (Meditech). Participants were collected at 16:30 on the first day and transported to the Metabolic Research Unit. After their arrival, participants were introduced to the experimental procedures in order to reduce anticipation stress [25]. Afterwards, participants enjoyed a standardised dinner and the last beverages were allowed at 20:30. They were advised to go to bed at 22:00, fasting overnight. The ABP monitors and the Actical® apparatuses were removed the following morning, after the last inflation of the cuff at 06:00. A registered nurse sampled fasting blood from their brachial vein branches with a sterile winged infusion set, whilst the participant reclined in a semi-Fowlers position.

Ultrasound structural endothelial assessment

A high-resolution ultrasound scan determined carotid intima media thickness (CIMT) images from at least two optimal angles of the left and right common carotid arteries (CCA), the carotid bulb and the internal carotid arterial (ICA) segments were obtained using a SonoSite Micromaxx ultrasound system (SonoSite Inc, Bothell, WA, USA) and 6- 13 MHz linear array transducer

according to the Rudy Meijer[17] protocol. The digitised images were imported into the Artery Measurement System automated software for analysis of the CIMT. A maximal 10 mm segment with good image quality was chosen for analysis. The program automatically identifies the borders of the CIMT of the near and far walls and the inner diameter of the vessel. The lumen diameter of both the left and right common carotid arteries were calculated between the near and far walls of the lumen-intima interface and the averages were calculated. Subsequently, the carotid cross-sectional wall area (CSWA) was calculated by using the equation CSWA = π (lumen diameter/2 + IMT$)^2 - \pi$ (lumen diameter/2)^2 (ref. 18-20).

Biochemical analyses

Overnight fasting blood samples were processed according to standard laboratory procedures and stored at -80°C for the analysis of biochemical markers. Fasting sodium fluoride (glucose) and serum samples for total- and high- density lipoprotein (HdL) cholesterol, triglycerides, high-sensitivity C-reactive protein (Hs-CRP) and gamma glutamyl transferase (GGT) were analysed using two sequential multiple analysers (Konelab 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800- Beckman and Coulter®, Germany). Fasting 8 hour overnight urinary Albumin and Creatinine levels were determined by means of the Turbidimetric method with a Unicel DXC 800 - Beckman and Coulter (Germany). Glycated Hemoglobin (HbA1c) was determined by a turbidometric inhibition immunoassay using the Integra 400 apparatus, Roche, Switzerland. Serum BDNF was determined through quantikine colorimetric-sandwich immunoassays from R & D Systems (Catalogue number: DBD00). A serum separator tube (SST) was used and samples were allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Serum was removed and stored at -20 °C until batch assay. The intra-assay and inter-assay precision was 3.8-6.2 % and 7.6-11.3 % respectively. HIV/AIDS screening was done with antibody tests, namely the First Response® kit (RPM Plus, Colonia, New Jersey, USA) and the confirmatory

Pareekshak test (Bhat Biotech, India). Alcohol abuse was indicated by increased GGT levels.[21] Serum cotinine is a reliable and valid circulating biochemical marker of nicotine exposure in the past 24 hours.[22] Cotinine levels were measured through homogeneous immunoassay, with the Roche Modular system (Roche, Basil, Switzerland).

Anthropometric measurements

Participants' body mass was determined with a digitally calibrated scale and rounded to the nearest 0.1 kg. The maximum stature was measured to the nearest 0.1 cm with a stadiometer while the participant's head was on the Frankfort plane, the heels together and the buttocks and upper back touching the stadiometer.[23] The listed anthropometric measurements were performed in triplicate by registered level II anthropometrists according to standardised procedures. The intra- and inter-observer variability was less than 10 %.

Statistical analyses

Data analyses were performed with Statistica 11 (Statsoft Inc. STATISTICA for Windows, Tulsa, OK, Statsoft, Inc, 2011). Departure from normality was evaluated through Shapiro-Wilks' analyses and Hs-CRP and γ-GT were normalised. Descriptive characteristics were computed with independent t-tests and indicated the covariates to be utilized in comparison models. Chi-square tests determined proportions. A single 2 x 2-way analysis of covariance (ANCOVA) determined interaction between main effects (BDNF median split x gender) and cardiometabolic risk markers, independent of covariates. Possible down-regulation in BDNF may be evident if BDNF is inversely associated to structural endothelial dysfunction. Subsequent single ANCOVAs were employed to compare BDNF median split gender groups independent of confounders.

Multiple unadjusted and adjusted linear regression analyses were computed. Linear forward stepwise regression analyses models identified the variables that best predicted the relationship between dependent variables; structural endothelial dysfunction (L-CIMT, L-CSWA, ACR) and independent cardiometabolic markers in model 1 for the total group and in model 2 for the BDNF gender groups. Independent variables for model 1 (total group) included gender, confounders, log GGT, Systolic BP (SBP), HbA1c, BDNF, cholesterol and log CRP. Independent variables considered for entry into model 2 (separate BDNF gender groups) were confounders, SBP, HbA1c, BDNF, cholesterol and log CRP. A p-value of 0.05 or less was considered statistically significant and tendencies were indicated by $p \leq 0.1$. For regression analyses, adjusted R^2 values > 0.25 were considered to be significant.

Sensitivity analyses

Log GGT was added as independent variable in the adjusted regression models to predict structural endothelial dysfunction.

Results

Table 1 summarises the characteristics of the black African cohort. On average, male participants were overweight (BMI 25-30kg/m^2) and abused more alcohol.[21] African men revealed a vulnerable cardiometabolic profile with values exceeding cut–points (European Society of Hypertension).[24] These men demonstrated increased acute and chronic glucose (HbA1c) levels indicating a pre-diabetic state; as well as a disturbed lipid profile with lower HdL and increased triglycerides.[24] Overall BDNF levels were lower than reference ranges (6.97 – 42.6 ng/ml).[25] The men revealed mean lower BDNF levels, ambulatory BP values exceeding guideline cut-points (ambulatory SBP > 130mmHg; DBP > 80mmHg)[24] as well as a hypertensive state compared to their female counterparts.[24] Pertaining to structural endothelial dysfunction, the mean ACR value in men exceeded normal laboratory values
(< 3.5mg/mmol).[26,27] The African women displayed an obese state with low grade inflammation (CRP, 12.27 ± 11.67mg/l).

A single two-way ANCOVA interaction on main effects (BDNF median split x Gender) demonstrated significant interaction for CIMTf [F (1,164); 3.99, p=0.05] and cholesterol [F (1,164); 4.12, p=0.05]. Therefore, a median split approach was followed which stratified gender groups into lower (≤ 1.37 ng/ml) and higher BDNF levels (>1.37 ng/ml).

In Table 2, the low BDNF men revealed higher cholesterol than the high BDNF group, independent of BMI and age. Only the low BDNF women indicated significantly higher values for structural vascular markers (p< 0.05) than the high BDNF female group.

Unadjusted and adjusted associations

No unadjusted or adjusted association was found between BDNF and ACR.

Figure 1 and Table 3 display the unadjusted and adjusted inverse association between BDNF and CSWA in the total African cohort. Table 3 represents forward stepwise regression analysis indicating associations between structural remodelling and cardiometabolic markers in the total and the BDNF gender groups. In the total cohort, gender and BDNF were negatively and SBP positively associated with structural remodelling, independent of covariates. Only SBP in the low BDNF gender groups as well as HbA1C in the high BDNF women, predicted structural remodelling in our models, independent of covariates. Adding Log GGT as independent variable in the adjusted regression models did not change the outcome of the findings.

Discussion

The present study aimed to assess possible associations between BDNF and structural endothelial dysfunction in an urban back African gender cohort. To our knowledge, this is the first study to investigate the role of BDNF in functional and structural changes of the vascular system, particularly in an African population. Overall, attenuated or possible down-regulated BDNF levels indicated hypertrophic remodelling of the carotid artery. Cardiovascular risk in both gender groups, as well as metabolic risk in women, was related to these changes. Different underlying mechanisms may underpin impaired neurotrophin health in men and women.

The neurotrophic factor, BDNF, has been a subject of considerable interest over the last decade, especially in the maintenance of synaptic plasticity and as a possible neuroprotective agent in the central nervous system.[28] Altered BDNF levels have therefore been associated with an array of neurological and psychiatric diseases. Even though very little is known about the specific role of BDNF in cardiovascular disease, outside the central nervous system, BDNF has been associated

with various lifestyle factors that increase the risk of developing cardiovascular disease.[29] On the other hand, certain lifestyle factors may be cardioprotective by increasing BDNF levels and include exercise[30] and reduced smoking.[5]

The lower serum BDNF levels demonstrated in the black Africans, support the notion of increased cardiometabolic-, oxidative stress-[29] and psychological distress risk in black Africans.[1,2] Their BDNF levels were remarkably lower than reference ranges (6.97 – 42.6 ng/ml).[25] Low levels of circulating BDNF were demonstrated in persons with type II diabetes and with chronic hyperglycaemia (HbA1c), which may impact on the development of the metabolic syndrome.[31] Thus lower levels of BDNF may further be detrimental to the normal functioning of the brain, especially with regard to functions such as cognition, memory, and the development of depression.[32] Indeed, in our sub-sample, metabolic and redox risk markers were associated with serum BDNF levels in the African gender groups presenting with symptoms of depression.[29] The clinical relevance of these findings is that changes in redox and metabolic status may represent counter-regulation by BDNF or alternatively indicate that BDNF may mediate undesirable redox and metabolic changes that are associated with the development of cardiometabolic risk and or a mood disorder.[31]Findings in our sub-study demonstrated that cardiometabolic risk factors predispose participants to disturbed structural endothelial function, increasing their sub-clinical atherosclerotic risk. These results may contribute to the rising body of evidence that supports the important central neural regulatory role of BDNF in the periphery, impacting on the cardiometabolic system.

Black African Male Participants

Lower levels of serum BDNF showed significant correlations with cardiovascular risk factors, such as systolic blood pressure, which is positively associated with structural wall abnormalities.[33] Serum BDNF plays a role in cardiometabolic health[33] and it is plausible to argue

that low levels of BDNF are potentially harmful for cardiovascular health in the male cohort. It is uncertain which factors might trigger attenuation and possible down-regulation of BDNF. Our findings suggest the involvement of BDNF in the pathology of sub-clinical atherosclerosis but it can also be as a compensatory response to an underlying pathology. A recent study investigating the association between serum BDNF and peripheral markers of metabolic and redox status concurred with the latter suggestion.[29]

Disturbances in metabolic markers and higher levels of alcohol abuse in the low BDNF males may explain the higher ambulatory systolic blood pressure responses, supporting increases in vascular tone and hyperkinetic sympathetic nervous system drive. Previous studies have demonstrated that ethanol consumption increases plasma levels of catecholamines, renin, and aldosterone, each of which may cause systemic arterial vasoconstriction.[34] Hamer *et al*.[35] have revealed that GGT demonstrated an odds ratio of 3.1 (95 % CI 0.6- 15.5) to develop structural vascular disease in the Africans from the SABPA study.[35] Current findings of the higher alcohol abuse, hypertensive and hyperglycemic status in the low BDNF males might decrease the BDNF levels[36] and the central neural regulatory cardioprotective effect of BDNF. Another study confirmed the impact of increased GGT and down-regulation of BDNF independently.[35] A proposed mechanism is suggested, where high BP might act as homeostatic mechanism when alcohol abuse is apparent, hence enhancing chronic hyperglycaemia, hypertriglyceridemia and subsequent attenuation or possibly down-regulation of BDNF. This may increase cardiometabolic risk, if chronic abuse is evident. However, prospective studies need to be conducted in order to determine cause and effect as well as confirm the underlying mechanism.

The *inverse* association between BP and structural endothelial remodelling in the low BDNF groups might support a role of down-regulation in BDNF, compensating for the higher blood pressure levels. The current sub-sample also displayed higher sympathetic activity with time domain depressed heart rate variability.[37] As autonomic pathways and cardiometabolic responses

imply a central neural regulatory role for BDNF, they might be involved in a counter-regulatory manner. Therefore, we cautiously suggest that the systemic effects of BDNF may influence the cardiovascular system at a secondary level; hence the significant associations between metabolic markers, blood pressure and secondary end points. This may also imply a chicken-or-egg situation as these men were found to be psychophysiological more vulnerable[1-2, 38-39]which may impair neurtrophin health.

Black African Female Participants

Pertaining to BP, similar trend as in men was evident in the women. Ambulatory SBP, independent of confounders, was positively associated with structural remodelling risk markers, supporting the findings of Sander *et al.*[33] However, an additional risk emerged, as chronically elevated levels of blood glucose (HbA1c) were associated with hypertrophic remodelling within higher levels of BDNF. Tonra *et al*.[37] demonstrated that higher levels of BDNF enable HbA1c and fasting glucose prevalence levels to be maintained near those of non-diabetics. We could not replicate these findings as no direct association existed between BDNF and glucose or renal function (ACR) in the total sample or in the separate gender groups independent of covariates. BDNF may have the potential ability to act as a long-term modulator of blood glucose levels.[36] Despite the higher circulating level of BDNF, it is still is much lower than reference ranges[25] and might not be beneficial to African women as their HbA1C indicated a pre-diabetic state. Congruent with this notion, Suwa *et al*.[36] predicted the development of obesity and type 2 diabetes mellitus if low levels of BDNF persisted. It may suggest a central systemic neural mediatory influence of BDNF rather than a local effect.

This may suggest involvement of BDNF as a possible metabolic factor responsible for regulating long term blood glucose levels. Clearly more research is needed to describe the ambivalent characteristics of BDNF.

Conclusion

In conclusion, we accept our hypothesis, as hypertrophic remodelling of the carotid artery was associated with lower BDNF levels. This may imply attenuated or possibly down-regulated BDNF levels acting as a compensatory mechanism for the mean higher BP levels. In women, metabolic risk and hypertrophic remodelling were evident within higher circulating levels of BDNF, underpinning different underlying mechanisms for impaired neurotrophin health in men and women. Novel findings of BDNF revealed the impact of central neural regulation on the circulatory system, which may contribute to cardiometabolic risk in Africans.

Limitations

Limitations of the study include the relatively small sample group size and no comparison sample with different ethnicity or differing health profile to generalize findings. The cross-sectional design of the sub-study cannot infer causality. Strengths include the fact that we had a population aged between 21 and 62, which represents a generalized well-spread range where clinical assessments were obtained within a well-controlled experimental set-up. Furthermore, the unique study population was representative of a target population group including black Africans from both gender groups.

Conflict of Interests

There are no conflicts of interests to be declared.

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Disclaimer

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Figure Legends

Figure 1

A scatterplot to show the association between serum brain derived neurotrophic factor (BDNF) in ng/ml and a structural endothelial marker, cross sectional wall area (CSWA) in mm^2, in a black African gender cohort with 162 participants.

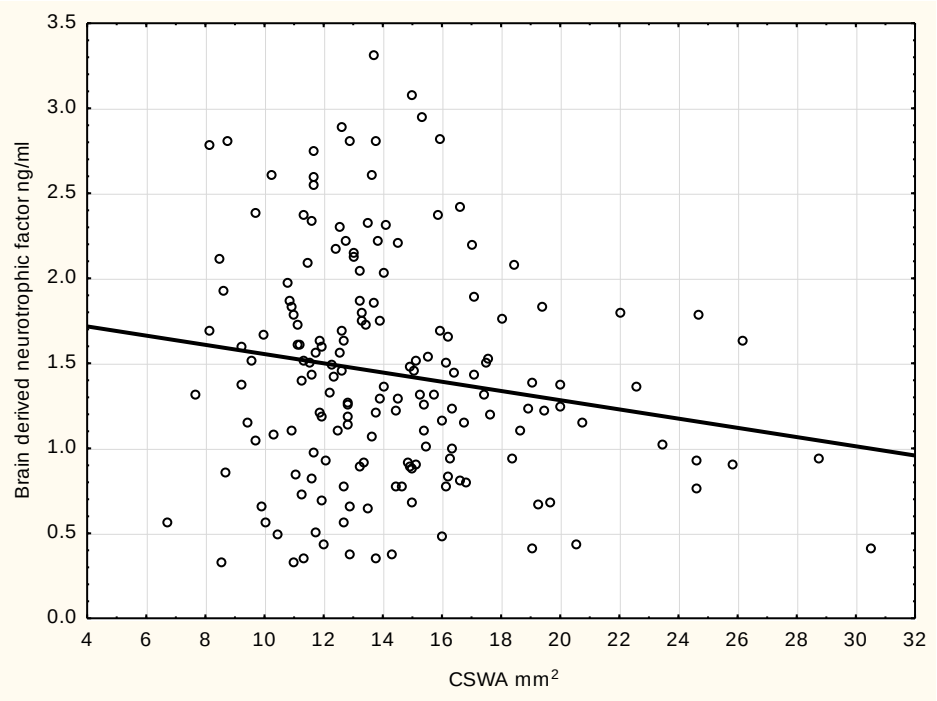

Figure 1: Association between serum brain derived neurotrophic factor (BDNF ng/ml) and structural endothelial marker, cross sectional wall area (CSWA mm^2) in a black African gender cohort (N=162).

Table 1: Characteristics of the male and female participants

Variables	Males (n = 82)	Females (n = 90)	p-value
Age (years)	42.81 ± 8.42	45.32 ± 8.10	0.047*
Lifestyle factors			
Physical activity (kcal/24h)	2719.78 ± 818.83	2669.01 ± 804.80	0.680
Cotinine (ng/ml)	24.79 ± 47.77	18.06 ± 55.25	0.400
GGT (u/l)	76.42 ± 72.07	48.10 ± 69.75	< 0.001*
Body Mass Index (BMI) (kg/m^2)	27.64 ± 5.91	32.78 ± 7.29	< 0.001*
Waist Circumference (cm)	93.51 ± 16.31	93.48 ± 15.68	0.98
Cardiometabolic profile			
Glucose (mmol/l)	6.0 ± 2.11	5.0 ± 1.10	< 0.001*
Glycated hemoglobin (HbA1C) (%)	6.23 ± 1.28	5.76 ± 0.61	0.003*
C-Reactive Protein (mg/l)	4.89 ± 7.63	12.27 ± 11.67	< 0.001*
Cholesterol (mmol/l)	4.90 ± 1.20	4.43 ± 1.15	0.021*
HDL (mmol/l)	1.08 ± 0.38	1.21 ± 0.31	0.014*
Triglycerides (mmol/l)	1.80 ± 1.69	0.99 ± 0.54	< 0.001*
BDNF (ng/ml)	1.27 ± 0.65	1.59 ± 0.64	0.001*
Ambulatory Blood Pressure (mmHg)			
Systolic	138 ± 16.83	128 ± 15.16	< 0.001*

Diastolic	88 ± 11.43	78 ± 8.98	< 0.001*
Heart Rate (bpm)	79 ± 11	80 ± 10	0.383
Structural Endothelial dysfunction risk markers			
Albumin Creatinine Ratio (mg/mmol/l)	3.63 ± 17.33	1.39 ± 1.08	0.224
CIMTf (mm)			
Left	0.710 ± 0.13	0.663 ± 0.18	0.056
Right	0.697 ± 0.13	0.677 ± 0.16	0.380
Mean	0.70 ± 0.16	0.67 ± 0.13	0.160
CSWA (mm^2)			
Left	15.74 ± 5.37	12.98 ± 3.22	< 0.001*
Right	15.52 ± 4.80	13.34 ± 3.28	< 0.001*
Mean	15.46 ± 4.70	13.13 ± 3.13	< 0.001*
Hypertensive, n (%)	59 (72)	50 (56)	0.025*
Hypertension medication, n (%)	14 (17.07)	21 (23.33)	0.310
Statins, n (%)	1 (1.22)	1 (1.09)	0.95
ACE inhibitors, n (%)	7 (8.54)	11 (11.96)	0.43
Thiazides, n (%)	7 (8.54)	14 (15.22)	0.16
Calcium Channel blockers, n (%)	6 (7.32)	7 (7.61)	0.91

Values are arithmetic mean ± SD, or number of subjects (%).Where: GGT, glutamyl transferase; HDL, High density lipoprotein; CIMTf, carotid intima media thickness far wall; CSWA, cross sectional wall area. *P-values ≤ 0.05 are regarded as significant.

Table 2: Adjusted comparisons of African men and women in lower vs. higher BDNF median split groups (mean ± 95 % CI or mean ± SD).

	AFRICAN MEN			**AFRICAN WOMEN**		
	[BDNF] ≤ 1.37 ng/ml	**[BDNF] > 1.37 ng/ml**	**p-value**	**[BDNF] ≤ 1.37 ng/ml**	**[BDNF] > 1.37 ng/ml**	**p-value**
N	**50**	**32**		**35**	**54**	
*Age (years)	42 ± 9	44 ± 8	0.38	46 ± 7	45 ± 9	0.36
GGT (u/l)	81.10 (60.4, 101.8)	69.2 (43.1, 95.3)	0.49	48.32 (24.2, 72.5)	47.89 (28.8, 67.1)	0.97
Cardiometabolic profile						
BDNF (ng/ml)	0.86 (0.75, 0.97)	1.90 (1.77, 2.04)	< 0.001*	0.99 (0.85)	1.98 (1.87, 2.09)	< 0.001*
Glucose (mmol/l)	6.02 (5.4, 6.6)	6.00 (5.2, 6.7)	0.91	5.17 (4.8, 5.5)	4.93 (4.6, 5.2)	0.29
HbA1C (%)	6.12 (5.8, 6.5)	6.39 (5.9, 6.9)	0.36	5.84 (5.7, 6.0)	5.70 (5.6, 5.9)	0.26

C-Reactive Protein (mg/l)	5.54 (3.4, 7.7)	3.87 (1.2, 6.6)	0.35	13.39 (9.5, 17.3)	11.56 (8.5, 14.6)	0.46
Cholesterol (mmol/l)	5.06 (4.7, 5.4)	4.52 (4.1, 4.9)	0.05*	4.27 (3.9, 4.7)	4.53 (4.2, 4.9)	0.29
Heart Rate (b/min)	79 (75, 81)	80 (75, 83)	0.57	80 (76, 83)	81 (78, 83)	0.68
Ambulatory Blood Pressure (mmHg)						
Systolic	138 (134, 142)	138 (133, 143)	0.94	129 (124, 134)	129 (125, 134)	0.97
Diastolic	88 (85, 91)	88 (84, 92)	0.98	79 (76, 82)	80 (76, 81)	0.69
Risk markers potentially affecting the structural endothelium						
ACR	6.37 (0.1, 12.6)	1.87 (-3.1, 6.9)	0.27	1.22 (0.9, 1.5)	1.70 (1.3, 2.0)	0.41
Carotid intima media thickness far wall (mm)						
Mean	0.71 (0.7, 0.8)	0.68 (0.6, 0.7)	0.36	0.71 (0.7, 0.7)	0.65 (0.6, 0.7)	0.02*

Left	0.72 (0.7, 0.8)	0.69 (0.6, 0.8)	0.41	0.70 (0.70, 0.7)	0.64 (0.6, 0.7)	0.01*
Right	0.71 (0.7, 0.8)	0.67 (0.6, 0.7)	0.19	0.71 (0.7, 0.8)	0.66 (0.6, 0.7)	0.07
Cross sectional wall area (mm^2)						
Mean	15.76 (14.6, 17.0)	15.00 (13.5, 16.5)	0.44	14.07 (13.2, 15.0)	12.70 (12.00, 13.40)	0.01*
Left	15.77 (14.3, 17.30)	15.68 (13.8, 17.5)	0.94	13.98 (13.0, 15.0)	12.41 (11.6, 13.2)	0.01*
Right	15.56 (14.3, 16.8)	15.45 (13.9, 17.0)	0.91	14.13 (13.2, 15.1)	12.84 (12.1, 13.7)	0.05*

Where, CIMTf, carotid intima media thickness far wall and CSWA, cross sectional wall area. Covariates included age and BMI. *P-values ≤ 0.05 are regarded as significant.

Table 3: Independent associations between structural endothelial dysfunction and cardiometabolic risk markers in a total and African gender cohort.

	CIMTf (mm)	CSWA (mm)	ACR (mg/mmol/l)
Total African cohort (N=166)			
Adjusted R^2	**0.46**	**0.37**	**<0.10**
ß (± 95% CI)			
24h SBP	NS	0.27 (0.13, 0.41)**	-
Gender	NS	-0.27 (-0.43, -0.11)**	-
BDNF	NS	-0.13 (-0.25, -0.01)*	-
African Men Low BDNF (N = 48)			
Adjusted R^2	**0.44**	**0.42**	**<0.10**
ß (± 95% CI)			-
24h SBP	0.27 (0.02, 0.52)*	0.39 (0.12, 0.66)*	-
African Women Low BDNF (N = 33)			
Adjusted R^2	**0.18**	**0.20**	**0.19**
ß (± 95% CI)			
24h SBP	NS	0.54 (0.13, 0.95)*	-
African Women High BDNF (N = 53)			
Adjusted R^2	**0.37**	**0.38**	**<0.09**
ß (± 95% CI)			
HbA1C	NS	0.27 (0.03, 0.51)*	-

ß denotes standardised regression coefficient. Covariates included in L-CIMT, L-CSWA and ACR dependent variable models: Age, BMI, log GGT, HbA1c, log CRP and cholesterol. Where: **P ≤ 0.001; *P ≤ 0.05.

Table 4: Summary table

What is known about the topic	What this study adds
• In urban Africans, cardiovascular disease is a major health problem.	• Brain derived neurotrophic factor is implicated in cardiovascular disease in urban Africans. An inverse association was evident between BDNF and structural endothelial changes
• High Blood pressure may lead to alterations in vascular function.	• A hypertensive state and vascular remodeling may impair neurotrophin health.
• Chronic states of high blood glucose level and impaired structural endothelial function, leads to an increase in cardiovascular disease	• Chronic states of high blood glucose was associated with structural remodelling underpinning different underlying mechanisms in gender groups for neurotrophin health

CHAPTER 3

GENERAL CONCLUSIONS AND RECOMMENDATIONS

INTRODUCTION

In this chapter, the main findings from this study is summarised. The results are discussed, interpreted, explained and compared to the relevant literature. Conclusions are drawn and recommendations are made to researchers who wish to pursue further research regarding the role of BDNF and its association with cardiovascular disorders and structural vascular disease.

SUMMARY OF THE MAIN FINDINGS

Attenuated Brain-derived Neurotrophic Factor and hypertrophic remodelling: The SABPA Study

This study aimed to discover the relationship between BDNF and various risk factors of cardiovascular disease in black South African men and women. Men and women were stratified into groups according to levels of serum BDNF. BDNF median split x Gender interaction effects for structural ED justified stratification of BDNF into low and high (≤ / > 1.37 ng/ml) gender groups.. Within these groups, we found several correlations indicating the possible role that BDNF might play in cardiovascular health. Significant inverse associations between CIMT and SBP were found in both gender groups presenting with low serum levels of BDNF. We predict that males with low BDNF, with chronically high levels of blood glucose, may develop an increase in BP. We also noted that females in the same category might be at risk for vascular remodelling if high levels of HbA1c persist. This study therefore suggests that the influence of BDNF on cardiometabolic health might be gender specific and that low BDNF levels may act as a protagonist to increase cardiometabolic disease.

COMPARISON TO RELEVANT LITERATURE

The results of this study are unique since no other data on the possible relationship between BDNF and the cardiovascular system of black Africans exists. However, some of our data

support data presented by other authors. We found that BDNF levels in male subjects was significantly lower than that of females (Male: 1.27 ± 0.65and Female: 1.59 ± 0.64) supporting the notions of Golden *et al.* [1] and Bus *et al.* [2]. Since research suggested a relationship between low levels of BDNF and HbA1c exists, we can propose that low BDNF may indeed be associated with diabetes, as described by Krabbe *et al.* [3].

In animal studies, the injection of BDNF into the brain leads to an increase in blood pressure [4, 5], although studies in humans has indicated that DBP will increase following an increase in BDNF [4]. Araya *et al.* [5] found that exercising for 10 weeks increased the level of BDNF and decreased the BP. In this study, we have found no association between the level of BDNF and blood pressure.We therefor recommend that further studies be undertaken into the possible relationship between BDNF and blood pressure in human subjects.

LIMITATIONS

It is imperative to reflect on some of the factors that might have adversely affected the results in this study. There are some methodological issues that could have caused weaknesses, and therefore might have influenced the outcomes of this study. Some limitations include the relatively small sample group size as well as the cross-sectional design of the sub-study, which cannot infer causality.Overall this can be classified as a controlled study consisting of an initial 200 participants, all selected from the same socio-economic class. Our exclusion criteria included an ear temperature above 37.5 °C, use of psychotropic substances, use of α- and β-blockers, blood donation or individuals vaccinated in the past 3 months. Further exclusion took place to assimilate the population of the sub-study, which included participants on medication for diabetes mellitus (n=9), and those who were HIV positive (n=19). An important limitation to consider is the fact that we cannot determine whether the actions of BDNF and subsequently whether our findings reflect the actions of CNS- or peripheral BDNF, or both.

CONFOUNDERS

By adjusting for age, BMI, and additionally adjusting for BP, cholesterol and CRP, for structural endothelial dysfunction models, there still might be a possibility that these covariates could have influenced the results by causing over or underestimation of the associations between BDNF and indicators of cardiovascular function investigated in this study. An important point to remember is that it is necessary to interpret all the statistical results from a physiological perspective, which entails that not all statistically significant changes are necessarily of physiological significance.

DISCUSSION OF THE MAIN FINDINGS

The rise in the prevalence of cardiovascular diseases worldwide, especially in South Africa, is a major health concern [6]. The onset of cardiovascular pathologies such as hypertension and atherosclerosis in the black South African population can be caused by many factors [7]. Relatively novel findings suggest that BDNF may play a major role in the health of the human body especially of the cardiovascular system [8]. Therefore, the focus of this study fell on the investigation of the possible relationship between BDNF and structural vascular disease in black African men and women in order to understand the possible role that BDNF might play in cardiovascular health.

In the presence of low BDNF levels, BP contributed to increases in CSWA,which directly contributes to vascular remodelling. However, with a rise in the level of serum BDNF, no relationship between vascular changes and BP could be found, however a positive association between HbA1c and CSWA existed. Although the findings cannot be extrapolated to the entire black South African population, it could provide a reference for future studies.

CONCLUSION

In both low BDNF gender groups, SBP appears to be the driving force to increase vascular remodelling which in turn may lead to structural vascular disease. Attenuated and possibly down-regulated BDNF levels were associated with a state of chronic hyperglycaemia, hyperkinetic BP and structural endothelial dysfunction. A novel finding is the demonstration of neurotrophin involvement in the regulation on the circulatory system, and which may contribute to cardiometabolic risk in black Africans. We suggest that the influence of BDNF on cardiometabolic health might be gender specific and that low BDNF levels may increase cardiometabolic morbidity.

RECOMMENDATIONS

We would recommend larger population samples to study the impact of BDNF on the cardiovascular system. Since BDNF has a rather profound effect on the human physiology, it is important to consider the interactions that variables might have on each other and that adjustments should be made accordingly. Prospective studies on BDNF and the cardiovascular system are important, especially in humans. More variables need to be taken into consideration (e.g., in females: phase of the menstrual cycle, pregnancy, hormone replacement therapy, etc.) if the effect of BDNF in a gender specific group is being studied.

Another recommendation is to study the effects of BDNF on a depressed vs. non-depressed cohort since depressed subjects have been found to present with cardiovascular abnormalities such an altered sympathovagal balance.

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