Brain-derived neurotrophic factor, vascular alterations, and depression: The SABPA study

Renè Smit

orcid.org/0000-0003-3474-6217

Dissertation submitted in fulfilment of the requirements for the degree *Master of Health Sciences* in Cardiovascular Physiology at the North-West University

Supervisor: Prof Leandi Lammertyn

Co-supervisor: Dr Esmé Jansen van Vuren

Co-supervisor: Dr Hannes Holm

Co-supervisor: Prof Martin Magnusson

Student Number: 28832299

PREFACE

The manuscript presented in this Master of Science (MHSc) study, constitutes four chapters, encompassing the following information. Chapter 1 contains a comprehensive literature overview of the topic including a detailed discussion of the mechanism of depression as well as neurotrophic factors, specifically brain-derived neurotrophic factor and its relationship to vascular alterations. In this chapter, the aims and hypothesis are also included. Chapter 2 contains a detailed discussion, which includes Sympathetic activity and Ambulatory Blood Pressure (SABPA) study protocol and methods of data collection. Chapter 3 includes the manuscript of the study, entitled: Brain-derived neurotrophic factor, carotid intima-media thickness, and depression: the SABPA study. In Chapter 4, a summary of the main findings of the MHSc study as well as a conclusion is presented. The hypotheses are also accepted and rejected in this chapter as well as recommendations for future research. The Vancouver referencing style was used throughout the dissertation. Each chapter's references are available at the end of the respective chapter.

Figures and artistic images were created by the author, R Smit, using Microsoft® Word, CorelDraw®. Anatomical images were used from a certified medical website, SERVIER® which provided images without copyright. The necessary permission was obtained from the corresponding authors for all copyright images used in this study.

ACKNOWLEDGEMENTS

I would like to convey a special word of thanks to the following people who contributed by sharing knowledge or applauded and motivated me in achieving my personal goal.

My parents, Johan Smit and Elsabè Smit, thank you for your sympathetic ear and understanding. I will always be grateful for your love and support. I would like to thank you for always believing in me even though it was not always easy. Thank you for always drying my tears on days when everything felt too much. There are not enough words to express how grateful I am to you for everything you have done for me. Dad, you've always been the backbone of my life. I've never seen you cry, even in the darkest moments of your life. Mom, you're the most amazing person on earth; you have always taught me to be kind to others. You taught me to be considerate and to maintain composure when dealing with someone in various types of challenges, which made me the person I am today.

My husband, Ockert Taljaard, thank you for engaging me in thought-provoking conversations and offering enjoyable distractions to give my mind a break from my research. I could never fully express the depth of my love for you. Thank you for believing in me and supporting me through this journey. Thank you for your patience. Thank you for always being there for me and going through the difficulties with me. You not only accompany me through these experiences, but you patiently talk me through them, never giving up until I am feeling better again. I feel incredibly fortunate to be married to someone who not only comprehends my moods and anxiety but also cherishes me as I am. I love you so much.

My mother-in-law, Naomi Taljaard, without your contribution, this journey could not have been completed. Your good actions and words are forever engraved in my heart. Thank you for what you have taught me during this journey. I know I do not say it nearly often enough but thank you for all the times you have been there for me.

My family, I would like to thank my you for your love and care during the time of this study, particularly my cousin, Dries Smit, who aided with editorial details on several images. I appreciate your kindness and loving heart.

My sister, Amey Klopper, thank you for your beautiful heart, your merciful temper and your gentle nature towards me when I was in times of need. I can never forget the love, care, support and concern you showed towards me and the direction and guidance you provided me during editorial details on several images. In times of sadness, you always motivated me and encouraged me to keep going. I am so grateful that you are my sister.

My supervisor, Prof Leandi Lammertyn and Dr Esmè Jansen van Vuren, thank you for all your professional contributions, help, insight and patience. Thank you for your encouragement and constructive criticism throughout mt academic years. I am very grateful for all the support that you extended to me during this time. I deeply appreciate how you continuously encouraged and guided me in the last three years and how you were always friendly and supportive of all my efforts and during all my struggles.

My co-supervisor and co-authors, Prof Ruan Kruger, Dr Hannes Holm and Prof Martin Magnusson, thank you for your encouragement with my research and for allowing me to grow. Your insightful feedback helped me sharpen my thinking and knowledge and brought my work to a higher level.

The National Research Foundation for financial support for this study.

Ms Clarina Vorster, thank you for your involvement as the language editor of this dissertation. I truly appreciate your patience and punctuality throughout the work on this dissertation.

Finally, I want to thank **God.** It is You who helped me to overcome all obstacles and hold on to my dreams and passion. I am immensely grateful for You blessing me with this amazing triumph. I experienced your guidance and love every day of this journey. You are the One who guided me through this time and I will always trust You with my future endeavors.

"Twenty years from now you will be more disappointed by the things that you didn't do than by ones you did do. So, throw off the bowlines. Sail away from the safe harbour. Catch the trade winds in your sails. Explore. Jump. Dream. Discover" – Mark Twain.

AUTHORS CONTRIBUTIONS

The role of each researcher involved in this study is as follows:

Ms R Smit (BSc Hons) was responsible for compiling a research proposal and obtaining Ethics approval for the current MHSc study. Ms Smit was primary responsible for the writing of all the chapters included in this dissertation and conducted all the statistical analyses with the guidance of her supervisory team.

Prof L Lammertyn (PhD) as supervisor, contributed to the initial planning and collection of data of the main Sympathetic Activity and Ambulatory Blood Pressure in Africans study. Assisted with the initial planning of this MHSc study as well as the ethics application, supervised the statistical analyses as well as the writing of the dissertation. Additionally, Prof Lammertyn was responsible for the monitoring of the study and submitting regular progress reports to the relevant committees.

Dr E Jansen van Vuren (PhD) as co-supervisor supervised the proposal compilation, statistical analyses and writing of the dissertation.

Prof M Magnusson (MD) as co-supervisor provided input into the interpretation and explanation of the results in the dissertation.

Dr H Holm (MD) as co-supervisor, provided input into the interpretation and explanation of the results in the dissertation.

Prof R Kruger (PhD) as co-author of the manuscript, provided input on the writing of the manuscript presented in chapter three.

I, René Smit, hereby declare that the statement confirms the actual contribution and that I have permission that this manuscript may be published as part of the dissertation of the degree Master of Science in Cardiovascular Physiology.

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

APPENDICES

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

LIST OF ABBREVIATIONS

Symbols/Abbreviation	Description
A	Alpha
B	Beta
y	Gamma
°C	Degree Celsius
%	Percentage
ABPM	Ambulatory blood pressure monitor
ACTH	Adrenocorticotropin hormone
AVP	Arginine vasopressin
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CIMT	Carotid intima-media thickness
Cm	Centimeter
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DSM-IV	Diagnostic Statistical Manual of Mental Disorders
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetate
GGT	Gamma-glutamyl transferase
HbA1c	Glycosylated haemoglobin A1c
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HRV	Heart rate variability
IL-6	Interleukin-6
Kcal	Kilocalorie
kg/m^2	Kilogram per square meter
LDL	Low-density lipoprotein
MAP	Mean arterial pressure
mg/l	Milligram per liter

Mm	Millimeter
mmHg	Millimeter of mercury
mmol/l	Millimoles per liter
MMP-9	Matrix metalloproteinase 9
m/s	Meter per second
N	Total participants
NADPH	Nicotinamide adenine dinucleotide phosphate
NE	Norepinephrine
Ng	Nano-gram
NGF	Nerve growth factor
p75	Pan neurotrophic factor
Pg	Pico-gram
PHQ-9	Patient health Questionniare-9
PI	Principle investigator
PP	Pulse pressure
PTSD	Post-traumatic stress disorder
PWV	Pulse wave velocity
SABPA	Sympathetic Activity and Ambulatory Blood Pressure in Africans
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor-alpha
Trk	Tropomyosin-related kinase family receptor
U/l	Units per liter
WC	Waist circumference
WHO	World Health Organization

SUMMARY

Motivation:

Several researchers suggest that neurotrophic factors play an important role in depression. Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors that has been investigated and it is reported that low BDNF levels occur in individuals with depression.

Brain-derived neurotrophic factor has been shown to have diverse effects on the cardiovascular (CV) system, which include angiogenesis, protective effects against oxidative stress, proinflammation and regulation of vascular tone and function. Adequate levels are therefore linked to vascular protection while low levels are associated with endothelial dysfunction and vascular remodeling. The low levels observed in individuals with depression may increase the cardiovascular disease (CVD) risk. In our study population, it has already been shown that specifically the Black population has decreased BDNF levels and increased carotid intima-media thickening when compared to their white counterparts. Many studies that originated from our study population reported significant results regarding depression. They predicted that chronic depression was associated with CV changes, such as microvasculature regulation and perfusion deficits.

Based on these prior results, we wanted to investigate whether BDNF associates with markers of vascular remodeling and function in Black individuals with and without moderate to severe symptoms of depression of the SABPA study population.

Aim and Objectives

Our aim was to investigate differences in BDNF levels as well as markers of vascular remodeling, carotid intima-media thickness (CIMT) and pulse wave velocity (PWV) in Black individuals with and without depression and whether relationships between these markers exist in each group.

Methodology

The study sample included 193 Black teachers (mean age of 44; 50% women) from the Dr Kenneth Kaunda Education district in the North West Province, South Africa. Depressive symptoms were obtained by the Patient Health Questionnaire-9 (PHQ-9). The recommended cut-off point of ≥10 indicated the presence of depression. For this study, individuals were grouped according to their depressive symptoms. The recommended PHQ-9 cut-off point of ≥10 indicated the presence of moderate-to-severe depression; a score below 10 indicated individuals without depression. A high-resolution ultrasound scan with CIMT images from at least two angles of the left and right common carotid artery was obtained by using a SonoSite Micromax ultrasound

system. Pulse wave velocity was obtained with the carotid-dorsalis pedis PWV using Complior SP Acquisition System (Artech-Medical, Pantin, France). Serum BDNF was measured by Quantikine Colorometric Sandwich Immunoassay, with intra-assay precision between 3.8% and 4.2% and inter-assay coefficient of variation (CV) <7.6% and 11.3%.

Results and Conclusion

We observed that 44% of the population had moderate to severe depressive symptoms. The groups with and without depression characteristics were similar as no statistical differences were observed in the variables under investigation. Despite this, single regression analyses indicated an inverse relationship exist between CIMT and BDNF in individuals without depression, but not in individuals with depression (non-depressive: r=-0.214; p=0.027); depressive: r=-0.030; p=0.781). After performing multiple regression analyses adjusted for age, sex, waist circumference, glucose, CRP, LDL cholesterol, MAP and GGT an independent inverse association was confirmed between CIMT and BDNF in individuals without depression (β=-0.19; p=0.026). No significant results were observed between BDNF and PWV in both groups.

Based on our results, we rejected our hypotheses since we were unable to replicate earlier findings that suggested that depression was accompanied by lower levels of BDNF and higher measures of CIMT and PWV. Despite this, our results indicate the possibility that BDNF has a protective role in maintaining endothelial barrier integrity and promoting smooth muscle cell activity in individuals without depression. The absence of the expected results between BDNF, CIMT and PWV in individuals with depression may indicate that the interplay between BDNF and the vasculature could be compromised, although further investigation is required.

Keywords: cardiovascular disease, neurotrophic factor, endothelial dysfunction, arterial stiffness, vascular structure.

TABLE OF CONTENTS

CHAPTER 1

1. Introduction

Depression is a mental health disorder characterized by persistently depressed mood, or loss of interest in daily activities affecting an individual's health and function in society (1). Depression is complex in measure and international standards for guiding the diagnosis of mental illness have been established as the Diagnostic Statistical Manual version 5 (DSM-V) (2). Several possible theories have been proposed to play a role in major depressive disorders. These theories include the monoamine deficiency, altered HPA axis regulation, neuroinflammation and the neurotrophic hypothesis. In this MHSc study, we focused on the neurotrophic marker, brain-derived neurotrophic factor (BDNF) and its role in cardiovascular disease (CVD) in individuals with moderate to severe depressive symptoms (3-5).

Brain-derived neurotrophic factor is an important protein in the process of developing depression and the treatment thereof (5). Brain-derived neurotrophic factor is a protein found in the brain and spinal cord where it maintains the survival of the nerve cells by its role in the growth, differentiation and maintenance of these cells (6). Brain-derived neurotrophic factor is commonly found in brain regions such as the hippocampus, amygdala, cerebellum, and cerebral cortex, whereas small amounts are also found in non-neuronal tissues such as the liver, heart, and lungs (7). According to Rajan et al. (2020) and Waitzfelder et al. (2018), depression have received significant attention in the past as an independent risk for CVD (8, 9). Lower BDNF is linked to cardiovascular (CV) risk factors which include lipid levels, elderly age, male sex, smoking, diabetes mellitus, and physical inactivity (10-13), possibly leading to the development of CVD.

Cardiovascular disease, including heart disease and stroke forms part of the leading causes of disabilities and death worldwide, accounting for 17 million deaths per year. However, CVD is among the top three causes of deaths in sub-Saharan Africa (14). This might have been due to the rapid urbanization which resulted in an upsurge of coronary heart and coronary artery disease (CAD) and metabolic disorders (15). Further studies have reported that black Americans have a higher risk to develop CVD, compared to many other ethnic groups in America (16).

Large amounts of literature have shown that there are many discrepancies in the prevalence and impact of cardiovascular disease and depression among different racial and ethnic groups, including black and white (17, 18). These health risks are complex and are influenced by various factors including socio-economic status, access to healthcare, lifestyle, genetics and system racism. Blacks have higher rates of certain risk factors for CVD such as hypertension, diabetes mellitus and obesity, which increases their prevalence of CVD (18-20). Cardiovascular disease and psychiatric disorders are linked and investigators have shown that race is associated with cardiovascular disease and general anxiety disorder (20). Black Africans from the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) cohort demonstrated higher

prevalence of depressive symptoms compared to their white counterparts (21). Further literature originated from the SABPA cohort, reported significant results of depression and BDNF and the affect it had on the CV system in the black population (4, 22-24).

This chapter constitutes a literature review that focuses on the possible interactions between depression, BDNF and vascular remodelling and function markers, specifically in Black individuals from developing countries, such as South Africa.

1.1 Depression

Depression is a widespread chronic mental illness that can affect thoughts, mood, and physical health (25). There has been significant research regarding the understanding and treatment of major depressive disorder; however, despite these advances, depression is one of the most common mental disorders and can cause tremendous challenges and burden for individuals and families. It also carries a large economic burden. The economic burden of major depression among American adults was estimated to exceed 230 billion dollars in 2018 (26), compared to the estimated 10.2 billion dollars for 2016 from CVD (27). The prevalence of depression in black developing countries can be based on factors such as socio-economic status and access to health care. Depression is a significant health concern in the sub-Saharan Africa. In these regions, factors such as poverty, unemployment, exposure to violence and limited access to mental health services can contribute to the development of depression (28).

Depression is often persistent despite changes of circumstances and causes feelings that are intense, chronic, and not proportional to a person's circumstances (29). Depression is characterized by symptoms of loss of interest and enjoyment, low mood and reduced energy (29). There are many possible causes of depression which include stressful life events, genetic vulnerability, environmental factors such as trauma or lack of social support and additional mental health conditions such as bipolar disorders and schizophrenia (30). Depression may also develop due to the use of certain medication such as isotretinoin (treatment of acne), antiretroviral drugs (treatment of human immunodeficiency virus), and corticosteroids (treatment of rheumatoid arthritis, inflammatory bowel disease, asthma, and allergies) (31). To be diagnosed with depression an individual must present with at least five depressive symptoms set out by the DSM-V criteria in **Table 1.1** (32). These symptoms should persist every day, nearly all day, for at least two weeks (33).

Table 1.1. **Symptoms for the diagnosis of depression as described by Kocsis (33)**

1	Depressed mood most of the day. This includes symptoms such as sadness and hopelessness in young individuals, an irritable mood could also be classified as depressive symptoms.

2	Loss of interest or pleasure in most of the activities of the day.
3	Symptoms such as weight gain or weight loss. Other symptoms include an increase and/or a decrease in appetite.
4	Insomnia or hypersomnia daily.
5	Psychomotor agitation which includes pacing around the room, tapping toes, or rapid talking. These symptoms often occur with anxiety disorders and mania. Other symptoms include psychomotor retardation which involves slowing down of thoughts and physical movement.
6	Fatigue or loss of interest daily.
7	Feelings of worthlessness or inappropriate guilt.
8	Decrease ability to think or concentrate.
9	Suicidal ideation, thoughts about death, or suicide attempt.

The Patient Health Questionnaire-9 (PHQ-9) is a tool for determining the severity of depressive symptoms. The PHQ-9 is based on the nine criteria for major depressive disorder outlined in the DSM-V, each of which is scored 0-3 providing a severity score of 0 to 27. Minimal depression is indicated with a score of 0-4, mild depression is indicated with a score of 5-9 and moderate depression is indicated with a score of 10-14. Moderately severe depression is indicated with a score of 15-19, and severe depression is indicated with a score of 20-27 (34). A score of 10 or higher is often considered a potential indicator of clinically significant depressive symptoms (35). It is recommended that a health care professional should consider the individual's overall mental health, medical history and other relevant factors before making a diagnosis and recommending appropriate treatment options (34).

It is important to note that individuals can suffer from depressive symptoms but not necessarily be diagnosed with major depressive disorder, as seen in any other mental health disorder (36). Therefore, in this dissertation, we grouped our individuals as pointed out above. A higher total score indicates a higher level of symptom severity, and by extension, a greater impact on an individual's daily life (34).

To understand depression, previous investigators explored the physiology and anatomy of the brain to explain changes that arose during depression (37). Possible factors leading to depression includes the involvement of BDNF and its receptor, tyrosine kinase B (TrkB) which mediates atrophy and structural changes in the brain (38, 39).

Possible brain areas that could be involved in depression are the prefrontal cortex, the limbic structure, basal ganglia, and the brainstem structure (37). The limbic structure includes the amygdala, hippocampus and brainstem **(Figure 1.1)**. Untreated depression may lead to

neurodegenerative changes in the brain; however, these changes are only seen in individuals with depression for more than ten years (40).

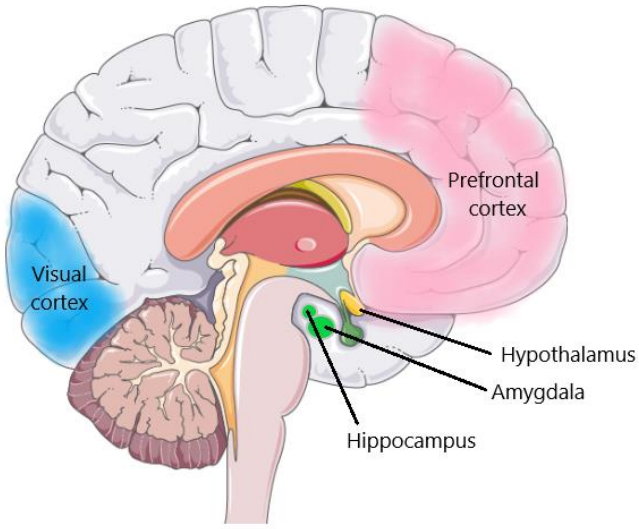


Figure 1.1 Schematic representation of brain regions affected by major depressive disorder.

1.1.1 Prefrontal cortex

The prefrontal cortex is known for its function in high-order guidance of thoughts, attention, behaviour and emotions (37, 41). The prefrontal cortex can be divided into two regions, namely the dorsolateral regions which is important during regulation and cognition and ventrolateral regions responsible for emotions **(Table 1.2)**. The dorsolateral prefrontal cortex is responsible for its major role in cognitive processes such as planning, cognitive flexibility and working memory (42). This area is known for problem solving and maintaining attention during certain tasks. Functional and structural prefrontal cortex abnormalities lead to increased prevalence of major depressive disorder (43); however, when the prefrontal cortex is damaged, it could also possibly lead to problems regulating emotions, further leading to psychiatric disorders such as depression (41). The ventromedial prefrontal cortex is involved in emotion, rewards, motivation, threat detection and fear (44).

Table 1.2. **Differences between the ventrolateral and -dorsolateral prefrontal cortex**

Ventrolateral prefrontal cortex	**Dorsolateral cortex**
During major depressive disorder and suicidal thinking, there is a decrease in the volume of the ventrolateral prefrontal cortex. It was also reported that there is inefficient processing of	The dorsolateral prefrontal cortex may contribute to depression due to its involvement in emotional levels (46). The dorsolateral prefrontal cortex has been found to be

emotions which leads to poorly dangerous behaviours **(45)**.	underactive in major depressive disorder (47). The prefrontal cortex also connects to other areas that are involved in mood regulation which include the insula, anterior cingulate cortex, and the amygdala (47)

The prefrontal cortex has been researched with regard to major depressive disorder (43), revealing clear evidence of structural and functional alterations in the prefrontal cortex (45).

1.1.2 Amygdala

The amygdala is commonly known for the processing of fearful and threatening stimuli, which include the detection of threats and the activation of fear-related behaviours in response to these stimuli (48). The amygdala is also responsible for emotions and emotional behaviours such as fear, anger, aggression, stress and motivation (49). Functional imaging studies conducted during depression have suggested alterations in the amygdala's function, demonstrating elevated activity that tends to normalize with the use of anti-depressant drugs (50). However, untreated depression leads to increased activity which enlarges the amygdala (50). Due to the higher activity of the amygdala, it connects several other brain regions that sharpen the behavioural response to the emotional stimuli such as an examination, divorce, death of a loved one, moving from houses and loss of job (51).

1.1.3 Hippocampus

The hippocampus is a complex brain structure that has an important role in learning, long-term memory and spatial navigation (52). This indicates the association between the hippocampus and the limbic structure, which also forms part of the brain that regulates our behaviour and emotional responses (53). The hippocampus contains high levels of glucocorticoids, which makes it more vulnerable to depression than other parts of the brain. While glucocorticoids are essential for healthy functioning of the body, depression often involves an excessive activation of these hormones, resulting in hippocampus atrophy. The atrophy of the hippocampus results from neuronal loss, inhibition of neurogenesis, dendritic atrophy, or reduction of neurotrophic factors (54).

1.1.4 Basal ganglia (not pictured)

The basal ganglia are associated with numerous functions which include control of movement, habit learning, conditional learning, eye movement, cognition and emotions (55). A decrease in the basal ganglia volume has previously been reported in individuals with major depressive disorder (56).

1.1.5 Brainstem

The brainstem is an essential part of the brain that plays a role in the central nervous system (CNS) and controls many subconscious body function, like breathing and heart rate (HR) (57). Other functions include consciousness, blood pressure and sleeping patterns (58). Song et al. (2014) found that, within the context of depression, the association between the brainstem and the amygdala is altered, indicating a potential mechanism that contributes to the development of depression (59).

1.2 Physiological Mechanisms Involved in Major Depressive Disorder.

1.2.1 Neurotransmitters in Depression

The different brain regions use chemical substances as messengers to communicate with other parts of itself and with the nervous system (60). These messengers are known as neurotransmitters and are released by several different neurons (60). Several researchers indicates that there are three primary monoamine neurotransmitters. They include dopamine, serotonin and norepinephrine, which play a role in the etiology of major depressive disorder. In the past, the monoamine deficiency theory served as the pathophysiological basis of depression due to the depletion of the neurotransmitters such as serotonin, norepinephrine, or dopamine in the CNS of depressed individuals (3). However, more recent evidence has suggested that low levels of monoamines do not necessarily induce or worsen symptoms of depression in individuals with and without depression. This means that monoamine deficiency alone is not sufficient for a clinical diagnosis (61). Further, dopamine plays a crucial role in mood, leading to positive feelings associated with reward; however, alterations in its release, reuptake or receptor sensitivity plays an important role in various psychiatric effects of the brain such as Parkinson's disease and schizophrenia (62). Research indicated that decreased levels in dopamine levels contribute to depression in some individuals (62). The norepinephrine hypothesis states that decreased activity or availability of norepinephrine in certain brain regions may be associated with the development of depression (63). One of the oldest hypotheses states that depression occurs due to the deficiency of serotonin and/or norepinephrine in the brain, which leads to increased possibility of depression. Genetic studies showed that mice with genetically engineered enhancements of norepinephrine protected the mice from stress-induced depression-like behaviours, whereas depression of norepinephrine results in depressive symptoms (63). Serotonin is the most extensively researched neurotransmitter in depression. A reductions in serotonin levels leads to the development of depressive symptoms in subjects prone to depression (3). The best evidence that indicates serotonin's involvement in the pathophysiology of depression is tryptophan depletion, which reduces central serotonin synthesis (3). Reduced serotonin concentrations have

also been associated with several other psychiatric conditions including anxiety, aggression, compulsive behaviour, substance abuse, season affective disorders, mania, schizophrenia and behavioural disorders (64).

1.2.2 Neuroinflammation in Depression

Neuroinflammation refers to inflammation that occurs in the central nervous system (CNS), which includes the brain and spinal cord. Neuroinflammation can be triggered by various factors including the overproduction of different cells which includes cytokines, chemokines, reactive oxygen and secondary messengers; where they are produced by microglia and astrocytes, endothelial cells and peripherally derived immune cells (65). Neuroinflammation has been associated with the pathophysiology of major depressive disorder through an increase in proinflammatory cytokines and is known to affect 27% of patients with major depressive disorder (66). Neuroinflammation is also seen in many different neurological disorders that have high rates of comorbidity with major depressive disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, stroke and migraine (67-70).

The neuroinflammation hypothesis of depression posits that inflammation in the CNS, particularly in the brain, plays a significant role in the development and maintenance of depression. This is induced by an elevated inflammatory response in the CNS, which contributes to the development of depression through the effects of proinflammatory cytokines and several other metabolites from inflammatory processes (65). Clinical studies have reported associations between depression and increased levels of various markers of immune activation, including circulating immune cells such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (71, 72). Lima Giacobbo et al. have reported that patients with depression have been known for abnormal BDNF levels which may be due to chronic inflammation; therefore, neuroinflammation is known to affect BDNF-related signalling pathways (73).

Research had further indicated that during the treatment of inflammation there is an improvement of depressive symptoms to a certain extend (74). Repeated stress does not only influence synaptic plasticity and neurotransmitter dysregulation, but also increase levels of pro-inflammatory cytokines in the brain and blood, which supports the hypothesis of depression and inflammation (75).

1.2.3 Hypothalamic-Pituitary-Adrenal Axis in Depression

During the HPA axis activation, there is an increased secretion of the corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus; however, during disease states such as depression, this process is aggravated (76). With overactivation of the HPA axis, individuals may develop further depressive symptoms

where CRH itself leads to a further increase in the sympathetic nervous system activity and resting HR. Corticotropin-releasing hormone activates the pituitary gland to secrete adrenocorticotropin hormone (ACTH) in the bloodstream (77). From here on, ACTH binds to its receptor on the adrenal cortex, leading to increased glucocorticoid levels that may result in the reduction of BDNF expression in the hippocampus (78). Excessive glucocorticoids supress the binding between BDNF and its receptor, TrkB which leads to a decrease in BDNF signalling (78).

1.3 Neurotrophic Factors

Levi-Montalcini (1987) identified the first neurotrophic factor known as nerve growth factor (NGF), which plays a protective role in developing the sympathetic nervous system. Nerve growth factor is important in the development of sympathetic, sensory and forebrain cholinergic neurons (79, 80). Together with NGF, other neurotrophic factors are also part of the family of biomolecules which include neurotrophin-3 (NT-3), NT-4, NT-5, NT-6, NT-9, ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF) and BDNF (81-83). In this dissertation, we only focused on BDNF and its diverse effects on depression and the cardiovascular system.

1.3.1 Brain-derived neurotrophic factor

Brain-derived neurotrophic factor is most known to act on certain neurons in the central and peripheral nervous system, helping to support survival of neurons and enhancing the growth and differentiation of new neurons and synapses (84-88). Brain-derived neurotrophic factor occur first as a precursor protein that undergoes intracellular cleaving to form a mature protein consisting of 118-120 amino acids (89). The precursor proBDNF (preproBDNF) is synthesized in the endothelial reticulum, where it is converted to proBDNF and transported to the Golgi for organisation into secretory vesicles (90, 91). The second mechanism incorporates a key candidate known as matrix metalloproteinase-9 (MMP-9) which is highly expressed in the hippocampus and has shown to convert proBDNF to mature BDNF (92).

Every neurotrophic factor exerts its function through several types of receptors (93). For many years, it has been reported that proBDNF binds to low affinity pan neurotrophic factor (p75), while mature BDNF preferentially activates the Trk receptors (93). There are various subtypes of Trk receptors which include TrkA, TrkB, and TrkC, derived from different messenger ribonucleic acid (mRNA) splicing events (82, 94). Each isoform is specifically made to support different neurotrophin. It has been reported that TrkA is primarily associated with NGF signalling that promotes the survival and differentiation of sensory- and sympathetic neurons. TrkB receptors are activated by BDNF, NT-4, NT-5 where it plays an important role in the survival and differentiation of various neurons, as well as various cardiovascular systems **(Figure 1.2)**. Lastly, TrkC receptors is activated by NT-3 which has an important role in sensory and motor neurons

(95). Importantly, Trk receptors are also present in the vascular endothelial cells and vascular smooth muscle cells of the CV system and suggests an autocrine control for CV cell functions by neurotrophins (96).

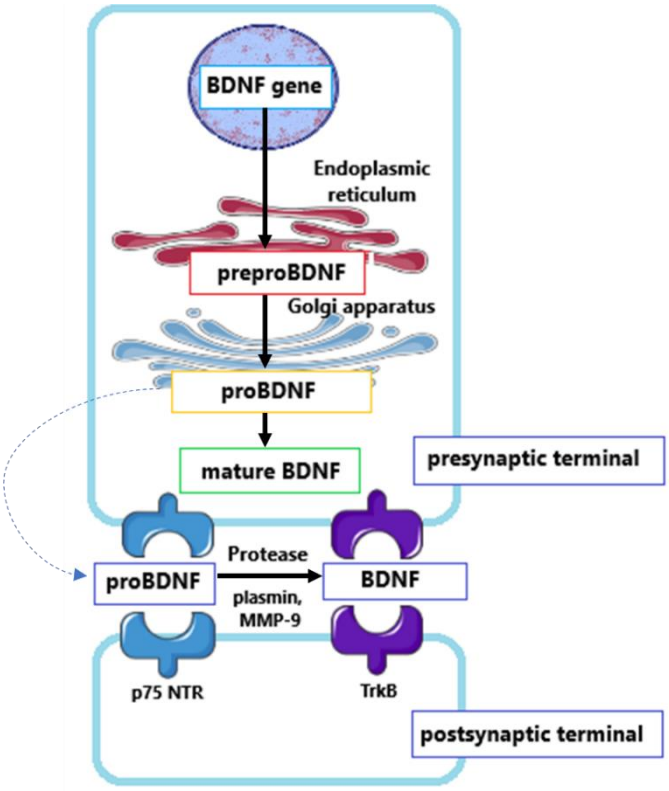


Figure 1.2. Brain-derived neurotrophic factor and synthesis and binding. Where: BDNF, brain-derived neurotrophic factor; p75, pan neurotrophic factor; TrkB, tyrosine kinase B; MMP-9, matrix metalloproteinase-9.

Brain-derived neurotrophic factor is structurally like NT-3, NT-4, and NT-5, which all binds to the TrkB receptor. Brain-derived neurotrophic factor is normally found in the hippocampus, amygdala, cerebellum and cerebral cortex in both humans and animals, with the highest levels found in the hippocampal neurons (7). Studies reported the following:

- *Amygdala*: there is reduced plasma BDNF in individuals suffering from major depressive disorder and the neurotrophic model proposes that stress decrease the expression of BDNF in the limbic regions, contributing to the development of depression (97).
- *Hippocampus:* there are studies that reported that prolonged anxiety or depression, leads to a significant decrease and secretion of BDNF levels in the hippocampus (7, 98).
- *Brainstem:* during depression, there is an overactivation of the HPA axis activity as well as the release of glucocorticoids, which in turn leads to decreased levels of BDNF (99).

Despite BDNF's role in neuronal tissue, neurotrophic factors also exert unique CV activity. Brain-derived neurotrophic factor is secreted from several different cells, where it acts locally and over short distances in the CV system (100). Chacon-Fernandez et al. (2016) reported that BDNF is synthesized in the platelets that circulates in the bloodstream (101). This is discussed later.

1.3.2 The role of the Epigenetic Mechanism in Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor is regulated with the help of an epigenetic mechanism known as deoxyribonucleic acid (DNA) methylation, histone acetylation and other chemical alterations in gene promotor regions (102, 103). Moore et al. (2013) indicated that DNA methylation of cytosine in cytosine-guanine (CpG) dinucleotides is one of the major factors responsible for changes in the BDNF gene locus (104). The locus of BDNF is highly complex and consists of 11 axons and nine promotors (105). There is a definite relationship between increased CpG methylation and the BDNF gene and decreased BDNF secretion (103, 106, 107). In the general population, increased BDNF methylation also correlate with depression (103). Several other studies indicated that individuals suffering from mood disorders have higher DNA methylation percentages, leading to decreased BDNF (103, 105).

Deoxyribonucleic acid methylation is one of the important mechanisms, which change gene expression and is correlated with atherosclerosis (108-111). Several researchers reported on the key role it initiates on atherosclerosis (109, 112). Further research indicated that alterations in DNA methylation have been associated with chronic inflammatory profile diseases (109). Inflammatory conditions have associated with hypermethylation of BDNF which is connected to increased mortality rate in atherosclerotic-related and CAD (109).

1.3.3 Cardio-Protective Mechanism of Brain-Derived N Neurotrophic Factor

The effects of BDNF on cardiac development were first identified through a study done on BDNF deficient mice, which exhibited early postnatal lethality and presented with defects which include atrial septation and intramyocardial vessel fragility and haemorrhage, leading to reduced ejection fraction (113). Notably, a study from the Framingham cohort that prospectively investigating the association of circulating BDNF levels with CV events and mortality in 3687 participants (mean age 65 years, 2068 women) and then performing Mendelian randomization experiment in the CARDIoGRAM (Coronary ARtery DIsease Genome-Wide Replication and Meta-Analysis) consortium (20,000 CAD, >60,000 controls) suggested a casual protective role of BDNF in the pathogenesis of CVD (114).

Brain-derived neurotrophic factor is secreted from different cells such as cardiomyocytes, endothelial cells, vascular smooth muscle cells and fibroblasts, where it exerts locally and acts

over short distances in the CV system (101). Brain-derived neurotrophic factor is synthesized and packed in alpha granules of megakaryocytes, the platelet precursor (101). When platelets are activated, such as in response to injury or inflammation, the contents of their granules, including BDNF, can be released in high concentrations (115). Brain-derived neurotrophic factor's receptor, TrkB has been found to be expressed in endothelial cells. The activation of TrkB has been associated with the regulation of vascular permeability and may also play a significant role in maintaining endothelial barrier integrity and promoting smooth muscle cell activity (116). Donovan et al. (1995) found that vascular smooth muscle cells release Trk receptors (117). Cardiac endothelial cells in atherosclerotic lesions in humans also release TrkB receptors, whereas a decrease in TrkB is necessary for the regulation of vascular endothelial cadherin expression, a molecule responsible for cell proliferation and apoptosis (118). Hereby, it is reasonable to speculate that increased TrkB activity protects against CV atherosclerosis. From the latter, increased BDNF/TrkB play a cardio-protective role against the development of atherosclerosis, whereas it also decreases the risk for CVD (119).

1.4 Atherosclerosis and Arteriosclerosis

Atherosclerosis is a condition characterized by the build-up of plaque inside the arteries (120, 121). Atherosclerosis is a progressive disorder that involves large and medium-sized arteries and involves the accumulation of fatty deposits, cholesterol, calcium and other substances in the inner walls of the arteries (120, 121). These deposits, known as plaque, can gradually narrow and harden the arteries restricting blood flow, leading to reduced supply of oxygen-rich blood to vital organs (122, 123).

The arterial wall consists of three layers which include the intima (innermost layer), media (middle layer), and adventitia (outer layer). The endothelium is a thin layer of cells that play an important role in regulating vascular tone, blood flow and immune response (124). The endothelial cells further maintain a non-adhesive and anti-inflammatory surface, preventing the adhesion of white blood cells and platelets. Furthermore, the arterial wall is constantly exposed to lipids, where the arterial wall metabolizes and remove the excess lipids, preventing the build-up of plaque (125). During the development of atherosclerosis, normal physiological process is disrupted which leads to the accumulation of lipids and different inflammatory substances in the arterial wall (126). Despite numerous research being done, the mechanism regarding atherosclerosis is still controversial and not yet well understood.

Upon injury to the endothelial cells, the innermost layer of the arterial walls' white blood cells and several other inflammatory cells are recruited. These cells release different cytokines and other molecules that cause the endothelium to become permeable, allowing lipids and other substances to enter the arterial wall (127, 128). Low-density lipoprotein (LDL) cholesterol particles enter the

arterial wall, trapping them in the intima (129). Low-density lipoprotein cholesterol trapping increases the concentration of LDL cholesterol in the intima, which leads to immediate oxidation of LDL particles (129). Due to atherogenesis, monocytes, and T-lymphocytes also infiltrate into the vascular intima (129). All these events include the accumulation of cholesterol and other lipids in the arterial wall, forming a fatty streak (127, 128). Oxidized LDL cholesterol particles come into contact with several other LDL particles, subsequently attracting and activating white blood cells such as monocytes. Monocytes are now rapidly recruited to the arterial wall, where they differentiate into macrophages and dendritic cells (130). Macrophages are recruited to the injured site, resulting in macrophage-derived foam cells. Macrophage-derived foam cells in the intima die through a process known as apoptosis. Apoptosis further leads to the growth of atherosclerotic plaque, where monocytes lead to the production of cytotoxic substances such as tumour necrosis factor alpha (TNF-α), growth factors, and free radicals, which further lead to more damage to the endothelium cells, as well as more LDL cholesterol oxidation (129).

Further damage to the vascular cells occurs when smooth muscle cells and endothelial cells secrete peptides, including cytokines, and growth factors such as interleukin-1 (IL-1), IL-6, and TNF-α (129). Smooth muscle cells now migrate to form fibrous caps. Fibrous caps are composed of collagen fibre tissues, smooth muscle cells, macrophages and T lymphocytes, that all contribute to mature atherosclerosis **(Figure 1.3)** (129).

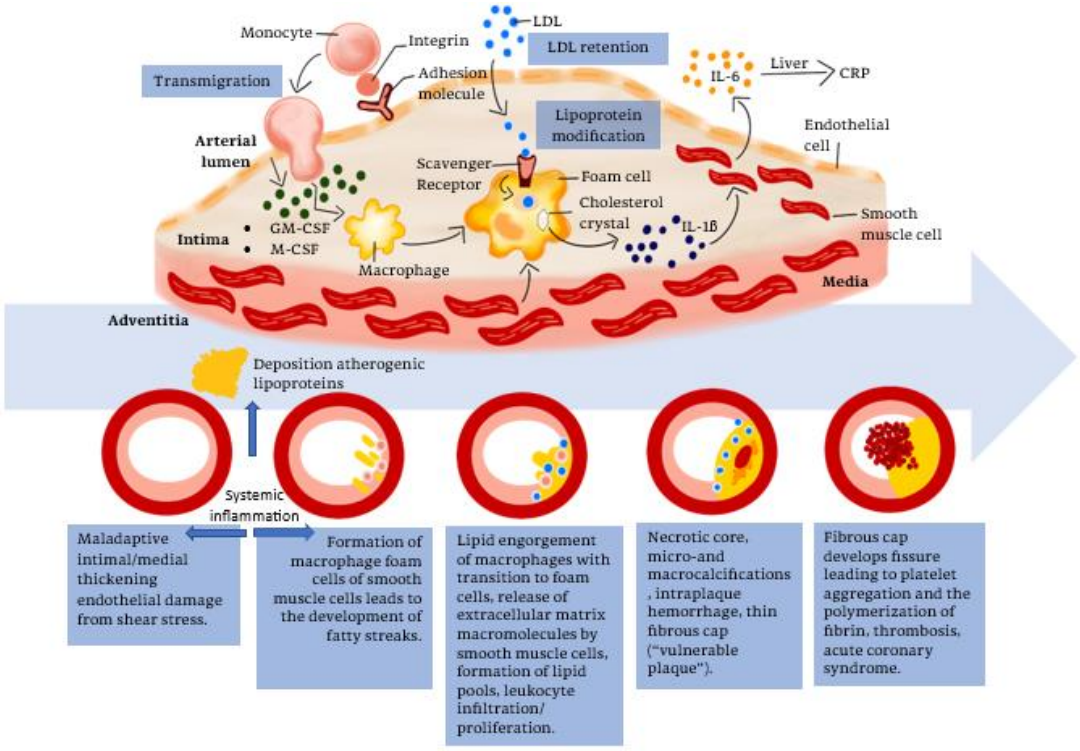


Figure 1.3. Schematic representation of the process of atherosclerotic disease.

Over time, plaque build-up tends to lead to hardening of the arterial wall, which reduces the ability of the arterial wall to expand and contract in response to several physiological changes resulting in the loss of elasticity (131).

Arterial stiffness or arteriosclerosis is mostly associated with but can also occur separate from atherosclerosis, which is an intimal abnormality that represents a consequence of arteriosclerosis. Therefore, atherosclerosis is literally the hardening of arteries (132). Arteriosclerosis is accompanied by aging and is associated with major adverse impacts on the CV system (132). Other risk factors include age, smoking, hypertension, diabetes mellitus, obesity and high cholesterol levels (133). The pathophysiological process of arteriosclerosis is a complex process that involves a combination of structural and functional changes in the arterial wall (134, 135). Arterial stiffness is further worsened by the tone of the smooth muscle cells in the arterial wall. Together with the above-mentioned CV risk factors, the smooth muscle cells become hypercontractile, leading to an increase in arterial tone and stiffness (133).

Another important risk factor includes inflammation. Inflammation contributes to the development of arterial stiffness, as it leads to the breakdown of elastin fibres and the deposition of collagen in the arterial wall (136). Several mechanisms of inflammation lead to arterial stiffness. These mechanisms include inflammation which is associated with endothelial dysfunction which in turn regulates smooth muscle tone. Inflammation also triggers an increased synthesis of matrix metalloproteinases, decreasing elastin and ultimately resulting in stiffening of arteries. Further several inflammatory mediators directly stimulate vascular calcification, which can lead to stiffening (137). During advanced stages of arteriosclerosis, deposition of inflammatory substances increases to such an extent that it leads to the hardening of the arterial wall, making the arterial wall less flexible (131, 133, 138).

1.5 Cardiovascular Risk Factors, Diseases and Brain-Derived Neurotrophic Factor

Cardiovascular risk factors are characteristics or behaviours that increase the likelihood of developing CVD. These risk factors could include lack of exercise, unhealthy eating, smoking, diabetes mellitus, age and family history. Hypertension is one of the strongest CV risk factors for the development of CVD (139). In addition, there is further evidence that suggests that depression and anxiety are known risk factors for developing CVD such as coronary heart disease (140). Furthermore, independent CV risk factors are known as hypertension, diabetes mellitus and cigarette smoking (140). Plasma BDNF levels was inversely associated with levels of triglycerides and LDL cholesterol, presence of diabetes mellitus, fibrinogen level, male sex and age, and positively associates with high-density lipoprotein (HDL) cholesterol and platelet count **(Table 1.3)** (11).

Table 1.3. **Association between BDNF and CV risk factors**

High BDNF levels	Low BDNF levels
↑ HDL cholesterol (11)	↑ LDL cholesterol (11)
↑ platelet count (11)	↑ total cholesterol
Daily exercise **(141)**	↑ triglycerides (11)
Healthy eating habits **(141)**	↑ blood pressure (142)
	↑ renin-angiotensin system activity (142)
	↑ fibrinogen (11)
	Bad eating habits (141)
	Old age (11, 118)
	Male sex (11)

Reduced BDNF levels have been observed in individuals with depression and these lower levels appear to affect various aspects of blood pressure, including low blood pressure, arterial baroreceptors, the renin-angiotensin system and endothelial nitric oxide (NO) synthase (141, 142). During normal conditions when blood pressure increases, baroreceptors reduce HR and blood pressure by a negative feedback mechanism. Pathophysiological changes influence this mechanism. Choe et al. reported that a high dietary salt intake can decrease the baroreceptor-mediated inhibition of vasopressin neurons through a BDNF-dependent activation of TrkB receptors (143). High salt intake has been a possible contributor to neuropsychiatric disease and has recently been associated with greater depressive symptoms severity in adolescents (144). The combination of prolonged stress together with a high salt intake has been recognized in humans as a predictor for hypertension, particularly in salt-sensitive individuals (145-147). Through these observations, stress-salt interactions are suspected to contribute to the association between stress-related neuropsychiatric diseases and CVD (148). Hereby, they reported that BDNF is required for normal baroreceptor function and HR regulation, but these effects can be reduced in pathophysiological conditions, which can lead to the development of hypertension (143).

Brain-derived neurotrophic factor and its receptor, TrkB, are expressed in various types of cells such as cardiomyocytes, endothelial cells, vascular smooth muscle cells and fibroblasts in the CV system. Here, BDNF/TrkB is associated with the development and outcome of CVD, including coronary artery disease, heart failure, cardiomyopathy, hypertension and metabolic disease (149). During the investigation of BDNF, it has been identified that low levels of BDNF are associated with endothelial dysfunction (141). Endothelial dysfunction has been recognized as the initial phases of atherosclerosis (150). Brain-derived neurotrophic factor is also expressed in

vascular endothelial cells, macrophages, and smooth muscle cells in atherosclerotic coronary arteries (117). Low BDNF levels also lead to plaque instability, due to the activation of oxidative stress which leads to the formation of superoxide utilizing nicotinamide, adenine dinucleotide phosphate (NADPH) oxidase system activation in several vascular structures (151). During diseases such as hypertension, diabetes mellitus, and atherosclerosis there is an increase in vascular superoxide radicals which indicates that oxidative stress is playing a role in atherosclerosis; therefore, correlating with CV risk factors and CVD (152). In accordance with the neurotrophic hypothesis of depression, depressive symptoms can be linked to stress-induced decreases in BDNF due to a mechanism that influence neuroplasticity and neurogenesis and promotes cell atrophy. This mechanism could possibly explain why decreased BDNF levels are often observed in individuals with depression (153). Han et al. reported a significant decreased BDNF serum levels in patients with coronary heart disease compared to healthy individuals (154), this was also the case in previous studies (11). Further, it has been proposed that depression could be linked to several CV risk markers which include bad eating habits, sedentary lifestyle, increased SNS activity, vascular inflammation and platelet activation (155).

Brain-derived neurotrophic factor has also been linked to arterial stiffness; however, the exact relationship is not fully understood (156). Arterial stiffness is also associated with CV risk factors such as aging, hypertension, diabetes mellitus, dyslipidaemia, obesity, and smoking (123). Arterial stiffness is measured using a non-invasive method that assess the speed at which a pulse wave travels along two different point. Pulse wave velocity (PWV) is the most used and well-established method for assessing arterial stiffness; also, it has been reported that PWV becomes higher in stiffened arteries (123). Brain-derived neurotrophic factor has been known to correlate with depression and arterial stiffness, as described previously, where low serum levels of BDNF associated with increased vascular resistance (156). Depressive symptoms further contribute towards subclinical atherosclerosis which results in impaired functional and structural markers of subclinical atherosclerosis (157). In addition, Dietz and Matthews reported in individuals with depression (low depressive symptoms n=52; moderate depressive symptoms n=55; high depressive symptoms n=50) had significant higher measures of PWV (152), indicating a higher risk for arterial stiffness (158). Also, Wu et al. reported on 5947 participants with depressive symptoms and 34,423 participants without depressive symptoms. When these groups were compared, individuals suffering from depressive symptoms showed a significantly higher PWV measures compared to individuals without depressive symptoms (157).

1.6 Problem Statement and Motivation

Depression is a mental health condition that affects several individuals worldwide, where it is reported that depression is one of the leading causes of disability, which significantly affects a

person's quality of life, functioning, and well-being (159). A contributing factor involved in the development of depression is alterations in neurotrophic factor levels. Studies have led to the formation of the neurotrophic hypothesis of depression (5). The neurotrophic hypothesis of depression posits that disruption in neurotrophic factor, particularly decreased levels of BDNF, play a role in the pathophysiology of depression (5). Support for the neurotrophic hypothesis has been tested by various studies, which include those showing that antidepressant medications can increase levels of BDNF and other neurotrophic factors in the brain (5). Animal studies demonstrated that reduce levels of BDNF leads to depressive-like behaviours, while increasing levels of BDNF can have antidepressant effects (160). Further research implicating BDNF's role in depression is still unknown.

Depression is more prevalent in black communities in developing countries, especially in women (161). The prevalence can be attributed to the fact that black individuals face different social pressures, which may make them more susceptible to depression (162). Socioeconomic status, educational opportunities, employment and neighbourhood conditions are important social determinants of health that play a pivotal role in the onset of depression (163). Black Africans from the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) cohort demonstrated higher prevalence of depressive symptoms compared to their white counterparts (21). Further literature originated from the SABPA cohort, reported significant results of depression and BDNF and the link it had on the CV system in the black population (4, 22-24). Higher levels of BDNF have been shown to have diverse effects on the CV system, including promoting angiogenesis, protecting against oxidative stress, inflammation, and regulation of vascular tone and function (124, 164). Studies have reported that there is an association between lower BDNF levels and increased risk for CVD, suggesting that BDNF plays a role in the development and progression of CVD (149, 165, 166). We aimed to explore whether this holds true within our population, focusing particularly on a black population among whom a higher prevalence of depressive symptoms, increased vascular remodelling and CV risk factors have already been documented.

1.7 The Research Aim, Objectives and Hypothesis

The aim of the study:

The overall aim of the study was to investigate differences in BDNF levels as well as markers of vascular remodeling and function, CIMT and PWV in individuals with and without depression and whether relationships between these markers exist in each group.

The objectives of the study:

i. To compare the BDNF, CIMT and PWV measures between individuals with and without depression.
ii. To investigate if independent associations exist between BDNF, CIMT and PWV in individuals with and without depression.

From the literature, we hypothesized that:

i. Individuals with depression exhibit lower BDNF levels and higher CIMT and PWV measures, compared to individuals without depression.
ii. Inverse independent associations will be present between BDNF, CIMT and PWV in individuals with depression, but not in individuals without depression.

1.8 References

1. Alexopoulos GS. Mechanisms and treatment of late-life depression. Transl Psychiatry. 2019;9(1):188.
2. King J. Measuring mental health outcomes in built environment research-Choosing the right screening assessment tools. Centre for Urban Design and Mental Health. 2018.
3. Hasler G. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? World J Psychiatry. 2010;9(3):155.
4. Mashele N. Depressive symptoms and cardiometabolic health in urban black Africans: the SABPA study 2014.
5. Bus BA, Molendijk ML. [The neurotrophic hypothesis of depression]. Tijdschr Psychiatr. 2016;58(3):215-22.
6. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. Arch Med Sci. 2015;11(6):1164-78.
7. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. Front Cell Neurosci. 2019;13:363.
8. Rajan S, McKee M, Rangarajan S, Bangdiwala S, Rosengren A, Gupta R, et al. Association of Symptoms of Depression With Cardiovascular Disease and Mortality in Low-, Middle-, and High-Income Countries. JAMA Psychiatry. 2020;77(10):1052-63.
9. Waitzfelder B, Stewart C, Coleman KJ, Rossom R, Ahmedani BK, Beck A, et al. Treatment Initiation for New Episodes of Depression in Primary Care Settings. J Gen Intern Med. 2018;33(8):1283-91.

10. Jin H, Chen Y, Wang B, Zhu Y, Chen L, Han X, et al. Association between brain-derived neurotrophic factor and von Willebrand factor levels in patients with stable coronary artery disease. BMC cardiovascular disorders. 2018;18(1):1-8.
11. Jiang H, Liu Y, Zhang Y, Chen ZY. Association of plasma brain-derived neurotrophic factor and cardiovascular risk factors and prognosis in angina pectoris. Biochem Biophys Res Commun. 2011;415(1):99-103.
12. Leckie RL, Oberlin LE, Voss MW, Prakash RS, Szabo-Reed A, Chaddock-Heyman L, et al. BDNF mediates improvements in executive function following a 1-year exercise intervention. Front Hum Neurosci. 2014;8:985.
13. Jung SH, Kim J, Davis JM, Blair SN, Cho H. Association among basal serum BDNF, cardiorespiratory fitness and cardiovascular disease risk factors in untrained healthy Korean men. Eur J Appl Physiol. 2011;111:303-11.
14. Loock M, Steyn K, Becker P, Fourie J. Coronary heart disease and risk factors in Black South Africans: a case-control study. Ethn Dis. 2006;16(4):872-9.
15. WHO. Global status report on noncommunicable diseases. 2014.
16. Lee H, Kershaw KN, Hicken MT, Abdou CM, Williams ES, Rivera-O'Reilly N, et al. Cardiovascular disease among Black Americans: comparisons between the US Virgin Islands and the 50 US states. Public Health Reports. 2013;128(3):170-8.
17. Weinstein AA, Abraham P, Diao G, Zeno SA, Deuster PA. Relationship between depressive symptoms and cardiovascular disease risk factors in african american individuals. Depress Res Treat. 2011;2011.
18. Clark LT, Ferdinand KC, Flack JM, Gavin JR, 3rd, Hall WD, Kumanyika SK, et al. Coronary heart disease in African Americans. Heart Dis. 2001;3(2):97-108.
19. Howard G, Safford MM, Moy CS, Howard VJ, Kleindorfer DO, Unverzagt FW, et al. Racial Differences in the Incidence of Cardiovascular Risk Factors in Older Black and White Adults. J Am Geriatr Soc. 2017;65(1):83-90.
20. Hackler III E, Lew J, Gore MO, Ayers CR, Atzler D, Khera A, et al. Racial differences in cardiovascular biomarkers in the general population. J Am Heart Assoc. 2019;8(18):e012729.
21. Hamer M, Frasure-Smith N, Lesperance F, Harvey BH, Malan NT, Malan L. Depressive Symptoms and 24-Hour Ambulatory Blood Pressure in Africans: The SABPA Study. Int J Hypertens. 2012;2012:426803.
22. Jansen van Vuren E, Malan L, Von Känel R, Magnusson M, Lammertyn L, Malan NT. BDNF increases associated with constant troponin T levels and may protect against poor cognitive interference control: The SABPA prospective study. Eur J Clin Invest. 2019;49(7):e13116.

23. Smith AJ, Malan L, Uys AS, Malan NT, Harvey BH, Ziemssen T. Attenuated brain-derived neurotrophic factor and hypertrophic remodelling: the SABPA study. J Hum Hypertens. 2015;29(1):33-9.
24. Schutte CE, Malan L, Scheepers JD, Oosthuizen W, Cockeran M, Malan NT. Cortisol:brain-derived neurotrophic factor ratio associated with silent ischaemia in a black male cohort: the SABPA study. Cardiovasc J Afr. 2016;27(6):387-91.
25. Cui R. Editorial: A Systematic Review of Depression. Curr Neuropharmacol. 2015;13(4):480.
26. Greenberg PE, Fournier A, Sisitsky T, Pike CT, Kessler RC. The economic burden of adults with major depressive disorder in the United States (2005 and 2010). J Clin Psychiatry. 2015;76(2):5356.
27. Ekinci G. Economic Impacts of Cardiovascular Diseases: An Econometric Evaluation in Turkey. Iran J Public Health. 2023;52(1):118-27.
28. Nyundo A, Manu A, Regan M, Ismail A, Chukwu A, Dessie Y, et al. Factors associated with depressive symptoms and suicidal ideation and behaviours amongst sub-Saharan African adolescents aged 10-19 years: cross-sectional study. Trop Med Int Health. 2020;25(1):54-69.
29. Buchwald AM, Rudick-Davis D. The symptoms of major depression. J Abnorm Psychol. 1993;102(2):197-205.
30. Beck AT, Alford BA. Depression: Causes and treatment: University of Pennsylvania Press; 2009.
31. Celano CM, Freudenreich O, Fernandez-Robles C, Stern TA, Caro MA, Huffman JC. Depressogenic effects of medications: a review. Dialogues Clin Neurosci. 2022.
32. Truschel J. Depression definition and DSM-5 diagnostic criteria. Retrieved from Psycom: https://www.psycom.net/depression/major-depressive-disorder/dsm-5-depression-criteria. 2020.
33. Kocsis RN. Book review: diagnostic and statistical manual of mental disorders: (DSM-5). Sage Publications Sage CA: Los Angeles, CA; 2013.
34. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16(9):606-13.
35. Gomez-Gomez I, Benitez I, Bellon J, Moreno-Peral P, Olivan-Blazquez B, Claveria A, et al. Utility of PHQ-2, PHQ-8 and PHQ-9 for detecting major depression in primary health care: a validation study in Spain. Psychol Med. 2023;53(12):5625-35.
36. Handy A, Mangal R, Stead TS, Coffee RL, Jr., Ganti L. Prevalence and Impact of Diagnosed and Undiagnosed Depression in the United States. Cureus. 2022;14(8):e28011.
37. Pandya M, Altinay M, Malone DA, Anand A. Where in the brain is depression? Curr Psychiatry Rep. 2012;14(6):634-42.
38. Dwivedi Y. Brain-derived neurotrophic factor: role in depression and suicide. Neuropsychiatr Dis Treat. 2009;5:433-49.

39. Yu H, Chen ZY. The role of BDNF in depression on the basis of its location in the neural circuitry. Acta Pharmacol Sin. 2011;32(1):3-11.
40. Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L, et al. Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. Lancet Psychiatry. 2018;5(4):339-47.
41. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci. 2009;10(6):410-22.
42. Hertrich I, Dietrich S, Blum C, Ackermann H. The Role of the Dorsolateral Prefrontal Cortex for Speech and Language Processing. Front Hum Neurosci. 2021;15:645209.
43. Pizzagalli DA, Roberts AC. Prefrontal cortex and depression. Neuropsychopharmacology. 2022;47(1):225-46.
44. Dean B. Changes in cortical gene expression in major depressive disorders: More evidence implicating inflammatory-related pathways in disease etiology. The Neuroscience of Depression: Elsevier; 2021. p. 89-100.
45. Zhang R, Wei S, Chang M, Jiang X, Tang Y, Wang F. Dorsolateral and ventrolateral prefrontal cortex structural changes relative to suicidal ideation in patients with depression. Acta Neuropsychiatr. 2020;32(2):84-91.
46. Koenigs M, Grafman J. The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. Behav Brain Res. 2009;201(2):239-43.
47. Yin H. Why is the dorsolateral prefrontal cortex (DLPFC) the favorite region to stimulate? Front Psychiatry. 2021.
48. Qi XR, Zhao J, Liu J, Fang H, Swaab DF, Zhou JN. Abnormal retinoid and TrkB signaling in the prefrontal cortex in mood disorders. Cereb Cortex. 2015;25(1):75-83.
49. van Beek J, de Moor MHM, Geels LM, Sinke MRT, de Geus EJC, Lubke GH, et al. The association of alcohol intake with gamma-glutamyl transferase (GGT) levels: evidence for correlated genetic effects. Drug Alcohol Depend. 2014;134:99-105.
50. van Eijndhoven P, van Wingen G, van Oijen K, Rijpkema M, Goraj B, Jan Verkes R, et al. Amygdala volume marks the acute state in the early course of depression. Biol Psychiatry. 2009;65(9):812-8.
51. Yin H. Neuroscience of depression and treatment. Front Psychiatry. 2021.
52. Anand KS, Dhikav V. Hippocampus in health and disease: An overview. Ann Indian Acad Neurol. 2012;15(4):239-46.
53. Rajmohan V, Mohandas E. The limbic system. Indian J Psychiatry. 2007;49(2):132-9.
54. Gradin VB, Pomi A. The role of hippocampal atrophy in depression: a neurocomputational approach. J Biol Phys. 2008;34(1-2):107-20.
55. Lanciego JL, Luquin N, Obeso JA. Functional neuroanatomy of the basal ganglia. Cold Spring Harb Perspect Med. 2012;2(12):a009621.

56. Lacerda AL, Nicoletti MA, Brambilla P, Sassi RB, Mallinger AG, Frank E, et al. Anatomical MRI study of basal ganglia in major depressive disorder. Psychiatry Res. 2003;124(3):129-40.
57. Vanderah T, Gould D. Nolte's The Human Brain E-Book: An Introduction to its Functional Anatomy: Elsevier Health Sciences; 2020.
58. Basinger H, Hogg JP. Neuroanatomy, brainstem. 2019.
59. Song YJ, Korgaonkar MS, Armstrong LV, Eagles S, Williams LM, Grieve SM. Tractography of the brainstem in major depressive disorder using diffusion tensor imaging. PLoS One. 2014;9(1):e84825.
60. Hyman SE. Neurotransmitters. Curr Biol. 2005;15(5):R154-8.
61. Brigitta B. Pathophysiology of depression and mechanisms of treatment. Dialogues Clin Neurosci. 2022.
62. Grace AA. Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. Nat Rev Neurosci. 2016;17(8):524-32.
63. Moret C, Briley M. The importance of norepinephrine in depression. Neuropsychiatr Dis Treat. 2011;7(Suppl 1):9-13.
64. Saldanha D, Kumar N, Ryali V, Srivastava K, Pawar AA. Serum Serotonin Abnormality in Depression. Med J Armed Forces India. 2009;65(2):108-12.
65. DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. J Neurochem. 2016;139:136-53.
66. Hassamal S. Chronic stress, neuroinflammation, and depression: an overview of pathophysiological mechanisms and emerging anti-inflammatories. Front Psychiatry. 2023;14:1130989.
67. Hirsch EC, Standaert DG. Ten unsolved questions about neuroinflammation in Parkinson's disease. Mov Disord. 2021;36(1):16-24.
68. Lassmann H. Multiple sclerosis pathology. Cold Spring Harb Perspect Med. 2018;8(3).
69. Crotti A, Glass CK. The choreography of neuroinflammation in Huntington's disease. Trends Immunol. 2015;36(6):364-73.
70. Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. Alzheimers Dement. 2016;12(6):719-32.
71. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. Mol Psychiatry. 2016;21(12):1696-709.
72. Lamers F, Milaneschi Y, Smit JH, Schoevers RA, Wittenberg G, Penninx BWJH. Longitudinal association between depression and inflammatory markers: results from the Netherlands study of depression and anxiety. Biol Psychiatry. 2019;85(10):829-37.

73. Lima Giacobbo B, Doorduin J, Klein HC, Dierckx R, Bromberg E, de Vries EFJ. Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. Mol Neurobiol. 2019;56(5):3295-312.
74. Kohler-Forsberg O, C NL, Hjorthoj C, Nordentoft M, Mors O, Benros ME. Efficacy of anti-inflammatory treatment on major depressive disorder or depressive symptoms: meta-analysis of clinical trials. Acta Psychiatr Scand. 2019;139(5):404-19.
75. Troubat R, Barone P, Leman S, Desmidt T, Cressant A, Atanasova B, et al. Neuroinflammation and depression: A review. Eur J Neurosci. 2021;53(1):151-71.
76. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008;31(9):464-8.
77. Juruena MF, Bocharova M, Agustini B, Young AH. Atypical depression and non-atypical depression: Is HPA axis function a biomarker? A systematic review. J Affect Disord. 2018;233:45-67.
78. Kunugi H, Hori H, Adachi N, Numakawa T. Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. Psyciatry Clin Neurosci. 2010;64(5):447-59.
79. Levi-Montalcini R. The nerve growth factor 35 years later. Science. 1987;237(4819):1154-62.
80. Aloe L, Rocco ML, Balzamino BO, Micera A. Nerve Growth Factor: A Focus on Neuroscience and Therapy. Curr Neuropharmacol. 2015;13(3):294-303.
81. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001;24(1):677-736.
82. Chao M, Casaccia-Bonnefil P, Carter B, Chittka A, Kong H, Yoon SO. Neurotrophin receptors: mediators of life and death. Brain Res Brain Res Rev. 1998;26(2-3):295-301.
83. Vega JA, Garcia-Suarez O, Hannestad J, Perez-Perez M, Germana A. Neurotrophins and the immune system. J Anat. 2003;203(1):1-19.
84. Pezet S, Malcangio M, McMahon SB. BDNF: a neuromodulator in nociceptive pathways? Brain Res Brain Res Rev. 2002;40(1-3):240-9.
85. Keefe KM, Sheikh IS, Smith GM. Targeting neurotrophins to specific populations of neurons: NGF, BDNF, and NT-3 and their relevance for treatment of spinal cord injury. Int J Mol Sci. 2017;18(3):548.
86. Kim H, Hong JT, Park MH. Centella asiatica enhances neurogenesis and protects neuronal cells against H2O2-induced oxidative injury. J Biomed Transl Res. 2015;16(3):121-8.
87. Kohman RA, Rhodes JS. Neurogenesis, inflammation and behavior. Brain Behav Immun. 2013;27(1):22-32.

88. Mansour SZ, Moawed FSM, Elmarkaby SM. Protective effect of 5, 7-dihydroxyflavone on brain of rats exposed to acrylamide or gamma-radiation. J Photochem Photobiol B. 2017;175:149-55.
89. Chao MV, Bothwell M. Neurotrophins: to cleave or not to cleave. Neuron. 2002;33(1):9-12.
90. Hashimoto K. Brain-derived neurotrophic factor (BDNF) and its precursor proBDNF as diagnostic biomarkers for major depressive disorder and bipolar disorder. Eur Arch Psychiatry Clin Neurosci. 2015;265(1):83-4.
91. Ilchibaeva TV, Kondaurova EM, Tsybko AS, Kozhemyakina RV, Popova NK, Naumenko VS. Brain-derived neurotrophic factor (BDNF) and its precursor (proBDNF) in genetically defined fear-induced aggression. Behav Brain Res. 2015;290:45-50.
92. Arango-Lievano M, Anastasia A, Jeanneteau F. ProBDNF biology and emerging roles in the CNS: The Unexpected Journey of Proneurotrophins. 2015.
93. Barbacid M. Neurotrophic factors and their receptors. Curr Opin Cell Biol. 1995;7(2):148-55.
94. Nagappan G, Lu B. Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. Trends Neurosci. 2005;28(9):464-71.
95. Schiro G, Iacono S, Ragonese P, Aridon P, Salemi G, Balistreri CR. A Brief Overview on BDNF-Trk Pathway in the Nervous System: A Potential Biomarker or Possible Target in Treatment of Multiple Sclerosis? Front Neurol. 2022;13:917527.
96. Caporali A, Emanueli C. Cardiovascular actions of neurotrophins. Physiol Rev. 2009;89(1):279-308.
97. Lorenzetti V, Costafreda SG, Rimmer RM, Rasenick MM, Marangell LB, Fu CHY. Brain-derived neurotrophic factor association with amygdala response in major depressive disorder. J Affect Disord. 2020;267:103-6.
98. Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A. Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. J Neurosci. 2011;31(12):4475-83.
99. Youssef MM, Underwood MD, Huang YY, Hsiung SC, Liu Y, Simpson NR, et al. Association of BDNF Val66Met Polymorphism and Brain BDNF Levels with Major Depression and Suicide. Int J Neuropsychopharmacol. 2018;21(6):528-38.
100. Harris NM, Ritzel R, Mancini NS, Jiang Y, Yi X, Manickam DS, et al. Nano-particle delivery of brain derived neurotrophic factor after focal cerebral ischemia reduces tissue injury and enhances behavioral recovery. Pharmacol Biochem Behav. 2016;150-151:48-56.
101. Chacon-Fernandez P, Sauberli K, Colzani M, Moreau T, Ghevaert C, Barde YA. Brain-derived Neurotrophic Factor in Megakaryocytes. J Biol Chem. 2016;291(19):9872-81.
102. Holliday R. DNA methylation and epigenetic mechanisms. Cell Biophys. 1989;15(1-2):15-20.

103. Kang HJ, Kim JM, Bae KY, Kim SW, Shin IS, Kim HR, et al. Longitudinal associations between BDNF promoter methylation and late-life depression. Neurobiol Aging. 2015;36(4):1764. e1-. e7.
104. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology. 2013;38(1):23-38.
105. Mitchelmore C, Gede L. Brain Derived Neurotrophic Factor: epigenetic regulation in psychiatric disorders. Brain Res. 2014;1586:162-72.
106. Chen D, Meng L, Pei F, Zheng Y, Leng J. A review of DNA methylation in depression. J Clin Neurosci. 2017;43:39-46.
107. Januar V, Ancelin ML, Ritchie K, Saffery R, Ryan J. BDNF promoter methylation and genetic variation in late-life depression. Transl Psychiatry. 2015;5(8):619.
108. Khyzha N, Alizada A, Wilson MD, Fish JE. Epigenetics of Atherosclerosis: Emerging Mechanisms and Methods. Trends Mol Med. 2017;23(4):332-47.
109. Tabaei S, Tabaee SS. DNA methylation abnormalities in atherosclerosis. Artif Cells Nanomed Biotechnol. 2019;47(1):2031-41.
110. Turunen MP, Aavik E, Yla-Herttuala S. Epigenetics and atherosclerosis. Biochim Biophys Acta. 2009;1790(9):886-91.
111. Xu S, Pelisek J, Jin ZG. Atherosclerosis is an epigenetic disease. Trends Endocrinol Metab. 2018;29(11):739-42.
112. Lee HT, Oh S, Ro DH, Yoo H, Kwon YW. The Key Role of DNA Methylation and Histone Acetylation in Epigenetics of Atherosclerosis. J Lipid Atheroscler. 2020;9(3):419-34.
113. Donovan MJ, Lin MI, Wiegn P, Ringstedt T, Kraemer R, Hahn R, et al. Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. Development. 2000;127(21):4531-40.
114. Kaess BM, Preis SR, Lieb W, Beiser AS, Yang Q, Chen TC, et al. Circulating brain-derived neurotrophic factor concentrations and the risk of cardiovascular disease in the community. J Am Heart Assoc. 2015;4(3):e001544.
115. Kermani P, Hempstead B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med. 2007;17(4):140-3.
116. Li L, Guo H, Lai B, Liang C, Chen H, Chen Y, et al. Ablation of cardiomyocyte-derived BDNF during development causes myocardial degeneration and heart failure in the adult mouse heart. Front Cardiovasc Med. 2022;9:967463.
117. Donovan MJ, Miranda RC, Kraemer R, McCaffrey TA, Tessarollo L, Mahadeo D, et al. Neurotrophin and neurotrophin receptors in vascular smooth muscle cells. Regulation of expression in response to injury. Am J Pathol. 1995;147(2):309-24.

118. Jiang H, Huang S, Li X, Li X, Zhang Y, Chen ZY. Tyrosine kinase receptor B protects against coronary artery disease and promotes adult vasculature integrity by regulating Ets1-mediated VE-cadherin expression. Arterioscler Thromb Vasc Biol. 2015;35(3):580-8.
119. Trombetta IC, DeMoura JR, Alves CR, Carbonari-Brito R, Cepeda FX, Lemos JR, Jr. Serum Levels of BDNF in Cardiovascular Protection and in Response to Exercise. Arq Bras Cardiol. 2020;115(2):263-9.
120. Douglas G, Channon KM. The pathogenesis of atherosclerosis. Medicine. 2014;42(9):480-4.
121. Mullenix PS, Andersen CA, Starnes BW. Atherosclerosis as inflammation. Ann Vasc Surg. 2005;19:130-8.
122. Bjorkegren JLM, Lusis AJ. Atherosclerosis: Recent developments. Cell. 2022;185(10):1630-45.
123. Kim HL, Kim SH. Pulse Wave Velocity in Atherosclerosis. Front Cardiovasc Med. 2019;6:41.
124. Tegos TJ, Kalodiki E, Sabetai MM, Nicolaides AN. The genesis of atherosclerosis and risk factors: a review. Angiology. 2001;52(2):89-98.
125. Linton MF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The role of lipids and lipoproteins in atherosclerosis. Endotext [Internet]. 2019.
126. Kaperonis EA, Liapis CD, Kakisis JD, Dimitroulis D, Papavassiliou VG. Inflammation and atherosclerosis. Eur J Vasc Endovasc Surg. 2006;31(4):386-93.
127. Libby P. Atherosclerosis: the new view. Sci Am. 2002;286(5):46-55.
128. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32(9):2045-51.
129. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med. 2014;5(8):927-46.
130. Yang J, Zhang L, Yu C, Yang X, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res. 2014;2(1):1-9.
131. Lacolley P, Regnault V, Segers P, Laurent S. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. Physiol Rev. 2017;97(4):1555-617.
132. Mitchell GF, Powell JT. Arteriosclerosis: a primer for “in focus” reviews on arterial stiffness. Am Heart Assoc; 2020. p. 1025-7.
133. Quinn U, Tomlinson LA, Cockcroft JR. Arterial stiffness. JRSM Cardiovasc Dis. 2012;1(6):1-8.
134. Lyle AN, Raaz U. Killing Me Unsoftly: Causes and Mechanisms of Arterial Stiffness. Arterioscler Thromb Vasc Biol. 2017;37(2):e1-e11.

135. Gopalan C, Kirk E. Biology of Cardiovascular and Metabolic Diseases: Academic Press; 2022.
136. Mozos I, Malainer C, Horbanczuk J, Gug C, Stoian D, Luca CT, et al. Inflammatory Markers for Arterial Stiffness in Cardiovascular Diseases. Front Immunol. 2017;8:1058.
137. Mäki-Petäjä KM, Wilkinson IB. Inflammation and large arteries: Potential mechanisms for inflammation-induced arterial stiffness. Artery Res. 2012;6(2):59-64.
138. Wilkinson IB, McEniery CM, Cockcroft JR. Arteriosclerosis and atherosclerosis: guilty by association. Am Heart Assoc; 2009. p. 1213-5.
139. Wood D, Joint European Societies Task F. Established and emerging cardiovascular risk factors. Am Heart J. 2001;141(2 Suppl):S49-57.
140. Dhar AK, Barton DA. Depression and the Link with Cardiovascular Disease. Front Psychiatry. 2016;7:33.
141. Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, et al. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol. 2001;15(10):1748-57.
142. Laszlo A, Lenart L, Illesy L, Fekete A, Nemcsik J. The role of neurotrophins in psychopathology and cardiovascular diseases: psychosomatic connections. J Neural Transm (Vienna). 2019;126(3):265-78.
143. Choe KY, Han SY, Gaub P, Shell B, Voisin DL, Knapp BA, et al. High salt intake increases blood pressure via BDNF-mediated downregulation of KCC2 and impaired baroreflex inhibition of vasopressin neurons. Neuron. 2015;85(3):549-60.
144. Mrug S, Orihuela C, Mrug M, Sanders PW. Sodium and potassium excretion predict increased depression in urban adolescents. Physiol Rep. 2019;7(16):e14213.
145. Henry JP. Stress, salt and hypertension. Soc Sci Med. 1988;26(3):293-302.
146. Staessen JA, Poulter NR, Fletcher AE, Markowe HL, Marmot MG, Shipley MJ, et al. Psycho-emotional stress and salt intake may interact to raise blood pressure. J Cardiovasc Risk. 1994;1(1):45-51.
147. Deter HC, Buchholz K, Schorr U, Schachinger H, Turan S, Sharma AM. Psychophysiological reactivity of salt-sensitive normotensive subjects. J Hypertens. 1997;15(8):839-44.
148. Gilman TL, Mitchell NC, Daws LC, Toney GM. Neuroinflammation Contributes to High Salt Intake-Augmented Neuronal Activation and Active Coping Responses to Acute Stress. Int J Neuropsychopharmacol. 2019;22(2):137-42.
149. Hang PZ, Zhu H, Li PF, Liu J, Ge FQ, Zhao J, et al. The Emerging Role of BDNF/TrkB Signaling in Cardiovascular Diseases. Life (Basel). 2021;11(1):70.
150. Hadi HA, Carr CS, Al Suwaidi J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. Vasc Health Risk Manag. 2005;1(3):183-98.

151. Poznyak AV, Grechko AV, Orekhova VA, Khotina V, Ivanova EA, Orekhov AN. NADPH oxidases and their role in atherosclerosis. Biomedicines. 2020;8(7):206.
152. Dietz LJ, Matthews KA. Depressive symptoms and subclinical markers of cardiovascular disease in adolescents. J Adolesc Health. 2011;48(6):579-84.
153. Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry. 2008;64(6):527-32.
154. Han W, Zhang C, Wang H, Yang M, Guo Y, Li G, et al. Alterations of irisin, adropin, preptin and BDNF concentrations in coronary heart disease patients comorbid with depression. Ann Transl Med. 2019;7(14):298.
155. Sobolewska-Nowak J, Wachowska K, Nowak A, Orzechowska A, Szulc A, Płaza O, et al. Exploring the heart–mind connection: unraveling the shared pathways between depression and cardiovascular diseases. Biomedicines. 2023;11(7):1903.
156. Alomari MA, Khabour OF, Maikano A, Alawneh K. Vascular function and brain-derived neurotrophic factor: The functional capacity factor. Vasc Med. 2015;20(6):518-26.
157. Wu Y, Sun D, Wang B, Li Y, Ma Y. The relationship of depressive symptoms and functional and structural markers of subclinical atherosclerosis: A systematic review and meta-analysis. Eur J Prev Cardiol. 2018;25(7):706-16.
158. Duprez D, DeBuyzere ML, Rietzschel ER. Arterial stiffness and cardiovascular disease. E-Journal of Cardiology Practice. 2002;1.
159. Bucciarelli V, Caterino AL, Bianco F, Caputi CG, Salerni S, Sciomer S, et al. Depression and cardiovascular disease: The deep blue sea of women's heart. Trends Cardiovasc Med. 2020;30(3):170-6.
160. Jaggar M, Fanibunda SE, Ghosh S, Duman RS, Vaidya VA. The neurotrophic hypothesis of depression revisited: new insights and therapeutic implications. Neurobiology of depression: Elsevier; 2019. p. 43-62.
161. Patel V, Abas M, Broadhead J, Todd C, Reeler A. Depression in developing countries: lessons from Zimbabwe. BMJ. 2001;322(7284):482-4.
162. Jones-Webb RJ, Snowden LR. Symptoms of depression among blacks and whites. Am J Public Health. 1993;83(2):240-4.
163. Freeman A, Tyrovolas S, Koyanagi A, Chatterji S, Leonardi M, Ayuso-Mateos JL, et al. The role of socio-economic status in depression: results from the COURAGE (aging survey in Europe). BMC Public Health. 2016;16(1):1098.
164. Usui T, Naruo A, Okada M, Hayabe Y, Yamawaki H. Brain-derived neurotrophic factor promotes angiogenic tube formation through generation of oxidative stress in human vascular endothelial cells. Acta Physiologica. 2014;211(2):385-94.

165. Chaldakov GN, Fiore M, Stankulov IS, Manni L, Hristova MG, Antonelli A, et al. Neurotrophin presence in human coronary atherosclerosis and metabolic syndrome: a role for NGF and BDNF in cardiovascular disease? Prog Brain Res. 2004;146:279-89.
166. Inoue N. Stress and atherosclerotic cardiovascular disease. J Atheroscler Thromb. 2014;21(5):391-401.

CHAPTER 2

2. Methodology

According to Babbie and Mouton (2001) research methodology refers to the design and methods of research. The research design focuses on the final product with the problem statement as a point of departure, while the research method is specific procedures done. Procedures include research design, target population, the sampling method, the methods and procedures of data collection, the method according to which data is analysed as well as specific measures to ensure that the results are ethically approved and valid (1). This section elaborates on the procedures and measurements used to obtain data required to answer the research question of our study.

2.1 Ethical Approval and Permission

Prior to commencement of the study, permission was obtained from the North-West University, Department of Education, the South African Democratic Teacher's Union, and the headmaster of respective schools. Ethical approval for the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study (number NWU-00036-07-A6) was obtained from the Health Research Committee of the North-West University, Potchefstroom Campus and written consent has been obtained for all volunteers prior to participation. All procedures adhered to the application institutional guidelines and terms, as stated by the Declaration of Helsinki, revised in 2008 (2).

Approval was granted by the North-West University's Health Research Ethics Committee (number NWU-0303-21-A1) for the completion of the master study **(Appendix A)**. Finally, a confidentiality agreement was signed by the student prior to the distribution of data.

2.2 Study Design and Sampling Population

This MHSc study is based on existing data from the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. The SABPA study was based on a cross-sectional approach defined as a type of research in which you collect data from many different individuals at a single point in time. In cross-sectional research, you observe variables without influencing them (3). The following actions were taken during 2008 and 2009 to recruit the population for purposes of this study.

The SABPA study was conducted on urban black and white teachers in the Dr Kenneth Kaunda District of the North West Province of South Africa **(Figure 2.1)**. This selection ensure that participants shared similar occupation and environmental statuses, but we could not control for

cultural diversity (4). Teachers (n=2170), aged 20-65 years, were invited and 471 participants were assessed for eligibility.

Figure 2.1. The Dr Kenneth Kaunda District of the North West Province of South Africa. Indicated in red the regions of teachers that participated in the Sympathetic activity and Ambulatory Blood Pressure in Africans.

2.3 Recruitment

Approximately three months prior to the commencement of the SABPA study, principles of 43 schools in the Dr Kenneth Kaunda district of the North West Province, South Africa, were informed about the study project by the principal investigator (PI), Professor Leone Malan. The principals were asked to notify their staff about the project. Hereafter, the SABPA research team held information sessions with the school personnel, at their respective schools, to explain the details of the project. These sessions were conducted in the three main languages spoken in the district which is either Afrikaans, English, and/or Setswana, the SABPA study's main aims and objectives, as well as planned measurements and procedures and their expected outcomes and expectations were discussed.

Teachers who indicated that they were not interested in participating were thanked for their time and not contacted again. Teachers that showed interest were screened to assess their eligibility to participate in the study according to the inclusion and exclusion criteria. The exclusion criteria

included individuals with a tympanum termperature higher than 37.5°C, those who used alpha and beta blockers, or pregnant and lactating women, also individuals who have been vaccinated or donated blood within three months prior to participation, and individuals depending on or abusing psychotropic substances. **Table 2.1** summarises the main inclusion and exclusion criteria and their justifications.

Table 2.1. **Inclusion and exclusion criteria.**

Inclusion criteria	**Justification**
Teachers from the Dr Kenneth Kaunda Education district aged 20-65 years.	To ensure socio-demographic homogeneity in the study sample.
Black and white ethnicity.	As part of the SABPA sutdy, the main aim was to investigate the ethnic differences related to the aspects of cardiovascular (CV) system. Hence, the study made use of both black and white teachers.
Exclusion criteria	**Justification**
A tympanic temperature of above 37.5°C.	Tympanic temperature above 37.5°C indicated the possibility of microbial infections (5).
Blood donors.	Blood donation in the preceding three months would exclude the participants having blood drawn due to the risk of anaemia (6).
Vaccination less than three months prior to data collection.	Vaccination artifically increases white blood cell count.
Individuals depending on or abusing psychotropic substances.	Psychotropic drugs would cause disturbances in biochemical measurements, e.g., anti-depressant treatment tends to increase serum brain-derived neurotrophic factor (BDNF), possibly decreasing the risk for depression, atherosclerosis, and arterial stiffness (7).
Pregnant or lactating women.	Hormonal changes related to pregnancy or lactating could influence various CV and biochemical measurements. Pregnant or lactating women may reduce wall tension or

	increase the exposure to collagen fibres, whereas it could lead to the development of vascular or venous disease (8).
Users of alpha and beta blockers	The use of alpha and beta blockers abuse can lead to disturbances in various measures, e.g., alpha and beta blockers can slow the progression of coronary atherosclerosis, hereby, decreasing the risk of atherosclerosis (9).

The teachers had the opportunity to discuss the study with their family for seven days. Teachers that notified the study team that they still had interest in participating were scheduled for participation. Informed consent was obtained by a registered nurse the day of participation before the commencement of any measurements. Participants were allowed to withdraw from the study at any given time.

2.3.1 Study Participants

Ultimately, 409 participants were finally included in the study: 200 black (101 men and 99 women) and 209 white (101 men and 108 women) **(Figure 2.2)**. Phase I of the study was conducted in February to May (2008 and 2009) to control for seasonal changes which could affect various physiolgoical measures such as inflammatory biomarkers, neurotrophic factor markers, and inflammatory markers (10, 11).

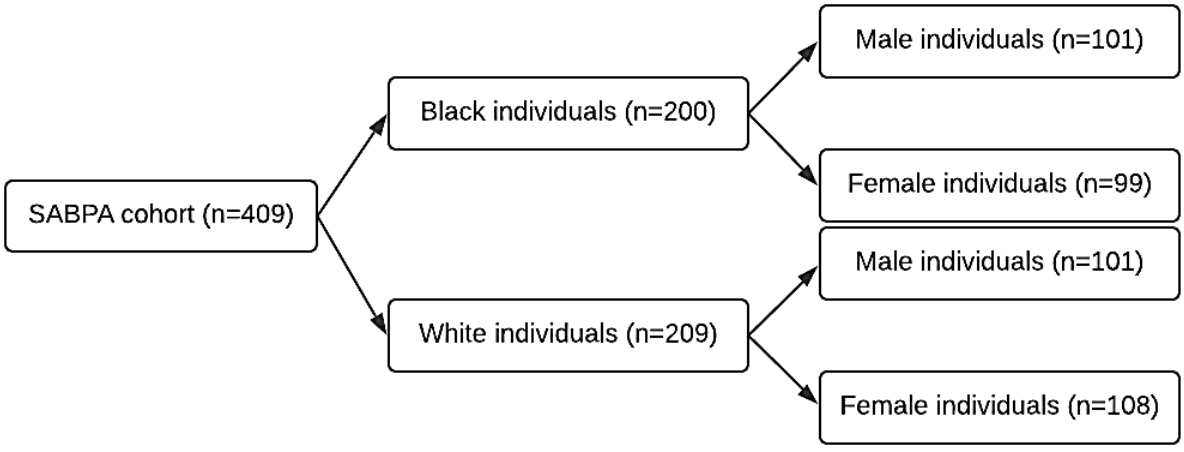


Figure 2.2. Diagram of the study from the Sympathetic activity and Ambulatory Blood Pressure in Africans study.

Only the research procedures relevant to this Master study will be discussed in the following section.

2.4 Study population

The Master's study included data from all 200 black teachers. Seven participants were excluded due to missing data for BDNF (n=1), carotid intima-media thickness (CIMT) (n=2), and pulse wave velocity (PWV) (n=4) **(Figure 2.3)**. We only included data from black teachers based on a large amount of literature originated from the SABPA cohort, reported significant results of depression, BDNF, and the affect it had on the CV system, in the black population (12-15). Schutte et al. later noticed that BDNF levels were lower in black individuals and hypothesized that lower BDNF levels could increase their vulnerability to depression (14). Malan et al. confirmed that chronic depression in the SABPA black teachers' cohort was associated with regulation and perfusion deficit (16). Further, the SABPA study also documented that the downregulation of BDNF levels is associated with hypertrophic remodeling of the carotid artery and may perhaps be a compensaotry mechanism for high blood pressure in black individuals (15).

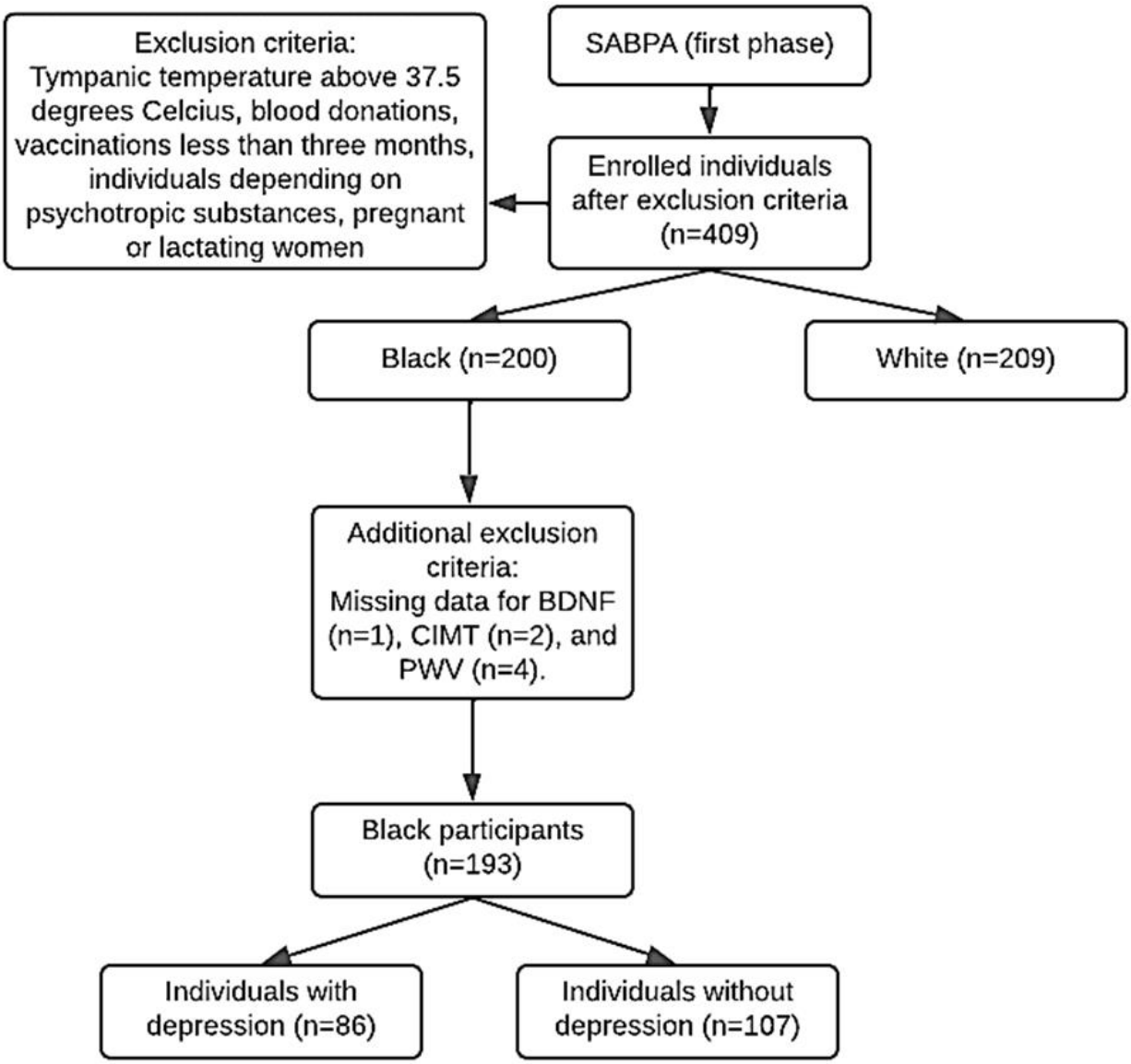


Figure 2.3. Flow chart of SABPA study phase I and the additional exclusion criteria for this MHSc study's population.

2.5 Data collection

The SABPA protocol was carried out over two days **(Figure 2.4)**. These two days included an overnight stay at the Metabolic Unit, where research participants took part on Tuesday, Wednesday, and Thursday, respectively. The Metabolic Unit of the North-West University, Potchefstroom Campus, is a research facility for human studies equipped with well-furnished bedrooms, a kitchen, two bathrooms, and a television room. On the first day, at 07:00, a validated

24-hour ambulatory blood pressure monitor (ABPM) was attached to each participant at their school to obtain ambulatory blood pressure measurements. Participants resumed with their normal daily activities for the day. Later the day, at approximately 16:30, participants were transported to the Metabolic Unit facility at the North-West University. The following day's procedures and experimental setup were explained to all the participants. A stadardised dinner was served at 18:00, whereafter, the questionnaires such as the General Health Questionnaire and Patient Health Questionnaire-9 followed. At 20:30, the participants received their last beverage and were requested to go to bed by 22:00.

On day two, the 24-hour ABPM was disconnected, followed by anthropometric measurements, CV measurements, which include resting blood pressure, PWV, and CIMT. Also, a resting sample of 65 ml was obtained by a registered nurse from the antebrachial vein of the dominant arm using a winged infusion set. Thereafter, the blood samples were sent to the laboratory for preparation and storage. Samples were prepared according to standardised methods and stored in a laboratory bio-freezer at -80°C. After the respective measurements were completed, participants received feedback. Participants in need of counselling were assisted, also, if there were any concern regarding their values, they were referred to a medical practitioner. Lastly, the participants were thanked for their time and given a takeaway breakfast.

Figure 2.4. Infographic of the SABPA protocol measurements that were carried out over two days.

2.6 Cardiovascular measurements

The 24-hour ABPM device was attached to the participant's non-dominant arm – seeing that we use our non-dominant arm less during day-to-day activities – with an appropriate cuff size for the participants. Participants were advised on optimising inflation during the measurement period, participants were advised to sit comfortably or stand still without engaging in coversation or walking whenever the cuff initiated inflation. The Cardiotens® apparatus (Meditech CEO®, Budapest, Hungary) was validated by the British Hypertension Society and was programmed to inflate every 30 minutes during the day (08:00-22:00) and every 60 minutess during night (22:00-06:00). The ABPM device was removed by 07:00 am for further analyses. These measurements included systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR). The data was downloaded onto a database with the help of the Cardiotens 1.9.0 personal software (Meditech). Sucessful inflation rates of 70% or more were required for a measurement to be regarded as adequate. If the inflation rates were below 70% the participants were asked to repeat the 24-hour ABPM measurements as soon as possible.

2.7 Carotid intima-media thickness

Carotid intima-media thickness (CIMT) is one of the biomarkers used to assess vascular structural changes (15). Carotid intima-media thickness can be used by medical professionals, as it is a biomarker for future cardiovascular disease (CVD) (17). The CIMT was measured with the help of SonoSite Inc., Micromax Ultrasound System (SonoSite Inc., Bothell, WA, USA) and 6-13 MHz linear array transducer, using the Rudy Meijer protocol (18). The carotid artery ultrasound was imaged from two different angles of both left and right artries. The images were then imported into the Artery System Automated Software (Gothenburg, Sweden) for dedicated analyses. The program automatically detects the borders of the intima-media of the near and far wall, as well as the inner diameter of the vessel and calculates CIMT from around 100 discrete measurements through the 10 cm segment. For this study the far wall CIMT measurements were used **(Figure 2.5)**.

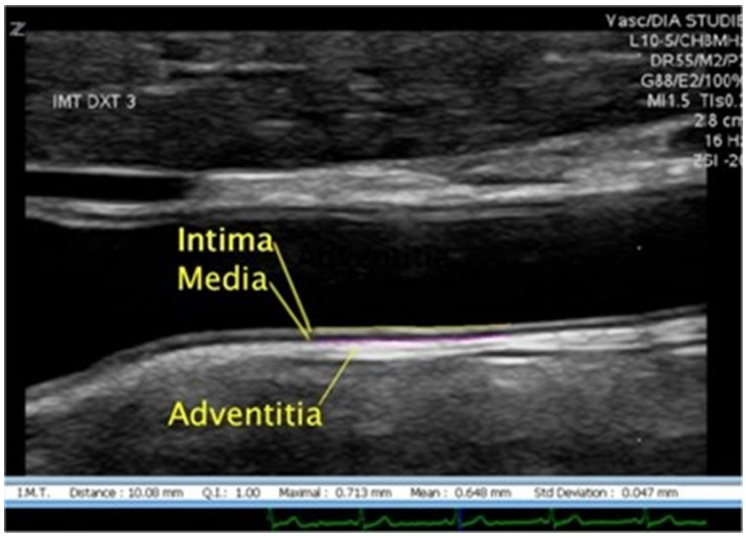


Figure 2.5. Schematic representation of the different parts of the carotid artery during the analyses of the carotid intima-media thickness ***(Appendix B)****.*

2.8 Pulse wave velocity

Pulse wave velocity (PWV) is a non-invasive method to determine the stiffness of both elastic and muscular arteries (19). For the study, we used the carotid-dorsalis pedis PWV using Complior SP Acquisition System (Artech-Medical, Pantin, France). The carotid-dorsalis pedis PWV is measured between the carotid artery and dorsalis-pedis. The PWV measurements were done in triplicate on the left side of each participant after 15-20 minutes in the supine position. The same two observers took all measurements, to ensure validity. However, the carotid-femoral PWV is the gold standard to assess aortic stiffness (20), as it is easy to use and has the best predictive value for CV events (21), but carotid-dorsalis pedis PWV is seen as a readily accessible alternative method for the carotid-femoral PWV (22). Pulse wave velocity measurement greater than 12 m/s were considered to indicate subclinical organ damage, which leads to increased morbidity and mortality (23).

2.9 Anthropometric measurements and questionnaires

2.9.1 Anthropometric measurements

All measurements were done in triplicate by trained anthropometrists, and the median was calculated for the final score and reported in the dataset. During these measurements, participants were requested to wear minimal clothing without shoes. Women participants were measured by a female biokineticist, and men by male biokineticist in a private room to ensure participant's comfort. Stature was measured with a stadiometer (Invicta stadiometer, IP, 1465,

London, UK) to the nearest 0.1 cm and weight to the nearest 0.1 kg with a health scale (Precision Health Scale; A & D Company, Tokyo, Japan). The waist circumference was measured to the nearest 0.1 cm using a non-stretchable flexible 7 mm wide metal tape (Holtain, Croswell, Wales). Body mass index (BMI) was calculated using the formula: weight/(height)^2.

2.9.2 Questionnaires

The General Health Questionnaires was completed to acquire self-reported data on age, sex, lifestyle factors, smoking, alcohol abuse, and medication use (anti-inflammatory, anti-coagulation, statins, and anti-hypertensives). Depressive symptoms severity was obtained by the Patient Health Questionnaire-9 (PHQ-9). The PHQ-9 is the international gold standard measurements tool for measuring the severity of depression (24). The PHQ-9 consisted out of nine items, each of which is scored 0-3 providing a severity score of 0 to 27. The PHQ-9 was validated in various ethnic groups for use in primary care settings (24). Minimal depression is indicated with a score of 0-4, mild depression is indicated with a score of 5-9, and moderate depression is indicated with a score of 10-14. Moderately severe depression is indicated with a score of 15-19, and severe depression is indicated with a score of 20-27 (24). The recommended and established PHQ-9 cut-off point of ≥10 indicates the presence of depression (24). Therefore, for this Master study, individuals with a total PHQ-9 score of ≥10 were considered as individuals with depression, and a score below 10 indicated individuals without depression.

2.10 Biochemical analysis

An experienced registered nurse drew blood from the antebrachial vein using a sterile infusion set from the participants before 10:00. The nurse adhered to all standard practise of care which include wearing latex gloves.

Standardised methods were followed in preparing serum, ethylenediaminetetraacetate (EDTA), and fluoride sample tests. Hereafter, the blood was taken to the laboratory by a runner, where blood samples were handled. Blood samples such as serum were left at room temperature for 20-45 minutes to allow coagulation; whereafter they were centrifuged. Plasma samples were aliquoted and placed into Eppendorf tubes for storage in a laboratory bio-freezer at -80°C until required for further analyses. Serum analyses of BDNF were done by Quantikine Colorometric Sandwich Immunoassay (R & D System, USA, Canada) with intra-assay precision between 3.8% and 4.2% inter-assay coefficient of variations (CV) <7.6% and 11.3%.

The EDTA whole blood samples were used to analyse glucosylated haemoglobin A1c (HbA1c) and sodium fluoride samples for fasting glucose determination. The percentage of HbA1c was determined using the turbidimetric inhibition immunoassay be means of the Roche Integra® 400 (Roche, Basil, Switzerland) apparatus. Additionally, fasting glucose samples were collected in

sodium fluoride tubes and analysed using the time-end method (Unicel DXC, Beckham, Coulter, Germany). Gamma-glutamyl transferase (GGT), an indicator of alcohol consumption (25), and serum cotinine, an indicator of nicotine levels (26), were analysed with the enzyme rate method. Tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were measured via a Quantikine HS Elisa Human Serum TNF-α Immunoassay (HS Elisa; R & D System, Minneapolis, MN, USA). Total cholesterol and high-density lipoprotein (HDL) cholesterol were determined via the time end-point method (Unicel DXC 800®, Beckham and Coulter, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the formula: LDL = TC – HDL – (0.45 x triglycerides)(27).

2.11 Power analysis and statistical analysis

2.11.1 Power analyses

We performed a-priori analyses during G*power 3.1.9.2 software (28) to determine the minimum required group size when performing a multiple regression **(Figure 2.6)**. The best family section was set as a t-test and the statistical test as linear multiple regression. A two-tailed model was performed, assuming a medium effect size of 0.3 and alpha 0.05 power and ten predictors. The results indicated that a sample size of 55 participants would be required per investigated group.

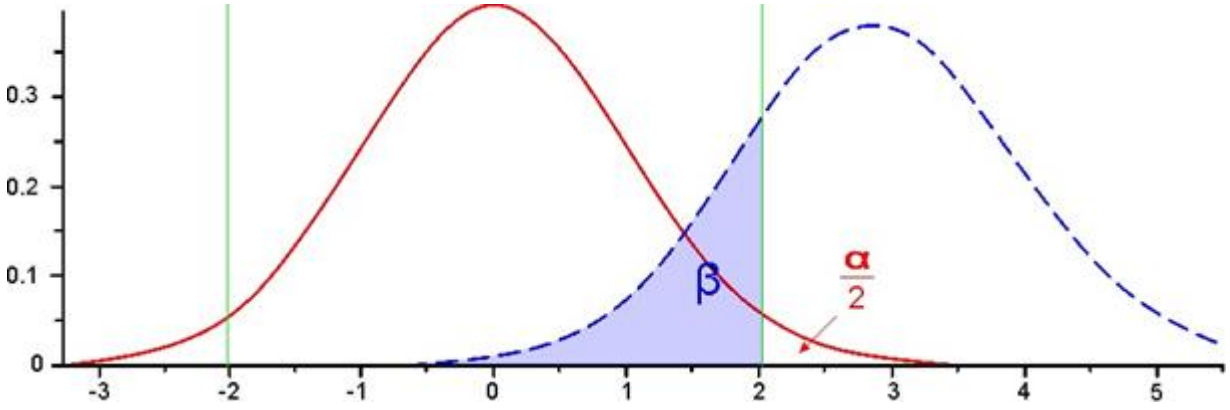


*Figure 2.6. Visual representation of the a-priori power analyses using G*power software.*

2.11.2 Statistical analysis

Data analyses and management were performed using IBM® SPSS® version 20 (IBM Corporation, Armonk, New York), Our sample population was divided into black individuals with and without depression, based on their PHQ-9 cut-off value of ≥10 (24). All variables that did not meet the criteria for normality (which include BMI, GGT, HbA1c, total cholesterol, TNF-α, IL-6, SBP, PP, PWV, TEE< cotinine, triglycerides, and glucose) were logarithmically transformed.

The central tendency and spread of the normal and log-transformed variables were represented by a geometric mean and the 25th and 75th percentile, while dichotomous variables were reported

as the total number of the population and percentage. Means and proportions were compared by an independent t-test and chi-square test, respectively. We used single and partial correlation (adjusted for age, sex, 24-hour mean arterial pressure, and waist circumference) to determine if a relationship exist between CIMT and BDNF in respective groups and to determine which covariates to include into the multiple regression. Also, adjustment made can also be supported by research which indicated that males, older age, hypertension, dyslipidaemia, and type II diabetes mellitus were identified as risk factors that contribute to the presence of carotid plaque. Further, visual representation has also been included in the form of different scatterplots regression analyses were performed to determine if an independent association exist between CIMT and BDNF, and PWV and BDNF in the respective groups. These covariates included in the final model were age, sex, waist circumference, glucose, CRP, LDL cholesterol, MAP, and GGT. All P values refer to a two-sided hypothesis.

2.12 Experienced during the MHSc academic years.

During my academic years as a postgraduate student at the Hypertension in Africa Research Team (HART), I gained practical experience by being involved with data collection for the various studies running at the time, which include the African Prospective study on the Early detection and Identification of the cardiovascular disease (African-PREDICT), Hypertension & Exercise Arterial Modulation and Nutrition in Youth South Africa (ExAMIN Youth SA) study. Laboratory work and experience were gained as the student was responsible for the following, (i) centrifuged blood samples, samples were aliquoted into Eppendorf tubes for further analyses and (ii) full blood count were analysed using Beckham, Coulter, Germany. Experience has been gained on various devices, including the plate reader, Unicel DXC 800, Beckham and Coulter, and the Cobas Integra 400 plus. Further courses such as ethical training and integrated research integrity management system training for postgraduate studies were completed.

2.13 References

1. Babbie E, Mouton J. The Practice of Social Research (Cape Town: Oxford University Press South Africa). 2001.
2. Williams JR. The Declaration of Helsinki and public health. Bull World Health Organ. 2008;86(8):650-2.
3. Kesmodel US. Cross-sectional studies–what are they good for? Acta obstetricia et gynecologica Scandinavica. 2018;97(4):388-93.
4. Malan L, Hamer M, Frasure-Smith N, Steyn HS, Malan NT. Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study. Int J Epidemiol. 2015;44(6):1814-22.

5. Varney SM, Manthey DE, Culpepper VE, Creedon JF, Jr. A comparison of oral, tympanic, and rectal temperature measurement in the elderly. J Emerg Med. 2002;22(2):153-7.
6. Armstrong KL. Blood donation and anemia. Can Fam Physician. 2016;62(9):730-1.
7. Björkholm C, Monteggia LM. BDNF–a key transducer of antidepressant effects. Neuropharmacology. 2016;102:72-9.
8. Gimunova M, Zvonar M, Kolarova K, Janik Z, Mikeska O, Musil R, et al. Changes in lower extremity blood flow during advancing phases of pregnancy and the effects of special footwear. J Vasc Bras. 2017;16(3):214-9.
9. Chobanian AV. Can antihypertensive drugs reduce atherosclerosis and its clinical complications. Am J Hyperten. 1994;7(10_Pt_2):119S-25S.
10. Liu B, Taioli E. Seasonal Variations of Complete Blood Count and Inflammatory Biomarkers in the US Population - Analysis of NHANES Data. PLoS One. 2015;10(11):e0142382.
11. Molendijk ML, Haffmans JPM, Bus BAA, Spinhoven P, Penninx BWJH, Prickaerts J, et al. Serum BDNF concentrations show strong seasonal variation and correlations with the amount of ambient sunlight. PloS one. 2012;7(11):e48046.
12. Jansen van Vuren E, Malan L, von Kanel R, Magnusson M, Lammertyn L, Malan NT. BDNF increases associated with constant troponin T levels and may protect against poor cognitive interference control: The SABPA prospective study. Eur J Clin Invest. 2019;49(7):e13116.
13. Mashele N. Depressive symptoms and cardiometabolic health in urban black Africans: the SABPA study 2014.
14. Schutte CE, Malan L, Scheepers JD, Oosthuizen W, Cockeran M, Malan NT. Cortisol: brain-derived neurotrophic factor ratio associated with silent ischaemia in a black male cohort: the SA BPA study: cardiovascular topics. Cardiovasc J Afr. 2016;27(6):387-91.
15. Smith AJ, Malan L, Uys AS, Malan NT, Harvey BH, Ziemssen T. Attenuated brain-derived neurotrophic factor and hypertrophic remodelling: the SABPA study. J Hum Hypertens. 2015;29(1):33-9.
16. Malan L, Hamer M, von Kanel R, Schlaich MP, Reimann M, Frasure-Smith N, et al. Chronic depression symptoms and salivary NOx are associated with retinal vascular dysregulation: The SABPA study. Nitric Oxide. 2016;55-56:10-7.
17. Simons PC, Algra A, Bots ML, Grobbee DE, van der Graaf Y. Common carotid intima-media thickness and arterial stiffness: indicators of cardiovascular risk in high-risk patients. The SMART Study (Second Manifestations of ARTerial disease). Circulation. 1999;100(9):951-7.
18. Liang Q, Wendelhag I, Wikstrand J, Gustavsson T. A multiscale dynamic programming procedure for boundary detection in ultrasonic artery images. IEEE Trans Med Imaging. 2000;19(2):127-42.

19. Kim HL, Kim SH. Pulse Wave Velocity in Atherosclerosis. Front Cardiovasc Med. 2019;6:41.
20. Covic A, Siriopol D. Pulse wave velocity ratio: the new “gold standard” for measuring arterial stiffness. Am Heart Assoc; 2015. p. 289-90.
21. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588-605.
22. Skilton M, Celermajer D, da Cruz E, Jagger J. Non-invasive assessment of arterial structure and function. Pediatric and congenital cardiology, cardiac surgery and intensive care London: Springer. 2014:531-45.
23. Gómez-Marcos MA, Recio-Rodríguez JI, Patino-Alonso MC, Agudo-Conde C, Gómez-Sánchez L, Rodríguez-Sánchez E, et al. Relationship between intima-media thickness of the common carotid artery and arterial stiffness in subjects with and without type 2 diabetes: a case-series report. Cardiovasc Diabetol. 2011;10(1):1-8.
24. Gomez-Gomez I, Benitez I, Bellon J, Moreno-Peral P, Olivan-Blazquez B, Claveria A, et al. Utility of PHQ-2, PHQ-8 and PHQ-9 for detecting major depression in primary health care: a validation study in Spain. Psychol Med. 2022:1-11.
25. van Beek J, de Moor MHM, Geels LM, Sinke MRT, de Geus EJC, Lubke GH, et al. The association of alcohol intake with gamma-glutamyl transferase (GGT) levels: evidence for correlated genetic effects. Drug Alcohol Depend. 2014;134:99-105.
26. Raja M, Garg A, Yadav P, Jha K, Handa S. Diagnostic Methods for Detection of Cotinine Level in Tobacco Users: A Review. J Clin Diagn Res. 2016;10(3):ZE04-6.
27. Wilson PW, Abbott RD, Garrison RJ, Castelli WP. Estimation of very-low-density lipoprotein cholesterol from data on triglyceride concentration in plasma. Clin Chem. 1981;27(12):2008-10.
28. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175-91.

CHAPTER 3

Journal of Affective Disorders

(https://www.elsevier.com/journal-of-affective-disorders/0165-0327/guide-for-authors)
Impact factor: 4.839

Scope

The journal of **Affective disorder** is a multidisciplinary and aims to bring together different approaches and fields of research, concern with psychiatric disorders such as depression, mania, mood disorders, emotions, and stress.

Author instructions for submission of original research

Title. The title should appear on the title page without the article type.

Authors name and affiliation. State all author's names. Each author is required to declare their contribution to the article.

Corresponding author. Indicate the corresponding author's name, address, telephone number, and email address.

Conflict of interest. The author must state all potential conflicts of interest.

Abstract

A structural abstract of no more than 250 words is required.

Keywords

The abstract should be followed by a list of three to six keywords, followed by a list of abbreviations used.

Article structure

The article must be divided into clearly defined sections (introduction, materials and methods, results, and discussion). Each heading should appear on its own separate line. The full-length article consists of approximately 5000 words, excluding the reference list.

Funding. The authors are required to identify who provided financial support for the conclusion of the research project and a brief description of the role of the sponsors are required. If the funding source was not involved in the study design, collection, analyses, and interpretation of data, it is recommended to state this.

Acknowledgements

Acknowledgements should be made in a separate section and at the end of the article, before the references to those who made a substantial contribution to the study.

References

The reference style, Vancouver, was used during the study. References should include the following elements: author(s) name(s), dataset title, data repository, version, year, and global persistent identifier.

Table. All tables should be numbered consecutively with Arabic numerals, cited in the text. Keep tables brief and place explanatory matter in the footnote below the table.

Figures. All figures should be a decent quality and a brief legend should be provided.

BRAIN-DERIVED NEUROTROPHIC FACTOR, CAROTID INTIMA-MEDIA THICKNESS, AND DEPRESSION: THE SABPA STUDY

René Smit[1], Hannes Holm[3,4], Esmè Jansen van Vuren[1,2], Ruan Kruger[1,2], Martin Magnusson[1,3-5], Leandi Lammertyn[1,2]

[1]*Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa*

[2]*South African Medical Research Council: Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa*

[3]*Department of Cardiology, Skåne University Hospital, Malmö, Sweden*

[4]*Department of Clinical Sciences, Lund University, Skåne University Hospital, Malmö, Sweden*

[5]*Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden*

Correspondence:

Leandi Lammertyn, PhD

Hypertension in Africa Research Team (HART)

Private Bag X1290

North-West University (Potchefstroom Campus)

Potchefstroom

2520

South Africa

Telephone: +27 18 299 2435

Email: leandi.lammertyn@nwu.ac.za

Conflict of interest. All authors declare no conflict of interest.

Word count: 3250

3. Abstract

*Due to the results of pulse wave velocity being insignificant, it was decided that these results will not be documented or reported in the research article, seeing that we are preparing the research article for publication. The absence of the pulse wave velocity results is however discussed in the concluding chapter **(Appendix C)**.*

Introduction: Brain-derived neurotrophic factor (BDNF) plays a cardio-protective role in the vascular system. Studies have shown that individuals with severe depression often have decreased BDNF which may increase their cardiovascular (CV) risk. With depression on the rise and non-communicable disease being the main cause of death in South Africa, we aimed to compare BDNF and carotid intima-media thickness (CIMT) and investigated the relationship between these factors in black individuals with and without depression.

Materials and methods: The study sample consisted of 193 black individuals (mean age of 44; 50% women). Depressive symptoms were determined with the Patient Health Questionnaire-9 (PHQ-9). Participants with a score of ≥10 were considered depressed. CIMT was obtained by linear vascular ultrasonography and serum BDNF determined by the Quantikine Sandwich Immunoassay kit.

Results: We observed that 44% of the study sample had moderate to severe depressive symptoms. The characteristics of the groups with and without depression were similar as no statistical differences were observed in variables under investigation. Despite this, correlation and multiple regression analyses indicated an inverse relationship between BDNF and CIMT (adjusted R^2=0.487; β=-0.19; p=0.026) in individuals without depression.

Conclusion: In individuals without depression, the inverse relationship found may be indicative of the protective effect on BDNF on the vascular structure. The absence of this relationship in individuals with depression suggested that BDNF's vascular effect may be altered in this group, but further studies investigating individuals with severe depression are required to investigate the role of BDNF in depression and subsequent cardiovascular disease risk.

Keywords: cardiovascular disease, atherosclerosis, neurotrophic factors, vascular dysregulation, mental disorders.

Word count: 250

3.1 Introduction

Mental disorders are increasingly being recognised as a serious health concern as people with severe mental health conditions, die on average 10 to 20 years earlier than the general population (1). According to the World Health Organization (WHO) most of these deaths are due to preventable diseases, especially cardiovascular disease (CVD) (2). Depression is considered as a risk factor for CVD and is correlated with increased mortality and poor quality of life in patients with CVD (3). Additionally, depression may play a role in CVD by means of depressed expression of BDNF, that could promote adverse vascular alterations to occur (4-6)

Brain-derived neurotrophic factor has been shown to play a protective role in maintaining CV homeostasis (7, 8). It is stored and released from platelets into plasma (9) and is present in endothelial cells, macrophages, vascular smooth muscle cells, as well as atherosclerotic plaque (10, 11). Normal levels of BDNF have been associated with lower levels of platelet activation, which lead to the prevention of atherosclerotic plaque (12-14). Notably, a Mendelian randomization study from the Framingham cohort that prospectively investigated the association of circulating BDNF levels with CV events and mortality in 3687 participants suggested a casual protective role of BDNF in the pathogenesis of CVD (12).

The Human Science Research Council (HSRC) reported that 33% of South Africans are depressed (15). In South Africa, non-communicable diseases (NCDs) are the primary causes of mortality, with CVD as one of the leading causes (16). Previous studies done in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) cohort has indicated that depression play a key role in CV conditions, such as vascular diseases, hypertension, coronary artery disease, atherosclerosis, and arterial stiffness (17-19). Although altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis functions in individuals with depression and lower levels of BDNF have already been reported in the black subgroups of the SABPA cohort (20), it is still unknown whether BDNF is related to vascular alteration in individuals with depression, and if the relationship will be different in this groups compared to individuals without depression.

3.2 Materials and methods

3.2.1 Study participants

The participants were recruited as part of the SABPA study. We included black schoolteachers (n=200), 26-65 years, from the Dr Kenneth Kaunda district in the North West Province of South Africa. They shared similar occupational and environmental statuses. Exclusion criteria involved: tympanic temperature of 37.5°C, blood donors, vaccination less than three months before data collection, pregnant or lactating women and individuals on or abusing psychotropic substances.

Additional exclusion criteria included participant with missing data on BDNF and CIMT as well as individuals using beta blockers. Therefore, 193 Black participants were included in this study.

3.2.2 Ethical consideration

Ethical approval was obtained from the Ethics Review Board of the North-West University, Potchefstroom, Campus (number NWU-0303-21-A1). All participants in the SABPA study signed consent forms prior to measurements. The study complied with all applicable international regulation and the Helsinki declaration for investigating human participants.

3.2.3 Research procedures and questionnaires

The SABPA protocol was conducted over two days. On day one of the procedures a 24-hour ambulatory blood pressure monitor (ABPM) apparatus (Meditech CR120, Cardiotens; Meditech, Budapest, Hungary) was attached to the participants at 07:00, at the start of the school day. At 16:30, participants were transported to the North-West University, where they were welcomed at the Metabolic Unit. Participants were notified of the procedures of the following day. The General Health Questionnaire were completed to capture data on age, sex, lifestyle factors, medication use (anti-inflammatory, anti-coagulation, and anti-hypertensive medication), as well as the PHQ-9 to determine depressive symptoms. At 20:30, the participants received their last beverage and were requested to go to bed by 22:00. On day two, the 24-hour ABPM were removed. Thereafter, the participants received breakfast and were transported back to their place of work.

3.2.4 Classification of depression

The PHQ-9 was used to assess and monitor depressive symptoms severity. The questionnaire assesses nine items, each of which is scored 0-3 providing a severity score of 0 to 27. Minimal depression is indicated with a score of 0-4, mild depression is indicated with a score of 5-9, and moderate depression is indicated with a score of 10-14 (21). Kroenke et al. (2001) reported that a PHQ-9 cut-off point of ≥10 indicated the presence of depression that requires some form of intervention such as counselling and/or pharmacotherapy or combination of both (21). For our study, we therefore referred to individuals with a total PHQ-9 score of ≥10 as individuals with depression (i.ee., those with moderate to severe depressive symptoms) and those with a score below 10 as individuals without depression (i.e., those without or with minimal or mild depressive symptoms).

3.2.5 Anthropometric measurements

Height was measured with a stadiometer (Invicta stadiometer, IP, 1465, London, UK) to the nearest 0.1 cm and weight to the nearest 0.1 kg with a health scale (Precision Health Scale, A & D Company, Tokyo, Japan). The waist circumference was measured to the nearest 0.1 cm using

a non-stretchable flexible 7 mm wide metal tape (Holtain, Croswell, Wales). Body mass index (BMI) was calculated using the formula: weight/(height)^2. Obesity is indicated according to the WHO guidelines, which indicates these obese individuals with a BMI ≥30 kg/m^2.

3.2.6 Cardiovascular measurements

The 24-hour ABPM apparatus was attached to the participant's non-dominant arm for approximately 24-hours. The monitor was programmed to inflate every 30 minutes during the day (08:00-22:00) and every 60 minutes during the night (22:00-06:00). The ABPM was removed the next day at 07:00. These measurements included systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR). The 24-hour ABPM data were downloaded onto a database with the help of the CardioVision 1.9.0 addition software (Meditech, Budapest, Hungary). The European Society of Cardiology criteria for hypertension was used (average 24-hour SBP of ≥130 mmHg and/or DBP of ≥80 mmHg). Each participant's MAP was calculated using the formula: diastolic blood pressure + ⅓ (systolic blood pressure – diastolic blood pressure).

3.2.7 Carotid intima-media thickness

The SonoSite Micromax Ultrasound System (SonoSite Inc., Bothell, WA, USA) was used together with a 6-13 MHz linear array transducer to obtain the CIMT from two different angles of the left and right arteries, as well as a 3-lead echocardiogram (ECG) for timing purposes. The images were digitized and imported into the Artery Measurement System automated software (Gothenburg, Sweden) for analyses of a segment was chosen for analyses. The program automatically identifies the border of the intima-media of the near and far wall. Also, the inner diameter of the vessels calculates the CIMT and diameter from around 100 discrete measurements through a 10 mm segment. For this study the far wall CIMT measurements were used.

3.2.8 Biochemical analyses

Fasting blood samples were obtained from the antebrachial vein branches of each participant's dominant arm with a sterile winged infusion set, by a registered nurse. Blood samples were dealt with in accordance with a standardised protocol, and serum and plasma were frozen at -80°C until analysed in duplicate. Serum analyses of BDNF were done by Quantikine Colorometric Sandwich Immunoassay (R & D System, USA, Canada) with intra-assay precision between 3.9% and 4.1% and inter-assay coefficient of variations (CV) <7.6% and 11.3%.

Ethylenediaminetetraacetate (EDTA) whole blood samples were used to analyse glycosylated haemoglobin (HbA1c) and sodium fluoride samples for fasting glucose determination. The percentage of HbA1c was determined by means of the Roche Integra® (Roche, Basil,

Switzerland) apparatus. Fasting blood glucose, total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol was determined with a turbidimetric inhibition immunoassay and a homogeneous enzymatic colorometric assay, respectively (Integra 400 plus, Roche, Switzerland). High sensitivity C-reactive protein (CRP) and interleukin-6 (IL-6) was analysed with two-sequential multiple analyses (Konelab 20i™, ThermoScientific Vantaa, Finland; Unicel DXC 800, Beckham and Coulter, Munich, Germany). Further, tumour necrosis factor-alpha (TNF-α) was measured via a Quantikine HS Elisa human serum TNF-α Immunoassay (HS ELISA, R & D System, Minneapolis, MN, USA). Low-density lipoprotein (LDL) cholesterol was calculated using the formula: LDL = TC – HDL cholesterol – (0.45 x triglycerides) (22). Gamma-glutamyl transferase (GGT), an indicator of alcohol abuse (23) and serum cotinine, an indicator of nicotine levels (24) was analysed with the enzyme rate method (Unicel DXC; Beckham and Coulter, Germany).

3.2.9 Statistical analyses

IBM® SPSS® version 28 (IBM Corporation, Armonk, New York) was used for database and statistical analyses. All variables were evaluated for normality (using QQ plots and inspection of skewness and kurtosis). Our study sample was divided into depressive and non-depressive black individuals according to the established cut-off point of the PHQ-9 scores. Means and proportions were presented by the geometric mean and the 25^{th} and 75^{th} percentile intervals, while dichotomous variables were reported as the total number of the population sample and percentages. Parameters that did not meet the criteria were logarithmically transformed for the regression analyses. We used single regression and partial correlations (adjusted for age, sex, 24-hour MAP and waist circumference) to determine of a relationship exist between CIMT and BDNF. Multiple regression was used to determine if an independent association exist between BDNF and CIMT in the respective groups. The covariates included in the final model was age, sex, waist circumference, glucose, CRP, LDL cholesterol, MAP, and GGT. All P values refer to a two-sided hypothesis (25).

3.3 Results

The population sample of 193 Black participants included 86 (44%) individuals (mean age of 44 years) with a depression score of ≥10, of which 52.3% were men **(Table 3.1)**. No statistical differences were observed in body composition, cardiovascular- and biochemical measurements as well as medication use between the two reported groups.

TABLE 3.1. Characteristics of the study population stratified by Black individuals with and without depression

	Individuals with depression N = 86	**Individuals without depression N = 107**	***P***
Age, years	44 (38; 51)	44 (39; 51)	0.669
Sex (men), N (%)	45 (52.3)	51 (47.7)	0.520
Depression Severity			
PHQ-9 scores	**13 (11; 17)**	**6 (4; 8)**	**<0.001**
Minimal depression	0 (0)	42 (39.3)	
Mild depression	0 (0)	65 (60.7)	
Moderate depression	52 (60.5)	0 (0)	
Moderately severe depression	23 (26.7)	0 (0)	
Severe depression	11 (12.8)	0 (0)	
Anthropometry			
Waist circumference, (men), cm	93.00 (83.39; 103.67)	93.07 (83.80; 100.60)	0.152
Waist circumference, (women), cm	99.73 (84.19; 105.40)	89.47 (80.47; 101.20)	0.152
Body mass index, kg/m^2	30.69 (25.76; 34.50)	29.30 (24.56; 33.76)	0.359
Obese individuals, N (%)	46 (53.5)	48 (44.9)	0.233
Cardiovascular variables			
CIMT mean, mm	0.68 (0.59; 0.77)	0.67 (0.60; 0.76)	0.913
24-hour Systolic BP, mmHg	131 (121; 145)	131 (122; 144)	0.886
24-hour Diastolic BP, mmHg	82 (75; 88)	82 (76; 91)	0.715
24-hour Mean arterial pressure, mmHg	98 (91; 107)	99 (91; 108)	0.864
Hypertensive individuals, N (%)	57 (66.3)	68 (63.6)	0.693
Biochemical variables			
Brain derived neurotrophic factor, ng/ml	1.37 (0.85; 1.80)	1.33 (1.01; 1.80)	0.566
Total cholesterol, mmol/l	4.41 (3.77; 5.51)	4.45 (3.72; 5.21)	0.381
LDL-cholesterol, mmol/l	2.71 (2.08; 3.71)	2.80 (2.10; 3.42)	0.593
Triglycerides, mmol/l	1.11 (0.76; 1.71)	1.08 (0.78; 1.56)	0.992
Glucose, mmol/l	5.18 (4.74; 5.66)	5.28 (4.58; 5.84)	0.613
C-reactive protein, mg/l	4.58 (1.97; 8.82)	5.10 (2.05; 12.77)	0.238
Gamma-glutamyltransferase, U/l	44.88 (31.01; 84.20)	39.81 (26.72; 61.50)	0.077
Medication use			
Anti-inflammatory, N (%)	5 (5.8)	11 (10.3)	0.263
Anti-hypertension, N (%)	19 (22.1)	25 (23.4)	0.834
Anti-depression, N (%)	1 (1.2)	0 (0)	0.263
Aspirin and anti-coagulants, N (%)	2 (2.3)	4 (3.7)	0.426

Data is reported as mean (25^{th} and 75^{th} percentile), or number of participants and (percentage). Only cotinine levels above or equal to 10 ng/ml are reported. BP, blood pressure; CIMT, carotid intima-media thickness; LDL, low-density lipoproteins; HDL, high-density lipoproteins; HbA1c, glycated haemoglobin; HIV, human immunodeficiency virus; BDNF, brain derived neurotrophic factor.

The single regression analyses indicated that an inverse relationship only exist between CIMT and BDNF in individuals without depressive symptoms (non-depressive: r=-0.214; p=0.026; depressive: r=-0.030; p=0.781) **(Figure 3.1)**. When partial regression was performed controlling for age, sex, waist circumference and MAP the significant inverse relation between CIMT and BDNF (r=-0.231; p=0.019) remained in the group without depression.

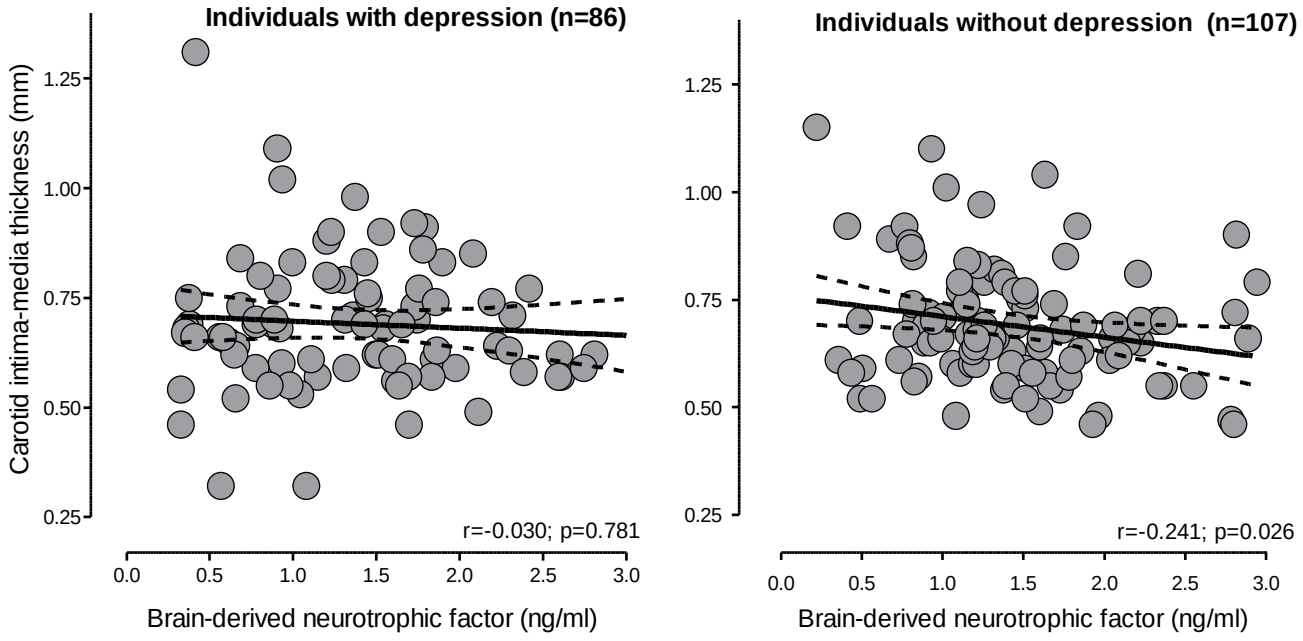


Figure 3.1. Schematic representation of correlations between brain-derived neurotrophic factor and carotid intima-media thickness in individuals with and without depression.

After performing multiple regression analyses **(Table 3.2)** adjusted for age, sex, waist circumference, glucose, CRP, LDL cholesterol, MAP and GGT, an independent inverse association was confirmed between CIMT and BDNF in individuals without depression (β=-0.19; p=0.026). Additional sensitivity analyses were performed in which the effect of additional covariates such as hypertensive medication was tested. These covariates did not influence the main outcomes of the multiple regression models.

TABLE 3.2. Associations between brain-derived neurotrophic factor and carotid intima-media thickness in individuals with and without depression

	Without depression (N=107)	***P***	**With depression (N=86)**	***p***
Adjusted R^2	0.487		0.310	
	β (±95% CI)	***P***	***β (±95% CI)***	***p***
Brain-derived neurotrophic factor, ng/ml	**-0.19 (-0.35; -0.02)**	**0.026**	-0.04 (-0.24; 0.16)	0.668
Age, years	**0.46 (0.29; 0.62)**	**<0.001**	**0.40 (0.18; 0.61)**	**<0.001**
Sex, women	0.10 (-0.10; 0.29)	0.321	-0.11 (-0.37; 0.15)	0.397
Waist circumference, mm	-0.01 (-0.18; 0.17)	0.940	0.02 (-0.25; 0.30)	0.864
Glucose, mmol/l	**0.21 (0.05; 0.37)**	**0.012**	0.13 (-0.09; 0.35)	0.254
C-reactive protein, mg/l	0.13 (-0.06; 0.32)	0.168	-0.06 (-0.31; 0.19)	0.638
LDL-cholesterol, mmol/l	0.14 (-0.01; 0.29)	0.063	0.18 (-0.04; 0.39)	0.105
Mean arterial pressure, mmHg	0.14 (-0.03; 0.31)	0.112	0.15 (-0.11; 0.40)	0.254
Gamma-glutamyltransferase, U/l	-0.10 (-0.25; 0.05)	0.190	-0.01 (-0.24; 0.22)	0.946

Abbreviations: CI, confidence interval; LDL, low-density lipoprotein.

3.4 Discussion

We aimed to compare BDNF and CIMT and investigated the relationship between these factors in Black individuals with and without depression. Overall, our groups with and without depression showed no significant differences in their CV health profiles, including BDNF in individuals without depression. A higher level of BDNF was associated with lower CIMT, further indicating BDNF's role in maintaining vascular health.

The neurotrophic factor, BDNF has been a subject of interest over the past decade, especially in the development of CVD and has a possible cardio-protective agent on the CV system (26). Lower BDNF levels have also been associated with an array of psychiatric disorders, including depression (27). The association of BDNF and atherosclerotic CVD is one of the main focuses in research (14, 28). Also, low BDNF levels have been detected in atherosclerosis and related to endothelial function suggesting BDNF's protective role to prevent further vascular injury (12, 29, 30) . We were unable to replicate earlier findings that depression severity is accompanied by lower levels of BDNF (31, 32). In fact, we did not observe lower levels of BDNF or larger CIMT measurements in the depression group. A possible explanation for these results may be that our study-sample were grouped according to depressive symptoms and not a clinically diagnosis of major depressive disorder, as was the case in the studies that did show an association (12, 33). Furthermore, during recruitment for the SABPA study, individuals receiving treatment for psychotropic substances were excluded thereby eliminating individuals with clinical depression.

No differences were observed between the groups with and without depression, however, we did observe an inverse association between BDNF and CIMT individuals without depression. This could indicate that BDNF have a protective role in the vascular structure in the absence of moderately to severe depressive symptoms. This neurotrophic hypothesis proposed that BDNF

levels are significantly lower in individuals with major depressive disorder (34). As previously reported, higher levels of BDNF are associated with cardio-protective effects on the vascular system (29, 30). Brain-derived neurotrophic factor and its receptor, tyrosine kinase B (TrkB) are synthesized in many non-neuronal tissues and cell types, which include endothelial cells, vascular smooth muscle cells, and atherosclerotic vessels (35). BDNF/TrkB has been found to be expressed in endothelial cells. The activation of TrkB has been associated with the regulation of vascular permeability and play a significant role in maintaining endothelial barrier integrity and promoting smooth muscle cell activity (36). Jiang et al. (2015) reported that TrkB activation promotes the synthesis of vascular endothelial cadherin, a molecule that plays a crucial role in the formation of endothelial cells in blood vessels (35, 37). Activation of TrkB promotes endothelial cell survival, proliferation, and angiogenesis, whereas vascular endothelial cadherin also plays an integral part of regulating endothelial cell proliferation (35, 37). Hereby, the upregulation of BDNF/TrkB plays a protective role in the macrovasculature, leading to decreased risk for CV system atherosclerosis (38). Further research is however needed in our population to explain the differences between CIMT and BDNF in our population groups. Therefore, we recommended that future studies consider additional variables such as haemostasis, redox system markers, or sympathetic activity markers which include heart rate variability, which has been shown to influence BDNF and CIMT (17, 39-42).

The strengths and limitations of this study should be considered when interpreting results. The strengths of this study include that groups were divided into individuals with and without depression using their PHQ-9 scores, whereas the PHQ-9 is considered as the national and international gold standard measurements tool for depression (6). Despite the small sample size at least 55 individuals were required per group. This is a well-designed study being based on cross-sectional data, and we could not determine changes in CIMT and BDNF or analyse the cause-and-effect in individuals with depression. When examining the potential effects of depression, it is important that our groups were not clinically diagnosed with depressive disorder, but only experience depressive symptoms. This can be seen as a limitation as studies that did find associations, reported in individuals suffering from major depressive disorder. Further, the sample population included a small sample size of teachers living in the North West Province from the Dr Kenneth Kaunda Education district; therefore, these results cannot be applied to the whole of South Africa.

3.5 Conclusion

Our groups with and without depression showed no significant differences in their health profiles. An independent relationship that possibly indicates BDNF’s role in maintaining vascular health was reported in individuals with depression are affected but requires further investigation.

3.6 References

1. Rothman SM, Griffioen KJ, Wan R, Mattson MP. Brain-derived neurotrophic factor as a regulator of systemic and brain energy metabolism and cardiovascular health. Ann NY Acad. 2012;1264(1):49-63.
2. WHO. Cardiovascular diseases (CVDs). World Health Organization. 2021.
3. Liu M, Jia J, Fan F, Sun P, Wu Z, Jiang Y, et al. Association of depressive symptoms with the progression of carotid intima-media thickness in a community-based cohort in Beijing, China. BMJ Open. 2021;11(8):e050254.
4. Tschorn M, Kuhlmann SL, Rieckmann N, Beer K, Grosse L, Arolt V, et al. Brain-derived neurotrophic factor, depressive symptoms and somatic comorbidity in patients with coronary heart disease. Acta Neuropsychiatr. 2021;33(1):22-30.
5. Pillay-van Wyk V, Msemburi W, Laubscher R, Dorrington R, E, Groenewald P, Matzopoulos R, et al. Second national burden of disease study South Africa: national and subnational mortality trends, 1997–2009. The Lancet. 2013;381:113.
6. Santos IS, Goulart AC, Brunoni AR, Kemp AH, Lotufo PA, Bensenor IM. Anxiety and depressive symptoms are associated with higher carotid intima-media thickness. Cross-sectional analysis from ELSA-Brasil baseline data. Atherosclerosis. 2015;240(2):529-34.
7. Fulgenzi G, Tomassoni-Ardori F, Babini L, Becker J, Barrick C, Puverel S, et al. BDNF modulates heart contraction force and long-term homeostasis through truncated TrkB. T1 receptor activation. J Cell Biol. 2015;210(6):1003-12.
8. Cai D, Holm JM, Duignan IJ, Zheng J, Xaymardan M, Chin A, et al. BDNF-mediated enhancement of inflammation and injury in the aging heart. Physiol Genomics. 2006;24(3):191-7.
9. Levi-Montalcini L, Cohen S, Leibrock J, Lottspeich F, Hohn A, Hofer M, et al. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. J Thromb Haemost. 2002;87(04):728-34.
10. Ejiri J, Inoue N, Kobayashi S, Shiraki R, Otsui K, Honjo T, et al. Possible role of brain-derived neurotrophic factor in the pathogenesis of coronary artery disease. Circulation. 2005;112(14):2114-20.
11. Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, et al. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett. 2000;470(2):113-7.
12. Kaess BM, Preis SR, Lieb W, Beiser AS, Yang Q, Chen TC, et al. Circulating brain-derived neurotrophic factor concentrations and the risk of cardiovascular disease in the community. J Am Heart Assoc. 2015;4(3):e001544.

13. Nemcsik J, Laszlo A, Lenart L, Eorsi D, Torzsa P, Korosi B, et al. Hyperthymic affective temperament and hypertension are independent determinants of serum brain-derived neurotrophic factor level. Ann Gen Psychiatry. 2016;15(1):17.
14. Pius-Sadowska E, Machaliński B. BDNF–A key player in cardiovascular system. J Mol Cell Cardiol. 2017;110:54-60.
15. HSRC. The mental health effects of the COVID-19 lockdown in South Africa. 2020.
16. Al-Mawali A. Non-Communicable Diseases: Shining a Light on Cardiovascular Disease, Oman's Biggest Killer. Oman Med J. 2015;30(4):227-8.
17. Smith AJ, Malan L, Uys AS, Malan NT, Harvey BH, Ziemssen T. Attenuated brain-derived neurotrophic factor and hypertrophic remodelling: the SABPA study. J Hum Hypertens. 2015;29(1):33-9.
18. Malan L, Hamer M, von Kanel R, Schlaich MP, Reimann M, Frasure-Smith N, et al. Chronic depression symptoms and salivary NOx are associated with retinal vascular dysregulation: The SABPA study. Nitric Oxide. 2016;55-56:10-7.
19. Mashele N, Van Rooyen JM, Malan L, Potgieter JC. Cardiovascular function and psychological distress in urbanised black South Africans: the SABPA study: cardiovascular topics. Cardiovasc J Afr. 2010;21(4):206-11.
20. Malan L, Schutte CE, Alkerwi A, Stranges S, Malan NT. Hypothalamic-pituitary-adrenal-axis dysregulation and double product increases potentiate ischemic heart disease risk in a Black male cohort: the SABPA study. Hypertens Res. 2017;40(6):590-7.
21. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16(9):606-13.
22. Wilson PW, Abbott RD, Garrison RJ, Castelli WP. Estimation of very-low-density lipoprotein cholesterol from data on triglyceride concentration in plasma. Clin Chem. 1981;27(12):2008-10.
23. van Beek J, de Moor MHM, Geels LM, Sinke MRT, de Geus EJC, Lubke GH, et al. The association of alcohol intake with gamma-glutamyl transferase (GGT) levels: evidence for correlated genetic effects. Drug Alcohol Depend. 2014;134:99-105.
24. Raja M, Garg A, Yadav P, Jha K, Handa S. Diagnostic Methods for Detection of Cotinine Level in Tobacco Users: A Review. J Clin Diagn Res. 2016;10(3):ZE04-6.
25. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175-91.
26. Okada S, Yokoyama M, Toko H, Tateno K, Moriya J, Shimizu I, et al. Brain-derived neurotrophic factor protects against cardiac dysfunction after myocardial infarction via a central nervous system-mediated pathway. Arterioscler Thromb Vasc Biol. 2012;32(8):1902-9.

27. Molendijk ML, Bus BA, Spinhoven P, Penninx BW, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. Mol Psychiatry. 2011;16(11):1088-95.
28. Chaldakov GN, Fiore M, Hristova MG, Aloe L. Metabotrophic potential of neurotrophins:implication in obesity and related diseases? Med Sci Monit. 2003;9(10):HY19-21.
29. Zierold S, Buschmann K, Gachkar S, Bochenek ML, Velmeden D, Hobohm L, et al. Brain-Derived Neurotrophic Factor Expression and Signaling in Different Perivascular Adipose Tissue Depots of Patients With Coronary Artery Disease. J Am Heart Assoc. 2021;10(6):e018322.
30. Kuhlmann SL, Tschorn M, Arolt V, Beer K, Brandt J, Grosse L, et al. Serum brain-derived neurotrophic factor and stability of depressive symptoms in coronary heart disease patients: A prospective study. Psychoneuroendocrinology. 2017;77:196-202.
31. Rana T, Behl T, Sehgal A, Srivastava P, Bungau S. Unfolding the Role of BDNF as a Biomarker for Treatment of Depression. J Mol Neurosci. 2021;71(10):2008-21.
32. Schroter K, Brum M, Brunkhorst-Kanaan N, Tole F, Ziegler C, Domschke K, et al. Longitudinal multi-level biomarker analysis of BDNF in major depression and bipolar disorder. Eur Arch Psychiatry Clin Neurosci. 2020;270(2):169-81.
33. Cavaleri D, Moretti F, Bartoccetti A, Mauro S, Crocamo C, Carra G, et al. The role of BDNF in major depressive disorder, related clinical features, and antidepressant treatment: Insight from meta-analyses. Neurosci Biobehav Rev. 2023;149:105159.
34. Bus BA, Molendijk ML. [The neurotrophic hypothesis of depression]. Tijdschr Psychiatr. 2016;58(3):215-22.
35. Jiang H, Huang S, Li X, Li X, Zhang Y, Chen ZY. Tyrosine kinase receptor B protects against coronary artery disease and promotes adult vasculature integrity by regulating Ets1-mediated VE-cadherin expression. Arterioscler Thromb Vasc Biol. 2015;35(3):580-8.
36. Li L, Guo H, Lai B, Liang C, Chen H, Chen Y, et al. Ablation of cardiomyocyte-derived BDNF during development causes myocardial degeneration and heart failure in the adult mouse heart. Front Cardiovasc Med. 2022;9:967463.
37. Dejana E, Giampietro C. Vascular endothelial-cadherin and vascular stability. Curr Opin Hematol. 2012;19(3):218-23.
38. Trombetta IC, DeMoura JR, Alves CR, Carbonari-Brito R, Cepeda FX, Lemos JR, Jr. Serum Levels of BDNF in Cardiovascular Protection and in Response to Exercise. Arq Bras Cardiol. 2020;115(2):263-9.
39. Han W, Zhang C, Wang H, Yang M, Guo Y, Li G, et al. Alterations of irisin, adropin, preptin and BDNF concentrations in coronary heart disease patients comorbid with depression. Ann Transl Med. 2019;7(14):298.

40. Harvey BH, Hamer M, Louw R, van der Westhuizen FH, Malan L. Metabolic and glutathione redox markers associated with brain-derived neurotrophic factor in depressed african men and women: evidence for counterregulation? Neuropsychobiology. 2013;67(1):33-40.
41. Podyma B, Parekh K, Guler AD, Deppmann CD. Metabolic homeostasis via BDNF and its receptors. Trends Endocrinol Metab. 2021;32(7):488-99.
42. Yang AC, Hong C, Tsai S. Heart rate variability in psychiatric disorders. Taiwanese Journal of Psychiatry. 2010;24(2):99-109.

CHAPTER 4

4. Introduction

The closing chapter for this study serves as a culmination of work, summarizing the main findings, conclusions, and the implications of the study. This chapter reflects on the significance of the work, highlighting its contributors to the field, and suggests avenues for future research.

4.1 Summary of the main findings integrated with acceptance or rejection of hypotheses.

The main aim of the study was to investigate differences in brain-derived neurotrophic factor (BDNF) levels as well as markers of vascular remodeling and -function, which included carotid intima-media thickness (CIMT) and pulse wave velocity (PWV) individuals with and without depression, and whether relationships between these markers exist in each group.

Our first objective was to compare BDNF, CIMT and PWV between individuals with and without depression. *We hypothesized that individuals with depression will exhibit lower BDNF levels, and higher CIMT and PWV measures, compared to individuals without depression.*

As previously reported, clinical depression is a mental disorder which is characterized by persistently depressed mood or loss of interest in daily activities, causing significant impairment in daily life activities (1). A suggested link between depression and BDNF has been identified, where it indicates that individuals with depression may have significantly lower levels of BDNF in their brains and blood compared to healthy individuals (2). Emerging research suggests that BDNF may have implications beyond mental health disorders, which includes BDNF's potential involvement in cardiovascular disease (CVD) (3, 4). Disturbances in lower BDNF levels is also linked to cardiovascular (CV) risk factors which include increased lipid levels, elderly age, male sex, smoking, diabetes mellitus and physical inactivity (5). All the above leads to an understanding that BDNF and its receptor, tyrosine kinase B (TrkB) are expressed in the CV system (6). During the investigation of BNDF, it has been identified that lower levels of BDNF is associated with endothelial dysfunction, the initial stages of atherosclerosis (7, 8). Over time, increased plaque build-up leads to hardening of the arterial wall, which reduces the ability of the arterial wall to expand and contract in response in physiological changes, resulting in the loss of elasticity (9), hereby increasing arterial stiffness (10).

We did not observe lower BDNF or higher CIMT and PWV measures in individuals with depression, hereby, we rejected this hypothesis. Since we were unable to replicate earlier findings suggesting that higher depressive symptoms are accompanied by lower levels of BDNF (11, 12). This might be due to our grouping criteria and the fact that our population sample was not clinically diagnosed with major depressive disorder, compared to other studies that reported on clinically depressed individuals. According to the Diagnostic Statistical Manual (DSM) criteria, individuals should experience depressive symptoms every day, all day, for at least two week, before

individuals are diagnosed with depression (13). Our study populations' depressive symptoms were determined using the Patient Health Questionnaire-9 (PHQ-9). It is important to note that the PHQ-9 is not a diagnostic tool but rather a screening instrument. A health care professional should evaluate the results and make a diagnosis based on a comprehensive assessment of the individual's symptoms and functioning. Previous research has examined individuals with chronic major depressive disorder, as well as individuals being treated (14), whereas less than one percent of our study population received treatment.

We further speculate that our population has not yet been diagnosed with atherosclerosis since our mean CIMT was 0.68 mm, whereas studies have reported that CIMT should be higher than 0.90 mm, before a patient can be diagnosed with atherosclerosis (15). Through this observation, it can be speculated that most individuals in our study still have good vascular health. The absence of any significant results with PWV in our study could be explained due to no clinical remodeling in the Sympathetic activity and Ambulatory Blood Pressure in South Africans (SABPA) cohort with regards to CIMT and PWV. It has been reported that arterial thickness leads to arterial stiffness by increasing the deposition of collagen in the arterial wall, which reduces the arterial wall to expand and contract in response to several physiological changes resulting in the loss of elasticity (16). We did not report any remarkable results on CIMT, and the above-mentioned information could be a possible explanation that we did not find any results when analyzing PWV in individuals with depression.

Our second objective was to investigate if independent associations exist between BDNF, CIMT, and PWV in individuals with and without depression. *We hypothesized that an inverse independent association will be present between BDNF, CIMT and PWV in individuals with depression, but not in individuals without depression.*

Studies has shown that BDNF may play a cardio-protective effects in maintaining endothelial barrier integrity and further promotes the survival of vascular smooth muscle cells, cardiomyocytes, endothelial cells and atherosclerotic vessels (17). Brain-derived neurotrophic factor is synthesized and packed in alpha granules of megakaryocytes, the platelet precursor (18). Brain-derived neurotrophic factor attached to platelets, circulate in the blood stream and upon injury, BDNF is released, which results into high concentrations of BDNF to the injured vessels (19). Brain-derived neurotrophic factor's receptor, TrkB may also have a significant role in the cardiovascular system (20). Cardiac endothelial cells in atherosclerotic lesions in humans express TrkB receptors, whereas a decrease in TrkB activity leads to vascular leakage (17). Another pathway for regulating endothelial cell include that TrkB is necessary for the regulation of vascular cadherin expression, a molecule responsible for cell proliferation and apoptosis (19). It is reasonable that increased TrkB activity protects against CV atherosclerosis, playing a cardio-

protective role against the development of atherosclerosis, whereas it also decreases the risk for CVD. The significant results between BDNF and CIMT in individuals without depression and the absence thereof in individuals with depression may infer that BDNF's beneficial role in maintaining vascular health in individuals with depression are affected. Hereby, we rejected the hypothesis.

The absence of results with PWV may be due to differences observed in accordance with previous studies and this Masters study. Previous studies that reported significance had the following contributing factors:

- Moh et al. (2011) reported in individuals (mean age of 64 years) with no-aortic stiffness (n=697) and aortic stiffness (n=261); they found that depressive symptoms correlated with PWV, leading to the conclusion that depressive symptoms are associated with PWV in individuals already diagnosed with aortic stiffness (21).
- Wu et al. (2018) compiled data from 38 full articles and meta-analyzed availability of data involving individuals with depressive symptoms (n=5947) and individuals without depression (n=34.423) provide evidence on the effects of depressive symptoms on arterial structure and – function. They reported that depressive symptoms were consistently associated with *more advanced function and structural vascular abnormalities* characterized by thicker CIMT and higher PWV in individuals with depression (22).

The above-mentioned studies all reported in individuals at risk for CVD, contradicting our current study. The SABPA cohort mostly consists of individuals with good vascular health as well as individuals without major depressive disorder. These previously reported studies showed that arterial stiffness was more strongly associated with major depressive disorder than with depressive symptoms; this could be attributed to the misclassification of depressive symptoms as major depressive disorder by the PHQ-9.

It is also possible that there are no differences within the healthy SABPA population for the variables tested; however, several mechanisms could explain the relationship between depression and subclinical atherosclerosis. Wu et al. (2018) reported on the two major pathways known as the hypothalamic-pituitary-adrenal (HPA) axis activity and the sympathetic nervous system (SNS). Depression leads to the activation of both systems, first the SNS then the HPA axis activity; hereafter, various hormones such as catecholamines, corticosteroids, glucagon and growth hormones are released. All these hormones play a crucial role in early progression and complications of atherosclerosis (22). We recommended that future studies consider these additional variables, which has shown to influence BDNF, CIMT and PWV in individuals with depression (22).

4.2 Limitations

When interpreting these results, it is imperative to reflect on the factors that could have affected the results in this study. Due to the cross-sectional design, it prevents the identification of physiological cause-and-effects. Since our population only included a small sample size of (n=193) of Black teachers living in the North West Province, from the Dr Kenneth Kaunda district, the results cannot be generalized to the whole of South Africa. We reported only in Black teachers, based on previous findings in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) cohort (23-25), but it has its limitations. In this study, we divided our groups according to depressive symptoms severity, hereby, our sample population have not yet been diagnosed with major depressive disorder. In addition, in this study we used the carotid-dorsalis pedis PWV. The carotid-dorsalis pedis PWV is measured between the carotid artery and the dorsalis pedis. However, the carotid femoral PWV is the current fold standard to assess aortic stiffness (26), as it is easy to use and has the best predictive value for CV events (27).

4.3 The element of chance

The element of chance should always be considered when interpreting the results from this study; this study was well-designed and was conducted under strict protocols. The study population was also selected from similar socio-economic backgrounds, also, a power analyses indicated that although we have a small population sample. All associations demonstrate that the study was genuine, significant and were investigated from a physiological perspective. A complete understanding of all mechanisms is crucial when interpreting the associations found during this research study.

We limited the possibility of chance by adjusting for several CV contributing factors. These covariates were included based on the literature and bivariate correlations and adjusted for age, sex, waist circumference, glucose, C-reactive protein (CRP), low-density lipoprotein (LDL) cholesterol, mean arterial pressure (MAP), and gamma-glutamyl transferase (GGT).

4.4 Recommendations

The importance of research in health sciences is to educate and inform most of the South African citizens about risk factors, treatment and public intervention and the use of the primary care centers and costs contributing to a living healthy lifestyle (28). The researchers will discuss recommendations for future recommendations for future research as well as for the public. Clinical studies of post-mortem teenage suicide victims reported on the significant decrease in BDNF/TrkB concentrations in the prefrontal cortex (29, 30). The financial burden on a country is influenced by suicide, the following monetary values have been reported in neighboring countries such as Mozambique with 42,397,600, Zambia with 32,396,104, Botswana with 56,953,746,

Namibia with 39,403,474, and South Africa has the largest amount of 1,260,115,686 international US dollars reported in 2019 (31). Companies suffer when individuals with depression have suicidal thoughts and suicide itself, due to the money that is spent during recruitment of other employees, therefore, it impacts the business directly as well as their families (32).

Recommendation for further studies

Considering our results, we found together with several other researchers that BDNF plays a crucial role in the development and protection of the CV system (2). Since our study was performed in a small group of Black teachers, we recommended that larger population samples diagnosed with major depressive disorder, to study the impact of depression, BDNF, and CVD. Different types or forms of BDNF have been identified and included proBDNF, mature BDNF and precursor BDNF (33, 34). It is advised to rather analyze mature BDNF, an active form of the protein. Researchers have reported on the role of mature BDNF in depression, by measuring BDNF levels in various brain regions or examination of BDNF with regards to depressive symptoms (35). Also, different variables have been known to interact with BDNF, since BDNF has various effects on the human body. It is also recommended that future studies document individuals who have been diagnosed with major depressive disorder, as diagnosed by psychologists and psychiatrists. Individuals with major depressive disorder experience higher severity of depressive symptoms compared to those with subclinical symptoms. This will allow researchers to investigate more pronounced cardiovascular changes and associations, providing a clearer picture of the impact of depression on biological factors like BDNF and CVD. To report significance in PWV it is recommended that the international gold standard, the carotid femoral PWV are used to assess arterial stiffness, as it is easy to use and has the best predictive value for CV events (26, 27).

Recommendation for the public

Depression has received significant attention in the past few years as an independent risk factor for CVD. One of the mechanisms linking depression to CVD is the hypothalamic-pituitary-adrenal (HPA) axis activity, and neurotrophic factors amongst others. Studies indicated that black individuals suffering from depression might be predisposed to alterations in the HPA axis activity (36). Stress induced hyperactivity of the HPA axis activity, together with an increase in glucocorticoids, reduces the expression of the neurotrophic factor, BDNF (37). Brain-derived neurotrophic factor plays a crucial role in the development of the CV system, where it activates TrkB receptors leading to the survival of endothelial cells and formation of cardiac vasculature (38). Brain-derived neurotrophic factor also has a positive relationship with regards to high-density lipoprotein (HDL) cholesterol and platelet count (39, 40), where it has been found to play a cardio-protective role in the CV system.

To increase BDNF will have positive effects on mental health as well as CV health, hereby, higher levels of BDNF will be observed during the following lifestyle changes:

- **Exercise:** Exercise such as swimming, cycling, and yoga has shown to effect the circulating BDNF levels (41), when exercising there is a consistently increase in BDNF levels in platelets as well as in serum BDNF levels in the hypothalamus, striatum and other cortical areas (42).
- **Deep sleep:** Your body is known to release high levels of BDNF during the deeper stages of sleep. Sleep mediates the plasticity-related changes and when sleep deprived, it could be linked to lower BDNF levels and depression (43).
- **Meditation:** Chronic stress and depression are known to decrease BDNF, however, during meditation there is a significant increase in BDNF levels, specifically in the brain regions responsible for memory, emotional control, happiness and attention (41).
- **Sunlight:** Exposure to sunlight also leads to an increase in BDNF; according to Molendijk et al. (2011) reported that BDNF concentration is significantly higher in spring-summer periods than in autumn-winter periods (44)

4.5 Reflection of the researcher

Childhood remarks towards my mother at the age of 6 months. “She has to be active for at least two hours a day, together with a good night’s rest to functioning normal without several panic attacks.” Throughout this study, I obtained an abundance amount of knowledge and insight into psychiatric disorders such as anxiety and depression. As a person suffering from severe anxiety, it was important to obtain a healthy lifestyle, which includes habits such as sleeping, exercising and eating healthy. Habits had to change, not saying no to chemical treatment, but rather have reduced treatment together with a healthier lifestyle.

“This will be my message to anyone suffering from psychiatric conditions such as anxiety and depression.”

4.6 Conclusion

Although no significant differences were found between individuals with and without depression, there is a strong possibility that BDNF might play an important role in the vascular system. Brain-derived neurotrophic factor has been found to play a cardio-protective role in the CV system of individuals without depression. However, the absence of these results in individuals with depression may infer that BDNF’s role in maintaining vascular health in individuals with depression are affected but requires further investigation. The research was evaluated, shortcomings were identified, and the researchers conclude with a reflection on the study.

4.7 References

1. Lopez AD, Murray CC. The global burden of disease, 1990-2020. Nat Med. 1998;4(11):1241-3.
2. Pius-Sadowska E, Machaliński B. BDNF–A key player in cardiovascular system. J Mol Cell Cardiol. 2017;110:54-60.
3. Rajan S, McKee M, Rangarajan S, Bangdiwala S, Rosengren A, Gupta R, et al. Association of Symptoms of Depression With Cardiovascular Disease and Mortality in Low-, Middle-, and High-Income Countries. JAMA Psychiatry. 2020;77(10):1052-63.
4. Waitzfelder B, Stewart C, Coleman KJ, Rossom R, Ahmedani BK, Beck A, et al. Treatment Initiation for New Episodes of Depression in Primary Care Settings. J Gen Intern Med. 2018;33(8):1283-91.
5. Jin H, Chen Y, Wang B, Zhu Y, Chen L, Han X, et al. Association between brain-derived neurotrophic factor and von Willebrand factor levels in patients with stable coronary artery disease. BMC Cardiovasc Disord. 2018;18(1):1-8.
6. Sirker A, Zhang M, Shah AM. NADPH oxidases in cardiovascular disease: insights from in vivo models and clinical studies. Basic Res Cardiol. 2011;106(5):735-47.
7. Alomari MA, Khabour OF, Maikano A, Alawneh K. Vascular function and brain-derived neurotrophic factor: The functional capacity factor. Vasc Med. 2015;20(6):518-26.
8. Poznyak AV, Grechko AV, Orekhova VA, Khotina V, Ivanova EA, Orekhov AN. NADPH oxidases and their role in atherosclerosis. Biomedicines. 2020;8(7):206.
9. Lacolley P, Regnault V, Segers P, Laurent S. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. Physiol Rev. 2017;97(4):1555-617.
10. Lyle AN, Raaz U. Killing Me Unsoftly: Causes and Mechanisms of Arterial Stiffness. Arterioscler Thromb Vasc Biol. 2017;37(2):e1-e11.
11. Rana T, Behl T, Sehgal A, Srivastava P, Bungau S. Unfolding the Role of BDNF as a Biomarker for Treatment of Depression. J Mol Neurosci. 2021;71(10):2008-21.
12. Schroter K, Brum M, Brunkhorst-Kanaan N, Tole F, Ziegler C, Domschke K, et al. Longitudinal multi-level biomarker analysis of BDNF in major depression and bipolar disorder. Eur Arch Psychiatry Clin Neurosci. 2020;270(2):169-81.
13. Kocsis RN. Book review: diagnostic and statistical manual of mental disorders: (DSM-5). Sage Publications Sage CA: Los Angeles, CA; 2013.
14. Cavaleri D, Moretti F, Bartoccetti A, Mauro S, Crocamo C, Carra G, et al. The role of BDNF in major depressive disorder, related clinical features, and antidepressant treatment: Insight from meta-analyses. Neurosci Biobehav Rev. 2023;149:105159.
15. Simova I. Intima-media thickness: appropriate evaluation and proper measurement. J Cardiol Pract. 2015;13(21):1-14.

16. Lacolley P, Challande P, Osborne-Pellegrin M, Regnault V. Genetics and pathophysiology of arterial stiffness. Cardiovasc Res. 2009;81(4):637-48.
17. Jiang H, Huang S, Li X, Li X, Zhang Y, Chen ZY. Tyrosine kinase receptor B protects against coronary artery disease and promotes adult vasculature integrity by regulating Ets1-mediated VE-cadherin expression. Arterioscler Thromb Vasc Biol. 2015;35(3):580-8.
18. Chacon-Fernandez P, Sauberli K, Colzani M, Moreau T, Ghevaert C, Barde YA. Brain-derived Neurotrophic Factor in Megakaryocytes. J Biol Chem. 2016;291(19):9872-81.
19. Kermani P, Hempstead B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med. 2007;17(4):140-3.
20. Li L, Guo H, Lai B, Liang C, Chen H, Chen Y, et al. Ablation of cardiomyocyte-derived BDNF during development causes myocardial degeneration and heart failure in the adult mouse heart. Front Cardiovasc Med. 2022;9:967463.
21. Moh MC, Low S, Ng TP, Ang SF, Ang K, Sum CF, et al. Association between depressive symptoms and pulse wave velocity is mediated by increased adiposity in older adults with type 2 diabetes. J Psychiatry Neurosci. 2021;46(1):E176-E83.
22. Wu Y, Sun D, Wang B, Li Y, Ma Y. The relationship of depressive symptoms and functional and structural markers of subclinical atherosclerosis: A systematic review and meta-analysis. Eur J Prev Cardiol. 2018;25(7):706-16.
23. Smith AJ, Malan L, Uys AS, Malan NT, Harvey BH, Ziemssen T. Attenuated brain-derived neurotrophic factor and hypertrophic remodelling: the SABPA study. J Hum Hypertens. 2015;29(1):33-9.
24. Schutte CE, Malan L, Scheepers JD, Oosthuizen W, Cockeran M, Malan NT. Cortisol: brain-derived neurotrophic factor ratio associated with silent ischaemia in a black male cohort: the SA BPA study: cardiovascular topics. Cardiovasc J Afr. 2016;27(6):387-91.
25. Malan L, Hamer M, Frasure-Smith N, Steyn HS, Malan NT. Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study. Int J Epidemiol. 2015;44(6):1814-22.
26. Covic A, Siriopol D. Pulse wave velocity ratio: the new “gold standard” for measuring arterial stiffness. Am Heart Assoc; 2015. p. 289-90.
27. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588-605.
28. Nass SJ, Levit LA, Gostin LO. The value, importance, and oversight of health research. Beyond the HIPAA privacy rule: enhancing privacy, improving health through research: National Academies Press (US); 2009.

29. Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Arch Gen Psychiatry. 2003;60(8):804-15.
30. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res. 2005;136(1-2):29-37.
31. Kirigia JM, Muthuri RDK, Muthuri NG, editors. The monetary value of human lives lost to suicide in the African continent: beating the African war drums. Healthcare; 2020: MDPI.
32. Woo JM, Postolache TT. The impact of work environment on mood disorders and suicide: Evidence and implications. Int J Disabil Hum Dev. 2008;7(2):185-200.
33. Hashimoto K. Brain-derived neurotrophic factor (BDNF) and its precursor proBDNF as diagnostic biomarkers for major depressive disorder and bipolar disorder. Eur Arch Psychiatry Clin Neurosci. 2015;265(1):83-4.
34. Ilchibaeva TV, Kondaurova EM, Tsybko AS, Kozhemyakina RV, Popova NK, Naumenko VS. Brain-derived neurotrophic factor (BDNF) and its precursor (proBDNF) in genetically defined fear-induced aggression. Behav Brain Res. 2015;290:45-50.
35. Psomiades M, Mondino M, Galvão F, Mandairon N, Nourredine M, Suaud-Chagny MF. Serum mature BDNF level is associated with remission following ECT in treatment-resistant depression. Brain Sciences. 2022;12(2):126.
36. Mashele N. Depressive symptoms and cardiometabolic health in urban black Africans: the SABPA study 2014.
37. Kunugi H, Hori H, Adachi N, Numakawa T. Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. Psyciatry Clin Neurosci. 2010;64(5):447-59.
38. Laszlo A, Lenart L, Illesy L, Fekete A, Nemcsik J. The role of neurotrophins in psychopathology and cardiovascular diseases: psychosomatic connections. J Neural Transm (Vienna). 2019;126(3):265-78.
39. Tasci I, Kabul HK, Aydogdu A. Brain derived neurotrophic factor (BDNF) in cardiometabolic physiology and diseases. Anadolu Kardiyol Derg. 2012;12(8):684-8.
40. Jiang H, Liu Y, Zhang Y, Chen ZY. Association of plasma brain-derived neurotrophic factor and cardiovascular risk factors and prognosis in angina pectoris. Biochem Biophys Res Commun. 2011;415(1):99-103.
41. Cahn BR, Goodman MS, Peterson CT, Maturi R, Mills PJ. Yoga, Meditation and Mind-Body Health: Increased BDNF, Cortisol Awakening Response, and Altered Inflammatory Marker Expression after a 3-Month Yoga and Meditation Retreat. Front Hum Neurosci. 2017;11:315.

42. Gyorkos A, Baker MH, Miutz LN, Lown DA, Jones MA, Houghton-Rahrig LD. Carbohydrate-restricted Diet and Exercise Increase Brain-derived Neurotrophic Factor and Cognitive Function: A Randomized Crossover Trial. Cureus. 2019;11(9):e5604.
43. Schmitt K, Holsboer-Trachsler E, Eckert A. BDNF in sleep, insomnia, and sleep deprivation. Ann Med. 2016;48(1-2):42-51.
44. Molendijk ML, Bus BA, Spinhoven P, Penninx BW, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. Mol Psychiatry. 2011;16(11):1088-95.

APPENDICES

Appendix A – Ethics Approval letter of Study

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: http://www.nwu.ac.za/

North-West University Health Research Ethics Committee (NWU-HREC)

Tel: 018 299-1206
Email: Ethics-HRECApply@nwu.ac.za (for human studies)

7 December 2021

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Health Research Ethics Committee (NWU-HREC) on 07/12/2021, the NWU-HREC hereby approves your study as indicated below. This implies that the NWU-HREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Brain derived neurotrophic factor and vascular alterations in depression: The SABPA study
Principal Investigator/Study Supervisor/Researcher: Dr L Lammertyn
Student: R Smit - 28832299

Ethics number:

N	W	U	-	0	0	3	0	3	-	2	1	-	A	1
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study
Commencement date: 07/12/2021
Expiry date: 28/02/2023

Risk: **Minimal**

Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of an annual monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of February annually until completion of the study.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-HREC:*
  - *Annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWU-HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for active monitoring.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-HREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*

Appendix A – Ethics Approval letter of Study

- *withdraw or postpone approval if:*
  - *any unethical principles or practices of the study are revealed or suspected;*
  - *it becomes apparent that any relevant information was withheld from the NWU-HREC or that information has been false or misrepresented;*
  - *submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
  - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-HREC can be contacted for further information via Ethics-HRECApply@nwu.ac.za or 018 299 1206*

Special conditions of the research approval due to the COVID-19 pandemic:

Please note: Due to the nature of the study i.e. (statistical analysis of previously collected data), this study will be able to proceed during the current alert level, following receipt of the approval letter. No additional COVID-19 restrictions have been placed on the study except that the researcher must ensure that before proceeding with the study that all research team members have reviewed the North-West University COVID-19 Occupational Health and Safety Standard Operating Procedure.

The NWU-HREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-HREC for any further enquiries or requests for assistance.

Yours sincerely,

Digitally signed by
Prof Petra Bester
Date: 2021.12.07
10:50:35 +02'00'

Chairperson NWU-HREC

Current details:(23239522) G:\My Drive\9. Research and Postgraduate Education\9.1.5.4 Templates\9.1.5.4.2_NWU-HREC_EAL.docm
20 August 2019
File Reference: 9.1.5.4.2

Appendix B – Schematic representation of the different parts of the carotid artery during the analyses of the carotid intima-media thickness.

customercare@copyright.com Oct 22, 2021, 11:27 PM (19 hours ago)

to me

Dear Rene Smit,

Thank you for contacting Copyright Clearance Center's Customer Service Department. My name is Manny and it will be my pleasure to assist you.

From my understanding, you would like to reuse content from the article, "Measurements of carotid intima media thickness in non-invasive high-frequency ultrasound images: the effect of dynamic range setting" I have researched this for you and I'm including the information on how to request permission through our platform.

In order to obtain permission, you can do the following:

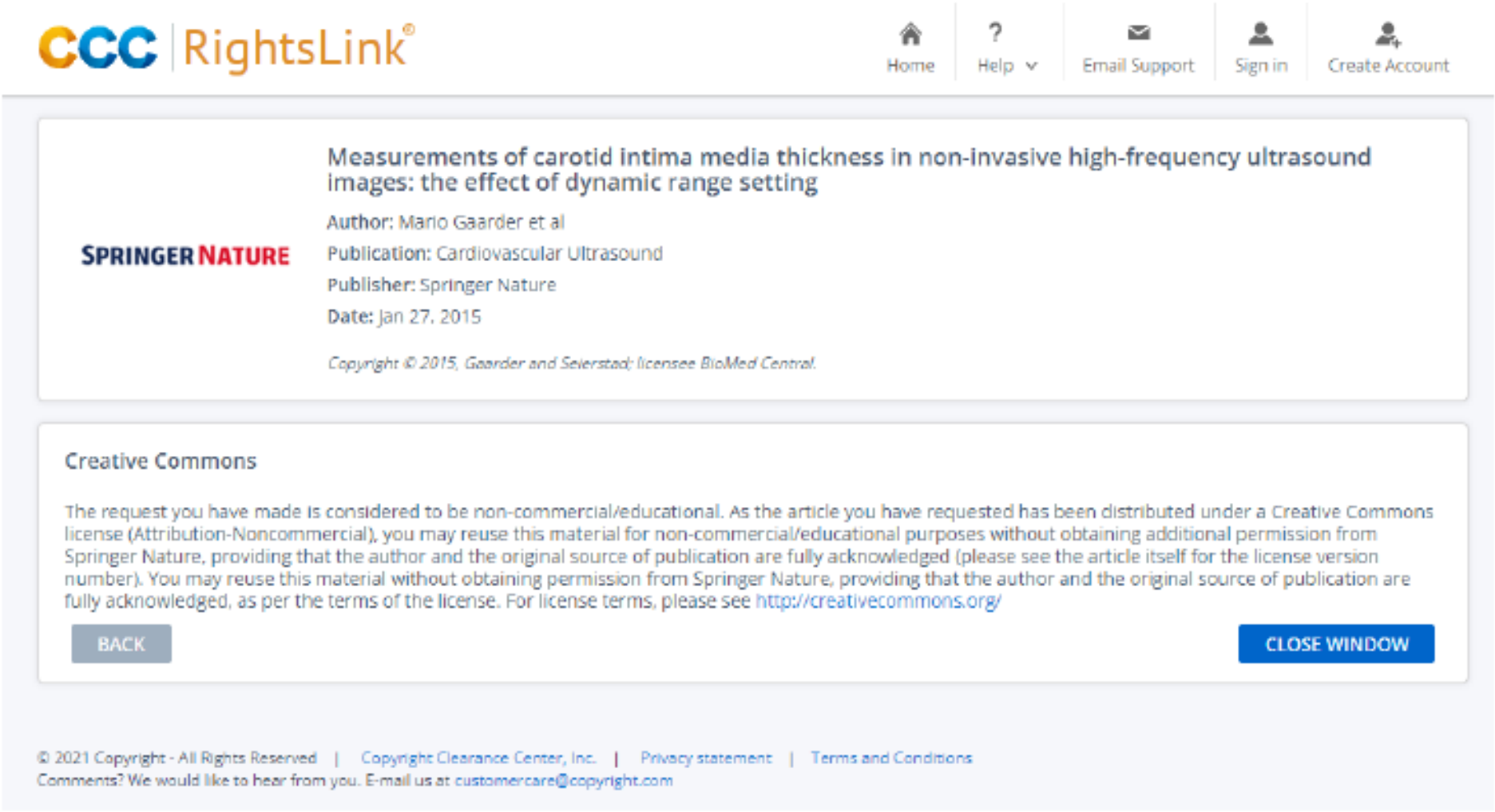
- Access this - LINK and you will be forwarded to Copyright Clearance Center's Rightslink form.
- Select the **Type of Use** from the drop-down menu.
- To complete the request, simply complete the fields on the **Quick Price Estimate page** and then click **Continue**.
- Most fields have an orange ? that you can click on at any time if you are not sure about that particular field during the process.
- **Kindly note that the pricing is dependent on the selection you make and all pricing is set by the publisher.**

I hope the above will help you obtain the necessary permission. Should you have any additional questions let me know and I'll be happy to help.

Warm Regards,
Manny

Manny Portillo
Customer Account Specialist
Copyright Clearance Center
222 Rosewood Drive
Danvers, MA 01923
www.copyright.com
Toll Free US +1.855.239.3415
International +1.978-646-2600

Appendix B – Schematic representation of the different parts of the carotid artery during the analyses of the carotid intima-media thickness.



Appendix C – Additional statistical analyses on the results of Pulse Wave Velocity

Contribution of pulse wave velocity:

SUPPLEMENTARY TABLE 1. Characteristics of the study population stratified by black individuals with and without depressive symptoms.

	Depressive individuals N = 86	Non-depressive individuals N = 107	*P*
Age, years	44 (38; 51)	44 (39; 51)	0.669
Sex (men), N (%)	45 (52.3)	51 (47.7)	0.520
Depression Severity			
PHQ-9 scores	13 (11; 17)	6 (4; 8)	<0.001
Minimal depression	0 (0)	42 (39.3)	
Mild depression	0 (0)	65 (60.7)	
Moderate depression	52 (60.5)	0 (0)	
Moderately severe depression	23 (26.7)	0 (0)	
Severe depression	11 (12.8)	0 (0)	
Anthropometry			
Waist circumference, (men), cm	93.00 (83.39; 103.67)	93.07 (83.80; 100.60)	0.152
Waist circumference, (women), cm	99.73 (84.19; 105.40)	89.47 (80.47; 101.20)	0.152
Body mass index, kg/m^2	30.69 (25.76; 34.50)	29.30 (24.56; 33.76)	0.359
Obese individuals, N (%)	46 (53.5)	48 (44.9)	0.233
Cardiovascular variables			
CIMT mean, mm	0.68 (0.59; 0.77)	0.67 (0.60; 0.76)	0.913
Pulse wave velocity, m/s	8.70 (7.50; 9.65)	8.50 (7.40; 9.60)	0.670
24-hour Systolic BP, mmHg	131 (121; 145)	131 (122; 144)	0.886
24-hour Diastolic BP, mmHg	82 (75; 88)	82 (76; 91)	0.715
24-hour Mean arterial pressure, mmHg	98 (91; 107)	99 (91; 108)	0.864
Hypertensive individuals (≥130/80 mmHg), N (%)	57 (66.3)	68 (63.6)	0.693
Biochemical variables			
Brain derived neurotrophic factor, ng/ml	1.37 (0.85; 1.80)	1.33 (1.01; 1.80)	0.566
Total cholesterol, mmol/l	4.41 (3.77; 5.51)	4.45 (3.72; 5.21)	0.381
LDL-cholesterol, mmol/l	2.71 (2.08; 3.71)	2.80 (2.10; 3.42)	0.593
Triglycerides, mmol/l	1.11 (0.76; 1.71)	1.08 (0.78; 1.56)	0.992
Glucose, mmol/l	5.18 (4.74; 5.66)	5.28 (4.58; 5.84)	0.613
C-reactive protein, mg/l	4.58 (1.97; 8.82)	5.10 (2.05; 12.77)	0.238
Gamma-glutamyltransferase, U/l	44.88 (31.01; 84.20)	39.81 (26.72; 61.50)	0.077
Medication use			
Anti-inflammatory, N (%)	5 (5.8)	11 (10.3)	0.263
Anti-hypertension, N (%)	19 (22.1)	25 (23.4)	0.834
Anti-depression, N (%)	1 (1.2)	0 (0)	0.263
Aspirin and anti-coagulants, N (%)	2 (2.3)	4 (3.7)	0.426

Data are reported as median (25th and 75th percentile), or number of participants and (percentage). Only cotinine levels above or equal to 10 ng/ml are reported. BP, blood pressure; CIMT, carotid intima-media thickness; LDL, low-density lipoproteins; HDL, high-density lipoproteins; HbA1c, glycated haemoglobin; HIV, human immunodeficiency virus; BDNF, brain derived neurotrophic factor.

Appendix C – Additional statistical analyses on the results of Pulse Wave Velocity

Supplementary table 2. Single and partial correlations between brain derived neurotrophic factor, carotid intima media thickness, and pulse wave velocity

	Carotid intima media thickness				Pulse wave velocity			
	Absence of depression (N= 107)		**Presence of depression (N=86)**		**Absence of depression (N=107)**		**Presence of depression (N=86)**	
Single correlations								
BDNF, ng/ml	r= -0.214	p= 0.027	r= -0.030	p= 0.781	r= -0.140	p= 0.150	r= 0.055	p= 0.614
Partial correlations (adjusted for age, sex, waist circumference, and mean arterial pressure)								
BDNF, ng/ml	r= -0.231	p= 0.019	r= -0.077	p= 0.491	r= -0.110	p= 0.271	r= 0.063	p= 0.574

BDNF, brain-derived neurotrophic factor

Supplementary Table 3. Residuals statistics between brain-derived neurotrophic factor and carotid intima-media thickness, stratified by black individuals with and without depression.

Individuals without depression (n=107)				
	Minimum	**Maximum**	**Mean**	**Std Deviation**
Predicted Value	-1.38	2.23	-0.001	0.70
Residual	-1.64	1.95	-0.014	0.70
Std Predicted Value	-1.98	3.19	-0.001	1.01
Std Residual	-2.20	2.60	-0.019	0.94
Individuals with depression (n=86)				
Predicted Value	-1.33	1.78	-0.003	0.56
Residual	-3.03	2.34	-0.009	0.83
Std Predicted Value	-2.40	3.20	-0.006	1.01
Std Residual	-3.44	2.66	-0.010	0.95

Std, standard; BDNF, brain-derived neurotrophic factor; CIMT, carotid intima-media thickness.