Monocyte chemoattractant protein-1 and large artery structure and function in young individuals: The African-PREDICT study

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This study is dedicated to my husband Johann Kriel.

With all my love

Preface

The article-format has been chosen for this dissertation. This is the format approved and recommended by the North-West University. The dissertation consists of a motivation, literature overview, methodology section, a manuscript to be submitted to a peer reviewed journal, namely *Atherosclerosis* and a concluding chapter which summarises the main findings and recommendations.

The layout of the dissertation is as follows:

Chapter 1: Background and motivation.

Chapter 2: Literature overview and detailed aim, objectives and hypotheses.

Chapter 3: Methodology.

Chapter 4: Manuscript for publication consisting of an abstract, introduction, materials and methods, results, discussion, conclusion and acknowledgements.

Chapter 5: Discussion of main findings, limitations, conclusions and recommendations.

References are provided at the end of each chapter according to an edited version of the Vancouver referencing style.

Contributions of the authors

The following researchers contributed to the article:

Mrs JI Kriel

Responsible for conducting the literature search. The candidate performed all statistical analyses, designed, wrote and compiled the manuscript. The candidate is also experienced with the detailed methodology of performing brachial and central blood pressures, and large artery stiffness measurements, using the Sphygmocor device and software.

Prof. AE Schutte

Supervisor

Supervised all stages of compiling the manuscript, was responsible for collection of data as principle investigator of the African-PREDICT study and gave general professional input.

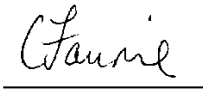
Dr. CMT Fourie

Co-supervisor

Provided recommendations on statistical analyses, writing of the manuscript and interpretation of results.

This is a statement from the authors confirming their individual contribution to the study and their permission that the manuscript may form part of this dissertation.

Prof. AE Schutte



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Summary

Monocyte chemoattractant protein-1 and large artery structure and function in young individuals: The African-PREDICT study

Motivation

In sub-Saharan Africa, the burden of cardiovascular diseases (CVD) is increasing at an alarming rate. This may be due to the rapid urbanisation of traditional black populations, leading to lifestyle changes (i.e. unhealthy diet, increased access to alcohol and a more sedentary lifestyle), which may increase their vulnerability to cardiovascular changes, such as hypertension, increased arterial stiffness and atherosclerosis. These changes in lifestyle can however not comprehensively account for the differences seen in cardiovascular disease development and progression between black and white populations. Black populations present with present with impaired vascular and endothelial function, as witnessed by greater hypertension and arterial stiffness, when compared to their white counterparts. The impaired endothelial function and differences in arterial function seen in black individuals may increase their vulnerability for cardiovascular disease.

In hypertension and established CVD the plasma levels of C-reactive protein and pro-inflammatory cytokines, as well as the chemokines, are all increased. The link between endothelial dysfunction, the inflammatory activation of the endothelium and the development of hypertension and ultimately CVD are well established. In the black population, not only blood pressure, but inflammatory markers are higher when compared to whites. Thus understanding the role of inflammation in the pathogenesis of arterial

stiffness, hypertension and CVD is of great importance for future development of treatment strategies and individual risk assessment.

In cardiovascular disease investigation the role of the chemokines and especially monocyte chemoattractant protein-1 and how it relates to an increased risk for hypertension and CVD are enjoying increasing attention. Chemoattractant proteins are part of the larger family of chemokines that direct the migration of monocytes from the blood to sites of inflammation. This arrest and transmigration of monocytes from the circulation by MCP-1 is induced under conditions of physiological shear force and by the pro-inflammatory cascade. MCP-1 is involved in the development of atherosclerosis through its promotion of the accumulation of lipids in the sub-endothelial intimal layer, as well as the differentiation of monocytes to macrophages and foam cells. MCP-1 correlates with carotid wall thickness, as is associated with hypertension and an increased risk of myocardial infarction, sudden death, coronary angioplasty and stent restenosis.

The pathological influence of MCP-1 in cardiovascular disease was however shown in largely elderly, white populations and little is known about MCP-1 levels in young, seemingly healthy black and white individuals, and how it may influence the development of cardiovascular disease risk and progression. This study will therefore attempt to demonstrate ethnic differences and plasma MCP-1 levels, and how it may play an important role in the early detection of vascular dysfunction and disease development in the South African population.

Aim

The central aim of this study was to determine whether MCP-1, as possible early marker of endothelial dysfunction, is associated with arterial stiffness and cIMT in young black and white individuals participating in the African-PREDICT study.

Methodology

This sub-study form part of the African-PREDICT study. We investigated 403 apparently healthy individuals aged 20-30 years, consisting of black (N=198) and white (N=205) men and women. Hypertensive individuals were excluded from the study. The study was reviewed by the Health Research and Ethics Committee (HREC) of the North-West University (Potchefstroom campus) and all participants signed informed consent prior to their enrolment in the study. Trained field workers gathered demographical data in the form of questionnaires and where necessary it was done in the participant's home language. Anthropometric measurements were taken, with calibrated instruments and included body weight, height, waist circumference, while body mass index was calculated as kg/m^2. Duplicate office brachial blood pressure measurement was taken on the left and right arm, with a 5 minute interval. Participants were fitted with a validated 24-hour ambulatory blood pressure apparatus. Carotid-femoral pulse wave velocity (cfPWV) were measured along the descending thoracic-abdominal aorta, suing the foot-to-foot velocity method, and central systolic blood pressure (SBP) were derived from the pulse wave analyses. B-mode ultrasonography was used to measure carotid intima-media thickness (cIMT). Plasma MCP-1 was determined using the quantitative sandwich enzyme immunoassay technique. Furthermore serum intercellular adhesion molecules (ICAM) and vascular cell adhesion

molecule (VCAM), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) were measured.

Results

cfPWV and cIMT were similar between the black and white groups, but black men and women showed higher central SBP and higher MCP-1 levels (both $p<0.001$) than their white counterparts. In addition, black women showed higher brachial SBP ($p<0.001$) and higher mean arterial pressure ($p=0.001$) than white women. We found a consistent positive association only in black women between cIMT and MCP-1 in single, partial and multiple regression analyses ($R^2=0.151$; $\beta=0.248$ [0.14; 0.35]; $p=0.021$).

Conclusion

In a young healthy bi-ethnic population, we found elevated central SBP and MCP-1 in blacks. In black women carotid wall thickness was related to early endothelial dysfunction (MCP-1), which may indicate an increased risk for early vascular deterioration in young black individuals.

Key words

Arterial stiffness, carotid intima-media thickness, inflammation, ethnicity, hypertension, adhesion molecules, central systolic blood pressure, atherosclerosis

List of abbreviations

ABI	ankle brachial index
African-PREDICT	African prospective study on the early detection and identification of cardiovascular disease and hypertension
apoE	apolipoprotein E
BMI	body mass index
cfPWV	carotid-femoral pulse wave velocity
cIMT	carotid intima media thickness
CRP	C - reactive protein
CSWA	cross sectional wall area
CVD	cardiovascular disease
DBP	diastolic blood pressure
EC	endothelial cells
eNOS	endothelial nitric oxide synthase
FRS	Framingham Risk Score
GGT	gamma glutamyltransferase
HDL-C	high density lipoprotein cholesterol
HIV	human immunodeficiency virus
hsCRP	high sensitivity c-reactive protein
ICAM-1	intercellular adhesion molecule-1
IDF	International Diabetes Federation
IL-17	interleukin-17
IL-1β	interleukin-1β
IL-4	interleukin-4
IL-6	interleukin-6
kg/m^2	kilograms per meter squared
LDL-C	low density lipoprotein cholesterol
MAP	mean arterial pressure
MCP-1	monocyte chemoattractant protein-1

MetS	Metabolic Syndrome
mg/L	Milligrams per liter
MI	myocardial infarction
mm	millimeter
mmHg	millimeter mercury
mmol/L	millimole per liter
MMP	matrix metalloproteinases
NAD(P)H	nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
ng/mL	nanograms per milliliter
NO	nitric oxide
oxLDL	oxidised low density lipoprotein
PAF	platelet activating factor
PDGF	platelet derived growth factor
PP	pulse pressure
RAAS	renin angiotensin aldosterone system
ROS	reactive oxygen species
SBP	systolic blood pressure
SD	standard deviation
SOD	superoxide dismutase
TC	total cholesterol
TNF-α	tumour necrosis factor alpha
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cells
WC	waist circumference
WHO	World Health Organization

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CHAPTER ONE

Introduction, Background and Motivation

1.1 BACKGROUND

In the United States the incidence and prevalence of hypertension in the black population is among the highest in the world.[1,2] Black populations develop high blood pressure earlier in life when compared to whites and their average blood pressures are higher.[3] African-Americans therefore continue to show disproportionately higher cardiovascular disease (CVD) morbidity and mortality in comparison to whites.[4-7]

As in the United States, the black population in sub-Saharan Africa also show a higher prevalence of hypertension and CVD.[8,9] From a cross-sectional survey done in four sub-Saharan rural and urban communities, it was estimated that eighty percent of global CVD occurs in these low- and middle income countries.[9] The South African black population shows a high prevalence of hypertension and CVD,[10-12] and South Africa has one of the highest rates of hypertension.[13] This may be explained by their transition from traditional rural living to more westernised, urban lifestyles.[14-16]

However, these observations and the increase in prevalence of CVD among blacks can only partly be explained by traditional cardiovascular risk factors such as lifestyle, hypertension and diabetes mellitus.[5] The black population presents with impaired vascular and endothelial function, accompanied by greater arterial stiffness when compared to whites.[17,18] Young healthy blacks have significantly impaired post-ischemic vasodilation and greater forearm vascular resistance than whites.[19,20] Blacks also demonstrated increased carotid intima-media thickness (cIMT) and stiffness.[21,22] The impaired endothelial function and differences in arterial structure seen in blacks may predispose them to CVD.[20,23] More detail on this subject is provided in the literature study (Chapter 2).

In essential hypertension and established CVD the plasma levels of the acute inflammatory marker C-reactive protein (CRP),[24,25] cytokines (tumour necrosis factor-α, interleukin-6 and interleukin-17),[26-28] adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1),[29,30] and chemokines such as monocyte chemoattractant protein-1 (MCP-1),[31-33] are all increased. The link between endothelial dysfunction, inflammatory activation of the endothelium and the development of essential hypertension and CVD are therefore well established,[34-36] with clear associations between increased inflammation, endothelial dysfunction and arterial stiffness.[34,37] Improving the inflammatory state reduced arterial stiffness.[37] In black populations not only blood pressure and arterial stiffness, but also inflammatory markers were higher when compared to whites.[16,38,39] Understanding the role of inflammation in the pathogenesis of arterial stiffness, hypertension and consequently CVD is therefore important for future development of preventative measures and treatment strategies, especially in vulnerable populations such as the South African black population.[40]

Although the acute phase inflammatory marker, CRP, is an independent predictor of CVD,[41-43] the research focus has shifted somewhat toward the chemokines and specifically MCP-1 and how it relates to increased risk for hypertension and CVD.[44-46] Chemokines are a large family of chemoattractants that direct migration of leukocytes from the blood to sites of inflammation.[47,48] This arrest and transmigration of leukocytes from the circulation by MCP-1 is induced under conditions of physiological shear force.[49,50] MCP-1 promotes the accumulation of lipids in the sub-endothelial intima layer, as well as the differentiation of monocytes to macrophages and foam cells.[47] Evidence suggests that MCP-1 and its receptor CCR2 are therefore involved in the development of atherosclerosis,[51] with MCP-1 also

correlating with cIMT.[52] Higher MCP-1 levels are associated with increased risk of myocardial infarction (MI), sudden death, coronary angioplasty and stent restenosis.[53]

MCP-1 is secreted from endothelial cells and vascular smooth muscle cells (VSMCs), it stimulates collagen expression, and enhances the expression of matrix metalloproteinases (MMPs) in cardiac myocytes and VSMCs.[44] MMP-1 and MMP-9 disrupt the cross-linking in elastin and collagen and results in stiffer, uncoiled collagen.[54] MCP-1 can activate MMP-1 through inflammatory responses and therefore control matrix deposition during inflammation, and further induce pro-inflammatory cytokines and MMPs.[55] Lui *et al.* (2011)[56] found that ambulatory arterial stiffness in pre-hypertensive subjects correlated positively with MCP-1. However, little is known about the role of MCP-1 levels in the general population. Findings in the study by McDermott *et al*[51] showed MCP-1 as a pathogenic factor in human CVD, but this study was largely done in a middle-aged to elderly white population. Literature on MCP-1 levels in African populations is very limited and to my knowledge no studies have reported MCP-1 levels in a seemingly healthy young African population or investigated its association with arterial structure and function. Given the direct effect of MCP-1 on the endothelium and VSMCs, it can be hypothesised that it may play an important role in the development of endothelial dysfunction, arterial stiffness, hypertension and ultimately CVD in black South Africans.

1.2 MOTIVATION

Due to the established high prevalence of essential hypertension and CVD in the black South African population, early detection and management of risk factors are essential. Urbanisation and lifestyle alone cannot fully explain the vulnerability of the black population to CVD in comparison with their white counterparts. The established link between

inflammation, endothelial dysfunction, hypertension and CVD is a strong indicator that study into inflammatory markers can provide new insight into the pathogenesis of arterial stiffness, essential hypertension and ultimately CVD. One such marker of inflammation is MCP-1.[46,56] This may provide answers to the differences in the disease profiles of blacks and whites, and may enable scientists and medical professionals to develop more effective treatment strategies.

1.3 AIM

The central aim of this study is to determine whether MCP-1, as a possible early marker of endothelial dysfunction, is associated with arterial stiffness and cIMT in young black and white individuals participating in the African-PREDICT study.

1.4 OBJECTIVES

The objectives of this sub-study are:

- to determine whether there are ethnic differences in MCP-1 levels;
- to compare the 24-hour blood pressure and measures of large artery structure (carotid intima-media thickness (cIMT)) and function (carotid-femoral pulse wave velocity (cfPWV)) between white and black participants;
- to determine whether arterial stiffness relates to MCP-1; and
- to determine whether cIMT relates to MCP-1 in both ethnic groups.

1.5 HYPOTHESES

With regard to the specific population of the African-PREDICT study, the following hypotheses were formulated:

1. Ethnic differences in circulating MCP-1 concentrations do exist, being higher in black than white participants.

2. Young black individuals have similar cIMT measurements as white participants;

3. Ethnic-specific differences exist regarding cfPWV, being higher in the black group;

4. Arterial stiffness is positively associated with MCP-1 in both the black and white groups.

5. Carotid wall thickness is positively associated with MCP-1 in both the black and white groups.

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CHAPTER TWO

Literature Review

2.1 BIOMARKERS OF CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality, not only in westernised countries, but increasingly also in developing countries.[1-5] This increase in CVD necessitates early detection and appropriate management, and this is especially true for developing countries where the disease burden is already high.[1,6-8] Biomarkers, as fast developing prognostic tools, are therefore an exciting research field.[9] In clinical practice, symptoms present only after an advanced disease state, resulting in a poorer prognosis. Biomarkers may provide a powerful tool for the early diagnosis of CVD and may strengthen the information obtained from traditional risk factors (hypertension, smoking, diabetes, dyslipidaemia). This will help to better identify high-risk individuals, to diagnose established disease, to better treatment strategies,[10] and may prove useful as a diagnostic tool.[9,11] Evidence also suggests that there are ethnic differences in biomarkers for cardiovascular disease.[12-16] However, the identification of biomarkers for the early diagnosis of CVD has not developed at the same pace as traditional risk factors.[17] Research into biomarkers may therefore provide a more precise estimation of their usefulness as a diagnostic tool for individual risk.[9,11]

A biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention."[18] According to this definition, a biomarker can either be a measurement variable (i.e. carotid intima-media thickness (cIMT) or carotid-femoral pulse wave velocity (cfPWV)), a macromolecule (bio-sample) or an imaging procedure (echocardiogram or CT-scan).[9,10] It is, however, generally considered to be a

macromolecule, often a protein.[9] The level of this molecule is associated with a pathophysiological process and may have clinical value for diagnoses and prognoses.

Biomarkers can indicate a range of healthy or diseased characteristics and can be grouped into categories, depending on their final purpose.[9,10] These include risk assessment (exposure to environmental factors and identifying risk of developing disease); screening markers (markers of sub-clinical disease); diagnostic markers (recognising established disease); staging markers (indicating disease severity); prognostic markers (predicting the probable course of the disease, including recurrence and type of therapy needed); stratification markers (indicators of response to therapy); and therapeutic monitoring (establishing efficacy of treatment and compliance).[9-11,19]

Biomarkers may also be utilised as research tools as they provide insight into different disease mechanisms[10] and variations in levels due to ethnicity, sex and age. The relation of the biomarker to known risk factors should also be examined.[20] One of the most extensively researched and interesting areas of biomarker biology and evaluation is CVD,[21] particularly those biomarkers involved in inflammatory cascades within the vascular wall.[22] Chemokine biology specifically is an area of interest as a novel marker for CVD risk assessment.[23]

2.2 CHEMOKINES – CHEMOTACTIC CYTOKINES THAT MEDIATE INFLAMMATION

Chemokines are a large family of small molecular weight (8 – 10 kilo dalton (kd)) proteins that regulate the migration of various cells in the body.[24,25] Hence their name, which is derived from 'chemotactic cytokines' as they are structurally related to cytokines, and induce chemotaxis in various cells.[24,26,27] Chemokines are subdivided into four groups based on the number and spacing of the cysteine residues in the N-terminus of the protein.[24,28]

They are named CXC, CC, CX_3C and C. Of the four families of chemokines, only two have been extensively characterised. They are named the α and β chemokines.[27] The α-chemokines have one amino acid separating the first two cysteine residues, and are called the CXC chemokines. In the β-chemokines, the first two cysteine residues are adjacent and form the CC chemokines.[24,26,27,29-31] β-chemokines attract monocytes, eosinophils, basophils, and lymphocytes with differing selectivity. Selectivity is determined by the N-terminal amino acid that precedes the CC residues and is therefore critical in the biologic activity and leukocyte selectivity of these chemokines.[27] MCP-1 falls under the CC sub-family (β-chemokine) and in previous classifications was known as CCL2, with its corresponding receptor being CCR2.[24,26,29,30](Table 1).

Chemokine receptors are G protein-coupled cell-surface receptors expressed on target sub-groups of leukocyte cells. Chemokine receptors are consistently expressed on different types of leukocytes, whereas on others it can be induced. In addition, some receptors are restricted to specific cells, while others are more widely expressed (i.e. CCR2 is expressed on monocytes, basophils, natural killer cells, and T cells).[27] There are 8 human CC receptors (CCR1 -8) and four human CXC receptors (CXCR1 -4) (Table 1). Once activated, these receptors trigger a set of cellular reactions that result in inositol triphosphate formation, intracellular calcium release and protein kinase activation.[27,32] Shape changes take place in the leukocytes after binding. Polymerisation and a breakdown of actin results in the formation of lamellipodia, which function like arms and legs for the migrating cells[31](see Figure 1).

Table 1 – CC Chemokines and their receptors

	Chemokine	Receptor	Cell Type
	MCP-3, -4; MIP-1α; RANTES	CCR1	**Eosinophil**
	MCP-3, -4; eotaxin-1; RANTES	CCR3	
CC	MCP-1, -2, -3, -4, -5	CCR2	**Basophil**
	MCP-3, -4; eotaxin-1, -2; RANTES	CCR3	
	MCP-3, -4; MIP-1α; RANTES	CCR1	**Monocyte**
	MCP-1, -2, -3, -4, -5	CCR2	
	MIP-1α, MIP-1β, RANTES	CCR5	
	I-309	CCR8	

(Adapted from Luster A. 1998).[27] In the β-chemokines the first two cysteine residues are adjacent to each other (CC), whereas in the α-chemokines the first two cysteine residues are separated by a single amino acid (CXC). Chemokine receptors are G protein-coupled proteins that are expressed on the subgroups of leukocytes. There are 8 human CC receptors. MCP – monocyte chemoattractant protein; MIP – macrophage inflammatory protein; RANTES – regulated upon activation normal T-cell expressed and secreted

Chemokines can also further be divided into groups based on their function.[24,26,30] Homeostatic chemokines are expressed in constant amounts, and is essential to various physiological processes. These chemokines fulfil the routine homeostatic circulation of leukocytes through the blood, tissues and lymphatic system.[24] The continuous renewal of circulating leukocytes brings them into lymph nodes, where they encounter antigens. The leukocytes are then transformed into memory leukocytes that can migrate to inflamed tissues to ensure normal immunological function.[27] During inflammation and infection, chemokines are also secreted by the inflamed tissue cells, resident and recruited leukocytes, and cytokine-activated endothelial cells.[27] There is a dramatic increase of chemokines during inflammation.[27] Leukocytes rolling on the endothelium come into contact with chemokines that are retained on the cell surface. Chemokines activate leukocyte integrins, which leads to firm adherence to the endothelium and migration into the tissue via diapedeses (Figure 1).[27,31,33-36] The main stimulus for inflammatory chemokine production is

the early pro-inflammatory cytokines (interleukin-1, interleukin-6 and tumour necrosis factor-α), bacterial products (lipopolysaccharide), and viral infections.[27,31,37,38]

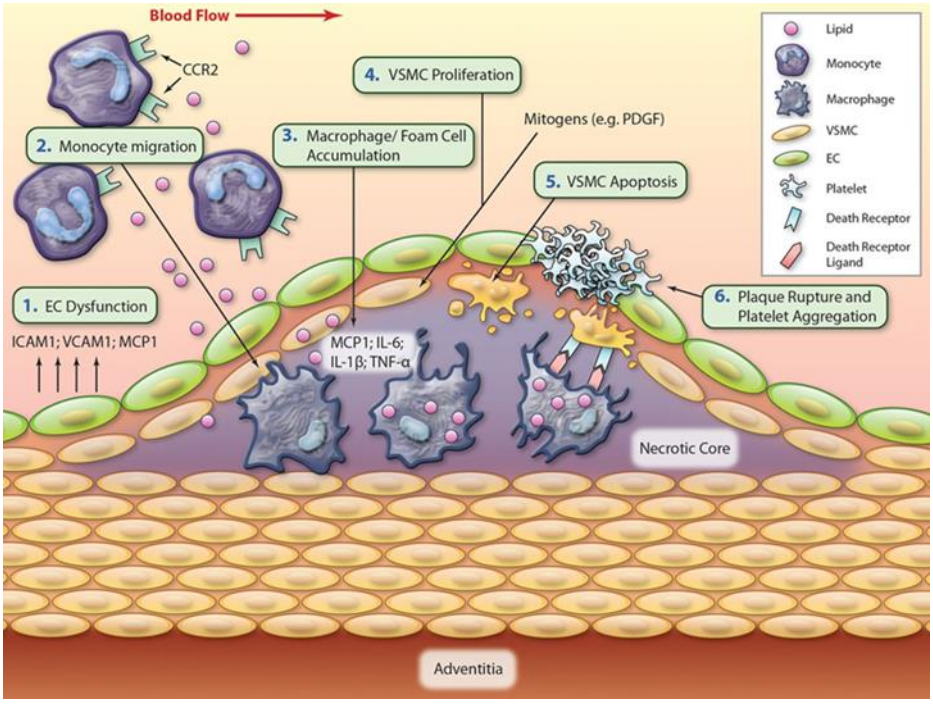

Figure 1. Schematic illustrating the role of MCP-1 in arterial stiffness (Taken from Wang et al. 2012).[39]
ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α, tumour necrosis factor-α; VSMC, vascular smooth muscle cells; EC, endothelial cells; PDGF, platelet-derived growth factor.

Chemokines have a role in embryonic development, wound healing, innate and adaptive immunity, homeostasis, and angiogenesis.[27,30] In addition to these physiological processes, chemokines also have a role in the pathophysiology of inflammation and disease.[23,30,40]

2.3 MONOCYTE CHEMOATTRACTANT PROTEIN-1

MCP-1 is produced by endothelial cells and vascular smooth muscle cells (VSMCs), among other cellular sources, in response to various stimuli.[37,41-45] Interleukin-1 (IL-1), interleukin-4 (IL-4), interleukin-6 (IL-6)[46-49] and tumour necrosis factor-α (TNF-α), amongst other factors, stimulate the expression of MCP-1 by vascular endothelial cells.

MCP-1 binds only to the CCR2 receptor,[27] and it is important to note that CCR2 is constantly expressed on monocytes.[27] MCP-1 is therefore a chemoattractant for human monocytes, and alone or in combination with other cytokines, attract monocytes to its site of release, and cause cellular activation of specific immunological functions related to immune defence.[37] This inflammatory response is necessary for removal of pathogens from the body, but without proper clearance, it leads to pathological inflammation and disease.[40] This may play a role in infectious disease (HIV infection)[24,26,40], pulmonary disease (asthma and chronic obstructive pulmonary disease),[30,40] autoimmune disease (multiple sclerosis and rheumatoid arthritis),[30,40] cancer,[24,37,40] renal disease[28,32,41] and vascular disease.[10,22,24,34,40,50]

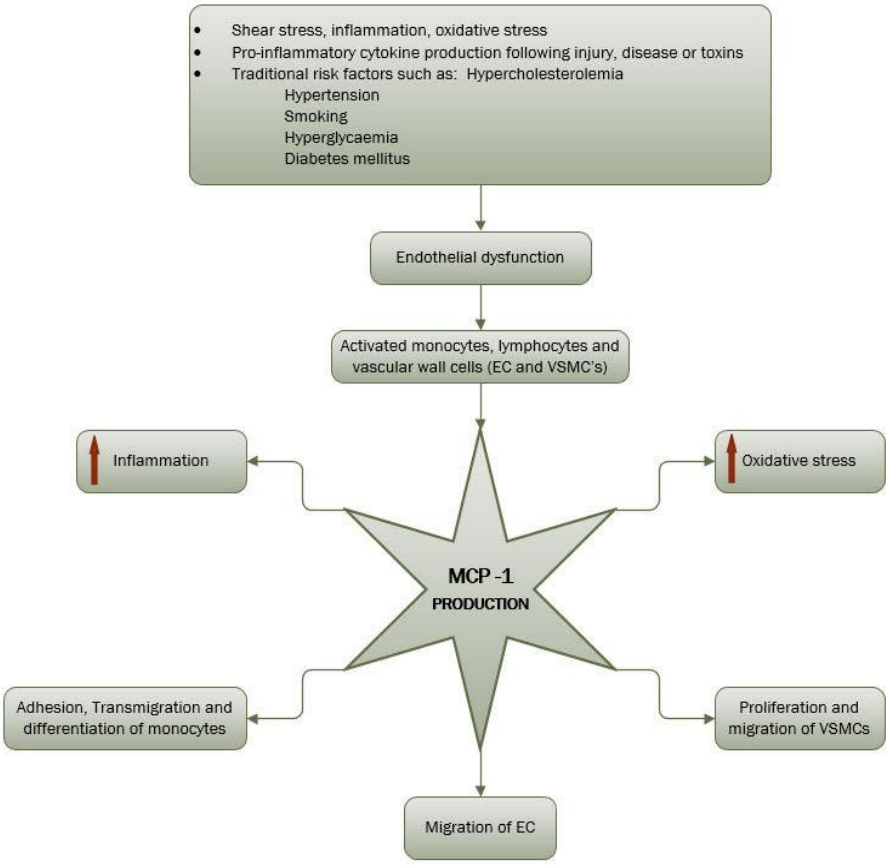

Figure 2. The role of the MCP-1 pathway in the pathogenesis of atherosclerosis and vascular remodelling (Adapted from Egashira 2003).[51] EC, endothelial cell; VSMCs, vascular smooth muscle cells.

2.4 LARGE ARTERY STRUCTURE AND FUNCTION, INFLAMMATION AND CARDIOVASCULAR RISK

The importance of large artery structure and function in our understanding of the development of hypertension and cardiovascular disease should be emphasised. Large arteries are not mere passive conduits of blood, but respond actively to the mechanical forces they are subjected to.[52] The large arteries, especially elastic arteries (i.e. aorta, carotid, etc.) serve as a buffering reservoir or "Windkessel" that store blood during systole and expel it during diastole to ensure a continuous flow of blood to the periphery.[53,54] This important function of large arteries provides a cushion against the pulsatile nature of blood ejected from the left ventricle, and ensures a constant perfusion of peripheral organs and tissues.[55]

2.4.1 Arterial stiffness and vascular ageing

For a given ventricular stroke volume, the central aortic pressure wave is composed of a forward-traveling wave and a delayed reflected wave arriving from the periphery.[56] Large artery wall structure and function are major determinants of the magnitude and propagation of these pressure waves.[57,58] Arterial stiffness in general refers to the compliance of the large conduit arteries, and can be measured non-invasively by tonometry.[59,60] Carotid-femoral pulse wave velocity (cfPWV), measured between the carotid and femoral arteries, is a regional assessment of aortic stiffness and considered to be the gold standard measurement of arterial stiffness.[61,62] The media and adventitia are considered to be responsible for arterial compliance through the ability to recoil to original dimensions when pressure returns to within normal range. This is also referred to as the elastic modulus of the artery.[63] Loss of elasticity of the arterial wall reduces the storage

capacity and buffering effect of the vessel, reducing its recoil capacity and subsequently increasing the velocity of the forward traveling wave, causing an earlier returning reflected wave, which increases central pulse pressure and therefore augments central systolic blood pressure.[53,60] The increase in central SBP leads to increased load on the left ventricle, which in turn increases the myocardial oxygen demand. Furthermore, central SBP, rather than brachial SBP, is associated with a greater risk for cardiovascular disease and mortality,[64-66] and arterial stiffness is associated with left ventricular hypertrophy, which is a known risk factor for cardiovascular disease in both normotensive and hypertensive subjects.[67] These factors form the basis for the underlying mechanisms of gradual increase of SBP with age, leading to isolated systolic hypertension in the elderly and an increased cardiovascular risk.[57,58] Increased arterial stiffness is an independent predictor of cardiovascular disease and may therefore be an important endpoint for determining cardiovascular risk[68-70] and end-organ damage.[58,71,72]

Stiffening of the arteries is commonly related to changes in the mechanical properties of the arterial wall. The main structural components of the media, in the large conduit arteries, are elastin, collagen and VSMCs.[73] Remodelling of the main structural components of arteries as they age or become diseased leads to changes in their composition and the manner in which shear and distending forces are distributed within their walls.[74] In normal physiology arterial remodelling is a response to the changes in shear and circumferential stress to restore normal flow and wall tension. Prolonged increase in shear and circumferential stress, such as characterised by hypertension, leads to pathological changes in the arterial wall, which irreversibly alter the geometrical and mechanical properties and leads to arterial stiffness.[74,75]

2.4.2 Arterial stiffness and inflammation

Arterial stiffness and inflammation are both factors that attenuate cardiovascular disease, and inflammatory pathways are implicated in vascular remodelling and disease.[76-78]

The walls of stressed vessels exhibit increased inflammatory mediators such as adhesion molecules (ICAM-1 and VCAM-1) and chemokines, including MCP-1. These cascades also lead to the production of pro-inflammatory cytokines, which trigger oxidative stress that in turn will attenuate the inflammatory response further.[78,79] Multiple immune cells take part in this process and are from innate and adaptive immunity, which interact in the pathophysiology of arterial stiffness, hypertension and cardiovascular disease.[39,80,81] Previous studies have indicated that systemic inflammation may be involved in the process of arterial stiffening and the development of hypertension.[82-85]

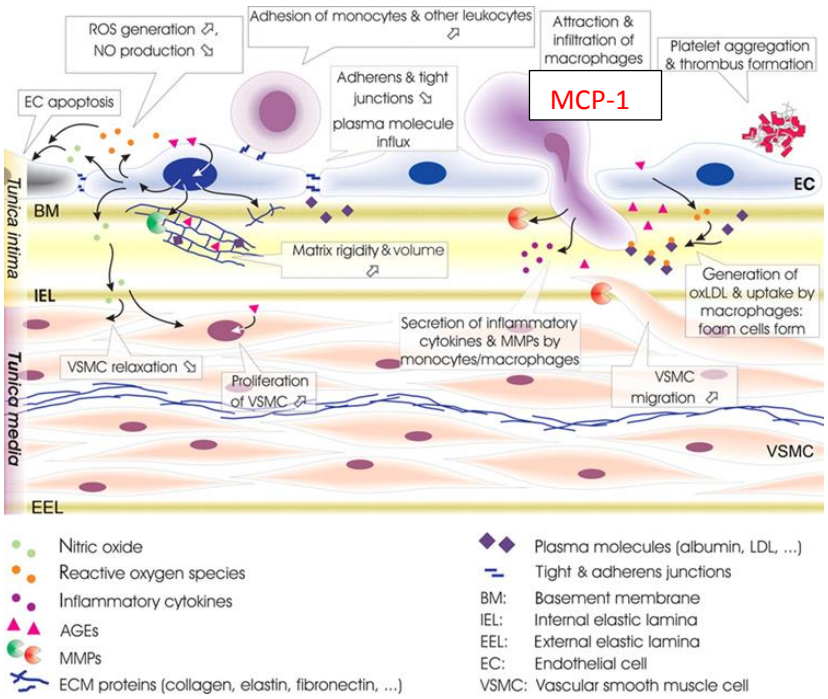

Figure 3. Alterations within intima and media during inflammation, leading to arterial stiffness (Taken from Spinetti et al. 2008).[86]
EC, endothelial cells, ROS, reactive oxygen species; NO, nitric oxide; VSMC, vascular smooth muscle cells; MMPs, matrix metalloproteinases; MCP-1, monocyte chemoattractant protein-1; oxLDL, oxidised low density lipoprotein.

Similarly, differences have been established in the endothelial function of black and white populations, with endothelial dysfunction being higher in the black population. This may predispose black individuals to the development of arterial stiffness, hypertension and cardiovascular disease.[87,88] Inflammation is a major cause of vascular dysfunction and may sustain this dysfunction and pro-atherosclerotic processes.[76] Studies have also reported that large artery stiffening occurs earlier or is more advanced in black populations.[61,89,90] The link between inflammation, vascular dysfunction, arterial stiffness, the development of hypertension and ultimately cardiovascular disease cannot be disputed. Additionally, it seems that black populations overall may be more vulnerable, having higher dysfunction in all these areas. However, little is known in the South African black population about the role of MCP-1 and this cascade of inflammation and vascular dysfunction.

2.4.3 Carotid intima-media thickness

As with increased arterial stiffness, increased carotid intima-media thickness (cIMT) is associated with increased vascular risk factors. Endothelial dysfunction and increased cIMT are considered to be early signs of atherosclerotic vascular disease and have been used in epidemiological studies as a surrogate marker for sub-clinical atherosclerosis.[91,92] Measured with B-mode ultrasound, cIMT represents the combined thickness of the medial and intimal layers of the carotid artery.[93] The measurement of cIMT is also a non-invasive, repeatable and relatively easy measurement that is suitable for large population-based or epidemiological studies.[94,95] Additionally, the incidence of vascular events in young populations is rare, which makes cIMT an attractive end-point to use as a dependable baseline measurement.[96,97] The Framingham Study and the subsequent development of the Framingham risk score (FRS) emphasises the importance of a multivariate risk profile for the

prediction and prevention of CVD. However, the relationships between the FRS and cIMT in young blacks and whites have enjoyed limited scrutiny. Such scrutiny could be useful in terms of cardiovascular epidemiology and with regard to preventative medicine.[98] Data concerning the differences in cIMT scores between black and white populations are contradictory. In some studies increased cIMT were demonstrated in black populations,[96,99] while others showed marked lower incidences of carotid atherosclerosis in black groups compared to whites, despite having a higher prevalence of hypertension, diabetes and smoking.[100] In a group of young black and white study participants it was demonstrated that multiple risk factors have a great impact on the early stages of atherosclerosis. The FRS was significantly associated with cIMT in both black and white young individuals, and supported a multiple risk factor profile regardless of ethnicity.[98] An important part of this multiple risk factor profile may be the inclusion of inflammatory markers. Although a novel marker, MCP-1 levels in particular have been associated with cIMT, suggesting that the role MCP-1 plays in the development of atherosclerosis may identify MCP-1 as an useful biomarker in combination with cIMT to predict future CVD.[101]

2.5 BIOCHEMICAL VARIABLES, INFLAMMATION AND CARDIOVASCULAR RISK

2.5.1 MCP-1

The pathogenesis of arterial stiffness involves the accumulation of fibronectin, collagen and VSMCs in the intimal layer of the vascular wall, with an increase in MMP-1 and angiotensin-II expression.[102,103] Stiffening of arteries is accompanied by VSMC proliferation and migration with this migration, as well as the migration of EC and macrophages, facilitated by MCP-1.[104] In fibroblasts, MCP-1 also induces the production of MMP-1.[105,106] Arterial stiffening is also characterised by an increase in NAD(P)H oxidase activity, which leads to an

increase in ROS production and a decrease in NO bioavailability. This is accompanied by increased pro-inflammatory cytokines.[107,108] Several studies have demonstrated the link between inflammatory markers and cfPWV.[79,109-111] In the Whitehall II study, involving 3769 white men and women, Johansen *et al.* (2012)[112] demonstrated a strong link between IL-6 and CRP and cfPWV, with central obesity being a strong predictor for aortic stiffness. Liu *et al.* (2011)[111] found a correlation between ambulatory arterial stiffness index and MCP-1 in pre-hypertensive subjects.

Similarly, a number of studies also indicated associations between inflammatory markers and cIMT,[113-115] and in a large community-based study involving 6017 people, Beck *et al.* (2001)[116] demonstrated the link between inflammation and carotid wall thickness. In addition, was cIMT measurements >1 mm linked with significantly higher levels of MCP-1 in hypertensive patients.[117]

2.5.2 Cytokines

Cytokines are small proteins that are primarily involved in the physiological response to disease or infection. They have been compared to hormones, but where hormones are produced by highly specialised tissues; cytokines are produced by nearly every living cell.[118] Some cytokines act to worsen disease, and are called pro-inflammatory, while others serve to reduce inflammation and are known as anti-inflammatory. Pro-inflammatory cytokines move to increase their own production and stimulate the production of inflammatory mediators such as platelet-activating factor (PAF) and MCP-1, as well as reactive oxidative species (ROS), and they recruit and stimulate the cellular components of the immune system.[119] The vascular effects of cytokines are multiple. The majority of cytokines stimulate immune cell proliferation and differentiation, and in the vasculature they stimulate growth

and migration, with both IL-6 and TNF-α inducing vascular endothelial growth factor (VEGF) expression in murine and human models.[120] In addition, many of the effects of cytokines on the vasculature may involve the production of ROS, with ROS generated at sites of inflammation and injury and being necessary for the regulation of cell activities, such as cell growth. At prolonged high concentrations, ROS may cause cellular injury and death, which plays an important role in the pathophysiology of vascular disease.[81] In addition, cytokines have specific effects on endothelial cells, VSMCs and endothelial cell matrix. This may affect the mechanism of vascular tone, the signalling pathways controlling vasoconstriction and –dilation, and through vascular cell growth and proliferation may lead to structural changes in the vessel wall, altering structure and function.[81] Furthermore, pro-inflammatory cytokines reduce nitric-oxide (NO) synthesis in the vascular endothelial cells by down-regulating the expression of endothelial NO synthase (eNOS).[121] Both IL-6 and TNF-α induce and sustain MCP-1 production, monocyte migration and differentiation to macrophages and the migration of VSMCs into the intima.[122]

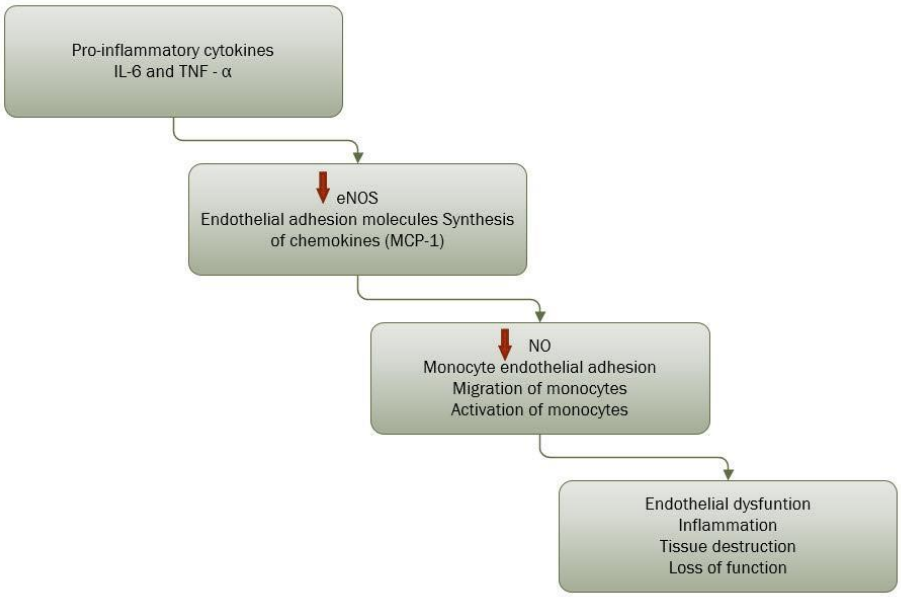

Figure 4. The inflammatory cascade triggered by IL-6 and TNF-α (Adapted from Dinarello 2000).[118]
IL-6, interleukin-6; TNF-α, tumour necrosis factor-α; eNOS, endothelial nitric-oxide synthase; NO, nitric oxide.

Originally it was thought that cytokines are very specific in their function. However, it has emerged that most cytokines have multiple functions, mediating the same or similar processes.[121] The best examples of multifunctional cytokines are IL-6, interleukin-1 (IL-1) and TNF-α, which display a wide range of biological functions, such as B-cell differentiation, T-cell activation and differentiation, and macrophage differentiation, amongst others.[123] The expression of IL-6 and TNF-α produces fever, inflammation, tissue destruction, and in severe cases, shock and death.[118] Ethnicity seems to have an influence on the expression of pro-inflammatory cytokines. Black Americans differed markedly from whites in the distribution of genotypes for IL-6[124] and TNF-α[125] and are therefore predisposed to elevated levels of pro-inflammatory cytokines. Although elevated levels of inflammatory markers have been observed in the South African black population when compared to whites,[16] data about the differences in pro-inflammatory cytokines is scant.

2.5.2.1 Interleukin-6

IL-6 was at first identified as a B-cell differentiation factor, and therefore one of the major functions of IL-6 is to induce antibodies (IgM, IgG and IgA production).[121,123] Activated leukocytes in the vessel wall or at the site of infection are considered to be the main source of circulating IL-6, with additional secretion by fibroblasts and endothelial cells. Furthermore, it has been shown that almost 30% of total circulating IL-6 is derived from adipose tissue.[126] During inflammation IL-6 has been found to be involved in the acute-phase reaction, and recombinant IL-6 induces various acute-phase proteins in the liver, including, importantly, high sensitivity C-reactive protein (hsCRP).[50,121] IL-6 also causes endothelial activation and up-regulates the expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-

1).[127] A study by Tieu *et al.* (2009)[128] suggests the existence of an IL-6 – MCP-1 amplification loop that enhances vascular inflammation. IL-6 skews monocyte differentiation towards macrophages, and further differentiates macrophages into a pathogenic type within the vascular wall. Along with enhanced production of MCP-1 and matrix metalloproteinases (MMPs), the upregulation of VCAM-1 and ICAM-1 creates an environment where macrophages can attach, migrate and remodel tissue within the vascular wall, recruiting more monocytes to the endothelium and amplifying the inflammatory process.[128]

2.5.2.2 Tumour necrosis factor-α

TNF-α is produced primarily by macrophages in response to a wide variety of pathological agents, particularly bacterial endotoxins.[129] Historically, TNF-α was identified as an endotoxin-induced factor that causes haemorrhagic necrosis in certain tumours.[130] Other functions of TNF-α include tumouricidal activity, inhibition of lipoprotein-lipase, as well as induction of bone resorption, myeloid leukemic cell differentiation, pro-coagulant activity, growth and differentiation of B cells, and ICAM-1 expression. TNF-α is a potent inducer of IL-6, and inversely, IL-6 regulates TNF-α expression.[123] Additionally, TNF-α may also induce apoptosis and increase endothelial cell permeability.[130] This increase in the permeability of the vascular endothelial cells alters the barrier properties and functioning of the vessel wall and leads to alterations in the vascular tone. TNF-α is implicated in the pathology of cardiovascular diseases, including atherosclerosis, acute myocardial infarction (MI)[131] and chronic heart failure.[119]

2.5.3 Adhesion molecules

VCAM-1 and ICAM-1 are two members of the Ig-gene superfamily of adhesion molecules, and they are closely related in structure and function.[132] During inflammation the increase in several cytokines, including IL-6 and TNF-α, up-regulate the expression of adhesion molecules. Endothelial cell expression of VCAM-1 and ICAM-1 in response to the inflammatory response mediates the interaction between the endothelium and blood cells and is central to the development of atherosclerosis. VCAM-1 and ICAM-1 play key roles in the firm adhesion and trans-endothelial migration of leukocytes, whereas selectins mediate the initial rolling of leukocytes along the endothelium[133] (Figure 1). Elevated ICAM-1 levels have been reported in patients with CHD and were shown to be a predictor of cardiovascular events in those without any prior history of coronary artery disease or other vascular disease, independent of traditional risk factors. VCAM-1 and ICAM-1 have also been associated with carotid atherosclerosis and increased cIMT and cfPWV.[132,134-137] Additionally, markedly higher ICAM-1 levels were reported for smokers compared to non-smokers.[132,133] Cockerill *et al.*[138] demonstrated that increased HDL-cholesterol levels decreased the expression of VCAM-1 and ICAM-1 levels, suggesting that the protective influence of HDL-cholesterol can decrease the effects of the inflammatory process.[138] VCAM-1 is different to ICAM-1 regarding the ligand it binds to, the time and duration of its expression, and the cell and tissue type in which it is expressed.[133] In an animal model studied by Walpola *et al.,*[139] VCAM-1 was upregulated by low shear stress, whereas ICAM-1 was down-regulated, but high shear stress upregulated both ICAM-1 and VCAM-1. This may suggest that VCAM-1 plays a role in early atherogenesis and less of an important role in advanced, complex lesions.[133] Cybulsky *et al.*[140] found that the deficiency of VCAM-1 diminished early foam cell

formation associated with atherogenesis, and further suggest that VCAM-1 has a major role in the initiation of atherosclerosis. In contrast, ICAM-1 deficiency did not influence early foam cell formation. ICAM-1 therefore may have a role in lesion progression and VCAM-1 a prominent role in lesion initiation and development.[140,141] This major role of VCAM-1 in atherosclerotic lesion formation likely shows a prominent function for VCAM-1 in the recruitment, activation, proliferation and apoptosis of intimal monocytes/macrophages, as well as lesion expansion and progression.[142] In a bi-ethnic study VCAM-1 and ICAM-1 associated with lower ankle brachial index (ABI) and the presence of atherosclerotic disease in blacks without known coronary heart disease compared to whites. This association was independent of conventional risk factors.[137]

2.5.4 C-reactive protein

C-reactive protein (CRP) is an acute phase reactant and marker of systemic inflammation, which increases markedly following infection or tissue injury and plays a key role in the innate immune response. It is synthesised mainly in the liver upon stimulation by interleukin-6 and other pro-inflammatory cytokines.[34,50,143-145] Although CRP is a non-specific inflammatory marker, it is established as an independent predictor of cardiovascular risk, and employed widely as an affordable biomarker.[146-148] A single baseline measurement of high sensitivity CRP (hsCRP) was shown to be a significant predictor of future myocardial infarction (MI) or stroke in apparently healthy individuals, independent of traditional risk factors.[149] Data showed that it is an even greater predictor of risk for first cardiovascular events than LDL-cholesterol.[150] In both a cross-sectional study in general practice[151] and a longitudinal study, namely the US Physicians Health Study,[149] CRP levels predicted cardiovascular events and mortality during follow-up, which suggests that atherosclerosis

progression may be indicated by raised CRP levels.[118] This furthermore suggests the role of inflammation in the development of atherosclerosis, as well as in the risk of an acute cardiovascular event.[152] However, the reference values, where levels of <1.1 to 3, and >3 mg/L represent low-, moderate-, and high-risk groups, were largely derived from a white population and although ethnic differences in CRP values were found,[14,153] little is known about the marker in black populations.[154] In the South African black population previous studies showed that black women presented with higher CRP levels when compared to white women.[16]

2.6 THE METABOLIC SYNDROME

Several cardiovascular risk factors were identified over the past decades, and include aspects such as hypertension, hyperglycaemia and obesity.[155] Over the years there have been suggestions to group these risk factors in a combined syndrome. The first description of the metabolic disturbances known as the Metabolic Syndrome (MetS) was by Kylin, E (1923),[156] and was described as the combination of hypertension, hyperglycaemia and gout. Later it was noted that visceral adiposity, or android type obesity, was most often linked with the metabolic abnormalities associated with diabetes and CVD.[155] After its first description, several phrases or terms have been coined to describe the condition, including 'Syndrome X', 'The deadly quartet' and 'The Insulin Resistance Syndrome'. Today the term Metabolic Syndrome remains the most accepted term for this combination of cardio-metabolic risk factors, with the International Diabetes Federation defining the core components of MetS as obesity, insulin resistance, dyslipidaemia and hypertension.[155,157,158]

2.6.1 **Obesity and adiposity**

Body mass index (BMI) provides the most practical population-based measure of overweight and obesity, and obesity is defined by the World Health Organization (WHO) as having a BMI of greater than or equal to 30 kg/m² and over-weight as a BMI of greater than or equal to 25 kg/m². A BMI between 25 kg/m² to 29.99 kg/m² is classified as pre-obese (see Table 2). Abdominal or visceral obesity is defined as a waist circumference of greater than 102 cm in men, and greater than 88 cm in women, and is associated with increased cardiovascular and other obesity-associated pathology.[159,160] Furthermore, visceral obesity is more strongly correlated with pathology than over-all obesity, and may be a simpler measure for identifying the need for tighter weight management.[161] Lean *et al.* (1995),[162] however, suggested that the threshold for WC, above which there is an increased risk for disease development, should be 94 cm for men and 80 cm for women, and further advised that a WC of 94–102 cm in men and 80–88 cm in women, should be a warning not to gain further weight.

Table 2: The international classification of adult underweight, overweight and obesity according to BMI

Classification	**BMI(kg/m^2)**	
	Principal cut-off values	**Additional cut-off values**
Underweight	**<18.50**	**<18.50**
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	**18.50 - 24.99**	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99

Obese class II	35.00 - 39.99	35.00 - 37.49 37.50 - 39.99
Obese class III	≥40.00	≥40.00

(Adapted WHO 2004.)[163]

In the United States an analysis done by the Centre for Disease Control (CDC) showed that the black population had a 51% greater prevalence of overweight and obesity, compared to whites and Hispanics. Black women had the highest prevalence, followed by black men.[164] South Africa presents with a similar scenario. According to the demographic and health survey of 1998,[165] 56.6% of women were obese, with mean BMI values of 27.1 kg/m², and 42% of women had abdominal obesity. Black women had a higher prevalence of overweight and obesity compared to white women (31.8% versus 22.7%), as well as markedly higher prevalence of abdominal obesity (15.5% versus 8.3%).[165] This is in line with findings by Mollentze (2006)[166], which show that urban black women had the highest prevalence of obesity with 35.7%. Kruger *et al.* (2001)[167] found a high prevalence of obesity among black women in the North West province of South Africa, with a strong correlation between BMI and WC in these women. They also demonstrated that obesity correlated with the risk for non-communicable disease (NCD) development in these women, and that WC should preferably be used as a measure of abdominal obesity, since it seems to be a greater predictor of NCD risk.

Obesity is increasingly associated with a state of chronic low-grade inflammation, with elevated levels of circulating adipocytokines and pro-inflammatory mediators, among others IL-6, TNF-α and MCP-1[168] and the levels of these circulating inflammatory markers associate with a higher BMI.[169]

Adiposity refers to the part of total body mass that is comprised of neutral lipids stored in adipose tissue.[170,171] Physiologically adipose tissue is an active participant in fat storage and release, and adipocytes play a role in other physiological and pathophysiological processes. Adipose tissue represents endocrine cells with the capacity to synthesise and secrete a diverse number of factors, collectively called adipokines.[172] These adipokines are involved in the regulation of several physiological functions, including insulin sensitivity, angiogenesis, blood pressure and the immune response, and is central in the maintenance of metabolic homeostasis in healthy individuals.[168] The increase in adipose tissue mass associated with overweight and obesity relates to changes in the endocrine function of adipose tissue and therefore links increased adiposity to alterations in systemic physiology.[160] Excess adiposity and increased levels of circulating inflammatory markers, such as hsCRP, TNF-α, IL-6 and MCP-1 are closely related and suggest that adipose tissue itself is a source and site of low-grade inflammation.[161] At tissue level, TNF-α was the first cytokine identified in the adipose tissue of rodents and this indicated the existence of a state of metabolic inflammation.[173] TNF-α seems to be over-expressed in adipose tissue, and is considered to be a factor that makes the functional link between inflammation and obesity.[174] Obese adipose tissue is infiltrated by macrophages, and these adipose tissue macrophages is considered to be the primary source of TNF-α and other pro-inflammatory cytokines, which lead to the activation of local and systemic immune systems. This demonstrates the close relationship between immune and metabolic cells.[161,172] Macrophage accumulation in adipose tissue is shown to be directly proportional to BMI and adiposity. Additionally, adipocytes are able to synthesise and secrete MCP-1, and increased adiposity may increase MCP-1 release into the circulation. The increased expression of MCP-1 in the circulation then recruits circulating

monocytes, and creates an environment that is favourable for monocyte differentiation and migration into the vascular endothelium.[161,175]

Whatever the initial stimulus may be, an increase in pro-inflammatory cytokines and a state of systemic low-grade inflammation adversely affects the endothelium.[176] Over-production of pro-inflammatory cytokines by adipose tissue in overweight and obese individuals, and especially the over-production of cytokines from the macrophages that reside within the adipose tissue, leads to endothelial dysfunction and vascular inflammation, which ultimately leads to the development of atherosclerosis and CVD.[174,175,177]

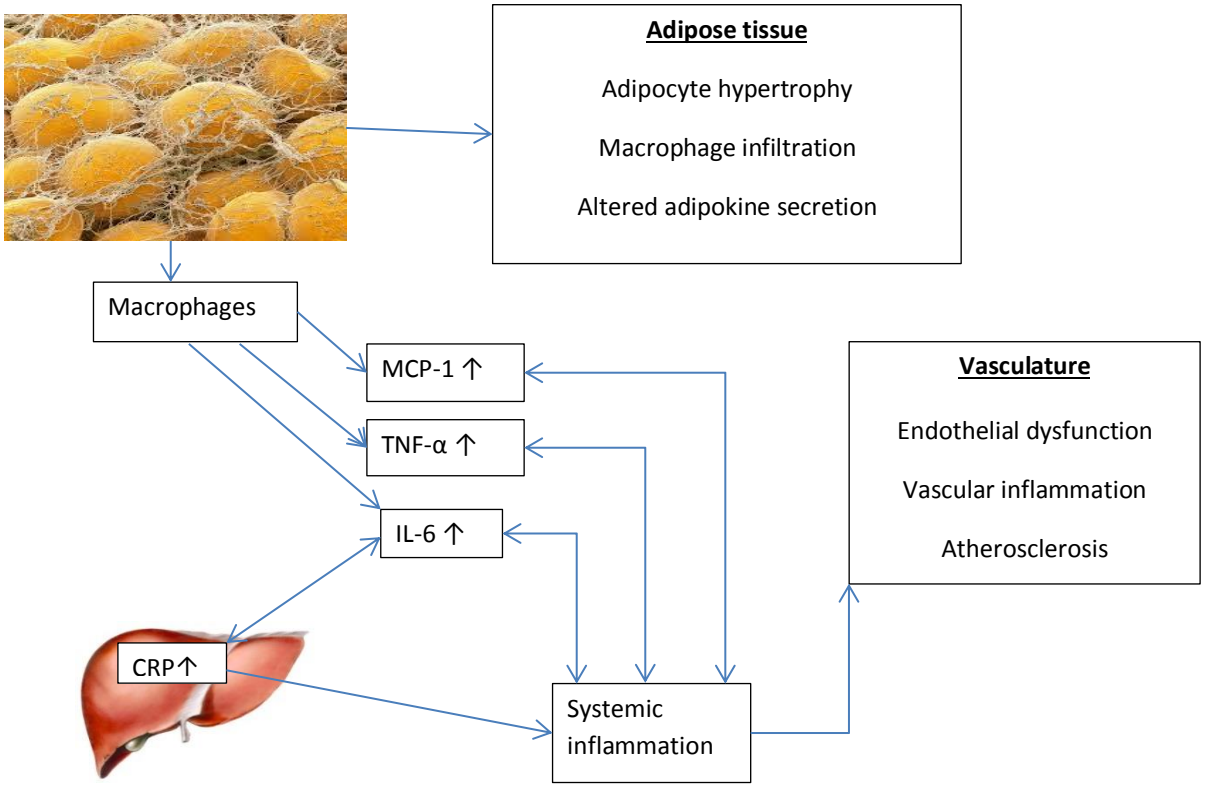

Figure 5. Pro-inflammatory adipokine secretion by adipose tissue and macrophages triggers endothelial dysfunction and vascular inflammation (Adapted from Bastard *et al.*).[174]

2.6.2 Dyslipidaemia

Serum lipid and lipoprotein profiles play a central role in the development of CVD.[178] Raised low density lipoprotein (LDL) cholesterol and reduced high density lipoprotein (HDL)

cholesterol levels are the main focus in current guidelines for the determination of cardiovascular risk because of its critical role in the development of atherosclerosis.[178] However, atherothrombosis often occurs in the absence of dyslipidaemia, which suggests that other mechanisms may have a role.[179] It was suggested that CRP may be a better predictor of cardiovascular endpoints compared to LDL-cholesterol, but the combined evaluation of these biochemical markers proved to be a superior predictor of cardiovascular risk compared to either of the markers alone.[150] In addition, Karabacak *et al* (2015)[180] showed that HDL-cholesterol inhibits inflammation associated with the development of atherosclerosis.[180,181] MCP-1 as the main chemokine involved in atherogenesis regulates plaque infiltration by inflammatory cells, induces migration and proliferation of VSMCs, induces matrix metalloproteinases (MMP) expression, the migration of endothelial cells, oxidative stress and thrombosis.[51] LDL-cholesterol and monocyte CCR2 expression is greatly increased in hypercholesteraemic individuals.[182] HDL-cholesterol, in contrast, is effective in inhibiting MCP-1 production in activated VSMCs and in human macrophages, and elevated HDL-cholesterol decreased, or even reversed, the effects of LDL-cholesterol on CCR2 expression, both in vivo and in vitro.[181,183,184] Additionally, HDL-cholesterol also inhibits NADPH oxidase dependent ROS generation, which further assists in the inhibition of the systemic inflammatory cascade.[184] In a murine model elevation of apolipoprotein AI (apoAI), the major structural protein of HDL-cholesterol significantly reduced the plaque expression of MCP-1 in apolipoprotein E (apoE)-deficient mice.[185]

Differences are evident in the lipid profiles of black and white individuals and these differences have not yet been fully explained by factors such as difference in diet or other lifestyle factors.[186] Although higher levels of hypertension, diabetes and obesity were

reported in black populations, they have particularly higher HDL-cholesterol levels compared to whites, which may provide them with a measure of cardiovascular protection.[100,186]

2.6.3 Hypertension

Hypertension is one of the greatest risk factors for cardiovascular morbidity and mortality in adult populations worldwide, contributing to all of the principle atherosclerotic disease outcomes, including ischemic heart disease, non-fatal and fatal stroke and increasing the risk 2- to 3-fold.[171] One in three American adults has hypertension, defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg (Table 3).[187,188] Hypertension forms part of the collection of metabolic abnormalities namely obesity, insulin resistance, dyslipidaemia and glucose intolerance and occurs in isolation in only 20% of individuals with the cumulative and ultimate risk depending on the number of linked risk factors present.[171] Blood pressure is involved in the specific function of large arteries, which includes the conduit function supplying blood to the periphery and organs using a pressure gradient, and the cushioning function, compensating for the pulsatile nature of blood flow ejected from the left ventricle, ensuring a constant flow of blood to the body. Hypertension affects the functional capacity of large arteries, contributing to structural change by stimulating vascular remodelling and arterial stiffness.[189]

Table 3. Definitions and classification of blood pressure (BP) levels (mmHg)

Category	Systolic		Diastolic
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89

Category	Systolic		Diastolic
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

(Taken from Mancia et al. 2013).[188]
Isolated systolic hypertension should be graded (1, 2, 3) according to systolic blood pressure values in the ranges indicated, provided that diastolic values are <90 mmHg. Grades 1, 2 and 3 correspond to classification in mild, moderate and severe hypertension, respectively.

Additionally, hypertension links to inflammation where cross-sectional studies showed that, compared to normotensives, essentially hypertensive participants with no clinical evidence of CVD had higher plasma levels of inflammatory markers, such as CRP, IL-6, TNF-α, adhesion molecules (VCAM-1 and ICAM-) and MCP-1.[83,190] Ethnicity seems to play a role in the development and progression of hypertension, with blacks developing hypertension earlier in life and their average blood pressures being higher when compared to whites.[187] In South Africa surveys showed that the country has a large burden from hypertension and subsequent CVD,[8] and as in other sub-Saharan countries, the greatest increase in hypertension is found in urban townships, which may be attributed to rapid urbanisation and lifestyle changes that make black South Africans particularly vulnerable to the development of atherosclerosis and cardiovascular disease.[191] In addition, the rapid urbanisation may be accompanied by obesity, and the combination of obesity and hypertension may be conducive to cardiovascular vulnerability, especially in women.[192]

2.7 LIFESTYLE, INFLAMMATION AND CARDIOVASCULAR RISK

2.7.1 Smoking

The disease burden attributable to smoking is considerable, and a third of deaths from smoking is secondary to CVD, with the 2014 report of the US Surgeon General stating that "there is no safe level of exposure to tobacco smoke."[193,194] Smoking causes endothelial cell activation and dysfunction through an increase in oxidative stress and reduced availability of nitric oxide (NO) that is caused by the inactivation of endothelial nitric oxide synthase (eNOS).[195] Macrophage and platelet activity are increased, and pro-inflammatory cytokines, such as IL-6 and TNF-α, with MCP-1, VCAM-1, ICAM-1 and CRP are all elevated in smokers[195]. Smoking therefore attenuates the vascular inflammatory process that leads to atherosclerotic disease initiation and progression.[193,196]

In South Africa, smoking is a great public health concern, exacerbating the huge disease burden on public health authorities.[197] Smoking prevalence was the highest among coloured men, followed by black men, and data shows that smoking accounts for 12-15 percent of deaths from preventable disease in adults, with many people still underestimating the health risks associated with smoking.[198]

2.7.2 Alcohol use

The harmful effect of excessive alcohol use on several disease conditions is well established. However, conflicting views regarding the potential beneficial effects of light to moderate alcohol consumption on the risk of coronary heart disease and stroke remain.[199] Regular alcohol consumption, in moderation, is associated with a lower incidence of myocardial infarction and individuals with hypertension appear to benefit from moderate alcohol

consumption.[200] The mechanisms supporting the potential benefit of moderate alcohol consumption include increased HDL-cholesterol levels, decreased platelet aggregation and coagulation factors and a beneficial effect on endothelial function and inflammation.[199,201] In contrast, alcohol consumption increases blood pressure in a dose-dependent fashion at intakes of more than two drinks per day, and excessive consumption of alcohol increases the risk for stroke.[200]

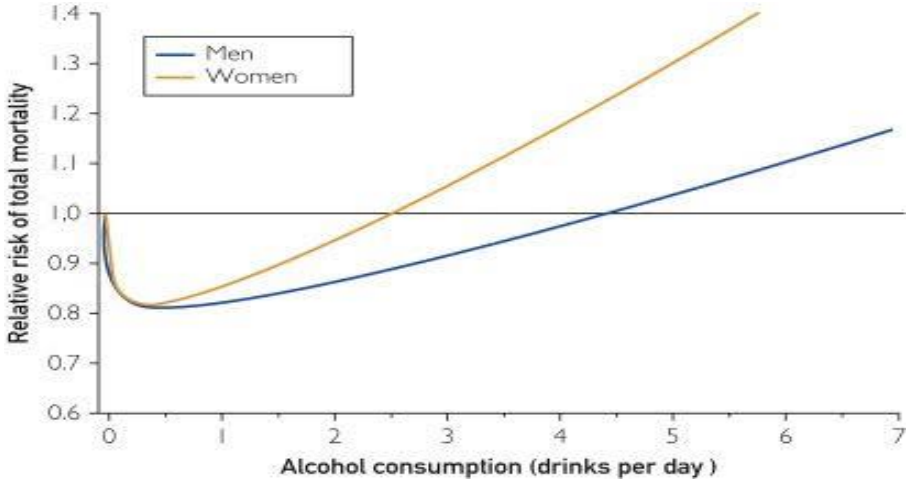

Figure 6. Alcohol and all-cause mortality. The relationship of daily alcohol consumption to the relative risk of all-cause mortality in men and women (Taken from DiCastelnuovo *et al*).[202]

Based on the J-shaped curve representing the association between alcohol and a wide range of disease outcomes (Figure 5), it is suggested that daily alcohol consumption may be cardio-protective when done in moderation and responsibly.[202]

2.7.3 Physical activity

Physical activity promotes beneficial health effects by reducing the detrimental effects of CVD such as hypertension, coronary artery disease, atherosclerosis and diabetes mellitus, and a healthy lifestyle is consistently associated with increased physical activity. Exercise training improved endothelial function in patients with coronary artery disease, as well as

increasing NO levels and bio-availability. In addition, exercise increases the activity of superoxide dismutase (SOD), leading to a decrease oxidative stress and inflammation.[203] (Figure 6)

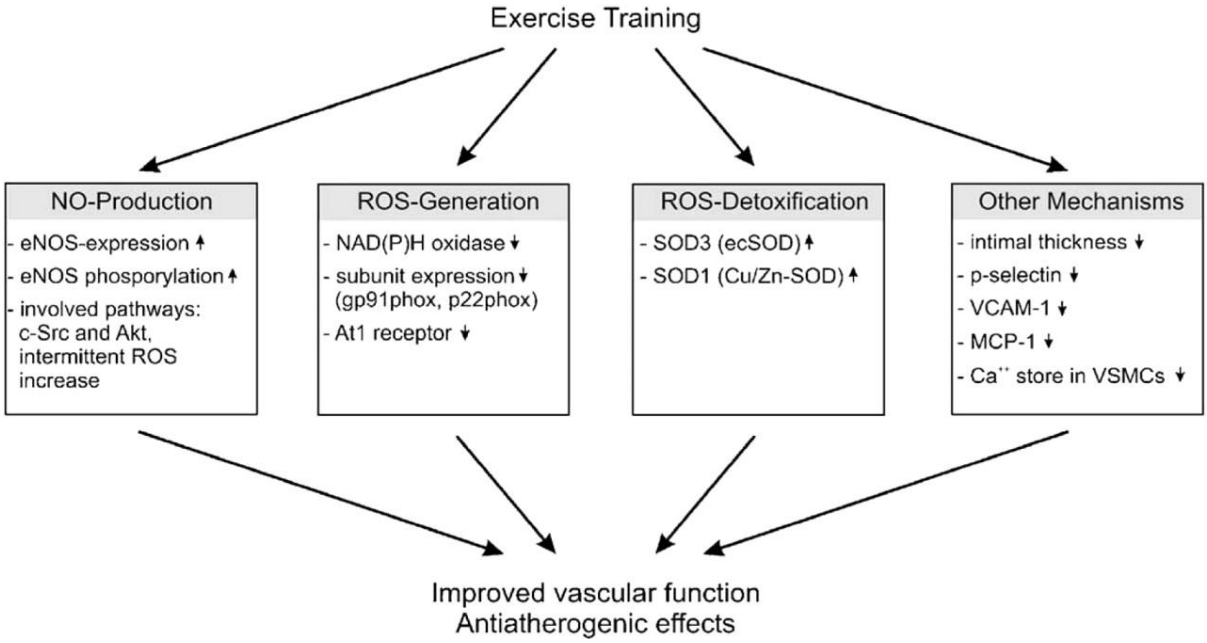

Figure 7. Mechanisms through which exercise training improves endothelial function (Taken from Kojda G & Hambrecht R. 2005).[204]
eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ROS, reactive oxygen species; NAD(P)H, nicotinamide adenine dinucleotide phosphate-oxidase; SOD, supreroxide dysmutase; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemoattractatn protein-1; VSMCs, vascular smooth muscle cells.

2.8 THE SOUTH AFRICAN CONTEXT

The World Economic Forum estimates that in developing countries non-communicable diseases (NCD) such as CVDs are a severe threat to economic development due to the long-term cost of treatment and the negative impact on productivity.[205] South Africa has a double burden, with an epidemic of infectious diseases and an alarming rise in NCD, such as hypertension, type-2 diabetes mellitus and CVD. The prevention and treatment of these non-communicable diseases do not receive sufficient attention due to the high prevalence of communicable diseases (HIV/AIDS and tuberculosis).[1] Black South Africans already

present with greater hypertension and arterial stiffness rates compared to their white counterparts,[191,206,207] and although black South Africans in the past presented with lower incidences of atherosclerosis, cIMT scores and coronary heart disease, this trend seems to be rapidly changing largely due to urbanisation and changes in lifestyle.[208,209] Additionally, the prevalence of obesity is of great concern among the black South African community and remains a challenge for health care providers, as increased weight carries social significance in black communities, representing wealth and good health.[210] The combination of earlier and more severe hypertension, arterial stiffness and rapidly developing atherosclerotic disease in the black population may predispose this already vulnerable population to increased CVD risk.

2.9 SUMMARY

Inflammation plays a central role in the initiation and progression of endothelial dysfunction and atherosclerotic disease, with the role of MCP-1 in the development and progression of atherosclerosis well established. It is, however, evident that no single pathway can account for all cardiovascular events and that the interaction between inflammatory markers and more traditional risk factors such as hypertension, smoking, obesity, diabetes mellitus and low levels of physical activity may be more or less important for individual risk assessment. No single inflammatory marker will likely be the perfect predictor of CVD. It may therefore be necessary to use several markers. Especially markers such as MCP-1, which has a function in atherosclerosis development, in combination with traditional risk scores, may indicate early vascular changes and assist in diagnosis and monitoring of CVD progression. Ethnicity does seem to play an important role in CVD development and there are differences in the inflammatory profiles of different ethnicities.

2.10 AIM, OBJECTIVES AND HYPOTHESES

2.10.1 Aim

The main aim of this study is therefore to determine whether MCP-1, as a possible early marker of endothelial dysfunction, is associated with arterial stiffness and cIMT in young black and white individuals participating in the African-PREDICT study.

2.10.2 Objectives

The objectives are:

- to determine whether there are ethnic differences in MCP-1 levels;
- to compare the 24-hour blood pressure and measures of large artery structure and function (cIMT and cfPWV) between black and white participants;
- to determine whether arterial stiffness relates to MCP-1; and
- to determine whether cIMT relates to MCP-1 in both black and white groups.

2.10.3 Hypotheses

With regard to the specific population of the African-PREDICT study, the following hypotheses were formulated:

1. Ethnic differences in circulating MCP-1 concentrations do exist, being higher in black than white participants.

2. Young black individuals have similar cIMT measurements as white participants;

3. Ethnic-specific differences exist regarding cfPWV, being higher in the black group;

4. Arterial stiffness is positively associated with MCP-1 in both the black and white groups.

5. Carotid wall thickness is positively associated with MCP-1 in both the black and white groups.

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CHAPTER THREE

Methodology

3.1 METHODOLOGY

The main aim of the umbrella study, **A**frican **PR**ospective study on the **E**arly **D**etection and **I**dentification of **C**ardiovascular disease and Hyper**T**ension: the **African-PREDICT** study, is to identify early markers or predictors for the development of cardiovascular disease in young black South Africans. The study aimed to track and monitor change in young, normotensive black and white individuals (N=1200) over 20 years. To achieve this, detailed cardiovascular and biomarker assessments will be done every 5 years in order to identify and understand early changes in cardiovascular function, and specific predictors contributing to the development of hypertension and target organ damage.

Potential participants for the African-PREDICT study are being sourced through various means. These include the media, for example the local radio stations and newspapers, by contacting local community leaders and using referrals from local medical staff at clinics. These persons are asked to contact the HART-clinic on the Potchefstroom campus of the North-West University and specific appointments are then established. Transport to the clinic is arranged for potential participants if needed. All participants that report to the clinic first undergo a screening process to determine whether they comply with the inclusion criteria of the study.

Inclusion criteria:

1. admitted to clinic and completed the screening step;
2. black and white ethnicity;
3. aged 20-30 years;
4. men and women (equally distributed);
5. apparently healthy; and
6. normotensive and pre-hypertensive (SBP < 140 and DBP < 90 mmHg), based on the average of 4 blood pressure measurements in one day.

Exclusion criteria:

1. age >30 or <20 years;
2. hypertensive;
3. indian, Asian or mixed ethnicity;

4. not a permanent resident of Potchefstroom or surrounding area (i.e. intend on moving to another area);
5. type 1 or 2 diabetes mellitus;
6. elevated glucose levels >5.6 mmol/L and confirmed glycated haemoglobin (HbA1c) ≥6.5%;
7. HIV-infected;
8. fever (internal ear temperature >37.5°C on the research day);
9. known liver disease, cancer, tuberculosis or renal disease;
10. microalbuminuria >30 mg/ml in spot urine or proteinuria;
11. medication use for chronic disease (i.e. antihypertensive, anti-diabetic, antiretroviral or anti-inflammatory medication);
12. pregnant or lactating women;
13. recent surgery or trauma (within the past three months); and
14. previous history of stroke, angina pectoris or myocardial infarction.

The central aim of this substudy was to determine whether monocyte chemoattractant protein-1 (MCP-1), as possible early marker of endothelial dysfunction, is associated with arterial stiffness and carotid intima-media thickness (cIMT) in the first 400 young black and white individuals participating in the African-PREDICT study. All methods that were used to obtain secondary data correspond with the methodology of the umbrella project and no additional measurements or assessments were done.

3.1.1 METHODOLOGY APPLICABLE TO THE SUBSTUDY

Information leaflets were distributed to participants a few days prior to the measurements. Upon arrival at the HART clinic the procedures were explained to the participants, using good clinical practice. It was explained in the participants' own language by skilled and experienced field workers. Participants were given the opportunity to ask questions before signing informed consent. Participants were then asked to complete the general health questionnaire.

General Health Questionnaire – Completed online on a web-based program and involved demographic information, employment information, alcohol and tobacco use, medication use and family history.

Blood pressure measurements - Duplicate office brachial blood pressure measurements were taken on the left and the right arm, while the participant was in a rested and sitting position using the Dinamap® Procare 200 blood pressure monitor (GE Medical Systems, Milwaukee, WI, USA). Participants were also fitted with a 24-hour ambulatory blood pressure device and an ECG apparatus (CardioXplore®, CE0120, Meditech, Budapest, Hungary, BHS validated). An appropriately sized cuff was fitted to the participant's non-dominant arm. Instructions were given on how to ensure successful inflation rates. An ambulatory diary card was completed by the participants during the measurements.

Biological sampling - Participants were required to be fasting for at least 8 hours. A registered and trained research nurse took a blood sample from the ante-brachial vein, using a winged infusion set. An early morning spot urine sample was also taken. All samples were taken to the on-site laboratory immediately for appropriate allocation into cryovials and stored in biofreezers at -80°C for short- and long-term (screw cap) storage.

Biochemical analysis – For the first 400 participants an immediate basic serum analysis was done. These analyses included total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), glucose, C-reactive protein (CRP), creatinine, liver enzymes, albumin and uric acid. Urinary albumin and creatinine were also determined and albumin-to-creatinine ratio calculated (Cobas Integra 400plus, Roche, Basel, Switzerland). Cotinine was analysed by using the Chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Full blood count was estimated on the day of sample collection (Coulter AcT5 diff OV Haematology analyser, Beckman Coulter, Brea, CA, US).

Plasma monocyte chemoattractant protein-1 (MCP-1) was analysed in duplicate using a human CCL2/MCP-1 immunoassay ELISA kit (R&D Systems, Inc., Minneapolis, US). This assay uses the quantitative sandwich enzyme immunoassay technique. This sandwich ELISA is developed to detect this highly specific monocyte chemotactic peptide and was developed since it is often challenging to determine the quantitative contribution of a monocyte chemotaxin. Additionally the kit has the capacity to determine endothelial cell-derived MCP-1, which renders it significant for cardiovascular studies.[1] Furthermore, serum intracellular-adhesion molecule-1 (ICAM), vascular cell adhesion molecule-1 (VCAM), interleukin-6 (IL-6)

and tumour necrosis factor-α (TNF-α) were measured using Quantikine® ELISA kits (R&D Systems, Inc., Minneapolis, US).

Anthropometric measurements – Anthropometric measurements were taken using calibrated instruments. This included body height (SECA 213 Portable Stadiometer, Hamburg, Germany), weight (SECA 813 Electronic Scale, Hamburg, Germany), and waist and hip circumferences (Lufkin Steel Anthropometric Tape, Apex Tool Group, Maryland, US). All anthropometric measurements complied with the International Standards for Anthropometric Assessment.[2]

Assessment of arterial stiffness and carotid wall thickness (sub-clinical organ damage) – Carotid-femoral pulse wave velocity (cfPWV) and pulse wave analyses (PWA) were performed by using the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia), according to the manufacturer's instructions. Central blood pressure and the augmentation index were derived from the PWA. The cfPWV were measured along the descending thoracic-abdominal aorta, using the foot-to-foot velocity method.[3,4] Measurements were taken in duplicate.

Carotid intima-media thickness (cIMT) and plaque scores were determined on the left and right common carotid artery, as well as the internal carotid artery (General Electric Vivid E9, GE Vingmed Ultrasound A/S, Horten, Norway). The images were digitised and imported into the Artery Measurement Systems Software for dedicated analyses (Gustavvson, Sweden). One observer did the analysis of all images. We calculated carotid cross-sectional wall area ($CSWA = \pi (d/2 + CIMT)^2 - \pi (d/2)^2$, where d denotes luminal diameter).[5]

Experienced and trained clinical technologists and researchers took all the cardiovascular measurements, using good clinical practice. These measurements were done in private rooms to ensure the participants' comfort and privacy. A maximum of 4 participants were measured per day to ensure personal contact and a comfortable experience.

Urinary albumin-to-creatinine ratio – Albumin and creatinine were determined using the spot urine samples obtained early in the morning when the participants arrived at the research facility (Roche Integra 400).

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CHAPTER FOUR

Manuscript for publication

Monocyte chemoattractant protein-1 and large artery structure and function in young individuals: The African-PREDICT study

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4.1 Abstract

Background

Black populations are known to have vulnerable cardiovascular profiles, but there is limited evidence concerning the influence of circulating chemokines to this vulnerability. We therefore set out to determine whether monocyte chemoattractant protein-1 (MCP)-1, an early marker of endothelial dysfunction, is associated with arterial stiffness and carotid wall thickness in a young bi-ethnic population.

Methods

We investigated 403 apparently healthy individuals aged 20-30 years, consisting of black (N=198) and white (N=205) men and women. Carotid-femoral pulse wave velocity (cfPWV) and central systolic blood pressure (SBP) were measured using the SphygmoCor® XCEL device. B-mode ultrasonography was used to measure carotid intima-media thickness (cIMT). Plasma MCP-1 was determined with the Quantikine® immunoassays (R&D Systems).

Results

cfPWV and cIMT were similar between the black and white groups, but black men and women showed higher central SBP and higher MCP-1 levels (both $p<0.001$) than their white counterparts. In addition, black women showed higher brachial SBP ($p<0.001$) and higher mean arterial pressure ($p=0.001$) than white women. We found a consistent positive association only in black women between cIMT and MCP-1 in single, partial and multiple regression analyses ($R^2=0.151$; $\beta=0.248$ [0.14; 0.35]; $p=0.021$).

Conclusion

In a young healthy bi-ethnic population, we found elevated central SBP and MCP-1 only in blacks. In black women carotid wall thickness was related to early endothelial dysfunction (MCP-1), which may indicate an increased risk for early vascular ageing in young black individuals.

Keywords

Arterial stiffness, carotid intima-media thickness, inflammation, ethnicity, hypertension, adhesion molecules, central systolic blood pressure, atherosclerosis

4.2 Introduction

Hypertension and cardiovascular disease (CVD) have reached epidemic proportions in Sub-Saharan Africa,[1,2] and as with the black population in the United States[3], the South African black population shows a high prevalence of hypertension and an increased risk of developing CVD.[4,5] This elevated cardiovascular risk can, however, only partly be explained by traditional risk factors such as lifestyle, diabetes mellitus and smoking.[4,6] Large artery stiffness is recognised as an independent predictor of cardiovascular disease morbidity and mortality.[7,8] The black population presents with impaired vascular and endothelial function, accompanied by greater arterial stiffness when compared to whites.[9] In African-Americans increased carotid intima-media thickness (cIMT) and stiffness were also demonstrated.[10,11]

When viewing endothelial dysfunction in black populations, evidence indicates that their increased blood pressure is characterised by elevated plasma levels of inflammatory markers, adhesion molecules and chemokines when compared to whites.[12-14]

Biomarkers and their association with arterial stiffness and atherosclerosis has increasingly been the subject of efforts to make risk stratification and early detection of CVD more efficient.[15,16] Monocyte chemoattractant protein-1 (MCP-1) is an extensively investigated chemokine with regard to its relationship with increased risk for hypertension and CVD.[17-19] Apart from hypertension, elevated MCP-1 and its receptor CCR2 are also involved in the development of atherosclerosis,[20] and are associated with increased risk of myocardial infarction,[20] coronary angioplasty[21] and stent restenosis.[22]

Literature on MCP-1 in black populations is scant and to our knowledge, no studies have reported MCP-1 levels in a healthy young black population to evaluate its potential

usefulness as early indicator of carotid wall thickness or large artery stiffness. We therefore investigated the association of cIMT and large artery stiffness with MCP-1 in a young and apparently healthy black and white population.

4.3 Methods

4.3.1 Study Design

This sub-study forms part of the larger African **PR**ospective study on the **E**arly **D**etection and **I**dentification of **C**ardiovascular Disease and Hyper**T**ension (African-PREDICT). Inclusion criteria were apparently healthy, black and white, men and women aged 20-30 years, with SBP <140 mmHg and <90 mmHg. After the procedures of the study were explained to all participants, they gave written informed consent and participated voluntarily. The Human Research Ethics Committee of the North-West University approved the study (NWU-00001-12-A1). In this cross-sectional sub-study we included the first 403 participants, consisting of black (N=198) and white (N=205) men and women.

4.3.2 Questionnaires

Demographic and lifestyle questionnaires and the global physical activity questionnaire (GPAQ)[23] were used to assess alcohol use, smoking habits and physical activity level.

4.3.3 Anthropometric measurements

Anthropometric measurements were done according to standard methods described by Marfell-Jones *et al.*[24], and included height (SECA 213 Portable Stadiometer, Hamburg, Germany), weight (SECA 813 Electronic Scale, Hamburg, Germany), and waist circumference (WC)(Lufkin Steel Anthropometric Tape, Apex Tool Group, Maryland, USA).

4.3.4 Cardiovascular measurements

Duplicate office brachial blood pressure (BP) measurements conducted made on the left and on the right arm with a 5-minute interval while the participants were seated and in a rested state, using the Dinamap® Procare 200 BP Monitor (GE Medical Systems, Milwaukee, WI, USA). Participants were also fitted with a validated 24-hour ambulatory blood pressure (ABPM) device (CardioXplore® CE120, Meditech, Budapest, Hungary).

Arterial stiffness was assessed according to the manufacturer's instructions to determine carotid-femoral pulse wave velocity (cfPWV) using the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). The cfPWV was measured along the descending thoracic-abdominal aorta, using the foot-to-foot velocity method. Measurements were taken in duplicate, and the mean value reported.

B-mode ultrasonography was used to determine the cIMT of the left and right common carotid artery (General Electric Vivid E9, GE Vingmed Ultrasound A/S, Horten, Norway). The images were digitised and imported into the Artery Measurement Systems Software for dedicated analyses (Gustavvson, Sweden) by a single observer. The mean cIMT values of the images taken were reported.

4.3.5 Biochemical analyses

Participants were required to be fasting for at least 8 hours. A registered nurse took a blood sample from the ante-brachial vein using a winged infusion set. An early morning spot urine sample was taken. All samples were immediately taken to the on-site laboratory and appropriately allocated into cryovials and stored in biofreezers at -80°C until analyses. Basic serum analyses included total cholesterol (TC), high density lipoprotein cholesterol (HDL-C),

low density lipoprotein cholesterol (LDL-C), triglycerides (TG), glucose, high sensitivity C-reactive protein (hsCRP), creatinine, gamma glutamyltransferase (GGT), albumin and urea (Cobas Integra 400plus, Roche, Basel, Switzerland). Total serum peroxides were determined with a high-throughput spectrophotometric kinetic assay as an indicator of reactive oxygen species (ROS). The assay measures the generic peroxide-induced modification of the chromogen *N*, *N*-diethyl-*para*-phenyl-endiamine expressed as units (1 unit equalling 1 mg H_2O_2/L).[25] Cotinine was analysed using the chemiluminescent method on the Immulite nicotine metabolite assay (Siemens, Erlangen, Germany), and MCP-1 with a Quantikine® Human CCL2/MCP-1 immunoassay (R&D Systems, Inc., Minneapolis, USA). This assay uses the quantitative sandwich enzyme immunoassay technique.[26] Furthermore, serum intercellular adhesion molecules (ICAM), vascular cell adhesion molecule (VCAM), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) were measured using Quantikine® ELISA kits (R&D Systems, Inc., Minneapolis, USA).

4.3.6 Statistical Analyses

For database management and statistical analyses, we used Statistica software version 12 (Statsoft, Inc., Tulsa, OK). We tested for the interaction of ethnicity and sex regarding the associations of cfPWV and cIMT with MCP-1. Means and proportions were compared between blacks and whites using independent t-tests and Chi-square tests, respectively. The biochemical variables with a skewed distribution (MCP-1; TNF-α; hsCRP; IL-6; ROS; total cholesterol; triglycerides and GGT) were normalised with natural logarithmic transformation. Continuous data were presented as arithmetic means ± standard deviation or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. We determined ethnicity- and sex-specific Pearson and partial correlation

coefficients between cardiovascular measurements (cfPWV and cIMT) and MCP-1, with adjustment for age and mean arterial pressure. Multiple regression analyses were performed with either cfPWV or cIMT as dependent variables and MCP-1 as the main independent variable. Independent variables included in the model were age, mean arterial pressure, LDL-cholesterol, waist circumference, glucose, TNF-α, VCAM-1 and ROS. Other covariates considered for entry in the models were IL-6, hsCRP, ICAM-1, HDL-cholesterol, central SBP, 24-hour blood pressure, and BMI. A $p<0.05$ was regarded as statistically significant.

4.4 Results

We found a significant interaction with sex for the association of cfPWV and MCP-1 ($p=0.042$), and a significant interaction with ethnicity for the association of cIMT and MCP-1 ($p=0.007$). Based on these interactions, as well as our research aim and previous studies confirming differential development of hypertension in black and white ethnicities,[1,27] we compared the ethnic and sex groups in subsequent analyses.

The characteristics of the study population are presented in Table 1. While the study population included young adults aged 20-30 years, the mean ages of white men ($p=0.014$) and women ($p=0.015$) were older than their black counterparts. The black men had lower indices of obesity (BMI, $p=0.001$ and WC, $p=0.001$) and total cholesterol ($p<0.001$) than white men, but showed both higher central SBP ($p=0.006$) and higher MCP-1 levels ($p<0.001$). cfPWV and cIMT were similar between the black and white men.

Black women had higher indices of obesity (BMI, $p=0.008$ and WC, $p=0.031$), but lower total cholesterol levels ($p<0.001$) than white women. Similar to the men, black women had higher

central SBP ($p<0.001$) and higher MCP-1 levels ($p<0.001$) than white women, and cfPWV and cIMT were similar between black and white women. Despite similar 24-hour blood pressure, black women had higher office brachial SBP and DBP (both $p<0.001$) and mean arterial blood pressure ($p=0.001$) when compared to white women.

Table 1. Characteristics of participants (n=403)

	Black men (n=91)	White men (n=80)	p-value	Black women (n=107)	White women (n=125)	p-value
Age (years)	24.5 ± 3.01	25.6 ± 2.90	0.014	24.5 ± 3.41	25.5 ± 2.85	0.015
Anthropometric indices						
Body mass index (kg/m²)	21.9 ± 3.40	28.0 ± 5.64	0.001	26.6 ± 5.81	24.6 ± 5.42	0.008
Waist circumference (cm)	74.5 ± 8.81	91.5 ± 14.1	0.001	79.6 ± 11.9	76.1 ± 12.6	0.031
Cardiovascular measures						
bSBP (mmHg)	126 ± 11.6	125 ± 8.42	0.59	116 ± 10.4	110 ± 10.4	<0.001
bDBP (mmHg)	82.5 ± 8.82	79.9 ± 6.67	0.044	79.1 ± 7.60	74.4 ± 7.27	<0.001
bMAP (mmHg)	96.9 ± 9.13	95.0 ± 6.50	0.12	91.5 ± 7.95	86.2 ± 7.89	0.001
24hr SBP (mmHg)	121 ± 9.12	124 ± 6.73	0.011	118 ± 8.64	118 ± 8.79	0.87
24hr DBP (mmHg)	71.7 ± 6.90	70.7 ± 6.12	0.98	69 ± 5.66	68.2 ± 5.71	0.93
cSBP (mmHg)	115 ± 10.2	111 ± 8.55	0.006	109 ± 8.09	103 ± 8.75	<0.001
cPP (mmHg)	36.1 ± 5.40	37.7 ± 5.58	0.066	31.8 ± 5.17	32.5 ± 4.21	0.24
bPP (mmHg)	43.3 ± 7.71	44.9 ± 7.09	0.16	37.9 ± 7.25	35.3 ± 6.43	0.027
cfPWV (ms^{-1})*	6.85 ± 0.74	6.73 ± 0.75	0.34	6.04 ± 0.78	6.09 ± 0.78	0.54
cIMT (mm)*	0.48 ± 0.05	0.48 ± 0.05	0.81	0.45 ± 0.04	0.46 ± 0.04	0.091
Biochemical variables						
Total cholesterol (mmol/l)	3.89 (3.70;4.07)	4.73 (4.49;4.98)	<0.001	3.83 (3.67;3.99)	4.73 (4.56;4.90)	<0.001
HDL-C (mmol/l)	1.34 ± 0.35	1.12 ± 0.26	<0.001	1.21 ± 0.32	1.61 ± 0.40	<0.001
LDL-C (mmol/l)	2.36 ± 0.83	3.30 ± 1.04	<0.001	2.50 ± 0.81	2.90 ± 0.83	0.001
Triglycerides (mmol/l)	0.94 (0.82;1.06)	1.21 (1.07;1.34)	0.001	0.77 (0.71;0.82)	1.03 (0.91;1.14)	<0.001
ROS (units)	156 ± 45.91	140 ± 40.70	0.035	228 ± 70.63	206 ± 101.4	0.003
MCP-1 (pg/ml)	190 (181;200)	154 (145;163)	<0.001	169 (160;178)	136 (127;145)	<0.001
hsCRP (mg/l)	1.61 (0.79;2.44)	2.36 (1.20;3.52)	0.091	3.84 (2.96;4.72)	3.18 (2.12;4.25)	0.001
Interleukin-6 (pg/ml)	1.02 (0.78;1.27)	0.98 (0.78;1.17)	0.85	1.45 (1.20;1.70)	0.95 (0.76;1.15)	<0.001
TNF-α (pg/ml)	1.86 (1.63;2.10)	1.91 (1.80;2.03)	0.071	1.94(1.58;2.29)	1.81 (1.67;1.95)	0.57
Serum albumin (g/L)	46.9 ± 3.92	48.8 ± 2.87	<0.001	44.7 ± 3.79	46.2 ± 3.10	0.003
sICAM-1 (ng/ml)	140 ± 72.84	187 ± 62.9	<0.001	152 ± 82.3	181 ± 56.7	0.002
sVCAM-1 (ng/ml)	544 ± 124	552 ± 147	0.70	537 ± 143	599 ± 185	0.005
Serum creatinine (umol/L)	73.7 ± 13.2	88.2 ± 11.3	<0.001	58.1 ± 10.2	66.9 ± 9.89	<0.001

	Black men (n=91)	White men (n=80)	p-value	Black women (n=107)	White women (n=125)	p-value
Serum urea (mmol/L)	3.94 ± 1.10	5.29 ± 1.13	<0.001	3.35 ± 0.95	4.38 ± 1.21	<0.001
Glucose (mmol/L)	3.81 ± 0.96	5.01 ± 0.71	<0.001	3.90 ± 0.74	4.53 ± 0.86	<0.001
GGT (U/L)	38.0 ± 38.3	30.3 ± 27.2	0.14	28.1 ± 22.9	16.6 ± 12.7	0.001
Cotinine (ng/ml)	1.14 ± 1.16	0.63 ± 0.97	0.002	0.36 ± 0.73	0.31 ± 0.68	0.85
Self-reported lifestyle variables						
Intense exercise, MET minutes	3.37 ± 0.40	3.18 ± 0.41	0.031	3.25 ± 0.41	3.10 ± 0.37	0.042
Current smoking, n/total %	51/91 (56.0)	19/80 (23.8)	0.001	12/107 (11.2)	13/125 (10.4)	0.84
Current drinking, n/total %	68/91 (74.7)	58/80 (72.5)	0.74	55/107 (51.4)	78/125 (62.4)	0.091

bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; bMAP, brachial mean arterial pressure, cPP, central pulse pressure; bPP, brachial pulse pressure; cfPWV, carotid-femoral pulse wave velocity; cIMT, carotid intima media thickness; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ROS, reactive oxygen species; hsCRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; ICAM, Intercellular adhesion molecule-1; VCAM-1, Vascular cell adhesion molecule-1; TNF-α, Tumour necrosis factor-α; GGT, Gamma-glutamyl tranferase. MET minutes calculated using WHO guidelines for GPAQ data analyses.[23] Data are expressed as arithmetic mean ± standard deviation or geometric mean with 95% percentile intervals for logarithmically transformed data, or % of n.
*Adjusted for mean arterial pressure.

In single and partial regression analyses (Table 2) and multiple regression analyses (Table 3), we found a consistent positive association between cIMT and MCP-1 in black women (R^2=0.151; β=0.248 [0.14; 0.35]; p=0.021). However, we found no significant associations of cfPWV or cIMT with MCP-1 in any of the other groups. In addition to the independent association between cIMT and MCP-1 in the black women (supplementary table S1), cIMT also associated positively with VCAM-1 (R^2=0.151; β=0.251 [0.14; 0.36; p=0.022) only in the black women, and independently of MCP-1. Although the participants were relatively young, cfPWV showed a positive association with age in the black men (R^2=0.161; β=0.250 [0.13; 0.37]; p=0.039) and women (R^2=0.184; β=0.396 [0.28; 0.51]; p=0.001) but not in the white men (R^2=0.308; β=0.51[0.02; 0.24]; p=0.24) and women (R^2=0.059; β=0.171 [0.08; 0.27]; p=0.078).

Table 2. Pearson and partial correlations of cfPWV and cIMT with MCP-1

	cfPWV	cIMT
Black men	r = 0.11; p = 0.29	r = 0.14; p = 0.20
White men	r = 0.16; p = 0.18	r = -0.17; p = 0.14
Black women	r = -0.15; p = 0.14	**r = 0.22; p = 0.021**
White women	r = 0.03; p = 0.72	r = 0.007; p = 0.93
Adjusted for age and mean arterial pressure		
Black men	r = 0.03; p = 0.77	r = 0.12; p = 0.28
White men	r = 0.09; p = 0.44	r = -0.13; p = 0.31
Black women	r = -0.17; p = 0.10	**r = 0.31; p = 0.002**
White women	r = -0.03; p = 0.76	r = -0.03; p = 0.77

MCP-1, monocyte chemoattractant protein-1; cfPWV, carotid-femoral pulse wave velocity; cIMT, carotid intima media thickness. Bold values indicate p < 0.05.

Table 3. Multiple regression analyses

Black men				
	Std β (95 % CI)			
	cfPWV	**p**	**cIMT**	**p**
R^2/Adj R^2	0.260/0.161		0.101/-	
bMAP	**0.401 [0.29; 0.51]**	**0.001**	0.146 [0.02; 0.27]	0.24
Age	**0.250 [0.13; 0.37]**	**0.039**	0.100 [-0.03; 0.23]	0.45
MCP-1	0.001 [-0.11; 0.11]	0.99	0.107 [-0.02; 0.23]	0.39
TNF-α	0.004 [-0.11; 0.11]	0.97	-0.020 [-0.14; 0.11]	0.87
VCAM-1	0.011 [-0.09; 0.12]	0.92	0.053 [-0.07; 0.17]	0.66
ROS	0.093 [-0.02; 0.21]	0.39	-0.079 [-0.19; 0.04]	0.51
LDL-cholesterol	-0.169 [-0.29; -0.05]	0.17	0.127 [-0.01; 0.26]	0.36
Glucose	-0.108 [-0.22; 0.01]	0.34	-0.111 [-0.24; 0.01]	0.38
Waist circumference	-0.031 [-0.15; 0.09]	0.81	-0.195 [-0.33; -0.06]	0.16
White men				
R^2/Adj R^2	0.398/0.308		0127/0.009	
bMAP	**0.511 [0.39; 0.63]**	**< 0.001**	-0.015 [-0.15; 0.12]	0.91
Age	0.129 [0.02; 0.24]	0.24	0.131 [0.01; 0.25]	0.29
MCP-1	0.113 [-0.01; 0.23]	0.35	-0.004 [-0.14; 0.13]	0.98
TNF-α	0.076 [-0.03; 0.18]	0.48	0.081 [-0.04; 0.21]	0.51
VCAM-1	-0.048 [-0.16; 0.06]	0.67	-0.215 [-0.34; -0.09]	0.09
ROS	-0.067 [-0.18; 0.04]	0.55	-0.034 [-0.16; 0.09]	0.79
LDL-cholesterol	0.029 [-0.08; 0.13]	0.78	-0.028 [-0.15; 0.09]	0.82
Glucose	0.117 [-0.82; 1.1]	0.28	0.036 [-1.08; 1.11]	0.77
Waist circumference	0.055 [-0.06; 0.17]	0.65	-0.199 [-0.34; -0.06]	0.15
Black women				
R^2/Adj R^2	0.273/0.184		0.238/0.151	
bMAP	**0.267 [0.16; 0.38]**	**0.016**	**0.309 [0.21; 0.42]**	**0.005**
Age	**0.396 [0.28; 0.51]**	**0.001**	0.118 [0.01; 0.23]	0.29
MCP-1	-0.138 [-0.24; -0.03]	0.19	**0.248 [0.14; 0.35]**	**0.021**
TNF-α	-0.122 [-0.23; -0.02]	0.25	-0.051 [-0.16; 0.05]	0.63

VCAM-1	-0.086 [-0.19; 0.02]	0.43	**0.251 [0.14; 0.36]**	**0.022**
ROS	-0.0006 [-0.11; 0.11]	0.99	0.069 [-0.04; 0.17]	0.51
LDL-cholesterol	-0.072 [-0.18; 0.04]	0.51	0.079 [-0.03; 0.19]	0.46
Glucose	-0.034 [-0.14; 0.07]	0.75	-0.197 [-0.31; -0.09]	0.07
Waist circumference	**-0.241 [-0.35; -0.13]**	**0.036**	-0.152 [-0.26; -0.04]	0.18
White women				
R^2/Adj R^2	0.134/0.059		0.054/-	
bMAP	**0.328 [0.22; 0.44]**	**0.003**	0.111 [-0.01; 0.22]	0.33
Age	0.171 [0.08; 0.27]	0.078	0.126 [0.03; 0.23]	0.21
MCP-1	-0.009 [-0.11; 0.09]	0.93	-0.012 [-0.11; 0.09]	0.91
TNF-α	-0.099 [-0.19; -0.01]	0.31	0.073 [-0.03; 0.17]	0.46
VCAM-1	-0.028 [-0.12; 0.07]	0.77	0.141 [0.04; 0.24]	0.16
ROS	-0.077 [-0.17; 0.02]	0.43	0.141 [0.04; 0.24]	0.17
LDL-cholesterol	-0.015 [-0.11; 0.08]	0.88	0.023 [-0.08; 0.12]	0.82
Glucose	0.116 [0.02; 0.21]	0.22	0.037 [-0.06; 0.13]	0.71
Waist circumference	-0.086 [-0.21; 0.03]	0.46	-0.127 [-0.25; -0.01]	0.29

bMAP, brachial mean arterial pressure; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumour necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; ROS, reactive oxygen species; LDL-cholesterol, low-density lipoprotein cholesterol.

Bold values indicate $p < 0.05$.

4.5 Discussion

We investigated the association of large artery structure and function with MCP-1 in a young bi-ethnic population. The main finding was that carotid wall thickness associated positively with MCP-1 in black women, but in none of the other groups. Furthermore, the cardiovascular profiles of the black men and women seemed more vulnerable due to elevated MCP-1 and central SBP compared to their white counterparts. In the black women the link between cIMT and MCP-1 was supported by a consistent association between cIMT and the endothelial activation marker, VCAM-1. Although cfPWV was similar between the black and white groups, it associated positively with age in the black population only, which may support the notion of early vascular ageing in this group.[28,29]

Even though all participants were screened for normal office brachial blood pressure, central SBP was higher in the black men and women compared to whites. Brachial blood pressure is a strong indicator of cardiovascular outcome, although central SBP gives a better measure of the load on central and carotid arteries, and may therefore be a better indicator of vascular damage[30] and cardiovascular outcome.[30,31] Despite the increased central SBP in blacks, their cfPWV and cIMT were similar to the white groups. Where cIMT is a measure of the structural components within the carotid arteries and therefore an important marker of early atherosclerotic build-up.[10] cfPWV is a measure of arterial stiffness, which portrays both the structure and function of large arteries, and therefore is associated with arteriosclerosis.[8,32] According to the ESH/ESC guidelines[33] the black and white groups presented with cIMT and cfPWV well below the cut-off values of 0.9 mm for cIMT and 10 ms^{-1} for cfPWV.

A prominent finding from our study is the positive association between cIMT and MCP-1 in the black women. Possible reasons for this standout finding may be explained by better characterising this specific group. The black women presented with an increased inflammatory profile (MCP-1, hsCRP and IL-6) and a consistent positive association between cIMT and VCAM-1 (Table S1). Increased levels of MCP-1, hsCRP, IL-6 and VCAM-1 are all established independent risk factors for atherosclerosis and coronary heart disease development.[34] The positive association of cIMT with MCP-1 and VCAM-1 may be an indication of early endothelial cell activation during the initial stages of sub-clinical atherosclerosis, as MCP-1 promotes the accumulation of lipids in the sub-endothelial intimal layer.[18] Dansky *et al.*[35] suggested a major role for VCAM-1 in the initiation of atherosclerosis through its recruitment of monocytes to the arterial intima and early foam cell formation. Although the lipid profile of the black women was more favourable compared to the white women, the black women had lower HDL-cholesterol ($p<0.001$). HDL-cholesterol promotes cholesterol efflux from the vascular wall and is inversely associated with cardiovascular disease risk.[36] Added to this, HDL-cholesterol inhibits inflammation associated with the development of atherosclerosis.[37] The lower HDL-cholesterol of the black women may therefore indicate a decreased cardio-protective effect and increased oxidative stress, which is confirmed by their higher ROS levels ($p=0.003$). Increased ROS in black South Africans was previously reported[38] and it links with endothelial cell activation, dysfunction and inflammation.[39]

Traditional risk factors, including BP, diabetes, smoking and obesity, are independently linked to atherosclerosis and CVD.[32,40] Age and BP are the most prominent determinants of arterial stiffness,[32] as seen in the black men and women in the present study, with the

traditional risk factors representing a less important role. These risk factors may, however, over time promote arteriosclerosis through changed haemodynamics.[41]

The black women further presented with higher GGT levels (p=0.001), although their self-reported alcohol use was similar to the white women. Elevated GGT levels also associate strongly with the development of CVD,[42] and apart from the association of GGT with excessive alcohol intake, it is influenced by factors such as age, obesity and non-alcoholic fatty liver disease (NAFLD).[43] Obesity is furthermore most prevalent among South African black women, and they are at a greater risk of developing NAFLD.[44] In addition, both overweight and NAFLD positively associate with MCP-1 levels.[45] Although the black women included in our study were young, they presented with higher indices of obesity compared to whites, and may already be at risk of developing Metabolic Syndrome, accompanied by NAFLD and inflammation, and its associated CVD risk.[46]

Hypertension is a strong risk factor in black South Africans, but they traditionally present with lower incidences of atherosclerosis and coronary heart disease.[47] The cardiovascular profiles of black South Africans are, however, deteriorating at a greater pace compared to whites,[47] possibly due to rapid urbanisation and associated dietary and lifestyle changes. This may lead to an increased risk for atherosclerotic disease in urban blacks. The underlying mechanisms relating to early endothelial cell activation seen in the young black women is not easily understood, although this early association of cIMT with MCP-1 may suggest that these women form part of a new generation of black South Africans with elevated atherosclerotic disease risk.

The relationships between inflammation, endothelial dysfunction, early vascular deterioration and arterial stiffness are well established, although the precise sequence of

events is complex.[34] Increased arterial stiffness is an independent predictor of CVD and therefore an important endpoint for determining cardiovascular risk[7] and end-organ damage.[8] In studies including older populations, blacks presented with increased arterial stiffness profiles compared to whites, and therefore greater cardiovascular risk.[27,29] Although there were no difference in the cfPWV between our young normotensive study groups, it may be important to note that in addition to their higher central SBP and MCP-1 levels, cfPWV in the black men and women associated positively with age. This may suggest early arterial aging in the black group and support previous findings of increased arterial stiffness profiles seen in the South African black population at older ages.[14,29]

Evidence for MCP-1 as a risk marker or indicator of vascular function and hypertension come from several studies,[17-19] and its role in the early development of atherosclerosis is well established.[17,34] Although ethnic differences in cardiovascular inflammatory markers do exist,[14] little is known about MCP-1 levels in different ethnic groups, especially young and apparently healthy individuals. The interesting finding of higher MCP-1 levels in blacks compared to whites adds value to our understanding of the role that chemokines and inflammatory markers may play in early vascular deterioration in blacks.

This study must be interpreted within the context of its strengths and potential limitations. Our study population consisted of young, apparently healthy men and women, which limits the possibility of a link between large artery structure and function with MCP-1. The cross-sectional study design may be a further limitation as association does not prove cause and effect.

In conclusion, we found that cIMT associated independently and positively with MCP-1 and VCAM-1 in young apparently healthy black women, but not in men or in white individuals.

Significantly elevated central SBP and MCP-1 concentrations in black compared to white individuals suggest that despite normotensive brachial blood pressures, young Africans may be at increased risk for early vascular deterioration and development of hypertension at younger ages.

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CHAPTER FIVE

Summary, Concluding remarks and Recommendations

5.1 INTRODUCTION

In this chapter the main findings are summarised and compared to the relevant literature. The original hypotheses (as set in Chapter 2) are compared with the results of this study, and finally conclusions are drawn. This is followed by recommendations for future research regarding the link between large artery structure and function and monocyte chemoattractant protein-1 (MCP-1).

5.2 SUMMARY OF THE MAIN FINDINGS AND A COMPARISON WITH THE RELEVANT LITERATURE

Due to the various pathophysiological mechanisms involved in CVD development and progression, the main focus of this study was to determine the association between large artery structure and function (carotid intima media thickness (cIMT) and carotid femoral pulse wave velocity (cfPWV)) and MCP-1 and whether ethnic differences exist in MCP-1 levels. This was done by analysing the cross-sectional data of the first 403 participants of the African-PREDICT study. We compared the groups based on both sex and ethnicity, and observed higher central SBP and MCP-1 levels in the black men and women compared to their white counterparts, even though they showed similar 24-hour blood pressure profiles. In addition, we found a consistent positive association of cIMT with MCP-1 in the black women, but in none of the other groups. Of further interest, we found that cfPWV associated with age in the black men and women, but not in the white groups. Considering these results, the hypotheses are now addressed.

5.2.1 Hypothesis 1: *Ethnic differences in circulating MCP-1 levels do exist, being higher in black than white participants*

We indeed found black men and women to have higher MCP-1 levels than whites. The first hypothesis is therefore accepted.

Black populations present with a higher incidence of hypertension, with disease progression of particular concern in this population.[1,2] In addition, ethnic differences in systemic inflammatory markers have also been demonstrated, with black populations presenting with higher circulating C-reactive protein (CRP).[3-5] CRP, however, is the most extensively researched inflammatory marker in cardiovascular disease (CVD)[6] and there is a lack of studies focussing on novel biomarkers, such as MCP-1 between different ethnicities. Several studies suggest different mechanisms responsible for the higher prevalence of hypertension in black individuals compared to whites.[7-9] By accepting this hypothesis we may potentially indicate that the role of inflammation and specifically the role of the chemokine MCP-1 in early disease development are clearly evident in young blacks.[10-13] The black participants displayed elevated plasma MCP-1 levels despite having similar 24-hour BP measurements as whites. Therefore, MCP-1 may be an early indicator of vascular endothelial dysfunction, even before clinical manifestation of dysfunction is observed in brachial arteries. Even though the 24-hour brachial BP levels were similar between the ethnic groups, the black group presented with higher central SBP levels. Arterial hypertension has a negative impact on endothelial function and is associated with greater plasma levels of MCP-1, VCAM-1 and ICAM-1.[14] Central SBP is associated with cIMT, the progression of coronary atherosclerosis and left ventricular hypertrophy.[15,16] Additionally, studies found that central SBP demonstrated a greater correlation with end-organ damage than brachial blood pressure.[17-

[19] MCP-1 expression is upregulated with increased SBP,[20,21] and the link between central SBP, vascular inflammation and early development cardiovascular disease may therefore be of importance in the black South African population, who is prone to developing hypertension.

5.2.2 Hypothesis 2: *Young black participants have similar cIMT measurements as white participants*

We found that young black and white participants had similar cIMT measurements. The hypothesis is therefore accepted.

The data regarding the differences in cIMT scores between black and white populations have been contradictory, with some studies reporting higher cIMT scores in black populations,[22,23] while others found a marked lower incidence of carotid atherosclerosis in black groups when compared to whites.[24] In the South African context the black population has traditionally presented with lower cIMT scores and a lower incidence of coronary heart disease. This is, however, rapidly changing as rural communities are increasingly disappearing and urbanisation occurs at a very rapid pace.[1,25] The measurement of cIMT is a non-invasive, repeatable procedure, with cIMT considered to be a substitute measurement for sub-clinical atherosclerotic disease.[26,27] Vascular events in young individuals are rare, and cIMT is therefore an attractive end-point to use as a dependable baseline measurement in large longitudinal studies.[28] When considering the literature, it was expected that the cIMT measurements between young healthy black and white participants will be similar, and therefore this hypothesis can be accepted.

5.2.3 Hypothesis 3: *Ethnic-specific differences exist regarding cfPWV, being higher in the black group*

cfPWV as a measurement of arterial stiffness is considered to be the gold standard.[29,30] Several studies have reported higher cfPWV measurements in black participants,[29,31,32] even in young populations.[31,33] Large artery stiffening seems to occur earlier and is more advanced in black populations.[31,34] In light of this data it was expected that the young black participants in this study, despite their healthy normotensive status, will have higher cfPWV measurements than whites. However, they had similar cfPWV measurements, and therefore this hypothesis is rejected. It is also important to mention that the African-PREDICT study's eligibility criteria indicate that hypertensive individuals should be excluded. It is furthermore noteworthy that arterial stiffness (cfPWV) is highly dependent on arterial pressure,[35-37] and that black individuals have higher blood pressures than white individuals in the general population.[2,38] As this study population forms part of the larger African-PREDICT study that aims to track these individuals over the next 10 years, it will be worthwhile to determine whether their stiffness profiles will remain the same, or whether an ethnic divergence in these profiles will be seen.

5.2.4 Hypothesis 4: *Arterial stiffness is positively associated with MCP-1 in both the black and white groups*

We found no association between MCP-1 and cfPWV in either the black or white participants. The hypothesis is therefore rejected. In previous studies, investigating both murine and human aortas, MCP-1 associated positively with arterial stiffness.[39,40] It was therefore expected that in our study population MCP-1 would associate with cfPWV. Increased arterial stiffness[39,41,42] and endothelial dysfunction[20,40,43,44] are closely related to

hypertension and increased age. The eligibility criteria of this study excluded older and hypertensive participants from both ethnic groups, and this may account for the difficulty in establishing a link between MCP-1 and cfPWV.

In both the black men and women there was, however, an independent positive association between cfPWV and age, which was not demonstrated in the white group. This was seen despite the fact that both the black men and women were slightly younger than their white counterparts. Arterial stiffness is strongly associated with age,[37,39,42] and the early association of cfPWV with age in the black population may indicate an already greater cardiovascular vulnerability in this population.

5.2.5 Hypothesis 5: *Carotid wall thickness is positively associated with MCP-1 in both the black and white groups*

We found a positive and consistent association between cIMT and MCP-1 in black women, but in none of the other groups. The fifth hypothesis can therefore be partially accepted.

This positive and consistent association may indicate early inflammatory and atherosclerotic changes in black women. cIMT and MCP-1 have been linked in previous studies,[45,46] with cIMT considered as a surrogate marker for sub-clinical atherosclerosis.[26,27,47] MCP-1 directs the migration of leukocytes from the blood to the vascular wall and promotes the accumulation of lipids in the sub-endothelial intimal layer, as well as the differentiation of monocytes and macrophages to foam cells. Therefore, taking into consideration the central role of MCP-1 in the early development and progression of atherosclerosis,[48-50] the positive and consistent association of MCP-1 with cIMT in the black women may indicate early atherosclerotic changes in these women.

It is peculiar that this link was only and specifically found in black women. The mechanisms responsible for this early association in this group cannot be easily explained due to the cross-sectional nature of this study. To speculate on some potential mechanisms, our characterisation of the participant groups indicated black women to present a high cardiovascular risk profile. They presented with lower HDL-cholesterol levels than white women. HDL-cholesterol has cardio-protective properties, with a proven anti-inflammatory effect.[51-53] The loss of this effect may also be exacerbated by the higher ROS seen in black women. Higher ROS is associated with endothelial dysfunction and increased cardiovascular risk.[54] In addition, black women presented with higher GGT levels, although their self-reported alcohol intake was low. Elevated GGT levels is associated with age, obesity and non-alcoholic fatty liver disease (NAFLD) with increased GGT levels linked to an increased CVD risk.[55,56] Obesity and NAFLD were linked to increased MCP-1 levels.[57,58] It can therefore be speculated that the young black women may already be at risk of developing several of the cardio-metabolic risk factors accompanied by obesity, NAFLD, inflammation and increased CVD risk.[59]

The lack of association between cIMT and MCP-1 in the other groups may again be ascribed to the specific inclusion criteria of the present study. Vascular endothelial dysfunction and atherosclerosis are not expected in young individuals and the specific criteria of this study may eliminate any chance of demonstrating inflammation, endothelial dysfunction and atherosclerotic changes.

5.3 DISCUSSION OF THE MAIN FINDINGS

It is well known that altered endothelial function and inflammation are linked to an increased risk of CVD.[44,50,60-62] Multiple mechanisms are responsible for this increased risk

and they interact to contribute to an augmented response.[62,63] The structure and function of large conduit arteries form a pivotal part of CVD mechanisms, and contribute to normal physiological function by regulating homeostasis through its mechanical properties and normal endothelial function.[64]

Inflammation has been associated with arterial stiffness and the development of atherosclerosis,[40,62] and may be triggered by a variety of stimuli, including acute infection, toxins, and chronic inflammatory conditions.[12,50,65] Ethnic differences in inflammatory markers[5,66,67] and the development and progression of hypertension and CVD are also well established.[1,2,23,29,68] Investigations on inflammation, specifically in CVD, are multiple and ongoing, with MCP-1 being the most established chemokine associated with hypertension and CVD.[12,20]

In the present study the plasma levels of MCP-1 differed markedly between the black and white men and women. Previous studies have demonstrated ethnic differences in CRP[4,5] and pro-inflammatory cytokines,[66] but data concerning MCP-1 levels specifically, are scant. MCP-1 is associated with the early development of atherosclerosis.[12,48] We demonstrated a significant and independent association between carotid (cIMT) and MCP-1 in young black women, as well as a consistent association between cIMT and VCAM-1, which may be indicative of early endothelial activation.[69] In addition, the black women demonstrated a particularly vulnerable cardiovascular profile, including markedly lower levels of HDL-cholesterol and higher plasma levels of ROS. HDL-cholesterol is known to be anti-inflammatory[52] and the loss of this protective effect may already be evident in the black women. Higher indices of obesity were also observed in the black women. Obesity positively link with increased inflammatory markers,[70] and an increased risk of CVD.[71]

Although there was no association between large artery stiffness (cfPWV) and MCP-1 in this study, cfPWV did associate with age in the black men and women. It is well established that age is strongly associated with arterial stiffness.[37] However, this early association between cfPWV and age in the black population may be an indication of an increased cardiovascular vulnerability to early vascular ageing in this population.

Based on the results of this study and the existing literature, we can speculate that the black women are predisposed to the development of cardiovascular disease, especially vascular inflammation, endothelial dysfunction, hypertension and subclinical atherosclerosis. Vascular inflammation, arterial stiffness and atherosclerosis, however, are multifactorial diseases and their development and progression are influenced by mechanisms that differ by age, sex, body composition and ethnicity. Our study suggests that there are ethnic differences in the plasma levels of inflammatory markers, as well as differences in the early activation of the vascular endothelium. Further studies could shed more light on the different mechanisms involved and the long term predictive value of MCP-1 as a marker for individual risk.

5.4 LIMITATIONS, CHANCE AND CONFOUNDING FACTORS

It is important to consider factors that may have influenced the results of this study. These include methodology, statistical analyses and the interpretation of the results.

The cross-sectional design of this study only reflects the current state of cardiovascular health and associations found, and can therefore not imply causality. In addition, a single measurement of MCP-1 may not reflect the long term status, whereas arterial stiffness and carotid wall thickness progress over time.

All participants were from Potchefstroom or the urban areas surrounding Potchefstroom in the North West Province and can as such not be regarded as a representative sample of the entire young South African population. Participation was voluntary and results may be biased towards individuals interested in their health or healthy behaviours. The specific inclusion criteria of this study, namely young, normotensive individuals, may also have limited the chances of associations being demonstrated, as excluded participants were likely to have elevated arterial stiffness, carotid wall thickness and elevated inflammatory profiles. The screening procedures for eligibility further included HIV-testing. Due to HIV-stigmatisation in South Africa,[72] many individuals refrained from participating due to this test.

The study was well designed, followed a strict protocol and was carried out under controlled conditions within the Hypertension Clinic on the Potchefstroom Campus. A wide range of cardiovascular and biochemical measurements were done, using high quality equipment and methods.

The possibility of chance findings should also be taken into consideration. Despite using multivariate regression analyses, there is a possibility that associations found may be due to chance. The likelihood of chance findings is, however, low due to our main findings being consistent in single, partial and multiple regression analyses where other covariates were included.

The socioeconomic profiles of the participants could have had an important confounding effect on the MCP-1 levels observed. Young adult SEP is largely determined by educational attainment and occupation or income.[73] Generally, a higher socioeconomic profile is associated with a more favourable CVD risk profile with socioeconomic status inversely

associating with MCP-1 levels.[74] The major mechanism suggested through which education may influence inflammatory markers is through an increase in healthy behaviours with an increase in SEP. A low SEP and educational attainment is associated with a higher prevalence of smoking, alcohol use, obesity and lower levels of leisure-time physical activity.[73,74] This research attempted to include these potential confounders in multiple regression models.

In addition, there are human genetic factors that may contribute to susceptibility to or protection from CVD. MCP-1 polymorphisms may influence susceptibility to CVD.[75] The MCP-1-2518G variant appears to be a genetic risk factor for coronary artery disease. This genotype of MCP-1 is associated with elevated Lp(a) levels.[76] Inflammatory traits seem to be heritable, with estimated CRP concentration heritability being at least 20%.[77] The genetic contribution to systemic concentrations of inflammatory markers, however, remain largely unresolved.[77] Since we did not include genetic analyses in the present study, it remains to be determined whether our findings may have a genetic foundation.

5.5 CONCLUSION

The association of large artery structure and function with MCP-1 was investigated in a young bi-ethnic South African population. The main findings were that MCP-1 levels were higher in the black population and that carotid wall thickness associated with MCP-1 levels in black women, but in none of the other groups. Although cfPWV was similar between the black and the white groups, it associated positively with age in the black population only, which may support the notion of early vascular ageing in this group. These results are particularly relevant to South Africa as there is an increasing disease burden attributable to hypertension and cardiovascular disease in the black population. This predisposition and

vulnerability are believed to increase over time, unless significant interventions are undertaken. The present study provides new information in evaluating the usefulness of MCP-1 as biomarker for early changes in large artery structure and function in healthy individuals. The novel findings regarding the differences in plasma levels of MCP-1 between black and white individuals and how it may affect endothelial function, especially in black women, warrants further study. Causality should be investigated in prospective and experimental studies.

5.6 RECOMMENDATIONS

- The African-PREDICT study is a longitudinal study, aiming to track a total of 1200 black and white individuals over 10 years. It is recommended that the changes in large artery structure and function be evaluated during follow-up to assess the development of arterial stiffness and subclinical atherosclerosis and the prognostic value of MCP-1.
- Investigation of the microvasculature, especially in the kidney, and the association of MCP-1 with the renin angiotensin aldosterone system (RAAS) and salt-sensitive hypertension could provide more insight into the link between MCP-1, hypertension and vascular dysfunction.
- Future studies could focus on the long-term predictive value of MCP-1 in large populations, ranging from young to the elderly, providing the opportunity to determine how behaviours and the environment (such as socioeconomic status) affect MCP-1 concentrations.

- Genetic polymorphisms of MCP-1 and their association with arterial stiffness and carotid wall thickness should be investigated. Investigation into different MCP-1 polymorphisms may explain ethnic differences in MCP-1 levels.
- Country-wide random sampling may provide more insight into the impact of urbanisation on MCP-1 levels and its association with large artery structure and function.

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Appendix A

Monocyte chemoattractant protein-1 and large artery structure and function in young individuals: The African-PREDICT study

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INSTRUCTIONS FOR AUTHORS

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Original articles should report original research not previously published or being considered for publication elsewhere. Manuscripts should be written in the English language, using either American or British spelling consistently throughout. An original research paper has a main body of text, not exceeding 4000 words, and abstract (not exceeding 250 words) and no more than 50 references. The abstract should be structured and divided into sections to include Background and Aims, Methods, Results and Conclusions. The results section must contain quantitative data and statistical significance. Longer articles may be accepted by the discretion of the editor. An original research paper should comprise of the following sections:

Title page

Title should be concise and informative and abbreviations and formulae should be avoided. Given name(s) and family name(s) of each author must be provided as well as their affiliation addresses, below the names. All affiliations should be indicated with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Full postal address for each affiliation, including the country name and, if available, the email address of each author. The corresponding author should be clearly indicated.

Keywords

A list of keywords (3-7 items) should be included. Authors are encouraged to choose their own keywords, but in doubt which items to select, Medical Subject Headings (issued with the January Index Medicus, 1969) may be used as a guideline.

Main text

The main text should be divided into sections headed by a caption (e.g. Abstract, Introduction, Materials and Methods, Results and Discussion. Si units must be used throughout. A short paragraph of conclusions should be included at the end of the text, indicating the relevance of the study with regards to aspects of atherosclerosis. A statement of the source of funding, conflicts of interest and disclosures of financial support is highly recommended.

References

References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is encouraged.

Tables, figures and illustrations

No more than 5 figures and tables in total. Tables with titles and legends should be on separate pages, with double spacing. The number of tables and/or figures must be listed on the title page. Additional figures and tables may be added as supplementary appendixes. For examination purposes all tables and figures will be included within the text.