

# **Exploring the link between left ventricular remodelling and the leukocyte profile of a young black and white population: The African-PREDICT study**

**MI Kirstein**

 **orcid.org: 0000-0002-9050-5285**

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Supervisor:	Prof R Kruger
Co-supervisor:	Dr S Botha
Co-supervisor:	Prof HW Huisman

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Student Number: 23394528

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## SUMMARY

**Background and motivation:** Early vascular deterioration is associated with additional strain on the heart which is linked to cardiac remodelling and increased left ventricular mass (LVM). Increased LVM contributes to cardiovascular morbidity and mortality in a general population. Unhealthy lifestyle risk factors such as smoking, alcohol abuse and physical inactivity, all contribute to low-grade inflammation and in turn lead to increased levels of leukocytes. In result to the low-grade inflammation and a higher leukocyte count, early vascular changes may occur. Leukocytes are involved in the process of cardiac remodelling and for instance associated with left ventricular mass index (LVMI) in elderly populations with known hypertensive heart disease. Limited literature is available in young South African populations regarding the links of leukocytes and LVMI.

**Aim:** The aim of this study was to investigate the link between LVMI and leukocyte count in a young South African population, free from overt cardiovascular diseases.

**Methodology:** Cross-sectional data of 800 participants from the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) was used. Healthy black and white men and women between the ages of 20-30 years, with normal brachial blood pressures were included. Participants with left and right bundle branch blocks were identified with electrocardiogram data and excluded from this study. All participants completed a general health questionnaire from which their socio-economic status scores were determined. Anthropometric measurements were performed to determine body height, body weight and waist circumference, as well as body mass index and body surface area. Physical activity was measured and active energy expenditure (AEE) was calculated and normalised for weight. Twenty-four-hour ambulatory blood pressure measurements were performed. LVM was measured by echocardiography and calculated with a standard formula, normalised for body surface area and defined as LVMI. EDTA whole blood samples were analysed for leukocyte counts and neutrophil to lymphocyte ratio (NLR) was calculated additionally. Statistical analyses were performed with the IBM® SPSS® Statistics, Version 24.

**Results:** Socio-economic score, body surface area, systolic blood pressure (SBP), mean arterial pressure, monocyte count and NLR were higher in the white group (all  $p \leq 0.017$ ) compared to the black group. The white group had lower interleukin-6 and AEE (both  $p < 0.001$ ), as well as higher anti-inflammatory medication use ( $p = 0.036$ ) than their black counterparts. In single regression analyses, a negative correlation was found between LVMI and leukocyte count ( $r = -0.16$ ;  $p = 0.003$ ), monocytes ( $r = -0.13$ ;  $p = 0.015$ ) and NLR ( $r = -0.13$   $p = 0.014$ ) in the white population only. These results were confirmed with partial correlation analyses after adjusting for age and sex. In multiple regression analysis, an inverse association of LVMI with leukocyte count (Model 1: Adjusted  $R^2 = 0.201$ ;  $\beta = -0.08$ ;  $p = 0.017$ ) and monocytes (Model 2: Adjusted  $R^2 = 0.205$ ;  $\beta = -0.11$ ;  $p = 0.002$ ) was found in the total group. After stratification by sex and ethnicity, the inverse association between LVMI and leukocyte count (Model 1: Adjusted  $R^2 = 0.094$ ;  $\beta = -0.30$ ;  $p = 0.001$ ) as well as NLR (Model 3: Adjusted  $R^2 = 0.053$ ;  $\beta = -0.19$ ;  $p = 0.025$ ) existed in white men only, whereas an inverse association between LVMI and monocytes was seen in white men (Model 2: Adjusted  $R^2 = 0.075$ ;  $\beta = -0.25$ ;  $p = 0.004$ ) and white women (Model 2: Adjusted  $R^2 = 0.060$ ;  $\beta = -0.21$ ;  $p = 0.005$ ). After additionally adjusting for interleukin-6, the inverse association between LVMI with leukocyte count and monocytes remained in all cases, but disappeared between LVMI and NLR.

**General conclusion:** In this study, LVMI and leukocytes, specifically monocytes and NLR, were inversely associated in the white population. The inverse association between LVMI and monocytes may indicate potential premature onset of monocyte depletion and reduced capacity of cardiac repair in this young white South African population.

**Keywords:** Left ventricular mass index, leukocytes, race, neutrophil to lymphocyte ratio, monocytes, inflammation

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Figure 2.1: This diagram gives the exact numbers of participants that are included and excluded in the study from recruitment phase to data analyses.

## **ABBREVIATIONS**

<b>AEE</b>	Active energy expenditure
<b>African-PREDICT</b>	African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension
<b>CKD-EPI</b>	Chronic Kidney Disease Epidemiology Collaboration
<b>CVD</b>	Cardiovascular disease
<b>DBP</b>	Diastolic blood pressure
<b>eGFR</b>	Estimated glomerular filtration rate
<b>ExAMIN Youth</b>	Exercise, Arterial Modulation and Nutrition in Youth South Africa
<b>HART</b>	Hypertension in Africa Research Team
<b>IVSd</b>	Interventricular septal thickness at end-diastole
<b>LVEDD</b>	Left ventricular end diastolic dimension
<b>LVM</b>	Left ventricular mass
<b>LVMi</b>	Left ventricular mass index
<b>MHSc</b>	Masters in Health Science
<b>NLR</b>	Neutrophil to lymphocyte ratio
<b>SBP</b>	Systolic blood pressure
<b>SES</b>	Socio-economic score
<b>TC:HDL</b>	Total cholesterol to high-density lipoprotein cholesterol

## PREFACE

This dissertation, submitted for the fulfilment of the requirements for the degree *Master of Health Sciences in Cardiovascular Physiology*, contains four chapters.

Chapter 1 contains the background, motivation, literature review concerning left ventricular remodelling and different leukocytes, as well as the aim, objectives and hypotheses of this study.

Chapter 2 is the methodology chapter containing all methods and procedures that were followed to acquire the data used in this study.

Chapter 3 is the research article written according to the author instructions of the *International Journal of Cardiology*. All instructions were followed, except in Table 3 and 4, where single spacing was used for optimal formatting purposes.

Chapter 4 summarises the main findings of the study and includes a reflection of the hypotheses. Acknowledgements, recommendations for future studies, limitations and final conclusions are also included in this chapter.

All references at the end of each chapter are indicated according to the style of the designated journal.

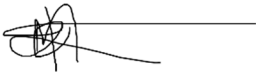
## DECLARATION OF AUTHORS

**Miss MI Kirstein:** Responsible for literature review, writing of the manuscript, statistical analyses, interpretation of results and writing of all sections of this dissertation.

**Prof R Kruger:** Intellectual and technical input, data collection and evaluation of statistical analyses. Supervised writing of the manuscript and initial design and planning of the dissertation.

**Dr S Botha and Prof HW Huisman:** Supervised analyses of data, writing of the manuscript and planning of the dissertation.

The following statement from the co-authors confirms their individual roles in this study and gives their permission that the relevant research article may form part of this dissertation:



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**MI Kirstein**



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**Prof R Kruger**



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**Dr S Botha**



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**Prof HW Huisman**

# Chapter 1

*Background and literature review*

## 1. Background

Cardiovascular disease (CVD) is categorised under non-communicable diseases and contributes largely to morbidity and mortality worldwide (1, 2). Non-communicable diseases are currently a leading cause of death in South Africa (3). Unhealthy lifestyle factors such as alcohol use, smoking and lack of exercise (4), lead to early vascular changes and play a major role in the development and progression of CVD.

Low-grade inflammation has been associated with early vessel diseases (5) which could lead to atherosclerotic disease progression and in turn to cardiovascular events (6). Long-term inflammation is closely related to vascular complications such as arterial stiffness, atherosclerosis, vascular calcification, peripheral artery disease and aortic sclerosis, which are all known to exert an excess overload on cardiac function (7-10). These vascular complications cause cardiac wall stress and may lead to cardiovascular events such as myocardial infarction (10-15). Leukocytes may be increased as a result of vascular diseases and myocardial infarction (16-21). Leukocytes are circulating cells that protect the body against infections and foreign invaders by secreting cytokines such as tumor necrosis factor alpha and interleukin-6 (22). Neutrophils and monocytes have phagocytic functions and destroy pathogens (23), whereas lymphocytes remove antigens (24). Monocytes infiltrate into the injured heart and aid in the recovery process through phagocytosis and tissue formation (25), differentiating into macrophages and releasing anti-inflammatory markers such as interleukin-4 and interleukin-10 for cardiac remodelling and repair (26).

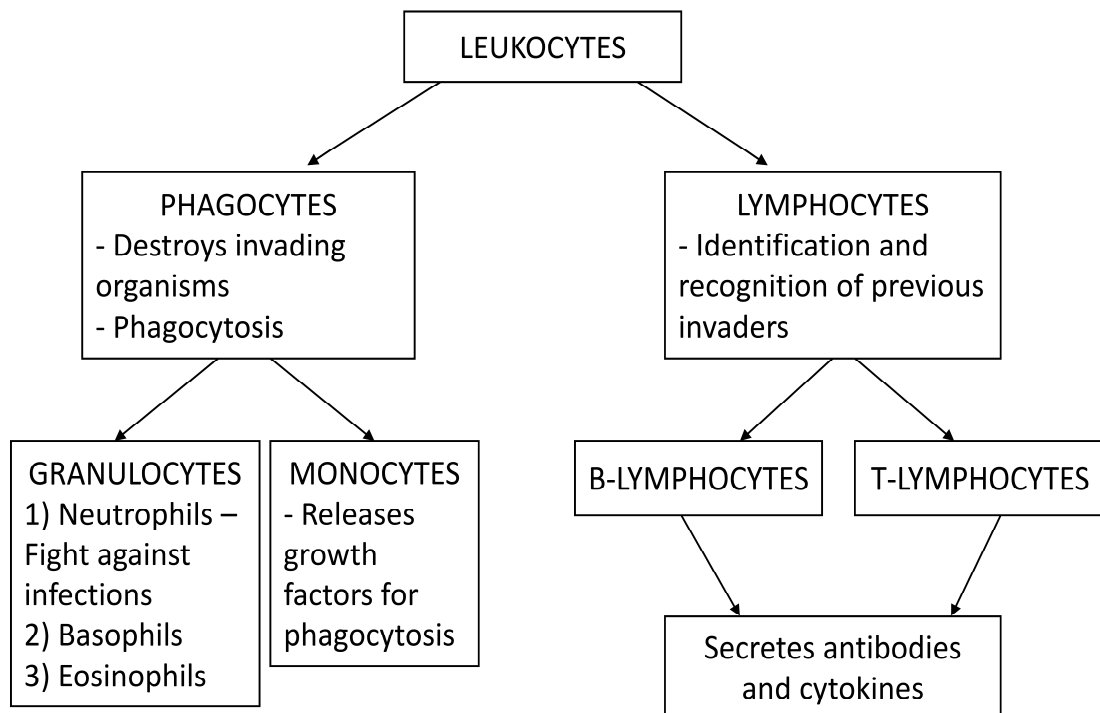
Cardiac remodelling is associated with increased left ventricular mass (LVM) (27), which is a risk factor for CVD in the general population (28), but more so in essential hypertensive patients (29). In addition, left ventricular mass index (LVMI) is also evident in young masked hypertensive individuals (30). Adverse cardiovascular changes begin during childhood due to the contribution of family history of CVD as well as environmental and/or lifestyle factors (31, 32). These factors increase the risk for susceptibility to earlier changes in cardiovascular structure and function in young individuals (33). An extensive description of CVD development and the role of leukocytes in cardiac remodelling were well documented



in elderly and diseased populations, but limited literature is available about the relationship in young individuals.

## **2. Inflammation**

Leukocytes, also called white blood cells (WBC) (34), function as the body's defense system. Leukocytes are formed in the bone marrow or lymph tissue (35-38) and are involved in counteracting foreign substances (39). Leukocyte count have been found to be higher in white compared to black individuals (40, 41). There are two basic categories of leukocytes, namely phagocytes and lymphocytes (Figure 1) (34). Phagocytes play a role in immunity by destroying invading organisms and removing apoptotic cells through a process called phagocytosis (42). Granulocytes, also referred to as polymorphonuclear cells, are a specific type of phagocyte and are divided into three subclasses, namely neutrophils, basophils and eosinophils (34). Monocytes, another type of phagocyte, are involved in the defense against bacteria and other invading organisms (34). Lymphocytes, on the other hand, identify and recognize previous invaders (24) and are part of the adaptive immunity response which develops throughout the course of life (42, 43). There are two types of lymphocytes, B-lymphocytes and T-lymphocytes, and both types can secrete a variety of antibodies and cytokines for the regulation of immune responses (34, 44).



**Figure 1:** This figure shows the classification of leukocytes and their function in the human immune system.

A young population with unhealthy lifestyle behaviours, such as smoking, alcohol abuse, physical inactivity and poor diet are likely to be more susceptible to low-grade inflammation (45-49). Inflammation is an important process in physiological and pathological states. This process consists of a series of responses to injuries or abnormal stimulation; caused by physical, chemical or biologic agents (50-54).

Macrophages, mast cells and dendritic cells are tissue sentinel cells that detects damaged cell signals and release local pro-inflammatory mediators (55). These mediators include cell adhesion molecules, such as selectins and integrins (56), which are present on the interacting cell surface and initiate the recruitment process (51, 56). Leukocytes and plasma mediators migrate through the vascular wall into the interstitial tissue and are finally recruited to the injured area where monocytes then differentiate into macrophages (37, 53, 57-60). The mediators at the inflammatory sites activate metabolic processes of phagocytic cells by binding to specific phagocytic receptors on the surface of the cell (52). The process of phagocytosis of particulate material may also initiate this process (52). Macrophages

promote foam cell formation, which is a hallmark of atherosclerotic lesions (60, 61). Macrophages are found in atherosclerotic lesions, along with sub-endothelial lipid deposition (61-64). As these macrophages accumulate into a large number of lipoproteins, they differentiate into foam cells (61-64).

Arteriosclerosis, the process during which the compliance and elasticity of blood vessels decrease (65), develops through the invasion of leukocytes (66) as a result of low-grade inflammation, hypertension with underlying extracellular matrix remodelling and lifestyle risk factors (47, 57, 67, 68). Atherosclerosis, on the other hand has been associated with interleukin-6 as this pro-inflammatory cytokine decreases lipoprotein lipase, which increases macrophage lipid uptake (69). The macrophage foam cell and smooth muscle cell then express interleukin-6 which has also been associated with the pathogenesis of coronary heart disease (69). Atherosclerosis is also linked with arterial stiffness (70) which may lead to additional strain on the heart, negatively affecting cardiac systolic and diastolic function (71). As the heart compensates for the additional strain, cardiac remodelling occurs, leading to increased LVM (72).

### **3. Left ventricular mass**

LVM is estimated from intracardiac dimensions derived from M-mode and 2-dimensional echocardiography (73). Echocardiography provides real-time imaging and direct visualization of the myocardium (28). The images of the left ventricle are obtained and LVM is then calculated (73). Formulas to calculate LVM were developed based on the regression equations of the calculated mass to autopsy findings for M-Mode and 2-dimensional echocardiography (74, 75). However, it was shown that 3-dimensional echocardiography is more accurate than 2-dimensional or M-mode echocardiography, as it removes the assumption of shape and wall thickness (76, 77). In addition, 3-dimensional echocardiography avoids using formulas and can thus be used for the direct measurement of LVM, although these techniques are much more difficult and not yet fully validated (78). Under pathological conditions, LVM can be used as a measure of left ventricular hypertrophy

and an independent predictor of adverse cardiovascular events and premature death (29, 79, 80). Left ventricular mass index (LVMI) is documented as the standard to estimate LVM as it avoids artefactual findings of the relationship between LVM and body height or body surface area (81). In a study on healthy individuals between the ages of 16-68 years, LVMI was on average  $105 \pm 14$  g/m for men and  $78 \pm 8$  g/m for women (82).

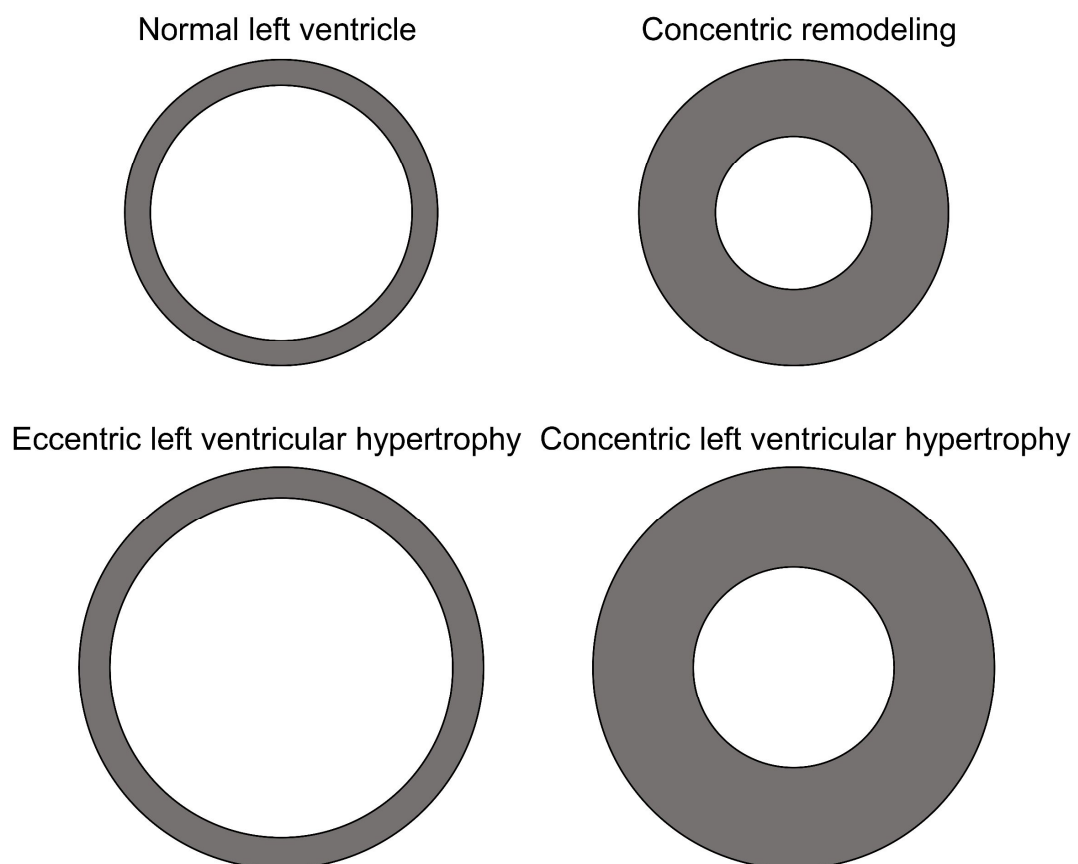
### *3.1 Left ventricular remodelling*

Left ventricular remodelling refers to changes in left ventricular size, structure, shape and function (83) for instance after myocardial injury or cardiac wall stress (84, 85). Cardiac remodelling, which includes increased LVM, occurs after pressure- or volume overload of the heart muscle (27). The endocardium, which is the inner layer of the heart tissue (86), covers both atria and ventricles of the heart and helps with blood flow through these chambers (87). The endothelial layer of the endocardium is attached to the endothelium of the larger blood vessels (88). The endothelium is considered as a crucial component in the structure and function of the cardiovascular system (89). The endothelium plays a role in homeostasis of the cardiovascular system by regulating vascular permeability, altering the diameter of the blood vessels and maintaining blood fluidity (90). Dysfunction of the endothelium is a potential risk factor for increased LVM (91). During the remodelling process of an unhealthy heart “death” of myocytes occur (92). Necrotized myocytes are then replaced with fibroblasts (92, 93). Collagen formation produced from fibroblasts then increases in response to the remodelling process throughout the heart (92, 93). This process leads to fibrosis and scar tissue formation, which could cause the apoptosis of even healthy myocytes (94, 95).

### *3.2 Types of left ventricular remodelling*

Left ventricular remodelling can be characterized as either physiological, a reversible condition, or pathological, in which case remodelling of the left ventricle is detrimental to heart function (96). Several left ventricular geometric adaptations occur during remodelling (Figure 2), such as eccentric left ventricular hypertrophy, concentric left ventricular hypertrophy and concentric remodelling (97). Eccentric left ventricular hypertrophy is due to

volume overload and presents with increased LVM and normal relative wall thickness (97-99). Concentric remodelling is caused due to pressure overload where a normal LVM is sustained, but an increased relative wall thickness occurs (80). Concentric left ventricular hypertrophy presents with a normal left ventricular cavity size, however, an increase in both LVM and relative wall thickness is evident (80). LVM increases mainly due to pressure overload (100), but also due to volume overload which can be observed by left ventricular dilation (101).



**Figure 2:** Normal left ventricle (normal LVM; normal relative wall thickness), concentric remodelling (normal LVM; increased relative wall thickness;), eccentric left ventricular hypertrophy (increased LVM; normal relative wall thickness), concentric left ventricular hypertrophy (increased LVM; increased relative wall thickness)

### *3.2.1 Physiological and pathological left ventricular remodelling*

Physiological left ventricular remodelling is labelled as 'normal cardiac remodelling' and is often called 'athletes heart' (102). Normal cardiac remodelling refers to normal adaptation of cardiac structure and function to compensate for increased cardiac workload (96). Physiological left ventricular hypertrophy occurs due to cardiac adaptations, usually caused by endurance or strenuous exercise (103). With physical activity, heart rate and blood pressure would increase and lead to cardiac changes, such as left ventricular hypertrophy (98). The type of structural changes in the heart however depends on the type of exercise involved (27).

Pathological remodelling includes a prolonged inflammatory response, leading to detrimental cardiac remodelling conditions (104). Pathological left ventricular remodelling is linked to cardiac dysfunction, interstitial fibrosis and increased risk for cardiovascular mortality (96). Detrimental cardiac remodelling can be caused by hypertension and several vascular conditions such as endothelial dysfunction, arterial stiffness and atherosclerosis which causes left ventricular afterload (98, 105). Systemic inflammation and oxidative stress are linked to arterial stiffness (106-108), atherosclerosis (57, 109) and endothelial dysfunction (110, 111) which may relate to cardiac afterload (112). Hypertension exerts additive strain on the left ventricle leading to dilation and hypertrophy (113). Hypertension-related left ventricular hypertrophy is a risk factor for a variety of cardiovascular events such as heart failure, myocardial infarction, sudden cardiac death, and stroke (113-115). An increase in the venous return leads to high filling pressure of the heart, contributing to the development of left ventricular hypertrophy (116). With the heart compensating for hemodynamic overload, LVM would increase (117). This elevation of LVM is because of existing myocytes undergoing hypertrophy (118). Increased LVM is associated with elevated atherosclerotic lesions in the vasculature of the heart and is linked with increased arrhythmogenesis (119). Atherosclerosis in individuals with left ventricular hypertrophy can also cause impaired coronary blood supply, as some factors linked to myocardial

hypertrophy can promote the formation of fatty deposits in the arteries (120). In addition, coronary blood flow decreases when LVM increases in hypertensive patients as a result of reduced coronary vasodilator capacity (121).

#### **4. Leukocytes and cardiac remodelling**

##### *4.1 Neutrophils*

In the presence of bacterial infections, neutrophils comprise of 65%-70% of the leukocyte count in the body and is essential for fighting against these infections (34). Neutrophils, developed in bone marrow, migrate to the ischemic endothelium in the heart to destroy particles such as invading pathogens and cell debris (38). Neutrophils were seen to play a central role in repairing cardiac tissue after a heart attack (104). These neutrophils are required early in the repair process, followed by monocytes and lymphocytes (104). Elevated levels of neutrophils however have a destructive effect on the heart after acute myocardial infarction (104). Some studies showed a strong positive correlation between a high neutrophil count and the risk for atherosclerotic ischemic events (122-126). Another study done on young healthy females found that activated neutrophils release substances such as cytotoxic material, protease and hydrolytic enzymes, which contribute to vascular and ischemic injury (127).

##### *4.2 Monocytes*

Monocytes account for 10% of the total peripheral leukocytes in adaptive and innate immune responses (128). Monocytes play an important role in the recovery process by releasing growth factors for the phagocytosis of dead cardiomyocytes and granulation tissue formation (25). Monocytes are released from bone marrow and become monocyte-derived macrophages when penetrating the endothelium of the heart (37). These macrophages are activated during inflammatory processes through tumor necrosis factor alpha, interferon  $\gamma$ , granulocyte-monocyte colony stimulating factor, extracellular matrix proteins and other chemical mediators (129). Type M2 macrophages secrete anti-inflammatory markers, including interleukin-4 and interleukin-10, and clear apoptotic cells

(26). These Type M2 macrophages complete the healing and cardiac remodelling processes after myocardial infarction by releasing proteases and promoting proliferation (130). On the other hand, Type M1 macrophages have pro-inflammatory functions as these cells release interleukin-6 and tumor necrosis factor alpha that provoke atherosclerosis (131, 132). Studies have shown that there is a relationship between an increase in C-reactive protein and interleukin-6 and the risk of coronary heart disease (132, 133). Tumor necrosis factor alpha lowers endothelial nitric oxide levels which causes endothelial dysfunction, chronic vasoconstriction and high blood pressure (134, 135). Tumor necrosis factor alpha delays the apoptosis process of cardiac myocytes after ischemia (136) and is also associated with myocardial infarction (137).

#### *4.3 Lymphocytes*

Lymphocytes, found in the lymphatic system, bind to specific antigens in response to the immune system when they mature and play a role in antibody production (138, 139). The greater part of the lymphocyte development is in the central lymphoid organs, namely the bone marrow and the thymus gland (139). There are two types of lymphocytes, depending on where they mature; B-lymphocytes mature in the bone marrow and T-lymphocytes in the thymus gland (139). Lymphocytes are also critical in the regulation of cardiac repair as they are recruited to the infarcted heart along with monocytes for the repair process (36). B-lymphocytes were shown to play a role in recruiting pro-inflammatory monocytes to the injured heart (36). B-lymphocytes function as scavenger cells for antigens and secrete antibodies to weaken antigens for phagocytosis (140). T-lymphocytes destroy the invaders which B-lymphocytes have identified (24). T-lymphocytes are involved in cardiac remodelling after chronic pressure-overload conditions (141, 142). These T-lymphocytes have positive effects on different cell types found in the cardiac repair and remodelling processes (143, 144). T-lymphocytes were however also shown to accelerate atherosclerosis (145) which is associated with heart failure (146). Less is however known regarding their role in young, healthy individuals in relation to cardiac structure.



#### *4.4 Neutrophil to lymphocyte ratio*

The neutrophil to lymphocyte ratio (NLR) is an inflammatory marker and an important measure of systemic inflammation (147). It has been shown that NLR could be a possible marker of immune responses when a stress stimulus is present (148) and is also closely related to immune suppression (148). NLR predicts cardiovascular events, including acute ischemic stroke (149), and is associated with poor outcome in individuals with coronary heart disease or chronic left ventricular heart failure (150-152). The normal range for NLR is between 0.78 and 3.53 (153) where a higher NLR has been associated with frequent congestive heart failure and long-term mortality (154). A study conducted in 2016 found that NLR is independently associated with left ventricular remodelling following ST-elevation myocardial infarction (155).

The link of left ventricular remodelling and leukocytes was previously found in diseased individuals with myocardial infarction (156, 157), whereby leukocytes contributed to cardiac structural changes (158). Little information on left ventricular remodelling and its link with the leukocyte profile in young, apparently healthy individuals is evident.

### **5. Left ventricular mass and other cardiovascular risk factors**

#### *5.1 Gender*

In non-hypertensive adults without CVD, after correcting for body size, LVM is higher in men compared to women (159). By indexing LVM for height<sup>2.7</sup> (where 2.7 is an allometric exponent), it allows us to use a cut-point of 51g/m<sup>2.7</sup> for both men and women (81) which lessens the influence of gender in left ventricular hypertrophy inference in African-Americans (160).

Women, even after correction for body size, have increased parietal hypertrophic responses to pressure overload (161, 162). Women with aortic stiffness have greater concentric remodelling and higher left ventricular hypertrophy than men (163). Studies done on spontaneously hypertensive rats showed that hypertrophy development is initially less in female than in male rats, but that concentric remodelling is more extensive and greater in

female rats (164, 165). In male rats, heart failure begins earlier in response to pressure overload, showing that concentric remodelling and an increase in LVM is similar in male and female rats in the beginning. However, left ventricular cavity dilation, decreased concentric remodelling and increased wall stress become evident in male rats three months later (166, 167). This proves the presence of early pathologic remodelling and the process of transitioning to heart failure in male rats (166, 167).

## *5.2 Race*

There is a high prevalence of left ventricular hypertrophy in African-Americans (168, 169) which was more evident when indexing for height than indexing for body surface area (170). African-Americans with hypertension have a higher relative wall thickness than hypertensive white individuals (171). This results in increased incidence of concentric remodelling in black individuals with the same LVM estimates (172, 173). A study done on young adults found that African-American and white men had higher LVM than their female counterparts, but the LVM of African-American men was higher than that of white men (174). African-Americans were also shown to have a higher body weight than their white counterparts (175), and was especially evident in females (176). In addition, white men were shown to be taller than white women and African-American men and women (177). Race disparities do however arise in this case as studies have proved that the taller and the more a person weighs, the higher the left ventricular mass would be (81).

## *5.3 Age*

During infancy and adolescence, cardiac size increases parallel to an increase in body size, where gender differences become noticeable (178). A study showed that the age associated changes in LVM is effected by body size and blood pressure (179). Another study done on individuals aged 18 to 39, found that nearly 30% of younger individuals with hypertension had left ventricular hypertrophy, as a result of the high prevalence of obesity (180). Early abnormalities in left ventricular structure and function may have important implications for the explanation of myocardial dysfunction that is related to elevated

cardiovascular morbidity and mortality (181).

#### **5.4 Obesity**

Body size is linked to LVM and other left ventricular dimensions (182). This related increase in LVM through obesity is said to be more than just a physiologic adaption (182). Additionally it was shown that increased body mass index during childhood has a negative impact on left ventricular hypertrophy later in adult life (183). A study done on obese and non-obese women found an association between young, otherwise-healthy obese women and concentric remodelling, as well as lower systolic and diastolic function (181). Obesity is independently associated with left ventricular hypertrophy (184) and predicts cardiovascular morbidity and mortality (185, 186). Another study however (187) established that uncomplicated obesity is not a risk factor for left ventricular hypertrophy when indexing for either body surface area or height<sup>2.7</sup>. Thus, adjusting for height<sup>2.7</sup> is more appropriate as some individuals may be falsely classified as obese if height is not taken into consideration (182). However, according to the guidelines, indexing for body surface area is the most common practice.

### **6. Motivation**

Increased LVM is known to contribute to cardiovascular morbidity and mortality worldwide (28). Early vascular changes can be ascribed to unhealthy lifestyle factors in young individuals (4) contributing to low-grade inflammation and the over-expression of leukocytes (35, 45-49). Low-grade inflammation and higher leukocyte counts may result in adverse strain on the cardiac myocytes, leading to increased LVM and increased left ventricular remodelling (72).

## **7. Aim**

The central aim of this study was to explore the relationship between LVMI and leukocytes in a young black and white South African population.

## **8. Objectives**

In the study population of young black and white men and women (ages 20–30 years), the objectives were to:

- i. compare LVMI and the leukocyte profile;
- ii. determine the associations of LVMI with the leukocyte profile; and
- iii. determine whether the association between LVMI and the leukocyte profile are dependent or independent of interleukin-6.

## **9. Hypotheses**

In a study population of young black and white men and women, the following hypotheses were proposed:

From the first objective, it was hypothesized that:

- LVMI would be higher in the white compared to the black group; and
- the leukocyte profile (neutrophils, monocytes and neutrophil to lymphocyte ratio (NLR)) would be higher in white compared to black participants.

From the second objective, it was hypothesized that:

- LVMI would associate adversely with leukocytes in both black and white groups.

From the third objective, it was hypothesized that:

- the association of LVMI with the leukocyte profile of both black and white groups, would be dependent of interleukin-6.

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# Chapter 2

*Methodology*

## **1. Study design and population sample**

This Master of Health Sciences (MHSc) study is embedded in the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension development (African-PREDICT). The African-PREDICT study is a longitudinal study, conducted in the Potchefstroom area, North West province, South Africa, where 1200 black and white participants between the ages of 20-30 years are followed for 20 years. The purpose of the African-PREDICT study is to detect early markers or predictors of cardiovascular disease (CVD) in young, apparently healthy South Africans.

### *1.1 Inclusion criteria for the African-PREDICT study*

As the African-PREDICT study is a longitudinal study, participants had to be a permanent resident in Potchefstroom or surrounding areas or had to return regularly to this area to be included. Both men and women (equally distributed) were included in this study to determine whether sex differences exist. Only normotensive or pre-hypertensive individuals were included, as this study aimed to detect early phases of hypertension and CVD development. Participants with normal brachial blood pressure, were included in the second phase of the study, whereby 24-hour ambulatory blood pressure monitoring were performed according to the current hypertension guidelines of the European Society of Hypertension and European Society of Cardiology (1). Participants of self-reported black and white races were included, providing the opportunity to investigate racial differences, as previous South African studies indicated that black participants have a higher risk for developing hypertension at an earlier age compared to white participants (2, 3). This MHSc study followed the same inclusion criteria as set out in the protocol of the African-PREDICT study.

### *1.2 Exclusion criteria for the African-PREDICT study*

Participants were excluded if they had any known risk factors that could influence cardiovascular health. These risk factors included either self-reported and/or confirmed

screening results of Type 1 or Type 2 Diabetes Mellitus, elevated glucose of  $>5.6$  mmol/L, confirmed glycated hemoglobin (HbA1c) of  $\geq 6.5\%$ , human immunodeficiency virus infected individuals, participants with a fever (ear temperature of  $>37.5^{\circ}\text{C}$  on the participation day), self-reported previous diagnosis of liver disease, cancer, tuberculosis or renal disease and participants with microalbuminuria (albumin $>30$  mg/ml) or proteinuria (protein $>300$  mg), as measured from morning spot urine samples. Participants with self-reported previous history of stroke, angina pectoris or myocardial infarction, as well as individuals who had undergone recent surgery or trauma (within the past three months) were also excluded. Those on medication for chronic diseases, i.e. antihypertensive, anti-diabetic, or antiretroviral, were excluded. Participants who were pregnant or breastfeeding at the time of participation were excluded as hormone levels change, weight increases, glomerular filtration rate increases and the risk of developing pre-eclampsia or pregnancy hypertension is higher, which all have an influence on normal physiology (4-7). English proficiency was preferred for the completion of questionnaires; however participants were assisted in their home language by field workers.

Additional exclusion criteria for this MHSc study was the exclusion of individuals with left and right bundle branch blocks ( $n=53$ ) since this could influence the association of left ventricular mass index (LVMI) and leukocytes. With left bundle branch blocks, the cardiac rhythm abnormalities are known to influence the accuracy of left ventricle mass (LVM) determination from internal cardiac dimensions (8).

## **2. Organizational procedures**

The African-PREDICT study (NWU-00001-12-A1), as well as this MHSc study (NWU-00068-17-A1), conformed with the ethical aspects, as outlined in the revised Declaration of Helsinki (2008) and was approved by the North-West Province Department of Health in South Africa and the Health Research Ethics Committee of the North-West University, Potchefstroom. Participants were recruited continuously for the African-PREDICT study until the full baseline sample of 1200 participants were included.

Participants were recruited using various approaches in Potchefstroom and surrounding areas in South Africa. Recruitment procedures included active contact via field workers and actively approaching possible candidates at their workplace or on North-West University campus, after which they were contacted to schedule appointments for participation if they wished to participate. Employers were contacted and appointments were made to gain access to their employees for recruitment into the study. Advertisements, approved by the Health Research Ethics committee, were placed on the radio, noticeboards and in local newspapers. Transport was offered to and from the research unit for participants who wished to participate in the study. Refreshments were offered to each participant after blood samples have been obtained. Each participant also received a R50 Checkers voucher at the end of the day as a token of appreciation.

The participants first went through a screening phase to determine their eligibility for participating in the larger African-PREDICT study, based on the inclusion and exclusion criteria. Screening took place in the Hypertension Research and Training Clinic of the Potchefstroom campus of the North-West University and at other locations (e.g. at the participants' workplace). All procedures of the screening process were explained in the participant's home language and participants could ask questions if they had any uncertainties. Informed consent forms were offered to participants and signed after which measurements began. Procedures performed in the screening phase included: glucose and cholesterol testing, urine dipstick analysis, blood pressure measurements, determining blood groups and human immunodeficiency virus testing. A nurse provided participants with feedback on their results. If participants were eligible, they were invited to participate in the advanced phase of the African-PREDICT study. Participants who were deemed unsuitable for progression to the advanced stage were offered counselling with regards to any undesirable test results.

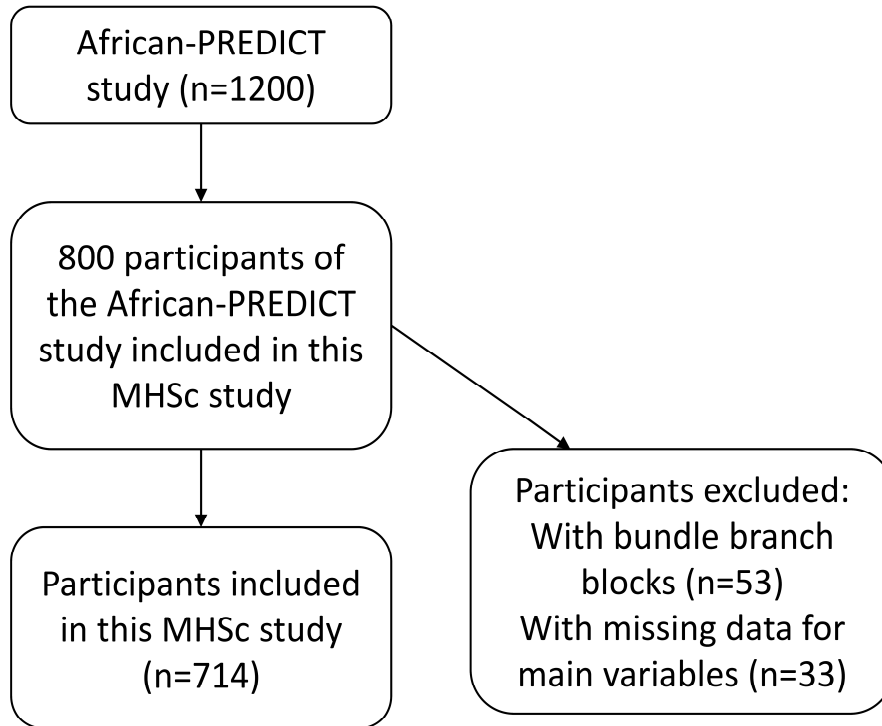
All procedures for data collection in the advanced phase took place in the Hypertension Research and Training Clinic of the Potchefstroom campus of the North-West University under the supervision of a registered research nurse. On the scheduled

morning of participation, transport was provided to the Hypertension Research and Training Clinic if requested, or participants were received at the clinic at 07h45. Participants were shown around to ensure they were familiar with their surroundings. The details and procedures were again explained, after which participants had the opportunity to ask questions. The informed consent forms were handed to them. Measurement procedures began after written informed consent was obtained.

The participants firstly had to provide a spot urine sample and were then taken to a private room where blood samples were collected by a registered research nurse. A research nurse also helped participants to each measurement station throughout the day. Validated questionnaires, including a General Health and a Global Physical Activity Questionnaire were completed between measurements. All researchers performed measurements according to good clinical practice (9). The study did not cause exposure of mental, physical or emotional risks to the participant and the participants had the opportunity to withdraw at any stage.

For both screening and advanced phase purposes, only four participants were scheduled per day to ensure quality and detailed measurements. Participants were required to fast overnight for eight to ten hours prior to the participation day to eliminate the influence of food or liquids on measurements. All measurements were done in private, temperature-controlled rooms for the participant's privacy and comfort.

Cross-sectional data from the African-PREDICT study was used for this MHSc study. From the first 800 participants in the African-PREDICT study, 714 participants were included according to the inclusion and exclusion criteria of this MHSc study. Thirty-three participants were excluded due to missing echocardiography or leukocyte profile data.



**Figure 1:** This diagram gives the exact numbers of participants that are included and excluded in the study from recruitment phase to data analyses.

### 3. Methodology

#### 3.1 Questionnaires

A research nurse, trained postgraduate students or a trained research assistant helped with the completion of the questionnaires and was done one-on-one in a quiet area in the clinic. Basic information regarding each participant's current health and family history was collected by giving a standard General Health Questionnaire for the participant to complete. The self-reported data collected included tobacco use, alcohol intake, anti-inflammatory medication use, age and sex. The Health Questionnaire took about 15 minutes to complete. Demographic data included information on the participant's level of education, household income and skill level. Participants were scored into three categories: skill level, household income and education level. The socio-economic score was calculated from the South African Standard Classification of Occupation. Participants were then categorized into low, middle and high socio-economic groups. Lower socio-economic

status is associated with increased risk for CVD (10). It is thus important to recruit a range of subjects from low, middle and high socio-economic groups.

### *3.2 Body composition*

Overweight and obesity can have an impact on the development of left ventricular hypertrophy (11). Therefore, information on body height, body weight, body surface area and waist circumference are needed when investigating the association of left ventricular mass index (LVMI) in relation to the main independents of interest. Anthropometric measurements were performed according to standard procedures, as prescribed by the International Society for the Advancement of Kinanthropometry (12). These measurements included body height (m), determined by the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), and weight (kg) using the SECA 813 Electronic Scales with weighing capacity up to 200kg (SECA, Hamburg, Germany). Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm and used for the calculation of body mass index ( $\text{weight (kg)/height (m)}^2$ ), rounded to one decimal. Body surface area ( $\text{m}^2$ ) was calculated by using the Mosteller equation (13). Body surface area, together with waist circumference could be a better indicator of either body size or abdominal obesity than body mass index, as body mass index is considered inaccurate especially among a population that performs excessive exercise (14, 15). Therefore, waist circumference was measured using a non-flexible tape measure (Holtain, Crymych, UK) for accurate readings and rounded to the nearest 0.1 cm. Waist circumference cut-off points of 91cm for African men and 84cm for African women, 97cm for white men and 84cm for white women (16) were used. Furthermore, waist circumference was shown to be closely related to inflammation and thus indirectly to leukocyte count (17). Measurements were done three times and the median was used for subsequent analyses.

### *3.3 Physical activity measurements*

Physical activity was measured continuously using a compact, accelerometer device that is placed on the chest and records heart rate, inter-beat-interval and physical activity in

a combined unit. The ActiHeart device (CamNtech Ltd., England, UK) was used to capture heart rate variability and to calculate total and activity induced energy expenditure. This also gives an estimate of the participant's physical activities for seven days. Active energy expenditure (AEE) (kCal/day) was calculated using the branched model equation (18). This equation was used as the combination of heart rate and computer science and applications improve the AEE estimates (18). AEE was standardized for weight (kCal/kg/day), as the population sample consists of various body sizes. AEE differs between lean, tall, overweight and muscular individuals, thus groups can be equally compared when normalizing for body weight.

### *3.4 Blood pressure and electrocardiography*

Twenty-four-hour ambulatory blood pressure was used as it is considered as the gold standard for blood pressure measurement and provides a general measurement of blood pressure during all common activities for 24-hours (19). Normotensive or pre-hypertensive cut-off points for 24-hours were regarded as systolic blood pressure (SBP) <130mmHg and/or diastolic blood pressure (DBP) <80mmHg (20), based on the average of four measures in one day. Participants were fitted with a validated Cardio(X)plore device (MediTech, Hungary) for the collection of 24-hour ambulatory blood pressure and electrocardiography measurements. 24-hour SBP and 24-hour DBP were obtained with the ambulatory blood pressure monitor that was programmed to record blood pressure every 30 minutes during the day (6am to 10pm) and every hour during the night (10pm to 6am). Individuals with left and right bundle branch blocks were identified with the ambulatory electrocardiogram that was recorded every five minutes for 20 seconds. The ambulatory blood pressure device was fitted on each participant's non-dominant arm, at approximately the same time every day (late morning), using an appropriate sized cuff. Instructions were given to the participant about how to ensure successful inflations over the 24-hour time period. According to the guidelines of successful inflations, only data above 70% inflation (19) was used.



### 3.5 Cardiac measurements

A clinical technologist, registered with the Health Professions Council of South Africa, performed a standard transthoracic echocardiography procedure for each participant, while the participant was in a partial left decubitus position with the head of the examining table modestly elevated. The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway) was used along with the 2.5 to 3.5 MHz transducer and a single electrocardiogram-lead for timing purposes. Standardized methods were employed to obtain high quality recordings according to the current recommendations, as outlined in the guidelines of the European Association of Echocardiography and the American Society of Echocardiography (21, 22). LVM was calculated by a standard formula: (Formula 1) (23) and normalized for body surface area (expressed as an index of LVM) (24). In this formula, 1.04 refers to the specific gravity of the myocardium (g/cm<sup>3</sup>) whereas 0.8 is a corrected coefficient. A constant, 0.6, is derived from the corrections from necropsy findings (25).

**Formula1:**  $0.8 \times 1.04 \times [(\text{Ventricular Septal Thickness at end-diastole} + \text{Left Ventricular Internal Diameter at end-diastole} + \text{Posterior Left Ventricular Wall Thickness at end-diastole})^3 - \text{Left Ventricular Internal Diameter at end-diastole}^3] + 0.6$

### 3.6 Biochemical analyses

Biochemical samples involved the collection and preparation of different blood samples for the analyses of biochemical data. A registered research nurse drew blood with a sterile needle from the anti-brachial vein. The antebrachial vein is the most prominent vein, close to the surface of the skin and has lower blood pressure than the arteries, which makes this vein well suited for drawing blood. All samples were prepared following standard procedures and stored at –80°C until analysed.

Serum analyses, including total cholesterol, glucose, high-density lipoprotein cholesterol, triglycerides, cystatin-C and creatinine concentrations were determined using the Cobas Integra® 400 plus (Roche, Basel Switzerland) apparatus. Serum cotinine analyses were done with the chemiluminescence method on an Immulite (Siemens, Erlangen, Germany) apparatus. An EDTA whole blood sample was analysed by a Coulter

AcT5 diff OV Hematology analyser (Beckman Coulter, Brea, CA, US) to determine total leukocyte counts (neutrophils, lymphocytes and monocytes). Neutrophil to lymphocyte ratio (NLR) was calculated additionally, as NLR is a marker of subclinical inflammation and prognostic of cardiovascular disease (26). Interleukin-6 was analysed from serum using the high-sensitivity Quantikine ELISA kit (R&D systems, Minneapolis, MN USA) on a Synergy H4 hybrid micro plate reader (Biotek, Winooski, VT, USA). Estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine + Cystatin C formula (Formula 2) (27). Renal dysfunction could influence LVM (28) and the resultant increase in LVM could in turn stimulate inflammation, thereby influencing kidney function (29).

**Formula2:** eGFR in ml/min/1.73<sup>2</sup>

$$\begin{aligned} & (130 \text{ if female and } 135 \text{ if male}) \times \text{minimum} (\text{Scr}/\kappa, 1)^{\alpha} \\ & \times \text{maximum} (\text{Scr}/\kappa)^{-0.601} \text{minimum} (\text{Cys}/0.8, 1)^{-0.375} \\ & \times \text{maximum} (\text{Cys}/0.8, 1)^{-0.711} \times 0.993^{\text{Age}} \times 1.018 \text{ [if female]} \\ & \times 1.159 \text{ [if black]} \end{aligned}$$

#### 4. Data management

The REDCap system was used for data capturing. The REDCap system is free, secure web-based, electronic database software and can be developed and customized for any study in a user-friendly manner. This system can be used for the collection and tracking of participant information and data, as well as to schedule participant visits (30).

The database manager, managed the REDCap system. The database is password protected and the identity of the participant is not exposed. The accuracy of data entered into the database was controlled by importing data directly into SPSS from automatically generated Excel sheets by the REDCap data management. All laboratory specimens, evaluation forms, reports, data and other records are stored in the REDCap system to ensure the confidentiality of each participant. In the case where data had to be captured manually, data entry was double checked. A confidentiality agreement with the Hypertension in Africa Research Team (HART) was signed by the student to further ensure the protection

of data. Only data from the participants and variables of interest for this MHSc study was provided to the student after ethical approval.

## **5. Statistical analyses**

Data analyses were done with IBM® SPSS® Statistics, Version 24 (IBM Corporation, Armonk, New York) software, which is powerful statistical software that is internationally recognized. All variables were tested for normality by visual inspection (Q-Q Plots) and skewness and kurtosis tests. If plots deviated from the regression line, skewness was less than -0.8 or more than 0.8 or if kurtosis was less than -3 or more than 3, these variables were classified as non-Gaussian. Non-Gaussian variables were logarithmically transformed and rechecked for normality. Due to log transformations, a Gaussian statistical approach was followed. When values were reported into tables, they were anti-logged and 5<sup>th</sup> and 95<sup>th</sup> quartiles were reported. For the purpose of this study, data was separated between black and white participants. From the multiple regression analyses, a strong association of LVMi with sex was observed in the total group, as well as in both black and white participants. Thus, we additionally separated the data between black and white, men and women to investigate the same multiple regression analyses in the four groups. Descriptive data was used to phenotype each group regarding their cardiovascular and inflammatory profiles and general demographic information. Such data was obtained in table format by performing independent t-tests. Normally distributed data was presented as arithmetic mean  $\pm$  standard deviation, whereas logarithmically transformed variables were presented as geometric mean with 5<sup>th</sup> and 95<sup>th</sup> percentiles. Proportions were determined with cross-tabs with significant differences indicated by Chi-square tests and presented as number and percentage. The link of LVMi with leukocytes (monocytes and NLR) were explored with Pearson and partial correlations and regression coefficients and p-values were reported. Standard and forward stepwise multiple regression analyses were done to test the independent relationships between the main dependent and independent variables. LVMi was used as main dependent and monocytes, NLR and leukocytes as main independent

variables. Covariates that were considered included age, SBP, self-reported alcohol use, glucose, socio-economic score and total cholesterol to high-density lipoprotein cholesterol ratio (TC:HDL).

## **6. Student contributions**

The student was involved in the following research activities during her MHSc:

- In the screening phase of the African-PREDICT study, I centrifuged blood samples, separate blood and urine samples into eppendorfs according to standard procedures and was responsible for packing blood and urine samples into bio-freezers. I was also responsible for laboratory Human Immunodeficiency Virus tests to confirm participant's Human Immunodeficiency Virus status.
- For the Exercise, Arterial Modulation and Nutrition in Youth South Africa (ExAMIN Youth) study in 2017 study I assisted in data collection (pulse wave velocity measurements), saliva and urine. I also had to deliver saliva and urine samples to the laboratory. I then prepared the laboratory for packaging of samples and then allocated the saliva and urine samples into eppendorfs according to standard procedure and packed it into bio-freezers. In addition, urine dipstick analyses for the confirmation of any defects in urine samples were also done.
- With regards to statistical analyses training, I completed the following modules: STTN 111, STTN 124 and FLGX 615. In 2016 and 2017 I had SPSS training and performed statistical analyses for my Honours and MHSc projects.
- I applied for ethics clearance for my MHSc project and attended the certified Ethics in Health research training.

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# Chapter 3

*Research article*

# Left ventricular mass and its adverse association with leukocytes in young South Africans: The African-PREDICT study

Marika I Kirstein<sup>a</sup>, Shani Botha<sup>a,b</sup>, Hugo W Huisman<sup>a,b</sup>, Ruan Kruger<sup>\*a,b</sup>

<sup>a</sup>*Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa  
(This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation)*

<sup>b</sup>*Medical Research Council: Research Unit for Hypertension and Cardiovascular Disease, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa.*

## **\*Corresponding author:**

Ruan Kruger, PhD  
Hypertension in Africa Research Team (HART)  
North-West University  
Potchefstroom, 2531  
South Africa  
Phone: +27 18 299 2904  
Fax: +27 18 285 2432  
Email: [ruan.kruger@me.com](mailto:ruan.kruger@me.com)

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\*Author instructions seen in Appendix A

## **Abstract**

Cardiac overload increases left ventricular remodelling and left ventricular mass. Leukocytes were previously implicated in left ventricular remodelling in elderly and diseased populations. However, limited knowledge on the relationship between left ventricular mass index (LVMI) and leukocytes exists in young, apparently healthy populations. The researchers aimed to explore the link of LVMI with leukocytes in 20–30 year old South Africans, free from overt cardiovascular disease. This was a cross-sectional study on 800 apparently healthy individuals from the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension. LVMI was calculated from echocardiographic data and leukocyte counts were measured. Monocytes and neutrophil to lymphocyte ratio (NLR) were higher in the white compared to black group (both  $p < 0.001$ ). LVMI showed an inverse association with leukocytes (Adj. $R^2 = 0.094$ ;  $\beta = -0.30$ ;  $p = 0.001$ ) and NLR (Adj. $R^2 = 0.053$ ;  $\beta = -0.19$ ;  $p = 0.025$ ) in white men, as well as with monocytes in white men (Adj. $R^2 = 0.075$ ;  $\beta = -0.25$ ;  $p = 0.004$ ) and white women (Adj. $R^2 = 0.060$ ;  $\beta = -0.21$ ;  $p = 0.005$ ). However, the association obtained in single regression analyses between LVMI and NLR disappeared, while the rest of the associations remained after adjusting for traditional risk factors. This study showed LVMI associates inversely with leukocytes, specifically monocytes and NLR in the white population. The inverse association of LVMI with monocytes may indicate an early decline in monocytes which could contribute to impaired cardiac repair in this young, white South African group.

*Word count: 230*

## 1. Introduction

According to the World Health Organization, non-communicable diseases account for 36 million deaths per year (1). A study done on young individuals reported that high blood pressure, obesity, increased levels of glucose and cholesterol, as well as unhealthy lifestyle choices such as smoking, drug abuse, excessive alcohol and physical inactivity are all risk factors that could increase the risk of developing non-communicable diseases (2). Cardiovascular disease (CVD) is a non-communicable disease accounting for 17.3 million deaths globally each year (1, 3). In addition, unhealthy lifestyle was reported to contribute to low-grade inflammation, known to early cardiovascular disease development (4). Leukocytes maintain the processes of inflammation (5), which is a major risk factor for cardiovascular morbidity and mortality (6).

Hypertension, arterial stiffness and atherosclerosis are all linked to endothelial dysfunction (7-12). Together with inflammation; hypertension, arterial stiffness and atherosclerosis contribute to cardiac overload, which causes an adverse strain on the left ventricle and increases left ventricular mass (LVM) (7-12). Leukocytes were found to play a role in cardiac hypertrophy after myocardial infarction (13). Inflammatory activation and recruitment is promoted at the damaged myocardium during the inflammatory phase (14), whereas the reparative phase is initiated by the resolution of inflammation, myofibroblast proliferation, scar formation and neovascularization (15, 16). After myocardial infarction, dead cardiomyocytes are cleared by leukocytes such as neutrophils, lymphocytes and monocytes (17). Nonetheless, an overactive and prolonged post-infarction inflammatory response may cause adverse left ventricular remodelling which contributes to increased LVM (18).

Unhealthy lifestyle choices and family history are risk factors for early vascular changes in a young population (19). Early vascular changes are associated with increased LVMi in the overall population (20). Leukocytes have been associated with LVMi in elderly and diseased populations (21, 22), however, limited literature has been documented on this association in young populations without known CVD. Reverse cardiac remodelling is

however a possibility at a young age; the focus should thus be on the prevention of CVD risk factors, such as obesity and atherosclerotic plaque, in young individuals. Therefore, we investigated the potential link between LVMI and leukocytes in young black and white South Africans population free from self-reported CVD.

## **2. Methods**

### *2.1 Study design and population sample*

Cross-sectional baseline data from 800 participants who participated in the African prospective study on the early detection and identification of CVD and hypertension (African-PREDICT) was used. Apparently healthy black and white individuals of both genders were included in the study. Participants with a brachial systolic blood pressure (SBP) of <140mmHg and diastolic blood pressure (DBP) of <90mmHg, who were not infected with the human immunodeficiency virus and did not have any medical history of chronic diseases, as well as non-pregnant or lactating women, were included. Participants with missing echocardiography or leukocyte count data (n=33) or with left and right bundle branch blocks (n=53) were excluded. This study conformed to the ethical aspects outlined in the revised Declaration of Helsinki (2008) and was approved by the North-West Province Department of Health in South Africa and the Health Research Ethics Committee of the North-West University, Potchefstroom (NWU-00068-17-A1). All participants gave written informed consent prior to any measurements performed.

### *2.2 Questionnaire*

A validated general health questionnaire was completed to obtain information about self-reported tobacco use, alcohol intake, anti-inflammatory medication use, age, sex, household income, level of skill and employment. A socio-economic score was calculated from the South African Standard Classification of Occupation register.

### *2.3 Body composition and physical activity measurements*

Anthropometric measurements included body height and weight, measured according to standard procedures, as prescribed by the International Society for the

Advancement of Kinanthropometry (23). Body mass index was calculated, along with body surface area using the Mosteller equation (24). A non-flexible tape measure (Holtain, Crymych, UK) was used to measure waist circumference. Physical activity was measured using the ActiHeart device (CamNtech Ltd., England, UK). Active energy expenditure (AEE) was calculated using the branched model equations and normalized for weight.

#### *2.4 Blood pressure and electrocardiography measurements*

Twenty four-hour blood pressure measurements were collected by fitting participants with a validated CardioXplore device (CardioXplore, MediTech, Hungary), which was programmed to record blood pressure every 30 minutes during the day (6am to 10pm) and every hour during the night (10pm to 6am). Electrocardiogram data was also recorded every five minutes for 20 seconds and were used for the identification of participants with left and right bundle branch blocks.

#### *2.5 Echocardiography*

A standard transthoracic echocardiography procedure was followed and conformed to the guidelines of the European Association of Echocardiography and the American Society of Echocardiography (25, 26). The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway) was used along with a 2.5 to 3.5 MHz transducer and a three-lead electrocardiogram for timing purposes. LVM was measured with echoPAC software by a trained sonographer and calculated with a standard formula (Formula 1) (27). LVM was normalised for body surface area (expressed as LVMi) (26).

**Formula 1:**  $0.8 \times 1.04 \times [(\text{Ventricular Septal Thickness at end-diastole} + \text{Left Ventricular Internal Diameter at end-diastole} + \text{Posterior Left Ventricular Wall Thickness at end-diastole})^3 - \text{Left Ventricular Internal Diameter at end-diastole}^3] + 0.6$

#### *2.6 Biochemical analyses*

Participants were requested to fast for at least eight hours prior to participation. Blood samples were collected with a sterile needle from the antebrachial vein. Standardized procedures were followed to prepare all samples and to store samples at  $-80^{\circ}\text{C}$  until

analysed.

Analyses of serum samples, including total cholesterol, glucose, high-density lipoprotein cholesterol, triglycerides, cystatin-C and creatinine were determined using the Cobas Integra® 400 plus (Roche, Basel Switzerland) apparatus. Analyses of serum cotinine were done with the chemiluminescence method on an Immulite (Siemens, Erlangen, Germany) apparatus. For the determination of neutrophils, lymphocytes and monocytes, an EDTA whole blood sample was analysed by a Coulter AcT5 diff OV Hematology analyzer (Beckman Coulter, Brea, CA, US). The neutrophil to lymphocyte ratio (NLR) was calculated additionally. Interleukin-6 was analysed from serum using the high-sensitivity Quantikine ELISA kit (R&D systems, Minneapolis, MN USA) on a Synergy H4 hybrid micro plate reader (Biotek, Winooski, VT, USA). Estimated glomerular filtration rate (eGFR) was determined with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine + Cystatin C formula (28).

## *2.7 Statistical analyses*

All statistical analyses were performed with the IBM® SPSS® Statistics, Version 24 (IBM Corporation, Armonk, New York). For the aim of this study, the data was originally separated between racial groups. However, due to the effect of sex that was found during multiple regression analyses, participants were additionally grouped by men and women before repeating the multiple regression analysis. Descriptive statistics were obtained by performing independent t-tests and presented as the arithmetic mean  $\pm$  standard deviation. Variables were tested for normality by visual inspection (Q-Q plots), and calculation of skewness and kurtosis. Non-Gaussian variables were logarithmically transformed and expressed as geometric mean with 5<sup>th</sup> and 95<sup>th</sup> percentiles. Pearson and partial correlations were performed to explore correlations of LVMi with leukocytes, monocytes and NLR. For multiple regression analyses, the following covariates were considered for entry into the models: age, SBP, alcohol use, glucose, socio-economic score and total cholesterol to high-density lipoprotein cholesterol ratio.

### 3. Results

Table 1 shows the descriptive characteristics of the study population. The white group was older, had a higher socio-economic score, body surface area, SBP, mean arterial pressure, monocyte count and NLR (all  $p \leq 0.017$ ) compared to the black group. Interleukin-6 and AEE were lower in the white group than in the black group (both  $p < 0.001$ ), while the white group reported higher anti-inflammatory medication use ( $p = 0.036$ ) than the black group.

In single regression analysis (Supplementary Table 1), LVMi correlated negatively with leukocyte count ( $r = -0.16$ ;  $p = 0.003$ ), monocytes ( $r = -0.13$ ;  $p = 0.015$ ) and NLR ( $r = -0.13$ ;  $p = 0.014$ ) in the white group only. After adjusting for age and sex (Table 2), the previous inverse correlations between LVMi with leukocyte count ( $r = -0.16$ ;  $p = 0.004$ ), monocytes ( $r = -0.20$ ;  $p < 0.001$ ) and NLR ( $r = -0.12$ ;  $p = 0.027$ ) remained in the white group.

In multiple regression analyses (Table 3), the independent associations of LVMi with leukocyte count, monocytes and NLR were tested in the total group and in black and white participants, respectively. An inverse association of LVMi existed with leukocyte count (Model 1: Adjusted  $R^2 = 0.201$ ;  $\beta = -0.08$ ;  $p = 0.017$ ) and monocytes (Model 2: Adjusted  $R^2 = 0.205$ ;  $\beta = -0.11$ ;  $p = 0.002$ ) in the total group. Moreover, an inverse association of LVMi with leukocyte count (Model 1: Adjusted  $R^2 = 0.199$ ;  $\beta = -0.18$ ;  $p = 0.001$ ) and monocytes (Model 2: Adjusted  $R^2 = 0.213$ ;  $\beta = -0.22$ ;  $p < 0.001$ ) was found, as well as with NLR (Model 3: Adjusted  $R^2 = 0.182$ ;  $\beta = -0.11$ ;  $p = 0.036$ ) in the white group only. Additionally, LVMi associated with sex and SBP in the total group, as well as in both black and white groups.

To account for the effect of sex on the association between LVMi and leukocytes, the groups were additionally stratified by both sex and race (Table 4).

LVMi associated inversely with leukocyte count (Model 1: Adjusted  $R^2 = 0.094$ ;  $\beta = -0.30$ ;  $p = 0.001$ ) and NLR (Model 3: Adjusted  $R^2 = 0.053$ ;  $\beta = -0.19$ ;  $p = 0.025$ ) in the white men only, whereas LVMi inversely associated with monocytes in both white men (Model 2: Adjusted  $R^2 = 0.075$ ;  $\beta = -0.25$ ;  $p = 0.004$ ) and white women (Model 2: Adjusted  $R^2 = 0.060$ ;  $\beta = -0.21$ ;  $p = 0.005$ ).



In sensitivity analyses, the researchers additionally adjusted for AEE, eGFR, anti-inflammatory medication use, and interleukin-6, respectively. The previous inverse association of LVMi with leukocyte count and monocytes remained in all cases. Similarly, the inverse association between LVMi and NLR remained when the researchers additionally adjusted for anti-inflammatory medication use ( $\beta=-0.186$ ;  $p=0.033$ ) and eGFR ( $\beta=-0.199$ ;  $p=0.022$ ), respectively. However, this association disappeared after additionally adjusting for AEE ( $\beta=-0.181$ ;  $p=0.065$ ) and interleukin-6 ( $\beta=-0.182$ ;  $p=0.058$ ), respectively (data not shown).

**Table 1.** Descriptive characteristics of the study population.

	<b>White (n=336)</b>	<b>Black (n=378)</b>	<b>p-value</b>
Age, years	25.2 ± 2.89	24.5 ± 3.24	0.001
Sex (men), n (%)	150 (44.6)	144 (38)	0.076
Socio-economic score	24.2 ± 5.66	17.7 ± 5.79	<0.001
Body surface area, m <sup>2</sup>	1.92 ± 0.26	1.72 ± 0.20	<0.001
Waist circumference, n (%)	77 (22.9)	88 (23.3)	0.91
eGFR, ml/min/1.73m <sup>2</sup>	103 ± 13.4	127 ± 15.3	<0.001
<b>Cardiovascular measurements</b>			
Systolic blood pressure, mmHg	118 ± 9.82	115 ± 9.14	<0.001
Diastolic blood pressure, mmHg	69.1 ± 5.88	68.9 ± 5.93	0.62
Mean arterial pressure, mmHg	88.7 ± 6.74	87.5 ± 6.63	0.017
Left ventricular mass index, g/m <sup>2</sup>	74.4 ± 16.0	72.8 ± 17.4	0.21
<b>Leukocyte profile</b>			
Leukocytes, 6.3/ηL	6.09 ± 2.03	5.43 ± 1.69	<0.001
Monocytes, 6.3/ηL	0.44 (0.22;0.92)	0.30 (0.15;0.56)	<0.001
Neutrophil:Lymphocyte, 6.3/ηL	1.51 (0.68;3.54)	1.25 (0.57;2.83)	<0.001
<b>Biochemical markers</b>			
Interleukin-6, pg/ml	0.87 (0.28;2.94)	1.17 (0.43;3.76)	<0.001
TC:HDL, mmol/L	3.61 (2.22;6.53)	3.03 (1.97;4.85)	<0.001
Triglycerides, mmol/L	0.98 (0.45;2.30)	0.73 (0.37;1.41)	<0.001
Glucose, mmol/L	4.90 ± 0.75	4.37 ± 0.85	<0.001
<b>Lifestyle measurements</b>			
Smoking, n (%)	80 (23.8)	96 (25.4)	0.62
Alcohol consumption, n (%)	200 (59.5)	217 (58.2)	0.72
Anti-inflammatory medication use, n (%)	8 (2.4)	2 (0.5)	0.036
Active energy expenditure, kCal/kg/day	5.38 ± 2.71	6.61 ± 3.16	<0.001

Values are arithmetic mean ± standard deviation and geometric mean (5<sup>th</sup> and 95<sup>th</sup> percentile) or number of participants (n).

**Abbreviations:** eGFR – Estimated glomerular filtration rate; Neutrophil:Lymphocyte – Neutrophil to lymphocyte ratio; TC:HDL – Total cholesterol to high-density lipoprotein cholesterol ratio.

**Table 2.** Partial correlations of left ventricular mass index with variables of interest within black and white participants.

	Left ventricular mass index (g/m <sup>2</sup> )		
	Total group (n=714)	White (n=336)	Black (n=378)
Waist circumference, n (%)	r= 0.02 p= 0.57	r= 0.05 p= 0.33	r= 0.006 p= 0.91
eGFR, ml/min/1.73m <sup>2</sup>	r= -0.04 p= 0.31	r= -0.02 p= 0.71	r= -0.06 p= 0.27
Systolic blood pressure, mmHg	r= 0.15 p< 0.001	r= 0.13 p= 0.018	r= 0.18 p= 0.001
Diastolic blood pressure, mmHg	r= -0.02 p= 0.66	r= -0.09 p= 0.10	r= 0.05 p= 0.35
Leukocytes, 6.3/ηL	r= -0.07 p= 0.054	r= -0.16 p= 0.004	r= 0.001 p= 0.99
Monocytes, 6.3/ηL	r= -0.10 p= 0.010	r= -0.20 p< 0.001	r= -0.04 p= 0.46
Neutrophil:Lymphocyte, 6.3/ηL	r= -0.05 p= 0.19	r= -0.12 p= 0.027	r= 0.01 p= 0.83
Interleukin-6, pg/ml	r= 0.04 p= 0.29	r= 0.05 p= 0.37	r= 0.05 p= 0.30
TC:HDL, mmol/L	r= -0.01 p= 0.77	r= 0.07 p= 0.21	r= -0.06 p= 0.23
Triglycerides, mmol/L	r= -0.02 p= 0.60	r= -0.03 p= 0.58	r= -0.01 p= 0.79
Glucose, mmol/L	r= 0.06 p= 0.14	r= 0.006 p= 0.91	r= 0.10 p= 0.045
AEE, kCal/kg/day	r= -0.02 p= 0.74	r= 0.03 p= 0.66	r= -0.03 p= 0.63

Adjustments were applied for age and sex.

*Abbreviations:* AEE – Active energy expenditure; eGFR – Estimated glomerular filtration rate; Neutrophil:Lymphocyte – Neutrophil to lymphocyte ratio; TC:HDL – Total cholesterol to high-density lipoprotein cholesterol ratio.

**Table 3.** Multiple regression analysis of left ventricular mass index with leukocyte counts in the total, as well as in black and white groups.

	Left ventricular mass index, g/m <sup>2</sup>					
	Total group (n=714)		White (n=336)		Black (n=378)	
	$\beta$ ( $\pm 95\text{CI}$ )	p	$\beta$ ( $\pm 95\text{CI}$ )	p	$\beta$ ( $\pm 95\text{CI}$ )	p
<b>MODEL 1</b>						
<i>Adj R<sup>2</sup></i>	0.201		0.199		0.224	
Leukocytes, 6.3/ $\eta\text{L}$	-0.08 (-1.32;-0.13)	0.017	-0.18 (-2.24;-0.59)	0.001	–	–
Socio-economic score	–	–	–	–	-0.12 (-0.66;-0.07)	0.016
Systolic blood pressure, mmHg	0.17 (0.16;0.43)	<0.001	0.15 (0.06;0.44)	0.011	0.18 (0.15;0.53)	<0.001
Sex, n	0.34 (8.99;14.10)	<0.001	0.28 (4.89;13.0)	<0.001	0.36 (9.25;16.4)	<0.001
Glucose, mmol/L	–	–	–	–	0.11 (0.31;4.18)	0.023
<b>MODEL 2</b>						
<i>Adj R<sup>2</sup></i>	0.205		0.213		0.226	
Monocytes, 6.3/ $\eta\text{L}$	-0.11 (-14.37;-3.28)	0.002	-0.22 (-26.6;-9.53)	<0.001	–	–
Socio-economic score	–	–	–	–	-0.12 (-0.65;-0.06)	0.020
Systolic blood pressure, mmHg	0.18 (0.18;0.44)	<0.001	0.16 (0.08;0.46)	0.006	0.18 (0.16;0.54)	<0.001
Sex, n	0.35 (9.52;14.5)	<0.001	0.32 (6.19;14.1)	<0.001	0.36 (9.38;16.5)	<0.001
Glucose, mmol/L	–	–	–	–	0.11 (0.32;4.19)	0.023
<b>MODEL 3</b>						
<i>Adj R<sup>2</sup></i>	0.195		0.182		0.224	
Neutrophil:Lymphocyte, 6.3/ $\eta\text{L}$	–	–	-0.11 (-15.4;-0.52)	0.036	–	–
Socio-economic score	–	–	–	–	-0.12 (-0.66;-0.07)	0.016
Systolic blood pressure, mmHg	0.16 (0.15;0.41)	<0.001	0.13 (0.02; 0.41)	0.028	0.18 (0.15;0.53)	<0.001
Sex, n	0.35 (9.38;14.4)	<0.001	0.30 (5.61;13.7)	<0.001	0.36 (9.28; 16.4)	<0.001
Glucose, mmol/L	–	–	–	–	0.11 (0.31; 4.18)	0.023

All models included age, systolic blood pressure, alcohol consumption, glucose, socio-economic score, sex and total cholesterol to high-density lipoprotein cholesterol ratio, as well as either leukocyte count (model 1), monocytes (model 2) or neutrophil to lymphocyte ratio (model 3).

*Abbreviation:* Neutrophil:Lymphocyte – Neutrophil to lymphocyte ratio.

**Table 4:** Multiple regression analysis of left ventricular mass index with leukocyte counts in black and white men and women.

	Left ventricular mass index, g/m <sup>2</sup>							
	White men (n=150)		Black men (n=144)		White women (n=186)		Black women (n=234)	
	$\beta$ ( $\pm 95\text{CI}$ )	p	$\beta$ ( $\pm 95\text{CI}$ )	p	$\beta$ ( $\pm 95\text{CI}$ )	p	$\beta$ ( $\pm 95\text{CI}$ )	p
<b>MODEL 1</b>								
<i>Adj R<sup>2</sup></i>	0.094		0.088		0.029		0.027	
Leukocytes, 6.3/ $\eta\text{L}$	-0.30(-3.72;-0.99)	0.001	–	–	–	–	–	–
SES	–	–	-0.31(-1.54;-0.39)	0.001	–	–	–	–
SBP, mmHg	0.20(0.08;0.74)	0.015	0.21(0.10;0.82)	0.014	–	–	0.19(0.08;0.51)	0.006
TC:HDL, mmol/L	–	–	–	–	–	–	–	–
Glucose, mmol/L	–	–	0.18(0.23;6.93)	0.036	–	–	–	–
<b>MODEL 2</b>								
<i>Adj R<sup>2</sup></i>	0.075		0.089		0.060		0.030	
Monocytes, 6.3/ $\eta\text{L}$	-0.25(-34.69;-6.67)	0.004	–	–	-0.21(-26.33;-4.71)	0.005	–	–
SES	–	–	-0.30(-1.53;-0.37)	0.001	–	–	–	–
SBP, mmHg	0.20(0.07;0.73)	0.018	0.21(0.10;0.84)	0.013	–	–	0.19(0.10;0.51)	0.005
TC:HDL, mmol/L	–	–	–	–	–	–	–	–
Glucose, mmol/L	–	–	0.18(0.27;6.96)	0.034	–	–	–	–
<b>MODEL 3</b>								
<i>Adj R<sup>2</sup></i>	0.053		0.087		0.019		0.027	
NLR, 6.3/ $\eta\text{L}$	-0.19(-24.8;-1.71)	0.025	–	–	–	–	–	–
SES	–	–	-0.31(-1.55;-0.39)	0.001	–	–	–	–
SBP, mmHg	–	–	0.21(0.09;0.82)	0.015	–	–	0.19 (0.09;0.51)	0.006
TC:HDL, mmol/L	–	–	–	–	–	–	–	–
Glucose, mmol/L	–	–	0.18(0.21;6.93)	0.037	–	–	–	–

All models included age, systolic blood pressure, alcohol consumption, glucose, socio-economic score and total cholesterol to high-density lipoprotein cholesterol ratio, as well as either leukocyte count (model 1), monocytes (model 2) or neutrophil to lymphocyte ratio (model 3).

*Abbreviations:* NLR – Neutrophil to lymphocyte ratio; SES – Socio-economic score; SBP – Systolic blood pressure; TC:HDL – Total cholesterol to high-density lipoprotein cholesterol ratio.

#### 4. Discussion

In this study, the aim was to investigate the relationship between LVMI and leukocytes in a young, black and white population. The findings showed inverse associations of LVMI with leukocytes, monocytes and NLR in the white group only. The findings furthermore indicated that a decrease in leukocytes, specifically monocytes and NLR, may contribute to an increase in LVM in this white South African population.

Although the study included healthy individuals, free from overt CVD, the findings are in accordance with a study conducted on 149 patients with acute myocardial infarction, which found that monocyte depletion causes an increase in cardiac ischaemia, contributing to the development of cardiac hypertrophy (29). In the young population, the inverse association of LVMI with monocytes may thus indicate potential early changes in LVM due to a depletion of monocytes.

Monocytes are involved in immune defenses, inflammation and remodelling through antigen processing and presentation, as well as phagocytosis and cytokine production (30). In an inflammatory milieu, monocytes have the ability to differentiate into macrophages that function as phagocytic cells for the clearance of unwanted cell debris, invading pathogens and other foreign substances by endocytosis (31, 32). Monocytes and activated macrophages play a major role in myocardial repair after cardiac injury, as for instance after myocardial infarction (33, 34). These cells also promote angiogenesis in and around the healing tissue through releasing vascular endothelial growth factors and pro-inflammatory cytokines (13, 35, 36). A reduction in monocytes and activated macrophages could therefore contribute to early cardiac changes, causing increased LVM. The finding resembled that of a previous study in 2009, conducted on hypertension-prone rats, which discovered that a deficiency in macrophages contributed to early myocardial dysfunction development (37). However, the findings were in normotensive individuals and further investigation on this matter is thus needed in a larger study population.

LVMI was further associated with SBP. A study done on healthy high school children similarly found elevated SBP to be associated with increased LVMI (38). The white group, in

addition, had higher (although not hypertensive) SBP levels than the black group. It is thus speculated that the sub-clinically higher SBP in this white group may contribute to monocytes being less activated. In the setting of higher blood pressure and lower monocyte activation, potential increases in cardiac structural changes may be evident in this young population.

In addition, it was found that an inverse association between LVMI and NLR in white men existed. Results from a previous study showed that an increased NLR is directly associated with increased LVMI, which is in contrast with our finding. However, this was found in patients with chronic hemodialysis (39) and a scarcity of information exists relating to normotensive individuals. Furthermore, the initial inverse association between LVMI and NLR disappeared after additionally adjusting for interleukin-6, which may suggest that the association between LVMI and NLR may be dependent on an inflammatory state. Clarification on this matter, with the inclusion of a larger inflammatory profile, is however needed.

The current study was well-designed, had a sample size with sufficient power and used gold-standard measurements such as echocardiography. A bi-racial population was examined and differences between the two different racial groups could thus be explored. Additionally, the study focused on young individuals and the researchers adjusted for multiple confounders which strengthened the results. This was however a cross-sectional study, which limited the study to observe potential causative trends. Basophils and eosinophils were not considered in this study, however, these leukocytes were shown to be involved in allergic reactions (40), which was not the focus of the study.

In conclusion, this study reported that LVMI inversely associates with leukocytes, specifically monocytes and NLR, only in the white population group. The findings indicated a potential mediating effect of blood pressure contributing to the link between LVM and leukocytes, especially in white men. The inverse correlation of LVMI with monocytes may indicate premature monocyte attenuation, which may consequently contribute to future impaired cardiac repair among this young white South African population.

## **5. Acknowledgements**

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**Supplementary Table 1.** Single regression analyses between left ventricular mass index and variables of interest in black and white groups.

	Left ventricular mass index (g/m <sup>2</sup> )		
	Total group (n=714)	White (n=336)	Black (n=378)
Age, years	r= 0.05 p= 0.21	r= 0.11 p= 0.052	r= −0.005 p= 0.92
Waist circumference, n (%)	r= −0.04 p= 0.24	r= 0.06 p= 0.28	r= −0.13 p= 0.01
eGFR, ml/min/1.73m <sup>2</sup>	r= −0.03 p= 0.46	r= −0.02 p= 0.74	r= 0.02 p= 0.72
Systolic blood pressure, mmHg	r= 0.32 p< 0.001	r= 0.32 p< 0.001	r= 0.31 p< 0.001
Diastolic blood pressure, mmHg	r= 0.06 p= 0.12	r= 0.02 p= 0.74	r= 0.09 p= 0.080
Leukocytes, 6.3/ηL	r= −0.10 p= 0.008	r= −0.16 p= 0.003	r= −0.06 p= 0.25
Monocytes, 6.3/ηL	r= −0.04 p= 0.25	r= −0.13 p= 0.015	r= −0.01 p= 0.79
Neutrophil:Lymphocyte, 6.3/ηL	r= −0.08 p= 0.035	r= −0.13 p= 0.014	r= −0.05 p= 0.33
Interleukin-6, pg/ml	r= −0.03 p= 0.48	r= 0.05 p= 0.34	r= −0.08 p= 0.13
TC:HDL, mmol/L	r= 0.10 p= 0.010	r= 0.26 p< 0.001	r= −0.08 p= 0.12
Triglycerides, mmol/L	r= 0.09 p= 0.019	r= 0.10 p= 0.12	r= 0.07 p= 0.18
Glucose, mmol/L	r= 0.09 p= 0.015	r= 0.12 p= 0.024	r= 0.05 p= 0.34
AEE, kCal/kg/day	r= −0.13 p= 0.002	r= −0.14 p= 0.020	r= −0.10 p= 0.082

*Abbreviations:* AEE – Active energy expenditure; eGFR – Estimated glomerular filtration rate; Neutrophil:Lymphocyte – Neutrophil to lymphocyte ratio; TC:HDL – Total cholesterol to high-density lipoprotein cholesterol ratio.

# Chapter 4

*Summary, conclusion and recommendations  
for future studies*

## 1. Introduction

A summary of the main findings from the research article is presented in this conclusive chapter. The results from this article in relation to the relevant literature are also explained. Conclusions are drawn and recommendations proposed for future studies to researchers investigating the relationship between left ventricular mass index (LVMI) and leukocyte count.

## 2. Summary of the main findings

We aimed to explore the relationship between LVMI and leukocytes in young black and white South Africans.

1) From the **first objective**, it was firstly hypothesized that LVMI would be higher in the white compared to the black group.

In descriptive statistics, no difference in LVMI between the black and white groups was observed. Previous studies have however seen larger increases in LVMI in black than white healthy populations (1-3). Additionally, body mass and systolic blood pressure (SBP) were higher in the white study population, which are known factors that influence left ventricular mass (LVM) (2). High body mass (obesity) causes increased preload and afterload on the heart (4) and high SBP causes left ventricular afterload (5). Both these factors, along with the increase in overload on the heart, lead to increased LVM (6, 7). In contrast to this study, other studies showed that black individuals have a higher SBP and increased prevalence of obesity or increased body weight than white individuals (8, 9). Even though no difference in LVMI between the race groups was found, it is speculated that the white population may be subjected to the development of early subclinical left ventricular hypertrophy as a result of the depletion of monocytes. This may be supported by the increased SBP and body composition (although not clinically regarded as pathological), in this population. The study was however done on young individuals and these types of differences are not generally evident in a young population.

This hypothesis is thus rejected as no differences in LVMI were seen between racial groups in the South African study population.



It was secondly hypothesized that the leukocyte profile (neutrophils, monocytes and neutrophil to lymphocyte ratio (NLR)) would be higher in white compared to black participants.

In descriptive analyses, the white group had higher leukocyte counts, specifically monocytes and NLR, than the black group. Other studies also consistently found the leukocyte count to be higher in white compared to black individuals (10, 11). Leukocyte count is known to positively associate with numerous cardiovascular disease risk factors such as body weight, SBP, smoking and glucose levels, and negatively with high-density lipoprotein cholesterol, family income, alcohol consumption and physical activity (12-14). Unhealthy lifestyle risk factors such as smoking, alcohol abuse and obesity are risk factors contributing to early vascular changes in young individuals and cause young individuals to be more susceptible to low grade inflammation (15). Though, to the researchers' knowledge, no apparent reason has been identified for the higher leukocyte count in the white compared to the black groups and this matter should thus be investigated further.

The second hypothesis was therefore accepted, as the white group had higher leukocyte counts than the black group.

2) From the **second objective**, it was hypothesized that LVMI would associate adversely with leukocytes in both the black and white groups.

With statistical analyses, it was found that LVMI associated inversely with leukocyte count, monocytes and NLR in white men and only with monocytes in white women. However, LVMI did not associate with any leukocytes in black men or women. Previous studies found that left ventricular remodelling and left ventricular dilation associates with inflammation, which leads to increased LVM (16-18). However, these studies were conducted in older and diseased populations (16-18). With SBP being higher in the white group (although not hypertensive), this might suggest that hemodynamic changes could be present in this group and might cause inflammation. In return, early cardiac changes might contribute to an imbalance between anti- and pro-inflammatory inflammation (19). However, if impaired cardiac repair is present it may be explained by a depletion of monocytes (20). It is thus speculated that the LVM is not

pathologically increased in this healthy population but that, on the contrary, the monocyte count may be attenuated by the potential mediating effect of SBP.

The third hypothesis was thus partially accepted as LVMI associated with leukocytes, but this association was inverse and present only in the white and not the black population.

3) From the **third objective**, it was hypothesized that the association of LVMI with the leukocyte profile in both the black and white groups, would be dependent on interleukin-6.

In the study, it was found that the association between LVMI and leukocytes was only evident in the white population. In white men LVMI associated with leukocyte count, monocytes and NLR while, in white women, LVMI associated with monocytes only. No association was seen in the black population. In sensitivity analyses where the researchers additionally adjusted for interleukin-6, the association of LVMI with leukocyte count and NLR in the white men disappeared, thus suggesting that the association is dependent of interleukin-6 and thus possibly, on an inflammatory state. However, the association between LVMI and monocytes in both white men and women did not disappear after additionally adjusting for this confounder, which may in turn suggest that this association with monocytes is independent of interleukin-6.

The fourth hypothesis was therefore partially accepted as the association of LVMI with leukocyte count and NLR, but not with monocytes, was dependent on interleukin-6, and only in the white group.

### **3. Limitation and, chance and confounding**

This cross-sectional study was done in a young population from the North West province; thus, results cannot be generalized to the entire South African population of various ages. However, the number of participants included in this study provided sufficient statistical power to test the hypotheses. For the purpose of this study, young South Africans were investigated. To the best of the researchers' knowledge, this association was previously investigated in older individuals only. Other inflammatory markers, such as C-reactive protein and tumor necrosis factor alpha, were not included in this study, as there was no correlation between LVMI and C-reactive protein or tumor necrosis factor alpha in exploratory analyses.

The researchers adjusted for contributing factors (including age, SBP, alcohol consumption, glucose, socio-economic score and total cholesterol to high-density lipoprotein cholesterol ratio) in order to account for as many factors as possible that may have influenced the results. After additionally adjusting for multiple confounders, the relationship with monocytes remained, suggesting that this finding is likely to be robust. However, the association of LVMI with leukocyte count and NLR disappeared, proposing that inflammation could be mediating these associations.

#### **4. Recommendations for future studies**

As the study confirmed the results of other studies where the leukocyte count is higher in white compared to black populations, it is recommended that future studies further investigate the reason for this matter, as no information has been published thus far.

It is suggested that this study should be investigated using 3D echocardiography as it does not rely on geometric adaptations only and adds a depth dimension on the left ventricle, rendering more accurate measurements.

Further research is suggested on young infants to investigate the degree to which family history may influence LVMI, independent of the influence of environmental factors.

Studies on the association between LVMI and the leukocyte count could further clarify whether this link is present in not only obese, but also in non-obese individuals. This is important as prevention strategies to reduce CVD risk factors, such as obesity, which may increase inflammation could contribute to reverse cardiac remodelling in young individuals.

It is further recommended that future studies be conducted on the relationship between left ventricular remodelling and the leukocyte profile comparing a young population, where left ventricular hypertrophy is present, to a population with no evidence of left ventricular hypertrophy.

This study should be followed up to determine if changes in lifestyle factors, the leukocyte profile or LVMI occur, as left ventricular remodelling is known to be reversible in young individuals. In such a study, the age where left ventricular hypertrophy is most likely to develop, could also be explored.

Future studies are also recommended in larger areas in South Africa, with a larger population group, in order to increase statistical power.

## **Final conclusions**

This study indicated that LVMI inversely associated with monocytes and NLR in white individuals only. Unhealthy lifestyle choices in a young population may contribute to inflammation, leading to early vascular changes and in turn lead to increased LVMI. Adverse cardiac remodelling can however be reversible at a young age, thus suggesting that the focus should shift to prevention strategies to prevent risk factors for CVD, such as obesity and atherosclerotic plaque, in young and healthy individuals. The inverse association of LVMI with monocytes in the young population could however suggest a potential decline in monocytes that may contribute to early impaired cardiac repair, mediated by increases in blood pressure, especially in the setting of low-grade inflammation in this young, white South African group.

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# **Appendix A**

*Instructions for authors*

## International Journal of Cardiology

The following summary of author's instructions was adapted from:

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- The text should have  $\leq 3500$  words,  $\leq 50$  references and  $\leq$  four tables/figure
- Double spaced
- Structure of the article
  - Title page
  - Abstract
  - Keywords
  - Introduction
  - Methods
  - Results
  - Discussion
  - Acknowledgements
  - References
- Title page (page 1) should include
  - Title of  $\leq 25$  words
  - All authors along with a numbered footnote stating each author's academic affiliation followed by the following statement "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation" – any author unable to make this statement should state their specific contribution to the manuscript.
  - Corresponding author should be included followed by their contact details
  - Acknowledgement of grant support
  - Any possible conflict of interest
  - 3-6 keywords
- Page 2



- Abstract of  $\leq 250$  words with sufficient information
- Page 3 – the start of the main article, and should include the following sections:
  - Introduction
  - Methods
  - Statistical methods
  - Results
  - Discussion
  - Limitation subsection
  - Conclusion
  - References

Vancouver and should include 'author(s) initials and surname(s), article title/chapter title, journal title/book title, year of publication, volume and issue/book chapter and paginations.

All authors should be listed when  $\leq$  six, however more than seven authors, only the first three should be listed, followed by et al.

- Tables
  - Double spacing
  - Each table should be on a separate paper
  - Numbered sequentially with Arabic numbers
  - Can only contain horizontal lines
  - Must have a short descriptive heading at the top of the table and footnotes and/or explanations at the bottom of the table

# **Appendix B**

*Ethics approval*



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NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom,  
South Africa, 2520

Tel: (018) 299-4900

Web: <http://www.nwu.ac.za>

**Institutional Research Ethics Regulatory Committee**

Tel: +27 18 299 4849

Email: [Ethics@nwu.ac.za](mailto:Ethics@nwu.ac.za)

## ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Health Research Ethics Committee (HREC)** on **12/07/2017** after being reviewed at the meeting held on **14/06/2017**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

**Study title:** Exploring the link between left ventricular remodelling and the leukocyte profile of a young black and white population: The African-PREDICT study.

**Study Leader/Supervisor:** Prof R Kruger

**Student:** MI Kirstein-23394528

**Ethics number:**

N	W	U	-	0	0	0	6	8	-	1	7	-	A	1
Institution				Study Number						Year			Status	

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

**Application Type:** Single Study

**Commencement date:** 2017-07-12

**Risk:**

**Minimal**

**Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.**

### Special conditions of the approval (if applicable):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

### General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
  - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
  - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviation from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
  - request access to any information or data at any time during the course or after completion of the study;
  - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
    - any unethical principles or practices of the study are revealed or suspected,
    - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
    - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
    - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for further information or any report templates via [Ethics-HRECAppl@nwu.ac.za](mailto:Ethics-HRECAppl@nwu.ac.za) or 018 299 1206.

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

Yours sincerely

**Prof LA  
Du Plessis**

Digitally signed by  
Prof LA Du Plessis

Date: 2017.08.05  
11:32:33 +02'00'

**Prof Linda du Plessis**

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

# **Appendix C**

***Turn it in and language editor's report***

## Untitled

### ORIGINALITY REPORT

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STUDENT PAPERS

### PRIMARY SOURCES

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Submitted to North West University

Student Paper

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2

Caitlynd Myburgh, Hugo W. Huisman, Catharina M. C. Mels. "The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: the African-PREDICT study", Journal of Human Hypertension, 2018

Publication

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3

"Monday, 31 August 2015", European Heart Journal, 2015.

Publication

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4

Thompson, Jane E S, Wayne Smith, Lisa J Ware, Carina M C Mels, Johannes M van Rooyen, Hugo W Huisman, Leone Malan, Nico T Malan, Leandi Lammertyn, and Aletta E Schutte. "Masked hypertension and its associated cardiovascular risk in young individuals: the African-PREDICT study", Hypertension Research, 2015.

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8	Kriel, Johanna I., Carla M. T. Fourie, and Aletta E. Schutte. "Monocyte Chemoattractant Protein-1 and Large Artery Structure and Function in Young Individuals: The African-PREDICT Study", Journal of Clinical Hypertension, 2016. Publication	<1 %
9	Blessing Ahiante, Wayne Smith, Leandi Lammertyn, Aletta Schutte. "Leptin and its Relation to Autonomic Activity, Endothelial Cell Activation and Blood Pressure in a Young Black and White Population: The African-PREDICT study", Hormone and Metabolic Research, 2017 Publication	<1 %
10	Barnard, Sunelle A., Marlien Pieters, Cornelia Nienaber-Rousseau, and Herculina S. Kruger. "Degree of obesity influences the relationship	<1 %

of PAI-1 with body fat distribution and metabolic variables in African women", Thrombosis Research, 2016.

Publication

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<div style="background-color: #000080; color: white; display: inline-block; width: 30px; height: 30px; text-align: center; line-height: 30px;">11</div>	<p>Michél Strauss, Wayne Smith, Wen Wei, Olga V. Fedorova, Aletta E. Schutte.</p>	<p>&lt;1 %</p>
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"Marinobufagenin is related to elevated central and 24-h systolic blood pressures in young black women: the African-PREDICT Study", Hypertension Research, 2018

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2010.

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Karin Malmqvist. "Evaluation of various electrocardiographic criteria for left ventricular hypertrophy in patients with stable angina pectoris: influence of using modified limb electrodes", Clinical Physiology, 3/2001

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Aumann, Nicole, Sebastian E. Baumeister, André Werner, Henri Wallaschofski, Anke Hannemann, Matthias Nauck, Rainer Rettig, Stephan B. Felix, Marcus Dörr, Henry Völzke, Wolfgang Lieb, and Sylvia Stracke. "Inverse association of estimated cystatin C- and creatinine-based glomerular filtration rate with left ventricular mass: Results from the Study of Health in Pomerania", *International Journal of Cardiology*, 2012.

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Richard B. Devereux, Jonathan Bella, Kurt B. "Echocardiographic Left Ventricular Geometry in Hypertensive Patients with Electrocardiographic Left Ventricular Hypertrophy: The LIFE Study", *Blood Pressure*, 4/1/2001

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and its Relationship with Retinal Microvascular Calibres in Young Individuals: The African-PREDICT Study", Heart Lung and Circulation, 2016.

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- 51 K R Westerterp. "Body composition is associated with physical activity in daily life as measured using a triaxial accelerometer in both men and women", International Journal of Obesity, 08/2008

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Pan, Huanglei, Xingxing She, Hongli Wu, Jun Ma, Difeng Ren, and Jun Lu. "Long-term regulation of the local renin–angiotensin system in the myocardium of spontaneously hypertensive rats by feeding bioactive peptides derived from *Spirulina platensis*", *Journal of Agricultural and Food Chemistry*

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R B Devereux. "Association of left bundle branch block with left ventricular structure and function in hypertensive patients with left ventricular hypertrophy: the LIFE study", *Journal of Human Hypertension*, 06/2004

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Nabeel P M, Jayaraj Joseph, Mohanasankar Sivaprakasam. "A Magnetic Plethysmograph Probe for Local Pulse Wave Velocity Measurement", *IEEE Transactions on Biomedical Circuits and Systems*, 2017

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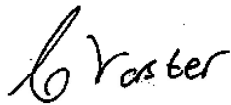
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## DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a dissertation written by Ms M Kirstein from the North-West University (student number 23394528).

Title of the dissertation: Exploring the link between left ventricular remodelling and the leukocyte profile of a young black and white population: The African PREDICT study



9 April 2018

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C Vorster

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Date