The Use of Ultrasonography for the Early Detection of Cardiovascular Risk in Adolescents.

By

Rabia Shah

Submitted in fulfilment for the degree

Magister Scientiae

(Medical Bioscience)

in the Department of Medical Bioscience, Faculty of Science

at the University of the Western Cape

Bellville South Africa

Supervisor: Prof. A. Oelofse

Co-supervisor: Dr JJ. De Smidt

DECLARATION

I, **Rabia Shah**, declare that **"The Use of Ultrasonography for the Early Detection of Cardiovascular Risks in Adolescents"** is my own work, that it has not been submitted before for any degree or assessment in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

27/01/2022

R Shah **Date Signed**

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Prof Andre Oelofse and Dr Juley de Smidt for all their support through this exceptional time in my life. For all their help, guidance, words of encouragement and just being there whenever I needed them. I was very fortunate to have supervisors like you.

I would then like to thank my parents for all their love and support throughout the years, never doubting me and always pushing me to go further. I love you.

Lastly, I'd like to thank my friends for being there through all my ups and downs with a shoulder to cry on and an ear to bite off. I appreciate you.

ABSTRACT

Cardiovascular disease (CVD) is the major cause of mortality and morbidity globally and is the leading cause of death in South Africa second to HIV/AIDS. CVD is on the rise not only in adults but also in children. The presence of modifiable CVD risk factors, such as, smoking, alcohol intake, diet quality, physical activity and obesity, during adolescence increases the chances of having a poor cardiovascular risk profile in adulthood. Therefore, the prevention of CVD in adolescents is important to prevent premature death from CVD. The aim of this study was to assess whether ultrasound measurements of intima-media thickness could indicate CVD risk and an association with modifiable risk factors in adolescent student population. A cross sectional study was conducted using ultrasonography to detect early increase in carotid and aorta intima-media thicknesses as an indication of early plaque formation in young adults aged 19 to 26. Additional measures of CVD risk were collected to seek associations with ultrasound findings. In the current study, 63 participants aged 19-26 years where included in the study by meeting the inclusion criteria. Anthropometric measurements, lipid profiling and ultrasound measurements of the abdominal aorta intima-media thickness (AIMT) and carotid intima-media thickness (CIMT) where recorded. Positive significant differences in low density lipoproteins (LDL) ($p = 0.018$) and waist-to-hip ratio ($p = 0.008$) were observed between males and females. Males had higher LDL (2.37±2), total cholesterol (4.37±3) and triglyceride (1.67±1.2) levels than females. Ultrasound measurements showed a positive significant association between age and the left CIMT ($p=0.025$). There was also a positive significance in the comparison of waist circumference and blood pressure ($p<0.001$), the right CIMT ($p=0.002$) and the left CIMT ($p<0.001$) as well as a significance between waist-hip ratio to blood pressure ($p<0.001$). The current study found that ultrasonography was a useful, non-

invasive tool to detect changes in the intima-media thickness of arteries indicating increased CVD risk in adolescents.

KEYWORDS**:** Cardiovascular Disease (CVD), Ultrasound, Adolescents, Abdominal Aorta Intima-media Thickness (AIMT), Carotid Intima-media Thickness (CIMT), Lipid Profile, Waist Circumference, Blood Pressure.

CONTENTS

DECLARATION....1

ACKNOWLEDGEMENTS....2

ABSTRACT....3

Keywords....4

ABBREVIATIONS....7

CHAPTER 1....8

INTRODUCTION....8

1.1. Background....8

1.2. Problem Statement....10

1.3. Rationale....10

1.4. Research Aims and Objectives....10

1.5. Hypothesis....11

CHAPTER 2....12

LITERATURE REVIEW....12

2.1. Coronary Artery Disease....12

2.2. Abdominal Aorta- And Carotid Intima-Media Thickness....14

2.3. CVD Risk Factors....18

2.4. Ultrasonography....28

CHAPTER 3....32

PROPOSED METHODOLOGY....32

3.1. Recruitment....32

3.2. Ethical Approval....32

3.3. Anthropometric Measurements....32

3.3.4. Skinfold Measurement....33

3.4. Blood Pressure Measurement....34

3.5. Haematology....34

3.6. Ultrasounds....34

3.7. Statistical Analyses....35

CHAPTER 4....37

RESULTS....37

4.1. Differences Between the Variables Measured..37

4.2. Correlation Between the Variables Whilst Controlling for Age, BMI, Smoking and Birth Weight 52

4.3. Relationships Between Means of Variables Compared to BMI (Grouped).......56

4.4. Waist and Waist-To-Hip Ratio Compared to Variables for Increased Risk of CVD...56

4.5. Chapter Summary 57

CHAPTER 5 58

DISCUSSION...58

5.1. Introduction 58

5.2. A Review on CIMT and AIMT with Regards to Age...59

5.3. Relationship Between BMI and Tested Variables 61

5.4. Relationship Between Blood Pressure and Lipid Profile and Blood Glucose Levels 62

5.5. The Role of Central Obesity (Increased Waist Circumference) and BMI, Blood Pressure (BP), Lipid Profile and Blood Glucose as Cardiometabolic Risk Factors.63

CONCLUSION...65

REFERENCES...66

ABBREVIATIONS

AIMT	Abdominal Aorta Intima-media Thickness.
BMI	Body Mass Index
CAD	Coronary Artery Disease
CIMT	Carotid Artery Intima-media thickness
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
HDL	High Density lipoproteins
IMT	Intima-media Thickness
LDL	Low Density Lipoproteins
PR	Pulse Rate
SBP	Systolic Blood Pressure
TC	Total Cholesterol
Trig	Triglycerides

CHAPTER 1

INTRODUCTION

1.1. Background

Cardiovascular disease (CVD) is the primary cause of morbidity and mortality in the world (Khanna et al., 2019) signifying 31% of all global deaths. In 2017, there were an estimated 422.7 million cases of CVD and 17.9 million CVD deaths worldwide (Roth et al., 2017). Out of the 17 million premature deaths (under the age of 70) due to non-communicable diseases in 2017, 82% were in low- and middle-income countries and 37% were caused by CVDs (Keates et al., 2017). In 2019 the stats remained the same with a 17.9 million on average death rate worldwide (Cardiovascular diseases (CVDs), 2021). In South Africa, CVD is the leading cause of death second to HIV/AIDS and responsible for almost 1 in 6 deaths (17,3%) annually. Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as smoking, unhealthy diets, obesity, physical inactivity and excessive consumption of alcohol (Msemburi et al., 2014). Those who are at high Cardiovascular risk require early detection and management through medication in order to reverse the effects of the disease (Roth et al., 2018).

Global death rates due to CVD are anticipated to increase as the occurrence of cardiac risk factors continue to increase. Although CVD generally manifests in adulthood, the process can begin in childhood (Brown et al., 2019). This was previously considered to be a major problem only in developed countries but has manifested in developing countries as well. This could be due to the notable increase in the prevalence of childhood obesity worldwide (Ho et al., 2019 and Keates et al., 2019). Paediatric cardiac death (PCD) occurs in about 1 to 6 per 100 000 children annually (Rodday et al., 2012). Prevention of CVD has been shown to be less costly than treating its complications (Barton et al., 2011), therefore, detection of this disease in the asymptomatic phase may have a great effect on public health and the cost thereof (Baber et al., 2015).

Historically, sub-Saharan Africa has reported low levels of atherosclerotic cardiovascular disease (CVD). Low levels of atherosclerotic CVD in populations of African descent were, in part, accredited to low levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) and high levels of high-density lipoprotein (HDL-C). However, as these populations undergo epidemiological transition; this may change (Sliwa et al., 2012) i.e. increased modern food consumption and lifestyle habits. It appears a great deal of the burden of CVD in those of African descent can be attributed to hypertension, rather than dyslipidaemia (Akinboboye & Akinkugbe, 2003).

A previous study showed that the degree of fatty streaks and plaques in the aorta and coronary arteries increased with age. The relationship between fatty streaks and plaques was much stronger in the coronary arteries ($r=0.60$, $P<0.001$) than in the aorta ($r=0.23$, $P=0.03$) (2-39 years) (Berenson, 1998). Studies have suggested that atherosclerosis beginning in childhood in the abdominal aorta and later involves the carotid arteries (Guzman et al., 1968). Screening for cardiac diseases, such as Atherosclerosis, through use of ultrasonography in asymptomatic children and adolescents should be considered a way to detect early signs of atherosclerosis due to its potential in measuring abdominal aorta intima-media thickness (AIMT) and carotid intima-media thickness (CIMT) (Dawson et al., 2009).

1.2. Problem Statement

Cardiovascular disease remains a major cause of death and disability. In addition, CVD is on the rise at a faster rate at earlier ages. Though CVD develops earlier, the disease remains asymptomatic in the majority of individuals. Ultrasound has successfully been used in detecting heart conditions in individuals already diagnosed with heart disease or who have previously had a heart attack. This study will aim to use this diagnostic technique in order to detect early signs of plaque build-up in arteries as indicated by an increase in the arterial intima-media thickness. Early detection of CVD will greatly contribute to prevent the increase in adolescent populations.

1.3. Rationale

CVD is a disease that is generally diagnosed after a patient has their first cardiac arrest at a later age in their life. Recent studies have shown that even though CVD is diagnosed later in life, the manifestation occurs in much earlier years. This was proven through autopsy studies on children and adolescents. Even though this was discovered, what was missing were studies on diagnostic tools for non-invasive detection of early signs of CVD. This paucity in research on preventive diagnostics prompted this study where non-invasive sonography, which can detect early signs of plaque and/or fatty streak build up in arteries. This allows for early lifestyle changes which may decrease CVD events.

1.4. Research Aims and Objectives

1.4.1. Aim

The aim of this study was to assess whether ultrasound measurements of intima-media thickness could indicate CVD risk and an association with modifiable risk factors in adolescent student population.

1.4.2. Objectives

Our objectives were:

- To use ultrasonography to detect increased aorta and carotid intima-media thickness indicating increased plaque formation in adolescents.
- To determine the blood lipid levels of the study population.
- To determine the relationship between BMI and IMT and lipid profile.
- To find a difference in the mean values of SFT, WC, BMI, IMT, Lipid profiles, BP between the sexes.

1.5. Hypothesis

Ultrasonography is an effective tool in detecting early signs of cardiovascular disease in adolescents.

Sex, age, body mass index (BMI), lipid profile and cholesterol influenced the intima-media thickness (IMT) of arteries in adolescents aged 19 to 26 years.

Ultrasonography of the intima-media artery walls is positively associated with the lipid profile of adolescents.

CHAPTER 2

LITERATURE REVIEW

2.1. Coronary Artery Disease

The most common form of CVD is coronary artery disease (CAD). Ischemic heart disease, i.e., atherosclerosis, is the leading cause of death in CVD health worldwide (Dong et al., 2022). Atherosclerosis begins in childhood with the build-up of lipid or plaque in the intima of arteries. Data from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study proved that 20% of 15- to 34-year-olds have increased fatty streaks in the abdominal aorta on autopsy and the presence of these lesions is related to cardiovascular risk factors. Predominantly after age 25, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) cholesterol levels showed a positively significant effect, and high-density lipoprotein (HDL) cholesterol levels showed a negative effect on fatty streaks in the aorta and right coronary artery (McGill and McMahan, 1998). The start of lipid build-up occurs in the aorta of practically every child over the age of 3 years (Holman et al., 1958). Later, autopsy studies in children have shown that atherosclerotic lesions begin to develop in the intima of the aorta before any other region of the vascular system (Dawson et al., 2009). McGill et al showed a tendency for atherosclerotic lesions to develop within the affected arteries of individuals aged 15 to 34 years (McGill et al., 2008). Atherosclerotic lesions in the coronary and carotid arteries occur later in life compared to its manifestation in the aorta (Guzman et al., 1968). Another study demonstrated that CAD began at a young age and that lesions were present in 1 of 6 teenagers. These findings voiced the need for intensive efforts of coronary disease prevention in adolescents (Tuzcu et al., 2001). Recent studies have focused on preventive actions for atherosclerosis beginning during childhood to reduce the risk of heart disease, stroke and peripheral vascular disease later in life (Davis et al., 2010). If prevention is to begin early in life, it would be useful to have a non-invasive method to detect early signs of atherosclerosis so that interventions could be focused on those at a higher risk (Slyper, 2004).

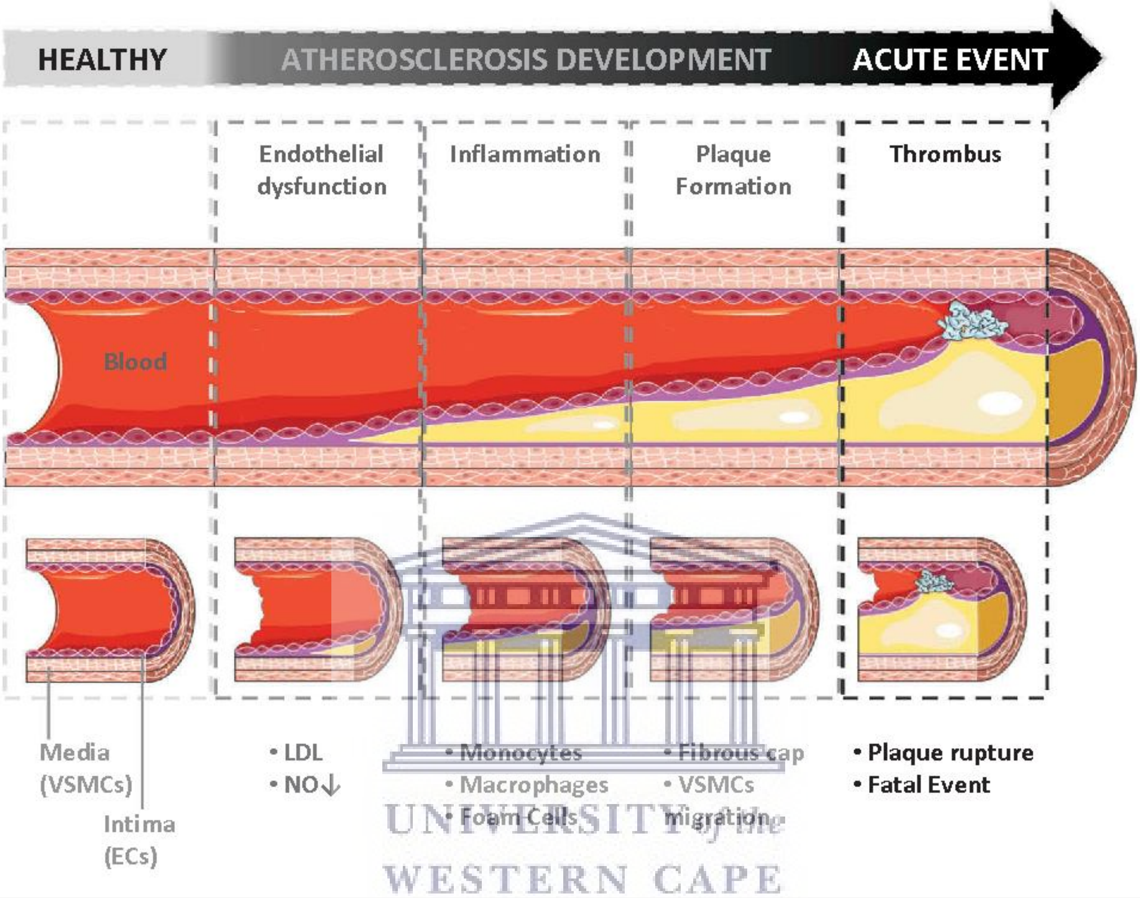


Figure 1: Schematic view of atherosclerosis progressive development inside the artery. Atherosclerosis development starts with an endothelial dysfunction, internalisation of monocytes in the intima and their differentiation to macrophages. VSMCs migrate from media layer to intima contributing to form a fibrous cap which surrounds the lipid core and thus constitute the plaque. The plaque could remain stable or release thrombogenic material which at the end triggers a fatal event. (EC: Endothelial cells; LDL: Low-density lipoprotein; NO: Nitric oxide; VSMCs: Vascular smooth muscle cells) (Martin-Lorenzo et al., 2015).

2.2. Abdominal Aorta- And Carotid Intima-Media Thickness

Atherosclerosis begins in childhood in the distal abdominal aorta and later involves the carotid arteries. Non-invasive screening to detect these lesions may allow early intervention (Davis et al., 2010). Developments in ultrasound techniques have made it possible to visualise early atherosclerotic lesions within the intima-media of the abdominal aorta and the carotid artery, noninvasively, in living asymptomatic patients (Järvisalo et al., 2001, Dawson et al., 2009). In older adults, this method has been commonly used to assess the carotid arteries and increased carotid intima-media thickness (CIMT) which may predict the early development of peripheral vascular disease, stroke and other cardiac problems. Dawson et al proved that both AIMT and CIMT are associated with cardiovascular risk factors. Measurement of AIMT and CIMT may help identify those at risk for premature cardiovascular disease (Dawson et al., 2009). In a case-control study of AIMT and CIMT conducted in children at high risk for CAD, 16 children with hypercholesterolemia and 44 with type 1 diabetes were compared with 28 healthy control subjects. There was a greater increase in AIMT than CIMT in high-risk children, although both were significantly thicker than in the control children (Jarvisalo et al., 2001).

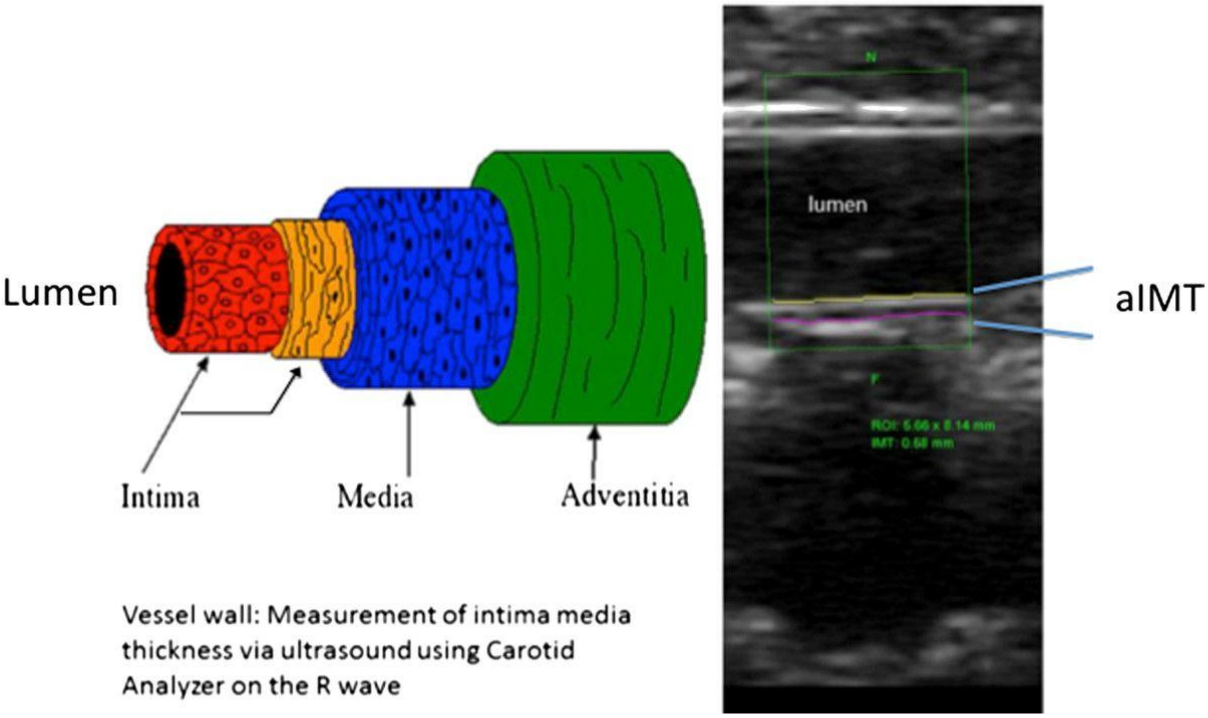


Figure 2: Vessel wall that is used for measurement of intima-media thickness via ultrasound, starting at the pink line and ending at the yellow line (West, 2005).

CIMT is associated with CVD risk factors (Young et al., 1960, Roman et al., 2006). It is proven that carotid intima-media thickness (CIMT) increases with advancing age, even in the absence of CAD, because of thickening of both the intimal and medial layers. In human beings, CIMT increases nearly 3-fold between the ages of 20 and 90 years (Nagai et al., 1998).

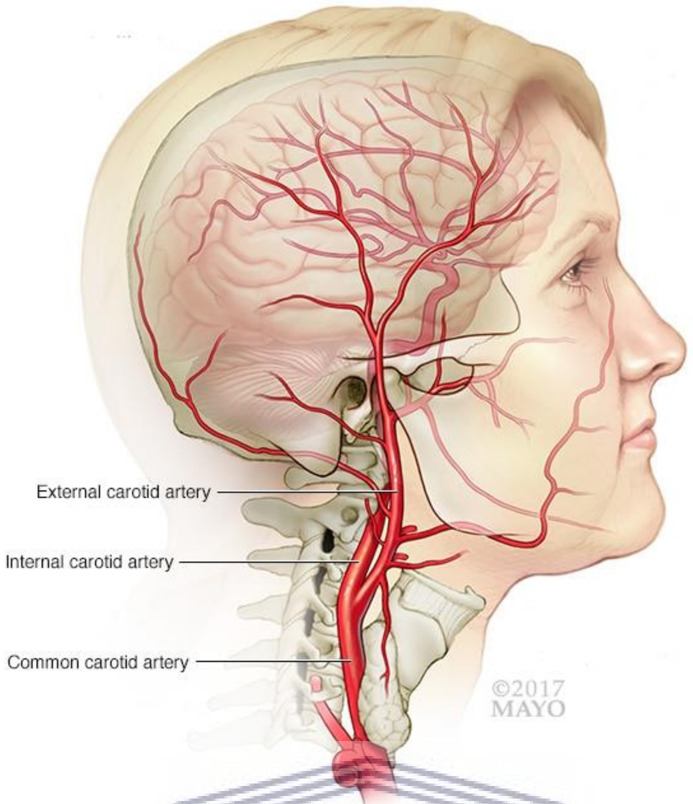


Figure 3: Image showing the Carotid Arteries.

Progression of AIMT and CIMT may be reduced or reversed with risk factor interventions which in turn reduces the risk of future CVD events (Hodis et al., 1998, Espeland et al., 2005). These findings provide support to the concept that CIMT measurements can be used as an indicator of CAD. Post-mortem studies indicate that age-associated increases in carotid wall thickening is mainly caused by an increase in intimal thickening (Virmani et al.,1991). These changes include many factors that have been implicated in the pathogenesis and progression of atherosclerotic plaques, such as endothelial dysfunction; increased endothelial cell adhesiveness and permeability; increases in procoagulant, vasoconstrictive, and inflammatory molecules; increases in cytokines and chemokines; increased oxidative stress and proliferation and migration of smooth muscle cells (Li et al.,1999, Asai et al., 2000). It represents subclinical vascular disease, the pathophysiologic substrate that explains why CIMT is a risk factor and a marker of CVD risk.

In a study by Dawson et al, a correlation was found between risk factors and AIMT and CIMT in two different age groups, teenagers (11-17 years) and adolescents (18-34 years). Mean (SD) values of AIMT and CIMT were 0.63 (0.14) and 0.49 (0.04) mm, respectively. In the teenage group, AIMT was associated with triglycerides, blood pressure, body mass index (BMI), and waist/hip ratio, after adjusting for age, sex, and height. In the adolescent group, AIMT was associated with those same 5 risk factors, plus HDL and pulse pressure. In the teenagers, CIMT was associated with systolic blood pressure (SBP), pulse pressure, heart rate, BMI, and waist/hip ratio. In adolescents, CIMT was associated with total cholesterol, LDL, triglycerides, SBP, diastolic blood pressure (DBP), BMI and waist/hip ratio (Dawson et al., 2009).

2.3. CVD Risk Factors

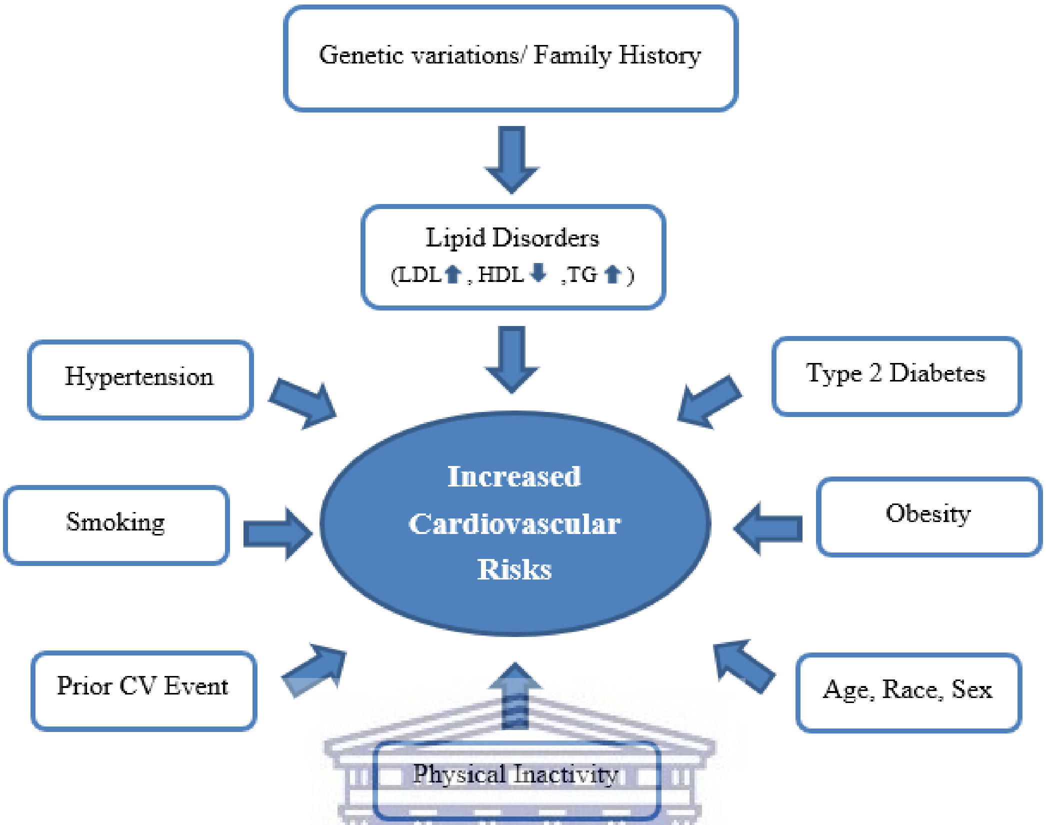


Figure 4: CVD risk factors.

2.3.1. Hypertension

Hypertension is one of the most important modifiable risk factors for cardiovascular diseases (CVD). Hypertension is the leading risk factor for stroke in South Africa, responsible for 1 in 2 (50%) strokes and 2 in 5 (42%) heart attacks (Norman et al., 2007). In South Africans, 1 in 3 of those 15 years and older have high blood pressure (Cape Town: Southern African Labour & Development Research Unit, 2009). To add to this problem, more than 50% of people with high blood pressure are unaware of their condition. Of the people diagnosed with high blood pressure, only a third is on treatment, and of those, only a third has adequate control of their blood pressure (Steyn et al., 2001). The complications of hypertension account for 9.4 million deaths worldwide every year. Hypertension is responsible for at least 45% of the deaths from CVD (Hien et al., 2018).

High blood pressure in children and adolescents is a growing health problem that is often goes unnoticed by doctors. Overweight and obesity are strongly related with primary hypertension in children. High blood pressure in childhood and adolescence may lead to hypertension in adult-hood (Chen and Wang, 2008). Children with hypertension could have evidence of target organ damage, including left ventricular hypertrophy and pathologic vascular changes (Sorof et al., 2003 and Brady et al., 2008). Previous studies have found a linear relationship between systolic blood pressure (SBP) and CIMT in both hypertensive and non-hypertensive individuals (Bots et al., 1993, Juonala et al., 2010, Ferreira et al., 2016 and Magnussen. 2017). A previous study described SBP and not diastolic blood pressure (DBP) as an independent predictor of increased CIMT (Di Bello et al., 2009). This is the reason only SBP (not DBP) was correlated with increased CIMT. Likewise, another study found that SBP was significantly associated with CIMT, even after adjustment for age, sex, and smoking. In that study, DBP was again not associated with CIMT measurement (Zanchetti et al., 2001). These findings have also been reported in other studies (Lusiani, Visonà and Pagnan, 1990, Mannami et al., 1997, Salonen and Salonen, 1999 and Lakka et al., 1999) and propose that SBP may prompt higher pressure overload and therefore induce more arterial hypertrophy or hyperplasia than DBP. Also, some authors claimed that SBP may be a more important risk factor for atherosclerosis and CVD than DBP (Mannami et al., 1997, Lakka et al., 1999 and Ferreira et al., 2016).

Juonala et al showed that children and adolescents with elevated blood pressure have higher adult CIMT, with the exclusion of other risk factors, compared to those without elevated blood pressure (Juonala et al., 2010, Juonala et al., 2013 and Ferreira et al., 2016). Clinical trials of antihypertensive medications have shown CIMT to regress or progress at a decreased rate among those receiving best therapy compared with those on placebo (Ferreira et al., 2016 and Magnussen. 2017). Providing evidence that introduction of a treatment may decrease the magnitude of disease later in life.

2.3.2. Lipid Profile

(mmol/l)	Normal	Intermediate	High Risk
LDL	<3.36	3.37-4.11	>4.12
HDL	>1.55	1.03-1.54	<1.02
Total Cholesterol	<4.1	4.2-6.2	>6.3
Triglycerides	<1.69	1.7-2.25	>2.26

Table 1: Criteria for cholesterol (Healthy Cholesterol Level; TC, HDL-C, LDL-C & TG Normal Ranges, 2012)

Risk factors associated with CVD include Total cholesterol, LDL, HDL, Triglycerides, Diastolic blood pressure (DBP), Systolic blood pressure (SBP), Pulse pressure, Heart rate, BMI, Waist/hip ratio and Smoking. A major CVD risk factor is dyslipidaemia which is said to be the primary cause of CVD, helping it to progress. The prevalence and pattern of dyslipidaemia differs between populations, ethnicities, geographic location, and socioeconomic development (Steinhagen-Thiessen et al., 2008 and Zhang et al., 2010). In 1901, Windaus revealed that the aortas of patients with atherosclerosis contain more cholesterol than aortas of healthy people (Windaus, 1910 and Keizer, 2012). Later in 1913, Anitschkow showed that feeding cholesterol to rabbits increased their plasma cholesterol and caused atherosclerosis. In subsequent years, he found that the process of atherosclerosis starts with the formation of fatty streaks. Fatty streaks consist of white blood cells which have penetrated the arterial wall. Later work of Anitschkow showed that these fatty streaks further develop into advanced lesions containing connective tissue. These lesions displayed a great comparison to early atherosclerotic lesions in humans (Anitschkow, 1913 and Keizer, 2012). A CVD susceptible lipid profile consists of high traces of triglycerides (TG) and low-density lipoprotein cholesterol (LDL) and decreased amounts of high-density lipoprotein cholesterol (HDL). Atherosclerosis is the primary cause of stroke and heart attack. Early observations that cholesterol is a key component of arterial plaques gave rise to the cholesterol hypothesis for the pathogenesis of atherosclerosis. The cholesterol hypothesis accepts that LDL is a main factor that gives rise to atherosclerosis. Population studies have established that increased levels of LDL cholesterol and apolipoprotein B (apoB) 100, the main structural protein of LDL, are directly associated with risk for atherosclerotic occurrences (Linton et al., 2019). Previous studies have demonstrated that LDL is the primary atherogenic lipoprotein (Nakamura et al., 2006 and Cannon et al., 2004) and that HDL is the predominant anti-atherosclerotic lipoprotein (Yang et al., 2014). Therefore, measurements of total cholesterol, HDL, and LDL are widely recommended for the early detection of plaque and/or fatty streak formation in arteries (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). In a past study, simvastatin was used to reduce plasma cholesterol levels. Simvastatin is an inhibitor of HMGCoA reductase, which is the rate limiting enzyme of the mevalonate pathway which is responsible for the formation of cholesterol. In the study plasma cholesterol was decreased by about 25% and reduced the risk of death due to coronary heart disease by 42% (Scandinavian Simvastatin Survival Study Group, 1994). Cheng et al also came to a similar conclusion where patients received complete CVD risk reduction therapy, delipidation of carotid plaque and decreases in CIMT mainly occurred within 2 years, and correlated with changes in risk factors/biomarkers (Cheng et al., 2016).

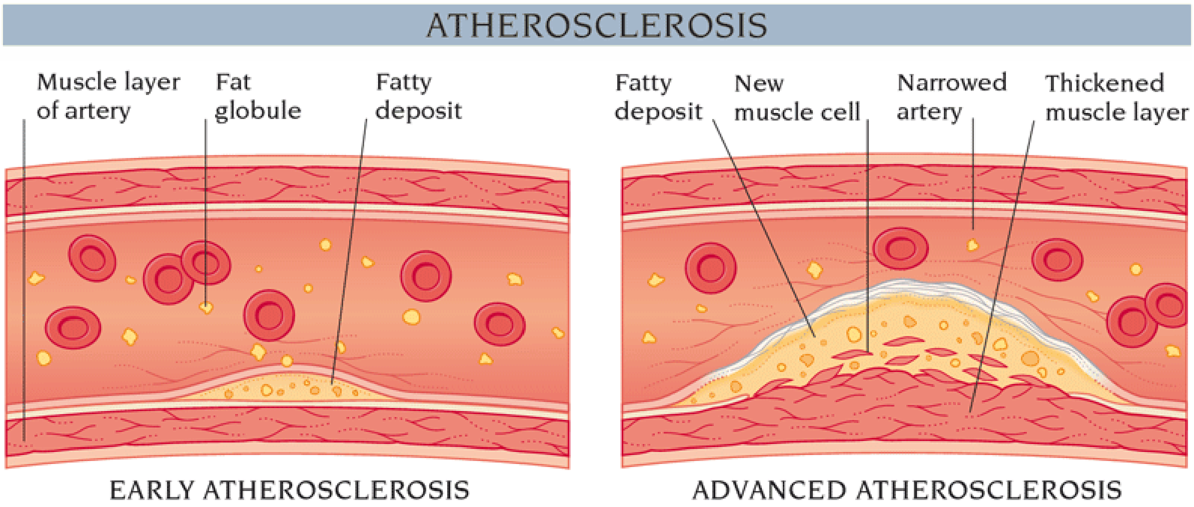


Figure 5: Figure showing advances in atherosclerosis, in an artery, from an early stage to a more advanced stage.

2.3.3. Ethnicity

Race is said to play a role in CVD development. The causes of ethnic difference in levels of CVD risk factors include genetic, environmental and cultural factors (Zaninotto et al., 2007). There are several factors that contribute to the development of dyslipidaemia, including genetic factors (Cohen et al., 1994) and acquired factors (Ruixing et al., 2008) such as overweight and obesity (Brown et al., 2000), physical inactivity (Berg et al., 1997; Hardman, 1999), cigarette smoking (Arslan et al., 2008; Batic-Mujanovic et al., 2008), high fat intake (Hennig et al., 2001; Tanasescu et al., 2004), very high carbohydrate diets (> 60% of total energy) (McNamara and Howell 1992) and certain drugs (such as beta-blockers, anabolic steroids and progestational agents) (Middeke et al., 1990; Stone 1994). Caucasians usually have higher mean total cholesterol (TC) concentrations than people of African descent (Tolonen et al., 2005). African men have been proven to exhibit a more favourable lipid profile compared with Caucasian men, regardless of having the highest overall death rates from CVD. African men have similar or lower LDL and TG but higher HDL levels compared with Caucasian men in general. The difference in TG may be related to increased activity of lipoprotein lipase in African individuals

(Sumner et al., 2005). Lipids and lipoproteins abnormalities are major metabolic disorders, which commonly include increased levels of TC, LDL and TG and decreased levels of HDL. Total fat (and saturated fat) intake has been shown to adversely affect total cholesterol concentrations in children, adolescents, and young adults (Post et al., 1997).

2.3.4. Type 2 Diabetes

A previous study showed that in most of the ethnic groups, individuals detected with undiagnosed diabetes had a worse lipid profile than diagnosed cases. Ethnic differences in the risk of CVD and type 2 diabetes have consistently been identified, with the most studies comparing the risk between African and Caucasian individuals. Age-, cohort- and BMI adjusted mean TC, LDL and TG increased while the mean HDL decreased with more pronounced glucose intolerance in most of the ethnic groups in individuals without a prior history of diabetes. 65% to 80% of people with diabetes will die from heart disease (Grundy et al., 1999). Compared to people without diabetes, people with type 1 and type 2 (especially women) are at higher risk of developing heart disease, which is usually asymptomatic, and at an earlier age. Type 2 diabetes is appearing as a new clinical hurdle within paediatric practice. Insulin resistance is the anticipated link between obesity and vascular stiffness (Montagnani and Quon, 2000). Although, there continues to be an increase in the frequency of type 2 diabetes mellitus in children and adolescents even if the incidence of obesity does not rise (Reinehr, 2013). In patients with type 2 diabetes, it is most commonly characterised by elevated TG and reduced HDL (Kendall, 2005). Cross-sectional studies have found positive associations of atherosclerotic vascular disease with TC (Jurado et al., 2009), LDL (Agarwal et al., 2009; Jurado et al., 2009), non-HDL (Jurado et al., 2009), and TG (Gomes et al., 2009), but inverse associations with HDL (Smaoui et al., 2004; Gomes et al., 2009; Jurado et al., 2009). The United Kingdom Prospective Diabetes study (UKPDS) has established that elevated LDL and decreased HDL are potentially modifiable risk factors for coronary artery disease (CAD) in patients with type 2 diabetes (Turner et al., 1998). Results from the Multiple Risk Factor Intervention Trial (MRFIT) (Stamler et al., 1993), in which 356,499 non-diabetic and 5163 diabetic men without CVD at baseline were studied for 12 years, indicated that serum cholesterol is an independent predictor of CVD mortality in men with diabetes. Rosengren et al., (1989) showed similar results in a study of 6897 middle aged diabetic men. Patients with TC > 7.3 mmol/l had a significantly higher incidence of CVD during the 7-year follow up than those with TC ≤ 5.5 mmol/l (28.3% vs. 5.4%, $p<0.05$).

2.3.5. Anthropometry

The most basic method of assessing body composition are anthropometric measurements (Roche, 1996). Anthropometric measurements describe body mass, size, shape, and level of fatness. Because body size changes with weight gain, anthropometry provides a suitable estimate of the general adiposity of an individual.

Body weight is the most frequently used measure of obesity. One would say that a person with a high body weight typically has higher amounts of body fat. However, weight changes with age in children as they grow and in adults as fat accumulates. Increased body mass and adiposity has been associated with increased intima-media thickness in children and teenagers. This association is present in children not considered overweight, highlighting the need for the continued promotion of adequate nutritional and physical exercise behaviour during childhood (Levitan, Wolk and Mittleman, 2009).

Body weight taken without other measures of body size can be misleading because a person's weight is greatly correlated to their build. Stature is measured simply with a range of wallmounted equipment (Chumla et al., 1998). One way to overcome the lack of specificity in body weight is to use the body mass index (BMI). BMI is a descriptive guide of the physique of the body that contains both the lean and the obese (WHO, 1995) and is formulated as weight divided by height squared (kg/m^2). Overweight is defined as a BMI of 25.0 to 29.9 kg/m^2 (WHO, 1998) obesity is defined as a BMI $\geq$30.0 kg/m^2 (Chumlea and Guo, 2000). An important advantage of BMI is the availability of extensive national reference data and its recognised connections with levels of body fatness, morbidity, and mortality in adults (WHO, 1995). BMI is mainly useful in monitoring the treatment of obesity, with a weight change of about 3.5 kg required to produce a unit change in BMI.

Obesity is frequently associated with increased amounts of intra-abdominal fat. Abdominal obesity has been shown to be a risk factor for CVD worldwide. Obesity may be associated with hypertension, dyslipidemia, diabetes, or insulin resistance, and elevated levels of fibrinogen and C-reactive protein, all of which are associated with an increased risk of cardiovascular disease (Akil and Ahmad, 2011). A generalised fat pattern is linked with the occurrence of both intra-abdominal (visceral fat) and subcutaneous abdominal adipose tissue (Smith et al., 2001). Abdominal circumference is an imperfect indicator of intra-abdominal adipose tissue, as it consists of subcutaneous fat deposition, as well as visceral adipose tissue (Pouliot et al., 1994). Persons in the upper percentiles for abdominal circumference are considered obese and at increased risk for morbidity, specifically type 2 diabetes, and the metabolic syndrome as well as mortality (Nicklas et al., 2004). There has been a steady increase in the prevalence of high abdominal circumference in the general population from 10% to 20% in the 1960s to between 40% and 60% in the year 2000 (Okosun et al., 2004). Circumferences of other body segments such as the arm and leg are possible (Lohman et al., 1992), but there are few reference data available for comparative purposes. Furthermore, the calculation of fat and muscle areas of the arm is not accurate or valid in the obese.

Criteria for Abdominal Circumference		
Risk Category	**Females**	**Males**
Very Low	<70cm	<80cm
Low	70-89cm	80-99cm
High	90-109cm	100-120cm
Very High	>110cm	>120cm

Table 2: Criteria for Abdominal Circumference (Lean, Han and Morrison, 1995).

Visceral fat likely contributes to increases in systemic inflammation and insulin resistance. A research group discovered that visceral fat in the abdomen was secreting high levels interleukin-6 (IL-6) into portal vein blood. Increased IL-6 levels correlated with concentrations of an inflammatory substance called C-reactive protein (CRP) in the body. High CRP levels are related to inflammation, and chronic inflammation is associated with insulin resistance, hypertension, type 2 diabetes, atherosclerosis, and cancer, among other things (Dryden, 2007). The strong relationship between inflammation and cancer is shown by the high IL-6 levels in the tumour microenvironment, where it promotes tumour growth by regulating all factors for cancer metastasis. IL-6 protects the cancer cells from therapy-induced DNA damage, oxidative stress and apoptosis by enabling the repair and initiation of countersignalling (antioxidant and anti-apoptotic/pro-survival) pathways (Kumari et al., 2016).

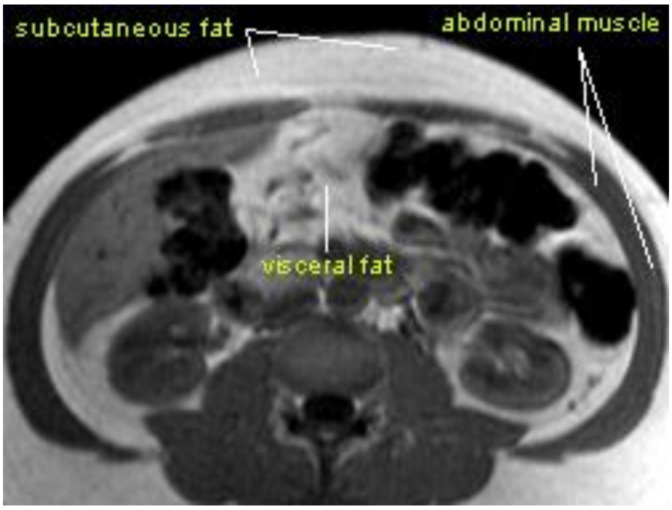


Figure 6: In this abdominal MRI scan, it is possible to see subcutaneous fat around the abdomen, surrounding abdominal muscles. Visceral fat is deeper inside the abdomen, surrounding internal organs. It is the visceral fat that secretes IL-6, strongly suggesting a mechanistic link to systemic inflammation (Dryden, 2007).

The ratio of abdominal circumference (often referred to incorrectly as "waist" circumference) to hip circumference is a rudimentary index for describing adipose tissue distribution or fat patterning (Chumlea et al., 1992). Abdomen-to-hip ratios greater than 0.85 represent a centralised distribution of fat. Most men with a ratio greater than 1.0 and women with a ratio greater than 0.85 are at increased risk for cardiovascular disease and diabetes (Seidell et al., 1987).

Body fat is stored in the connective tissue known as adipose tissue. Body fat is thus a useful metric to track the progress of an exercise and diet regimen. Skinfold measurements are used to characterise subcutaneous fat thickness at various regions of the body, but it should be noted that it has limited value in the overweight or obese adult. A few skinfold callipers take large measurements, but this is not a significant improvement due to the difficulty of grasping and holding a large skinfold while reading the calliper dial. The majority of national reference data available are for skinfolds at the triceps and subscapular locations. The triceps skinfold varies considerably by gender and can reflect changes in the underlying triceps muscle rather than an actual change in body fatness. Skinfolds are particularly useful in monitoring changes in fatness in children because of their small body size, and most fat is subcutaneous even in obese children (Malina and Bouchard, 1988), the statistical relationships between skinfolds and percent or total body fat in children and adults are often not as strong as that of BMI (Roche et al., 1981). Also, the true upper distribution of subcutaneous fat measurements remains unknown because most obese children and adults have not had their skinfolds measured.

2.4. Ultrasonography

Since the method was first proposed in the mid-1980s (Pignoli et al., 1986), the ultrasonic evaluation of the intimal layers of the common carotid arteries have gathered significant scientific and clinical support as an early, preclinical, vascular marker (Magnussen, 2017). Ultrasound imaging, also called ultrasound scanning or sonography, involves the use of a small transducer (probe) and ultrasound gel placed directly on the skin. High-frequency sound waves are transmitted from the probe through the gel into the body. The transducer collects the sounds that bounce back and a computer then uses those sound waves to create an image. Ultrasound examinations do not use ionising radiation (as used in x-rays), thus there is no radiation exposure to the patient. Because ultrasound images are captured in real-time, they can show the structure and movement of the body's internal organs, as well as blood flowing through blood vessels. Ultrasonography can be used as a tool to assess the structural and functional properties of the ventricles, including ventricular masses, volumes, and function (Moran and Thomson, 2020). The carotid arteries and abdominal aorta allows itself to be studied by high-resolution ultrasound devices because of its superficial location, is quite stationary, and runs parallel to the surface of the neck. Atherosclerotic plaque can be defined as a measured protrusion of the intima-media thickness (IMT) of >1.5 mm into the lumen (Polak et al., 2011). Intima-media thickness (IMT) is the area of tissue starting at the luminal edge of the artery and ending at the boundary between media and adventitia, showing a double-line pattern Figure 7). Using B-mode ultrasound is useful in representing the combined width of the carotid artery intima and media that can be visualized in nearly all subjects (Polak et al., 2010). Some studies have also indicated that CIMT <0.8 mm is associated with normal healthy individuals, and a value of CIMT at or above 1 mm is associated with atherosclerosis and a significantly increased CVD risk in any age group (Simon et al., 2002). Kumar et al., 2009 observed that CIMT of healthy controls were 0.73 mm and < 0.7 mm, respectively.

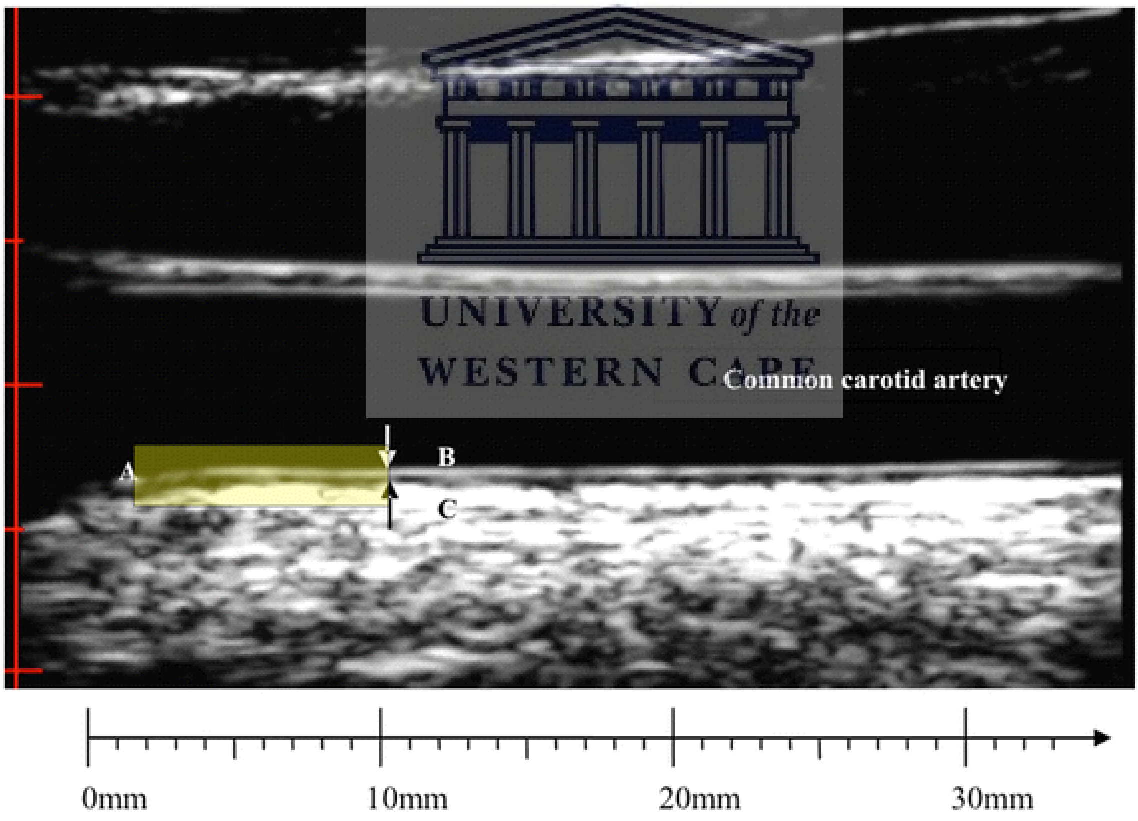


Figure 7: An ultrasound image of the common carotid artery (CCA): (**a**) border of carotid bulb widening (0 mm), (**b**) CCA far wall lumen-intima interface, (**c**) media-adventitia interface. Definitions of the CCA segment for measurement differ depending on study and convention

but are typically measured in the vicinity 0–10 mm proximal to the border of the bulb widening (**a**) (measurement area highlighted by *yellow box*). CCA IMT is taken as the distance between (**b**) and (**c**) (Magnussen, 2017).

In 1986, Italian investigators described that a characteristic B mode image of the arterial wall composed of two parallel echogenic lines separated by a hypoechoic space. The distance between the two lines did not differ significantly from the intima–media thickness (IMT) measured on pathologic examination, leading the investigators to suggest that B mode imaging could present a useful approach to the measurement of IMT in vivo (O'Leary and Bots, 2010). In another study of 1118 Spanish patients over 30 years of age, Aguilar-Shea et al identified 320 subjects as low-intermediate cardiovascular risk using the European Systematic Coronary Risk Evaluation (SCORE). After an ultrasound was done, 18.4% of the subjects were reclassified to the high-risk category, suggesting that CIMT measurement could be a useful preventive tool (Aguilar-Shea et al., 2011). An article by Hecht has further addressed the importance of imaging tools in the primary prevention of atherosclerosis (Hecht, 2011). Carotid arteries are usually narrowed by a build-up of plaque which is made up of fat, cholesterol, calcium and other substances that circulate in the bloodstream. Early diagnosis and treatment of a narrowed artery can decrease stroke risk. Doctors may recommend a carotid ultrasound if you have medical conditions that increase the risk of stroke, including: High blood pressure, diabetes, high cholesterol, family history of stroke or heart disease, recent ischemic attack or stroke, abnormal sound in carotid arteries, detected by your doctor using a stethoscope and coronary artery disease.

The major advantages of ultrasonography are that it can be repeated as often as required, is entirely non-invasive and it provides a continuous measure, since all subjects have a measurable artery wall. It is also moderately inexpensive to perform, and the technology is widely available. Increased IMT has been shown consistently. Ultrasound imaging can contribute to the detection of atherosclerosis by detecting advanced and early plaques, evaluating the consequences of atherosclerosis in the vessel wall, and measuring plaque neovascularization. For all these reasons, external ultrasound should likely continue to be amongst the most widely employed and popular imaging techniques for the prediction of future vascular events and quantification and monitoring of early detection of atherosclerosis helping to prevent CVD later in life.

CHAPTER 3

PROPOSED METHODOLOGY

3.1. Recruitment

63 Male and female participants aged 19 to 26 was recruited from The University of the Western Cape in Cape Town, South Africa. A cross-sectional and retrospective study was designed to determine if ultrasound is an appropriate tool in detecting changes in the IMT of adolescents, as well as how IMT is changed by lipid profiles and anthropometric measurements. Those with previous or current cardiovascular, systemic or metabolic disease or on medication for such diseases (except oral contraceptives, hormone replacement therapy or oral incontinence medication) were excluded from the study. In addition, those that were on specific diets known to impact the test results, were pregnant or anaemic were also be excluded. Written consent was obtained from each participant.

3.2. Ethical Approval

Ethical approval was obtained by the ethics committee of the University of the Western Cape (#14/9/54).

3.3. Anthropometric Measurements

3.3.1. Weight measurement

Weight measurements was taken using an electric scale (scale name and accuracy to 0.1 kg) (Frisard et al., 2005). BMI was then calculated using calculator.net with the formula: BMI = kg/m^2.

3.3.2. Abdominal and Hip Circumference

All measurements were measured while the subjects were wearing light clothing and not wearing shoes. Waist and hip circumference measurements was taken using a flexible 1 cm wide tape. The waist circumference was measured at the midpoint between the lateral iliac crest and the lowest rib, and the hip circumference was measured at the maximal protrusion of the greater trochanter.

3.3.3. Height Measurement

The height of each participant was measured using a stadiometer attached to a wall. The participants were wearing no shoes and/or caps. The measurements were done to the nearest 0.01 m.

3.3.4. Skinfold Measurement

Skinfold measurements was done using a skinfold calliper.

We measured:

Triceps skinfold thickness - We had the participant bend the elbow to 90 degrees and mark the point midway between the top of the shoulder and elbow. Then, measured a vertical fold (with the callipers at a 90° angle) was measured at that midway point with the arm hanging naturally at the subject's side.

Subscapular - Measurements of the subscapular area were taken as a diagonal fold (callipers held at a 45° angle) across the back, just below the shoulder blade.

3.4. Blood Pressure Measurement

BP was measured 3 times in all participants, at 1-minute intervals, using the Microlife electronic sphygmomanometer. The monitoring cuff was placed around the participant's non-dominant arm. BP was calculated as the mean of the 3 measurements.

3.5. Haematology

Fasting blood sample were drawn by me, the researcher, through the finger prick technique. This sample was taken for measurement of serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) using CardioCheck Plus by pts Diagnostics. Estimated values of serum LDL was calculated from the TC, TG and HDL, using Frieldwald's equation (Frieldwald et al., 1972).

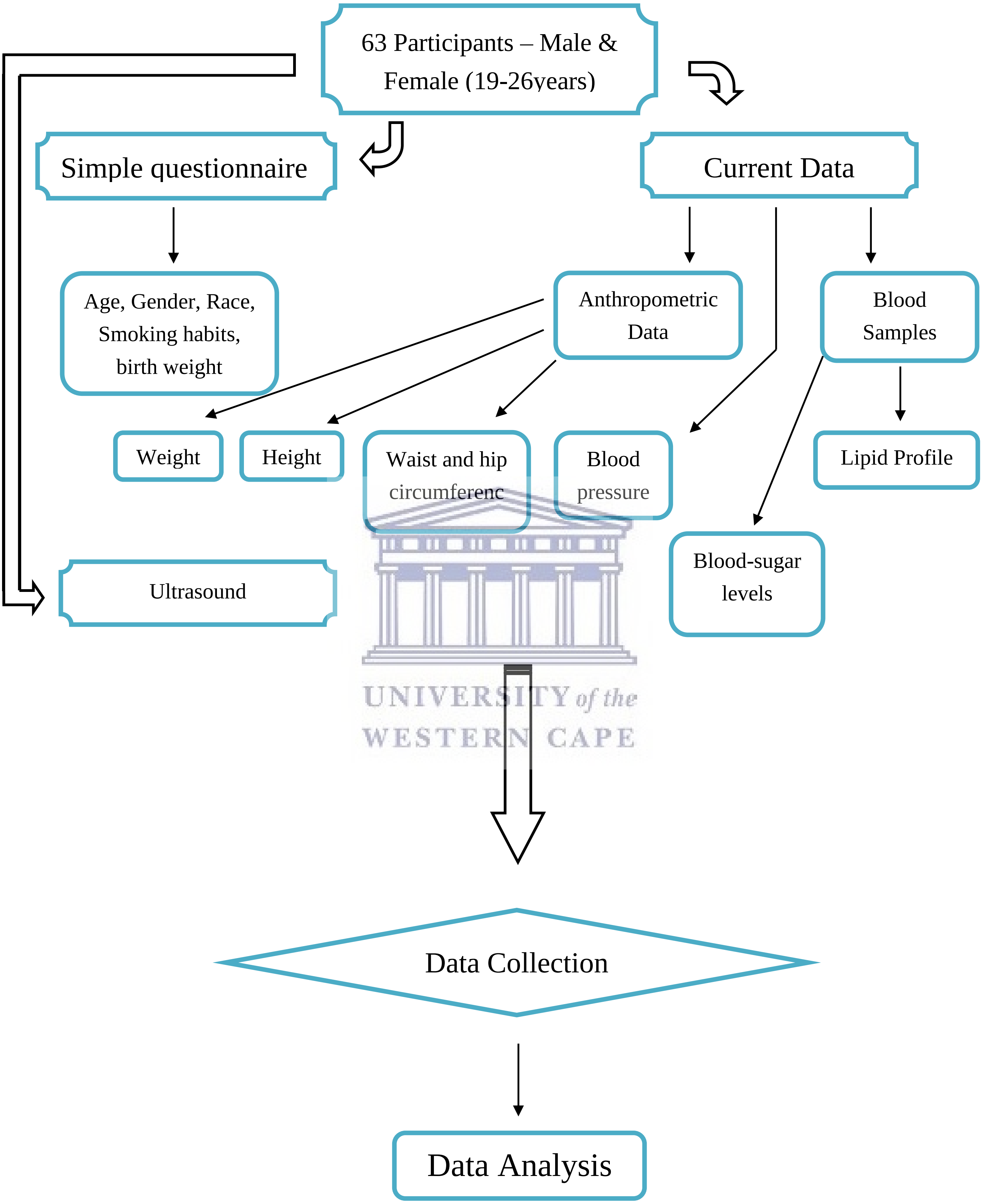
3.6. Ultrasounds

For ultrasound of the carotid artery and abdominal aorta, the participant was asked to avoid eating for 8 to 12 hours before the test. The participant was positioned lying in the supine position on an examination table. Participants may have been turned to either side to improve the quality of the images. After being positioned on the examination table, sonographer applied a warm water-based gel to the area of the body being studied. The gel helped the transducer make secure contact with the body and eliminated air pockets between the transducer and the skin that can block the sound waves from passing into the body. The transducer was placed on the body and moved back and forth over the area of interest until the desired images were captured. There is usually no discomfort from pressure as the transducer is pressed against the area being examined.

3.7. Statistical Analyses

Data were compiled in MS excel Office 2010 and analysis was performed using the Statistical Package for Social Science (SPSS) version 22. Descriptive statistics was generated to describe the relationships between the different variables in order to verify a common ground between the results, proving the hypothesis correct or invalid. Data were expressed as mean values ± standard deviations (SD) for continuous variables. T-test was used to compare means between groups. Pearson correlation was used to study the relationship between the variables. P-value of <0.05 was considered to be significant.

Methods (compacted)



CHAPTER 4

Results

The total number of participants for this study was 63. There were 17 males (27%) and 46 females (73%) in the sample. The mean ages of males and female subjects were 21.2 ± 2.5 and 21.6 ± 2.1 years, respectively. Among subjects 29% of males and 37% of females had high BMI values and 24% were smokers but none were diabetic. The mean BMI values were 24.60 ± 5.23 kg/m2 and mean WC values of 77.43 ± 14.50 cm.

The mean values of LDL cholesterol, HDL cholesterol, TC and Glucose in mg/dl among all the subjects (entire group) were 1.88 ± 1.22 and 1.41 ± 0.58, 3.74 ± 1.69 and 4.21 ± 0.62, respectively. And the mean values of right CIMT, left CIMT, and AIMT in mm among all the subjects (entire group) were 0.64 ± 0.12 and 0.63 ± 0.11, 1.06 ± 0.23, respectively, as shown in Table 4.1.

4.1. Differences Between the Variables Measured

Table 4.1. Mean values for anthropometric ,clinical and ultrasound measurements for the whole group

Variables	Mean	Standard Deviation	Median	Min	Max
Hip (cm)	100.659±	10.764	98.750	78.5	121
Waist (cm)	77.429±	14.495	74.250	61.5	116
Skinfold Arm (cm)	19.786±	8.848	19	4	45
Skinfold Scapular (cm)	18.889±	9.487	17	7	47

BMI (kg/m2)	24.5738±	5.228	23.7	17.2	36
LDL (mg/dl)	1.8807±	1.218	1.7	0.03	7
HDL (mg/dl)	1.4123±	.583	1.29	.72	3.11
Cholesterol (mg/dl)	3.7403±	1.684	3.54	0.89	10.36
Triglycerides (mg/dl)	1.4119±	1.071	1.03	0.7	4.41
Glucose (mg/dl)	4.2115±	.619	4.2	3	6
Right Carotid Artery (mm)	.640±	.115	0.6	0.5	1.1
Left Carotid Artery (mm)	.628±	.111	0.6	0.4	1
Abdominal Aorta (mm)	1.055±	.227	1	0.8	1.8

T-tests were used to determine the differences between the variables measured. As per table 4.2, skin fold thickness ($p<0.001$) measurements were significantly higher in females compared to males. It was observed that there was not a significant difference between the gender of participants except for skinfold thicknesses of the arm ($p<0.001$), females have more subcutaneous fat compared to males.

Table 4.2. Differences between waist hip ratio, triceps and subscapular skinfold thickness, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate (PR), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (Trig), total cholesterol (TC), glucose, abdominal aorta, right carotid vein and left carotid vein.

Variables	**Male**			**Female**			**Sig.**
	N	**Mean ± SD**	**CI (95%)**	**N**	**Mean ± SD**	**CI (95%)**	**(Between groups)**
Hip (cm)	17	98.85±9.5	93.98(103.73)	46	101.33±11.2	98(104.66)	.423
Waist (cm)	17	78.65±11	4.69(64.77)	46	78.44±12.4	74.76(82.11)	.951
Skinfold Arm (mm)	17	12±6.1	1.48(8.86)	46	22.66±8	1.17(20.3)	**<.001**
Skinfold Scapula (mm)	17	13.79±6.7	1.62(10.36)	46	20.77±9.7	1.43(17.88)	**.008**
BMI (kg/m^2)	17	23.29±4.7	1.13(20.89)	46	25.05±5.4	23.45(26.65)	.238
SBP (mmHg)	17	127.59±18.8	117.91(137.27)	46	120.63±15.2	116.12(125.14)	.136
DBP (mmHg)	17	82.71±12.8	76.12(89.29)	46	77.22±9.8	74.32(80.11)	.074
PR (min)	17	75.06±13.8	67.97(82.15)	46	80.07±10.8	76.85(83.28)	.136
LDL (mg/dl)	16	2.37±2	1.31(3.42)	42	1.7±0.7	1.48(1.91)	.060
HDL (mg/dl)	17	1.58±0.8	1.16(1.2)	44	1.35±0.5	1.2(1.49)	.173

Trig (mg/dl)	17	1.67±1.2	1.04(2.3)	44	1.31±1	1.01(1.62)	.246
TC (mg/dl)	17	4.37±3	2.85(5.89)	44	3.5±0.7	3.29(3.71)	.071
Glucose (mg/dl)	17	4.4±0.7	4.03(4.77)	44	4.14±0.6	3.97(4.31)	.141
Right CIMT (mm)	16	0.68±0.2	0.6(0.76)	43	0.63±0.1	0.6(0.66)	.102
Left CIMT (mm)	16	0.66±0.1	0.58(0.73)	43	0.62±0.1	0.59(0.65)	.252
Abdominal AIMT (mm)	16	1.09±0.3	0.95(1.24)	43	1.05±0.2	0.99(1.11)	.499

Abbreviations: BMI- Body Mass Index, SBP- Systolic Blood Pressure, DBP- Diastolic Blood Pressure, PR- Pulse Rate, LDL- Low Density Lipoproteins , HDL- High Density lipoproteins, Trig- Triglycerides, TC- Total Cholesterol, CIMT- Carotid Artery Intima-media thickness, AIMT- Abdominal Aorta Intima-media Thickness.

There was a significant positive correlation between the following variables whilst controlling for gender: Right CIMT and BMI (r^2=0.092, p=0.020), Right CIMT and LDL (r^2=0.076, p=0.044), Right CIMT and HDL (r^2=0.087, p=0.026), Right CIMT and Cholesterol (r^2=0.018, p<0.001), Right CIMT and Triglycerides (Trig) (r^2=0.18, p<0.001). Left CIMT and BMI (r^2=0.081, p=0.030), Left CIMT and LDL (r^2=0.12, p=0.009), Left CIMT and HDL (r^2=0.08, p=0.031), Left CIMT, Left CIMT and Cholesterol (TC) (r^2=0.18, p<0.001), and Triglycerides (r^2=0.07, p=0.049). Abdominal AIMT and Triglycerides (r^2=0.08, p=0.032), according to Table 4.3.

Table 4.3. Bivariate Correlation of variables whilst controlling for gender

		BMI	LDL	HDL	TC	Trig	Glucose
Right CIMT	**R**	.303	.275	.295	.428	.423	.144
	Sig.	**.020**	**.044**	**.026**	**.001**	**.001**	.286
Left CIMT	**R**	.284	.350	.286	.423	.263	.082
	Sig.	**.030**	**.009**	**.031**	**.001**	**.049**	.546
Abdominal AIMT	**R**	.119	.073	-.004	.048	.285	.007
	Sig.	.371	.599	.977	.725	**.032**	.959

Abbreviations: CIMT- Carotid Artery Intima-media thickness, AIMT- Abdominal Aorta Intima-media Thickness.

There was a significant positive correlation between the following variables whilst controlling for gender and BMI: Right CIMT and Cholesterol (r^2=0.18, p=0.002), Left CIMT and Cholesterol (TC) (r^2=0.14, p=0.007), Left CIMT and Right CIMT (r^2=0.45, p=0.001) and

Abdominal AIMT and Left CIMT (r^2=0.1, p=0.026), HDL and LDL (r^2=0.22, p=0.001), Cholesterol and LDL (r^2=0.76, p=0.001), Cholesterol and HDL (r^2=0.42, p=0.001), Glucose and LDL (r^2=0.4823, p=0.001) and Glucose and Cholesterol (r^2=0.17, p=0.003), according to Table 4.4.

Table 4.4. Correlation of variables while controlling for the effect of gender and BMI

Control Variables			**LDL**	**HDL**	**TC**	**Trig**	**Glucose**
Gender & BMI	**Right CIMT**	**r**	.172	.270	.342	.416	.040
		sig	.224	.053	**.013**	**.002**	.776
	Left CIMT	**r**	.272	.270	.372	.241	.021
		sig	.051	.053	**.007**	.086	.880
	Abdominal AIMT	**r**	.009	-.072	-.038	.166	-.046
		sig	.949	.610	.789	.238	.747

There was a significant positive correlation between the following variables whilst controlling for gender: Glucose and LDL (r^2=0.24, p=0.001), Glucose and Cholesterol (r^2=0.18, p=0.002), Right CIMT and Cholesterol (r^2=0.15, p=0.004), Right CIMT and Triglycerides (r^2=0.21, p=0.001), Left CIMT and LDL (r^2=0.1, p=0.022), Left CIMT and Cholesterol (r^2=0.17, p=0.002), Left CIMT and Triglycerides (r^2=0.09, p=0.031), Left CIMT and Right CIMT (r^2=0.5, p=0.001), Scapula Skinfold and Tricep Skinfold (r^2=0.71, p=0.001), HDL and LDL (r^2=0.22, p=0.001), Cholesterol and Weight (r^2=0.1, p=0.019), Cholesterol and LDL (r^2=0.77, p=0.001), Cholesterol and HDL (r^2=0.41, p=0.001), Abdominal AIMT and Tricep Skinfold (r^2=0.13, p=0.011) and Abdominal AIMT and Left CIMT (r^2=0.11, p=0.015), shown on Table 4.5.

Table 4.5. Correlation of variables while controlling for the effect of gender

Control Variables			Skinfold Arm	Skinfold Scapular	LDL	HDL	TC	Trig	Glucose
Gender	Right CIMT	R	.249	.165	.230	.265	.388	.462	.087
		Sig	.072	.239	.098	.055	**.004**	**.001**	.537
	Left CIMT	R	.192	.147	.315	.269	.412	.296	.062
		Sig	.169	.295	**.022**	.052	**.002**	**.031**	.658
	Abdominal AIMT	R	.346	.189	.035	-.066	-.009	.192	-.027
		Sig	**.011**	.176	.802	.639	.951	.169	.846

Abbreviations: CIMT- Carotid Artery Intima-media thickness, AIMT- Abdominal Aorta Intima-media Thickness.

Females have a higher waist measurement and tricep (22.66±8 mm) and scapular (20.77±9.7 mm) skinfold thickness. This is due to females having higher amounts of subcutaneous fat than males, as shown in figure 4.1. The higher weight of males was possibly due to males being taller than females, shown in the BMI measurement, males have a lower BMI.

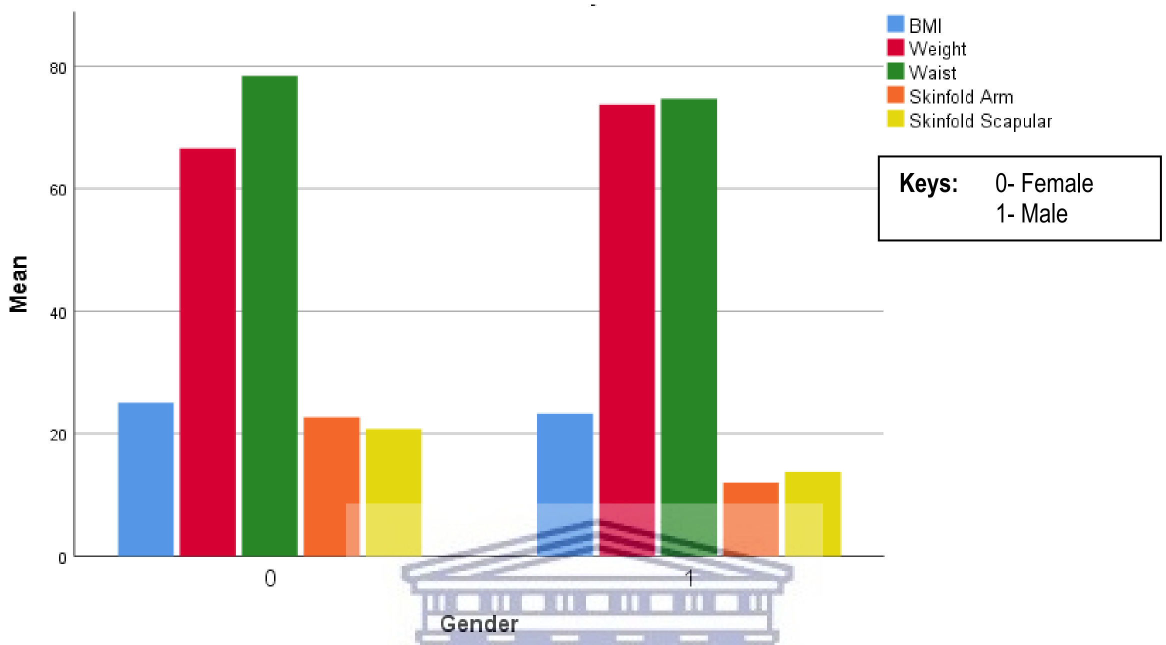


Figure 4.1: Bar graph showing the mean values of BMI, Weight, Waist measurement and Tricep and Scapular skinfold thickness based on Gender.

Males tended to have much higher levels of LDL, cholesterol and triglyceride. The intima media thicknesses were basically the same for both genders showing that gender does not influence its thickness, as shown in figure 4.2.

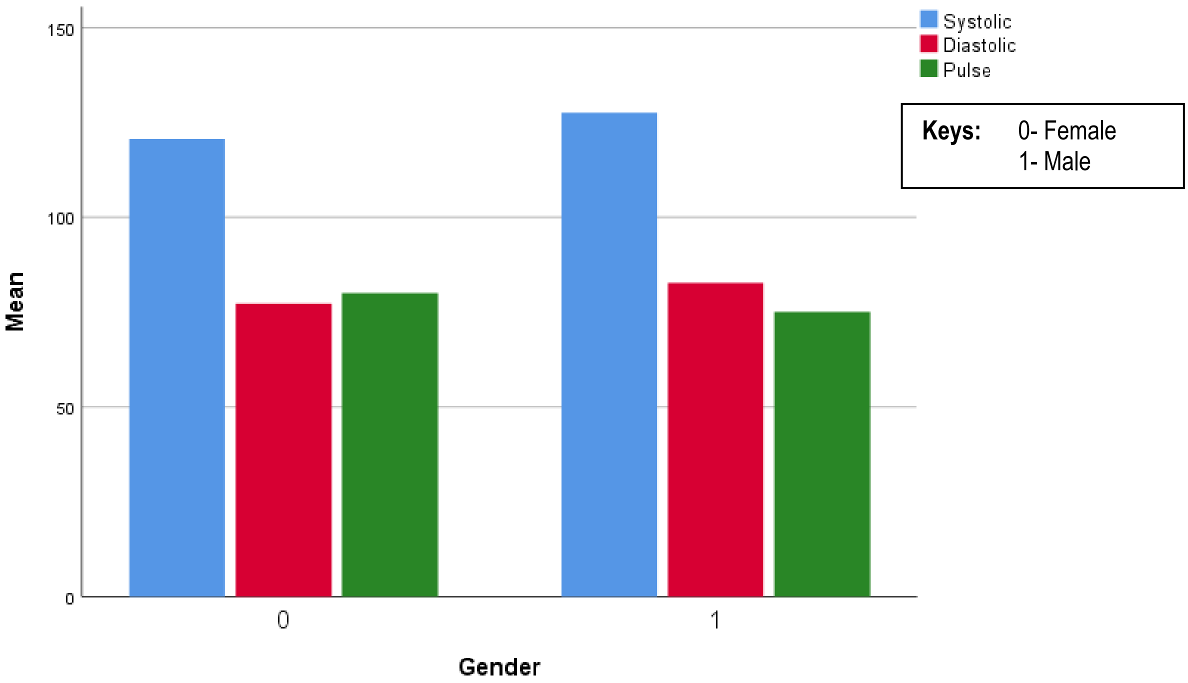


Figure 4.2: Bar graph showing the mean Systolic, Diastolic and Pulse Rate according to Gender.

Glucose measurements and the Right and Left CIMT and the Abdominal AIMT readings based on, Gender, as viewed in Figure 4.3.

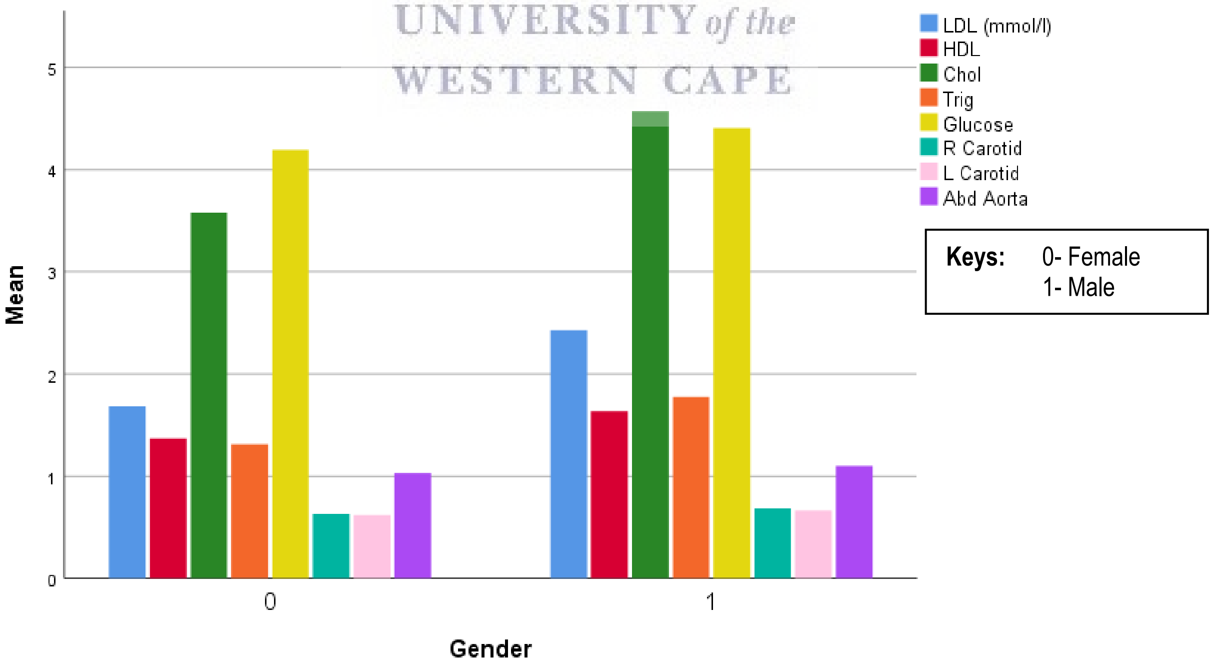


Figure 4.3: Bar graph showing the mean LDL, HDL, Total Cholesterol, Triglycerides and

Males and females both had results that were skewed to the right, implying that 50% of the participants fell into waist measurements in a more condensed lower range and 50% of the participants' results were more scattered, according to figure 4.4.

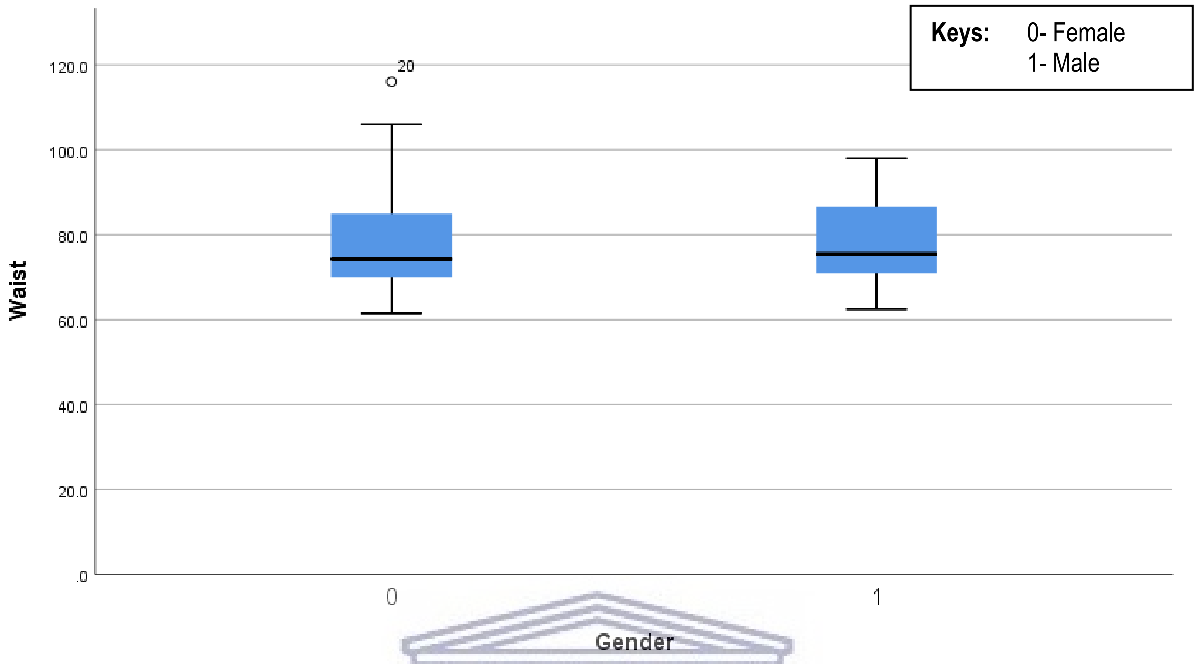


Figure 4.4: Box plot showing waist measurement according to gender.

Females had a higher variation in results, the data was uniformly distributed. Male values were skewed to the right, where 50% of males fell into the lower quartile range showing that more males have similar lower LDL levels than females, except for the outliers, shown in figure 4.5.

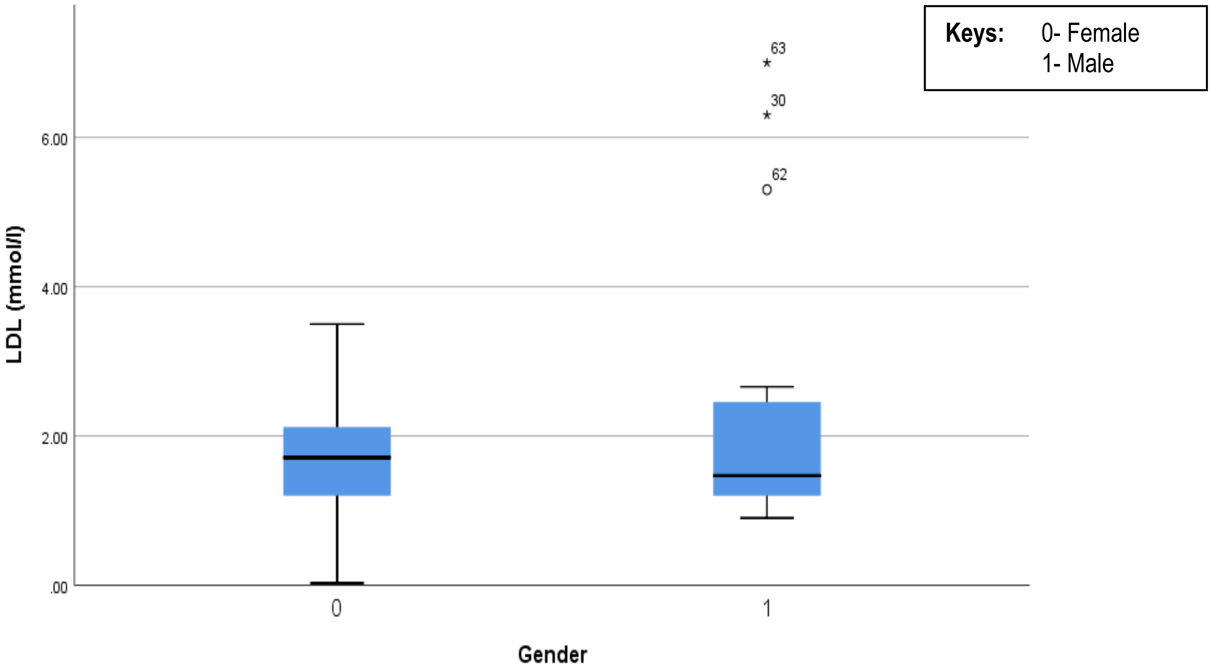


Figure 4.5: Box plot showing LDL levels according to gender.

Both male and female plots were skewed to the left. The results explain that 50% of the participants fell into lower ranges of HDL values. Females had a more consistent distribution of results compared to males, in figure 4.6,

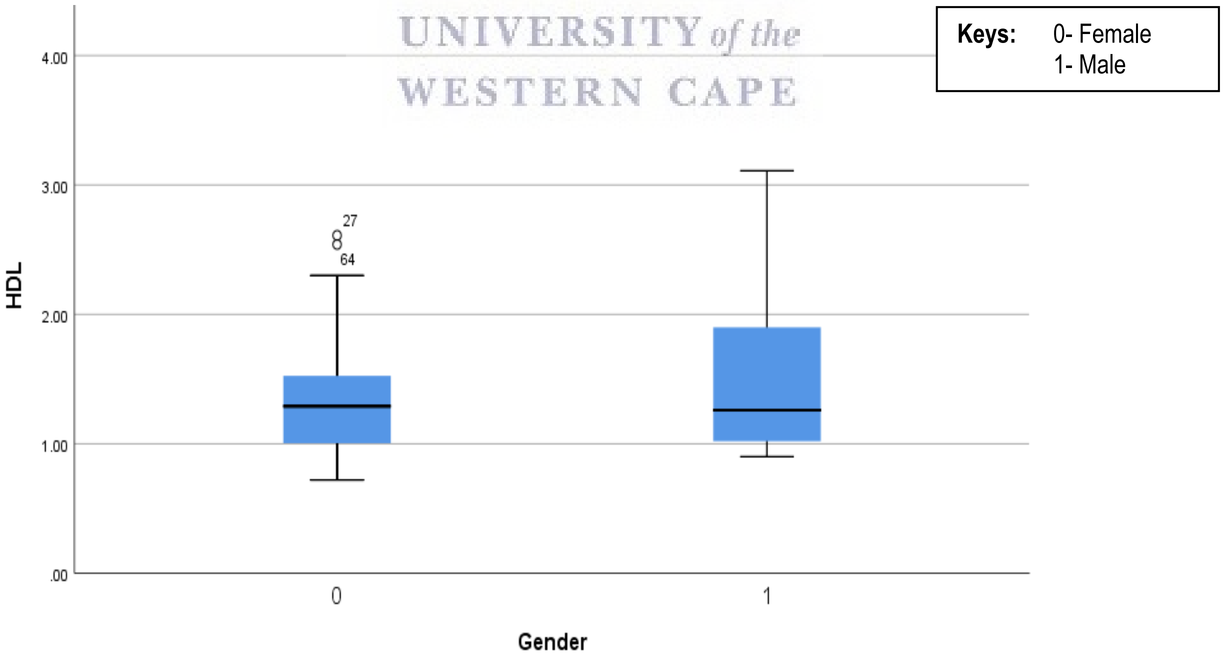


Figure 4.6: Box plot showing HDL levels according to gender.

Males have a lower clustered cholesterol reading compared to females. Both groups were skewed to the left, as per figure 4.7

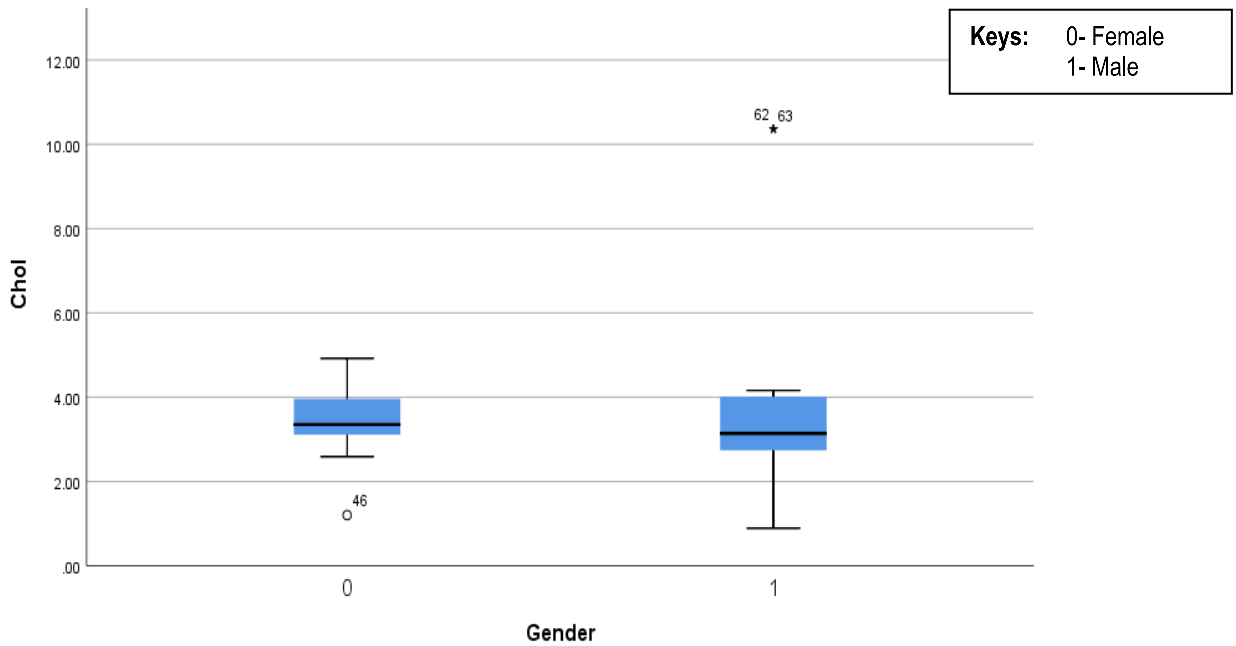


Figure 4.7: Box plot showing total cholesterol levels according to gender.

Both females and males were skewed to the right, implying that 50% of the results had scattered results. Females, in general, had lower triglyceride levels than male, shown in figure 4.8,

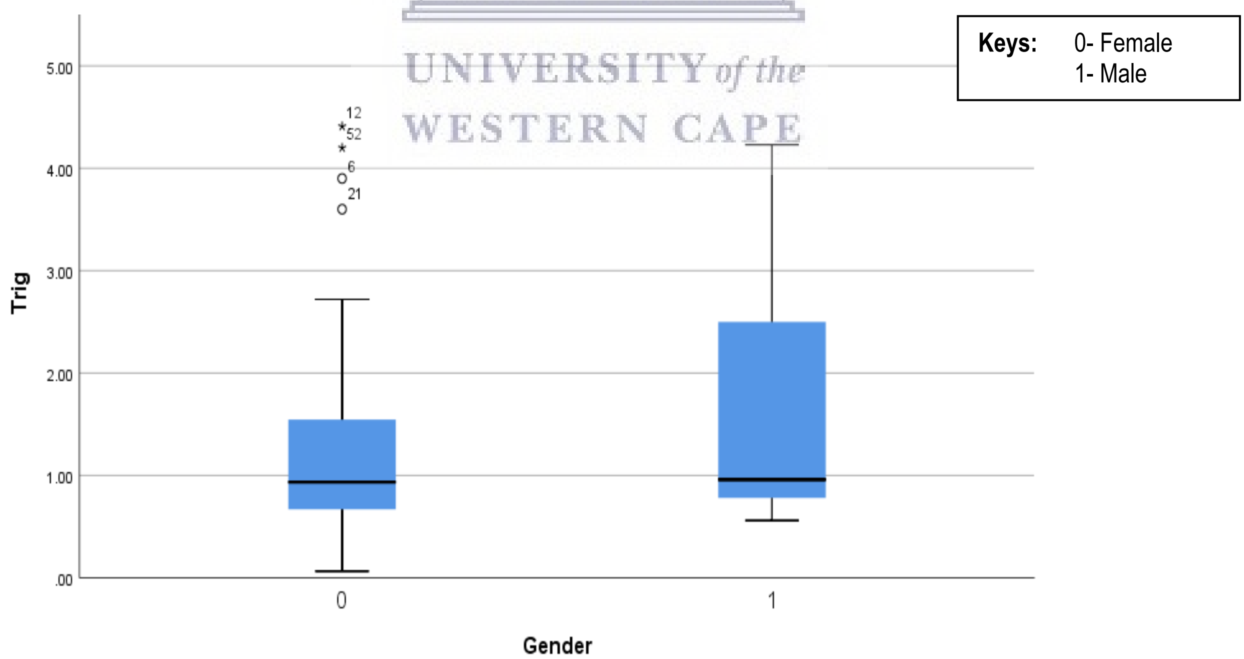


Figure 4.8: Box plot showing triglyceride levels according to gender.

Females' results were skewed to the right, implying that the values were clustered in the higher glucose amounts. Females tended to have lower glucose readings than males. Male results were skewed to the left, thus having the opposite reflection of results to females, according to figure 4.9.

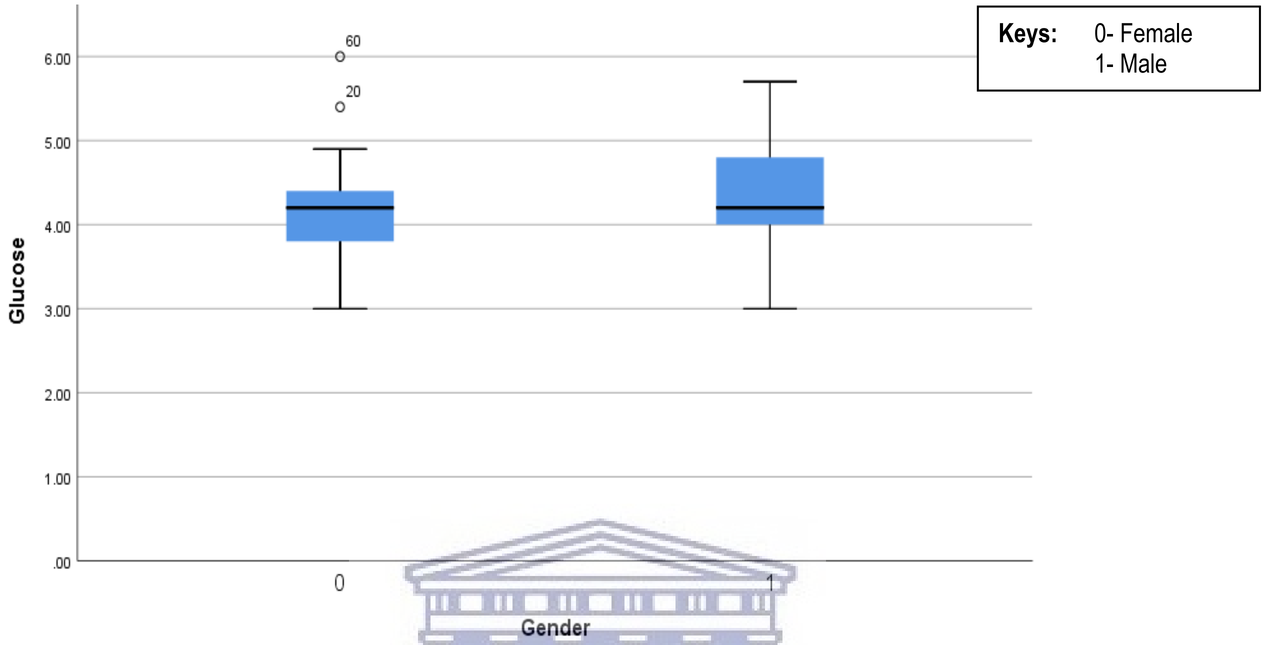


Figure 4.9: Box plot showing glucose levels according to gender.

Male and female readings where in similar proportions, not much change in the values of right CIMT. Both were skewed to the right, as shown in figure 4.10.

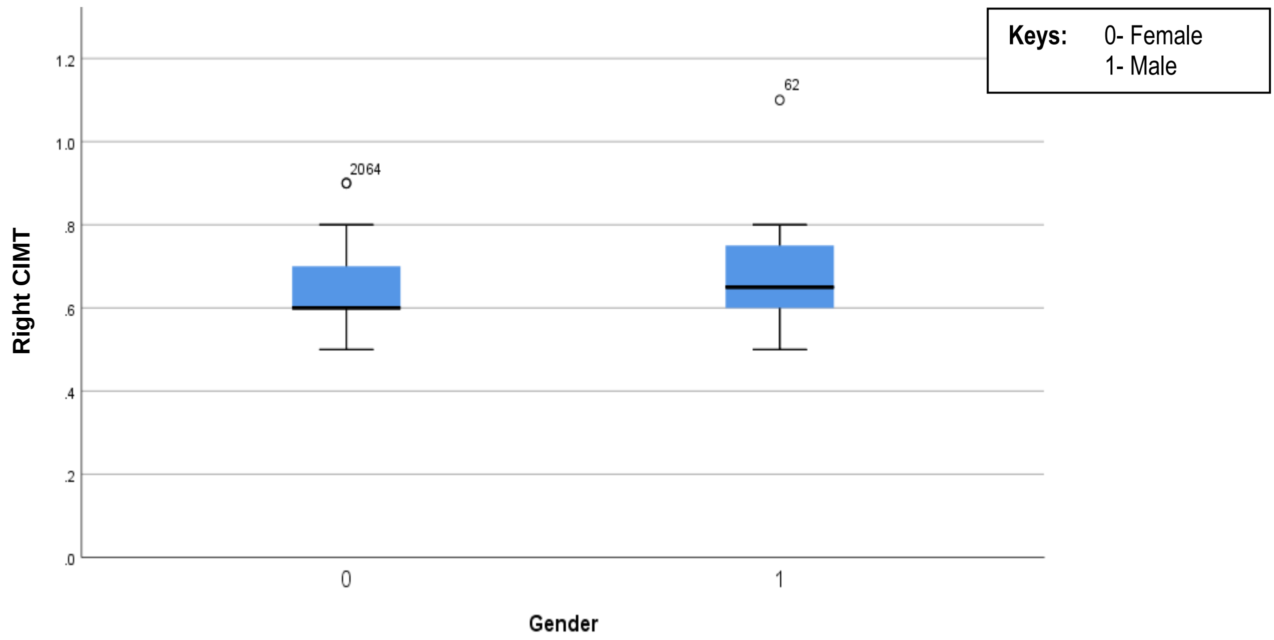


Figure 4.10: Box plot showing right carotid intima media thickness according to gender.

As per figure 4.11, male and female have consistent low and high values with skewed results. Males have more variation in their results and skewed to the left, implying that 50% of males are clustered into higher IMT measurements. 50% of females were more disposed to lower IMT values, being skewed to the right.

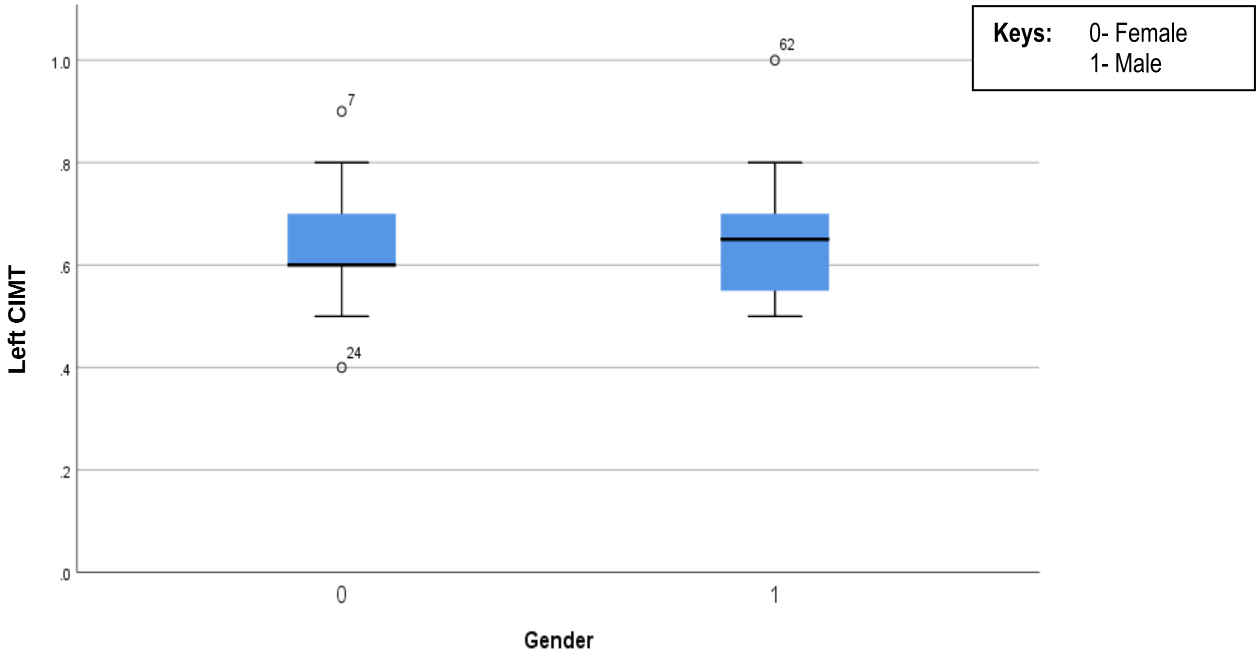


Figure 4.11: Box plot showing left carotid intima media thickness according to gender.

As shown in figure 4.12, males were skewed to the right; females were slightly skewed o the right but more evenly distributed than males. Females had lower abdominal aorta IMT values than males.

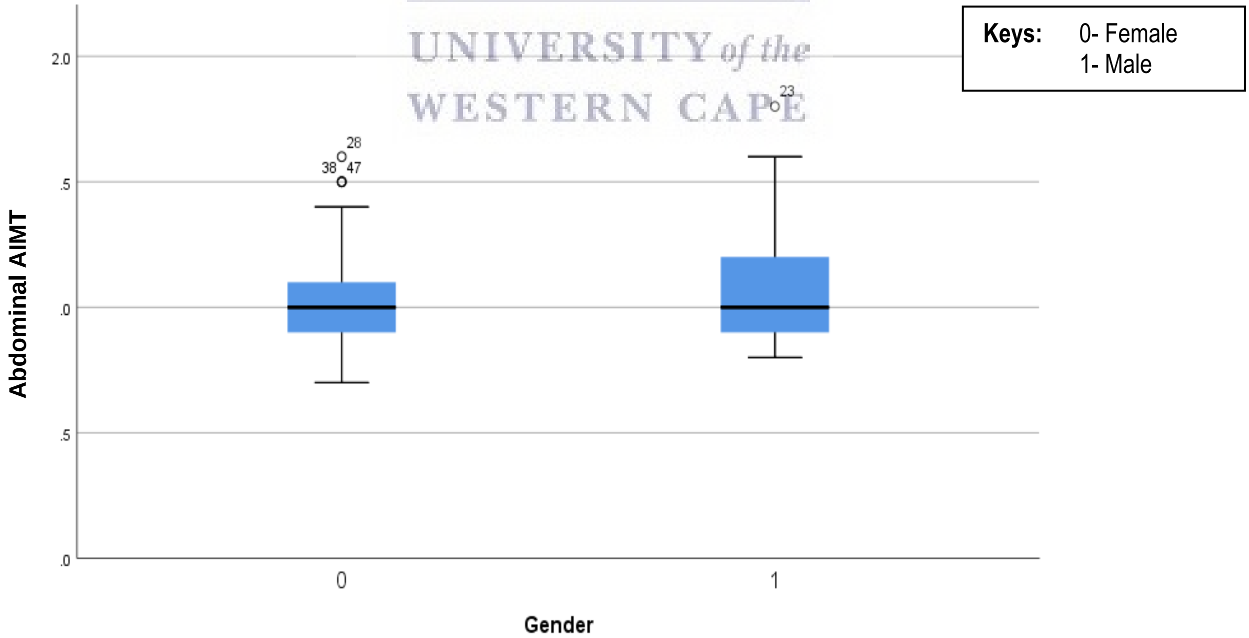


Figure 4.12: Box plot showing abdominal aorta intima media thickness according to gender.

4.2. Correlation Between the Variables Whilst Controlling for Age, BMI, Smoking and Birth Weight

The objectives of the study were to find relationships between BMI, and Birth Weight and the variables. Correlations were analysed and controlled for the above-mentioned variables to find relationships between them and the variables.

Table 4.6. Correlation between Age and body mass index (BMI), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (Trig), total cholesterol (TC), glucose, abdominal aorta, right carotid vein and left carotid vein.

		BMI	**LDL**	**HDL**	**TC**	**Trig**	**Glucose**
Age	**R**	.232	.151	-.055	.102	.119	-.232
	Sig	.068	.258	.676	.432	.361	.072

A significant result between age and the left CIMT (p=0.025) as shown in Table 4.7. No other significance was found.

Table 4.7. Correlation between Age and IMT of the arteries.

Age		**N**	**Correlation**	**Sig.**
	Right CIMT	60	.129	.327
	Left CIMT	60	.290	**.025**
	Abdominal AIMT	60	.053	.687

There was a significant positive correlation between the following variables whilst controlling for birth weight: Glucose and Scapula Skinfold (r^2=0.16, p=0.033), Left CIMT and Right CIMT (r^2=0.48, $p<0.001$), Scapula Skinfold and Tricep Skinfold (r^2=0.74, $p<0.001$), Cholesterol (TC) and LDL (r^2=0.21, p=0.013) and Triglyceride and HDL (r^2= 0.14, p=0.04). Triglycerides and HDL which had a negative correlation but is significant. There was no significance between the IMTs and the risk factors according to birth weight, according to Table 4.8.

Table 4.8. Correlation of variables while controlling for the effect of birth weight

Control Variables			Skinfold Arm	Skinfold Scapular	LDL	HDL	TC	Trig	Glucose
Birth Weight	Right CIMT	R	.247	.034	-.042	-.002	.111	.340	-.286
		Sig	.196	.862	.830	.993	.568	.071	.132
	Left CIMT	R	.203	.055	.147	.057	.124	.043	-.205
		Sig	.292	.777	.448	.768	.522	.823	.285
	Abdominal AIMT	R	.341	.152	.268	-.261	.044	.167	-.051
		Sig	.070	.433	.160	.171	.821	.386	.794

There was a significant positive correlation between the following variables whilst controlling for smoking: Scapula Skinfold and Tricep Skinfold (r^2=0.71, p=0.001), HDL and LDL (r^2=0.25, p=0.001), Cholesterol (TC) and LDL (r^2=0.79, p=0.001), Cholesterol (TC) and HDL (r^2=0.45, p=0.001), Glucose and LDL (r^2=0.26, p=0.001), Glucose and Cholesterol (TC) (r^2=0.2, p=0.001), Right CIMT and LDL (r^2= 0.07, p=0.039), Right CIMT and HDL (r^2=0.13, p=0.009), Right CIMT and Cholesterol (TC) (r^2=0.19, p=0.001), Right CIMT and Triglycerides (r^2=0.23, p=0.001), Left CIMT and LDL (r^2=0.13, p=0.009), Left CIMT and HDL (r^2=0.12, p=0.014), Left CIMT and Cholesterol (r^2=0.2, p=0.001), Left CIMT and

Triglycerides (r^2=0.1, p=0.02), Left CIMT and Right CIMT (r^2=0.52, p=0.001), Abdominal AIMT and Right CIMT (r^2=0.12, p=0.014) and Abdominal AIMT and Left AIMT (r^2=0.14, p=0.006), according to Table 4.9.

Table 4.9. Correlation of variables while controlling for the effect of smoking

Control Variables			Skinfold Arm	Skinfold Scapular	LDL	HDL	TC	Trig	Glucose
Smoke	Right CIMT	R	.099	.101	.285	.357	.439	.477	.114
		sig	.481	.471	.039	**.009**	**<.001**	**<.001**	.417
	Left CIMT	R	.064	.087	.355	.336	.449	.318	.089
		sig	.647	.535	**.009**	**.014**	**<.001**	**.020**	.528
	Abdominal AIMT	R	.225	.119	.063	-.104	.012	.245	.007
		sig	.105	.395	.656	.457	.932	.076	.962

4.3. Relationships Between Means of Variables Compared to BMI (Grouped)

The results show a significance in cholesterol (p= .004), blood pressure (p<0.001), the left carotid artery (p=0.007), waist measurements (p=0.037) and waist-to-hip ratio (p=0.027) compared to BMI.

	BMI	
	F	**Sig.**
LDL	1.623	.148
HDL	1.257	.318
Cholesterol (TC)	3.521	**.004**
Triglycerides	1.216	.345
Glucose	.751	.778
Blood Pressure	16.686	**<.001**
Right CIMT	2.062	.116
Left CIMT	4.411	**.007**
Waist	2.281	**.037**
Waist-Hip Ratio	2.445	**.027**

Table 4.10. Analysis of variance between means of variables

4.4. Waist and Waist-To-Hip Ratio Compared to Variables for Increased Risk of CVD

Waist circumference measurements and waist-to-hip ratio both associated with BP measurements (p<0.000 and p<0.000, respectively), the right CIMT (p=0.002 and p=0.039, respectively) and the left CIMT (p<0.001 and p=0.002, respectively) of the participants. Waist-hip measurements associated with total cholesterol (TC) (p=0.046), as shown in Table 4.11.

Table 4.11. Bivariate correlation of variables

	LDL	HDL	TC	Trig	Glucose	BP	Right CIMT	Left CIMT	Abdominal AIMT
Waist	.106	.469	.064	.129	.621	**<.000**	**.002**	**<.001**	0.304
Waist-Hip	.067	.176	**.046**	.912	.324	**<.000**	**.039**	**.002**	.919

4.5. Chapter Summary

This chapter defines the severity of lipid levels of participants by comparing it to IMT. The chapter also summarizes the influence gender, race and other factors has on the different variables through the use of correlation analysis. Multiple statistical analyses were done to prove the effectiveness of ultrasound in early detection of plaque/fatty streaks in the intima-media of arteries.

CHAPTER 5

DISCUSSION

5.1. Introduction

The aim of the study was to examine changes in lipid profile and compare it to ultrasound measurements of AIMT and CIMT in an adolescent population, with regards to gender, race and body mass index (BMI).

Cardiovascular disease is the leading cause of mortality and morbidity worldwide (Khanna et al., 2019). The first clinical manifestation of cardiovascular disease often arises in a stage of well-advanced atherosclerosis. Around 90% of cardiac related deaths are due to cardiovascular risk factors such as race, gender, age, diabetes, smoking, BMI, hyperlipidaemia, and hypertension (McEvoy et al., 2015). However, the conventional risk factors do not explain morphological changes in the blood vessels, such as an increase in the intima-media thicknesses (IMT) of arteries (Schlendorf et al., 2009). Males are said to have a higher incidence rate as compared to females (Howard et al., 1993, Bots et al., 2017). Ultrasounds are a common tool for a non-invasive and cost-effective procedure that provides visualisation of blood vessels and the atherosclerotic plaque and increases fat layer deposited within the vessel wall (intima-media layer) in patients already diagnosed with a heart condition (Suri et al., 2005). Not much is known about the use of ultrasounds as an effective way to measure the IMT of arteries of those not diagnosed with a predisposed CV problem. In this study we aimed at detecting early signs of CVD by viewing the IMT of the carotid and abdominal aorta by means of an ultrasound.

5.2. A Review on CIMT and AIMT with Regards to Age.

Previous studies have considered the relationship between CIMT and AIMT and its risk factors (Heilman et al., 2009). A well-known subclinical marker of atherosclerosis is CIMT and a strong predictor of cardiovascular events (Soliman et al., 2010). According to Davis et al., AIMT increased with age at a greater rate than CIMT (Davis et al., 2010). The build-up of lipids in the intimal layer of arteries forming fatty streaks is said to be an early sign of the atherosclerotic process, which leads to morphological changes that has an influence on vascular function. This can ultimately lead to coronary artery disease (CAD) and stroke (Gooty et al., 2018). The intima-media layer of the carotid and abdominal aorta thicknesses are also important markers for atherosclerosis (Davis et al., 2010 and Abd El Dayem et al., 2016), which indicates the presence of the disease and its development (Awad and Abbas, 2017). Studies have been focusing on preventive measures for atherosclerosis beginning during childhood to reduce the risk of heart disease later in life (McGill, Jr. et al., 2008 and Davis et al., 2010). A close relationship of IMT with several cardiovascular risk factors has been found. A previous study had shown that both the carotid and femoral IMTs increased significantly with age, and the IMT was greater in men than in women (Ando et al., 2000). The CIMT was estimated to increase by 0.04mm for every 10 years. These findings were supported by the EAS (The Edinburgh ARTERY Study) (Allen et al., 1997).

Childhood and adolescent obesity continue to be a major health issue due to its considerable health implications and to the economic burden that occur from treating this disease and its complications. Being overweight as a child can cause health problems, and children may be affected psychologically. Latest studies show that in the adult population, cardiovascular disease is the main cause of mortality and morbidity among obese patients. These risk factors will then continue and become increasingly worse if obese youth remain obese as they reach adulthood. (Brown et al., 2019 and Ho et al., 2019). A study suggested that obesity in childhood and adolescence increased the incidence of cardiovascular disease risk factors which, in turn, increases the risk of cardiovascular morbidity, including ischemic heart disease, stroke, but also non-ischemic heart disease-related cardiac pathologies, and mortality in adulthood (Sommer and Twig, 2018).

This study focused on the adolescent age group (21.5 ± 2.2 years), taking one ultrasound reading of the right and left carotid arteries and the abdominal aorta, respectively, and viewing those images for any early signs of plaque and/or fatty streak build up in the intima. There was no relation between age and signs of plaque and/or fat manifestation in the artery walls of the right CIMT and the AIMT but there was a significance between age and the left CIMT (p = 0,025) [Table 4.7]. This was possibly due closeness in the age range in the study, as was stated before that the IMT increases by 0.04 mm every 10 years (Allen et al., 1997). Our age range was possibly too small (<10 years) to compare the fact that there was an increase within that time bracket. The other factor may have been that average number of participants in the group, which was only 63, was too small of a sample size. In this study, age did not show any significant correlation with body mass index (BMI) (r^2=0.05, p=0.068), low density lipoproteins (LDL) (r^2=0.02, p=0.258), high density lipoproteins (HDL) (r^2=0.003, p=0.676), triglycerides (Trig) (r^2=0.01, p=0.361), total cholesterol (TC) (r^2=0.01, p=0.432), glucose (r^2=0.05, p=0.072) [Table 4.6], abdominal aorta (p=0.627) and right carotid artery (p=0.387) [Table 4.7]. Because our group of participants had a small age difference, there was almost no change in the values. Previous studies suggest more women with carotid plaque have a normal

IMT. This may explain why IMT failed to be predictive of CV risk in women (Matangi et al., 2019). Plaque build-up in the IMT is predictive of increased CVD risk in both male and females.

5.3. Relationship Between BMI and Tested Variables

In medical practice, anthropometric measurements such as BMI and waist circumference are frequently used to measure general and abdominal adiposy. In most existing literature on children, associations of IMT and adiposity are compared between lean and obese youth (Park et al., 2015). In a more current study, even though most of the youth in the study were not obese and/or generally healthy, BMI correlated with CIMT measurements. This showed that maintaining normal fat levels and other risk factors may be useful in preventing early changes associated with atherosclerosis (Gooty et al., 2018). Waist circumference is a composite measure of subcutaneous and visceral abdominal adipose tissue (van den Munckhof et al., 2017). A higher cardiovascular mortality was observed in men with an increased abdominal diameter (Wirth and Steinmetz, 1998). Prior epidemiologic studies have shown that increasing body mass index (BMI) is associated with higher total cholesterol and low-density lipoprotein cholesterol (LDL) (Shamai et al., 2010). Numerous epidemiologic studies have demonstrated a direct correlation between increasing BMI and elevated TC, LDL, and triglycerides and an inverse relationship with HDL (Krauss et al., 1998). Our findings showed a significanct correlation between BMI and cholesterol ($p=0.004$), blood pressure ($p<0.001$), the left carotid artery ($p=0.046$), waist measurements ($p<0.001$) and waist-to-hip ratio ($p<0.001$) [Table 4.10]. The results express that increasing body mass index may lead to increases in cholesterol, blood pressure and, in turn, cardiovascular events, suggesting the need for better preventative measures for the increase in BMI, as well as the treatment of hypertension and better lifestyle methods (Linderman et al., 2018). Results expressed that higher BMI was associated with higher cholesterol (TC) levels in the participants.

The current study was one of the firsts performed in South Africa in early detection of increased IMT, in university students (adolescents aged 19-26 years), through means of ultrasonography and comparing them to the risk factors associated with CVD. By statistically comparing the variables, we came to the conclusion that they are interrelated and could predict risk for CVD later in life. More could have been done in the current study, but since we had a small sample size and the ratios of male to female and race were unproportioned and not all questionnaires were returned, results slightly varied from previous studies.

5.4. Relationship Between Blood Pressure and Lipid Profile and Blood Glucose Levels

Hypertension is one of the biggest risk factors for increased occurrence of CVD worldwide. In recent studies, high blood pressure (BP) and prehypertension have also been proven as risk factors for CVD (Kokubo and Kamide, 2009). Increased BP is the most probable cause of CVD and stroke (Imano et al., 2009). The prevalence of glucose intolerance has shown to be a key problem as well as a risk factor for CVD (Doi et al., 2010).

Assessment of the collective influence of these two risk factors is vital in preventing CVD because elevated BP is a detrimental risk factor of CVD incidence (Kokubo et al., 2010). There have been a few population studies on the association between the occurrence of hypertension together with DM and the risk of stroke and coronary heart disease (CHD) (Hu, Jousilahti and Tuomilehto, 2007). However, few population cohort studies have evaluated the impact of the combination of BP and fasting glucose on the risk of CVD (Kokubo et al., 2010). Our study aided in filling those missing blocks. Our findings show that BMI had a positive significance associated with blood pressure (p<0.001) [Table 4.10].

5.5. The Role of Central Obesity (Increased Waist Circumference) and BMI, Blood Pressure (BP), Lipid Profile and Blood Glucose as Cardiometabolic Risk Factors

For the past 30 years the occurrence of obesity has been on the increase worldwide, possibly because of calorie consumption increase and decreased physical activity (Swinburn et al., 2011). Both BMI and WC are extremely age-dependent in adolescents. BMI and WC are frequently used factors to define obesity and central adiposity. Obesity and abdominal adiposity have been shown in studies to be risk factors for cardiovascular disease (Yoo, 2016). Waist circumference and waist to hip ratio are more accessible universal screening tools than BMI to detect health risks and is cheaper and easier to use.

Our study showed a significant relationship between waist to hip measurements and waist circumference measurements on BP measurements (p<0.000 and p<0.000, respectively), the right CIMT (p=0.002 and p=0.039, respectively) and the left CIMT (p<0.001 and p=0.002, respectively) of the participants. Waist-hip measurements had a significant influence on total cholesterol (TC) (p=0.046) [Table 4.11]. This correlates with the association of waist circumference and CIMT and BP.

In conclusion, according to the results of our study it was shown that lipoproteins do in fact play a role in the thickness of arteries. If the participant is found to have a higher-than-average IMT and does not change their lifestyle habits, this could result in heart disease later in life.

Previous studies have shown that changing lifestyle habits may work in the favour of the participant when plaque and fatty streak build up in arteries are found in the early stages.

CONCLUSION

As CVD continues to rise not only in South Africa but worldwide, more aggressive and readily available approaches are required to manage CVD in South Africa and other countries. The current study found that ultrasounds are confirmed to be a useful non-invasive, low cost and easily accessible tool to detect even the smallest changes in the intima-media thickness of arteries. Results from this study showed that CVD is directly associated to CVD risk factors and lipid levels in the body. Previous studies have shown that if lifestyle habits changed, the risk for CVD would drop significantly. Our study was not done over a period of time therefore this hypothesis could not be included in the study. Also, further research would aid in identifying CVD before it progresses, by urging lifestyle changes and subsequently a decrease in risk factors and in turn decrease the severity of the disease later in life. Prevention is better than cure.

REFERENCES

Abd El Dayem, S., Battah, A. and El Bohy, A. (2016). Assessment of Increase in Aortic and Carotid Intimal Medial Thickness in Type 1 Diabetic Patients. *Open Access Macedonian Journal of Medical Sciences*, 4(4), p.630.

Agarwal, A. K., S. Singla, R. Singla, A. Lal, H. Wardhan, et al., (2009). Prevalence of Coronary Risk Factors in Type 2 Diabetics without Manifestations of Overt Coronary Heart Disease. *Journal of Associated Physicians in India*, 57, pp.135-142.

Aguilar-Shea, A., Gallardo-Mayo, C., Garrido-Elustondo, S., Calvo-Manuel, E. and Zamorano-Gómez, J. (2011). Carotid Intima-Media Thickness as a Screening Tool in Cardiovascular Primary Prevention. *European Journal of Clinical Investigation*, 41(5), pp.521-526.

Akil, L. and Ahmad, H. (2011). Relationships between Obesity and Cardiovascular Diseases in Four Southern States and Colorado. *Journal of Health Care for the Poor and Underserved*, 22(4A), pp.61-72.

Akinboboye, O., Idris, O., Akinkugbe, O. (2003). Trends in Coronary Artery Disease and Associated Risk Factors in Sub-Saharan Africans. *Journal of Human Hypertension*, **17**(6), pp.381–387.

Allan, P., Mowbray, P., Lee, A. and Fowkes, F. (1997). Relationship Between Carotid Intima-Media Thickness and Symptomatic and Asymptomatic Peripheral Arterial Disease. *Stroke*, 28(2), pp.348-353.

Ando, F., Takekuma, K., Niino, N. and Shimokata, H. (2000). Ultrasonic Evaluation of Common Carotid Intima-Media Thickness (IMT) Influence of Local Plaque on the Relationship Between IMT and Age. *Journal of Epidemiology*, 10(1sup), pp.10-17.

Anitschkow, N. (1913). Ueber Experimentelle Choleserinsteatose Und Ihre Bedeutung Fur Die Entstehung Einiger Pathologischer Prozesse. *Zentralbl Allg Pathol*, 24, pp.1-9.

Arheden, H. (2009). Age and Gender Specific Normal Values of Left Ventricular Mass, Volume and Function for Gradient Echo Magnetic Resonance Imaging: A Cross Sectional Study. *BMC Medical Imaging*, 9:2 doi:10.1186/1471-2342-9-2.

Arslan, E., Yakar, T., and Yavasoglu, I. (2008). The Effect of Smoking on Mean Platelet Volume and Lipid Profile in Young Male Subjects. *Anadolu Kardiyol Derg*, 8(6), pp.422-425.

Asai, K., Kudej, R., Shen, Y., Yang, G., Takagi, G., Kudej, A., Geng, Y., Sato, N., Nazareno, J., Vatner, D., Natividad, F., Bishop, S. and Vatner, S. (2000). Peripheral Vascular Endothelial Dysfunction and Apoptosis in Old Monkeys. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 20(6), pp.1493-1499.

Awad, I. and Abbas, H. (2017). Ultrasound Evaluation of Carotid Artery Intima-Media Thickness in Patients with Risk Factors for Cardiovascular Disease. *International Journal of Diagnostic Imaging*, 4(2), p.16.

Baber, U., Mehran, R., Sartori, S., Schoos, M., Sillesen, H., Muntendam, P., Garcia, M., Gregson, J., Pocock, S., Falk, E. and Fuster, V. (2015). Prevalence, Impact, and Predictive Value of Detecting Subclinical Coronary and Carotid Atherosclerosis in Asymptomatic Adults. *Journal of the American College of Cardiology*, 65(11), pp.1065-1074.

Barton, P., Andronis, L., Briggs, A., McPherson, K. and Capewell, S. (2011). Effectiveness and Cost Effectiveness of Cardiovascular Disease Prevention in Whole Populations: Modelling Study. *BMJ*, 343(jul28 1), pp.4044-d4044.

Batic-Mujanovic, O., Beganlic, A., Salihefendic, N., Pranjic, N. and Kusljugic, Z. (2008). Influence of Smoking on Serum Lipid and Lipoprotein Levels among Family Medicine Patients. *Med Arh* , 62(5-6), pp.264-267.

Berenson, G., Srinivasan, S., Bao, W., Newman, W., Tracy, R. and Wattigney, W. (1998). Association between Multiple Cardiovascular Risk Factors and Atherosclerosis in Children and Young Adults. *New England Journal of Medicine*, 338(23), pp.1650-1656.

Berg, A., Halle, M., Franz, I. and Keul, J. (1997). Physical Activity and Lipoprotein Metabolism: Epidemiological Evidence and Clinical Trials. *European Journal of Medical Research*, 2(6), pp.259-264.

Bots, M., Hofman, A., de Bruyn, A., de Jong, P. and Grobbee, D. (1993). Isolated Systolic Hypertension and Vessel Wall Thickness of the Carotid Artery. The Rotterdam Elderly Study. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*, 13(1), pp.64-69.

Bots, S., Peters, S. and Woodward, M. (2017). Sex Differences in Coronary Heart Disease and Stroke Mortality: A Global Assessment of the Effect of Ageing between 1980 and 2010. *BMJ Global Health*, 2(2), p.298.

Brady, T.M., Fivush, B., Flynn, J.T., Parekh, R. (2008). Ability of Blood Pressure to Predict Left Ventricular Hypertrophy in Children with Primary Hypertension. *Journal of Pediatrics*, 152(1), pp.73-78.

Brown, C. D., Higgins, M., Donato, K. A., Rohde, F. C., Garrison, R. et al. (2000). Body Mass Index and the Prevalence of Hypertension and Dyslipidemia. *Obesity Research*, 8(9), pp.605-619.

Brown, T., Moore, T., Hooper, L., Gao, Y., Zayegh, A., Ijaz, S., Elwenspoek, M., Foxen, S., Magee, L., O'Malley, C., Waters, E. and Summerbell, C. (2019). Interventions for Preventing Obesity in Children Cochrane Systematic Review.

Cannon, C., Braunwald, E., McCabe, C., Rader, D., Rouleau, J., Belder, R., Joyal, S., Hill, K., Pfeffer, M. and Skene, A. (2004). Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes. *New England Journal of Medicine*, 350(15), pp.1495-1504.

Cape Town: Southern African Labour & Development Research Unit (2009). *National Income Dynamics Study Health: Analysis of the NIDS Wave 1 Dataset. Discussion Paper No. 2*. Cape Town.

Chen, X., Wang, Y. (2008). Tracking of Blood Pressure from Childhood to Adulthood. *Circulation*, 117(25), pp.3171-3180.

Cheng, H., Patel, B., Martin, S., Blaha, M., Doneen, A., Bale, B. and Jones, S. (2016). Effect of Comprehensive Cardiovascular Disease Risk Management on Longitudinal Changes in Carotid Artery Intima-Media Thickness in a Community-Based Prevention Clinic. *Archives of Medical Science*, 4, pp.728-735.

Chumlea, W. and Guo, S. (2000). Assessment and Prevalence of Obesity: Application of New Methods to a Major Problem. *Endocrine*, 13(2), pp.135-142.

Chumlea, W., Guo, S., Wholihan, K., Cockram, D., Kuczmarski, R. and Johnson, C. (1998). Stature Prediction Equations for Elderly non-Hispanic White, non-Hispanic black, and Mexican-American Persons Developed from NHANES III Data. *Journal of the American Dietetic Association*, 98(2), pp.137-142.

Chumlea, WC., Baumgartner, RN., Garry, PJ., Rhyne, RL., Nicholson, C. and Wayne, S. (1992). Fat Distribution and Blood Lipids in a Sample of Healthy Elderly People. *International Journal of Obesity Related Metabolic Disorders,* 16(2), pp.125–133.

Cohen, J., Wang, Z., Grundy, S., Stoesz, M. and Guerra, R., 1994. Variation at the Hepatic Lipase and Apolipoprotein AI/CIII/AIV Loci is a Major cause of Genetically Determined Variation in Plasma HDL Cholesterol Levels. *Journal of Clinical Investigation*, 94(6), pp.2377-2384.

Davis, P., Dawson, J., Blecha, M., Mastbergen, R. and Sonka, M. (2010). Measurement of Aortic Intimal-Medial Thickness in Adolescents and Young Adults. *Ultrasound in Medicine & Biology*, 36(4), pp.560-565.

Dawson, J., Sonka, M., Blecha, M., Lin, W. and Davis, P., 2009. Risk Factors Associated with Aortic and Carotid Intima-Media Thickness in Adolescents and Young Adults. *Journal of the American College of Cardiology*, 53(24), pp.2273-2279.

Di Bello, V., Carerj, S., Perticone, F., Benedetto, F., Palombo, C., Talini, E., Giannini, D., La Carrubba, S., Antonini-Canterin, F., Di Salvo, G., Bellieni, G., Pezzano, A., Romano, M. and Balbarini, A. (2009). Carotid Intima-Media Thickness in Asymptomatic Patients With Arterial Hypertension Without Clinical Cardiovascular Disease: Relation With Left Ventricular Geometry and Mass and Coexisting Risk Factors. *Angiology*, 60(6), pp.705-713.

Doi, Y., Ninomiya, T., Hata, J., Fukuhara, M., Yonemoto, K., Iwase, M., Iida, M. and Kiyohara, Y., 2010. Impact of Glucose Tolerance Status on Development of Ischemic Stroke and Coronary Heart Disease in a General Japanese Population. *Stroke*, 41(2), pp.203-209.

Dong, C., Bu, X., Liu, J., Wei, L., Ma, A. and Wang, T., 2022. Cardiovascular Disease Burden Attributable to Dietary Risk Factors from 1990 to 2019: A Systematic Analysis of the Global

Burden of Disease Study. *Nutrition, Metabolism and Cardiovascular Diseases*, 32(4), pp.897-907.

Dryden, J. (2007). Belly Fat may Drive Inflammatory Processes Associated with Disease. [Blog] *The Source*. Available at: https://source.wustl.edu/2007/03/belly-fat-may-drive-inflammatory-processes-associated-with-disease/ [Accessed 12 Dec. 2019].

Espeland M.A., O'Leary, D.H., Terry, J.G., Morgan, T., Evans, G. and Mudra, H. (2005). Carotid Intimal-Media Thickness as a Surrogate for Cardiovascular Disease Events in Trials of HMG-CoA Reductase Inhibitors. *Current Control Trials for Cardiovascular Medicine*, 6, p.3.

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001). Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA: The Journal of the American Medical Association*, 285(19), pp.2486-2497.

Ferreira, J., Girerd, N., Bozec, E., Machu, J., Boivin, J., London, G., Zannad, F. and Rossignol, P. (2016). Intima–Media Thickness Is Linearly and Continuously Associated With Systolic Blood Pressure in a Population-Based Cohort (STANISLAS Cohort Study). *Journal of the American Heart Association*, 5(6).

Friedewald, W., Levy, R. and Fredrickson, D. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without use of Preparative Ultracentrifuge. *Clinical Chemistry*, 18(6), pp.499-502.

Frisard, M., Greenway, F. and DeLany, J. (2005). Comparison of Methods to Assess Body Composition Changes during a Period of Weight Loss. *Obesity Research*, 13(5), pp.845-854.

Gomes, M., Giannella-Neto, D., Faria, M., Tambascia, M., Fonseca, R., Rea, R., Macedo, G., Modesto-Filho, J., Schmid, H., Bittencourt, A., Cavalcanti, S., Rassi, N., Pedrosa, H. and Dib, S. (2009). Estimating Cardiovascular Risk in Patients with Type 2 Diabetes: A National Multicenter Study in Brazil. *Diabetology & Metabolic Syndrome*, 1(1), p.22.

Gooty, V., Sinaiko, A., Ryder, J., Dengel, D., Jacobs, D. and Steinberger, J. (2018). Association Between Carotid Intima Media Thickness, Age, and Cardiovascular Risk Factors in Children and Adolescents. *Metabolic Syndrome and Related Disorders*, 16(3), pp.122-126.

Grundy, S., Benjamin, I., Burke, G., Chait, A., Eckel, R., Howard, B., Mitch, W., Smith, S. and Sowers, J., 1999. Diabetes and Cardiovascular Disease. *Circulation*, 100(10), pp.1134-1146.

Guzmán, M., McMahan, C., McGill, H., Strong, J., Tejada, C., Restrepo, C., Eggen, D., Robertson, W. and Solberg, L. (1968). Selected Methodologic Aspects of the International Atherosclerosis Project. *Laboratory Investigation*, 18(5), pp.479-497.

Hardman, A. (1999). Physical Activity, Obesity and Blood Lipids. *International Journal of Obesity Related Metabolic Disorders*, 23(3): S64-71.

Healthy-ojas.com. 2012. *Healthy Cholesterol Level; TC, HDL-C, LDL-C & TG Normal Ranges*. [online] Available at: https://healthy-ojas.com/cholesterol/healthy-cholesterol-level.html

Hecht, HS. (2011). The Role of Atherosclerosis Imaging in Redefining Normal and Abnormal Cholesterol Values, and Risk Reduction in Primary Prevention Statin Trials. *Current Atherosclerosis Reports*, 13(5), pp.422–430.

Heilman, K., Zilmer, M., Zilmer, K., Lintrop, M., Kampus, P., Kals, J. and Tillmann, V. (2009). Arterial Stiffness, Carotid Artery Intima-Media Thickness and Plasma Myeloperoxidase Level in Children with Type 1 Diabetes. *Diabetes Research and Clinical Practice*, 84(2), pp.168-173.

Hennig, B., Toborek, M. and McClain, C. (2001). High-Energy Diets, Fatty Acids and Endothelial Cell Function: Implications for Atherosclerosis. *Journal of American College Nutrition*, 20(2), pp.97-105.

Hien, H., Tam, N., Tam, V., Derese, A. and Devroey, D. (2018). Prevalence, Awareness, Treatment, and Control of Hypertension and Its Risk Factors in (Central) Vietnam. *International Journal of Hypertension*, 2018, pp.1-12.

Ho, H., Maddaloni, E. and Buzzetti, R. (2019). Risk Factors and Predictive Biomarkers of Early Cardiovascular Disease in Obese Youth. *Diabetes/Metabolism Research and Reviews*, 35(4), p.3134.

Hodis, H., Mack, W., LaBree, L., Selzer, R., Liu, C., Liu, C. and Azen, S. (1998). The Role of Carotid Arterial Intima-Media Thickness in Predicting Clinical Coronary Events. *Annals of Internal Medicine*, 128(4), p.262-269

Holman, R.L., McGill, H.C Jr., Strong, J.P. and Geer, J.C. (1958). The Natural History of Atherosclerosis: The Early Aortic Lesions Seen in New Orleans in the Middle of the 20th Century. *American Journal of Pathology*, 34(2), pp.209-235

Howard, G., Sharrett, A., Heiss, G., Evans, G., Chambless, L., Riley, W. and Burke, G. (1993). Carotid Artery Intimal-Medial Thickness Distribution in General Populations as Evaluated by B-Mode Ultrasound. ARIC Investigators. *Stroke*, 24(9), pp.1297-1304.

Hu, G., Jousilahti, P. and Tuomilehto, J., 2007. Joint Effects of History of Hypertension at Baseline and Type 2 Diabetes at Baseline and During Follow-Up on the Risk of Coronary Heart Disease. *European Heart Journal*, 28(24), pp.3059-3066.

Imano, H., Kitamura, A., Sato, S., Kiyama, M., Ohira, T., Yamagishi, K., Noda, H., Tanigawa, T., Iso, H. and Shimamoto, T., 2009. Trends for Blood Pressure and Its Contribution to Stroke Incidence in the Middle-Aged Japanese Population. *Stroke*, 40(5), pp.1571-1577.

Jarvisalo, M., Jartti, L., Nanto-Salonen, K., Irjala, K., Ronnemaa, T., Hartiala, J., Celermajer, D. and Raitakari, O. (2001). Increased Aortic Intima-Media Thickness: A Marker of Preclinical Atherosclerosis in High-Risk Children. *Circulation*, 104(24), pp.2943-2947.

Juonala, M., Magnussen, C., Venn, A., Dwyer, T., Burns, T., Davis, P., Chen, W., Srinivasan, S., Daniels, S., Kähönen, M., Laitinen, T., Taittonen, L., Berenson, G., Viikari, J. and Raitakari, O. (2010). Influence of Age on Associations Between Childhood Risk Factors and Carotid Intima-Media Thickness in Adulthood. *Circulation*, 122(24), pp.2514-2520.

Juonala, M., Viikari, J. and Raitakari, O., 2013. Main findings from the prospective Cardiovascular Risk in Young Finns Study. *Current Opinion in Lipidology*, 24(1), pp.57-64.

Jurado, J., Ybarra, J., Solanas, P., Caula, J., Gich, I., Pou, J. and Romeo, J. (2009). Prevalence of Cardiovascular Disease and Risk Factors in a Type 2 Diabetic Population of the North Catalonia Diabetes Study. *Journal of the American Academy of Nurse Practitioners*, 21(3), pp.140-148.

Keates, A., Mocumbi, A., Ntsekhe, M., Sliwa, K. and Stewart, S. (2017). Cardiovascular Disease in Africa: Epidemiological Profile and Challenges. *Nature Reviews Cardiology*, 14(5), pp.273-293.

Keizer, H. (2012). The "Mevalonate Hypothesis": A Cholesterol-Independent Alternative for the Etiology of Atherosclerosis. *Lipids in Health and Disease*, 11(1).

Kendall, D. (2005). The Dyslipidemia of Diabetes Mellitus: Giving Triglycerides and Highdensity Lipoprotein Cholesterol a Higher Priority? *Endocrinology and Metabolic Clinics of North America*, 34(1), pp27-48.

Khanna, N., Jamthikar, A., Araki, T., Gupta, D., Piga, M., Saba, L., Carcassi, C., Nicolaides, A., Laird, J., Suri, H., Gupta, A., Mavrogeni, S., Kitas, G. and Suri, J. (2019). Nonlinear Model for the Carotid Artery Disease 10-Year Risk Prediction by Fusing Conventional Cardiovascular Factors to Carotid Ultrasound Image Phenotypes: A Japanese Diabetes Cohort Study. *Echocardiography*, 36(2), pp.345-361.

Kokubo, Y. and Kamide, K., 2009. High-Normal Blood Pressure and the Risk of Cardiovascular Disease. *Circulation Journal*, 73(8), pp.1381-1385.

Kokubo, Y., Okamura, T., Watanabe, M., Higashiyama, A., Ono, Y., Miyamoto, Y., Furukawa, Y., Kamide, K., Kawanishi, K., Okayama, A. and Yoshimasa, Y. (2010). The Combined Impact of Blood Pressure Category and Glucose Abnormality on the Incidence of Cardiovascular Diseases in a Japanese Urban Cohort: The Suita Study. *Hypertension Research*, 33(12), pp.1238-1243.

Krauss, R., Winston, M., Fletcher, B. and Grundy, S. (1998). Obesity: Impact on Cardiovascular Disease. *Circulation*, 98(14), pp.1472-1476.

Kumari, N., Dwarakanath, B., Das, A. and Bhatt, A. (2016). Role of Interleukin-6 in Cancer Progression and Therapeutic Resistance. *Tumor Biology*, 37(9), pp.11553-11572.

Kumar, V., Kumar, K., Lakshmi, A., Srinivasa Rao, P. and Das, G., 2009. Carotid Intima-Media Thickness in Patients with End-stage Renal Disease. *Indian Journal of Nephrology*, 19(1), p.13.

Lakka, T., Salonen, R., Kaplan, G. and Salonen, J. (1999). Blood Pressure and the Progression of Carotid Atherosclerosis in Middle-Aged Men. *Hypertension*, 34(1), pp.51-56.

Lean, M., Han, T. and Morrison, C., 1995. Waist Circumference as a Measure for Indicating Need for Weight Management. *BMJ*, 311(6998), pp.158-161.

Levitan, E., Wolk, A. and Mittleman, M., 2009. Consistency With the DASH Diet and Incidence of Heart Failure. *Archives of Internal Medicine*, 169(9), p.851.

Li, Z., Froehlich, J., Galis, Z. and Lakatta, E. (1999). Increased Expression of Matrix Metalloproteinase-2 in the Thickened Intima of Aged Rats. *Hypertension*, 33(1), pp.116-123.

Linderman, G., Lu, J., Lu, Y., Sun, X., Xu, W., Nasir, K., Schulz, W., Jiang, L. and Krumholz, H. (2018). Association of Body Mass Index with Blood Pressure Among 1.7 Million Chinese Adults. *JAMA Network Open*, 1(4), p.e181271.

Linton, M., Yancey, P., Davies, S., Jerome, W., Linton, E., Song, W., Doran, A. and Vickers, K. (2019). The Role of Lipids and Lipoproteins in Atherosclerosis. *Endotext*.

Lohman, T., Roache, A. and Martorell, R., 1992. Anthropometric Standardization Reference Manual. *Medicine & Science in Sports & Exercise*, 24(8), p.952.

Lusiani, L., Visonà, A. and Pagnan, A. (1990). Noninvasive Study of Arterial Hypertension and Carotid Atherosclerosis. *Stroke*, 21(3), pp.410-414.

Magnussen, C. (2017). Carotid Artery Intima-Media Thickness and Hypertensive Heart Disease: A Short Review. *Clinical Hypertension*, 23(1).

Malina, R. and Bouchard, C. (1988). Subcutaneous Fat Distribution During Growth. In Fat Distribution During Growth and Later Health Outcomes. pp63-84.

Matangi, M., Cases, M., Brouillard, D., Armstrong, D. and Johri, A., 2019. 3042 Gender Differences in the Prevalence of a Normal IMT with Increasing Severity of Carotid Disease. *European Heart Journal*, 40(Supplement_1).

Mannami, T., Konishi, M., Baba, S., Nishi, N. and Terao, A. (1997). Prevalence of Asymptomatic Carotid Atherosclerotic Lesions Detected by High-Resolution Ultrasonography and Its Relation to Cardiovascular Risk Factors in the General Population of a Japanese City. *Stroke*, 28(3), pp.518-525.

Martin-Lorenzo, M., Alvarez-Llamas, G., McDonnell, L. and Vivanco, F. (2015). Molecular Histology of Arteries: Mass Spectrometry Imaging as a Novelex Vivotool to Investigate Atherosclerosis. *Expert Review of Proteomics*, 13(1), pp.69-81.

McEvoy, J., Chen, Y., Nambi, V., Ballantyne, C., Sharrett, A., Appel, L., Post, W., Blumenthal, R., Matsushita, K. and Selvin, E., 2015. High-Sensitivity Cardiac Troponin T and Risk of Hypertension. *Circulation*, 132(9), pp.825-833.

McGill, H. and McMahan, C., 1998. Determinants of atherosclerosis in the young. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *The American Journal of Cardiology*, 82, pp.30-36.

McGill, H., McMahan, C. and Gidding, S. (2008). Preventing Heart Disease in the 21st Century: Implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study. *Circulation*, 117(9), pp.1216-1227.

McNamara, D. and Howell, W. (1992). Epidemiologic Data Linking Diet to Hyperlipidemia and Arteriosclerosis. *Seminars in Liver Disease,* 12(4), pp.347-355.

Middeke, M., Richter, W. O., Schwandt, P., Beck, B. and Holzgreve, H. (1990). Normalization of Lipid Metabolism after Withdrawal from Antihypertensive Long-Term Therapy with Beta Blockers and Diuretics. *Arteriosclerosis*, 10(1), pp.145-147.

Montagnani, M. and Quon, M J. (2000). Insulin Action in Vascular Endothelium: Potential Mechanisms Linking Insulin Resistance with Hypertension. *Diabetes Obesity Metabolism,* 2, pp.285– 292.

Moran, C. and Thomson, A., 2020. Preclinical Ultrasound Imaging—A Review of Techniques and Imaging Applications. *Frontiers in Physics*, 8.

Msemburi, W., Pillay-van Wyk, V., Dorrington, R., Neethling, I., Nannan, N., Groenewald, P., Laubscher, R., Joubert, J., Matzopoulos, R., Nicol, E., Notilana, B., Prinsloo, M., Sithole, N., Somdyala, N. and Bradshaw, D. (2014). Second National Burden of Disease Study for South

Africa: Cause-of-Death Profile for South Africa, 1997 2010. *Cape Town: South African Medical Research Council*, 978(1-920618), pp.34-36.

Nagai, Y., Metter, E., Earley, C., Kemper, M., Becker, L., Lakatta, E. and Fleg, J. (1998). Increased Carotid Artery Intimal-Medial Thickness in Asymptomatic Older Subjects with Exercise-Induced Myocardial Ischemia. *Circulation*, 98(15), pp.1504-1509.

Nakamura, H., Arakawa, K., Itakura, H., Kitabatake, A., Goto, Y., Toyota, T., Nakaya, N., Nishimoto, S., Muranaka, M., Yamamoto, A., Mizuno, K. and Ohashi, Y. (2006). Primary Prevention of Cardiovascular Disease with Pravastatin in Japan (MEGA Study): A Prospective Randomised Controlled Trial. *The Lancet*, 368(9542), pp.1155-1163.

Norman, R., Pieterse, D., Bradshaw, D., Levitt, N., Steyn, K., Laubscher, R. and Gaziano, T. (2007). Estimating The Burden of Disease Attributable to Diabetes in South Africa in 2000. *South African Medical Journal*, 97, pp.692-698.

Okosun, I., Chandra, K., Boev, A., Boltri, J., Choi, S., Parish, D. and Dever, G. (2004). Abdominal Adiposity in U.S. Adults: Prevalence and Trends, 1960–2000. *Preventive Medicine*, 39(1), pp.197-206.

O'Leary, D. and Bots, M. (2010). Imaging of Atherosclerosis: Carotid Intima-Media Thickness. *European Heart Journal*, 31(14), pp.1682-1689.

Park, M., Skow, Á., De Matteis, S., Kessel, A., Saxena, S., Viner, R. and Kinra, S. (2015). Adiposity and Carotid-Intima Media Thickness in Children and Adolescents: A Systematic Review. *BMC Pediatrics*, 15(1).

Pignoli, P., Tremoli, E., Poli, A., Oreste, P. and Paoletti, R. (1986). Intimal Plus Medial Thickness of The Arterial Wall: A Direct Measurement with Ultrasound Imaging. *Circulation*, 74(6), pp.1399-1406.

Polak, J., Pencina, M., Pencina, K., O'Donnell, C., Wolf, P. and D'Agostino, R. (2011). Carotid-Wall Intima-Media Thickness and Cardiovascular Events. *National English Journal of Medicine,* 365, pp.213–221.

Polak, J., Person, S., Wei, G., Godreau, A., Jacobs, D., Harrington, A., Sidney, S. and O'Leary, D. (2010). Segment-Specific Associations of Carotid Intima-Media Thickness with Cardiovascular Risk Factors. *Stroke*, 41(1), pp.41-47.

Poredos, P. (2004). Intima-Media Thickness: Indicator of Cardiovascular Risk and Measure of the Extent of Atherosclerosis. *Vascular Medicine*, 9(1), pp.46-54.

Post, G., Kemper, H., Twisk, J. and van Mechelen, W. (1997). The Association between Dietary Patterns and Cardiovascular Disease Risk Indicators in Healthy Youngsters: Results Covering Fifteen Years of Longitudinal Development. *European Journal of Clinical Nutrition*, 51(6), pp.387-393.

Pouliot, M., Després, J., Lemieux, S., Moorjani, S., Bouchard, C., Tremblay, A., Nadeau, A. and Lupien, P. (1994). Waist Circumference and Abdominal Sagittal Diameter: Best Simple Anthropometric Indexes of Abdominal Visceral Adipose Tissue Accumulation and Related Cardiovascular Risk in Men and Women. *The American Journal of Cardiology*, 73(7), pp.460-468.

Reinehr T. (2013). Type 2 Diabetes Mellitus in Children and Adolescents. *World J Diabetes*, 4(6), pp.270-281.

Roche, A. (1996) Anthropometry and Ultrasound. In: Roche A, Heymsfield S, Lohman T. *Human body composition*. Champaign, IL: Human Kinetics Press, pp.167–189.

Roche, A., Siervogel, R., Chumlea, WC. and Webb, P. (1981). Grading Body Fatness from Limited Anthropometric Data. *The American Journal of Clinical Nutrition*, 34(12), pp.2831–2838.

Rodday, A., Triedman, J., Alexander, M., Cohen, J., Ip, S., Newburger, J., Parsons, S., Trikalinos, T., Wong, J. and Leslie, L. (2012). Electrocardiogram Screening for Disorders that Cause Sudden Cardiac Death in Asymptomatic Children: A Meta-analysis. *Pediatrics*, 129(4), pp.e999-e1010.

Roman, M., Naqvi, T., Gardin, J., Gerhard-Herman, M., Jaff, M. and Mohler, E. (2006). Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. *Journal of American Society of Echocardiography*, 19, pp.943-54.

Rosengren, A., Welin, L., Tsipogianni, A. and Wilhelmsen, L., 1989. Impact of Cardiovascular Risk Factors on Coronary Heart Disease and Mortality Among Middle Aged Diabetic Men: A General Population Study. *BMJ*, 299(6708), pp.1127-1131.

Roth, G., Abate, D. and Abate, K. (2018). Global, Regional, and National Age-Sex-Specific Mortality for 282 Causes of Death in 195 Countries and Territories, 1980–2017: A Systematic Analysis for the Global Burden of Disease Study 2017. *Lancet*, 392(10159), pp.1736-1788.

Ruixing, Y., Jinzhen, W., Yaoheng, H., Jing, T., Hai, W., Muyan, L., Yiyang, L., Dongmei, F., Hanjun, Y. and Yuming, C. (2008). Associations of Diet and Lifestyle with Hyperlipidemia for Middle-Aged and Elderly Persons among the Guangxi Bai Ku Yao and Han Populations. *Journal of the American Dietetic Association*, 108(6), pp.970-976.

Salonen, R. and Salonen, J. (1991). Carotid Atherosclerosis in Relation to Systolic and Diastolic Blood Pressure: Kuopio Ischaemic Heart Disease Risk Factor Study. *Annals of Medicine*, 23(1), pp.23-27.

Scandinavian Simvastatin Survival Study Group: Randomised Trial of Cholesterol Lowering in 4444 Patients with Coronary Heart Disease: The Scandinavian Simvastatin Survival Study (4S). (1994). *The Lancet*, 344(8934), pp.1383-1389.

Schlendorf, K., Nasir, K. and Blumenthal, R. (2009). Limitations of the Framingham Risk Score are now much clearer. *Preventive Medicine*, 48(2), pp.115-116.

Seidell, J., Oosterlee, A., Thijssen, M., Burema, J., Deurenberg, P., Hautvast, J. and Ruijs, J. (1987). Assessment of Intra-Abdominal and Subcutaneous Abdominal Fat: Relation between Anthropometry and Computed Tomography. *The American Journal of Clinical Nutrition*, 45(1), pp.7-13.

Shamai, L., Lurix, E., Shen, M., Novaro, G., Szomstein, S., Rosenthal, R., Hernandez, A. and Asher, C. (2010). Association of Body Mass Index and Lipid Profiles: Evaluation of a Broad Spectrum of Body Mass Index Patients Including the Morbidly Obese. *Obesity Surgery*, 21(1), pp.42-47.

Simon, A., Gariepy, J., Chironi, G., Megnien, J. and Levenson, J. (2002). Intima–Media Thickness: A New Tool for Diagnosis and Treatment of Cardiovascular Risk. *Journal of Hypertension*, 20(2), pp.159-169.

Sliwa, K., Lyons J., Carrington, M., Lecour, S., Marais, A., Frederick, J., Raal, F. and Stewart, S. (2012). Different lipid profiles according to ethnicity in the Heart of Soweto study cohort of *de novo* presentations of heart disease. *Cardiovascular Journal of Africa*, 23(7), pp.389–395.

Slyper, A. (2004). What Vascular Ultrasound Testing Has Revealed about Pediatric Atherogenesis, and a Potential Clinical Role for Ultrasound in Pediatric Risk Assessment. *The Journal of Clinical Endocrinology & Metabolism*, 89(7), pp.3089-3095.

Smaoui, M., Hammami, S., Chaaba, R., Attia, N., Hamda, K., Masmoudi, A., Mahjoub, S., Bousslama, A., Farhat, M. and Hammami, M. (2004). Lipids And Lipoprotein(A)

Concentrations in Tunisian Type 2 Diabetic Patients; Relationship to Glycemic Control and Coronary Heart Disease. *Journal of Diabetes Complications,* 18(5), pp.258- 263.

Smith, S., Lovejoy, J., Greenway, F., Ryan, D., deJonge, L., de la Bretonne, J., Volafova, J. and Bray, G. (2001). Contributions of Total Body Fat, Abdominal Subcutaneous Adipose Tissue Compartments, and Visceral Adipose Tissue to the Metabolic Complications of Obesity. *Metabolism*, 50(4), pp.425-435.

Soliman, E., Ding, J., Hsu, F., Carr, J., Polak, J. and Goff, D. (2010). Association between Carotid Intima-Media Thickness and Pericardial Fat in the Multi-Ethnic Study of Atherosclerosis (MESA). *Journal of Stroke and Cerebrovascular Diseases*, 19(1), pp.58-65.

Sommer, A. and Twig, G. (2018). The Impact of Childhood and Adolescent Obesity on Cardiovascular Risk in Adulthood: A Systematic Review. *Current Diabetes Reports*, 18(10).

Sorof, J., Alexandrov, A., Garami, Z., Turner, J., Grafe, R., Lai, D. and Portman, R. (2003). Carotid Ultrasonography for Detection of Vascular Abnormalities in Hypertensive Children. *Pediatric Nephrology*, 18(10), pp.1020-1024.

Stamler, J., Vaccaro, O., Neaton, J. and Wentworth, D., 1993. Diabetes, Other Risk Factors, and 12-Yr Cardiovascular Mortality for Men Screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care*, 16(2), pp.434-444.

Steinhagen-Thiessen, E., Bramlage, P., Lösch, C., Hauner, H., Schunkert, H., Vogt, A., Wasem, J., Jöckel, K. and Moebus, S. (2008). Dyslipidemia in Primary Care – Prevalence, Recognition, Treatment and Control: Data from the German Metabolic and Cardiovascular Risk Project (GEMCAS). *Cardiovascular Diabetology*, 7(1), p.31.

Steyn, K., Gaziano, T., Bradshaw, D., Laubscher, R. and Fourie, J. (2001). Hypertension in South African adults: results from the Demographic and Health Survey, 1998. *Journal of Hypertension*, 19(10), pp.1717-1725.

Stone, N., 1994. Secondary Causes of Hyperlipidemia. *Medical Clinics of North America*, 78(1), pp.117-141.

Sumner, A., Vega, G., Genovese, D., Finley, K., Bergman, R. and Boston, R. (2005). Normal Triglyceride Levels Despite Insulin Resistance in African Americans: Role of Lipoprotein Lipase. *Metabolism*, 54(7), pp.902-909.

Suri, J., Yuan, C., Wilson, D. and Laxminarayan, S. (2005). Plaque Imaging: Pixel to Molecular Level, 113, p.488.

Swinburn, B., Sacks, G., Hall, K., McPherson, K., Finegood, D., Moodie, M. and Gortmaker, S., 2011. The Global Obesity Pandemic: Shaped by Global Drivers and Local Environments. *The Lancet*, 378(9793), pp.804-814.

Tanasescu, M., Cho, E., Manson, J. and Hu, F. (2004). Dietary Fat and Cholesterol and the Risk of Cardiovascular Disease Among Women with Type 2 Diabetes. *American Journal of Clinical Nutrition*, 79(6), pp.999-1005.

Tolonen, H., Keil, U., Ferrario, M., and Evans, A., (2005). Prevalence, Awareness and Treatment of Hypercholesterolaemia in 32 Populations: Results from the WHO MONICA Project. *International Journal of Epidemiology*, 34(1), pp.181-192.

Turner, R., Millns, H., Neil, H., Stratton, I., Manley, S., Matthews, D. and Holman, R., 1998. Risk Factors for Coronary Artery Disease in Non-Insulin Dependent Diabetes Mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *BMJ*, 316(7134), pp.823-828.

Tuzcu, E., Kapadia, S., Eralp Tutar, E., Ziada, K., Hobbs, R., McCarthy, P., Young, J. and Nissen, S. (2001). High Prevalence of Coronary Atherosclerosis in Asymptomatic Teenagers and Young Adults Evidence from Intravascular Ultrasound. *Circulation*,103, pp.2705-2710.

van den Munckhof, I., Holewijn, S., de Graaf, J. and Rutten, J. (2017). Sex Differences in Fat Distribution Influence the Association between BMI and Arterial Stiffness. *Journal of Hypertension*, 35(6), pp.1219-1225.

Virmani, R., Avolio, A., Mergner, W., Robinowitz, M., Herderick, E., Cornhill, J., Guo, S., Liu, T., Ou, D. and O'Rourke, M. (1991). Effect Of Aging on Aortic Morphology in Populations with High and Low Prevalence of Hypertension and Atherosclerosis: Comparison Between Occidental and Chinese Communities. *American Journal of Pathology*, 139(5), pp.1119-1129.

West, J. (2005). Biomimetic Strategies in Vascular Tissue Engineering. Frontiers of Engineering: Reports on Leading-Edge Engineering from the 2004 NAE Symposium on Frontiers of Engineering., 56-Washington, DC: The National Academies Press.

Who.int. 2021. *Cardiovascular diseases (CVDs)*. [online] Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

WHO. Obesity: Preventing and Managing the Global Epidemic. Geneva: World Health Organization Programme of Nutr; 1998. 6-3-1997.

WHO. Physical status: the use and interpretation of anthropometry. Geneva: WHO; 1995.

Wirth, A. and Steinmetz, B. (1998). Gender Differences in Changes in Subcutaneous and Intra-abdominal Fat during Weight Reduction: An Ultrasound Study. *Obesity Research*, 6(6), pp.393-399.

Windaus, A. (1910). Über den Gehalt normaler und atheromatöser Aorten an Cholesterin und Cholesterinestern. (About the Content of Normal and Atheromatous Aorta on Cholesterol and Cholesterol Esters). *Hoppe-Seyler´s Zeitschrift für physiologische Chemie*, 67(2), pp.174-176.

Yang, C., Sun, Z., Li, Y., Ai, J., Sun, Q. and Tian, Y. (2014). The Correlation between Serum Lipid Profile with Carotid Intima-Media Thickness and Plaque. *BMC Cardiovascular Disorders*, 14(1).

Yoo, E., (2016). Waist-To-Height Ratio as a Screening Tool for Obesity and Cardiometabolic Risk. *Korean Journal of Pediatrics*, 59(11), p.425.

Young, W., Gofman, J., Tandy, R., Malamud, N. and Waters, E. (1960). The Quantitation of Atherosclerosis III: The Extent of Correlation of Degrees of Atherosclerosis with and between the Coronary and Cerebral Vascular Beds. *American Journal of Cardiology*, 8, pp.300-308.

Zanchetti, A., Crepaldi, G., Bond, M., Gallus, G., Veglia, F., Ventura, A., Mancia, G., Baggio, G., Sampieri, L., Rubba, P., Collatina, S. and Serrotti, E. (2001). Systolic and Pulse Blood Pressures (but not Diastolic Blood Pressure and Serum Cholesterol) are Associated with Alterations in Carotid Intima–Media Thickness in the Moderately Hypercholesterolaemic Hypertensive Patients of the Plaque Hypertension Lipid Lowering Italian Study. *Journal of Hypertension*, 19(1), pp.79-88.

Zaninotto, P., Mindel,l J. and Hirani, V. (2007). Prevalence of Cardiovascular Risk Factors Among Ethnic Groups: Results from the Health Surveys for England. *Atherosclerosis*, 195(1), pp.48-57.

Zhang, L., Qiao, Q., Tuomilehto, J., Janus, E., Lam, T., Ramachandran, A., Mohan, V., Stehouwer, C., Dong, Y., Nakagami, T., Onat, A. and Söderberg, S. (2010). Distinct Ethnic Differences in Lipid Profiles across Glucose Categories. *The Journal of Clinical Endocrinology & Metabolism*, 95(4), pp.1793-1801.