MODULATION OF CARDIOVASCULAR FUNCTION BY ROOIBOS IN ADULTS AT RISK FOR CARDIOVASCULAR DISEASE

BY

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DECLARATION

I, Maria Diana Hartnick, declare that the content of this thesis represents my own unaided work and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

M.D. Hartnick  September 2024

Signed Date

ABSTRACT

Introduction: Cardiovascular disease (CVD), diabetes and stroke collectively represent the second leading cause of mortality among South African adults. These non-communicable diseases pose a significant global health threat, emphasising the importance of adopting and maintaining a healthy lifestyle with optimal dietary habits. Failure to address this issue will likely result in a substantial increase in the burden of non-communicable diseases (NCDs) in South Africa in the coming decades. In 2017, coronary heart disease and stroke emerged as the predominant non-communicable disease (NCD) globally and resulted in an estimated 17.8 million fatalities, with over 75% of these fatalities occurring in low-income and middle-income countries. This fact underscores the critical need for further investigation and interventions in the field of cardiovascular health. One of the root causes underlying many non-communicable diseases, including CVD, cancer, and diabetes, is a redox imbalance resulting in 'chronic' oxidative stress. To mitigate these risks and potentially enhance the quality of life and lifespan, the utilisation of bioactives, such as antioxidants to support the redox balance, is imperative.

Aim: This research aims to determine the prevalence and characteristics of CVD and its associated risk factors in a South African cohort, assess the impact of Rooibos (*Aspalathus linearis*) on cardiovascular function in adults at risk for CVD using Transthoracic Echocardiography (TTE), and establish an association between CVD findings and dietary interventions.

Method: Using a randomised, placebo-controlled parallel design, this study investigated the impact of regular consumption of two types of Rooibos on key cardiovascular parameters using TTE. The cohort of participants (n=219) included three parallel groups that were matched according to age and gender. They were randomly assigned to consume either the placebo or fermented Rooibos, or green Rooibos. The Rooibos capsules contained standardised water-soluble extracts, and participants were requested to consume one capsule (equivalent to two cups of Rooibos herbal tea) three times a day with meals (morning, midday, and evening) for 12 weeks. Cardiovascular function was assessed using TTE. All echocardiograms were performed according to the American Society of Echocardiography (ASE) guidelines, standards and recommendations.

Results: Objective 1: The results indicate that the population was moderately obese: Body Mass Index (BMI) = 31 kg/m²; the average systolic blood pressure (SBP) = 131 mmHg; average diastolic blood pressure (DBP) = 84 mmHg; total serum cholesterol = 5.5 mmol/l; fasting plasma glucose = 4.51 mmol/L, within normal limits. Of the 219 participants, 118 (53.9%) were at a lower risk for cardiovascular disease, and 101 (46.1%) were at a higher risk for cardiovascular disease. Objective 2: The effect of daily Rooibos consumption over 12

weeks on the cardiovascular system included the following outcomes: grade 1 diastolic dysfunction counts decreased from 63 (28%) to 47 (21.5%), grade 2 diastolic dysfunction decreased from 46 (21%) to 24 (11%) and grade 3 diastolic dysfunction decreased from 5 (2.3%) to 3 (1.4%). There was no effect on the aortic size (AO) in all the intervention groups, while the left atrium (LA) significantly (p=0.01) decreased in size from 3.83 cm ± 0.07 cm to 3.68 cm ± 0.07 cm. The LA / AO ratio did not change significantly in all the intervention groups. Interventricular septum diameter (IVSd) in the placebo group decreased significantly *(p=0.002)* from 1.33 cm ± 0.030 cm to 1.25 cm ± 0.03 cm with no positive change in the fermented rooibos group, while the green rooibos group demonstrated a significantly *(p=0.002)* decreased diameter from 1.28 cm ± 0.04 cm to 1.19 cm ± 0.03 cm. In all three intervention groups no significant changes for the left ventricle posterior wall (LVPWd) were detected. The placebo *(p=0.300)* and green rooibos *(p=0.292)* groups demonstrated no significant changes for the left ventricle mass (LVM), while the fermented rooibos group showed a significant *(p=0.015)* decreased mass (from 204.1 g ± 7.1 g to 191.4 g ± 6.7 g). No significant positive changes were seen in all the groups with regard to the left ventricle in diastole diameter (LVIDd), left ventricle in systole diameter (LVISd) and the ejection fraction (EF).

Conclusion: In general, this study reveals evidence suggesting that the regular consumption of Rooibos may be associated with cardiovascular protective effects, with specific focus on the left atrium (LA), interventricular septum (IVS) and left ventricular mass (LVM). These findings underscore the potential benefits of incorporating Rooibos into the daily dietary habits as a means to promote heart health and reduce the risk for cardiovascular diseases. Further research and clinical investigations are warranted to elucidate the precise mechanisms underlying these protective effects and to explore the long-term implications of Rooibos consumption on overall cardiovascular well-being.

PUBLICATIONS AND OUTPUTS

Below is a list of the Presentations and Publications for the Research conducted to date:

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2. Hartnick, M., Marnewick, J., Engel-Hills, P., Kemp, M. 2024. Modulatory Effect of Rooibos on Cardiac Parameters: An Echocardiography Analysis. *United Kingdom Imaging and Oncology Congress.* E-Poster Presentation, ACC Liverpool: United Kingdom. 10 – 12 June 2024.

3. Hartnick, M., Marnewick, J., Engel-Hills, P., Kemp, M., Pretorius, K., Uys, C & Lekata, S. 2024. Impact of Chronic Consumption of Herbal Rooibos on Cardiovascular Function in Adults with Cardiovascular Risk. *Journal of Medicinal Food*. Published Online: 3 July 2024. https://doi.org/10.1089/jmf.2024.0012.

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DEDICATED TO

My father, Willie Louw, who died on 11 May 2021. You have believed in me even when I did not believe in myself. You sacrificed so much for me to achieve every goal that I had set for myself. You are forever in my heart.

"I would have lost heart, unless I had believed that I would see the goodness of the Lord in the land of the living".

Psalms 27:13

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ABBREVIATIONS

2D	-	Two-Dimensional
A2C	-	Apical 2-Chamber
A4C	-	Apical 4-Chamber
ACC	-	Acetyl-CoA Carboxylase
ACE	-	Angiotensin-converting Enzyme
ACS	-	Acute Coronary Syndrome
AF	-	Atrial Fibrillation
ALARA	-	As Low As Reasonably Achievable
AMHBI	-	Applied Microbial and Health Biotechnology Institute
AMI	-	Acute Myocardial Infarction
AMPK	-	Adenosine Mono Phosphate activated protein Kinase
A. linearis	-	*Aspalathus linearis*
AO	-	Aorta
ASE	-	American Society of Echocardiography
Asp	-	Aspalathin
ATP	-	Adenosine Triphosphate
AV	-	Aortic Valve
BP	-	Blood Pressure
CAD	-	Coronary Artery Disease
CBF	-	Cerebral Blood Flow
CF	-	Colour Flow
CHD	-	Coronary Heart Disease
CK	-	Creatine Kinase
CO	-	Cardiac Output
CPT1	-	Carnitine Palmitoyltransferase 1
CPUT	-	Cape Peninsula University of Technology
CRP	-	C-reactive Protein
CT	-	Computer Tomography
CVA	-	Cerebrovascular Accident
CVD	-	Cardiovascular Disease
CWD	-	Continuous Wave Doppler
DM	-	Diabetes Mellitus
DBP	-	Diastolic Blood Pressure
ECG	-	Electrocardiograph
ECHO	-	Echocardiogram

EF	-	Ejection Fraction
FDA	-	Food and Drug Administration
FHDC	-	Faculty Higher Degrees Committee
FS	-	Fractioning Shortening
GMP	-	Good Manufacturing Practices
HB	-	Hemoglobin
Hcy	-	Homocysteine
HDL	-	High Density Lipoprotein
HF	-	Heart Failure
HPCSA	-	Health Professions Council of South Africa
HPT	-	Hypertension
hs-CRP	-	high-sensitivity C-reactive Protein
IFG	-	Impaired Fasting Glucose
IVS	-	Interventricular septum
IVSd	-	Interventricular septum thickness in diastolic
IVSs	-	Interventricular septum thickness in systolic
LA	-	Left Atrium
LDL	-	Low Density Lipoprotein
LV	-	Left Ventricle
LVH	-	Left Ventricular Hypertrophy
LVIDd	-	Left Ventricle Internal Diastolic diameter
LVISd	-	Left Ventricle Internal Systolic diameter
LVOT	-	Left Ventricular Outflow Tract
LVPWd	-	Left Ventricle Posterior wall diameter
MI	-	Myocardial Infarction
Mb	-	Myoglobin
MCD	-	Malonyl-CoA Decarboxylase
MR	-	Mitral Regurgitation
MRI	-	Magnetic Resonance Imaging
MS	-	Mitral Stenosis
MV	-	Mitral Valve
NCDs	-	Non-Communicable Diseases
Not	-	Nothofagin
ox-LDL	-	oxidised LDL
PA	-	Pulmonary Artery
PAI-1	-	Plasminogen Activator Inhibitor-1
PAD	-	Peripheral Arterial Disease

PWD	-	Pulse Wave Doppler
RA	-	Right Atrium
RNS	-	Reactive Nitrogen Species
ROS	-	Reactive Oxygen Species
RVOT	-	Right Ventricular Outflow Tract
SV	-	Stroke Volume
SARC	-	South African Rooibos Council
T1D	-	Type 1 Diabetes
T2D	-	Type 2 Diabetes
TC	-	Total Cholesterol
TEE	-	Trans-esophageal echocardiography
TG	-	Triglyceride
TIA	-	Trans Ischemic Attack
Trop T	-	Troponin T
TTE	-	Trans-Thoracic Echocardiography
TV	-	Tricuspid Valve
WHO	-	World Health Organisation

A SNAPSHOT OF THE THESIS

Title of the Study:	**MODULATION OF CARDIOVASCULAR FUNCTION BY ROOIBOS IN ADULTS AT RISK FOR CARDIOVASCULAR DISEASE**
Aim:	This research aimed to determine the prevalence and characteristics of CVD and its associated risk factors in a South African cohort, assess the impact of Rooibos (Aspalathus linearis) on cardiovascular function in adults at risk for CVD using Transthoracic Echocardiography (TTE), and establish an association between CVD findings and dietary interventions.
Research Question:	What is the effect of the chronic intake of rooibos herbal tea on cardiovascular function in adults at risk for cardiovascular disease as determined by Trans-Thoracic Echocardiography?
Research Design:	This research engaged in a 14-week randomised, parallel, placebo-controlled dietary intervention in which participants were administered capsules containing either standardised water-soluble rooibos herbal tea extracts or a placebo. To establish the initial health status and to measure the final outcomes, transthoracic echocardiography was conducted both at the commencement and conclusion of the study.
The philosophical basis for the research: A Quantitative Research study, which is based on answering the research question and measuring each objective in this study (Shields & Twycross, 2003:24).	
Paradigm: Framework	Positivism
Ontology: Nature of reality	The burden of cardiovascular disease in low- and middle-income countries, particularly in sub-Saharan Africa, carries substantial economic and social implications. The higher mortality rates observed among younger individuals can be attributed to inadequate prevention and management efforts. Addressing these challenges requires a multifaceted approach, prioritising prevention measures and improving access to effective treatments (Strong et al., 2005; Kaptoge et al., 2019).

Epistemology: Nature of knowledge	The research contributes to the understanding of rooibos herbal tea's potential health and/or therapeutic benefits in reducing cardiovascular risk factors and improving overall cardiovascular health (Marnewick et al., 2009; Marnewick et al., 2011; Van Wyk et al., 2011; Maleki et al., 2015; Ajuwon et al., 2018; Ito et al., 2019).
Axiology: Application of knowledge gained	This research is the first to report on the use of echocardiography to assess the effect of Rooibos on heart function to improve the health outcomes of individuals at risk for cardiovascular disease.

Objectives	**Literature Review**	**Methodology**	**Results**	**Recommendations**
1. Determine the prevalence and characteristics of cardiovascular disease and its associated risk factors.	Cardiovascular disease risk factors -Diabetes Mellitus - Hypertension - Athero-sclerosis - Life style	Data were collected from demographic and health questionnaires which the participants had to complete, screening results using point-of-care blood test kits, as well as anthropometric measurements that were done by registered medical personnel.	N=219: one was at higher risk across all seven risk categories, 19 at higher risk in six categories, 41 at higher risk in five categories, and 40 participants were at higher risk in four categories. Five participants were found to have no elevated risk across any of the seven categories	- Including waist-hip ratio and waist-height ratio to measure abdominal obesity

Objectives	Literature Review	Methodology	Results	Recommendations
			used in this study.	
2. Draw a comparison between the cardiovascular ultrasound findings in the traditional/fermented and green/unfermented rooibos and placebo groups of participants.	- Role of Echocardiography in assessing cardiovascular function -Aspalathus linearis health promoting properties	-12-week randomised, placebo-controlled dietary intervention trial using capsules containing standardised green and fermented rooibos herbal tea extracts or a placebo. - Transthoracic echocardiography to assess the cardiac structures	Echocardiography played a role in identifying cardiovascular disease in this group. - *Aspalathus linearis* (Rooibos) has been effective in modulating the cardiovascular function of participants in this research study.	- Consider LA volume measurement with LA diameter in future projects
3. Explore possible associations between the CVD findings and Rooibos interventions.	- Dietary intervention to prevent / modulate cardiovascular risks	- Analyse the cardiac function using Echocardiography in relation to the rooibos interventions.	- The study found that the interventions had an influence on some of the cardiac variables.	

Chapter 1 | Introduction

This study explores the potential health benefits of Rooibos, specifically its impact on cardiovascular function in individuals at risk for cardiovascular disease (CVD). Despite the growing body of research highlighting the therapeutic properties of Rooibos, there is a gap in clinical studies using diagnostic tools such as echocardiography to evaluate its effects on heart health. To my knowledge, this research is the first to employ echocardiography to investigate how Rooibos consumption might influence cardiovascular function in at-risk adults.

The research project, titled ‘Modulation of Cardiovascular Function by Rooibos in Adults at Risk for Cardiovascular Disease,’ is detailed in this chapter. Cardiovascular disease, which encompasses a variety of chronic conditions such as coronary artery disease, heart failure, and stroke, remains the leading non-communicable disease (NCD) globally and poses a significant health challenge in South Africa (Smith & Swart, 2018). The prevalence of CVD has escalated dramatically in South Africa over the past decades, contributing to a substantial burden on the healthcare system and highlighting the urgent need for early intervention and management strategies (Mayosi et al., 2009). Type 2 diabetes, cancer, chronic lung disease, and depression, CVD is a predominant NCD affecting both developing and developed regions, with an impact in low-income areas and countries in transition, such as South Africa Mayosi et al., 2009; Smith and Swart, 2018).

All patients should have a resting transthoracic echocardiogram at the time of their initial presentation of cardiovascular disease. Imaging plays a key role in diagnosing concurrent illnesses, facilitating risk stratification, and guiding therapy choices for patients with stable CAD, aligning with the insights shared by Tendera and Wojakowski (2015) and Votavová et al. (2015). TTE is the most utilised cardiovascular diagnostic test, followed by electrocardiography (ECG) and chest X-rays, to evaluate the left ventricular systolic and diastolic function regional wall motion anomalies and is crucial for making a diagnosis of acute coronary syndrome (ACS) (Esmaeilzadeh et al., 2013). According to Lang et al. (2015), the assessment of the size and function of the cardiac chambers is important when imaging the cardiac system. The ability to bring echocardiographic imaging to patients' bedsides makes the approach vital and its expertise indispensable for all cardiologists and technicians (Votavová et al.,2015). What adds more value to TTE is the fact that it is a non-invasive, accessible, affordable, and trustworthy imaging tool to assess the heart because it provides important diagnostic information regarding cardiac function and can identify CVD (Esmaeilzadeh et al., 2013; Tendera & Wojakowski, 2015).

According to the World Health Organization (2010), CVDs are a group of abnormalities of the heart and the blood vessels which include the following: CAD, cerebrovascular accident

(CVA), peripheral arterial disease (PAD), rheumatic heart disease (RHD), congenital heart disease (CHD), deep vein thrombosis (DVT) and pulmonary embolism (PE). Risk factors, such as diabetes, hypertension (HPT) and hypercholesterolemia, increase an individual's risk for developing CVDs (World Health Organization, 2017). There is, therefore, a definite need for early detection and/or intervention to mitigate their risk. The need for early intervention is confirmed by Park et al. (2021), who suggest that an individual's risk for developing cardiovascular disease increases because of additional risk factors, such as diabetes, hypercholesterolemia, and hypertension, and, as a result, early identification is necessary to enable risk-reduction measures. The World Health Organization launched the 2025 Global Action Plan in 2013, aiming to create a comprehensive strategy for nations to cut early deaths caused by NCDs by 25% by the year 2025, as these diseases represent the majority of global morbidity and mortality (Joseph et al., 2017). Mayosi et al. (2009) proposed that to address the increasing demand for chronic care for both communicable and non-communicable diseases, a unified care model equipped with an effective monitoring system is essential.

I acknowledge that while there is such a high demand for TTE, accessibility challenges with regard to the availability of echocardiography do exist, particularly in resource-constrained settings such as public healthcare systems. With that being said, it is crucial to continue advocating for initiatives that promote greater accessibility and affordability of echocardiography, as it can play an important role in enhancing healthcare outcomes for patients at risk for CVD.

This underscores the need for innovative approaches to mitigate these conditions. My study aims to fill this gap by exploring whether Rooibos, a widely consumed herbal tea with known antioxidant properties, can serve as a practical, dietary-based intervention to improve cardiovascular health. By integrating advanced echocardiographic techniques, this research not only seeks to provide evidence on the cardiovascular benefits of Rooibos but also aims to pave the way for future studies and interventions. The ultimate goal is to contribute to the broader scientific understanding of dietary impacts on heart health and to offer a potentially low-cost, natural intervention that can be easily adopted in daily life, especially in regions with limited access to advanced medical treatments. This research could thus play a pivotal role in shaping public health strategies and policies aimed at reducing the incidence and impact of CVD in vulnerable populations.

The contributions of the main causes of CVD are summarised below **(Table 1:1)** adapted from Common Cardiovascular Diseases, Related Mortality, and Disease Burden in 2015 (Joseph et al., 2017:679).

Table 1-1 The contributions of the main causes of CVD (adapted from Joseph et al., 2017:679)

	Total number of estimated global deaths in 2015	Cumulative percentage of CVD deaths	The age-standardised death rate per 100 000 person-years	Ranking based on disease burden measured by disability-adjusted life years
All deaths	55792		850	
All CVD deaths	17921	100%	285	
Ischaemic heart disease	8917	49.8%	142	1
Cerebrovascular disease	6326	85.1%	101	2 = other strokes 3 = ischaemic stroke
Hypertensive heart disease	962	90.4%	15.4	4
Cardiomyopathy and myocarditis	353	92.4%	5.4	4
Rheumatic heart disease	319	94.2%	4.8	6
Aortic aneurysm	168	96.2%	2.7	9
Endocarditis	84	96.7%	1.3	10
Peripheral vascular disease	52	97.0%	0.9	11
Other cardiovascular diseases	541	100.0%	8.6	5

Research spanning from 2003 to 2020 has highlighted significant shifts in the health landscape of South Africa and sub-Saharan Africa. Bradshaw et al. (2003) identified a combination of heart disease, diabetes, and stroke as the second leading cause of death among South African adults in 2003. Building on this, Maredza et al. (2016) reported in 2016 that rural regions in South Africa are undergoing a rapid health transition characterised by a notable increase in NCDs, particularly stroke. They emphasised the importance of understanding the relative impact of modifiable risk factors on disease occurrence for effective public health

prevention strategies and community-centred health promotion initiatives. Expanding beyond South Africa, Yuyun et al. (2020) documented a rising prevalence of CVDs and associated risk factors across sub-Saharan Africa. Their projections suggest that in the coming decades, CVDs and other NCDs will surpass communicable diseases as the primary causes of mortality in the region, with CAD and stroke being major contributors to this shift. These findings underscore the urgent need for targeted interventions and public health policies to address the growing burden of NCDs in the region.

As the world's top cause of mortality, CVD continues to pose a danger to global public health; therefore, maintaining healthy eating habits is crucial for reducing cardiovascular risk (Murray & Lopez, 1996; Park et al., 2021). It is predicted that if steps are not taken to reverse the trend, the burden of disease caused by NCDs is expected to rise significantly in South Africa over the next few decades (Mayosi et al., 2009). Oxidative stress, caused by reactive oxygen molecules and free radicals, is the root cause of many fatal diseases, such as cardiovascular diseases, cancer and diabetes, and antioxidant agents are needed to reduce them and increase lifespan (Janabi et al., 2020). Rooibos is rich in flavonoids, a group of polyphenolic compounds known for their antioxidant properties that play a significant role in reducing oxidative stress and inflammation, which are key contributors to cardiovascular disease (Breiter et al., 2011).

Rooibos herbal tea, derived from *Aspalathus linearis*, a legume and part of the Cape Fynbos, is indigenous to South Africa and grown in the Cederberg region and surrounding areas of the Western Cape Province of South Africa, and popular in many South African homes (South African Rooibos Council (SARC), 2011). It contains a variety of unique polyphenolics and flavonoids, which display a wide range of bioactivities, including the modulation of oxidative stress, glucose levels, lipid profiles and inflammatory markers in humans (Abbas et al., 2017). Additional foods considered to be major dietary flavonoid sources include citrus fruits, onions, apples, berries, cherries, soybeans, and leafy greens (Janabi et al., 2020) - all of which are important in the prevention and/or modulation of cardiometabolic diseases.

Regular intake of fermented Rooibos, in the form of herbal tea, among adults at risk for cardiovascular disease led to notable reductions in serum oxidative stress, improvements in High-density lipoprotein (HDL) cholesterol levels, and decreases in low-density lipoprotein (LDL) cholesterol and triglyceride levels (Marnewick et al., 2011). Several studies exist regarding the cardioprotective effects of *Aspalathus linearis* (Pantsi et al., 2011; Dludla et al., 2014). According to Janabi and colleagues (2020), in vitro laboratory experiments have shown that flavonoids demonstrated antioxidant capabilities, as well as anti-inflammatory, immune-modulator, antiviral, antiallergic and anticarcinogenic activities. The authors further state that

the anti-inflammatory and antioxidant effects of flavonoids may aid in preventing toxicity-induced stress and chronic diseases, which suggests that flavonoids are disease-preventing, health-promoting dietary supplements due to their unexpected biological health benefits. Supporting this, Hertog et al. (1993), Rimm et al. (1996), and McCullough et al. (2012) have conducted extensive studies demonstrating that the regular consumption of foods and beverages high in flavonoids is associated with a reduced risk of mortality from cardiovascular conditions. These studies highlight the potential health benefits of incorporating flavonoid-rich items, such as Rooibos, into one's diet as a preventive measure against heart disease. The evidence suggests that flavonoids can improve vascular function, reduce blood pressure, and lower cholesterol levels, all of which are crucial factors in maintaining cardiovascular health.

1.1. Aim of the study

With CVD being a long-term problem, early intervention is of the essence to alleviate the burden of cardiovascular disease on our health system (Kelly & Fuster, 2010). The current research study aims, firstly, to develop a risk assessment model to determine the prevalence and characteristics of cardiovascular disease and its associated risk factors in a South African cohort; secondly, to determine the effect of consuming the South African herbal Rooibos tea (*Aspalathus linearis*) on cardiovascular function in adults at risk for developing cardiovascular disease using TTE, a non-invasive and reliable imaging tool used widely to evaluate the heart structure and function; and, finally, to establish an association between the CVD findings and dietary interventions.

1.2. Research question

During a review done by Abdelatif et al. (2021) on the prevalence of coronary artery disease and stroke in South Africa these authors found that every year 31% of all fatalities worldwide can be attributed to cardiovascular diseases, with 15 million deaths due to coronary heart disease and stroke. Their review suggests that, because prevention is not prioritised and effective treatments are not widely accessible, the increased mortality rate is most likely due to insufficient prevention and management strategies. Within this context, the need arises for prevention plans to improve cardiovascular function in patients at risk for CVD. Therefore, the research question of this study is:

What is the effect of the chronic intake of Rooibos herbal teas on cardiovascular function in adults at risk for cardiovascular disease as determined by trans-thoracic echocardiography?

1.3. Research objectives

The following study objectives have been formulated for the study population:

1. Design a risk assessment model to determine the prevalence and characteristics of cardiovascular disease and to assess the associated risk factors for cardiovascular disease in this group of participants.
2. Compare the cardiovascular ultrasound findings of the traditional/fermented, the green/unfermented Rooibos and placebo groups of participants.
3. Explore possible associations between echocardiography findings and Rooibos tea consumption in the study interventions.

1.4. Significance of the Study

Over the past decade, there has been a 12.5% increase in the number of CVD-related deaths worldwide. Currently, about one-third of all fatalities worldwide are caused by CVD (Joseph et al., 2017). Ischemic heart disease (IHD), stroke, hypertensive heart disease, cardiomyopathy, RHD and atrial fibrillation (AF) account for more than 95% of all CVD deaths, according to Joseph and colleagues seen in (**Figure 1.1**). The substantial economic and social implications of CVD in low- and middle-income countries, specifically in sub-Saharan Africa, cannot be overlooked. The increased mortality rates among younger individuals in these regions can be traced back to insufficient efforts in prevention and management (Strong et al., 2005). To address these challenges effectively, a comprehensive approach is necessary, with a focus on prioritising preventive measures and enhancing accessibility to effective treatments.

Marnewick et al. (2011) demonstrated that fermented Rooibos herbal tea can reduce specific risk factors associated with cardiovascular disease in adults. This statement is confirmed by Dludla and colleagues (2017), who mentioned that the flavonoids in Rooibos have the potential to shield cardiac tissue against oxidative stress induced by conditions such as hyperglycaemia. These researchers conclude that a range of mechanisms may contribute, such as metabolic signalling pathways, responses to oxidative stress, and mitochondria stimulation. Despite the promising findings from these and previous studies, there remains a significant gap in understanding these mechanisms that bring about the protective effects. Maarman (2019), agrees and state that more information about the physiological mechanisms of Rooibos is needed that contribute to its potential cardio-protective effects. Limited work has also been done on the use of echocardiography to detect early cardiovascular changes and the potential modulation of these changes through increased consumption of dietary antioxidants like Rooibos herbal tea. By focusing on individuals at risk for cardiovascular disease, this study aims to provide new insights that could enhance health outcomes and contribute to preventive healthcare strategies. This research not only builds on existing knowledge but also reflects the commitment to bridging the gap between traditional dietary practices and modern medical diagnostics by improving health outcomes through the early detection and modulation of cardiovascular risk factors via dietary intervention.

1.5. Summary of the Chapters

In the scientific environment, little evidence exists regarding the potential modulation of the cardiovascular function (using echocardiography) by Rooibos in adults at risk for cardiovascular disease. This study seeks to enhance the existing understanding of Rooibos and its potential health benefits by employing echocardiography to evaluate participants' heart function before and after a 12-week period of Rooibos consumption. The intention is to take the lead and report on the possible effects of Rooibos on the cardiovascular function of adults at risk for developing cardiovascular disease.

1.5.1. Chapter one

This chapter introduces the concept of CVD, the impact of the disease on the health system, and the role of echocardiography in assessing heart function, as well as introducing Rooibos as a possible preventative strategy for CVDs.

1.5.2. Chapter two

Chapter two presents aspects of CVD and the associated risk factors, as well as the health benefits of Rooibos. Furthermore, literature related to echocardiography, Rooibos and cardiovascular disease will provide the reader with a deeper insight into the relevant concepts and aspects of cardiovascular disease.

1.5.3. Chapter three

The materials and methods used in the study are presented. This section, therefore, includes the study design, sampling technique, the instruments that were used to collect the data, as well as the strategies used to analyse the data.

1.5.4. Chapter four

This chapter includes the results as presented in the form of graphs and tables, diagrams, and pie charts, as well as the statistical analysis of the study data collected.

1.5.5. Chapter five

This chapter will be a critical discussion of the study results, within the context of existing studies.

1.5.6. Chapter six

Conclusions are drawn with regard to this study with suggested future research directions. Recommendations and limitations are presented. This section will be followed by a list of references.

Chapter 2 | Literature Review

2.1. Introduction

Echocardiography is a fast-expanding field of study used to quantify cardiac chamber size, ventricular mass, and cardiac function. Picture quality has improved significantly over the years as a result of the advent of higher-frequency transducers, harmonic imaging, and digital machines. Because of its mobility and adaptability, it is increasingly employed in emergency, operating and critical care departments (Lang et al., 2005). Several diagnostic imaging modalities, including catheter angiography, magnetic resonance imaging (MRI), and computed tomography (CT) together with echocardiography, are all used to identify CHD, but each modality has its advantages and disadvantages (Votavová et al., 2015).

Bu et al., (2016) indicates that TTE is the first-line diagnostic tool due to its non-invasive nature, convenience, and ability to identify cardiac morphology and measure blood flow velocities. In this chapter, the current literature on CVD and associated risk factors and the role of echocardiography in assessing the heart function for CVD will be discussed. All echocardiography images in this document have been generated by the researcher who followed the guidelines of the American Society of echocardiography's (ASE) guidelines for performing an echocardiogram. The primary goal of the ASE is to ensure that high-level quality echocardiographs are produced (Picard et al. 2010). They further state that this can only be achieved by adhering to set guidelines to ensure patients are satisfied and the desired outcome is achieved.

2.1.1. **Dendrogram topology (Author's construct)**

Dendrogram topology refers to the branching-pattern relationship that exists among individual items in a dendrogram. This branching pattern illustrates the hierarchical connections and similarities between the items, making it a critical concept when comparing different classifications Phipps,1971). The importance of understanding dendrogram topology to interpret and compare data clusters accurately is very important. These clustering methods are frequently used to illustrate similarities between objects (Forina et al., 2002). Dendrograms are important tools for visualising complex data as it effectively convey a vast quantity of information in a format that is intuitive and easily comprehensible. By organising data into a tree-like structure, dendrograms enable researchers to quickly identify patterns, relationships, and groupings within the data set (Wilke et al., 2013).

The dendrogram below adapted from Hartnick (2021) (**Figure 2:1**) is a schematic cluster analysis of this research study and will be discussed in detail in this chapter. This figure illustrates the hierarchical structure of the data and provides insights into the relationships among the studied elements. In the following chapters, I will discuss this dendrogram in detail,

exploring the observed clustering patterns and how they contribute to our overall understanding of the research findings and the complete data set. By analysing the dendrogram, we aim to uncover meaningful connections and derive conclusions that are grounded in the visualised data structure.

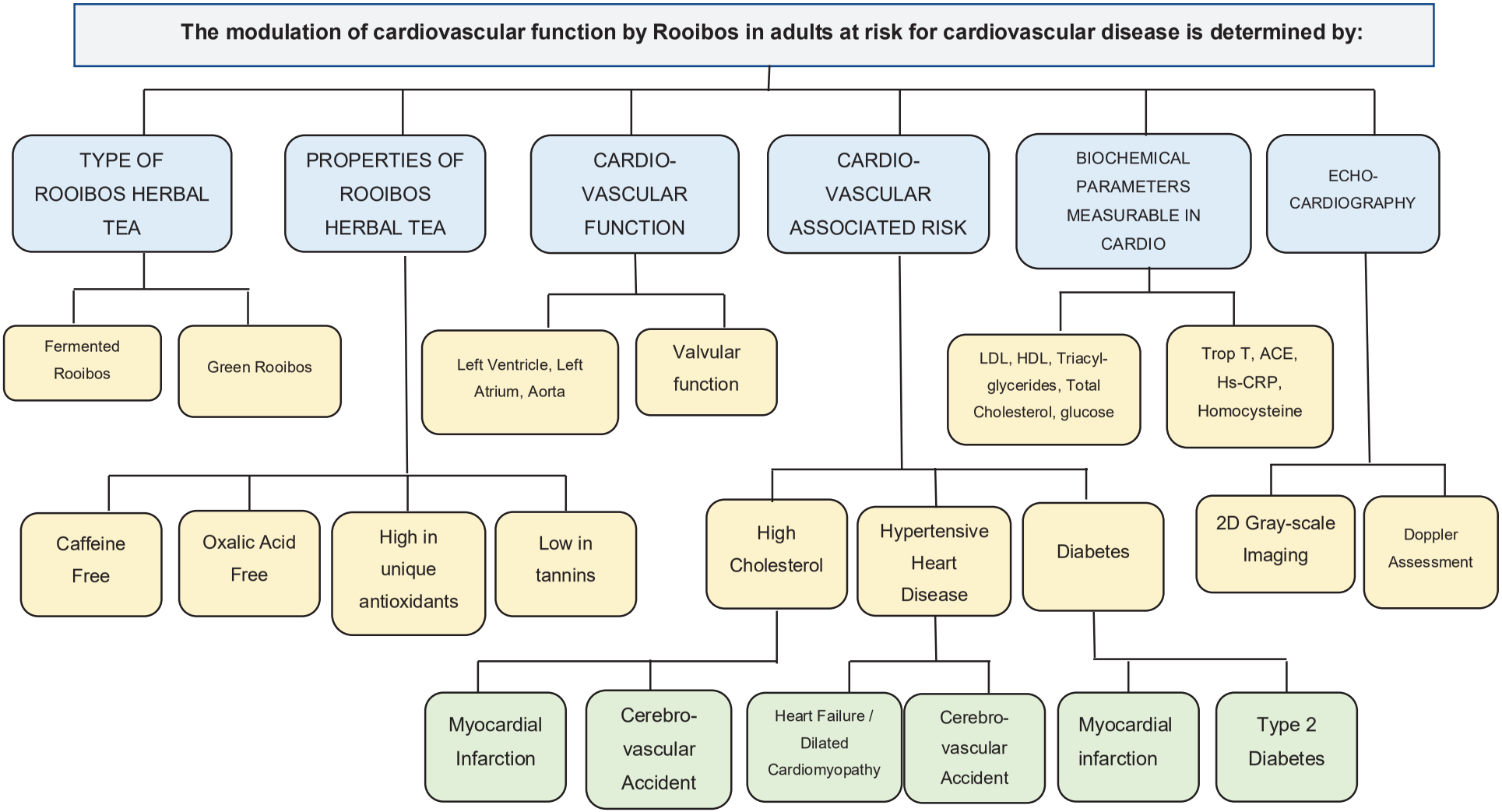

Figure 2-1: Schematic Cluster Analysis of this Research Study (Hartnick, 2021)

2.2. Echocardiography to investigate the cardiovascular function

2.2.1. Introduction

Echocardiography (ECHO) is a non-invasive medical procedure that uses sound waves to generate detailed images of the heart in multiple dimensions. This technique, complemented by Doppler examinations, provides accurate assessment of cardiac hemodynamics, making it an invaluable tool in diagnosing cardiovascular disease (Mohamed et al., 2010; Esmaeilzadeh et al., 2013). As noted by Capotosto et al. (2018) ECHO This enables the comprehensive evaluation of both left and right ventricular function, facilitating the assessment of myocardial performance and valvular function. With its ability to create multiple imaging windows of the heart, echocardiography allows for a multi-angled view of the heart, enhancing the diagnostic capabilities of the procedure (Mohamed et al., 2010). This widely adopted diagnostic test provides valuable insights into blood flow patterns within the heart and the functioning of heart valves. By analysing the images obtained from the echocardiogram, healthcare providers can accurately identify and diagnose various heart conditions, including heart disease (Mayo Clinic, 2019; American Heart Association (AHA), 2021).

Since its inception, ECHO has flourished into a well-established ‘tree’ with numerous ‘growing branches’ and multiple clinical applications widely used in the cardiovascular system. This development has been carefully nurtured and shaped by various pioneers to meet the needs of patients and medical professionals (Oh et al., 2006:2). **Figure 2:2** illustrates the diverse clinical applications of ECHO, encompassing colour-flow imaging (CFI), intracardiac ultrasound (ICU), intra-operative echocardiography (I-Op), intravascular ultrasound (IVUS), trans-oesophageal echocardiography (TEE), two-dimensional (2D), three-dimensional (3D) and four-dimensional (4D) imaging, Doppler assessments, haemodynamic evaluation, tissue Doppler imaging, stress echocardiography and harmonics.

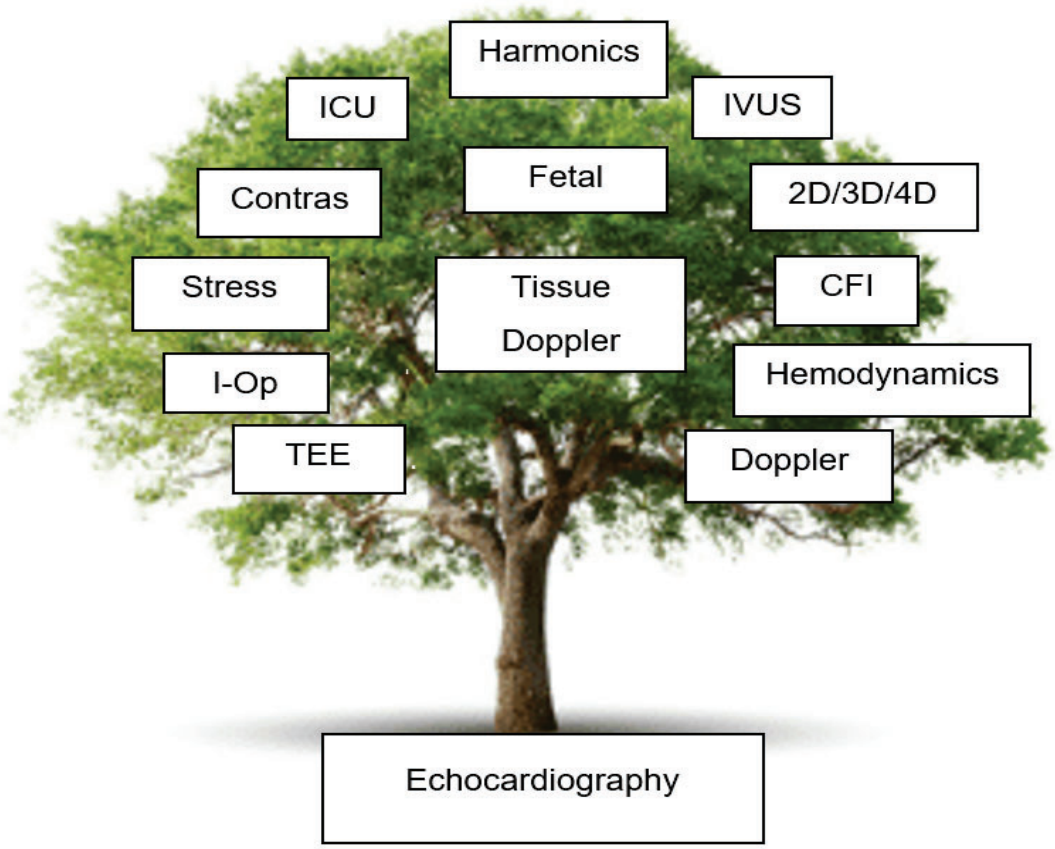

Figure 2-2 Clinical applications of echocardiography (Adapted from Oh et al., 2006:2)

According to Simova (2015), ECHO not only offers a non-invasive approach but also presents the additional advantages of being accessible and affordable. The modality serves as a reliable method for assessing coronary flow velocity reserve (CFVR) in epicardial coronary arteries, including the three major coronary arteries, both with and without stenosis. CFVR plays a crucial role in the identification of significant coronary artery stenosis. Trans-thoracic and trans-oesophageal provide detailed imaging of heart chambers, wall motion, and valvular blood flow, offering valuable clinical and experimental data for assessing viability and clinical applicability (Kovács et al., 2000). In contrast, other non-invasive imaging tools, such as cardiac MRI and cardiac nuclear imaging, while valuable for clinical research, have limited practical applications due to their complexity, time-consuming nature, high cost and limited availability (Simova, 2015). CHF is identified by specific symptoms and physical signs, and ECHO is commonly used to measure ejection fraction (EF) and assess whether systolic function is impaired or preserved. Additionally, Doppler echocardiography can directly evaluate diastolic function (Redfield et al., 2003; Bombardini, 2005). Canty et al. (2012) carried

out a prospective observational study to examine the impact of echocardiography on the anaesthetic treatment plans for 100 patients over 65 years old with suspected heart disease. The study found that echocardiography successfully identified cardiac illness in 31 patients. These results suggest that focused trans-thoracic echocardiography in a pre-operative clinic setting is both feasible and beneficial for guiding the management of patients with potential heart conditions.

While trans-thoracic echocardiography emerges as the preferred imaging modality for assessing the heart and ruling out cardiovascular disease, Popescu et al. (2020) however, highlight the limited availability of echocardiography in its early years, noting the absence of specific training requirements for conducting and interpreting echocardiographic studies. They emphasise that current echocardiography necessitates comprehensive training in the latest techniques and a dedication to maintaining quality standards for effective imaging.

In the current research study, ECHO was employed to evaluate individuals' hearts. As mentioned earlier, echocardiography encompasses various clinical applications. Specifically, the study explored the use of 2D echocardiography, motion-mode (M-Mode) echocardiography, and Doppler analysis, with comprehensive discussions provided in Chapter 3.

2.2.2. **Echocardiography**

2.2.2.1. **Two-Dimensional echocardiography**

Echocardiography plays a vital role in providing essential anatomical and haemodynamic information about cardiac chamber size, systolic and diastolic function, valvular function, and the presence of intracardiac and extracardiac masses and fluid collections. (Levy et al., 1990). Two-dimensional grey-scale imaging is a standard technique used to assess cardiac structures and LV function, providing valuable information on systolic and diastolic dysfunctions and is a valuable clinical tool for predicting CVD (Nagueh et al., 2016; Chang et al., 2009). The prevalence of confirmed CHF diagnoses rises with the severity of ventricular dysfunction. However, even among those with severe diastolic or systolic dysfunction, over half do not have a CHF diagnosis or have not received treatment (Redfield et al., 2003). Yildiz et al. (2020), in their manuscript, confirm the usefulness of echocardiography and state that it is widely used for evaluating the LV geometry, including LVM and the LV free walls. Marwick, (2015), states that the data from ECHO are fundamental in managing heart failure, offering unique versatility compared to other imaging techniques. While all imaging methods can determine ejection fraction, ECHO stands out by also providing detailed information on volumes, diastolic function, right ventricular function, hemodynamics, and valvular regurgitation. HF can present with a wide range of LV functional abnormalities, from normal LV size and preserved EF to severe dilation and significantly reduced EF. Most patients exhibit

both systolic and diastolic dysfunction, regardless of EF and ECHO has the ability to exclude systolic and diastolic dysfunctions. (Yancy et al., 2013).

A range of imaging views are possible with ECHO to evaluate various cardiac structures and functions. Each imaging view provides important diagnostic information of the heart. This includes the parasternal long-axis view, parasternal short-axis view, apical 4-chamber and 2-chamber view, subcostal view, and suprasternal view (Lang et al., 2015). In both longitudinal and short-axis planes, different measurements and dimensions are obtained to examine cardiac structures, such as the left atrium (LA), LV, right atrium (RA), right ventricle (RV), aorta, mitral valve (MV), tricuspid valve (TV) and aortic valve (AV), for abnormalities (Nagueh et al., 2016). Echocardiography also delivers high-resolution images of the heart from four typical transducer positions through manual rotation and angulation of the transducer, allowing for both quantitative and qualitative assessments of heart dimensions (Lang et al., 2005). While three-dimensional (3-D) echocardiography is gaining influence in clinical practice, 2D echocardiography remains the dominant technique (Votavová et al., 2015).

Chapter 3 will provide a comprehensive discussion of the methodology used for each echocardiographic image will be presented.

2.2.2.2. **Motion-mode**

Motion mode (M-mode) echocardiography offers exceptional temporal resolution, which is often underestimated in its clinical significance and potential. This technology allows for highly detailed assessment of cardiac dynamics and motion, providing critical insights into both myocardial function and structural characteristics (Feigenbaum, 2010). M-mode recordings serve as a valuable tool in capturing the movements of cardiac structures and are primarily used for detecting irregularities in cardiac motion, monitoring cardiac events, and measuring chamber size (Lang et al., 2005). Assessing cardiac chamber size and function is a fundamental aspect of cardiac imaging, as emphasised by Lang et al. (2005). By applying M-mode, early changes in the hearts of hypertensive patients can be detected, enabling 2-D measurements of the interventricular septum (IVS) (Capotosto et al., 2018). Left ventricular hypertrophy (LVH), characterised by the thickening of the interventricular septum, serves as an early predictor of hypertensive heart disease and a significant risk factor for cardiovascular morbidity.

Echocardiographers can measure the size of various cardiac structures, including the IVS, posterior wall (PW), LV, RV, aorta, LA and RA using this mode making it possible to assess and diagnosed several CVD predictors (Chang et al., 2009). As previously mentioned, as noted by Lang et al. (2005), M-mode facilitates the diagnosis of LVH, a complication of

hypertensive heart disease, through the measurement of the IVS and the PW thickness in both systole and diastole.

M-mode echocardiography efficiently captures multiple cardiac cycles, providing valuable recordings of valves and the IVS. This technique enhances the accuracy and comprehensiveness of echocardiographic assessments, contributing to cost-effective examinations (Feigenbaum, 2010).

2.2.2.3. **Doppler Echocardiography**

Echocardiography proved effective in diagnosing a variety of heart conditions, including hypertensive heart disease, valvular heart disease, cardiomyopathy, and ischemic heart disease (Talle et al., 2016). Spectral continuous wave (CW), pulsed wave (PW), and colour Doppler analyses facilitate the measurement of blood flow velocities across various heart valves, including the aortic valve (AV), mitral valve (MV), pulmonary valve (PV), and tricuspid valve (TV), to evaluate valvular dysfunction. The primary function of the heart is to supply oxygen and nutrients to the body through blood circulation. Doppler echocardiography plays a crucial role in assessing blood flow within the heart (Bombardini, 2005). Doppler echocardiography is a non-invasive imaging technique that utilises the reflection of ultrasound waves by moving red blood cells (Zoghbi et al., 2017). The Doppler principle is applied to derive velocity information from the reflected ultrasound. The frequency shift in the reflected ultrasound, relative to the transmitted ultrasound, is determined by the velocity and direction of blood flow, providing valuable haemodynamic information about the heart and blood vessels (Galie et al., 2015). Doppler echocardiography has proven to be particularly useful in assessing various cardiac conditions. By analysing the velocity and direction of blood flow, Doppler echocardiography can measure the severity of valvular narrowing (stenosis) and detect valvular leakage (regurgitation) (Lancellotti et al., 2010). Furthermore, it is effective in identifying intracardiac shunts, such as ventricular septal defects (VSDs) and atrial septal defects (ASDs) (Galie et al., 2015). These applications make Doppler echocardiography an invaluable tool for assessing cardiac function and diagnosing various heart conditions. Talle and colleagues (2016) conducted a three-year retrospective review at a tertiary hospital in northern Nigeria to identify heart diseases detected by echocardiography. Their findings revealed that hypertensive heart disease was the most common echocardiographic diagnosis among adults, aligning with trends observed in Nigeria and other African countries. Additionally, pulsed wave Doppler examinations of the mitral valve allow for the assessment of diastolic function, helping to track the progression of diastolic dysfunction (Nishimura et al., 1997).

The pathophysiology of diastolic dysfunction is demonstrated through the two-phase ventricular filling of the heart. Early diastole involves rapid, passive filling of the ventricle due

to ventricular relaxation, while late diastole includes additional filling from atrial contraction (Bombardini, 2005). Doppler imaging captures two main peaks: the E wave, representing ventricular relaxation, and the A wave, representing atrial contraction **(See Figure 3.15 in Chapter 3)**. Since the E wave reflects diastolic properties, a reduced E wave indicates diastolic dysfunction (Mottram and Marwick, 2005). Impaired diastolic function leaves more residual blood in the atrium, increasing the atrial contribution to ventricular filling, shown by a higher A wave. A reduced E/A ratio is indicative of diastolic dysfunction (Nagueh et al., 2016). This underscores the significance of echocardiography as a valuable tool for accurately diagnosing and assessing cardiac pathologies in clinical settings.

2.2.2.4. **Echocardiography features of cardiovascular disease**

The medical profession should be pivotal in assessing evidence regarding drugs, devices, and procedures to detect, manage, and prevent disease (Yancy et al., 2013). While echocardiography cannot prevent disease, it can detect it and thereby play a role in its management. Echocardiography offers a wide range of clinical applications, making it an excellent diagnostic tool for various cardiac conditions. It aids in the diagnosis of myocardial infarction, LVH characterised by a thickened septum, mitral valve regurgitation, enlargement of the atrium and aorta, as well as enlarged cardiac chambers, identification of thrombus, septal defects, and several other predictors of CVD (Savage et al., 1987). A study by Whelton (2018) indicates that a dilated aorta is associated with aortic regurgitation. Hypertension-induced remodelling of the LV leads to the development of LVH, which is a major risk factor for poor CVD outcomes. Kizer et al. (2006) conducted a population-based study among middle-aged and elderly individuals without clinical cardiovascular disease found that the LA diameter was a robust predictor of initial cardiovascular events, irrespective of clinical, echocardiographic, and inflammatory factors. Their findings suggest that LA dimension can identify individuals, without clinical illness yet, but at risk nevertheless, who may benefit from aggressive risk factor management.

Heart failure (HF) is a multifaceted clinical syndrome caused by structural or functional impairments in ventricular filling or blood ejection. As some patients may not exhibit signs or symptoms of volume overload, the term "heart failure" is now preferred over "congestive heart failure" (Yancy et al., 2013). The incidence of heart failure increases with age, from around 20 per 1,000 individuals aged 65 to 69 to over 80 per 1,000 individuals aged 85 and older (Curtis et al., 2008). Cardiovascular conditions like hypertension, coronary artery disease, and cardiomyopathies frequently result in both systolic and diastolic dysfunction of the ventricles. It's common for patients experiencing systolic dysfunction to also exhibit some level of concurrent diastolic dysfunction, typically characterised by compromised relaxation and ventricular function (Nishimura et al., 1997). As mentioned earlier, diastolic dysfunction is

linked to cardiovascular disease, therefore reinforcing that hypertension and coronary artery disease tend to cause this condition (Bella et al., 2002). Tsang et al. (2003), aimed at determining whether echocardiography enhances the prediction of the onset of the first age-related cardiovascular events, revealed that echocardiography can identify subclinical CVD risk markers, such as LV systolic or diastolic dysfunction, enlarged left atrium and increased LVM, thereby improving risk stratification for the onset of the first age-related cardiovascular events. Research by Lang et al. (2015) indicates that an enlarged left atrium is associated with an increased risk of atrial fibrillation and stroke, as well as a higher likelihood of major cardiac events or death in patients with diabetes mellitus (DM).

2.3. Cardiovascular disease risk factors

2.3.1. Diabetes mellitus

Diabetes mellitus is a global disease with a significant impact on morbidity and mortality, affecting 429 million people worldwide. Projections indicate that this number will increase to 578 million by 2030 and 700 million by 2045 (International Diabetes Federation, 2019). Alipour et al. (2012) highlight that DM is becoming a major health problem, affecting over 165 million individuals globally and leading to cardiovascular disease, nephropathy, retinopathy, and extensive illness of both the peripheral and central neurological systems. The development and progression of the disease is influenced by various behavioural, lifestyle and biological risk factors. These include obesity, sedentary lifestyle, advanced age, high blood pressure, unfavourable lipid profile, cigarette smoking and genetic predisposition (Defronzo et al., 2015).

DM is categorised into: Type 1 diabetes (T1D) and Type 2 diabetes (T2D), with T1D more prevalent in children and characterised by the immune system attacking the insulin-producing beta cells of the pancreas, resulting in the inability of the pancreas to produce insulin. T2D, is the most common form of diabetes seen in older individuals and is characterised by the body's cells not responding fully to insulin, leading to impaired insulin secretion (International Diabetes Federation, 2019; Grundy, 2016). Chronic hyperglycaemia can lead to the development of CVD and HF due to its influence on altering the myocardial substrate preference in cardiomyocytes and inducing the production of free radical species, leading to oxidative stress (Maarman, 2019; Dludla et al., 2017). Diabetic hearts often present with common diagnoses, such as diastolic and systolic dysfunction, left ventricular hypertrophy, myocardial interstitial fibrosis, increased apoptosis, and upregulation of oxidative stress (Yue et al., 2007; Palmieri et al., 2008). Dilated cardiomyopathy (DCM), occurring as a result of DM, is characterised by an enlarged LV and systolic and diastolic dysfunction, and is influenced by several factors, including oxidative processes, inflammation, cell death, fibrosis, and hypertrophy (Huynh et al., 2014; Shabab et al., 2021). The presence of diabetes mellitus

increases the risk of CVD, emphasising the crucial role of glycaemic management in lowering the risk of ACS.

2.3.2. **Hypertension**

In Sub-Saharan Africa, HPT is a significant risk factor for cardiovascular disease and the most prevalent cardiovascular condition, contributing to 13% of global fatalities (Cappuccio & Miller, 2016). Two-thirds of HPT cases worldwide are found in Sub-Saharan Africa and other developing regions. Projections indicate that by the year 2025, approximately 1.56 billion individuals will live with HPT (Talle et al., 2016). The classification of HPT has been redefined as per the 2017 American College of Cardiology (ACC)/American Hypertension Association (AHA) guidelines, with hypertension now defined as a SBP of ≥130 mmHg or a diastolic BP of ≥80 mmHg (Carey et al., 2017). The South African Hypertension Society (SAHS) estimates that almost 30.4% of the adult population is hypertensive (Seedat et al., 2014).

Increased blood pressure (BP) levels are associated with an elevated risk of cardiovascular disease, including strokes, CHD, chronic kidney disease, HF and death. Managing BP within appropriate levels can significantly reduce these risks (Gabb et al., 2016). Al-Shafei and El-Gendy (2019) also support the notion that CVD is a clinical complication of systemic HPT. The rise in childhood obesity and hypertension is a concerning trend, as these children face an increased risk of developing CVD. Early intervention is essential in addressing this issue (Ho et al., 2019). The association between elevated blood pressure and obesity (and caloric excess) has been explored extensively, with several mechanisms proposed to elucidate this relationship. These mechanisms include enhanced renal reabsorption of sodium, possibly due to insulin resistance (Reaven, 1991), expansion of intravascular volume, activation of the renin-angiotensin-aldosterone system and sympathetic nervous system, release of angiotensinogen from adipose tissue, and insulin resistance (Hall, 2000). It is currently widely accepted that these various factors operate synergistically to elevate blood pressure (Reaven, 1991; Hall, 2000).

Uncontrolled hypertension can have adverse effects on the left ventricle, leading to LVH due to pressure overload. If left untreated, this overload can impact left ventricle systolic function and lead to heart failure, arrhythmia, and sudden death (Al-Shafei & El-Gendy, 2019). LVH determined by echocardiography is strongly linked to cardiovascular morbidity and mortality (Liao et al., 1995). Research involving specific patient groups and population-based epidemiological research has demonstrated that an increase in LVM is an independent risk factor for cardiovascular morbidity and mortality. It is widely recognised that LVH is significantly influenced by body composition (Kuch et al., 2000). Differences in body size and composition can affect the absolute measurements of LVM, potentially resulting in misclassification if not properly adjusted. To address this, LVM is frequently indexed to body

surface area (BSA), providing a normalised value that reduces the confounding effects of variations in body size (Liao et al., 1995; Chirinos et al., 2010). Al-Shafei & El-Gendy, (2019) further state that small changes in BP levels can significantly influence the prevalence of hypertension and risk factors for cardiovascular disease.

Controlling blood pressure within appropriate levels is crucial for reducing the risk of cardiovascular complications, including LVH and its associated outcomes. Early intervention and proper management are essential to mitigate the adverse effects of hypertension and improve overall Cardiovascular health.

2.3.3. **Atherosclerosis**

The pathogenesis of insulin resistance, Type 2 diabetes, and metabolic syndrome (MetS) involves various cytokines and proteins with significant implications (De Rosa et al., 2019). Among these, IL-6, a potent inflammatory cytokine, is associated with insulin resistance and diabetes, with elevated levels observed in individuals with obesity and MetS (Rupérez et al., 2018). Additionally, tumour necrosis factor α (TNF-α), another proinflammatory cytokine, has links to cardiovascular conditions, such as atherosclerosis and heart failure (Yamagishi et al., 2001). Interestingly, TNF-α can also reduce insulin resistance in adipocytes (Connelly & Otvos, 2004). Furthermore, PAI-1, a serine protease inhibitor, exhibits prothrombotic properties and is increased in obese MetS patients and those with Type 2 diabetes (Farrell et al., 2013). Adipocyte dysregulation in MetS may be attributed to obesity-induced oxidative stress and increased production of reactive oxygen species (Connelly & Otvos, 2004). In contrast, adiponectin, an anti-inflammatory cytokine secreted by adipocytes, demonstrates reduced levels in MetS and plays a pivotal role in insulin sensitisation and anti-atherogenesis (Yamagishi et al., 2001). Moreover, the presence of elevated levels of oxidised low-density lipoprotein (OxLDL) in MetS contributes to oxidative stress and atherosclerosis (Connelly & Otvos, 2004) (See **Figure 2:3**). The interaction and interplay of these cytokines and proteins contribute to the complex pathophysiology of MetS and its associated cardiovascular complications (De Rosa et al., 2019).

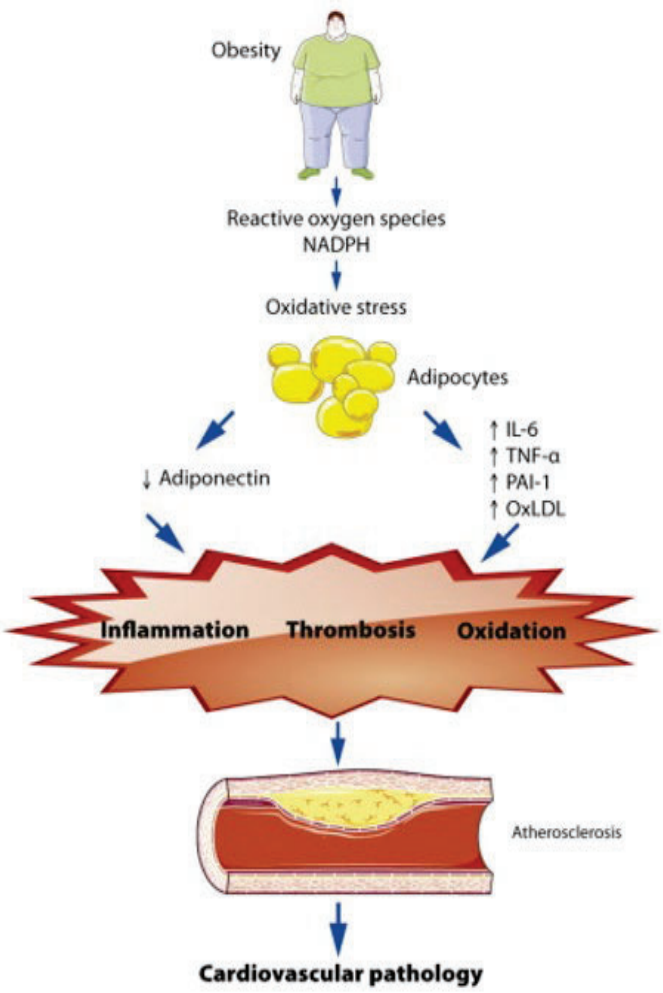

Figure 2-3 Pathogenesis of atherosclerosis (taken with permission from McCracken et al., 2018, Licence 5595830224641)

Atherosclerosis arises from an intricate interplay between circulating factors within the vessel wall, triggered by repetitive exposure to pathogenic elements. Elevated levels of LDL play a crucial role in atherosclerosis development (Badimon & Vilahur, 2012). This chronic inflammatory disease is driven by oxidative stress and inflammation, ultimately leading to endothelial dysfunction (Marchio et al., 2019). In particular, endothelial dysfunction represents a critical initial step in atherosclerosis formation, particularly in diabetic individuals, as hyperglycaemia associated with this metabolic disorder is closely linked to atherosclerotic plaque development (Steven et al., 2019). The connection between endothelial function in the human brachial artery and human coronary artery underscores the significance of endothelial health in vascular disease (Duffy et al., 2001). A study investigating the impact of long-term black tea consumption on endothelial dysfunction found that certain antioxidants present in black tea are associated with the reversal of endothelial dysfunction in individuals with

coronary artery disease (Duffy et al., 2001). While several factors contribute to endothelial dysfunction, oxidised LDL emerges as a particularly toxic element for endothelial cells, playing a pivotal role in atherogenesis development and maintenance (Stein et al., 1999). The above literature highlights the multifaceted nature of atherosclerosis development, involving factors such as LDL levels, oxidative stress, inflammation, and endothelial dysfunction. A deeper understanding of these interactions is vital for effective therapeutic strategies to combat atherosclerosis and its related cardiovascular complications.

2.3.4. **Oxidative Stress and cardiovascular disease**

Hypertension, high cholesterol, diabetes, and chronic smoking trigger the generation of reactive oxygen species (ROS) within the vascular wall (Wong et al., 2010). These ROS are produced by a range of oxidase enzymes, including NADPH oxidase, xanthine oxidase, uncoupled endothelial NO synthase (eNOS), cyclooxygenase, glucose oxidase, lipooxygenase, and mitochondrial electron transport (Tousoulis et al ., 2011). These enzymes contribute to ROS production through various cellular processes and pathways, influencing oxidative stress levels and cellular signalling and are a major contributor to cellular and tissue damage and the pathogenesis of various chronic diseases (Abbas et al., 2017). Reactive nitrogen species (RNS), like ROS, are recognised for their dual role, acting as both harmful and beneficial agents (Valko et al., 2004). ROS and RNS are generated as part of physiological and metabolic reactions by the central nervous system as well as other tissues and organ systems throughout the body. These reactive species are produced in various cellular processes, including mitochondrial respiration, immune responses, and enzymatic reactions in different organs (Sies & Jones, 2020; Valko et al., 2006). These radicals have the potential to induce lipid peroxidation, leading to cellular organelle and membrane destruction and, eventually, cell death (Alipour et al., 2012). When the production of oxidants surpasses the body's antioxidant capacity, oxidative stress occurs, contributing to the development of several chronic diseases, such as diabetes, cardiovascular disease, cancer, and renal disorders. These conditions pose significant global health challenges, and their uncontrolled prevalence will undoubtedly result in substantial financial burdens (Maleki et al., 2015).

Oxidative stress also plays a pivotal role in age-related disorders and is a prominent risk factor for the formation of atherosclerotic plaques and increased cardiovascular disease risk (Weidinger & Kozlov, 2015; Ito et al., 2019). In this context, herbal teas containing flavanones, iso-flavanones and other antioxidants have been recognised for their potential to elevate blood antioxidant levels, thereby offering protection against chronic illnesses (Maleki et al., 2015).

2.3.5. **Lifestyle and obesity**

As stated by Saklayen in 2018, the fundamental factors driving MetS involve the rise in the intake of high-calorie, low-fibre fast food and the decline in physical activity levels. This

syndrome contributes to the proliferation of conditions, such as Type 2 diabetes, coronary ailments, stroke, and various impairments. Suliga and colleagues (2022) conducted a study exploring the relationship between lifestyle factors and the risk of MetS in adults. The objective of their research was to provide an overview of current knowledge concerning the pathophysiological consequences of obesity and MetS. Their findings revealed that individuals with MetS are more likely to develop CVD and face a less favourable prognosis. The National Health and Nutritional Examination Survey (NHANES) estimates that obese individuals often exhibit concurrent risk factors associated with an increased risk of cardiovascular disease (Suliga et al., 2022).

The global prevalence and impact of metabolic syndrome and highlight the alarming rise in metabolic syndrome incidence worldwide and its significant implications for public health (Saklayen et al., 2018). Bastien and colleagues (2014) explored the complex relationship between obesity and cardiovascular disease, highlighting obesity as a major modifiable risk factor and underscoring the growing public health concern surrounding the obesity epidemic and its implications for cardiovascular morbidity and mortality. Reflecting on these findings, I am reminded of the critical importance of addressing obesity. This study reinforces my commitment to promoting healthy habits and interventions that can help mitigate the risk of cardiovascular disease among my patients. Individuals with MetS run a higher risk of developing cardiovascular disease compared to those without MetS (Suliga et al., 2022). The association between lifestyle factors and the risk of metabolic syndrome in adults, emphase the importance of understanding the pathophysiological consequences of obesity and MetS, as well as potential contributing mechanisms to adverse cardiovascular outcomes.

Early identification, assessment, and intervention to manage metabolic syndrome effectively is important especially with regard to lifestyle factors such as adopting a healthy diet and regular physical activity (Grundy et al., 2016). By addressing underlying mechanisms and risk factors, healthcare professionals can improve outcomes for patients with metabolic syndrome, reducing the risk of cardiovascular events and diabetes. These efforts are critical in promoting public health and enhancing the quality of life for affected individuals.

The diagram below (**Figure 2:4**) illustrates the association between energy excess or obesity and risk factors of the metabolic syndrome.

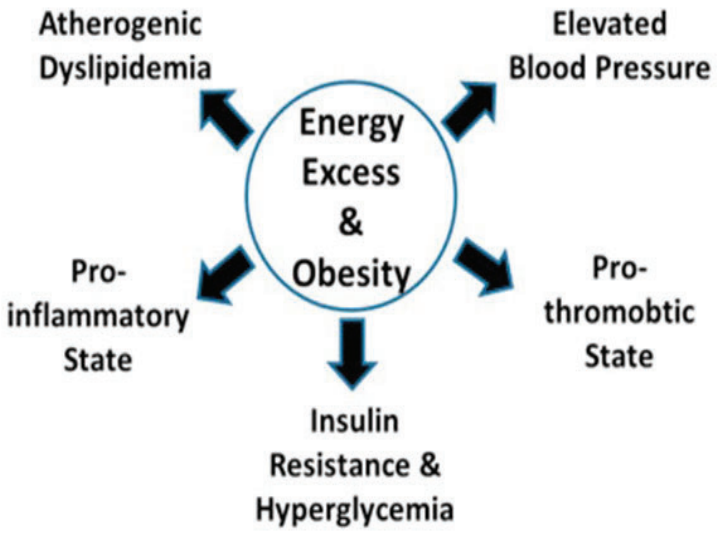

Figure 2-4 The association between energy excess and obesity and metabolic syndrome risk factors (With permission from Grundy et al. (2016), Licence 5595820043757)

2.4. *Aspalathus linearis* (Rooibos)

The indigenous Khoi people of South Africa have a long history of using Rooibos (*Aspalathus linearis*) as an herbal beverage, dating back to the late 1700s. The first known documentation of Rooibos was by the botanist Carl Thunberg in 1772 (Morton, 1983). However, it was Benjamin Ginsberg, a Clanwilliam trader, who recognised the commercial potential of Rooibos as an herbal tea and began promoting it in 1904. Later, in 1930, P. Le Fras Nortier, a medical practitioner and nature enthusiast from Clanwilliam, identified the agricultural benefits of Rooibos (Pretorius, 2007).

With the increasing popularity of Rooibos, there have been concerns about its impact on the biodiversity of the Greater Cederberg Biodiversity Corridor. To address these concerns, the South African Rooibos Council (SARC) collaborated with CapeNature to develop the Rooibos Biodiversity Initiative. One of the key objectives of this initiative was to implement a cooperative expansion planning approach to minimise the loss of vulnerable natural habitat (Pretorius, 2007; Joubert & De Beer, 2011). The map **Figure 2:5** below depicts the Cedarberg region where Rooibos is produced.

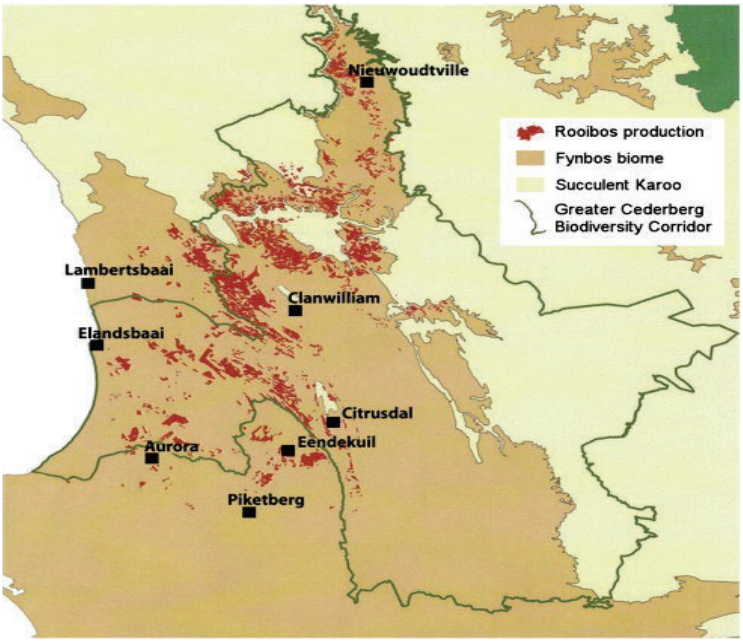

Figure 2-5 The Cederberg region where Rooibos is produced [1]

Rooibos, a popular herbal tea, is gaining momentum in terms of its popularity and consumption globally and is projected to become the second most widely consumed beverage globally, with an estimated 10.9 million households enjoying its benefits (Joubert & de Beer, 2011; SA Rooibos Council (SARC), 2011). Indigenous to South Africa, Rooibos thrives exclusively in the Cederberg region in and around Clanwilliam, situated in the Western Cape Province. As an integral part of Cape *fynbos*, the Rooibos plant, scientifically known as *Aspalathus linearis*, is a woody shrub that can reach a height of up to 2 meters. In South Africa, it is traditionally consumed in fermented or 'red' form by many individuals (Joubert & de Beer, 2011; SA Rooibos Council (SARC), 2011). To enhance reader comprehension, the visual representations depicting Rooibos tea, found in Figures 2.6 to 2.9, have been incorporated into this document.

[1] Map provided with permission from the South African Rooibos Council (SARC)

Figure 2-6 The Rooibos plant[2]

Figure 2-7 The harvested Rooibos plants[2]

[2] Photographs in Figures 2.6 to 2.9 were taken with permission at Muggiesdraai, Clanwilliam, in the Western Cape.

Figure 2-8 Harvested Rooibos plant material ready for processing[2]

Figure 2-9 Processed Rooibos ready for distribution[2]

The green/unfermented form of Rooibos has emerged in the market in recent years. This herbal tea is reputedly naturally caffeine-free and contains lower levels of tannins compared to traditional *Camellia sinensis* teas (green and black teas). Moreover, Rooibos possesses a unique composition of phytochemical constituents with various bioactivities, prominently including antioxidant activity (Ajuwon et al., 2018). These distinctive attributes make Rooibos an appealing candidate with the potential to impact CVD, specifically in terms of dietary prevention. Consequently, Rooibos has gained popularity due to its capacity for enhancing general human health and/or preventing diseases (Abdul & Marnewick, 2023).

In a study conducted by Yang et al. (2011), it was found that an increase of 2 grams in daily consumption of dry green tea leaves (roughly equivalent to the content of a tea bag) correlated with a 12% decrease in the risk of colorectal issues. The bioactivities of Rooibos are

multifaceted, encompassing antioxidant, anti-inflammatory, anti-carcinogenic, redox regulation, and anti-diabetic effects. Additionally, it exerts modulatory effects on the immune system, adrenal steroidogenesis, and lipid metabolism (Marnewick et al., 2011; Ajuwon et al., 2018; Smith & Swart, 2018). These diverse bioactive properties of Rooibos contribute to its potential as a beneficial dietary component for promoting human health and disease prevention.

The potential of fermented Rooibos to modulate specific risk factors associated with the development of cardiovascular disease (CVD) in individuals at risk has been previously documented (Marnewick et al., 2011). Although some studies have utilised experimental animal models, others have incorporated dietary intervention-type studies in human subjects (Persson et al., 2010; Marnewick et al., 2011). In the commercial market, fermented Rooibos is predominantly sold in South Africa, with the unfermented/green form targeted towards international markets, but also gaining popularity among local consumers (South African Rooibos Council (SARC), 2020). Notably, green Rooibos possesses higher antioxidant activity compared to the traditional/fermented Rooibos, making it a sought-after ingredient in cosmeceutical products (Tobin, 2018).

The processing of the harvested Rooibos plant material involves cutting, bruising, wetting, and stacking the plant leaves and stems in heaps exposed to the sun for approximately 12-14 hours. Although termed fermentation, it is primarily a chemical oxidation process. The bruising and hydration of the plant material create conditions favourable for enzymatic reactions, which are essential for developing characteristic sensory attributes, including the red-brownish colour of traditional/fermented Rooibos. In contrast, green Rooibos does not undergo the fermentation step; instead, the plant material is dried soon after harvesting, resulting in the yellowish, straw-like colour of green Rooibos. The distinctive colour and higher content of unique Rooibos phenolic compounds, such as Aspalathus, differentiate green Rooibos from its fermented counterpart (Tobin, 2018).

2.4.1. **Rooibos is caffeine-free**

Moderate caffeine consumption has been associated with various benefits, including improved physical performance, enhanced cognitive function, increased alertness, and vigilance (Ruxton, 2008). However, excessive caffeine intake can lead to negative effects, such as dehydration, anxiety, headaches, sleep disturbances and heart palpitations. Rooibos, on the other hand, has been recognised as a caffeine-free alternative, making it a suitable beverage for individuals sensitive to caffeine or even infants (Joubert & De Beer, 2011). Canda et al. (2014) support this claim and highlight that Rooibos is rich in polyphenol antioxidants and contains very few tannins. They also suggest that Rooibos has potential benefits, such as assisting with insomnia, alleviating allergies, reducing nervous breakdowns, and improving

appetite. Considering these qualities, Rooibos serves as a promising beverage option for individuals seeking to avoid caffeine-related adverse effects while still enjoying potential health benefits from the antioxidant properties it offers.

2.4.2. **Rooibos is oxalic acid free**

Oxalic acid, an organic compound present in various plants, green leaves, vegetables, and fruits, can have adverse effects on human health due to its ability to bind with calcium and other minerals. This binding with minerals may lead to the formation of stones when oxalates are excreted through the urinary system (Noonan, 1999). Goldfarb and colleagues (2005) conducted a study on the influences of genetics and dietary factors on kidney stone formation, revealing an interesting association between tea (*C. sinensis* teas) consumption and kidney stone formation. Their findings showed that individuals who consumed five or more cups of tea daily experienced a reduced incidence of kidney stone formation compared to those who did not consume tea at all.

Interestingly, when comparing Rooibos to Japanese green tea (*Camellia sinensis*), it was found that Rooibos contains very low levels of oxalic acid (Rodgers et al., 2016), suggesting that it may be a more favourable choice for individuals concerned about oxalate intake. Shu et al. (2019) found that regular tea consumption was associated with a 13% reduction in the incidence of kidney stone disease in women and a 22% reduction in men. This suggests that incorporating tea into one's diet may offer a protective effect against the development of kidney stones, particularly for both genders.

2.4.3. **Rooibos acting as an antioxidant**

Rooibos, both the fermented and green varieties, is known to contain a rich array of unique polyphenolic constituents, including Aspalathus (exclusive to Rooibos), nothofagin, rutin, luteolin and quercetin, among others (Joubert & de Beer, 2011). Studies have shown that polyphenols have a range of medicinal properties, including antioxidant, anti-inflammatory, anti-tumour, anti-allergic, antimicrobial, and neuro- and cardio-protective abilities (Catarino et al., 2014). When cells in the body utilise oxygen to produce energy, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated as by-products of cellular redox processes (Alipour et al., 2012; Ajuwon et al., 2015:172). While these reactive species play beneficial roles in cellular responses and immune function at moderate levels, excessive generation can lead to oxidative stress, resulting in damage to essential cellular components, including lipids, proteins, and genetic material (Pham-Huy & Hua He, 2008). Oxidative stress has been implicated in the development of various chronic and degenerative conditions, including cardiovascular disease (Bonnefoy et al., 2002). The human body possesses an endogenous antioxidant defence system to counteract oxidative stress. However, with ageing, this system becomes less effective, making it essential to supplement the body with

antioxidants through the consumption of foods and beverages rich in polyphenolic compounds with antioxidant properties (Pham-Huy & Hua He, 2008; Maleki et al., 2015:90; Ajuwon et al., 2015:171).

Polyphenols, particularly flavonoids and phenolic acids, are a widely distributed group of bioactive molecules found abundantly in tea, comprising approximately 30% of the dry content of tea leaves (Deka & Vita, 2011; Joubert & de Beer, 2011). Flavonoids are phenolic compounds found in various vascular plants, with more than 8000 known individual compounds. In plants, they function as antioxidants (Pietta et al., 2000). The primary phenolic constituents of unprocessed South African herbal teas are dihydrochalcones, such as aspalathin and nothofagin, which are present in rooibos (Marnewick et al., 2005). These polyphenols are crucial in preventing various diseases, such as hypercholesterolemia, hyperglycaemia, hyperlipidaemia, and cancer (Abbas et al., 2017). The polyphenols in Rooibos are low in caloric or stimulant components and according to Beltrán-Debón et al. (2011), Rooibos extract activates AMP-activated protein kinase (AMPK), a regulator of cellular energy balance, which may influence metabolic diseases. According to Zungu et al. (2011), AMPK is a heterotrimeric protein consisting of catalytic alpha, non-catalytic beta, and gamma subunits. Its primary role is to conserve Adenosine Triphosphate (ATP) or stimulate alternative ATP generation pathways. AMPK regulates myocardial energy metabolism by phosphorylating Acetyl-CoA Carboxylase (ACC) and Malonyl-CoA Decarboxylase (MCD). Chronic hyperglycemia activates AMPK, leading to ACC phosphorylation, which alleviates malonyl-CoA inhibition on CPT1, facilitating enhanced mitochondrial entry of Free Fatty Acids (FFAs) via Carnitine Palmitoyltransferase 1 (CPT1) for beta-oxidation. Abnormally elevated beta-oxidation can induce mitochondrial membrane damage via peroxyl radicals. The diverse polyphenolic compounds found in Rooibos play a crucial role in combating oxidative stress, which is implicated in the development of chronic conditions such as cardiovascular disease. As research advances, deeper understanding of these mechanisms could lead to innovative therapeutic strategies that harness the benefits of Rooibos polyphenols.

2.5. Dietary intervention and risk factor prevention

2.5.1. High cholesterol prevention

Interest is increasing in dietary flavonoids and their potential to enhance endothelial function by altering oxidative stress levels. Endothelial dysfunction results from an imbalance in vasodilatory and vasoconstrictive molecules produced by the endothelium, largely influenced by oxidative stress, which carries significant implications for cardiovascular events (Papageorgiou et al., 2013). Endothelial dysfunction, associated with coronary artery disease and oxidative stress, is characterised by increased Reactive Oxygen Species (ROS), proinflammatory agents, unbalanced vasodilation, vasoconstriction and reduced nitric oxide

(NO) bioavailability, leading to disrupted endothelial barrier permeability and contributing to the inflammatory response in cardiovascular diseases (Duffy et al., 2001; Sun et al., 2020). In an attempt to address endothelial dysfunction, Duffy et al. (2001) investigated the effect of long-term black tea (*Camellia sinensis*) consumption on participants with coronary artery disease and found that certain antioxidants in black tea may contribute to the reversal of endothelial dysfunction. Beverages, such as teas, rich in polyphenols have demonstrated a positive effect in reducing the risk of cardiovascular diseases, stroke, dementia, and cerebral vascular accidents (CVA) caused by an increased development of arteriosclerosis (Ghosh & Scheepens, 2009).

Polyphenolic compounds can also be found in various foods and beverages, such as cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and red wine, and have been associated with decreased risks of coronary artery disease, cancer and stroke (Kris-Etherton et al., 2002). These compounds provide antioxidant protection in the vascular endothelium and may reduce the risk of atherosclerosis (Grassi et al., 2005; Rusconi & Conti, 2010). Studies have shown that the powerful antioxidants present in wine, particularly in Petite Syrah wine, can prevent LDL oxidation, a critical step in the progression of atherosclerosis (Teissedre et al., 1996).

Epicatechin, a polyphenol found in cocoa and red wine, has also been reported to hinder LDL oxidation and potentially aid in preventing atherosclerosis (Kondo et al., 1996; Collins et al., 2009). Moreover, cocoa flavonoids have been associated with cardioprotective abilities, such as reducing LDL oxidation, increasing HDL levels, inhibiting platelet activation and aggregation, and displaying anti-inflammatory properties (Ding et al., 2006).

Black tea consumption has been linked to a reduction in CHD risk factors in continental Europe and the United States of America (USA), with an average decrease of 11% in CVD risk observed per increase of three cups of tea per day (Erdman et al., 2007). Stein et al. (1999) suggested that regular consumption of alcohol-containing beverages, such as purple grape juice, may be associated with a lower risk of cardiac mortality, including myocardial infarction and stroke. Previously, fermented Rooibos (*A. linearis*) consumption had been shown to improve the lipid profile and redox status of individuals at risk of developing CVD (Marnewick et al., 2011). Furthermore, quercetin, present in Rooibos, has demonstrated a remarkable effect in preventing inflammation in atherosclerotic progression *in vivo* (Xiao et al., 2017).

Coffee, another widely consumed beverage worldwide (Johanson et al., 2019), has attracted considerable interest due to its potential effects on the cardiovascular system. With over 1000 bioactive substances, coffee has been the subject of multiple studies investigating its association with cardiovascular diseases. Moderate coffee intake has been linked to a reduced risk of heart failure, while excessive consumption may reverse this trend (Grosso et al., 2012;

Loftfield et al., 2018). However, Senftinger et al. (2023) found no significant correlation between coffee consumption and major cardiovascular diseases.

2.5.2. **Hypertensive heart disease prevention**

According to existing epidemiological research, quercetin, a plant flavonoid with antioxidant properties also found in Rooibos supplementation, has demonstrated potential preventive effects on the cardiac function of patients or groups at risk for cardiovascular disease (Xiao et al., 2017). In a study conducted by Mennen et al. (2004), it was suggested that a diet rich in flavonoids may play a role in preventing cardiovascular disease, particularly in women who consumed flavonoid-rich foods and beverages such as apples, tea, chocolates, onions, citrus and red fruits. Additionally, Grassi et al. (2005) proposed that flavonoids found in dark chocolate not only improve lipid profiles but may also have beneficial effects on insulin resistance and hypertension. Their study involving participants with essential hypertension found that a diet high in flavonoids could reduce blood pressure in healthy, young individuals with isolated systolic hypertension. Dark chocolate contains flavonoids, such as epicatechin, catechin and procyanidins (Khan et al., 2014). Shiina et al. (2009) investigated the acute effect of flavonoid-rich dark chocolate on coronary circulation. They discovered that the oral intake of flavonoid-rich dark chocolate positively impacted coronary circulation in healthy adults compared to those who consumed non-flavonoid white chocolate. Their study suggests that cacao products possess a greater antioxidant capacity and higher flavonoid content than tea, red wine, fruits, and vegetables. These flavonoids may reduce the risk of cardiovascular disease due to their high antioxidant content, which has anti-platelet and anti-inflammatory properties, improving endothelial function, increasing HDL levels, and lowering blood pressure.

Another study conducted by Arazi et al. (2014) investigated the effects of green tea extract (GTE) supplementation on various cardiovascular parameters in response to an acute resistance exercise. Participants were administered GTE capsules twice daily for a duration of 3 weeks. The results indicated that GTE supplementation had no significant effect on systolic and diastolic blood pressure and heart rate (HR) in the participants. However, a notable improvement was observed in mean arterial blood pressure (MAP) and rate pressure product (RPP) response one hour following the acute resistance exercise. These findings suggest the potential of GTE in enhancing cardiovascular responses during physical activity. While three weeks of GTE ingestion did not influence SBP, DBP and HR, it may have a favourable effect on MAP and RPP responses to an acute resistance exercise during a 1-hour exercise recovery. As previously mentioned, in the study conducted by Shiina et al. in 2009, flavonoids demonstrated significant potential in reducing the risk of cardiovascular disease, primarily owing to their robust antioxidant properties.

2.5.3. **Ischaemic heart disease prevention**

A review conducted by Cao and colleagues (2019) indicates that tea derived from *Camellia sinensis* contains bioactive compounds that offer protection against various risk factors associated with CVD, such as hypertension and hypercholesterolemia. These bioactive components have been found to prevent and treat CVD and provide defence against ischemia and reperfusion injuries, which are primary contributors to myocardial infarctions (Kuriyama et al., 2006; Deka & Vita, 2011). Several studies support the notion that regular consumption of green and black teas offers cardioprotective effects and promotes cardiovascular health (Hartley et al., 2013 a & b; Cao et al., 2019; Abe et al., 2021).

In a study carried out by Huang et al. (2018), the primary objective was to investigate the potential beneficial effects of green tea extract supplementation on low-density lipoprotein cholesterol (LDL-C) levels in overweight and obese women with high cholesterol. The subjects were instructed to ingest one green tea extract capsule 30 minutes following a meal, thrice daily, for a duration of 6 weeks during each phase, while their LDL-C levels were closely monitored before and after the supplementation period. The results of the study revealed that green tea extract supplementation exhibited improvement in the LDL-C levels, with a notable improvement in the LDL-C levels compared to baseline measurements.

To investigate the hypothesis of their study that regular tea consumption may reduce the risk of ischemic stroke, Liang et al. (2009) conducted a study which demonstrated a significant decrease in ischemic stroke risk with the consumption of just one cup of tea daily, while a greater risk reduction was observed in individuals consuming two cups of green or oolong tea daily. The study was conducted between July 2007 and July 2008. Other studies conducted by (Tian et al., 2016; Pang et al., 2016) have also reported an association between increased green tea consumption and a reduced risk of death from cardiovascular disease.

2.5.4. **Diabetes mellitus prevention**

According to a review conducted by Okello et al. (2020), the prevalence of DM is projected to increase by 160% by 2030, primarily due to urbanisation and unhealthy lifestyles. In addressing this growing health concern, Rooibos has been identified as a potential contributor in the prevention, management, and treatment of T2DM (Ajuwon et al., 2018). Notably, Orlando et al. (2019) found that a diet enriched with aspalathin, a unique polyphenolic compound found exclusively in Rooibos, had a positive impact on hyperglycaemia in vervet monkeys.

Currently, the main treatment options for DM involve insulin and Metformin. However, Dludla et al. (2017) suggest that Rooibos flavonoids, which possess antioxidant properties, are being investigated for their potential effectiveness against various metabolic complications

associated with diabetes. Numerous other studies have also reported on the potential anti-diabetic properties of Rooibos and its major phenolic compound, aspalathin (e.g. Kawano et al., 2009; Johnson et al., 2018; Sasaki et al., 2018; Dludla et al., 2020). These findings highlight the promising role of Rooibos as a natural intervention for diabetes prevention and treatment. In a double-blind, placebo-controlled, randomised trial conducted by Ferreira et al. (2017), the researchers aimed to determine whether supplementation with green tea extract could offer superior effects compared to metformin in improving lipid levels and blood glucose control in overweight women. During the 12-week study period, 120 overweight women were randomly assigned to receive either GT capsules or a placebo. The results revealed that daily supplementation with GT capsules led to a significant reduction in total cholesterol and LDL levels, whereas the placebo group did not show significant improvements in these parameters. Moreover, the study reported that green tea extract supplementation demonstrated greater efficacy than metformin in improving the lipid profile and glycaemic control.

2.6. Echocardiography and dietary intervention

A number of studies have reported on the effect of dietary antioxidant supplementation on cardiac characteristics. In a study conducted by Jones and colleagues (2003), the role of cellular antioxidant enzymes in the pathogenesis of myocardial ischemia-reperfusion (MI/R) injury was examined using gene-targeted mice and echocardiography. The researchers found that the overexpression of manganese (Mn) superoxide dismutase (SOD) significantly attenuated myocardial necrosis after MI/R injury. Non-transgenic mice showed significantly depressed cardiac output and impaired anterior wall motion, while transgenic MnSOD-treated mice did not exhibit these impairments.

Hart et al. (2005) reported that reduced mitochondrial respiratory chain activity and elevated oxidative stress are associated with the aetiology of Friedreich ataxia (FRDA), suggesting that energy supplementation and antioxidant therapy might be effective treatments. They treated ten individuals with FRDA using a combination of coenzyme Q10 (400 mg/d) and vitamin E (2100 IU/d) for 47 months and assessed cardiac function using echocardiography. The study revealed a considerable increase in cardiac function and a long-lasting improvement in mitochondrial energy generation, which was linked to a slowing of the progression of certain clinical characteristics of the disease.

As previously mentioned, HPT is an important risk factor for CVD. Masoumi-Ardakani and colleagues (2022) utilised echocardiography to explore the effect of endurance training (ET) and Mitoquinone mesylate (MitoQ) on cardiac function and antioxidant serum levels in hypertensive subjects. The study found that ET improved endothelial function and exhibited antioxidant properties, which are crucial in the pathophysiology of CVDs. Additionally, MitoQ, a mitochondrial-targeted antioxidant supplement, was shown to enhance vascular function

and reduce angiotensin 2 levels in rats. The combination of MitoQ and ET resulted in a reduction in systolic blood pressure and LVH, which occurs due to increased blood pressure.

Research has highlighted questions about the similarity between animal and human responses to physiological stimuli. Goutianos et al. (2015), addressed the similarity between animal and human responses to physiological stimuli. Their study confirmed that rats closely mimic human responses to exercise in essential blood biochemical parameters, supporting the validity of using rat models in exercise physiology research.

2.7. Summary of the Literature Review

This chapter has explored the impact of CVD and its associated risk factors, as well as potential dietary interventions that can serve as early intervention strategies to reduce the risk of CVD. Additionally, valuable information has been provided on the use of echocardiography as an imaging tool for the early identification of CVDs in conjunction with standard risk blood biomarkers. Regarding the role of Rooibos, various studies have been conducted, including experiments using animal models and dietary intervention-type designs in humans at risk of developing CVD (Persson et al., 2010; Marnewick et al., 2011). Given the increasing burden of cardiovascular disease on the South African health system, and the projected rise in the prevalence of the disease over the coming decades, the usefulness of effective, non-invasive diagnostic tools, such as echocardiography, to complement invasive blood biomarkers for the early diagnosis of CVDs, is of paramount importance. Particularly noteworthy is the accessibility of echocardiography services in many healthcare facilities, including clinics, making it a valuable tool in addressing this pressing health concern. In the next chapter, we will discuss the research study's methodology.

Chapter 3 | Methodology

3.1. Introduction

This chapter outlines the design of the research project and presents the research process. In accordance with Gelo et al. (2008), the principal objective for formulating a research design is to guarantee that the information obtained equips the researcher with the means to tackle the research subject effectively while concurrently serving as a roadmap for both data collection and analysis. In this study, a randomised, parallel, placebo-controlled approach was chosen, involving 219 participants. A dietary intervention period of 12 weeks, preceded by a 2-week washout period, was employed.

The limitations of Randomised Controlled Trials (RCTs) have been addressed by Frieden (2017), who noted that RCTs may lack external validity and often have insufficient duration or size to assess long-term effects and rare adverse events. This can lead to a reliance on surrogate markers, which may not correlate with the outcome of interest, and hinder RCTs' ability to keep pace with clinical innovations and address urgent health issues, as they are limited in assessing the individualised effect of treatment. Another study by Batioka et al. (2023) evaluated the adherence of RCTs for the surgical management of stress urinary incontinence (SUI) to the Consolidated Standards of Reporting Trials of Patient-reported Outcomes (CONSORT-PRO) guidelines. Their comprehensive literature review assessed relevant RCTs using the CONSORT-PRO checklist and the Cochrane risk of bias tool, finding that 88.37% of RCTs had a risk of bias rating of concern. They concluded that many RCTs on SUI surgery poorly adhere to CONSORT-PRO guidelines. Improved adherence to these guidelines can enhance clinical decision-making and improve patient's quality of life.

Despite these critiques, there is reason to suggest that the interpretation of this study results is reasonable and supported by the statistical analysis. The measures below were implemented to ensure the internal validity of the study and to minimise bias, thereby supporting the reliability of the study's findings, as this study was a double-blinded Randomised Control Trial (RCT) designed with rigorous measures to minimise bias:

I. Randomisation was conducted by an external statistician, using a method that did not involve participant identification details, ensuring the process was impartial and free from selection bias. Additionally, the statistician classified participants into three groups using the Excel RAND function iteratively to avoid repetitions, ensuring random allocation was maintained. These measures were implemented to ensure the internal validity of the study and minimise bias, thereby supporting the reliability of the study's findings.

II. Blood tests were performed by external assessors (nursing staff) who did not have access to participant identification details, preventing any potential bias in the assessment of biochemical outcomes.

III. The echocardiography studies were performed by the researcher without access to participants' identification details during the procedures, ensuring unbiased imaging assessments.

The researcher was involved in all aspects of this study where echocardiography was mentioned, starting with writing the research proposal, obtaining the relevant ethics approval, providing the participants with information about the ECHO procedures and assisting with obtaining written informed consent after the information session to the participants, as well as to perform all echocardiography assessments before and after the Rooibos intervention. The researcher collected all echocardiography data and compiled it into an Excel spreadsheet, which was then analysed in consultation with a qualified statistician and interpreted by the researcher for the purposes of the objectives of the study.

The first aim of this research study was to determine the prevalence and characteristics of cardiovascular disease and its associated risk factors in a South African cohort using a prediction risk model. Secondly, the aim was to determine the effect of the consumption of South African herbal Rooibos tea on cardiovascular function in adults at risk for developing cardiovascular disease using TTE and, finally, to establish any association between the CVD findings and dietary interventions.

By incorporating echocardiography into the research project, the study seeks to enhance its capacity to detect and understand potential cardiovascular implications associated with Rooibos consumption in individuals at risk for cardiovascular disease. This approach can provide valuable insights into the cardiovascular effects of Rooibos, thereby contributing to the existing body of knowledge on heart health interventions.

This research study, titled "Modulation of Cardiovascular Function by Rooibos in Adults at Risk for Cardiovascular Disease," constitutes the echocardiography imaging component of the larger, ethically approved research study titled "Rooibos, Heart, and Cognitive Health." This research study aims, to determine the prevalence and characteristics of cardiovascular disease and its associated risk factors in a South African cohort using a prediction risk model. Secondly the aim was to determine the effect of consuming the South African herbal Rooibos tea on cardiovascular function in adults at risk for developing cardiovascular disease using TTE and, finally, to establish an association between the CVD findings and dietary interventions.

3.2. Study population

Between September 2021 and April 2022, a total of n = 721 adults, aged between 28 and 79 years, participated in a screening programme conducted at the Oxidative Stress Building of the Applied Microbial and Health Biotechnology Institute (AMHBI), situated on the Bellville campus of the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. This site was selected for its dedicated space for human intervention studies and its well-equipped laboratories with necessary analytical instruments. The recruitment of participants was carried out by an appointed trial coordinator in collaboration with research study nurses and the principal investigator.

From the initial screening, n = 412 were excluded (see below the schematic presentation of the Consolidated Standards of Reporting Trials (CONSORT) guidelines that were followed). A total of 309 participants were randomly assigned to consume either a Placebo (n = 103), fermented Rooibos (n = 100) or green Rooibos (n = 106) capsule. The composition of the capsule is seen in **Appendix A**. Following the baseline phase, n = 219 participants proceeded to complete the 12-week Rooibos intervention phase.

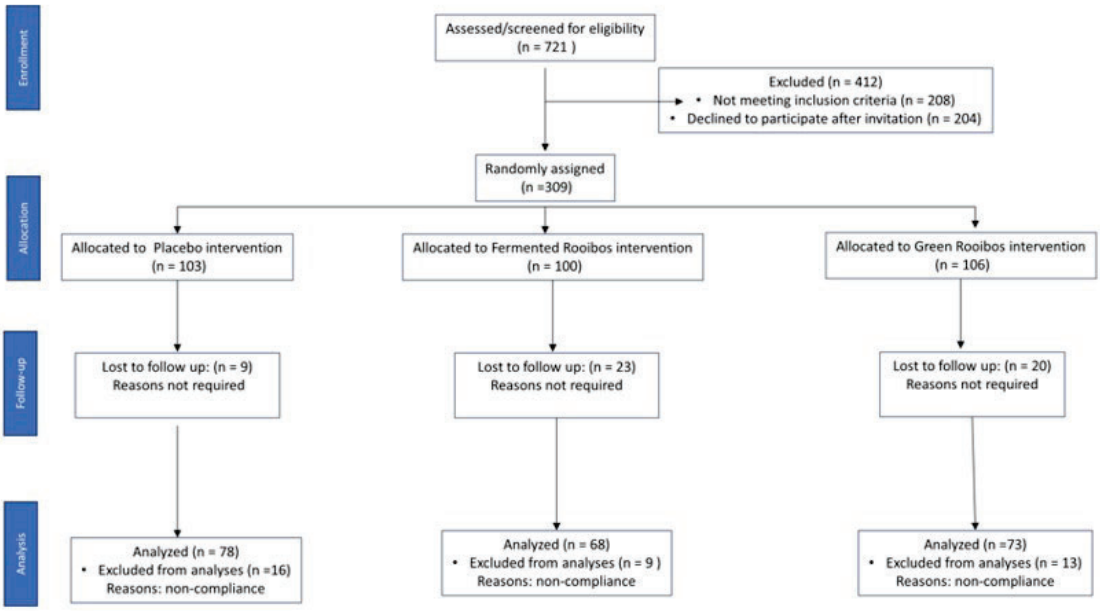

Figure 3-1 Schematic presentation OF THE CONSORT guidelines

The study participants consisted of both males and females who presented with at least two or more of the following modifiable cardiovascular disease (CVD) risk factors, as specified in the study's inclusion criteria: 1) hyperlipidaemia, 2) hypertension, 3) hyperglycaemia, 4) unhealthy lifestyle factors (such as tobacco misuse, unhealthy diet, obesity, physical inactivity) and 5) a family history of CVDs.

The study design and recruitment process aimed to identify individuals at risk for CVDs and to investigate the potential impact of Rooibos intervention on their cardiovascular health. By enrolling participants with specific CVD risk factors, the study sought to assess the effectiveness of Rooibos in modulating these risk factors and potentially mitigating the overall risk of cardiovascular diseases in this population.

3.2.1. **Inclusion and exclusion criteria**

The inclusion criteria for this study were established based on the findings and methodology of Marnewick et al. (2011), which investigated the "Effects of Rooibos (Aspalathus linearis) on Oxidative stress and biochemical parameters in adults at risk for Cardiovascular disease. The criteria were designed to include a specific demographic with relevant cardiovascular risk factors, thus ensuring the study's focus remained on individuals at risk for cardiovascular disease. The exclusion criteria were applied to eliminate potential confounding factors that could interfere with the research outcomes, ensuring a more accurate assessment of Rooibos's effects.

Inclusion Criteria:

The study aimed to include males and females aged between 28 and 79 years who presented with at least two or more of the following cardiovascular risk factors:

1. Hyperlipidaemia: Defined as raised cholesterol levels of >5.5 to 7.5 mmol/L and triglyceride levels of >1.7 to <2.5 mmol/L.
2. Hypertension: Characterised by increased blood pressure readings of >120-139/80-90 mmHg.
3. Hyperglycaemia: Indicated by elevated blood glucose levels >5.6 mmol/L
4. Lifestyle Factors: Participants with a body mass index (BMI) of > 25 to 38 kg/m², classified as overweight or obese, physically inactive, and following a high atherogenic diet, were considered for inclusion.

Exclusion Criteria:

Certain individuals were excluded from the study based on the following criteria:

1. Known medical disorders: Individuals with known renal, hepatic, endocrine or gastrointestinal disorders were excluded.
2. Alcohol consumption: Participants who consumed more than two alcoholic drinks per day were excluded.
3. Unusual dietary habits: Vegans and vegetarians were excluded from the study due to their distinct dietary patterns (usually aimed at an increased antioxidant intake).
4. Medication and supplement use: Participants on chronic/life-sustaining oral medications, antioxidant supplements, aspirin or any other drugs with antioxidant properties were excluded.

5. Pregnancy and lactation: Pregnant and lactating women were not included in the study.
6. Cardiac valvular disease: Patients with known or diagnosed cardiac valvular diseases, such as valvular stenosis and valvular regurgitation due to Rheumatic fever, were excluded from the study, as these conditions could influence echocardiography parameters.

These well-defined inclusion and exclusion criteria were applied to ensure that the study enrolled a specific target population with relevant cardiovascular risk factors and excluded potential confounding factors that could interfere with the research outcomes.

3.3. Study design

This clinical study was registered with the Pan African Clinical Trial Registry with registration number: **PACTR202108636334823**. The study design consisted of a 14-week randomised, parallel, placebo-controlled dietary intervention trial utilising capsules containing either a standardised Green or Fermented Rooibos water extract or a Placebo. After a 2-week washout phase, the participants were randomly assigned to one of the three intervention groups: Placebo, Fermented Rooibos, or Green rooibos. The composition of the Placebo, Fermented Rooibos and Green Rooibos extracts can be seen in **APPENDIX A: ROOIBOS CAPSULE INFORMATION**. The commercially available standardised water-soluble rooibos extracts were purchased from Rooibos Ltd (Clanwilliam, South Africa) and packaged into capsules by Verve Dynamix (Somerset West, South Africa), an FDA-registered facility (See **APPENDIX A: ROOIBOS CAPSULE INFORMATION**). The content of each capsule is equivalent to that of 2 cups of Rooibos herbal tea in the context of antioxidant content measured as total polyphenolics.

Each participant was instructed to take one capsule three times a day with meals (morning, midday, and evening) for the entire 12-week intervention period. Compliance was assessed by reviewing dietary records and counting the remaining capsules in the holders, which had to be returned for monitoring. To ensure double blinding, the researcher remained unaware of which capsules were being consumed by the participants (Fermented Rooibos, Green Rooibos or Placebo). Before the commencement of the 12-week intervention, they all followed their normal diet with certain dietary flavonoid restrictions that needed to be adhered to throughout the intervention.

A detailed food and beverage restriction list was provided to each participant before the commencement of the study. After the two-week wash-out period, baseline trans-thoracic echocardiography was performed. Following the 12-week intervention phase, the participants returned for a second trans-thoracic echocardiogram.

All echocardiograms were conducted by the same sonographer, following the guidelines, standards, and recommendations of the American Society of Echocardiography (ASE). Additionally, participants were instructed to maintain daily dietary records during specific indicated times throughout the wash-out and intervention phases to monitor compliance with dietary intake restrictions and capsule consumption.

By using randomisation, blinding and placebo control, the study aimed to minimise bias and provide reliable results for evaluating the impact of the Rooibos interventions on cardiovascular parameters.

3.4. Ethical considerations

3.4.1. Participant privacy and confidentiality

Ethical approval for this research project was obtained from the Human Research Ethics Committee of the Faculty of Health and Wellness Sciences at the Cape Peninsula University of Technology in 2021, with subsequent renewals in 2022 and 2023 (See **APPENDIX B: LETTER OF ETHICS APPROVAL)**. No changes or amendments were made to the study protocol. Renewals were applied only when the expiration date had passed. Additionally, permission was granted by the principal investigator (PI) of the comprehensive/mother study, titled "Rooibos, Heart and Cognitive Health," to include the study participants in this study, as mentioned previously. During an information session, all participants were thoroughly informed about the study, and the study team provided detailed explanations regarding their involvement. Participants were made aware of their right to withdraw from the study at any point without facing prejudice or any adverse impact. Written informed consent was obtained from all participants prior to the start of the study and data collection (See **APPENDIX C: PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT)**.

The Protection of Personal Information Act (POPIA), which became effective on July 1, 2021, governs the processing of personal information for research purposes, including the transfer of data beyond South African borders. The act is designed to ensure that any limitations on the right to privacy are justified and aimed at preserving other essential rights and interests. Sensitive personal information, such as health status, political beliefs, racial or ethnic origin, and criminal behaviour, is protected under POPIA.

To adhere to ethical standards of confidentiality and the POPIA regulations for the protection of personal information, each participant was assigned a unique participation code to safeguard their identity and privacy. Only the PI of the comprehensive/mother study, the study coordinator, and the study doctor had access to the participants' identities linked to the study codes. The researcher of this study was granted access to participants' identities for quality assurance purposes and to facilitate referral for clinical care in cases of abnormal

echocardiography findings. The unique code was used in all research-related data and documents for each participant. All information was treated with utmost confidentiality, and a password-protected computer was used to store all relevant data. Handwritten data were securely kept in a locked cabinet at the study site, with an access code required for electronic data access.

Trans-thoracic echocardiograms were conducted in a secure and private setting, with only the qualified nurse and the study doctor (medical practitioner) allowed to be present during the procedure to provide medical assistance if necessary. At the end of each day, the echocardiogram recordings were securely transferred to a safe location and stored in a closed and protected room. The information collected from this research project will be used exclusively for the purposes of this study and associated publications. The researcher was granted permission to use echocardiographic images of the participants in this document, with their identities protected by the unique participation codes.

3.4.2. **Risks and benefits related to the study**

The application of ultrasound as a non-invasive technique for examining human tissue is widely acknowledged. While diagnostic ultrasonic imaging is generally considered safe, adherence to the ALARA (as low as reasonably achievable) principle is essential during soft tissue examinations. For this research, a medical doctor registered with the Health Professional Council of South Africa (HPCSA) and a qualified study nursing sister were on-site to aid and manage any adverse effects experienced by participants. The contact details of the doctor and study coordinator were readily available to participants for addressing any research-related queries. In cases where abnormal values were detected in echocardiography, blood results, etc., the study doctor promptly referred the participants to the nearest medical facility. All medical personnel, including phlebotomists responsible for obtaining fasting blood samples, were qualified nursing staff with ample experience. Regarding the safety of Rooibos consumption, Marnewick et al. (2011) reported no known side-effects associated with the daily intake of six cups of rooibos by adults at risk for developing cardiovascular disease (CVD). Additionally, Canda et al. (2014) suggest that daily consumption of Rooibos herbal tea offers beneficial effects on human health. The dosage of Rooibos provided in one capsule was equivalent to two cups of Rooibos herbal tea and was considered safe, thus avoiding a ‘megadose’. Notably, during the study, none of the participants reported any adverse effects from taking the Rooibos capsules. None of the participants or staff contracted the COVID 19 virus during the recruitment process or during the data collection process.

3.5. Data Collection

The echocardiography examinations for this research were conducted by the researcher, a qualified sonographer holding a Master of Technology degree in diagnostic ultrasound and a specialist qualification in echocardiography from the American College of Cardiology, Washington, United States of America. The researcher is an experienced echocardiographer, as she previously worked in an established Cardiology Practice for many years having to perform echocardiography for diagnostic purposes. The ASE mandates that Sonographers attain and uphold the minimum standards in education and credentialing within two years of commencing employment, according to Picard et al. (2010).

All echocardiography assessments were performed using standardised TTE imaging protocols under consistent conditions. Due to the lack of sonographers with specialist expertise in echocardiography within the Western Cape region of South Africa, inter-rater reliability could not be determined. Nevertheless, the researcher ensured intra-rater reliability through the following measures:

I. The researcher's clinical training and experience in echocardiography equipped her with proficiency in conducting echocardiography studies. She worked closely with cardiologists and physicians for several years, learning correct techniques, understanding equipment nuances, and practising until achieving a high level of competency.

II. The research study followed the standardised echocardiography protocol of the American Society of Echocardiography for conducting echocardiography measurements. This involved consistent participant positioning, uniform equipment settings, and adherence to specific measurement techniques to minimise variability.

III. The researcher was blinded to previous results and measurements, ensuring unbiased and consistent assessments during each examination.

IV. To maintain quality control, measurements were repeated at intervals for validation, ensuring accuracy and reliability in the data obtained.

These measures collectively underscore the validity of the research findings despite the researcher being the sole performer of echocardiography examinations.

Following the guidelines of the ASE, the echocardiography equipment used for the examinations was equipped with two-dimensional, M-mode, and colour and spectral Doppler imaging capabilities, as recommended by Picard et al. (2011). The data collection involved the following grey-scale imaging and Doppler applications:

1. Two-dimensional imaging was utilised to assess cardiac structures and evaluate the LV function.

2. Motion mode was employed to provide one-dimensional morphological information on all moving cardiac structures.

3. Spectral and colour Doppler analysis was performed to assess the heart and valves for any abnormal blood flow patterns.

All participants' age, gender and echocardiogram results were carefully recorded on the research echocardiography datasheet (See **APPENDIX D: ECHOCARDIOGRAPHY REPORT**), and all retrieved information was entered into an electronic Microsoft Excel research data spreadsheet (See **APPENDIX E: EXAMPLE OF THE DATA SHEET WITH ECHOCARDIOGRAPHY MEASUREMENTS**). The research supervisors conducted quality control checks on the data, while the statistical analysis was performed by a qualified statistician employed at CPUT.

3.5.1. Echocardiography equipment

The E2 Series Digital Colour Doppler Ultrasound system with Brightness mode (B-mode), M-Mode, Doppler facilities (colour, pulsed-wave and continuous-wave Doppler), and a 2-8 MHz multi-frequency phased array transducer (**Figure 3-2)** with coupling gel was used to assess the cardiac structures **(Figure 3-3).** The ultrasound system is the Sonoscape E2 Serial number 720505620 Ultrasound machine manufactured by Medical Corporation based in China.

Figure 3-2 Echocardiography transducer (probe)[3]

[3] Figure 3.2 The image was taken by the author

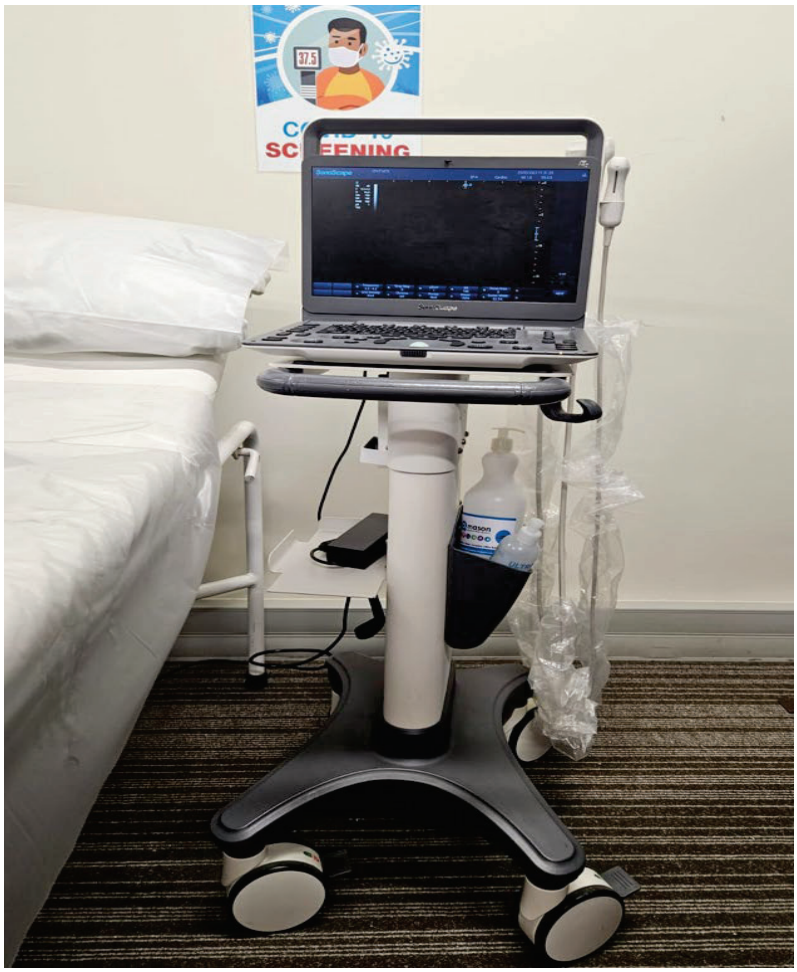

Figure 3-3 Echocardiography system[4]

The Ultrasound room at the study site was fully fitted with a moveable table, ultrasound system and accessories needed to perform the ultrasound, as seen in **Figure 3-3**.

3.5.2. Echocardiography scanning

The echocardiography examinations were conducted with participants at rest, lying on their left side on the examination bed, to acquire the specific echocardiography window **(Figure 3-4)**. The transducer was carefully positioned in the third or fourth intercostal space to the left of the sternum, with the index marker directed towards the right shoulder at approximately the 9 to 10 o'clock position. Adjustments were made as needed to accommodate individual anatomical variations. This positioning ensured that the LV appeared perpendicular to the ultrasound beam within the image field. All relevant data, including the participant's age, unique participation number and measurements (as recorded in **APPENDIX C: PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT**), were diligently

[4] Figure 3:3 The image was taken by the author.

documented for each participant with strict adherence to data privacy and confidentiality protocols. To facilitate comparisons with earlier investigations, it is crucial to maintain consistent conditions once laboratory protocols have been established, as recommended by Mitchell et al. (2019). Therefore, to ensure consistency throughout the study, the echocardiography assessments were performed by the same technician, during a similar time of the day on both study visit occasions.

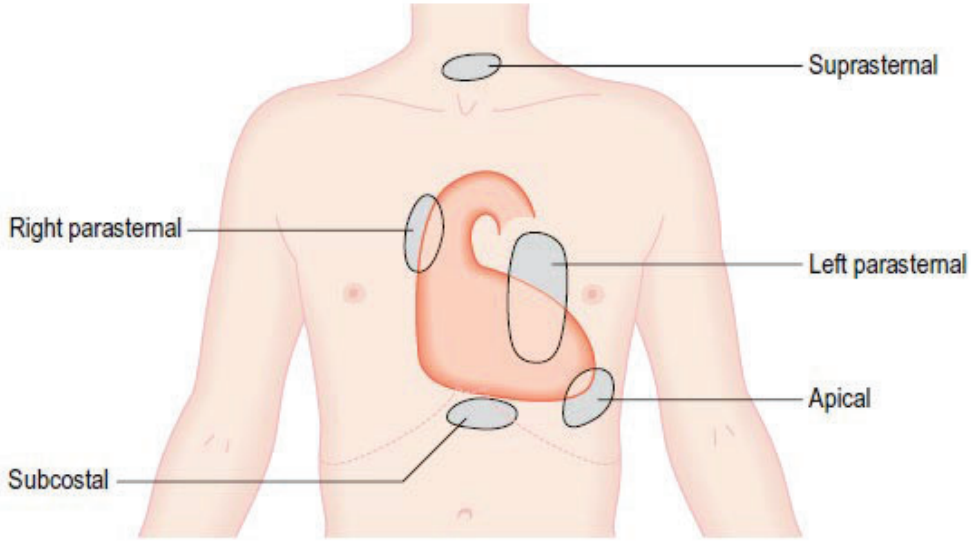

Figure 3-4 The placement of the transducer to obtain the main echocardiography windows (Taken from Kaddoura (2016) Licence number: 230814-020947)

Chapter 2 highlighted echocardiography as a versatile imaging modality for managing patients with chest pain and assessing various aspects of cardiac function, including left ventricular systolic and diastolic function, as well as myocardial and coronary perfusion. Echocardiography proves to be particularly valuable in diagnosing and prioritising patients with acute chest pain or dyspnoea. In support of these findings, Mohamed et al. (2010) concur that echocardiography offers the unique advantage of viewing the heart from multiple windows, each providing a distinct angle for comprehensive cardiac assessment **(Figure 3-5).**

The above statements are in line with the researcher's aims for this study which are to provide substantial evidence of echocardiography's reliability and accuracy and to demonstrate its efficacy as a trusted diagnostic tool in the field of medical imaging, particularly for diagnosing CVDs. Moreover, this research aims to highlight the potential benefits of early detection and management of cardiovascular diseases through echocardiography, ultimately contributing to improved patient outcomes and overall healthcare efficiency. By reaffirming echocardiography's effectiveness, this research strives to enhance its widespread adoption in

clinical practice and reinforce the importance of echocardiography as a vital component of comprehensive cardiovascular assessment and disease management protocols.

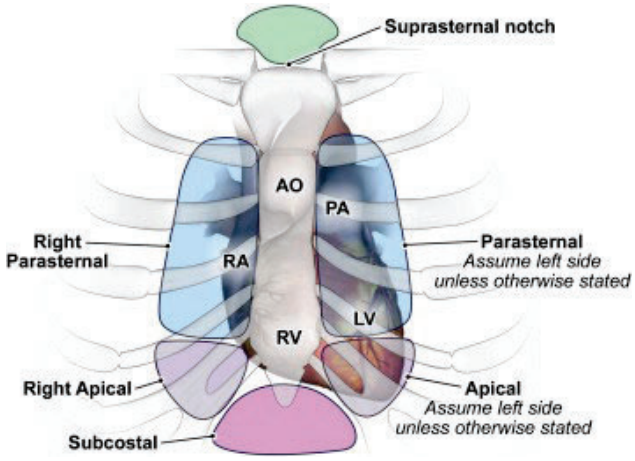

Figure 3-5 Echocardiographic windows to obtain images [5]

Achieving sufficient endocardial definition in 2D echocardiographic images is crucial for accurately determining cardiac morphology and motion. It is essential to visualise all relevant anatomical structures within the standard planes to ensure precise assessment (Picard et al., 2011; Mitchell et al., 2019. For this study, various 2D trans-thoracic echocardiographic planes were captured to assess cardiac structures and functions comprehensively. These planes facilitate a detailed evaluation of the heart's size, shape, and function, including the assessment of valves, chambers and blood flow patterns. By adhering to these standardised imaging protocols, this research aims to provide reliable and accurate data for its analysis and contribute to the understanding of cardiac health and function **(Figure 3-6)**:

1. Parasternal long-axis plane **(Figure 3-8)**
2. Parasternal short-axis plane **(Figure 3-9)**
3. Apical 4-chamber and 2-chamber plane **(Figure 3-10)**

[5] Taken from Mitchell et al., 2019, with permission: Licence Number 5550680988362

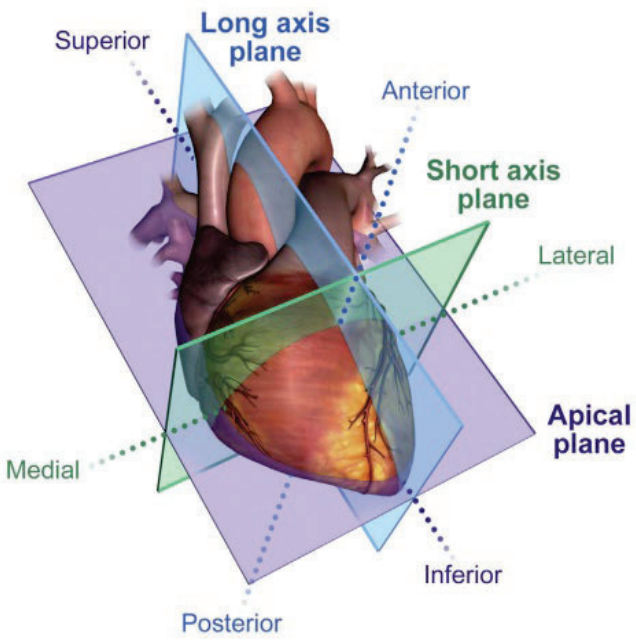

Figure 3-6 The five scanning planes of the heart that should be captured [6]

3.5.2.1. **Two-dimensional imaging**

The B-mode or grey-scale imaging mode was employed to examine the cardiac structures and evaluate the left ventricular function for systolic and diastolic dysfunctions. B-mode echocardiography provides real-time, two-dimensional images of the heart, allowing for the assessment of the LV's contractility during systole and relaxation during diastole.

The following measurements and dimensions were applied in the longitudinal and short axis to determine abnormalities of the cardiac structures **(Figure 3-7)**:

- Interventricular septum diastolic dimension (IVSd)
- Left Ventricle Internal End Diastolic dimension (LVIDd)
- Left Ventricular Posterior Wall Diastolic Thickness (LVPWd)
- Interventricular Septum Systolic Thickness (IVSs)
- Left Ventricular Internal End Systolic Dimension (LVIDs)
- Left Ventricular Posterior Wall Systolic Thickness (LVPWs)
- Aortic root
- Left Atrium (LA)
- Right Atrium (RA)
- Right Ventricular Outflow Tract (RVOT)
- Left Ventricular Outflow Tract (LVOT)

[6] Taken from Mitchell et al., 2019, with permission: Licence Number 5550680988362

- Right Ventricular Anterior Wall Diameter (RVAWd)
- Right Ventricular Internal End Diastolic Dimension (RVIDd)
- Mitral valve Diameter (MV Diam)
- Mitral Valve Area (MVA)
- Fractioning shortening (FS)
- Ejection Fraction (EF)

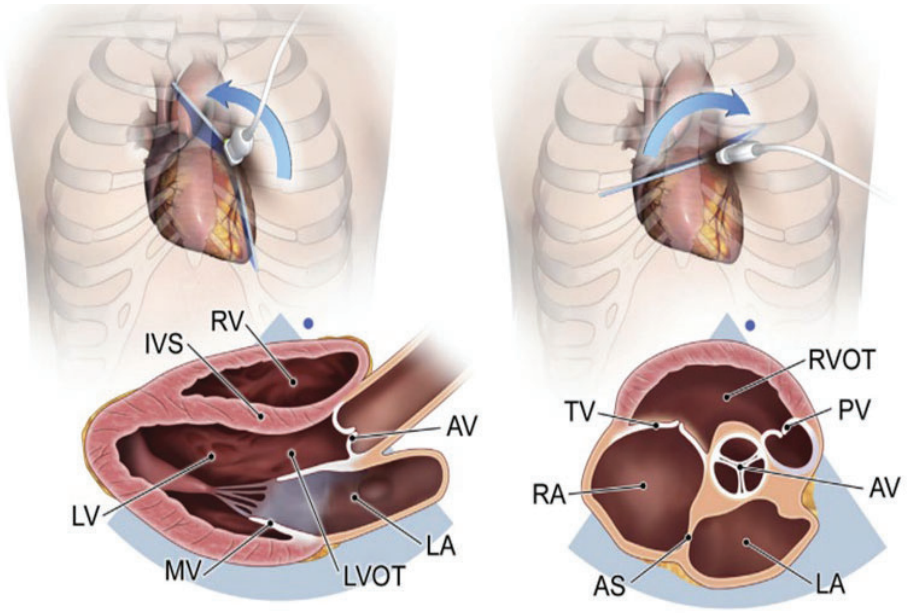

Figure 3-7 The rotation of the transducer to create the parasternal long-axis image (left) and the parasternal short-axis image (right) [7]

As recommended by the ASE, measurements in echocardiography should be carried out and reported only when they are precise and repeatable. This was ensured through regular calibration of the equipment and comprehensive training of the echo-cardiographer performing the assessments. In cases where measuring a specific cardiac structure proves challenging, the report should still include a qualitative assessment of that structure, unless such an assessment is equally impractical (Picard et al., 2010; Mitchell et al., 2019). For this study, all measurements and dimensions mentioned earlier were obtained during the resting phase of each participant's cardiac function. These calculations provide valuable insights into the participant's cardiac health and function during a period of minimal physiological stress, allowing for a comprehensive evaluation of their baseline cardiac performance.

[7]Taken from Mitchell et al., 2019, with permission: Licence Number 5550680988362

3.5.2.2. **Left parasternal long-axis window**

In this echocardiographic view, the placement of the transducer was carefully performed between the 2nd and 4th intercostal spaces, towards the left side of the sternum, ensuring optimal visualisation of the heart's structures. The marker of the transducer was directed towards the right shoulder of the participant to maintain consistency in orientation. A long-axis image was obtained, capturing tomographic slices from the base to the apex of the heart, providing a comprehensive and detailed view of the cardiac anatomy (**Figure 3-8)**. This imaging approach allows for the evaluation of various cardiac structures, including the left ventricle, aortic and mitral valve, left atrium, and the right ventricle, enabling precise assessment of cardiac function and morphology. The obtained images will play a crucial role in the comprehensive analysis of the participant's cardiac health in this study.

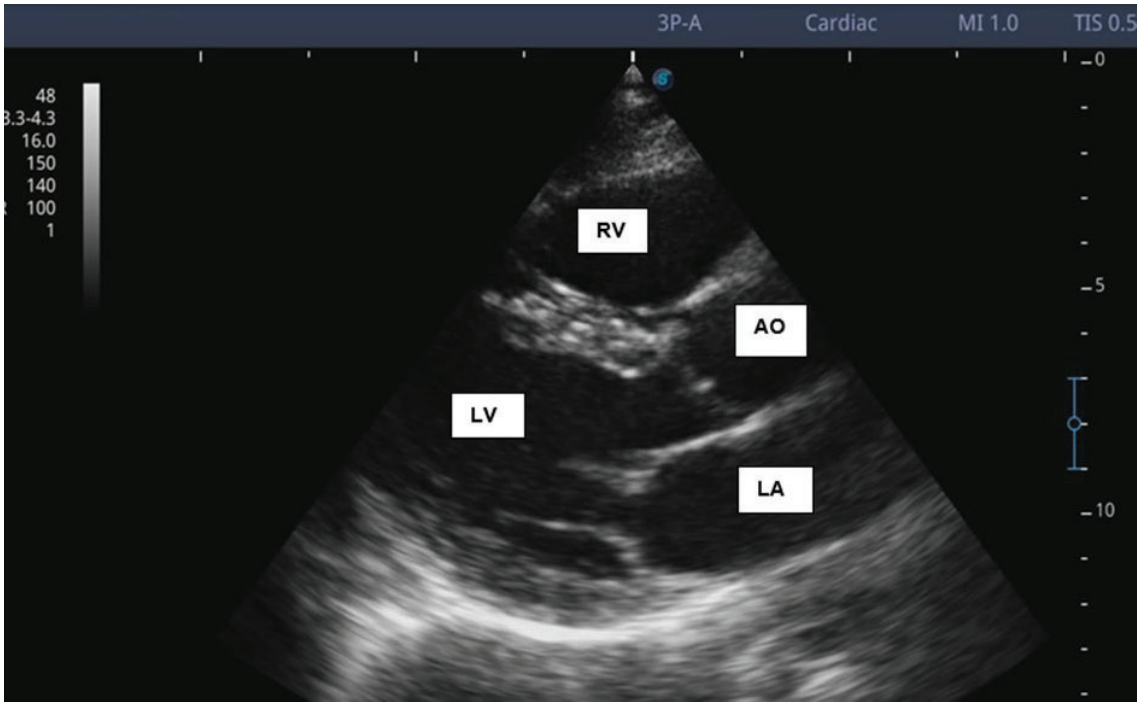

Figure 3-8 Parasternal long-axis view demonstrating the LV, AO, RV[8]

3.5.2.3. **Parasternal short-axis views**

In this view, the transducer remains in the same position on the chest as in the long-axis view. However, a 90º rotation of the transducer is made, and the marker is adjusted to point towards the left shoulder, enabling the acquisition of a transverse or short-axis image of the heart. By angling the transducer, various short-axis views can be obtained, providing a comprehensive assessment of different levels and segments of the heart's anatomy. The short-axis view below **(Figure 3-9)** displays the papillary muscles and the mitral valve. This imaging technique

[8] Figures 3:8 – 3:10 and 3:11,3.12 - 3.14, 3.16 taken by the author with permission

allows for the evaluation of the LV dimensions, LV wall thickness and regional wall motion. The short-axis view is an essential component of the echocardiographic examination, as it aids in the identification of any abnormalities in the cardiac structures and function. It also provides valuable insights into the 2-D aspects of the heart, contributing significantly to the comprehensive evaluation of the heart health in this study.

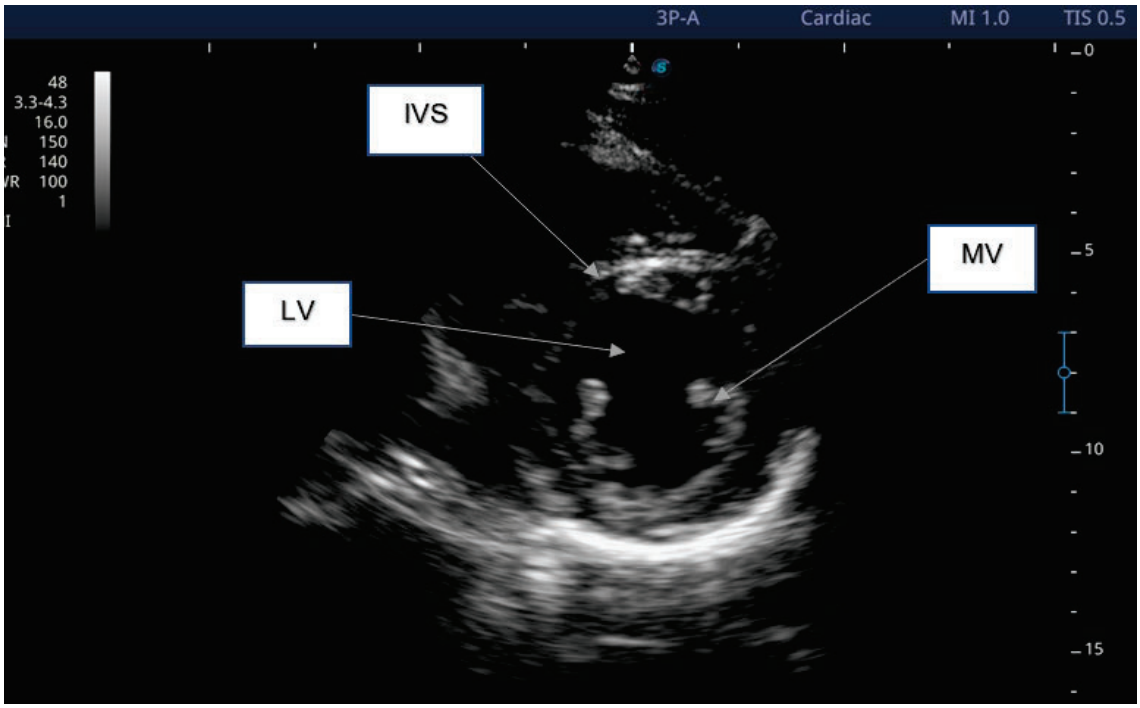

Figure 3-9 Parasternal short-axis view demonstrating LV, MV, IVS

3.5.2.4. **Apical windows**

In the apical windows, the transducer is positioned at the apex of the heart, with the marker directed downwards towards the left shoulder. By adjusting the transducer in this manner, a 4-chamber view is obtained, which displays the left ventricle, right ventricle, left atrium and right atrium. Furthermore, by angling the transducer more anteriorly towards the chest wall while maintaining the same position, a 5-chamber view can be obtained, revealing the aortic valve and the ascending aorta. This view holds significant clinical importance, as it allows for the exclusion of aortic valve stenosis and aortic valve regurgitation (**Figure 3-10**). Additionally, an apical long-axis 2-chamber view of the heart can be obtained, providing insights into various segments of the LV. The apical views offer a comprehensive assessment of all LV segments, enabling the evaluation of the apex for thrombus and aneurysms. Moreover, these views present an opportunity to assess the aortic valve, mitral valve, and tricuspid valve (Oh et al., 2006:13). Incorporating these apical views into the echocardiographic examination allows for a detailed assessment of the cardiac morphology and function.

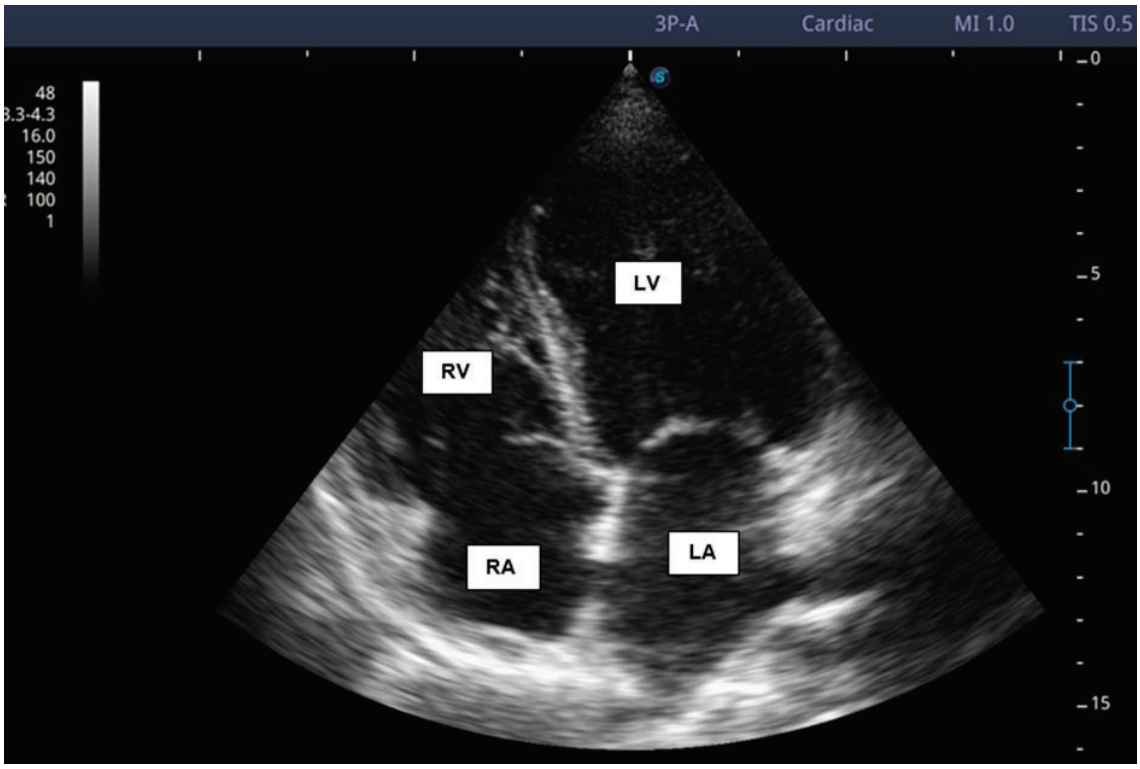

Figure 3-10 Apical 4-chamber view demonstrating the RV, RA, LV, and LA

For optimal assessment of the systolic function, the ventricle was divided into segments to evaluate regional LV function (**Figure 3-11)**. Each of these segments corresponds to specific coronary perfusion areas. For example, a segment showing hypokinetic thickening indicates reduced myocardial contraction, which could suggest ischemia in the corresponding coronary territory. Following the recommendations by Esmaeilzadeh et al. (2013), all segments were meticulously examined using multiple views. Each segment was then assigned a semiquantitative wall motion score, allowing for the calculation of the LV wall motion score index as the average of all segment values. To grade each wall region's motion, the following scoring system was applied: normal or hyperkinetic thickening, hypokinetic (reduced) thickening, akinetic (absence or minimal) thickening (e.g., scar), and dyskinetic systolic thinning or stretching (e.g., aneurysm). The scoring system used in this study is based on established criteria widely employed in echocardiography and cardiology practice, reflecting standard practices and protocols in the field. These criteria align with recommendations from Lang et al. (2015), which further validate the reliability and validity of the scoring system for assessing myocardial motion abnormalities. This comprehensive evaluation of regional LV function provides valuable insights into the heart's contractility and aids in detecting any abnormalities or areas of concern related to coronary perfusion and wall motion. The systematic assessment of these segments contributes significantly to a comprehensive understanding of cardiac health in the participants of this study.

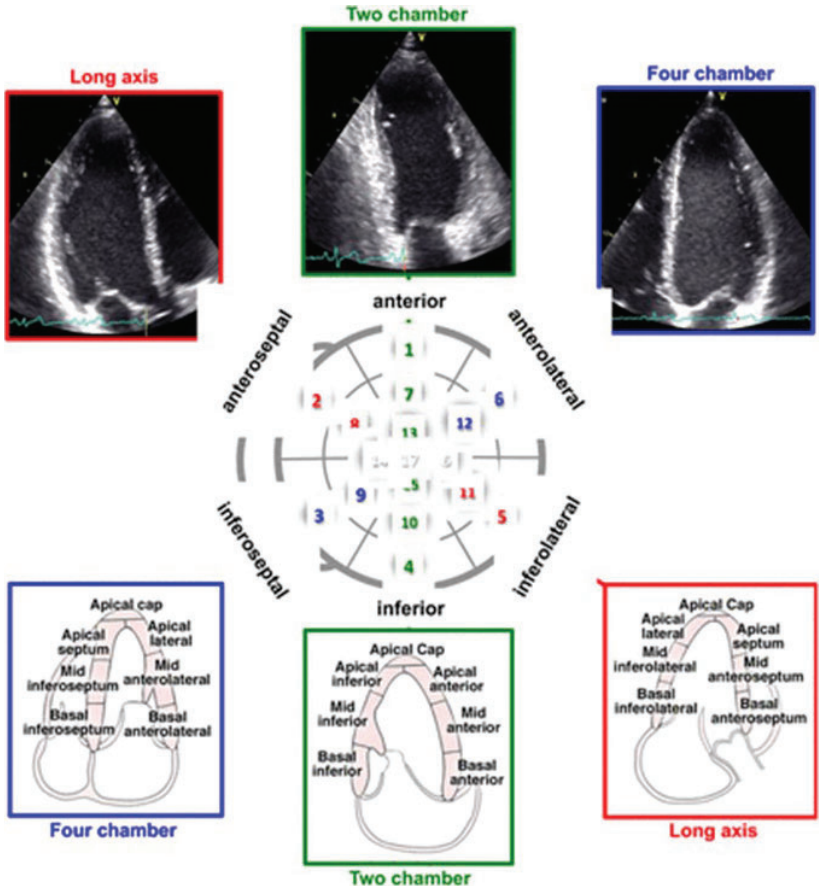

Figure 3-11 Orientation of apical four-chamber, apical two-chamber and apical long-axis views in relation to the bull's-eye display of the LV segments in the centre. The top panels provide the actual images and the bottom panels are a schematic depiction of the LV wall segments in each view. [9]

3.5.2.5. Motion mode imaging

The M-mode in echocardiography aims to provide a one-dimensional view of the heart, offering essential morphological and functional information about cardiac structures. Through M-mode, the echocardiographer can accurately measure the size of various cardiac structures, including the left ventricle, right ventricle, aorta, left atrium and right atrium. As stated by Oh et al. (2006:22), M-mode echocardiography complements 2D echocardiography by capturing movements of heart structures through a moving cursor placed over the area of interest.

In this study, to capture the movement of cardiac structures, a straight-line cursor was drawn from the transducer position, and two-dimensional guided M-mode tracings were used to measure cardiac dimensions. **Figure 3-12** illustrates an M-mode view used to measure the size of the RV, AO and LA. The diameter of the aortic root, LA and RV were obtained using a

[9] Taken with permission from Lang et al., 2015, Licence Number: 5535500366559

2D echocardiogram from the parasternal long-axis view, which displays the aortic root and the proximal ascending aorta. For LA size quantification, the LA diameter measurement approach was utilised, as suggested by Kizer et al. (2006), who deem it a viable alternative to LA volume measurement. Similarly, Lang et al. (2015) support this approach and recommend it for LA size quantification.

In accordance with the guidelines of the ASE and the European Association of Cardiovascular Imaging, normal left atrial anterior dimensions for females are considered to range from 2.7 to 3.8 cm, while in men the range is 3.0 to 4.0 cm (Lang et al., 2015). Any dimension exceeding these ranges is considered abnormal.

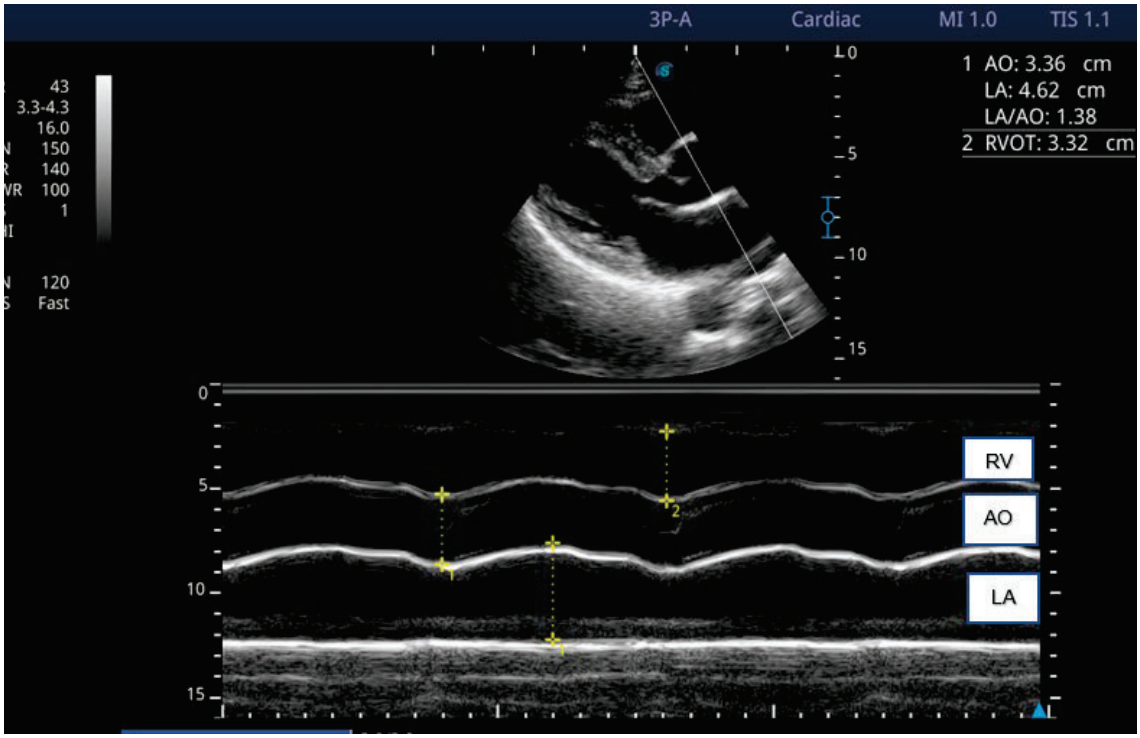

Figure 3-12 Parasternal long-axis m-mode demonstration of RV, AO and LA (Male) (Author image with permission)

In this thesis, the parasternal long-axis view, in combination with M-mode echocardiography, enables the measurement of important cardiac parameters, including the IVS, left ventricle and posterior wall. The interventricular septum is assessed to exclude LVH. The internal dimension and wall thickness of the left ventricle were measured at the end of diastole and systole, following the guidelines set by the American Society of Echocardiography. LVH and LVM are independent risk factors for cardiovascular disease and cardiovascular events (Bombelli et al., 2009). M-mode echocardiograms were obtained at the midpapillary level of the left ventricle, utilising both parasternal short-axis and long-axis two-dimensional perspectives. Within the parasternal long-axis view, linear internal measurements of the left

ventricle and its walls were taken. These measurements were obtained perpendicular to the LV long axis and at or just below the mitral valve leaflet tips (**Figure 3:12**). As previously indicated, LVH an abnormal increase in the mass of the myocardium of the left ventricle induced by a chronically elevated workload on the heart muscle (Bornstein et al., 2020; Simone et al., 2005). Hypertension causes LV overload, which leads to an increase in LV mass (Lee & Vasan, 2007). The accurate assessment of the LV mass is important to detect LVH (Perdix et al., 2011). Additionally, coronary artery disease has been shown to contribute to the development of LVH (Bornstein et al., 2020). The M-mode LV mass in grams was calculated using the following equations according to the American Society of Echocardiographers: LV mass g = 0.8[1.04(LVIDd + IVS + PW)³ – LVIDd³] + 0.6 g (Lang et al., 2015). The equation focuses on measurements of the left ventricular internal dimension at end-diastole (LVIDd), interventricular septal thickness (IVS), and posterior wall thickness (PW) to calculate LVM directly. This approach allows for the precise assessment of LV dimensions, RV and wall thickness, providing essential data for the comprehensive evaluation of the size and function of the mentioned structures.

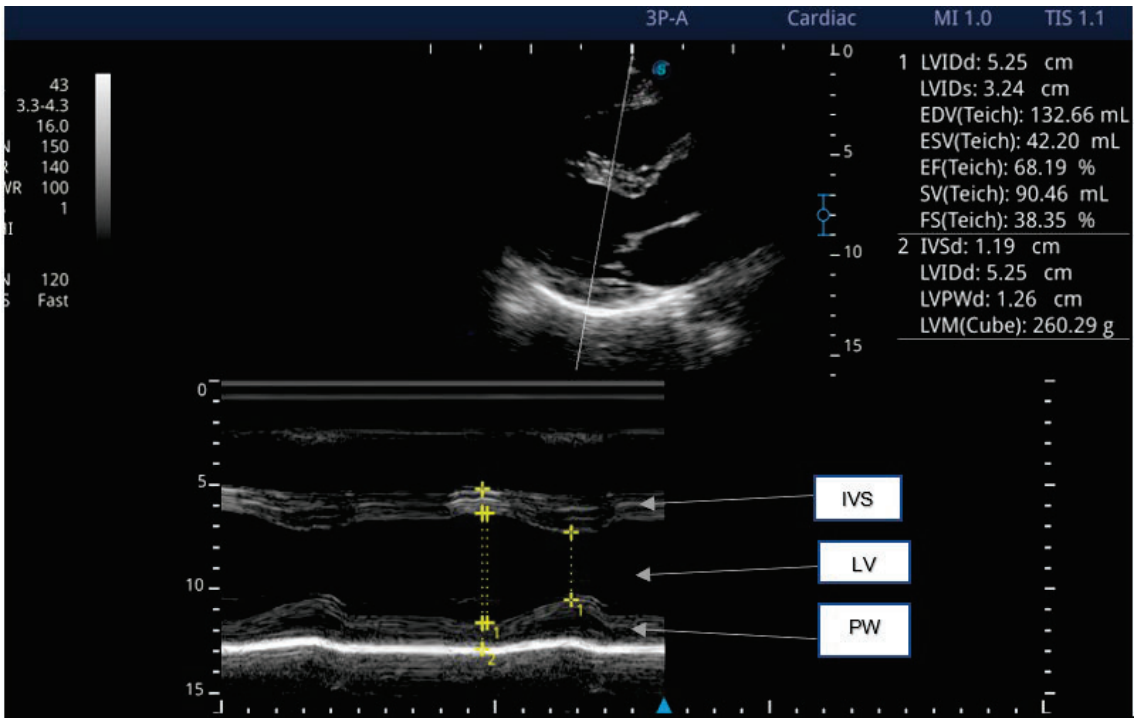

Figure 3-13 Parasternal long-axis M-mode demonstration of IVS, LV, LVED, LVES

M-mode size measurements were obtained using electronic callipers placed at specific junctions of the myocardial wall and cavities, as well as the wall and the pericardium. Internal dimensions were then measured using a 2D echocardiography-guided M-mode technique. The left ventricle ejection fraction (LVEF) is widely recognised as a representation of global LV function, and 2D echocardiography, together with M-mode, allows for qualitative

assessment of LV systolic function by observing the LV from various planes. LVEF was calculated from the computer-derived data collected by capturing and tracking the LV endocardial boundaries in systolic and diastolic frames. Oh et al. (2011) and Lang et al. (2015) note that skilled echocardiographers can visually evaluate LV systolic function, often referred to as an "eyeball" LV assessment. In this study, the ejection fraction (EF) was determined using the formula: EF=(EDV-ESV)/EDV, where EDV and ESV represent end-diastolic and end-systolic volumes, respectively. As suggested by Lang et al. (2015) and Lang et al. (2005), an LVEF of <52% for men and <54% for women indicates abnormal LV systolic function. New guidelines and recommendations for quantifying cardiac chambers have been set forth by the American Society of Echocardiography and the European Association of Cardiovascular Imaging, and these dimensions were adopted for the statistical analysis of the research data in this study seen in **Table 3-1**

Table 3-1 Left ventricle dimension normal values according to gender [10]

	Male				Female			
	Normal	Mildly abnormal	Moderately abnormal	Severely abnormal	Normal	Mildly abnormal	Moderately abnormal	Severely abnormal
LV Dimensions								
LV Diastolic diameter (cm)	4.2-5.8	5.9-6.3	6.4-6.8	>6.8	3.8-5.2	5.3-5.6	5.7-6.1	>6.1
LV Systolic diameter (cm)	2.5-4.0	4.1-4.3	4.4-4.5	>4.5	2.2-2.3	3.6-3.8	3.9-4.1	>4.1
LV Septal wall thickness	0.6-1.0	1.1-1.3	1.4-1.6	>1.6	0.6-0.9	1.0-1.2	1.3-1.5	>1.5
Posterior wall thickness	0.6-1.0	1.1-1.3	1.4-1.6	>1.6	0.6-0.9	1.0-1.2	1.3-1.5	>1.5
LV Mass (grams)	96-200	201-227	228-254	>254	66-150	151-171	172-193	>193
LV EF%	52-72	41-51	30-40	<30	54-74	41-53	30-40	<30

3.5.2.6. **Doppler Echocardiography**

Doppler echocardiography assesses blood flow, direction and velocity, aiding in the detection of regurgitation and its severity (Lancellotti et al., 2010 a & b). Colour-flow imaging, a practical method, is often utilised for determining the severity of mitral regurgitation (MR) (Chaliki et al., 1998). In the context of this research study, Colour Doppler was employed to exclude valvular regurgitation and provide an indication of the regurgitation severity (**Figure 3-14**). Additionally, it was used to exclude ventricular septal defects as suggested by Hoffmann et al. (2004), who stated that Colour Doppler echocardiography has been employed for evaluating cardiac blood flow in experimental environments. Blood flow velocities across all the valves were also assessed within the heart (aorta, mitral, pulmonary, and tricuspid) to identify valvular dysfunction and detect abnormal blood flow.

Echocardiography stands as the gold standard for diagnosing mitral valve regurgitation and plays a crucial role in determining its aetiology, mechanism, severity, progression, and consequences, while also aiding in the evaluation after valvular repair (Van de Heyning et al., 2012). To assess colour-flow Doppler in valvular regurgitation, the ASE recommends the use of a minimum of two imaging planes, and multiple views should be employed to assess the severity of valvular stenosis to achieve optimal flow velocity through the stenotic valve (Picard et al., 2010; Esmaeilzadeh et al., 2013). The researcher conducted the assessment of the valves using Colour Doppler, following the guidelines and recommendations established by the ASE. For primary valvular regurgitation evaluation, the European Association of Echocardiography (EAE) recommends TTE as the first-line approach, emphasising meticulous valve analysis and assessment (Lancellotti et al., 2010 a % b).

[10] Adapted from Lang et al., 2015

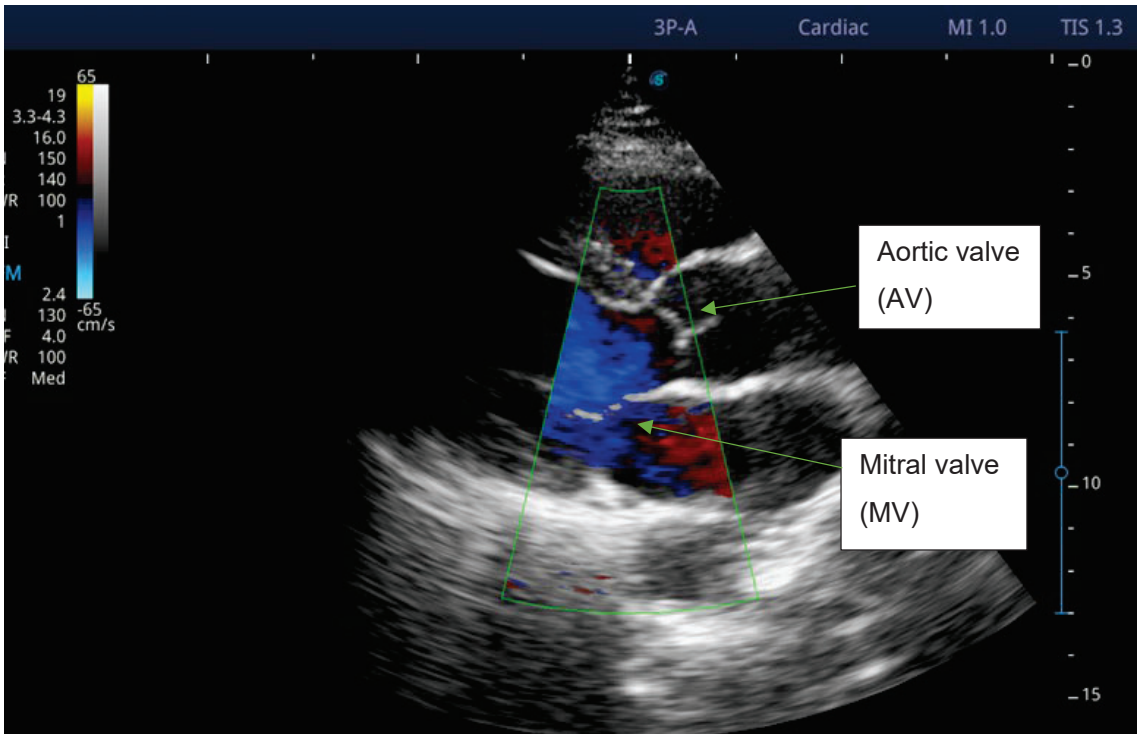

Figure 3-14 Colour Doppler flow velocity of the MV and AV

Spectral doppler was used to assess valvular stenosis by measuring velocities and estimating pressure gradients. The application of CW Doppler and PW Doppler allowed for the localisation of flow disturbances and the measurement of blood velocities from specific regions. Both CW and PW Doppler techniques provide a graphic representation of velocity over time, which is commonly referred to as spectral Doppler. Furthermore, spectral Doppler analysis can be used to diagnose diastolic dysfunction by interrogating the mitral valve inflow Doppler pattern.

Diastolic dysfunction refers to an anomaly in the heart's filling process during the diastolic phase, characterised by relaxation and chamber filling. Among the crucial parameters used to assess diastolic function is the E/A ratio, representing the ratio of early (E wave) to late (A wave) diastolic filling velocities of the mitral valve ((Mottram and Marwick, 2005). The E wave corresponds to passive early ventricular filling, primarily influenced by the pressure gradient between the left atrium and left ventricle during early diastole, while the A wave denotes atrial contraction during late diastole, contributing to the final ventricular filling before the next contraction. Doppler echocardiography, particularly spectral Doppler, is employed to calculate the E/A ratio by measuring the blood flow velocities across the mitral valve (Nagueh et al., 2016). A normal E/A ratio is typically greater than 1, indicating faster early filling than late filling. Deviations from the normal E/A ratio can signify impaired diastolic function and are linked to various cardiac conditions (Oh et al., 2011; Harris & Kuppurao, 2016). As a result,

the E/A ratio obtained through spectral Doppler assessment serves as a valuable diagnostic tool for evaluating diastolic function in clinical practice, as well as in this research setting. Little and Oh (2006) agree with the above statement and maintain that the diagnosis of diastolic dysfunction can be made by analysing the mitral valve inflow pattern through measuring the early (E wave) and late (A wave) diastolic filling velocities and calculating the E/A ratio. Abnormal E/A ratios may indicate impaired diastolic function **(Figure 3-15).**

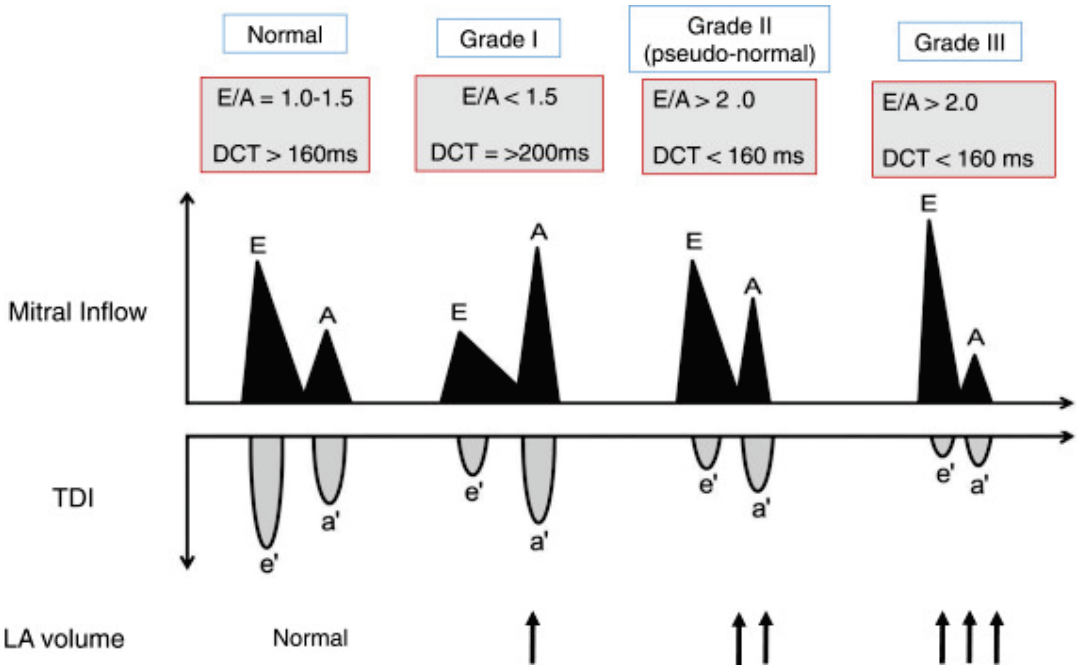

Figure 3-15 Diastolic dysfunction grading as estimated by spectral Doppler (taken with permission from Gorgis & Desai, 2023 with licence number 5601620150589)

Primary diastolic dysfunction is a prevalent condition observed in patients with hypertension, hypertrophic heart disease or restrictive cardiomyopathy **(Figure 3-15),** as well as various other clinical disorders (Marantz & Tobi, 1994; Fagerberg, 1998; Mandinov et al., 2000). Moreover, it exhibits a notably high prevalence in the elderly population. According to Zabalgoitia et al. (1997) and Zabalgoitia et al. (1998), the elderly population is particularly vulnerable to various factors that contribute to impaired diastolic function, including tachycardia, hypertension and ischemia. The researchers note that the aging process is linked to 'physiologic' diastolic dysfunction, characterised by an increase in LV muscle mass and alterations in the passive elastic properties of the myocardium.

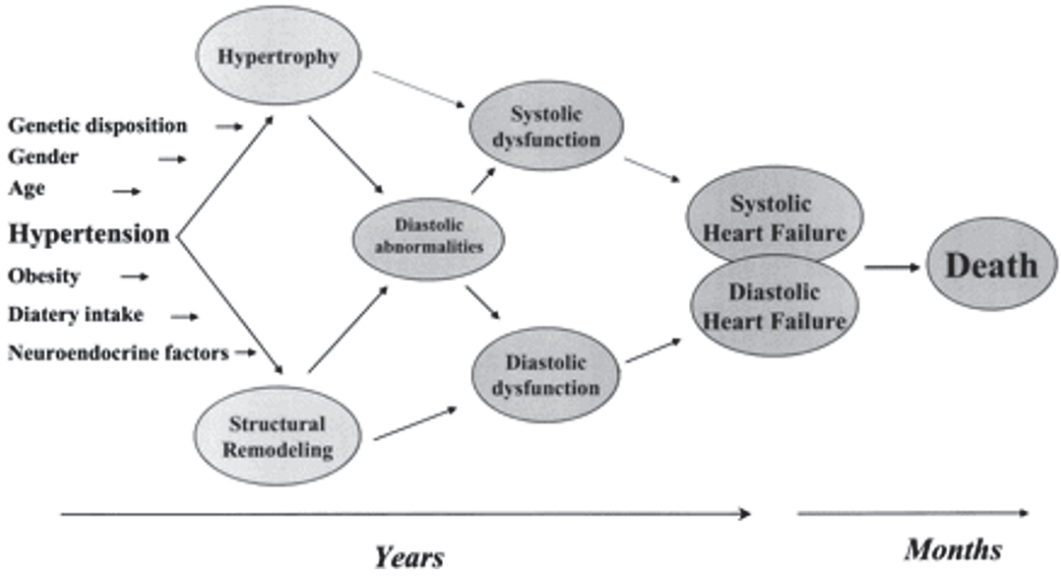

Figure 3-16 The progression from hypertrophy to diastolic heart failure (taken with permission from Mandinov et al. (2000) with licence number 5601590779031)

Spectral Doppler in echocardiography plays a crucial role in assessing blood flow patterns, identifying valvular abnormalities and diagnosing diastolic dysfunction, thereby providing valuable insights into cardiac function and contributing to comprehensive cardiovascular evaluation to prevent cardiovascular disease. See below **Figure 3-17** the mitral valve inflow pattern.

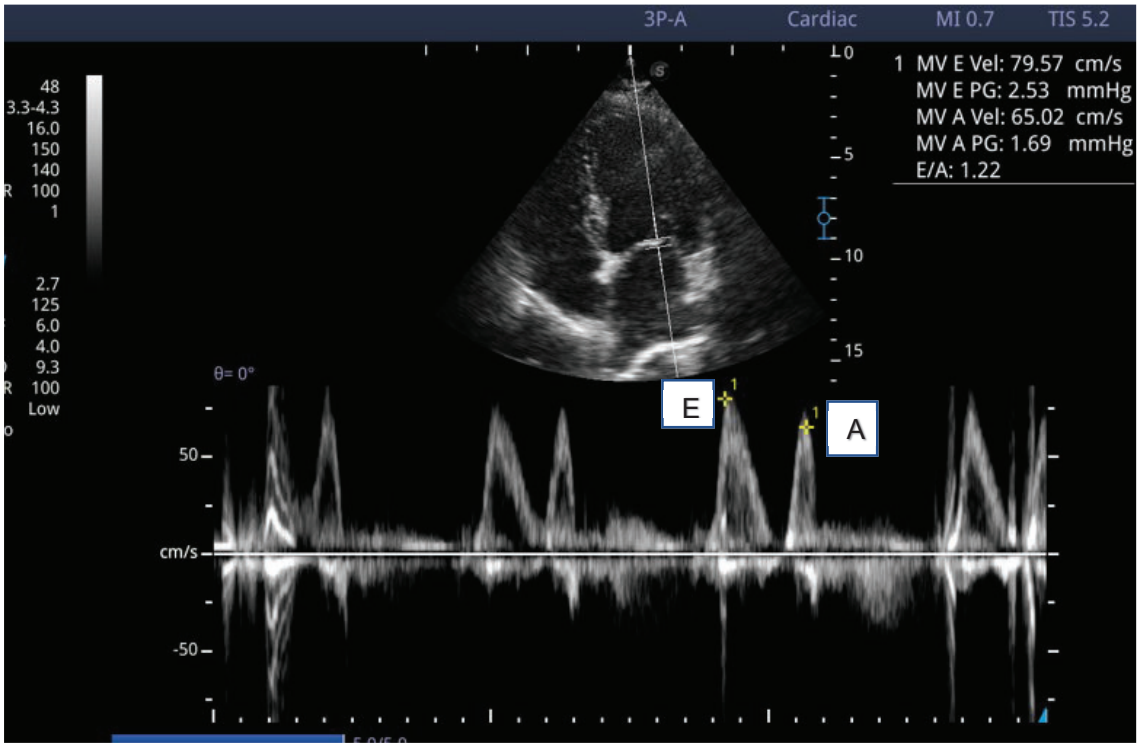

Figure 3-17 MV pulsed wave Doppler inflow pattern demonstrating the normal diastolic function

Additionally, spectral Doppler is instrumental in diagnosing cardiac tamponade, a condition characterised by fluid accumulation in the pericardial sac, which compresses the heart. By detecting changes in blood flow patterns and velocities associated with this condition, spectral Doppler aids in prompt diagnosis and management (Lang et al., 2015).

3.6. Statistical analysis

The sample size for this study was determined by a qualified, experienced statistician to eliminate a bias factor using Fisher's formula. To determine Objective 1, Descriptive statistics were calculated using Python. This included measures such as means, medians, standard deviations, and ranges to summarise the data. To determine Objective 2 and Objective 3, Generalized Estimating Equations (GEE) were used for the analysis. This technique, implemented using IBM SPSS, is particularly suited for handling repeated measurements where the dependent variable may not follow a normal distribution. GEEs account for the correlation between repeated measurements on the same subject, providing more accurate parameter estimates.

The rationale for choosing these statistical methods is as follows:

I. Descriptive Statistics: Using Python, we calculated basic descriptive statistics to provide an overview of the data distribution and identify any initial patterns or anomalies.

II. Generalised Estimating Equations (GEE): Software IBM SPSS was used for GEE due to its advanced capabilities in handling complex, non-normal data structures.

To answer the first objective, risk categories were created. The risk prediction model was designed to determine the risk factors for cardiovascular disease in this group of participants. In modern healthcare, particularly within the cardiovascular field, there exists a growing reliance on prediction models to supplement clinical judgment and decision-making (Mills et al., 2020). The integration of these models should serve as a compass for physicians' decision-making processes and individuals' behaviour, ultimately leading to enhanced individual outcomes and the cost-effectiveness of healthcare, as emphasised by Moons et al. (2012). O'Mahony et al. (2014) stated that a prediction risk model holds promise for enhancing patient management with the aim to complement, not replace, physicians' clinical judgment by providing objective, individualised prognostic information. Despite the existence of several risk prediction models for sudden cardiac death (SCD) in hypertrophic cardiomyopathy (HCM), Mills et al. (2020) aimed to derive and validate a new model. This model is designed to provide individualised risk estimates for SCD and enhance therapeutic strategies for patients with HCM. While established disease risk algorithms, such as the Framingham risk scores, are available, they may not always be fully applicable to the unique characteristics of our study population. Therefore, there is a need to design a unique risk prediction model tailored to our study population.

3.6.1. **Developing the risk categories**

The risk categories for this study were created using the screening data from our study population to ensure that they accurately reflect the specific demographics, health status, and risk factors present within this group. This tailored approach allows for a more precise assessment and categorisation of risk, which is crucial for the validity and relevance of our findings.

The tables below demonstrate the detailed procedure followed to create the risk categories using the screening data of the study population. The risk categories were created using the data of all the participants (n=219), and the example illustrated in the tables below is a demonstration of only the first 10 participants. Four steps were followed before creating the risk categories. The procedure employed to create each risk category, using the introduction of the sequential steps, is explained here below. This procedure is similar to using the interquartile range approach to look at extremely low and extremely high values (outliers).

Step one

The data for each variable were divided into quartiles (**Table 3-2.)** Quartiles were used to categorise the risk levels because they provide a simple and effective way to stratify

participants based on their relative position within the dataset**.** If the data observation fell below the lower quartile, the label 'Below Q1' was assigned, and if the data observation fell above the upper quartile, the label 'Above Q3' was assigned. A value below the lower quartile implies that the value is too low, and a value above the upper quartile implies that the value is too high. For instance, the lower quartile, which is Q1 (the 25th percentile), is the point when values range from 0% to 25%. The median, which refers to the middle of the dataset, is the second quartile, Q2 (the 50th percentile). The upper quartile, Q3 (the 75th percentile) is the point where values range from 75% - 100%.

Table 3-2 Developing the quartiles

No	BMI Class	WC Class	Glucose Class	HB class	Cholesterol Class	BP (Systolic) class	BP (Diastolic) Class
1	Below Q1	Below Q1	Below Q1	Above Q3	Above Q3	Between Q1 & Q2	Between Q2 & Q3
2	Below Q1	Below Q1	Between Q2 & Q3	Above Q3	Between Q2 & Q3	Below Q1	Below Q1
3	Between Q2 & Q3	Above Q3	Between Q2 & Q3	Between Q1 & Q2	Above Q3	Between Q2 & Q3	Between Q1 & Q2
4	Below Q1	Below Q1	Between Q1 & Q2	Below Q1	Between Q2 & Q3	Between Q1 & Q2	Between Q1 & Q2
5	Between Q2 & Q3	Between Q1 & Q2	Below Q1	Between Q1 & Q2	Above Q3	Below Q1	Between Q2 & Q3
6	Above Q3	Above Q3	Between Q1 & Q2	Between Q1 & Q2	Above Q3	Between Q2 & Q3	Above Q3
7	Above Q3	Above Q3	Above Q3	Between Q2 & Q3	Above Q3	Between Q2 & Q3	Between Q1 & Q2
8	Between Q1 & Q2	Between Q1 & Q2	Below Q1	Between Q2 & Q3	Above Q3	Between Q1 & Q2	Between Q2 & Q3
9	Between Q2 & Q3	Between Q1 & Q2	Below Q1	Between Q2 & Q3	Above Q3	Above Q3	Above Q3
10	Between Q2 & Q3	Between Q2 & Q3	Below Q1	Between Q1 & Q2	Below Q1	Between Q2 & Q3	Between Q1 & Q2

Q1=Lower quartile or 25th percentile, Q2=Middle / median quartile or 50th percentile, Q3= Upper quartile or 75th percentile.

Step two (See **Table 3-3**)

In this step, the risk categories were inserted. This step differs with each variable. With a glance at BMI, a value 'Below Q1' was further given the label 'Lower Risk' and a value 'Between Q1 & Q2' was given the label 'Medium Risk'. A value 'Between Q2 & Q3' was given the label 'Higher Risk' and a value 'Above Q3' was given the label 'Very High Risk'. With regard to glucose, a value 'Below Q1' was assigned the label 'Lower Risk', and a value 'Above Q3' the label 'Very High Risk'.

Table 3-3 Insertion of the risk categories

No	BMI Risk	Waist Circumference Risk	Glucose Risk	HB Risk	Cholesterol Risk	BP (Systolic) Risk	BP (Diastolic) Risk
1	Lower Risk	Lower Risk	Lower Risk	Very High Risk	Very High Risk	Lower Risk	Medium Risk
2	Lower Risk	Lower Risk	Higher Risk	Very High Risk	Higher Risk	Higher Risk	Higher Risk
3	Higher Risk	Very High Risk	Higher Risk	Medium Risk	Very High Risk	Medium Risk	Lower Risk
4	Lower Risk	Lower Risk	Medium Risk	Lower Risk	High Risk	Lower Risk	Lower Risk
5	Higher Risk	Medium Risk	Lower Risk	Medium Risk	Very High Risk	Higher Risk	Medium Risk
6	Very High Risk	Very High Risk	Medium Risk	Medium Risk	Very High Risk	Medium Risk	Very High Risk
7	Very High Risk	Very High Risk	Very High Risk	Higher Risk	Very High Risk	Medium Risk	Lower Risk
8	Medium Risk	Medium Risk	Lower Risk	Higher Risk	Very High Risk	Lower Risk	Medium Risk
9	Higher Risk	Medium Risk	Lower Risk	Higher Risk	Very High Risk	Very High Risk	Very High Risk
10	Higher Risk	Higher Risk	Lower Risk	Medium Risk	Lower Risk	Medium Risk	Lower Risk

Step three (See **Table 3-4.**)

In this step, codes were given (0 or 1) depending on whether the variable was identified as 'Lower Risk', 'Medium Risk', 'Higher Risk' or 'Very High Risk'. Binary codes (0 for Lower/Medium Risk and 1 for Higher/Very High Risk) were chosen for simplicity in logistic regression analysis

Table 3-4 Development of the risk category codes

No	BMI grade	Waist Circumference grade	Glucose grade	HB grade	Cholesterol grade	BP (Systolic) grade	BP (Diastolic) grade
1	0	0	1	1	1	1	0
2	0	0	1	1	0	0	1
3	1	1	1	0	0	0	1
4	0	0	0	0	0	0	1
5	1	0	1	0	1	1	1
6	1	1	0	0	0	1	1
7	1	1	1	1	0	0	0
8	0	0	1	1	1	0	0
9	1	0	1	1	1	1	1
10	1	1	0	0	1	1	1

Step four (See Section 4 of **Table 3-5**)

In this final step, the codes were summed, the percentages were calculated, and a decision was made on whether a participant was at risk based on the percentage risk. Any participant with a risk value below 50% was seen as 'Lower Risk', and anyone with a risk value above 50% was seen as 'Higher Risk' for developing cardiovascular disease. In this section, the risk categories for each participant were calculated. A sum of four suggests that, out of the seven risk categories, a participant is at risk in four of them. For example, the sum of risk factors for Participant 2 is 3. This participant is at risk for three of the seven risk factors: BMI, waist circumference and glucose.

Table 3-5 Development of the risk categories

No	Sum	Total	Sum/Total	% Risk	Binary Risk	Risk Status	
1	4	7	0.5714	57.1%	1	At Risk	
2	3	7	0.4286	42.9%	0	No Risk	
3	4	7	0.5714	57.1%	1	At Risk	
4	1	7	0.1429	14.3%	0	No Risk	
5	5	7	0.7142857	71.4%	1	At Risk	
6	4	7	0.5714285	57.1%	1	At Risk	
7	4	7	0.5714285	57.1%	1	At Risk	
8	3	7	0.4285714	42.9%	0	No Risk	
9	6	7	0.8571428	85.7%	1	At Risk	
10	5	7	0.7142857	71.4%	1	At Risk	

To further test the accuracy of the above 4-step model, the Logistic Regression Classifier (see below) was fitted to predict the risk categories in this data set and the probability of participants being at risk. The classification report (**Table 4.3**) indicates that the model used was 73 % accurate. This suggests that, of 100 participants, the model has correctly predicted the class ('higher risk' or 'lower risk').

In addition to the Logistic Regression Classifier, a statistical assessment calculation was performed to demonstrate the accuracy, precision, sensitivity and specificity percentages of the model that we used. According to the calculation (**4.1.1.3),** the risk algorithm model we designed and used demonstrated an accuracy of 72.8%, a precision of 77.8%, a sensitivity of 66.7%, and a specificity of 79.5%. While the acceptability of these metrics can vary depending on the context and application, they are within a range that is considered reasonable for many predictive models in clinical research. In our case, the balance between sensitivity and specificity indicates that our model is effective at identifying true positives while minimising false positives. Given the characteristics and risk factors of our study population, these performance metrics suggest that the model provides a reliable tool for risk assessment in this

specific context. This research is the first of its kind in this group of participants and used only the logistic regression classifier. Future research can build on this by exploring and employing different classification algorithms to improve the risk model.

To illustrate the acceptability of these performance metrics, it's helpful to compare them to benchmarks from similar predictive modelling studies, even if they are in different medical fields. For example, in a review by Alapat et al. (2022) on detecting pneumonia in chest X-ray images using neural networks, similar performance metrics were used to evaluate the effectiveness of neural networks. While our study focuses on cardiovascular risk assessment, the principles of model evaluation remain consistent. Therefore, the performance metrics achieved by our algorithm are within an acceptable range, demonstrating its effectiveness for the specific context of our research.

3.6.2. **The Generalized Linear Model**

The theoretical background to the Generalized Linear Model consists of three components: a random component, a systematic component and a link function (McCullagh & Nelder, 1989). The assumptions of the classic linear model, as outlined by these authors, are:

1) All components of Y, the dependent variable, are independent and normally distributed, and they all have a common variance (random component).
2) The covariates are combined to give the linear predictor (systematic component), $\eta_i = \alpha + \beta_1 X_{i1} + \beta_2 X_{i2} + \cdots + \beta_k X_{ik}$
3) The relationship between the random component and the systematic component is described using a link function which follows a specific probability distribution according to the characteristics of the dependent variable.

$$g(p_i) = \eta_i = \alpha + \beta_1 X_{i1} + \beta_2 X_{i2} + \cdots + \beta_k X_{ik}$$

The generalised linear model relaxes the first assumption to allow for dependent values that may be from one of the exponential family of distributions and to allow for variances that are not common. Link functions are chosen according to the data type and the context of the data. In the case of repeated (dependent) measurements, the Generalized Linear Model can be extended to the Generalized Estimating Equations application (Zeger & Liang, 1986:126). This application allows for the dependent variable to be numerical or categorical. In this study, the dependent variable is a binary variable, thus a logit link function $g(p) = \ln\frac{p}{1-p}$ is selected, where p, for example, is the probability (p) of a specific profile making a specific selection (Simonoff, 2003:366-367). If the p-value was less than 0.05, it would be indicative of statistical significance.

3.6.2.1. Logistic Regression Classifier - Predicting the risk status

This model was designed to predict the risk categories in this data set. After having created risk factors, the final decision was to consider whether an individual is at 'Higher Risk' or 'Lower Risk', which can also be given levels '1' (for 'higher risk') and '0' (for 'lower risk'). Hence the binary column with 0 and 1 was created. The logistic model used the seven risk factors as regressors and the binary column as the target variable.

To predict the probability of being at risk, an individual is considered to be at higher risk if his percentage risk is above 50%. The presence or absence of higher risk can be viewed as a Bernoulli trial, in which the success trial is the presence of higher risk (index 1) and the failure trial is the absence of higher risk (index 0). A participant is either classified as being at higher risk or lower risk; therefore, the risk index can serve as binary data. Considering Y_i as a Bernoulli random variable,

$$Y_i = \begin{cases} 1, & if\ higher\ risk (with\ probability\ \pi_i) \\ 0, & if\ lower\ risk\ (with\ probability\ 1-\pi_i) \end{cases}$$

In the logistic regression, we are interested in modelling how the probabilities of success (high risk) and failure (low risk) depend on a vector of regressors. According to Musa (2014), the binary response random variable is denoted as in **equation 3.6.1.1.1.**:

Equation 3.6.1.1.1.

$$\pi(X) = \frac{\exp(\beta_o + \beta_1 X_1 + \cdots + \beta_n X_n)}{1 + \exp\ (\beta_o + \beta_1 X_1 + \cdots + \beta_n X_n)}$$

$$= \frac{\exp(X\beta)}{1 + \exp(-X\beta)}$$

$$= \frac{1}{1 + \exp(-X\beta)}$$

where π is the probability that an observation is in a specified category of the binary variable. Hence, the odds ratio of favourable so that unfavourable cases can be calculated as shown in **equation 3.6.1.1.2.**

Equation 3.6.1.1.2.

$$\frac{\pi_i}{1-\pi_i} = \exp(X_i\beta)$$

The section above demonstrates a detailed account of how the risk prediction model for cardiovascular disease was developed. We described the creation of the risk categories based on various risk factors. These categories were crucial for accurately stratifying participants' risk for cardiovascular disease. The results from the above analyses guided the identification of significant predictors and their incorporation into the final model.

3.7. Summary of the Chapter

This chapter presents a comprehensive and detailed account of the research design and techniques employed to ensure objectivity and accuracy of data collection in the study. It

encompasses an overview of the research methods, ethical concerns, sampling procedures and data collection strategies. Special emphasis is placed on the echocardiography protocol, a critical element of the research tool, which is thoroughly discussed to assist the reader in comprehending the data-gathering process. This section explains how the risk categories were created. By meticulously designing and validating this risk prediction model, we aim to provide a robust tool for early identification and prevention of cardiovascular disease, thereby enhancing clinical decision-making and patient outcomes.

This section aims to describe the association between variables by conducting descriptive analysis to organise the raw data from the sample in a structured format. Descriptive statistics, as emphasised by Kaur et al. (2018), are an essential aspect of preliminary data analysis, laying the groundwork for comparing variables using inferential statistical tests. Therefore, it is critical to report the most relevant descriptive statistics systematically as part of good research practice to minimise the chance of providing misleading data. The findings presented in the next chapter correspond to each research objective, providing crucial statistical details on how each objective was measured.

Chapter 4 | Results

The anthropometric characteristics of the study population presented below in **Table 4-1** was collected by qualified and trained nursing staff. While not all this data formed part of this research study, it was included in this document to provide background information on the study population.

Table 4-1 Anthropometric characteristics of study participants (n=219)

Characteristics of the group	Placebo group n = 78	Fermented Rooibos group n = 68	Green Rooibos group n = 73
Age (years)	45.54 ± 10.98	45.9 ± 10.09	47.03 ± 10.77
Gender	Male: 30.8%; Female: 69.2%	Male: 22.1%; Female: 77.9%	Male: 23.3%; Female: 76.7%
Body weight (kg)	82.18 ± 19.84	77.29 ± 16.29	82.2 ± 21.82
Stature (m)	1.64 ± 0.09	1.63 ± 0.09	1.63 ± 0.08
BMI (kg/m^2)	30.72 ± 7.53	29.35 ± 6.69	31.04 ± 7.59
Resting Systolic Blood Pressure (mm Hg)	132.43 ± 17.44	133.42 ± 19.81	131.41 ± 18.54
Resting Diastolic Blood Pressure (mm Hg)	85.43 ± 11.39	84.58 ± 11.89	82.4 ± 11.04
Haemoglobin (g/dl)	14.31 ± 2.41	13.55 ± 2.44	14.22 ± 2.32
Glucose	4.28 ± 1.87	4.81 ± 1.93	4.47 ± 1.25
Cholesterol (n = 61; 58; 67)	5.39 ± 0.96	5.39 ± 0.91	5.5 ± 1.04
Smoking	No: 57.1%; Yes: 31.2%; Previous: 11.7%	No: 58.8% Yes: 29.4%; Previous: 11.8%	No: 54.8%; Yes: 24.7%; Previous: 20.5%
Family history of CVD	No: 35.9%; Yes: 59.0%; Unsure: 5.1%	No: 39.7%; Yes: 57.4%; Unsure: 2.9%	No: 42.5%; Yes: 52.1%; Unsure: 5.5%
Physical activity	Yes: 70.5%; No: 29.5%	Yes: 67.6%; No: 32.4%	Yes: 78.1%; No: 21.9%

Abbreviations: n = total number, kg = kilogramme, m = meter, kg/m^2 = kilogram per square metre, mmHg = millimetres of mercury, g/dl = grams per decilitre

4.1. Descriptive characteristics of the study population

This paragraph will discuss the characteristics of the study population as outlined in the table below **(Table 4-2)**. This table contains descriptive data of the study subjects using their screening data, which represent each variable's minimum value, maximum value, mean, mode, skewness kurtosis and quartiles. As mentioned before, 721 adults were screened, of

whom 413 satisfied the inclusion and exclusion criteria and were invited to a study information session. The information session was attended by 333 participants, of whom 257 provided written informed consent and completed the baseline and intervention phases of the study. Of the 257, 38 participants' data had to be removed from the study data as a result of non-compliance, leaving 219 study participants who successfully completed both baseline and intervention phases of the study. Participants consisted of males and females with two or more modifiable CVD risk factors as per the study inclusion criteria mentioned in Chapter 3 of this document.

According to the descriptive statistics table (**Table 4-2)**, which was generated to determine the screening characteristics of this study, the data indicate that the youngest participant in this study was 28 years old and the oldest was 79. The mean age of the participants was 46.7 years, the median (Q2) was 46 years, and the model age was 42 years. The data also indicate that the minimum weight was 43 kg, the maximum weight was 178 kg and the mean weight was 81 kg. The minimum waist circumference was 62 cm, the maximum was 149 cm and the mean was 98 cm. Regarding the BMI, data suggest that the minimum BMI was 16 kg/m², the maximum BMI was 59 kg/m² and the mean was 31 kg/m². The lowest blood glucose concentration was 1.7 mmol/L, the maximum was 16.9 mmol/L and the mean was 4.5 mmol/L. Concerning haemoglobin levels, the minimum level was 7.2 grams per decilitre (g/dl), the maximum 21g/dl and the mean 14 (g/dl). The minimum systolic blood pressure was 94 mmHg, the maximum systolic blood pressure 183 mmHg and the mean systolic blood pressure was 131 mmHg. The minimum diastolic blood pressure was 61 mmHg, the maximum diastolic blood pressure 115 mmHg and the mean diastolic blood pressure was 84 mmHg. The minimum cholesterol was 3 mmol/L, the maximum cholesterol 9 mmol/L and the mean cholesterol 5.5 mmol/L.

All variables are positively skewed distributed, meaning the mean, median and mode of the distribution are positive, except for haemoglobin, which is slightly skewed to the left suggesting that the mean is less than the median. Age, haemoglobin, and diastolic blood pressure variables have negative kurtosis, which implies that their tails would be modelled by a distribution lighter than a normal distribution. This means that the distribution of data points for these variables has tails that are lighter or less extreme than those of a normal distribution. Stated differently, the data for age, haemoglobin and diastolic blood pressure do not have as many extreme outliers or values that deviate significantly from the mean compared to a normal distribution. This, therefore, suggests that the data for these variables tend to cluster more closely around the mean and have fewer extreme values in the tails of the distribution, making them somewhat less skewed or more 'normal' in shape compared to a standard normal distribution.

The paragraph above reflects the statistics presented in the table below, offering a summary rather than a full discussion. A more comprehensive discussion of these statistics will be included in the Discussion chapter

Table 4-2 Descriptive characteristics of the study population using screening data

Categories	Min	Max	Mean	SD	Model	Q1	Q2	Q3	Skew	Kurt
Age	28.00	79.00	46.71	10.69	42.00	38.00	46.00	55.00	0.33	-0.70
Weight (kg)	43.30	177.50	80.67	19.57	54.00	67.10	78.80	91.55	0.87	2.21
Stature (m)	1.44	1.98	1.63	0.08	1.57	1.57	1.61	1.68	0.82	0.90
Waist circumference (cm)	62.00	149.00	97.70	15.23	87.00	87.00	97.00	107.30	0.30	0.14
BMI (kg/m²)	15.89	59.31	30.40	7.30	30.09	25.13	29.45	34.57	0.73	0.75
Glucose (mmol/L)	1.70	16.90	4.51	1.71	3.70	3.60	4.30	4.90	3.71	23.29
HB (g/dl)	7.20	21.10	14.04	2.40	14.70	12.45	14.00	15.55	-0.07	-0.13
Blood Pressure (S)	94.00	183.00	130.89	17.23	137.00	118.00	131.00	141.00	0.38	0.15
Blood Pressure (D)	61.00	115.00	83.90	10.65	83.00	76.00	83.00	91.00	0.24	-0.36
Cholesterol (mmol/L)	3.00	9.00	5.46	1.28	3.00	4.56	5.30	6.12	0.71	0.79

BMI=Body mass index, WC=Waist circumference, HB=Haemoglobin, BP=Blood pressure,
Q1 = lower quartile, Q2 = median quartile, Q3= upper quartile, SD= Standard Deviation, Kurt (kurtosis), Skew (skewness), AO=Aorta, LA=Left atrium, HB = haemoglobin, BMI = body mass index

4.1.1. OBJECTIVE 1: The Cardiovascular Disease risk categories of the study population

The classification report below indicates that our logistic classifier is 73% accurate. This model was designed to predict the risk categories in this data set as discussed in Chapter 3. This implies that 73 out of 100 participants it correctly predicts the class (higher risk or lower risk). The split of data was as follows: 60% of the data was used in model building and 40% of the data was used for prediction purposes.

Table 4-3 The Classification Report

	Precision	Recall	F1-score	Support
Lower risk	0.69	O.79	0.74	39
Higher risk	0.78	0.67	0.72	42
Accuracy			0.73	81
Macro average	0,73	0.73	0.73	81
Weighted average	O.73	0.73	0.73	81

Model Accuracy: 0.7283950617283951 (72.84%)

4.1.2. **Confusion Matrix**

More information on the accuracy of the prediction model was obtained with the confusion matrix below (**Figure 4-1**).

True Negatives	**False Positives**
False Negatives	**True Positives**

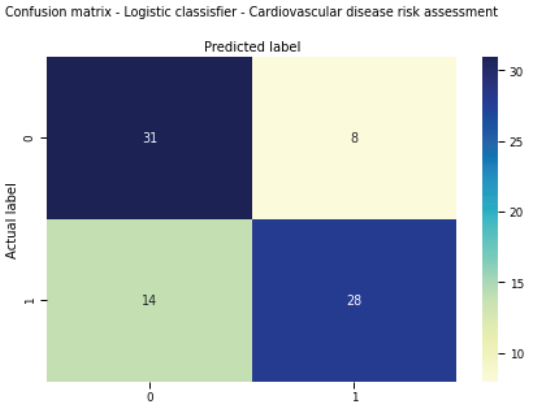

Figure 4-1 Confusion matrix for cardiovascular disease risk management

The binary classification confusion matrix shows the numbers of the following:

- The true negative predictions – 31 observations are predicted correctly as zeros (lower risk).
- The false negative predictions - 14 observations are wrongly predicted as zeros (lower risk).
- The false positive prediction – 8 observations are wrongly predicted as ones (higher risk).
- The true positive prediction - 28 observations are predicted correctly as ones (higher risk)

The statistical assessment (below) for accuracy and precision reflects the reliability of a statistical test, while specificity and sensitivity reflect the possibility of false negatives and false positives in a test. The calculation below **(equation 4.1.2.1.)** demonstrates the accuracy, precision, sensitivity and specificity percentage of the model used.

Equation 4.1.2.1.

$$\text{Accuracy} = \frac{TP + TN}{Total\ Numer\ of\ observations} = \frac{31+28}{81} = \frac{59}{81} = 72.8\%$$

$$\text{Precision} = \frac{TP}{TP + FP} = \frac{28}{28+8} = \frac{28}{36} = 77.8\%$$

$$\text{Recall / Sensitivity} = \frac{TP}{TP + FN} = \frac{28}{28+14} = \frac{28}{42} = 66.7\%$$

$$\text{Recall/Specificity} = \frac{TN}{TN + FP} = \frac{31}{31+8} = 79.5\%$$

According to the calculation, the model used suggests 72.8% accuracy, 77.8% precision, 66.7% sensitivity and 79.5% specificity.

4.1.3. The Receiver Operating Characteristic Curve

The Receiver Operating Characteristic Curve (ROC) was also used to measure visually the classification model performance. The ROC shows the performance of a classification model at various classification threshold levels by plotting the True Positives against the False Positives at different threshold levels. A glance at the curve shows that the ROC Area under the Curve (AUC) of the model is 0.8412 (84%), which is a value much closer to 1(100%). Therefore, the classifier performed well in predicting whether an individual is in the lower risk or higher risk category.

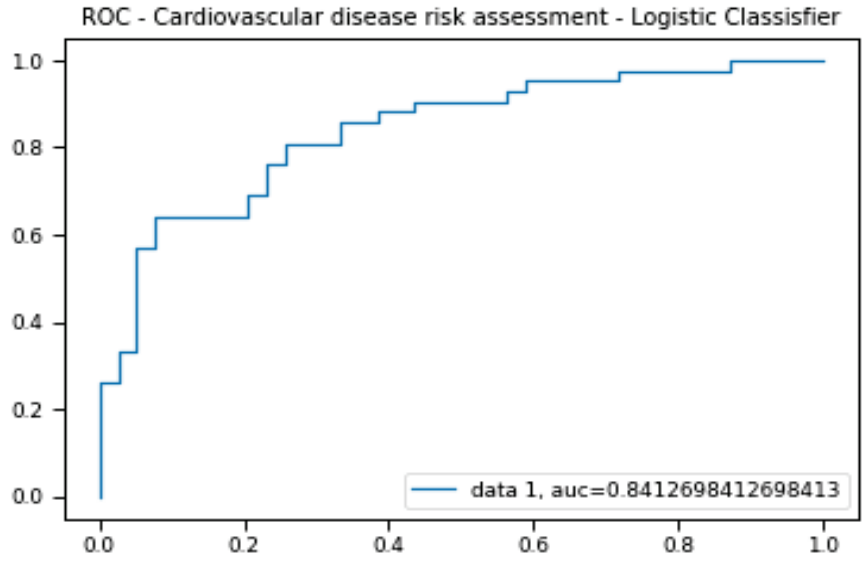

Figure 4-2 Receiver Operating Characteristic (ROC) Curve

4.2. Frequency of risk categories

Table 4-4 below represents the screening risk categories (Body Mass Index (BMI), Waist Circumference (WC), Glucose, Haemoglobin (HB), Cholesterol, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP)) and their risk frequencies.

According to the cardiovascular disease risk categories assessment model created (

Table 3-3), 219 participants formed part of the data set. With regard to the BMI, 56 (25.5%) were at lower risk, 54 (24.7%) at medium risk, 54 (24.6%) at higher risk, and 55 (25%) of the participants were at very high risk. The waist circumference demonstrated that 60 (27.4%) participants were in the lower risk frequency, 53 (24%) were at medium risk, 51 (23%) were at higher risk, and 55 (25%) of the participant were at very high risk. According to the blood glucose analysis, the model suggests 64 (29.5%) participants to be at lower risk, 53 (24.4%)

at medium risk, 49 (22.6%) at higher risk and 51 (23.5%) participants in the very high-risk frequencies. With regard to HB, 55 (25%) were at lower risk, 57 (26%) at medium risk, 52 (23.7%) at higher risk, and 55 (25%) were at very high risk. The cholesterol category demonstrates that 51 (25%) were at lower risk, 51 (25%) were at medium risk, 51 (25%) at higher risk, and 50 (25%) were at very high risk. With regard to systolic BP, 57 (26%) were at lower risk, 52 (24%) at medium risk, 56 (25.6%) at higher risk, and 54 (24.7%) of the participants were at very high risk. Regarding diastolic BP, 52 (23.7%) were at lower risk, 58 (26.5%) at medium risk, 58 (26.5%) at higher risk, and 51 (23.3%) participants were at very high risk.

Overall, these findings highlight the diverse risk profiles of the participants in this data.

Table 4-4 Categories and risk frequency counts and percentages (%) of the study population screening data

Categories and risk frequency counts							
Row Labels	BMI Risk	Waist Circumference Risk	Glucose Risk	HB Risk	Cholesterol Risk	BP (Systolic) Risk	BP (Diastolic) Risk
Lower Risk	56	60	64	55	51	57	52
Medium Risk	54	53	53	57	51	52	58
Higher Risk	54	51	49	52	51	56	58
Very High Risk	55	55	51	55	50	54	51
Grand Total	219	219	217	219	203	219	219
Categories and risk frequencies percentage							
Row Labels	BMI Risk	Waist Circumference Risk	Glucose Risk	HB Risk	Cholesterol Risk	BP (Systolic) Risk	BP (Diastolic) Risk
Lower Risk	25.6%	27.4%	29.5%	25.1%	25.1%	26.0%	23.7%
Medium Risk	24.7%	24.2%	24.4%	26.0%	25.1%	23.7%	26.5%
Higher Risk	24.7%	23.3%	22.6%	23.7%	25.1%	25.6%	26.5%
Very High Risk	25.1%	25.1%	23.5%	25.1%	24.6%	24.7%	23.3%
Grand Total	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%

BMI=Body mass index, HB=Haemoglobin, BP=Blood pressure, WC= Waist circumference

4.2.1. The risk category boxplots using the participant screening data

Below are the risk category boxplots of the variables that represent all the screening data according to each risk factor. The boxplots (**Figure 4-3 - Figure 4-8**) below display the distribution of data in an attempt to answer Objective one, which was to establish the prevalence and characteristics of cardiovascular disease and its associated risk factors, using the risk assessment model.

The boxplots indicate some outliers, meaning values which deviated significantly from the average value, as indicated in the graphs. The WC data (**Figure 4-3**) indicate that the medium

WC is below 100cm. The median WC for the lower-risk participants is the lowest at 80cm. Participants at very high risk had the highest median WC at 120cm. Visually, the boxplots form an increasing pattern, indicating that, when shifting from lower-risk to higher-risk participants, the median WC also increases.

The median BMI is slightly below 30 kg/m² (29.45), according to **Figure 4-3.** The maximum BMI measurement is close to 59.31 kg/m² as is indicated by the outlier and the minimum value is 15.89. The range, which is the maximum value minus the minimum value, equals 43.42 kg/m². This range appears to be high, which could suggest that some participants had very high BMI and some very low BMI measurements. Shifting from lower risk to very high risk, the medium for the categories also increases.

The haemoglobin (Hb) data (**Figure 4-5**) suggest the median Hb level in the lower risk group to be below 12 grams per decilitre (g/dl) and the median Hb level in the very high risk group was above 16 and below 18 grams per decilitre (g/dl). As mentioned before, there were levels outside the average levels.

The cholesterol data, according to the boxplot (**Figure 4-6**, indicate that the median blood cholesterol concentration in the lower-risk group is just above 4mmol/L, and the median concentration in the very high-risk group is just below 7mmol/L.

In the case of systolic BP, very high systolic BP indicates a participant who is at very high risk; similarly, very low systolic BP indicates participants who are at higher risk. If systolic blood pressure falls below the first quartile, it signifies a higher risk. Systolic blood pressure between the first and second quartiles suggests a lower risk, while systolic blood pressure between the second and third quartiles implies a medium risk. Finally, systolic blood pressure that exceeds the third quartile is classified as a very high risk. As shown in **Figure 4-7**, higher risk indicates participants with very low systolic blood pressure, where the medium is generally below 120 mmHg, with BP values below 100 mmHg, as indicated in the outlier observation in the higher-risk group in the same figure. Lower-risk and medium-risk groups have a median between 120 mmHg and 140 mmHg with absolutely no outliers.

With regard to the diastolic BP, (**Figure 4-8**), the median diastolic BP in the low risk group is below 80 mmHg while the median in the higher risk group is below 100 mmHg.

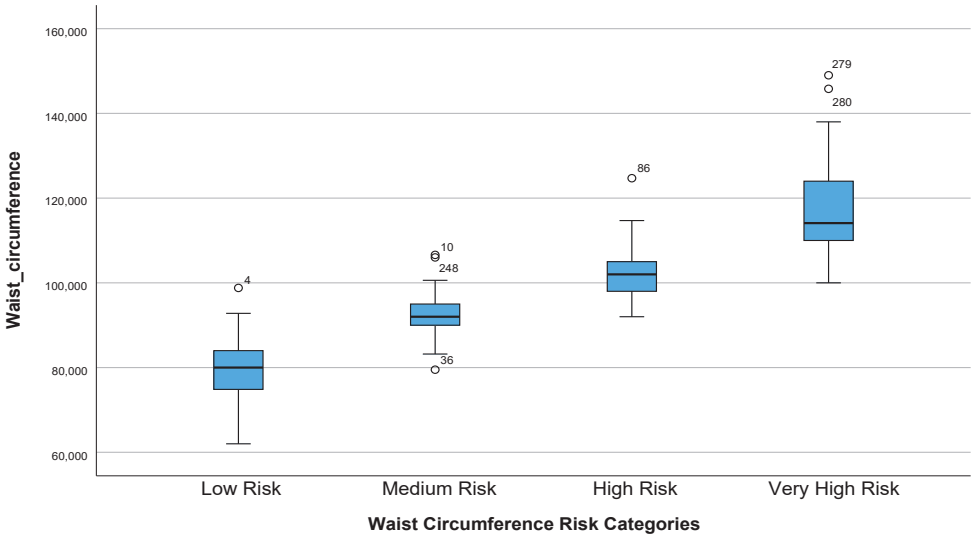

Figure 4-3 Screening Waist Circumference Risk Assessment

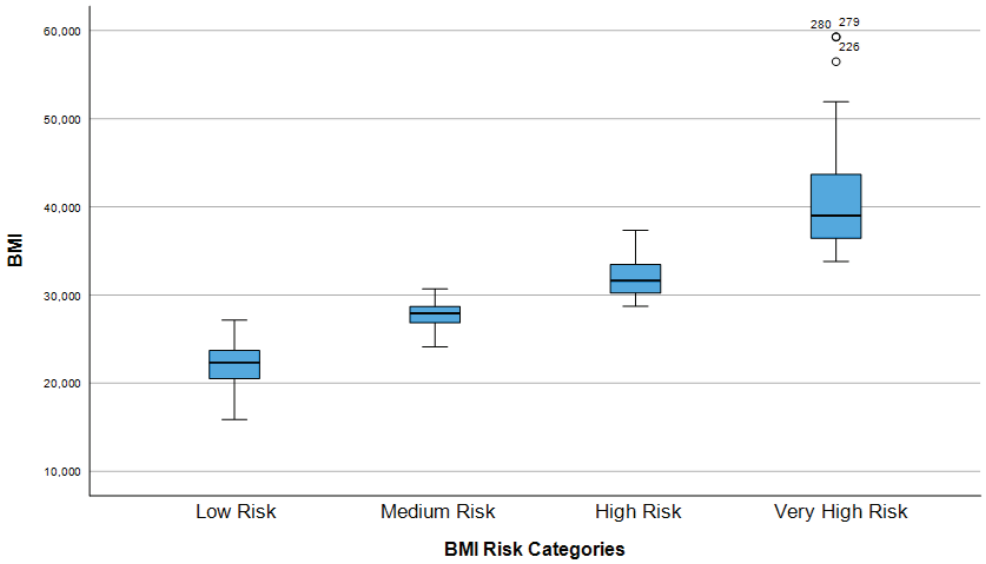

Figure 4-4 Screening Body Mass Index (BMI) Risk Assessment

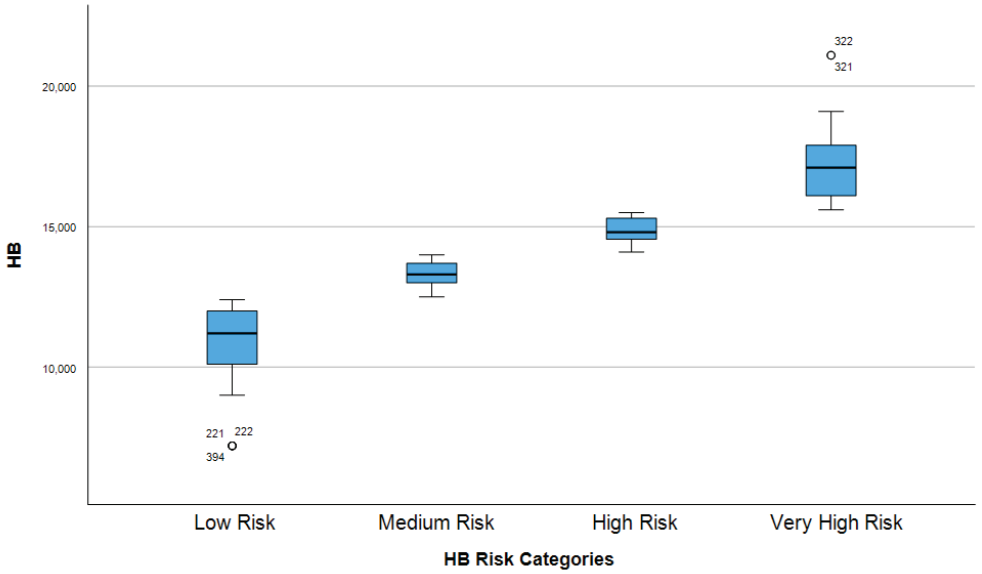

Figure 4-5 Screening Haemoglobin (HB) Risk Assessment

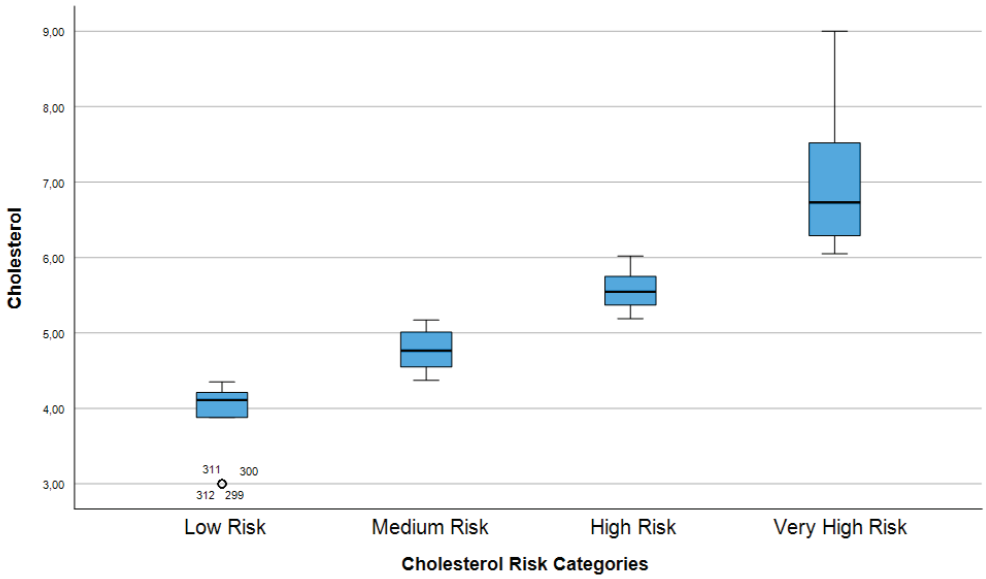

Figure 4-6 Screening Cholesterol Risk Assessment

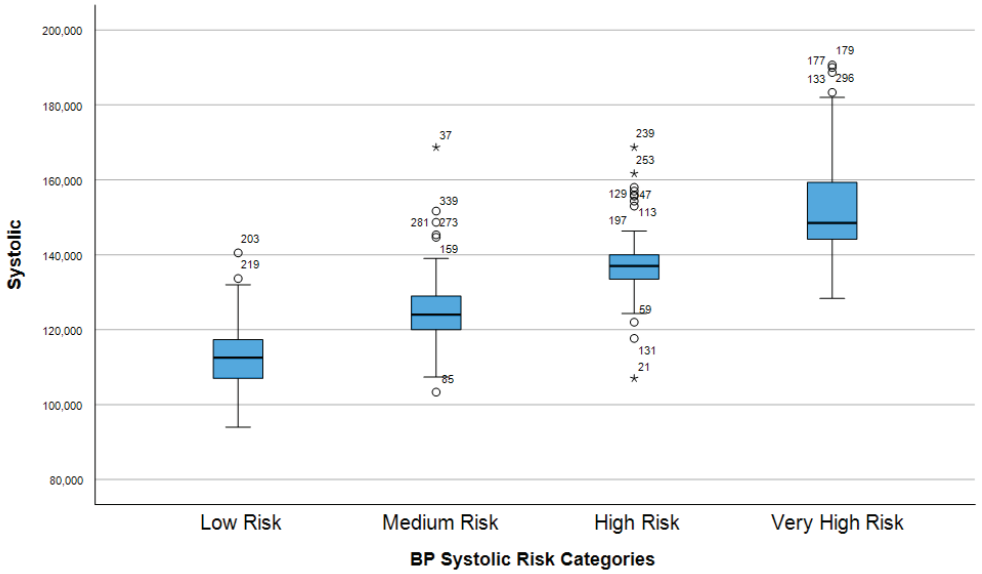

Figure 4-7 Screening Systolic Blood Pressure (BP) Risk Assessment

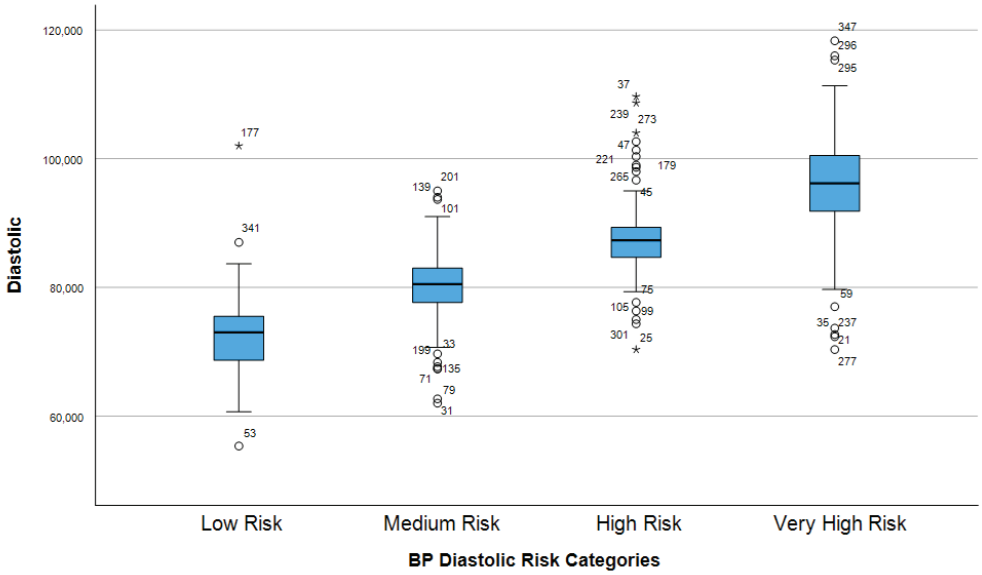

Figure 4-8 Screening Diastolic Blood Pressure (BP) Risk Assessment

4.3. OBJECTIVE 2: Comparison between the echocardiography findings in the traditional/fermented Rooibos, green/unfermented Rooibos and placebo groups of participants

Objective two, also the main objective, was to draw a comparison between the cardiovascular ultrasound findings in the different intervention groups that consumed either fermented or green Rooibos tea, or a placebo. First the results stemming from the descriptive statistical analysis of the echocardiography findings will be provided, followed by the quantitative analysis. The table (**Table 4-5 - Table 4-13**) below presents all the descriptive statistics of the

ECHO variables for the different interventions. The descriptive statistics include the Minimum, Maximum, Q1 (lower quartile), Q2 (middle quartile), Q3 (higher quartile), Mean, Variance, Standard Deviation (SD), Skewness and Kurtosis. In this table, 'Baseline' represents the first visit of the participants to the research centre, following a two-week wash-out period, and the 'Intervention' represents the second visit after a 12-week intervention with either green Rooibos, fermented Rooibos, or the placebo. Scatterplots that demonstrate the characteristics of the echocardiographic findings per intervention for each participant's visit are demonstrated as **APPENDIX G: SCATTERPLOTS** and are visual representations of the actual values in this data set.

4.3.1. Descriptive statistics of the echocardiography findings and Interventions

Based on the descriptive statistics presented in **Table 4-5** - **Table 4-13** the resulting patterns emerged when comparing the baseline data with their respective interventions. Following the 12-week green Rooibos intervention, echocardiography indicated a minor reduction in the aortic (AO) size, decreasing from 3.12 cm to 3.08 cm. Similarly, the left atrium (LA) size exhibited a decrease from 3.79 cm to 3.67 cm, along with the Interventricular septal (IVS), which decreased from 1.25 cm to 1.18 cm. Additionally, the left ventricular mass decreased from 193 g to 183 g. When considering the 12-week fermented Rooibos intervention, the echocardiography findings also revealed a reduction in LA size from 3.77 cm to 3.71 cm, but not to the same extent as with the green Rooibos intervention. Furthermore, there was a slight improvement in ejection fraction (EF%) in this group, with EF% increasing from 0.667% to 0.668%. It is noteworthy that the green Rooibos intervention had no observable effects on the echocardiography findings related to the left ventricular posterior wall, left ventricular internal diastole dimension, left ventricular internal systole dimension and EF%. Similarly, this was also evident in the fermented Rooibos intervention group concerning AO, LVPW, LVIDd and LVISd echocardiography findings. Considering the 12-week placebo intervention, the echocardiography findings revealed an increase from 3.16 cm to 3.20 cm in the AO size. Furthermore, a reduction from 3.95 cm to 3.85 cm could be seen in the LA size, as well as a reduction in the IVS diameter from 1.33 cm to 1.24 cm. Finally, the LMV in this group demonstrated a decrease from 207 g to 199 g.

The tables below represent the descriptive statistics of the echocardiography findings and the different study interventions. Improvement is demonstrated in each table below in the colour green.

Table 4-5 Echocardiography Variable Aorta (AO) in centimetre (cm)

ECHOCARDIOGRAPHY VARIABLE – Aorta (AO) in centimetre (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	2.2500	2.2900	2.4900	2.4100	2.5300	2.4500	2.2500
Maximum	4.6200	4.4600	4.1100	4.3800	4.6200	4.2300	4.3100
Q1	2.8800	2.9600	2.8400	2.8400	2.9200	2.8800	2.8400
Q2	3.1200	3.1600	3.0800	3.0400	3.1200	3.1600	3.0400
Q3	3.4000	3.3400	3.4300	3.3200	3.4000	3.4800	3.3200
Mean	3.1509	3.1719	3.1580	**3.1186**	3.1743	3.2015	**3.0797**
Variance	0.1553	0.1301	0.1528	0.1732	0.1499	0.1634	0.1647
SD	0.3941	0.3606	0.3910	0.4161	0.3871	0.4042	0.4059
Skew	0.6525	0.7597	0.2925	0.8652	0.9424	0.6052	0.7036
Kurt	0.5859	2.5108	-0.5986	0.6684	2.3740	-0.1936	0.6587

Table 4-6 Echocardiography Variable – Left atrium (LA) (cm)

ECHOCARDIOGRAPHY VARIABLE – Left atrium (LA) in centimetre (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	2.1300	2.7700	2.8800	3.0000	2.1300	2.8400	2.6500
Maximum	5.6500	5.2500	5.1400	5.6500	4.9000	5.4900	5.1400
Q1	3.4800	3.4600	3.6300	3.3600	3.4400	3.5200	3.4000
Q2	3.7500	3.7100	3.9100	3.7100	3.7100	3.7900	3.6700
Q3	4.1500	3.9900	4.3350	4.1500	4.0300	4.1500	3.9500
Mean	3.7911	**3.7658**	**3.9537**	**3.7907**	**3.7102**	**3.8503**	**3.6661**
Variance	0.2549	0.2324	0.2526	0.2649	0.3042	0.2228	0.2301
SD	0.5049	0.4821	0.5026	0.5147	0.5516	0.4720	0.4797
Skew	0.3380	0.5047	-0.1207	1.0606	-0.0354	0.4871	0.3957
Kurt	0.6612	0.8176	-0.4173	2.0501	0.4364	1.1170	1.4116

Table 4-7 Echocardiography Variable – LA/AO Ratio (cm)

ECHOCARDIOGRAPHY VARIABLE – LA/AO Ratio (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	0.7007	0.8343	0.8175	0.7841	0.7007	0.7470	0.7146
Maximum	1.8535	1.7045	1.7233	1.7958	1.5771	1.8300	1.8535
Q1	1.0889	1.0605	1.1397	1.1127	1.0417	1.0981	1.0882
Q2	1.2045	1.2219	1.2565	1.2043	1.1941	1.2027	1.1898
Q3	1.3390	1.3121	1.3680	1.3734	1.3693	1.2861	1.3045
Mean	1.2178	**1.2007**	**1.2660**	**1.2311**	**1.1827**	**1.2186**	**1.2053**
Variance	0.0393	0.0375	0.0382	0.0399	0.0430	0.0397	0.0372
SD	0.1983	0.1937	0.1955	0.1999	0.2075	0.1993	0.1928
Skew	0.2738	0.1949	0.2457	0.3993	-0.2494	0.6245	0.5123
Kurt	0.2893	-0.1169	-0.0924	0.2868	-0.5615	0.9917	1.5367

Table 4-8 Echocardiography Variable – Interventricular septum in diastole (IVSD) (cm)

ECHOCARDIOGRAPHY VARIABLE – Interventricular septum in diastole (IVSD) in centimetre (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	0.6700	0.7500	0.7900	0.6700	0.7500	0.7500	0.7100
Maximum	1.9000	1.7800	1.9000	1.8200	1.8200	1.8200	1.8600
Q1	1.0800	1.1100	1.1500	1.0700	1.1100	1.1500	1.0300
Q2	1.2200	1.2200	1.3000	1.2600	1.2200	1.2200	1.1900
Q3	1.4200	1.4200	1.5000	1.4600	1.3800	1.3400	1.3400
Mean	1.2497	1.2512	**1.3264**	**1.2525**	1.2543	**1.2409**	**1.1770**
Variance	0.0589	0.0519	0.0633	0.0823	0.0558	0.0414	0.0547
SD	0.2427	0.2277	0.2516	0.2868	0.2361	0.2036	0.2338
Skew	0.2187	0.2579	0.0797	0.0073	0.3071	0.3198	0.4364
Kurt	-0.1508	-0.2691	-0.4885	-0.7723	0.1299	0.9437	0.7113

Table 4-9 Echocardiography Variable – Left ventricle posterior wall in diastole (LVPWD) (cm)

ECHOCARDIOGRAPHY VARIABLE – Left ventricle posterior wall in diastole (LVPWD) in centimetre (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	0.5500	0.5900	0.5900	0.5500	0.5900	0.6700	0.6300
Maximum	1.7800	1.5400	1.5000	1.5800	1.5000	1.5800	1.7800
Q1	0.8700	0.8700	0.9100	0.8300	0.8300	0.9100	0.8700
Q2	0.9900	0.9500	0.9900	0.9900	0.9900	0.9900	0.9900
Q3	1.1450	1.0700	1.1500	1.1500	1.1500	1.1500	1.1100
Mean	1.0060	0.9617	1.0200	0.9981	1.0042	1.0366	1.0118
Variance	0.0418	0.0326	0.0331	0.0508	0.0458	0.0460	0.0430
SD	0.2044	0.1804	0.1820	0.2254	0.2140	0.2144	0.2074
Skew	0.4893	0.5975	0.2278	0.3568	0.0116	0.6872	0.9408
Kurt	0.5242	1.3664	0.5683	-0.1118	-0.7590	0.4310	2.2824

Table 4-10 Echocardiography Variable – Left ventricle mass (LVM) (g)

ECHOCARDIOGRAPHY VARIABLE – Left ventricle mass (LVM) in grams (g)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	49.7500	105.8000	89.6600	87.0300	102.2800	91.9600	49.7500
Maximum	377.9600	309.9000	342.3600	356.2300	312.2300	348.0300	377.9600
Q1	151.8875	142.3300	170.3200	146.4100	166.0200	161.7700	139.7400
Q2	188.5150	174.9000	204.8700	175.1900	197.5200	191.9200	179.0900
Q3	231.3700	217.3100	243.7500	240.1000	226.2500	230.9800	209.0700
Mean	194.0850	185.0666	**207.2068**	**192.5854**	199.7540	**197.5851**	**182.8625**
Variance	3260.8827	3028.7904	3506.0821	3623.2309	3079.9875	2789.0123	3420.7384
SD	57.1041	55.0344	59.2122	60.1933	55.4976	52.8111	58.4871
Skew	0.4878	0.5476	0.2067	0.5834	0.1652	0.6594	0.8622
Kurt	-0.0774	-0.5613	-0.4129	-0.2187	-0.6346	0.7411	1.5165

Table 4-11 Echocardiography Variable – Left ventricle in diastole diameter (LVIDd) (cm)

ECHOCARDIOGRAPHY VARIABLE – Left ventricle in diastole diameter (LVIDd) in centimetre (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	3.3200	3.5200	3.3200	4.0700	3.9100	3.6700	3.7500
Maximum	6.3600	5.7700	6.3600	5.6100	5.9700	6.2000	5.8500
Q1	4.5400	4.4800	4.5000	4.5400	4.3900	4.5000	4.5400
Q2	4.9000	4.9000	4.9800	4.8200	4.8600	4.9400	4.8600
Q3	5.2100	5.0600	5.3500	5.1800	5.2100	5.2100	5.2100
Mean	4.8658	4.7946	4.9254	4.8563	4.8608	4.8788	4.8762
Variance	0.2372	0.2373	0.3553	0.1604	0.2083	0.2437	0.2233
SD	0.4870	0.4871	0.5961	0.4004	0.4564	0.4937	0.4725
Skew	-0.1951	-0.4878	-0.3226	-0.1437	0.0582	-0.1788	-0.1175
Kurt	-0.0250	-0.0629	0.1838	-0.8493	-0.4894	-0.0070	-0.4653

Table 4-12 Echocardiography Variable – Left ventricle in systolic diameter (LVISd) (cm)

ECHOCARDIOGRAPHY VARIABLE – Left ventricle in systolic diameter (LVISd) (cm)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	1.6200	1.8200	1.9000	1.6200	1.9400	1.8600	2.2100
Maximum	4.2700	4.2700	4.1100	3.7900	4.0700	4.0700	4.2700
Q1	2.6900	2.6700	2.6700	2.7300	2.6900	2.5700	2.6900
Q2	3.0400	2.9200	3.0800	3.0400	3.0400	3.0000	3.0000
Q3	3.3600	3.3000	3.5600	3.3200	3.3200	3.4000	3.4000
Mean	3.0216	2.9810	3.0568	3.0275	3.0281	3.0011	3.0372
Variance	0.2598	0.2652	0.3274	0.1963	0.2170	0.3013	0.2595
SD	0.5097	0.5149	0.5722	0.4431	0.4658	0.5489	0.5094
Skew	0.0259	0.2101	-0.1219	-0.4868	-0.1801	0.0396	0.4652
Kurt	-0.4293	-0.0755	-0.8542	0.3951	-0.0933	-0.6411	-0.5276

Table 4-13 Echocardiography Variable – Ejection fraction in percentage (EF%)

ECHOCARDIOGRAPHY VARIABLE – Ejection fraction in percentage (EF%)							
Statistic Description	All data	Baseline Traditional Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Traditional Rooibos	Intervention Placebo	Intervention Green Rooibos
Minimum	0.3158	0.3158	0.4412	0.4262	0.5117	0.3884	0.4236
Maximum	0.8985	0.8902	0.8330	0.8985	0.8810	0.8477	0.8558
Q1	0.6037	0.5985	0.6069	0.6072	0.6013	0.6048	0.5882
Q2	0.6825	0.6865	0.6808	0.6752	0.6752	0.7027	0.6887
Q3	0.7465	0.7431	0.7618	0.7271	0.7429	0.7668	0.7445
Mean	0.6717	**0.6677**	0.6743	0.6705	**0.6684**	0.6820	0.6657
Variance	0.0105	0.0137	0.0109	0.0076	0.0088	0.0108	0.0117
SD	0.1026	0.1172	0.1046	0.0874	0.0936	0.1037	0.1080
Skew	-0.4677	-0.6623	-0.4795	-0.0232	0.2307	-0.7502	-0.5777
Kurt	0.0329	0.6741	-0.5414	0.4389	-0.6653	0.2214	-0.3175

Q1 = lower quartile, Q2 = median quartile, Q3 = upper quartile, SD = Standard Deviation, Kurt (kurtosis), Skew (skewness), AO = Aorta, LA = Left Atrium, IVSd = Interventricular Septum in diastolic, LVPWd = Left ventricular posterior wall diameter, LVM = Left ventricular mass, LVIDs = Left ventricular internal diameter in systolic, LVIDd = Left ventricle internal diameter in diastolic, EF = Ejection fraction

4.3.2. Diastolic function per intervention group

The descriptive statistics outlined in **Table 4-14** to **Table 4-18** revealed distinct trends in diastolic function. Notably, the green Rooibos intervention group displayed an increase in normal counts from 39 (54.2%) to 49 (68.1%), while the fermented Rooibos intervention group exhibited an increase from 32 (48.5%) to 45 (68.2%). The placebo group also showed an increase in normal counts from 34 (42%) to 51 (63%). Diastolic function was graded according to **Figure 3-15** as seen in Chapter 3. **Figure 4-9** and **Figure 4-10** below are the graphic outlines of the diastolic function pre and post intervention. The green Rooibos intervention group demonstrated no change in grade 1 diastolic dysfunction counts from baseline to intervention. However, grade 2 diastolic dysfunction counts decreased from 13 at baseline to 5 after intervention, with grade 3 diastolic dysfunction counts declining from 2 to 0 post intervention. In the traditional fermented group, grade 1 diastolic dysfunction counts decreased from 24 at baseline to 17 after intervention, while grade 2 diastolic dysfunction counts reduced from 10 at baseline to 4 post intervention. Grade 3 diastolic dysfunction counts remained consistent at 0 during and after intervention. In the Placebo group, grade 1 diastolic dysfunction counts decreased from 21 at baseline to 12 after intervention, and grade 2 diastolic dysfunction counts decreased from 23 at baseline to 15 after intervention. The counts for grade 3 diastolic dysfunction remained unchanged at 3 both during and after intervention.

Overall, these results suggest that the Rooibos interventions positively impact diastolic function, as demonstrated by the increase in normal counts and the reduction in diastolic dysfunction grades. This indicates the potential of Rooibos interventions as beneficial in managing diastolic function.

Table 4-14 Descriptive statistics of the diastolic function per intervention group

Visit				Intervention Group			Total
				Green Rooibos	Placebo	Fermented Rooibos	
Base-Line	Diastolic Function	Normal	Count	39	34	32	105
			% within Intervention Group	54.2%	42.0%	48.5%	47.9%
		Grade 1	Count	18	21	24	63
			% within Intervention Group	25.0%	25.9%	36.4%	28.8%
		Grade 2	Count	13	23	10	46
			% within Intervention Group	18.1%	28.4%	15.2%	21.0%
		Grade 3	Count	2	3	0	5
			% within Intervention Group	2.8%	3.7%	0.0%	2.3%
	Total		Count	72	81	66	219
			% within Intervention Group	100.0%	100.0%	100.0%	100.0%
Intervention	Diastolic Function	Normal	Count	49	51	45	145
			% within Intervention Group	68.1%	63.0%	68.2%	66.2%
		Grade 1	Count	18	12	17	47
			% within Intervention Group	25.0%	14.8%	25.8%	21.5%
		Grade 2	Count	5	15	4	24
			% within Intervention Group	6.9%	18.5%	6.1%	11.0%
		Grade 3	Count	0	3	0	3
			% within Intervention Group	0.0%	3.7%	0.0%	1.4%
	Total		Count	72	81	66	219
			% within Intervention Group	100.0%	100.0%	100.0%	100.0%
Total	Diastolic Function	Normal	Count	88	85	77	250
			% within Intervention Group	61.1%	52.5%	58.3%	57.1%
		Grade 1	Count	36	33	41	110
			% within Intervention Group	25.0%	20.4%	31.1%	25.1%
		Grade 2	Count	18	38	14	70
			% within Intervention Group	12.5%	23.5%	10.6%	16.0%
		Grade 3	Count	2	6	0	8
			% within Intervention Group	1.4%	3.7%	0.0%	1.8%
	Total		Count	144	162	132	438
			% within Intervention Group	100.0%	100.0%	100.0%	100.0%

Table 4-15 Chi-Square Tests

Visit		Value	df	Asymptotic Significance (2-sided)
Base-Line	Pearson Chi-Square	8.875[b]	6	.181
	N of Valid Cases	219		
Intervention	Pearson Chi-Square	14.699[c]	6	.023
	N of Valid Cases	219		
Total	Pearson Chi-Square	19.191[a]	6	.004
	N of Valid Cases	438		

a. 3 cells (25.0%) have expected count less than 5. The minimum expected count is 2.41.
b. 3 cells (25.0%) have expected count less than 5. The minimum expected count is 1.51.
c. 3 cells (25.0%) have expected count less than 5. The minimum expected count is .90.

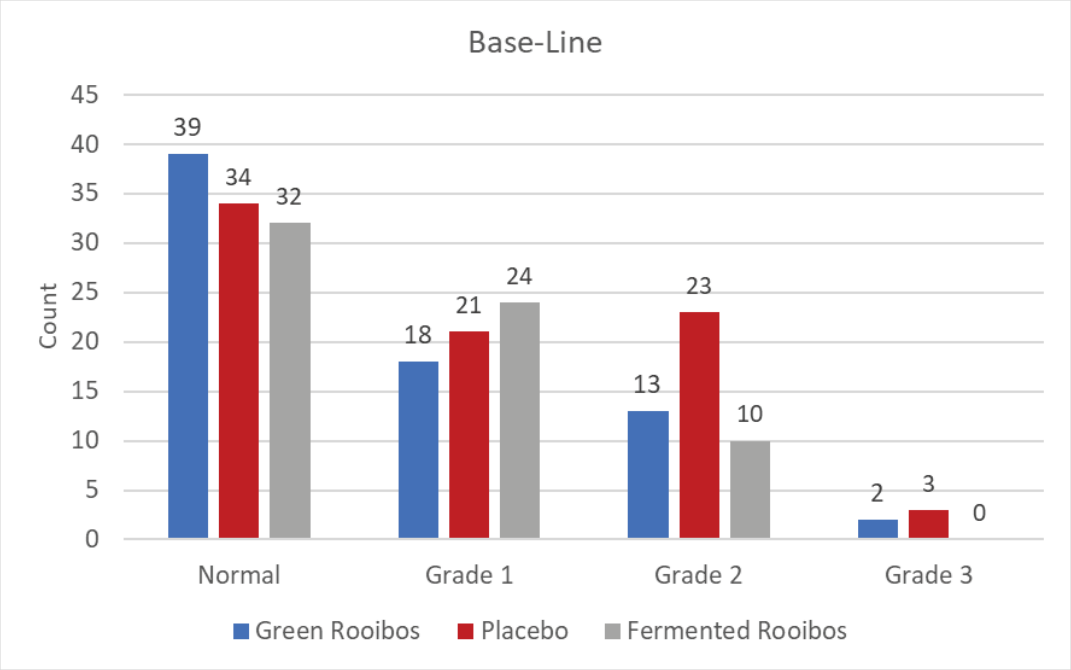

Figure 4-9 Baseline diastolic function per intervention group

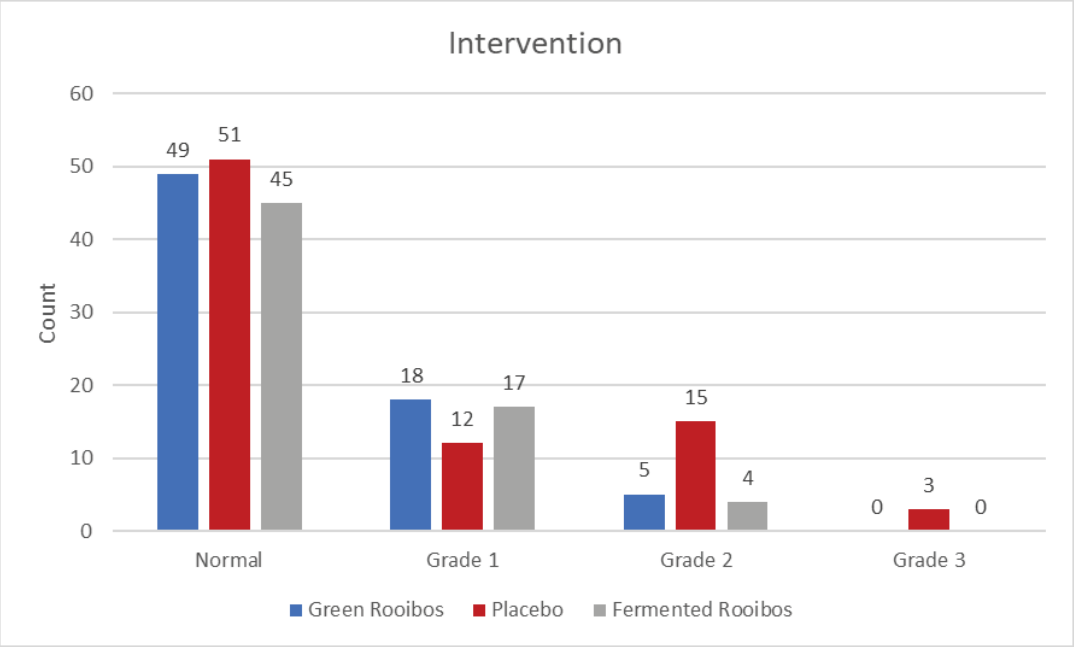

Figure 4-10 Intervention diastolic function per intervention group

4.4. OBJECTIVE 3: The Echocardiography findings and the dietary interventions

In this section, echocardiography factors were created using the dimensions of the different echocardiography variables. Normal intervals followed the description in **Table 3-1** (Chapter 3), and anything outside these intervals was considered as not normal.

4.4.1. Frequency of the echocardiography findings according to the interventions

The frequency distributions, presented in **Table 4-8** below, provide visual depictions of the percentage frequencies concerning normal and abnormal counts within the study. The table reveals that the normal AO distribution for fermented Rooibos (Baseline to Intervention) experienced a decrease in percentage counts (96.61% to 94.34%), suggesting a slight rise in abnormal outcomes. Consequently, the intervention of fermented Rooibos did not exhibit a positive influence on AO. In contrast, the green Rooibos intervention group displayed an increased percentage in normal counts (92.98% to 95.08%), indicating a reduction in abnormal counts from 7.02% to 4.93%. For LA, both fermented and green Rooibos groups maintained consistent normal counts between the baseline and the intervention, without a decline in abnormal counts. Notably, the green Rooibos group demonstrated an upward trend in normal counts between the baseline and the intervention for both IVSd (57.89% to 73.77%) and LVPWd (94.74% to 95.08%), suggesting a decline in abnormal counts. With regard to the LVM, the fermented Rooibos intervention group demonstrated an upward trend in the normal counts from 50.85% to 75.47%. The green Rooibos intervention group also demonstrated an upward trend in normal counts from 49.12% to 63.93%. All three interventions demonstrated

a decrease in normal counts in the LVID and LVIS dimensions, meaning none of them had an influence on these echocardiography variables. In the case of the fermented Rooibos intervention group, the percentage of normal counts for EF% increased (91.53% to 98.11%) from baseline to intervention, directly indicating improved systolic function and a reduction in abnormal EF% counts.

Table 4-16 Frequencies of the normal and abnormal counts per visit and intervention

Aorta diameter (AV)			
Baseline & Intervention	Abnormal range (%)	Normal range (%)	Grand Total
Baseline Fermented Rooibos	3.39%	96.61%	100%
Baseline Green Rooibos	7.02%	92.98%	100%
Baseline Placebo	1.69%	98.31%	100%
Intervention Fermented Rooibos	5.66%	94.34%	100%
Intervention Green Rooibos	4.92%	95.08%	100%
Intervention Placebo	9.23%	90.77%	100%
Grand Total	5.37%	94.63%	100%
Left atrium diameter (LA)			
Baseline & Intervention	Abnormal range (%)	Normal range (%)	Grand Total
Baseline Fermented Rooibos	35.59%	64.41%	100%
Baseline Green Rooibos	40.35%	59.65%	100%
Baseline Placebo	57.63%	42.37%	100%
Intervention Fermented Rooibos	35.85%	64.15%	100%
Intervention Green Rooibos	40.98%	59.02%	100%
Intervention Placebo	49.23%	50.77%	100%
Grand Total	43.50%	56.50%	100%
Interventricular septum in diastole (IVSD)			
Baseline & Intervention	Abnormal range (%)	Normal range (%)	Grand Total
Baseline Fermented Rooibos	32.20%	67.80%	100%
Baseline Green Rooibos	42.11%	57.89%	100%
Baseline Placebo	47.46%	52.54%	100%

Intervention Fermented Rooibos	35.85%	64.15%	100%
Intervention Green Rooibos	26.23%	73.77%	100%
Intervention Placebo	26.15%	73.85%	100%
Grand Total	34.75%	65.25%	100%
Left ventricle posterior wall in diastole (LVPWD)			
Baseline & Intervention	Abnormal range (%)	Normal (%)	Grand Total
Baseline Fermented Rooibos	5.08%	94.92%	100%
Baseline Green Rooibos	5.26%	94.74%	100%
Baseline Placebo	3.39%	96.61%	100%
Intervention Fermented Rooibos	3.77%	96.23%	100%
Intervention Green Rooibos	4.92%	95.08%	100%
Intervention Placebo	7.69%	92.31%	100%
Grand Total	5.08%	94.92%	100%
Left ventricle mass (LVM)			
Baseline & Intervention	Abnormal range (%)	Normal (%)	Grand Total
Baseline Fermented Rooibos	49.15%	50.85%	100%
Baseline Green Rooibos	50.88%	49.12%	100%
Baseline Placebo	33.90%	66.10%	100%
Intervention Fermented Rooibos	24.53%	75.47%	100%
Intervention Green Rooibos	36.07%	63.93%	100%
Intervention Placebo	26.15%	73.85%	100%
Grand Total	36.72%	63.28%	100%
Left ventricle internal dimensions in systole (LVIDs)			
Baseline & Intervention	Abnormal range (%)	Normal (%)	Grand Total
Baseline Fermented Rooibos	6.78%	93.22%	100%
Baseline Green Rooibos	10.53%	89.47%	100%
Baseline Placebo	18.64%	81.36%	100%
Intervention Fermented Rooibos	15.09%	84.91%	100%

Intervention Green Rooibos	21.31%	78.69%	100%
Intervention Placebo	21.54%	78.46%	100%
Grand Total	15.82%	84.18%	100%
Left ventricle internal dimensions in diastole (LVIDd)			
Baseline & Intervention	Abnormal range (%)	Normal (%)	Grand Total
Baseline Fermented Rooibos	11.86%	88.14%	100%
Baseline Green Rooibos	12.28%	87.72%	100%
Baseline Placebo	16.95%	83.05%	100%
Intervention Fermented Rooibos	26.42%	73.58%	100%
Intervention Green Rooibos	26.23%	73.77%	100%
Intervention Placebo	26.15%	73.85%	100%
Grand Total	20.06%	79.94%	100%
Ejection Fraction % (EF%)			
Baseline & Intervention	Abnormal range (%)	Normal (%)	Grand Total
Baseline Fermented Rooibos	8.47%	91.53%	100%
Baseline Green Rooibos	3.51%	96.49%	100%
Baseline Placebo	10.17%	89.83%	100%
Intervention Fermented Rooibos	1.89%	98.11%	100%
Intervention Green Rooibos	9.84%	90.16%	100%
Intervention Placebo	6.15%	93.85%	100%
Grand Total	6.78%	93.22%	100%

4.4.2. **Frequencies of the normal and abnormal diastolic function counts**

Table 4.9 and **Figure 4.11** above illustrate the percentage frequencies related to normal and abnormal counts of diastolic function. The instances of normal counts increased from 105 (47.9%) to 145 (66.2%). The counts for grade 1 diastolic dysfunction reduced from 63 to 47, corresponding to a decrease in percentage from 28.8% to 21.5%. Similarly, the counts for grade 2 diastolic dysfunction decreased from 46 to 24, resulting in a percentage decline from 21.0% to 11.0%. Additionally, the counts for grade 3 diastolic dysfunction decreased from 5 to 3, with the percentage decreasing from 2.3% to 1.4%. These results indicate an overall improvement in diastolic function, demonstrated by an increase in normal counts and a reduction in all grades of diastolic dysfunction.

Table 4-17 Frequencies of the normal and abnormal diastolic function counts per initial visit and intervention

			Baseline (Visit 1)	Intervention (Visit 2)	Total
Diastolic Function	Normal	Count	105	145	250
		% within Visit	47.9%	66.2%	57.1%
	Grade 1	Count	63	47	110
		% within Visit	28.8%	21.5%	25.1%
	Grade 2	Count	46	24	70
		% within Visit	21.0%	11.0%	16.0%
	Grade 3	Count	5	3	8
		% within Visit	2.3%	1.4%	1.8%
Total		Count	219	219	438
		% within Visit	100.0%	100.0%	100.0%

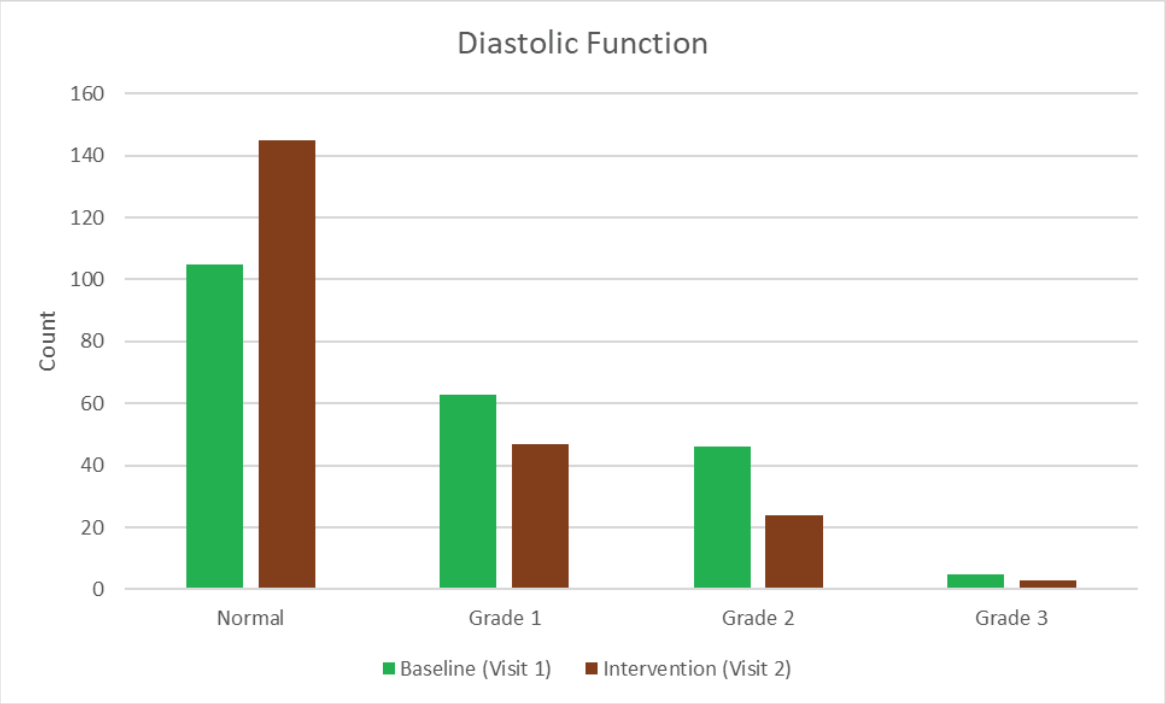

Figure 4-11 Diastolic function per intervention

4.4.3. The echocardiography findings for the different dietary interventions (Fermented Rooibos, Green Rooibos, Placebo)

Based on the Generalised Estimating Equations application demonstrated in **Table 4-10**, no significant relationships were observed between the AO, LVPWd, LVISd, LVIDd and EF%; however, an association was found between LA, IVSd and LVM. Where there is a relationship, it suggests that the intervention influences cardiovascular function, and where there is no relationship, it suggests that the interventions had no influence on the cardiovascular function. Detailed statistical analyses, including the corresponding tables, can be found in **APPENDIX I: GENERALISED ESTIMATING EQUATIONS APPLICATION**.

Table 4-18 Effects of the three different interventions on the various echocardiography measurements

ECHO Characteristics	Placebo		Fermented Rooibos		Green Rooibos	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
AO (cm)	3.245 ± 0.047	3.296 ± 0.056 (p=0.254)	3.205 ± 0.041	3.228 ± 0.049 (p=0.575)	3.206 ± 0.050	3.191 ± 0.053 (p=0.755)
LA (cm)	3.938 ± 0.063	3.848 ± 0.058 (p=0.060)	3.766 ± 0.059	3.731 ± 0.071 (p=0.615)	3.832 ± 0.071	3.675 ± 0.067 **(p=0.01)**
LA / AO Ratio	1.226 ± 0.023	1.185 ± 0.024 (p=0.076)	1.186 ± 0.023	1.169 ± 0.027 (p=0.520)	1.208 ± 0.024	1.165 ± 0.023 (p=0.091)
IVSd (cm)	1.334 ± 0.030	1.250 ± 0.025 **(p=0.002)**	1.267 ± 0.028	1.259 ± 0.031 (p=0.784)	1.282 ± 0.036	1.186 ± 0.029 **(p=0.002)**
LVPWd (cm)	1.007 ± 0.023	1.035 ± 0.026 (p=0.383)	0.970 ± 0.022	1.012 ± 0.028 (p=0.181)	0.998 ± 0.028	1.014 ± 0.027 (p=566)
LVM (g)	210.659 ± 7.029	203.350 ± 6.503 (p=0.300)	204.102 ± 7.102	191.394 ± 6.707 **(p=0.015)**	198.841 ± 7.636	191.120 ± 7.379 (p=0.292)
LVIDd (cm)	4.970 ± 0.064	4.925 ± 0.059 (p=0.461)	4.819 ± 0.061	4.929 ± 0.059 (p=0.059)	4.913 ± 0.049	4.967 ± 0.058 (p=0.356)
LVISd (cm)	3.071 ± 0.065	3.077 ± 0.067 (p=0.924)	3.004 ± 0.068	3.109 ± 0.062 (p=0.127)	3.075 ± 0.055	3.129 ± 0.065 (p=0.335)
EF (Teich) (%)	0.676 ± 0.013	0.668 ± 0.013 (p=0.563)	0.666 ± 0.015	0.658 ± 0.012 (p=0.634)	0.666 ± 0.010	0.657 ± 0.013 (p=0.549)

Aorta (AO), Left Atrium (LA), Interventricular Septum diameter (IVSd), Left Ventricle Posterior Wall Diameter (LVPWd), Left Ventricle Mass (LVM), Left Ventricle in Diastole diameter (LVIDd), Left Ventricle in Systole diameter (LVISd), Ejection Fraction (EF)

Table 4-10 above provides an overview of the associations between the Echocardiography (ECHO) findings and the three intervention groups in the present study. In the group receiving green Rooibos, there was a slight reduction in the size of the aortic (AO) from the baseline ECHO to the intervention ECHO. However, this change was not statistically significant (p = 0.75). Both the fermented Rooibos (p = 0.57) and placebo (p = 0.25) groups exhibited an increase in AO dimensions, which did not reach statistical significance. Regarding the left atrium (LA) dimensions, a significant reduction (*p = 0.01*) was observed between baseline echocardiogram dimensions and the intervention echocardiogram in the green Rooibos group.

In contrast, both the placebo and fermented Rooibos groups showed minor reductions in LA dimensions, although these changes were not statistically significant (fermented Rooibos, p = 0.061; placebo, p = 0.060). A slight, non-significant decrease (p > 0.05) was observed in the mean LA/AO ratio across all three intervention groups when comparing baseline with intervention echocardiograms. For interventricular septal thickness in diastole (IVSd), a significant decrease in dimension was noted in the groups having consumed green Rooibos and placebo (*p = 0.002*), while the fermented Rooibos intervention resulted in a decreasing trend without statistical significance (p = 0.784). Significant differences were observed in the interventricular septum thickness (IVSd) among females in both the green Rooibos group (*p = 0.001)* and the placebo group (*p = 0.015*). Significant differences were also observed in the interventricular septum thickness (IVSd) among males in the placebo group (p = 0.048). No significance was observed among females with regard to the AO, LA, LVPWd, LVM, LVIDd, LVISd and EF percentage. Where males are concerned, no significant differences were observed with regard to AO, LVPWd, LVIDd, LVISd and the EF percentage. The left ventricular posterior wall thickness in diastole (LVPWd) exhibited a slight increase between baseline and intervention echocardiograms in all three intervention groups, although none of these changes were statistically significant (green Rooibos, p = 0.56; placebo, p = 0.38; fermented Rooibos, p = 0.18). A significant decrease in LVM was observed in the fermented Rooibos group when comparing baseline and intervention echocardiograms (*p = 0.015*). However, the differences in LVM for the green Rooibos and placebo groups were not statistically significant (p > 0.05). No significant differences were found in the LVIDd or LVISd across all three intervention groups when comparing baseline and intervention measurements (p > 0.05). In terms of EF, no notable increase was observed in any of the three intervention groups when comparing baseline with intervention echocardiograms (p > 0.05).

4.4.4. **Effect sizes in repeated measures**

Effect sizes were calculated to quantify the magnitude of significance in the green Rooibos LA size, IVSd in the Placebo and green Rooibos group, as well as the Fermented Rooibos group. Below is an explanation of the provided effect sizes. (Please see **APPENDIX M** for a detailed explanation).

Effect sizes in repeated measures designs.
LA: effect size: d_{RM} = -.210 (Green) - adverse effect (a negative sign of correlation)
ISVd: effect size d_{RM} = -.224 (Placebo) - adverse effect (negative sign of correlation)
ISVd: effect size d_{RM} = 0.247 (Green) – Small effect
LVM: effect size: d_{RM} = .195 (Fermented) – Small effect

4.5. Summary of the Chapter

This chapter employed various statistical methods to address each of the study's objectives comprehensively. Firstly, for the assessment of the prevalence and characteristics of cardiovascular disease risk categories (Objective 1), the results revealed an evenly distributed representation of participants across Lower Risk, Medium Risk, Higher Risk, and Very High Risk categories. This distribution aligns with the study's inclusion criteria, which required participants to exhibit two or more risk factors for eligibility. Objective 2, aimed to compare echocardiography findings among the fermented Rooibos, green Rooibos and Placebo groups, a descriptive statistical approach was applied. This analysis demonstrated that both the green Rooibos and fermented Rooibos interventions had a discernible impact on cardiovascular function. It is noted that the placebo group also exhibited changes in one of the cardiovascular function parameters. Significant change was also found among both females and males in certain echocardiography parameters. In Objective 3, we investigated the association between echocardiography measurements and the three distinct dietary interventions, Chi-square tests were conducted to evaluate for statistical significance. These tests demonstrated that the null hypothesis can be rejected and that the various interventions did, indeed, influence specific cardiac variables. In the chapter that follows, we will embark on a comprehensive discussion of the findings pertaining to each specific objective of this study, elucidating their significance and implications in the context of cardiovascular health and dietary interventions.

The PYTHON CODE used for generating the results in this study can be found in **APPENDIX F: THE PYTHON CODE OF THE GENERATED RESULTS**, as well as additional graphs, scatterplots as **APPENDIX G: SCATTERPLOTS** and the heatmaps **APPENDIX H: HEATMAPS**

Chapter 5 | Discussion

In this Chapter, the statistical significance and implication of the results of this research study will be discussed. The chapter plays a pivotal role in this thesis, as it acts as the central theme around which the study revolves. Our primary aim is to clarify the innovative contributions and insights obtained during the course of this study. Additionally, we intend to address any limitations, provide recommendations, and extend an invitation for future research inquiries.

5.1. OBJECTIVE 1: The risk assessment model

The first objective was to establish the prevalence and characteristics of cardiovascular disease and its associated risk factors in the adult study population. The purpose of a risk assessment is not only to determine an individual's risk; rather, the risk assessment should guide appropriate health interventions and thereby enhance clinical outcomes (Hlatky et al., 2009). Jee et al. (2014) presented initial evidence indicating that the Framingham risk function overestimates the risk of coronary heart disease (CHD) in the Korean population, where CHD incidence is relatively low. The Framingham risk function predicted 3–6 times more CHD events than were actually observed. This underscores the need to explore alternative risk stratification tools, particularly in diverse population groups, despite the utility of the Framingham risk scoring. **Paragraph 3.6.1** in Chapter 3 provides a detailed demonstration of how the risk categories in this research study were created. The accuracy of the four-step risk category model was tested by fitting a Logistic Regression Classifier to predict the risk categories in this data set and the probability of being at risk.

The classification report indicates that the model used is 73 % accurate which, therefore, suggests that of 100 participants, the model has correctly predicted the class (higher risk or lower risk). According to data from the Framingham research, CVD risk is often calculated using data on age, gender, blood pressure, blood cholesterol and smoking status (Gaziano et al., 2013). The Framingham Risk Score and the Systematic Coronary Risk Evaluation (SCORE), both being techniques created for America and Europe, are the most widely used risk assessment methods to estimate cardiovascular risk (Jahangiry et al., 2022). While the Framingham risk scoring system is a well-established method for assessing cardiovascular disease (CVD) risk, it was not used in this study. The decision was based on the unique characteristics and specific needs of our study population. Our research aimed to develop a tailored risk algorithm that better reflects the demographics, health status, and risk factors present in our participants as mentioned to in chapter 3.

The increased prevalence of modifiable CVD risk factors, such as high blood pressure, diabetes, smoking and increased BMI, contributes to the burden of CVD (Wagner et al., 2021). This research study used the following non-laboratory (point of care test kits) baseline data to

calculate the risk profile: age, blood pressure, blood cholesterol, haemoglobin, blood glucose, BMI and waist circumference. Gaziano et al. (2013) used similar non-laboratory baseline data to compare the performance of simple non-laboratory-based CVD risk scores to laboratory-based risk scores. Their study found a high level of correlation between non-laboratory and laboratory risk scores. A similar study was conducted by Jahangiry et al. (2022) to predict the 10-year risk of cardiovascular diseases among Iranians. They used the Globorisk office-based (non-laboratory) model approach and found that their approach could identify risk categories in low- to middle-income nations with limited resources. These findings support the feasibility and effectiveness of using non-laboratory data for risk profiling in diverse populations.

According to the World Health Organisation (2000), the following BMI measurements can be used to determine whether a person is underweight or overweight. Severely Underweight: < 16 kg/m²; Underweight: 16.0 - 18.4 kg/m²; Normal Weight: 18.5 - 24.9 kg/m²; Overweight: 25.0 - 29.9 kg/m²; Moderately Obese: 30.0 - 34.9 kg/m²; Severely Obese: 35.0 - 39.9 kg/m²; Morbidly Obese: greater than or equal to 40.0 kg/m². The descriptive analysis of Body Mass Index data in this study reveals that the average BMI in the dataset stands at 31 kg/m². According to the World Health Organisation's classification provided above, the population under investigation in this research study falls within the category of moderate obesity. Nuttall (2015) suggests that the BMI measurement plays an important role in predicting future health issues, in particular when formulating public health policies. Ross et al. (2020) suggest that both BMI and waist circumference measurements are critical in the management of obesity and associated metabolic diseases. They further suggest that, when stratifying the health risk associated with obesity, the BMI should be considered. While BMI is a widely used and useful tool for predicting future health issues and formulating public health policies, it is not without its criticisms. One major limitation of BMI is that it does not differentiate between muscle and fat mass, potentially misclassifying muscular individuals as overweight or obese and underestimating the health risks for those with a normal BMI but high body fat percentage. Additionally, BMI does not account for the distribution of fat in the body, which is an important factor in assessing the risk of metabolic and cardiovascular diseases. For instance, abdominal fat is more strongly associated with health risks than fat distributed in other areas. Therefore, while BMI is a useful initial screening tool, it should be used in conjunction with other measurements, such as waist circumference, to provide a more comprehensive assessment of an individual's health risks (Prentice and Jebb, 2001; Okorodudu et al., 2010).

A 24-year follow-up systematic review and meta-regression analysis performed by Carmienke and colleagues (2013) indicated that waist circumference of over 95 cm for males and 80cm for women was related to a higher all-cause mortality. Hunt et al. (2004), who investigated clinical definitions of the metabolic syndrome in order to identify individuals with increased

cardiovascular risk, also suggest that the BMI and waist circumference be considered to identify individuals at risk for CVD. The 2017 American College of Cardiology/American Hypertension Association guidelines, classified HPT as systolic blood pressure (SBP) of ≥130 mmHg or a diastolic blood pressure (DBP) of ≥80 mmHg. Our study's average SBP measured 131 mmHg, while the DBP averaged 84 mmHg. These measurements slightly exceed the recommendations outlined by the American College of Cardiology and the American Heart Association. The average level of serum total cholesterol in this research study was 5.5 mmol/l, which is higher than the recommended normal level of 5.17 mmol/l, as suggested in the guidelines of the National Cholesterol Education Program (Kannel, 1995). Men with serum total cholesterol levels of ≥6.21 mmol/L had a higher risk of ischemic stroke and large-artery occlusive infarction compared to those with levels below 4.65 mmol/L (Cui et al., 2012).The World Health Organization and various other healthcare organisations utilise a lower threshold for fasting blood glucose levels to detect individuals at risk of developing diabetes. A fasting blood glucose measurement of 5.6 mmol/L (100 mg/dL) is frequently employed as a criterion for categorising individuals with impaired fasting glucose (IFG), which serves as a precursor to diabetes (WHO, 2006). The mean fasting plasma glucose in this study was 4.51 mmol/L which is still with in normal range according to the WHO. It is noteworthy that the risk of diabetes increases even when fasting plasma glucose (FPG) levels are within the normal range (WHO, 2006). This persistent risk, even in the presence of normal plasma glucose levels, is substantiated by Tirosh et al. (2005), who identified an elevated risk of diabetes in healthy Israeli men when fasting plasma glucose levels reached or exceeded 4.8 mmol/L, in contrast to those below 4.5 mmol/L. The results have shown that, of the n=219 participants, n=118 (53.9%) were in the lower risk frequency for cardiovascular disease and n=101 (46.1%) were in the higher risk frequency (see **Table 4-4**).

This analysis underscores the significance of multiple health indicators in assessing cardiovascular and overall health risks. Our findings align with the evidence presented by Carmienke et al. (2013); Hunt et al. (2004), and the guidelines from prominent health organisations, reinforcing the critical role of waist circumference, BMI, blood pressure, cholesterol levels, and fasting plasma glucose in predicting health outcomes. The prevalence of higher risk profiles in nearly half of our study participants highlights the urgent need for targeted interventions to address these modifiable risk factors, thereby mitigating the risk of cardiovascular disease and enhancing long-term health outcomes.

The section that follows discusses the second objective, which was to draw a comparison between the cardiovascular ultrasound findings in the fermented and green Rooibos and placebo groups of participants.

5.2. OBJECTIVE 2: Comparison between the echocardiography findings in the fermented Rooibos, the green Rooibos, and the placebo groups of participants

The study's second, and main, objective was to draw a comparison between the cardiovascular ultrasound findings in the fermented Rooibos, the green Rooibos and the placebo groups of participants. According to the echocardiography findings, a decrease was found in some of the echocardiography parameters in the fermented Rooibos group, the green Rooibos group, as well as the placebo group.

According to the descriptive statistics in **Table 4-5,** the aorta dimensions demonstrated no significant changes in the fermented Rooibos group and the placebo intervention group. In both these groups, the aorta dimensions had increased slightly. However, the mean aortic root dimensions were within normal limits in both these groups before and after the intervention. The maximum aorta dimensions in all three groups were above the normal limits, as suggested by the guidelines of the American Society of Echocardiography and the European Association of Cardiovascular Imaging. These two societies suggest that the normal aorta dimension for females ranges from 2.7cm to 3.8cm, while in men, the range is 3.0cm to 4.0 cm (Lang et al., 2015). The present research study aligns with existing literature by demonstrating statistically significant differences in specific echocardiography variables between male and female participants (see **APPENDIX J and K**). Any dimension exceeding these ranges is considered abnormal, meaning dilated. A dilated aortic root is an effective indication of increased cardiovascular risk related to target organ injury (Cuspidi et al., 2006). As mentioned previously in this document, Whelton (2018) mentions that a dilated aorta is associated with aortic regurgitation.

In the same descriptive statistics (**Table 4-6**) post-intervention decrease in the left atrium dimensions were demonstrated in all three intervention groups, with a significant change in only the Placebo and the green Rooibos group. The mean left atrium dimensions in all three groups reflect values that are within the normal limits. Kizer et al. (2006) discovered that the diameter of the left atrium (LA), whether measured as a continuous or categorical variable, was significantly linked to the occurrence of cardiovascular events. They suggested that LA dilatation could serve as an indicator for identifying individuals at higher risk, who may benefit from more intensive risk factor management. Considering this information, while a slight decrease in LA size was seen after the intervention, it is reassuring that the mean LA size in this group of participants remained within normal limits. This implies that the Rooibos intervention might play a role in preventing cardiovascular events, as mentioned by Kizer and colleagues.

Quan et al. (2019) examined the effects of the green tea extract catechin on cardiac function in twelve paediatric cardiomyopathy patients with diastolic dysfunction. Catechin was

administered for twelve months, with assessments including echocardiography, ECG, and laboratory tests conducted before and after treatment. Results showed a significant reduction in isovolumetric relaxation time, though ejection fraction, left ventricular wall thickness, and bi-atrial dimension remained unchanged. This implies that catechin may improve impaired relaxation in paediatric cardiomyopathy patients but does not affect LA, LV, or EF%. The current research study aligns with the findings of Quan et al. (2019) and others, supporting the idea that the catechin found in green tea may effectively address impaired relaxation in paediatric patients diagnosed with diastolic dysfunction as well as LA dimensions as mentioned earlier. Bella et al. (2002) demonstrated that diastolic dysfunction is strongly linked to the presence of cardiovascular disease (CVD), supporting the tendency of hypertension and coronary artery disease to cause diastolic dysfunction. This study indicates that the interventions employed in our research had a favourable impact on diastolic function. Specifically, the instances of grade 1 diastolic dysfunction counts decreased from 63 (28%) to 47 (21.5%). Similarly, cases of grade 2 diastolic dysfunction decreased from 46 (21%) to 24 (11%). Furthermore, instances of grade 3 diastolic dysfunction decreased from 5 (2.3%) to 3 (1.4). Gottdiener et al. (2002) found that as diastolic dysfunction becomes more severe, the prevalence of systolic dysfunction also increases, although participants with diastolic dysfunction typically exhibited a normal ejection fraction (EF). In this study the mean EF reflects normal EF % values throughout all the interventions, while the diastolic function reflects abnormalities from grade 1 diastolic dysfunction to grade 3 diastolic dysfunction. Gottdiener et al. (2002) also observed that severe isolated diastolic dysfunction was as prevalent as systolic dysfunction. In light of the above findings, it is reassuring to know that Rooibos might be beneficial in modulating diastolic function in this group of participants. Al-Shafei and El-Gendy (2019) discovered that regular consumption of green tea was associated with cardiovascular benefits, including reductions in systolic and diastolic blood pressure, pulse pressure (PP), and a reversal of LVH. In contrast, drinking only hot water had a minimal effect on blood pressure and did not reverse LVH. While the study does not clearly specify if hot water was used as a placebo, it was used as a comparison to evaluate the effects of green tea.

According to the American Society of Echocardiography and the European Association of Cardiovascular Imaging, the normal left atrium dimensions for females range from 2.7cm to 3.8cm, while in men the range is 3.0cm to 4.0cm (Lang et al., 2015). Lee and Park (2015), as well as Eshoo et al. (2009), state that increased LVM, reduced LV systolic performance, impaired LV diastolic function and increased left atrial size are all indicators of a poor prognosis linked with hypertension. Dreslinski et al. (1981), Suzuki et al. (2011) and Boyd et al. (2013) all documented that hypertension causes left ventricular hypertrophy, a decrease in

the LV diastolic function and LA enlargement. This is confirmed by Kizer et al. (2006) who also found that the left atrial diameter was a robust predictor of initial cardiovascular events among middle-aged and elderly individuals without clinical cardiovascular disease. In this current study, the maximum LA dimensions in the (fermented and green Rooibos) intervention groups were above the normal limits as indicated earlier. Research by Feigin et al. (2014) and Xu et al. (2020) has demonstrated that an enlarged left atrium is linked to a higher risk of ischemic stroke and overall mortality. It is projected that by 2030, there will be nearly 12 million deaths due to stroke and 70 million stroke survivors. According to the descriptive statistics, positive changes were demonstrated in both the fermented and the green Rooibos intervention groups after a 12-week consumption of an equivalent of 6 cups of Rooibos tea daily. In their study, Kizer et al. (2006) also suggest that LA dimensions, established through echocardiography, can identify individuals at risk - without clinical illness - and who may benefit from aggressive risk factor management.

LVH is a risk factor for cardiovascular disease and cardiovascular events (Bombelli et al., 2009). This study used the American Society of Echocardiographers calculation: LV mass g = $0.8[1.04(LVIDd + IVS + PW)^3 – LVIDd^3] + 0.6$ g to calculate the LVM. The LVM was calculated based on the dimensions obtained through echocardiography without the need to normalise for body surface area. However, normalisation for body surface area (BSA) or height might be applied separately after calculating LVM to adjust for body size differences among individuals. Normalising LVM by height to allometric powers (a mathematical method to account for body size differences) is more effective at identifying individuals at high risk for cardiovascular events compared to normalising LVM by BSA (Simone et al., 2005). This normalisation involves adjusting the measurement of LVM to account for individual differences in body size and therefore allows for a more accurate assessment of LVM in relation to body size, ensuring fair and meaningful comparisons between individuals (Bornstein et al., 2020). Clinicians can, therefore, better determine the presence of LVH and assess cardiovascular risk. This method is especially useful in populations with a high prevalence of obesity.

A positive change was observed in the green Rooibos and placebo intervention groups concerning IVSd. At baseline, the maximum IVSd diameter in all three intervention groups exceeded the normal measurement guidelines established by the ASE, as detailed in Chapter 2. Specifically, the maximum IVSd was greater than 1.8 cm in all groups. According to the ASE and the European Association of Cardiovascular Imaging, an IVSd greater than 1.6 cm is considered severely abnormal and indicative of LVH (Lang et al., 2015). No significant positive changes were observed following the consumption of fermented Rooibos. As previously discussed, hypertension-induced remodelling of the left ventricle results in the

development of LVH, a major risk factor for adverse cardiovascular disease (CVD) outcomes (Whelton, 2018).

Tsang et al. (2005) demonstrated that echocardiography enhances risk stratification for age-related cardiovascular events by identifying key CVD risk markers. Vakili et al. (2001) highlighted the strong association between LVH and adverse cardiovascular outcomes, emphasizing the clinical importance of detecting LVH. Furthermore, studies by Levy et al. (1990) and Kuwahara et al. (2014) documented a correlation between chronic hypertrophy and a significantly increased risk of heart failure, dilated cardiomyopathy, ischemic heart disease, and sudden death, contributing to higher cardiovascular mortality. Additionally, a well-established link exists between hypertension and AF, with elevated SBP, even within the upper limits of the normal range, serving as a long-term predictor for the development of AF. These discoveries imply that adopting a more proactive approach to blood pressure management could potentially mitigate the risk of developing new cases of AF, as highlighted in the study conducted by Okin et al. (2015). This is further confirmed by a study conducted by Kuwahara et al. (2014), who indicated that, as hypertrophy advances, the heart muscle sustains damage due to an irregularity in contractions or excessive expansion. Prolonged hypertrophy is linked to a notable rise in the likelihood of heart failure, dilated cardiomyopathy, ischemic heart disease and sudden death, consequently elevating the risk of cardiovascular mortality.

According to the descriptive statistics table mentioned earlier, the green Rooibos group demonstrated a decrease in the LVM from 193 g to 183 g. Lee and Park (2015), as well as Eshoo et al. (2009), noted that an increased LV mass is an indicator of a poor prognosis linked to hypertension. The investigation carried out by Bolognese et al. in 1994 proposes that left ventricular mass holds significance as an autonomous risk element in individuals with uncomplicated acute myocardial infarction linked to single-vessel coronary artery disease. In an article authored by Koren and colleagues in 1991, the researchers highlight that echocardiography-based assessment of left ventricular mass and geometry holds the ability to categorise risk among individuals with essential hypertension. Importantly, this categorisation is distinct from and holds greater significance than, blood pressure or other modifiable risk factors. These findings suggest that echocardiographic evaluation could aid in identifying the necessity for intensive treatment strategies.

5.3. OBJECTIVE 3: The association between the CVD findings and the dietary interventions

This section will provide a discussion of the third and final objective which was to establish an association between the CVD findings and the three 12-week dietary interventions. The

distributions of frequency, as illustrated in **Table 4-16**, represents the percentage frequencies of the normal and abnormal counts in the study, testing for the independence of Echo findings and interventions (fermented Rooibos, green Rooibos, placebo).

The Generalised Estimating Equations application, demonstrated in **APPENDIX I: GENERALISED ESTIMATING EQUATIONS APPLICATION**, allowed for the dependent variable to be numerical or categorical; therefore, the gamma probability with a log-link function was utilised in the analysis. Using this analysis, an association was found between LA *(p=0.01)*, IVSd *(p=0.002)* and LVM *(p= 0.015)* echocardiography variables and the interventions. The influence and association that the interventions had on the cardiovascular function corroborate research that suggests Rooibos to improve cardiovascular (Marnewick et al., 2009; Marnewick et al., 2011; Maleki et al., 2015; Ajuwon et al., 2018; Ito et al., 2019). It is widely recognised that oxidative stress-induced damage and chronic inflammation activate cell signal transduction pathways, resulting in unfavourable cardiac remodelling, including cardiomyocyte hypertrophy, fibrosis, apoptosis, and necrosis (Tsutsui et al., 2011). As mentioned, Rooibos is known for its good antioxidant and anti-inflammatory capacity and redox modulation in humans (Marnewick et al., 2011) and it is proposed that rooibos polyphenolic compounds could trigger protective cell signalling pathways in cardiomyocytes in addition to reducing inflammation that can lead to the suppression of ventricular remodelling, but these mechanisms still need to be confirmed and further elucidated.

Given its potent antioxidant and anti-inflammatory properties, RES was extensively evaluated in different murine models of cardiac remodelling and HF. The echocardiography variables that demonstrate an association with the interventions are associated with hypertensive heart disease (Dreslinski et al., 1981; Eshoo et al., 2009; Suzuki et al., 2011; Boyd et al., 2013; Lee & Park, 2015). According to these authors, an increased LV mass, reduced LV systolic performance, impaired LV diastolic function and increased left atrial size are all indicators of a poor prognosis linked to hypertension. They further state that hypertension causes left ventricular hypertrophy, a decrease in the LV diastolic function and LA enlargement.

With regards to the descriptive statistics according to gender, **APPENDIX J and APPENDIX K : DESCRIPTIVE DATA** indicated that Rooibos had no effect on the aorta across all three intervention groups for both genders. However, in the male group, Rooibos had a positive effect on left atrium dimensions, with positive changes seen in the green Rooibos and placebo groups. In the male group, positive changes in interventricular septum thickness were observed only in the green Rooibos intervention group. Left ventricular mass showed improvements in the male placebo and green Rooibos groups, while ejection fraction percentage improved in the male green Rooibos and placebo groups. Among females, positive changes were demonstrated in left atrium dimensions across all three intervention groups.

Additionally, interventricular septum thickness and left ventricular mass showed positive changes in the green Rooibos intervention group. These findings suggest that while Rooibos had no impact on aortic measurements, it did show beneficial effects on various cardiac dimensions in both males and females across different intervention groups.

In our study, we measured echocardiography variables related to cardiovascular function. The reduction in the size of certain echocardiography variables observed in our participants may suggest improvements in cardiac structure and function, which can indirectly reflect endothelial function and overall cardiovascular health. While our study did not directly measure lipid profiles or blood pressure, the changes observed in echocardiography parameters are indicative of potential benefits to endothelial function and cardiovascular outcomes. Deka and Vita (2011), as well as Joubert and de Beer (2011), also mention that flavonoids and phenolic acids play an important role in preventing disease. Rooibos serves, therefore, as a promising beverage option for individuals seeking to avoid caffeine-related adverse effects while still enjoying potential health benefits from the antioxidant properties it offers (Canda et al., 2014).

The present research study reveals that the placebo group experienced improvement with regard to the IVS diameter, as well as the LVM. This phenomenon of improvement in the placebo group was also seen in research conducted by Liebson et al. (1995) which involved a double-blind, placebo-controlled clinical trial with 844 participants who had mild hypertension. The participants were divided into two groups: one group received nonpharmacological – or nutritional-hygienic (NH) - intervention plus a placebo, while the other group received NH intervention along with one of five classes of antihypertensive medications. Changes in LVM were assessed using echocardiograms performed at baseline, three months and annually for four years. Both the pharmacologically treated group and the placebo/nonpharmacological (NH) intervention group showed significant reductions in LVM (10% to 15%) from baseline. Another study by Berman et al. (2004) aimed to investigate the impact of coenzyme Q10 (CoQ10) on patients with end-stage heart failure awaiting heart transplantation. The researchers conducted a double-blind, placebo-controlled, randomised study involving 32 patients. For three months, sixteen participants were given 60 mg/day of Ultrasome™ CoQ10, while the other sixteen received a placebo, along with their usual medications. The findings revealed that the group receiving CoQ10 experienced significant improvement; however, there were no significant changes in echocardiography measurements or blood levels of an atrial natriuretic factor (ANF) and tumour necrosis factor (TNF) after three months of treatment. While the precise mechanisms underlying placebo responses remain unclear, evidence suggests that these responses indicate a well-functioning brain structure. Cognitive factors, including the patients' perception of disease symptoms, together with their current cognitive state, emerge as prominent influencers of both the

presence and magnitude of placebo effects (Theodosis-Nobelos et al., 2021). While our study revealed significant change with regards to IVSd and LVM after intervention, it is important to note that differences in body size and composition can affect the absolute measurements of left ventricular mass, potentially resulting in misclassification if not properly adjusted.

In addition to the descriptive statistics, effect sizes were calculated to quantify the magnitude of significance for the green Rooibos LA size, IVSd in the Placebo and green Rooibos groups, as well as the LVM in the Fermented Rooibos group. Below is an explanation of the provided effect sizes. **Please see APPENDIX M** for the calculation.

LA: effect size: dRM = -0.210 (Green Rooibos)

This indicates a small adverse effect, as shown by the negative value suggesting a reduction in the left atrium (LA) measurement in the green Rooibos group over time.

ISVd: effect size: dRM = -0.224 (Placebo)

This also indicates a small adverse effect, as shown by the negative value suggesting a reduction in the interventricular septum in diastole (ISVd) measurement in the Placebo group over time.

ISVd: effect size: dRM = 0.247 (Green Rooibos)

This indicates a small positive effect, suggesting an increase in the ISVd measurement in the green rooibos group over time.

LVM: effect size: dRM = 0.195 (Fermented Rooibos)

This indicates a small positive effect, suggesting an increase in the left ventricular mass in the fermented Rooibos group over time. The above effect sizes indicate that there are small but meaningful changes in the measured variables over time within the different intervention groups.

5.4. Summary of the Chapter

The discussions in this section were aligned with the three objectives of the research study. In the first section of this chapter, the characteristics of the study population were discussed with a special focus on the design of the risk prediction model to assess the prevalence and characteristics of CVD risk factors. Furthermore, this section discussed the echocardiography findings in each of the intervention groups and provided significant positive and negative associations between the cardiovascular ultrasound variables and the impact of the herbal Rooibos interventions on the heart. In the last section of this chapter, the association between the CVD findings and the dietary interventions was discussed. The final chapter of this thesis, Chapter Six, presents the conclusion of the study, as well as recommendations for future research projects.

Chapter 6 | Conclusion and Recommendations

6.1. Conclusion

The aim of the research study was to determine the effect of consuming standardised water extracts of the South African herbal tea, Rooibos, in both its fermented and green form, on the cardiovascular function in adults using trans-thoracic echocardiography, which is a non-invasive and trustworthy imaging tool used widely to evaluate cardiovascular function. Clinical prediction models are valuable tools for estimating a patient's risk of developing a specific disease or experiencing a future event based on their current characteristics (Wynants et al., 2019). As presented in Chapter 3, a four-step risk prediction procedure model was created to determine the cardiovascular risk profile of the study participants. The Logistic Regression Classifier, generally used to predict the probability of an objective variable, was fitted to predict the risk categories in this data set and the probability of those being at risk. According to the classification report, the model used to determine the risk categories was (72.8%) 73 % accurate. According to the classification report, the model used to determine the risk categories achieved an accuracy of 73% (72.8%). Although this level of accuracy is relatively low, it represents a preliminary step towards predicting cardiovascular risks in this population. There is a need for model improvement, and the researcher aims to engage in further studies to enhance the predictive capabilities of the model. The following non-laboratory baseline data: age, blood pressure, blood cholesterol, haemoglobin, blood glucose, BMI and waist circumference were used to calculate the risk profile of participants in this research. A study done by Jahangiry et al. (2022) has found that both non-laboratory and laboratory testing correlate well and are effective risk assessment methods. Both BMI and waist circumference measurements are critical in the management of obesity and associated metabolic diseases.

Table 4-4 provides a breakdown of the study population based on various health risk factors, including BMI, Waist Circumference, Glucose, Haemoglobin, Cholesterol, Systolic Blood Pressure, and Diastolic Blood Pressure. The data is presented in terms of counts and percentages for each risk category: Lower Risk, Medium Risk, Higher Risk, and Very High Risk. The data presented highlights the distribution of various health risks within the study population. Each health risk metric has a relatively even distribution across the four risk categories, which suggests that the population exhibits a diverse range of risk profiles. With regards to the BMI Risk, the distribution indicates that the population is evenly spread across the BMI risk categories, suggesting no single category predominates. This implies a balanced representation of participants with varying BMI levels. Similar to BMI, the waist circumference risk is also evenly distributed, indicating a mix of participants with different waist sizes, which is a significant factor in assessing obesity-related health risks. The slightly higher counts and

percentage in the lower-risk category [n = 64 (29.5%)] compared to others might indicate a relatively lower prevalence of high glucose levels in the population. The haemoglobin distribution shows a slight variation, with the medium risk category being slightly higher, suggesting a more varied haemoglobin level within the population. The even distribution across all categories indicates a balanced spread of cholesterol levels among participants. The slight variations in the distribution of systolic and diastolic BP risks highlight the importance of considering both metrics when assessing cardiovascular risk.

The study population is evenly distributed across the various risk categories for each health metric, highlighting a balanced representation of risk factors. This distribution allows for comprehensive analysis and understanding of the health risks prevalent in the population. The even spread of participants across risk categories ensures that the study's findings are robust and can be generalised to a larger population. Future studies can consider these distributions to explore the relationships between different risk factors and health outcomes. The risk prediction model has shown that of n = 219 participants, n = 118 (53.9%) were in the lower risk frequency for cardiovascular disease, and n = 101 (46.1%) were in the higher risk frequency.

The 12-week interventions showed some beneficial effects on echocardiographic parameters, including minor reductions left atrium sizes, interventricular septal thickness, and left ventricular mass. However, the improvements were more pronounced with the green Rooibos intervention. The statistical analysis findings suggest that *Aspalathus linearis* (Rooibos) has been effective in modulating the cardiovascular function of participants in this research study and that Rooibos were shown to have an impact on the cardiovascular variables. It is reassuring to know that there was statistical significance between the cardiovascular function modulation of participants in this research study and consuming Rooibos. Smit et al. (2022) demonstrated that supplementation with green Rooibos extract for 6 weeks protected cardiovascular disease-compromised rat hearts from ischemia/reperfusion injury by reducing inflammation, oxidative stress, and heart rate. Similarly, Obasa et al. (2021) investigated the impact of green Rooibos extract on obese Wistar rats and observed that it mitigated cardiovascular risk factors in high-fat diet (HFD) animals, indicating potential therapeutic benefits of green Rooibos extract in obesity-related cardiovascular risks. The findings from these studies highlight the potential of green Rooibos extract as a protective agent against cardiovascular complications associated with both disease and diet-induced risk factors. The beneficial effect of Rooibos on cardiovascular health can be attributed to their rich content of antioxidants, anti-inflammatory compounds, and other bioactive substances that contribute to cardiovascular, metabolic health as discussed in Chapter 2. The clinical significance of these findings must still be further investigated to clearly understand the clinical usefulness of

Rooibos and cardiovascular health and how this might impact clinicians' decision-making. In light of these findings, it is noteworthy that no adverse events were reported in the studies involving Rooibos, and its side effect profile was found to be safe, suggesting that the risk-benefit of incorporating Rooibos into an existing treatment regimen is favourable.

APPENDIX N provides a clear and concise overview of the risk-of-bias assessment for this study based on the Revised Cochrane Risk-of-Bias Tool for Randomised Trials (RoB 2) according to Eldridge et al. (2016).

6.2. Recommendations

1. The risk prediction model developed to assess cardiovascular disease risk factors in this participant group achieved an accuracy rate of 73%. While no modifications were made to the model in this study, it is advisable to explore alternative statistical methods in future research to enhance accuracy.
2. A study done by Yusuf and colleagues (2005) to explore obesity and the risk of myocardial infarctions, discovered that BMI, waist circumference and waist–hip ratio were strongly and linearly associated with the risk of myocardial infarction. The present study considered only the BMI and waist circumference and the researcher would, therefore, recommend that future studies should include waist-hip ratio and waist-height ratio to measure abdominal obesity.
3. The current study used only the LA diameter measurement to quantify the LA size and, while research has shown that the LA diameter measurement is effective in quantifying LA size, the left atrium volume measurement should also be considered, together with the LA diameter measurement.
4. The effect of the BMI differences between the three intervention groups was not investigated in this study, the researcher therefore recommends that further studies be employed to investigate the effect of BMI across the three intervention groups.
5. While our study did not assess changes in blood pressure post-intervention, as it was not within the study's scope, positive changes were observed in specific echocardiography variables indicative of improved cardiac structure and function, commonly linked to markers of hypertension in echocardiography. Future research could investigate the potential relationship between Rooibos consumption and changes in blood pressure to enhance our understanding of its cardiovascular effects comprehensively.
6. It is important to acknowledge that both body mass index and waist circumference are commonly used indicators of obesity, and their high correlation could indeed introduce bias by potentially penalising more obese participants twice for the same

underlying risk factor. Despite their correlation, BMI and WC provide distinct information. BMI is a general measure of overall body fat, whereas WC specifically captures central adiposity, which is a stronger predictor of cardiovascular risk. Including both can therefore enhance the precision of risk stratification if handled correctly. It is acknowledged that by using both, it has the potential to create bias and it is therefore recommended that in future publications that focus on risk assessments this should be addressed.

6.3. Limitations of the study

1. The timeline of the initial study was influenced by COVID-19, but not the quality of the methodology as it was not compromised by a shift in the timeline.
2. Important risk factors for cardiovascular disease, such as physical activity, smoking, and alcohol intake were excluded from this study. The exclusion of these variables can limit the comprehensiveness of the risk assessment.
3. The relationship with BP was not explored in this study, which could have provided valuable information on the impact of Rooibos on BP.
4. This study used the LVM in gram formula to calculate LVM, meaning the BSA was excluded, while BSA allows for a more detailed understanding of LVH in relation to body composition.

6.4. Final Conclusion

Oxidative stress is considered a major factor in the development of various diseases, especially those related to ageing, and numerous cardiovascular risk factors are closely linked to increased oxidative stress-induced myocardial injury (Weidinger and Kozlov, 2015; Dludla et al., 2017). This study indicates that Rooibos herbal tea is an effective dietary source of antioxidants and that regular daily consumption may positively influence cardiovascular function in adults at risk for cardiovascular disease.

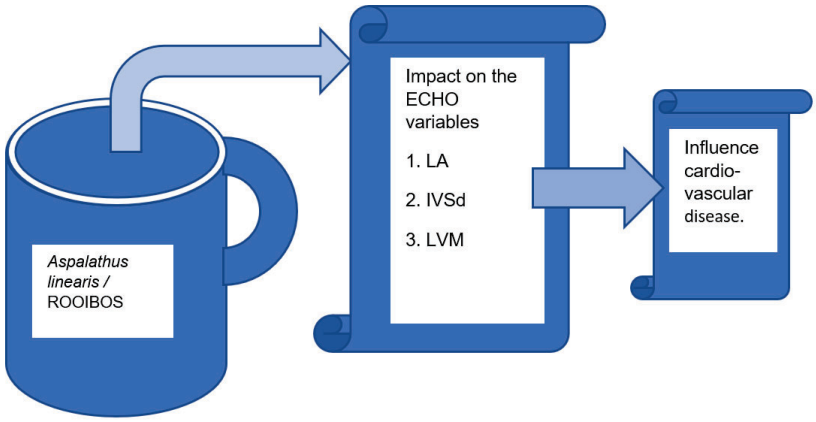

Figure 6-1 Schematic illustration of the findings of this research study[11]

As demonstrated in **Figure 6.1,** the findings are in line with statements made by Smith and Swart (2018), and many other researchers, that the bioactivities in *Aspalathus linearis* (Rooibos) have the potential to influence cardiovascular disease. In light of the global crisis posed by NCDs, which continue to claim countless lives and place immense strain on healthcare systems, it is evident that effective preventive measures are imperative. The profound socio-economic impact of NCDs, as highlighted by the World Health Organisation, underscores the critical importance of addressing this health challenge as a fundamental development priority in the 21st century. Moreover, the United Nations' recognition of NCDs in the 2030 Agenda for Sustainable Development reaffirms their status as a significant global concern. The results of this study contribute valuable insights into the potential role of Rooibos in mitigating NCDs, particularly in relation to cardiovascular health. While our findings demonstrated statistical significance, the clinical significance must still be determined and further research will contribute to understanding the relationship between Rooibos and cardiovascular disease prevention.

[11] Figure 6:1 Represents the author's construct

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APPENDIX A: ROOIBOS CAPSULE INFORMATION

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Cape Peninsula University of Technology

Certificate of Analysis

Assay for: Jeanine Marnewick

Results per g

Sample #	Batch	Total Polyphenols mg GAE/g	TEAC umol TE/g	FRAP umol AAE/g
Potato starch	1	N.D.	N.D.	N.D.
	2	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.
Topiaca starch	1	N.D.	N.D.	N.D.
	2	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.
Green Rooibos	1	280.80	2951.80	864.85
	2	315.48	3061.44	876.86
	3	271.80	2963.11	840.27
Fermented Rooibos	1	217.30	2400.50	561.67
	2	217.60	2175.98	504.09
	3	213.49	2222.09	474.13

Results per capsule

Sample #	Batch	Total Polyphenols mg GAE/capsule	TEAC umol TE/capsule	FRAP umol AAE/ capsule
Potato starch	1	N.D.	N.D.	N.D.
	2	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.
Topiaca starch	1	N.D.	N.D.	N.D.
	2	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.
Green Rooibos	1	183.00	1923.69	563.62
	2	202.44	1964.53	562.68
	3	177.46	1934.61	548.61
Fermented Rooibos	1	123.49	1363.20	319.19
	2	129.97	1299.71	301.10
	3	128.01	1332.37	284.29

Results per g

Sample #	Green Rooibos mg/g	Fermented Rooibos mg/g
Aspalathin	24.09	1.38
Orientin	4.93	2.20
Isoorientin	5.03	2.23
Isovitexin	1.46	0.55
Vitexin	1.28	0.65
Hyperoside/Rutin	0.22	0.30
Quercetin	1.77	0.53
Luteolin	0.09	0.13
Chrysoeriol	0.02	0.03

Results per capsule

Sample #	Green Rooibos mg/capsule	Fermented Rooibos mg/capsule
Aspalathin	15.70	0.78
Orientin	3.21	1.25
Isoorientin	3.28	1.32
Isovitexin	0.95	0.31
Vitexin	0.83	0.37
Hyperoside/Rutin	0.14	0.17
Quercetin	1.15	0.30
Luteolin	0.06	0.07
Chrysoeriol	0.01	0.02

Assay details:

The FRAP value was determined using the method as described by: Benzie IFF, Strain JJ. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, Volume 299, Pages 15-27.

Total polyphenols were determined using the method as described by: Singleton V.L. and Rossi, J.A. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture. **16**(3):144-158.

The TEAC assay was performed using the method as describe by: Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation assay. *Free radical Biology and Medicine*, **26**:1231-1237.

HPLC parameters

HPLC column : YMC - Pack Pro C18
Mobile phase A : Water with 300µl/L formic acid
Mobile phase B : Methanol with 300µl/L formic acid
Run Time : 30 minutes
Column Temperature : 21°C
Flow rate : 1.0 mL/min
UV detection : 287nm, 360nm
Injection : 20µL

Analysis date : 11/06/2021
Analysed by : G.S. Rautenbach
Authorised by : J.L. Marnewick

G.S. Rautenbach
Laboratory Manager

APPENDIX B: LETTER OF ETHICS APPROVAL

Cape Peninsula
University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (CPUT HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

22 February 2023
REC Approval Reference No:
CPUT HWS-REC 2021/H29 (Renewal)

Faculty of Health and Wellness Sciences

Dear Ms. M Hartnick

Re: APPLICATION TO THE CPUT HWS-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to **Ms. M Hartnick** for ethical clearance. This approval is for research activities related to research for **Ms. M Hartnick** at Cape Peninsula University of Technology.

TITLE: **Modulation of cardiovascular function by Rooibos in adults at risk for cardiovascular disease**

Supervisors: Prof. J von Metzinger and Prof .P Engel-Hills

Comment:

Approval will not extend beyond 23 February 2024. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the CPUT HWS-REC in December of that particular year, for the CPUT HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Ms. Carolynn Lackay
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

APPENDIX C: PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT

Applied Microbial and Health Biotechnology Institute, Cape Peninsula University of Technology

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Participant Information Sheet and Informed Consent Form

This Informed Consent Form is for men and women who we are invited to participate in research on Rooibos and its potential heart health benefits. The title of our research project is "Rooibos and Heart Health"

Name of Study leader: Prof JL Marnewick

Name of Organization: Applied Microbial and Health Biotechnology Institute

Name of Sponsor: Cape Peninsula University of Technology - Prestigious Project Funding & South African rooibos Council

Name of Project proposal: Modulation of cardiovascular function by Rooibos in adults at risk for cardiovascular disease

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with you)**
- **Certificate of Consent (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

I am Prof JL Marnewick, Director of the Applied Microbial and Health Biotechnology Institute of CPUT. We are doing research to establish the health benefits of Rooibos, which is a homegrown herbal tea in South Africa. I am going to give you information and invite you to be part of this research. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain it. If you have questions later, you can ask them of me, or any member of the study team present here today.

Purpose of the research

Oxidative stress has the potential to damage the cells in your body and contributes to the development of various important chronic diseases such as heart disease, cancer, and diabetes. This research study aims to address this issue of cell damage by focusing on herbal rooibos tea, as a new measure to reduce this oxidative stress-induced damage in your body.

What will be expected of you?

This research will involve you to drink 3 capsules containing either Fermented/traditional Rooibos or Unfermented/Green Rooibos or a placebo every day with your food for a period of twelve (12) weeks. Blood samples will be taken on four (4) occasions from your forearm during this study period. You will also be required to provide three (3) 24-hr urine samples during this study period.

Who can take part in the study?

- Adults within the age range of 30 to 80 years' old
- A stable body weight 6 months before the study starts.
- Female participant not pregnant or breast feeding.
- Individuals who consume less than 2 alcoholic beverages per day.
- Individuals who follows a regular diet and not a special diet (i.e. vegetarian)
- Individuals with no known heart disease, diabetes, kidney or liver disease.
- Individuals with no endocrine disorders.
- Individuals not taking any medication, vitamins or dietary supplements with established antioxidant properties.
- Participants with any two or more of the following risk factors: hyperlipidemia (raised cholesterol - >5.5-7.7 mmol/L), pre-hypertension (120-139/80-90 mm Hg), smoking, increased BMI (>25 to 38 kg/m^2), but not requiring any medication for these medical conditions and family history of cardiovascular disease. The BMI measurement can be obtained using your weight in kilograms (kg), divided by your height in meters squared, but not requiring any medication for these medical conditions and family history of cardiovascular disease.

Voluntary Participation

Your participation in this research study is entirely voluntary. It is your choice whether to participate or not. Even if you have agreed to take part in the study, you may still stop participating at any time you want without any consequences to you.

Procedures and Protocol

This project forms part of a human intervention study to determine the effect of rooibos consumption on antioxidant/oxidative stress, inflammation and certain genetic measures in the blood of volunteers at risk for developing heart disease. There are no known risks to participate in this study. If you volunteer to take part in this study, you will be asked to do the following:

1) Answer questions and complete questionnaires about your demography, health, diet/food intake, dietary supplements, and physical activity. The study nurse will also take my body measurements, (such as height, weight, and waist circumference) at 4 (four) occasions during the study.

2) Take part in a study over a 14 (fourteen) week period which includes the completion of a self-administered dietary record, based on your habitual dietary intake for the first 2 (two) weeks. During this 2-week period (known as the *wash-out period*) you will be requested to follow a flavonoid-restricted, omit/restrict certain antioxidant-containing food and beverages from your diet and continue to complete the dietary records. The following 12 (twelve) weeks is the *intervention period* in which you will consume either 3 capsules containing a green rooibos tea extract or 3 capsules containing a fermented rooibos tea extract or 3 capsules containing a placebo (does not contain any antioxidants) daily with meals, follow the same flavonoid-restricted diet and once again complete the dietary food records. You will receive training at the beginning of the study on how to complete the dietary records, flavonoid frequency questionnaire and the health questionnaire.

3) If you are willing, a qualified phlebotomist or nursing sister will take samples of your blood from your forearm, take your blood pressure measurements, while trained professionals will examine & evaluate the structures of your eyes, as well as do an ultrasound scan of your neck and chest area. These procedures will be done on 4 (four) occasions. The first blood sample (1 blood tube, approximately two teaspoons in volume) to be drawn will be for screening purposes before the study starts to determine if you are eligible to take part in the study, while the remaining 3 (three) multiple samples (4 blood tubes) will be drawn 2 weeks after the beginning of the study, after 8 weeks and again after 12 weeks/end of the study. Blood samples will be analysed to determine your general health, blood lipids, antioxidant/oxidative stress status, inflammatory& stress status, as well as include your metabolomic & genetic profiling with respect to coronary heart disease and the metabolic syndrome. Individuals with different genetic profiles react differently to dietary and lifestyle factors and genetic testing may explain why only a subset of the study participants may benefit from the rooibos intervention. The blood samples will only be used for the intended purpose of this study.

4) The questions and blood tests are not for diagnostic purposes; your blood will not be tested for HIV-AIDS. Should the study doctor deem it necessary after your blood test results are known, he will refer you to your personal physician or local clinic doctor.

5) To also supply 3 (three) 24 hour-urine samples on the same time points as the blood samples for antioxidant content, oxidative stress and related biochemistry analyses.

6) Someone from the study may call you to clarify your information.

7) A qualified sonographer will perform **two** echocardiography examinations (ultrasound of the heart) on you, one at the beginning of the study and one at the end of the 12 weeks' period. This is a painless procedure. You will be asked to lie on

a bed inside the examination room. A hospital gown will be given to you to cover your chest area. A small device or instrument will be place on your chest area to take images of your heart.

All these above procedures will probably be familiar to you as most of them occur when you visit your general practitioner.

Randomization

Because we do not know if the rooibos capsules will be better, we need to compare it to a placebo capsule (containing no antioxidants). To do this, we will place people taking part in this research into three groups. The groups are selected by chance, as if tossing a coin, also called *randomization.* Participants in one group will be given the green rooibos capsule while participants in the other two group will be given the fermented rooibos capsule or the placebo capsule. The placebo or inactive capsule will look like the rooibos capsule, but does not contain the active substances. It is important that neither you nor we know which of the three capsules you are given. This information will be in our files, but we will not look at these files until after the research is finished or unless deemed necessary by the study doctor. This is the best way we have for testing without being influenced by what we think or hope might happen. We will then compare which of the three has the best results.

Side Effects

We do not anticipate any side affects you may experience, but should you experience any unease or feel unsure during the study please contact the study doctor, Dr D van Velden (contact number 082 4676738) or study leader (Prof JL Marnewick at 0828979352) immediately and they will advise and guide you in this regard.

Risks

No risk is expected but you may experience some discomfort when your blood is drawn or when the researchers ask you questions about your health, nutrition, physical activity and smoking habits. The risks of drawing blood from your arm include the unlikely possibilities of a small bruise or localized infection and bleeding. These risks will be reduced by using a qualified phlebotomist or nursing sister to draw the blood.

Benefits

Apart from getting to know certain of your health indicators such as blood pressure, cholesterol and blood glucose levels, there are not any direct benefit for you but your participation is likely to help us find the answer to the research question.

Costs

You will not be given any money or gifts to take part in this research study nor will you be charged any costs for the results of the brief health report, which will include your cholesterol level, glucose level, blood pressure and body mass index (BMI). However, you

will be given a small amount of money to cover your travelling expenses to and from the research facility.

Confidentiality

Your personal information we collect during this research study will be kept confidential, it will be stored in a locked filing cabinet and/or a password protected computer to which only the study leader, Prof JL Marnewick will have access. Any information about you will have a number on it instead of your name. Only the study leader will know what your number is, it will not be shared with or given to anyone except Dr Van Velden, the study doctor, if and when deemed necessary.

Sharing the Results

The knowledge that we get from doing this research will be shared at public meetings such as conferences and we will also publish the results in order that other interested people may learn from our research. Confidential information will not be shared. You should note that this is a long process and may take up to 4 years after the study has been completed.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

Who to Contact?

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact the study coordinator, Dr Nicole van der Merwe at 0828761064 or email: Rooibostrial@cput.ac.za or the study PI, Prof JL Marnewick at 021-9538416 or email marnewickj@cput.ac.za at the Oxidative Stress Research Centre of the Cape Peninsula University of Technology, Bellville Campus, Corner of Symphony way and Robert Sobukwe Drive, Bellville East, Bellville).

This research study has been approved by the Faculty of Health and Wellness Research Ethics Committee (H&W REC), Cape Peninsula University of Technology; a committee tasked to make sure that research participants are protected from harm. If you wish to find more about the H&W REC, contact The Chairperson, H&W REC, Tel: +27 21 959 6917; email: sethn@cput.ac.za.

PART II: Certificate of Consent

Declaration by study participant:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research. I may choose to leave this study at any time and will not be penalised or prejudiced in any way. I

may be asked to leave the study before it has finished if the study doctor or study leader feels it is in my best interest or if I do not follow the study plan as agreed to.

Place_____

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

Signature of witness_____

Declaration by investigator:

I declare that I have explained the information in this document to the study participants and encourage them to ask questions and took adequate time to answer them. I am satisfied that he/she adequately understands all aspects of the research study as discussed above. I did/did not use an interpreter. (If an interpreter is used the interpreter must sign the declaration below).

Place_____

Print Name of Investigator_____

Signature of Investigator _____

Date _____Day/month/year

Signature of witness_____

Declaration by interpreter:

I declare that I assisted the investigator to explain the information in this document to the participant using the language medium of Afrikaans/Xhosa. We encouraged him/her to ask questions and took adequate time to answer them. I conveyed a factual correct version of what was related to me. I am satisfied that the participant fully understands the content of this information and informed consent document and has had all his/her questions satisfactorily answered.

Place_____

Print Name of Interpreter_____

Signature of Interpreter _____

Date _____Day/month/year

Signature of witness_____

Thumb print of participant

INFORMATION FOR PARTICIPANTS HAVING AN ECHOCARDIOGRAM (ULTRASOUND OF THE HEART)

This leaflet aims to answer your questions about having an Echocardiography Ultrasound scan. It explains the benefits, risks as well as what you can expect. If you have any further questions, please ask the nursing sister available.

What is an ultrasound scan?

An ultrasound machine uses high frequency sound waves to image parts of the human body including the heart. This study will use such a machine to look at the function of your heart. You will receive two ultrasound scans of your heart, one at the start of the study and the final scan at the end of the 12-week study period. The ultrasound will be performed at the Oxidative stress building of the AMHBI on the Bellville campus at CPUT. A room suitable for performing Ultrasound examinations has been allocated that is fully fitted with a moveable table, ultrasound system and accessories needed to perform the ultrasound.

What happens during an ultrasound scan of the heart?

This ultrasound examination takes about 30 minutes. It is a painless examination and requires you to lie on your back while a small instrument called a probe is moved across the chest area to take pictures and recordings of your heart. A water-based gel is first applied to the skin so the probe has good contact and can move smoothly over the skin. Gel which might get onto your clothes will wash out easily. Some patients find this test relaxing. Images of the heart are captured onto the Ultrasound system and reviewed by the sonographer.

What are the risks?

There are no known risks to ultrasound imaging. Please advise the staff if you may be allergic to the silicone gel.

How can I prepare for my ultrasound scan?

Ensure that you wear loose fitting clothes around your chest area to allow adequate access for the scan, for example if possible if you are a female do not wear a bra. Please remove all jewellery such as earrings, studs, or necklaces before the scan.

Will I feel pain?

This procedure is not painful, but you will need to remain still for the duration of the scan. The gel may feel a bit cold and wet.

What happens after I have had the ultrasound scan?

- After the examination you will be able to return to work or home
- You should have no after-effects
- You can eat and drink and carry on with all your normal activities

If you have any questions, please speak to our nursing sister or study staff.

APPENDIX D ECHOCARDIOGRAPHY REPORT TEMPLATE

ECHOCARDIOGRAPHY REPORT

Participants number:

Study Date:

Gender: DOB: Age :

2D Mode Dimensions

LV (Left ventricle)

IVS ED:

IVS ES:

LVPW ED:

LVPW ES:

LVID ED:

LVID ES:

EF (Ejection Fraction):

LA (Left Atrium):

AO root (Aorta):

AO/LA Ratio:

Valvular Function:

Mitral valve (MV):

Aorta valve (AV):

Tricuspid valve (TV):

Pulmonary Valve (PV):

Echocardiography Findings

LV size and function:

……

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RV size and function:

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Left Atrium:

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Right Atrium:

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Aortic root:

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Valves:

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Pericardial / Pleural effusion:

……

……

Final Comments:

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……

Echocardiographer:

APPENDIX E: EXAMPLE OF THE DATA SHEET WITH ECHOCARDIOGRAPHY MEASUREMENTS

CARD Report

Patient Information

Patient Name:	Patient ID: 713fu	Exam Date: 08/04/2022
Birth Date:	Gender: Unknown	Accession#:
BP(mmHg):		
Description:		

Exam Information

Exam Type: CARD	Height(cm):	Weight(kg):
Heart Rate(bpm):	BSA(m^2):	
Sonographer:	Referring.M.D:	Performing.M.D:
Chief Complaint:		
Past History:		
Comments:		

Measurements

M Mode

Parameter	M1-M5	Value	Unit
AO	2.80	2.80 (Last)	cm
LA	3.83	3.83 (Last)	cm
LA/AO		1.37	
RVOT			
RVOT	3.08	3.08 (Last)	cm
LV Mass (Cube)			
IVSd	1.42	1.42 (Last)	cm
LVIDd	4.98	4.98 (Last)	cm
LVPWd	1.26	1.26 (Last)	cm
LVM(Cube)		272.54	g
LVMI(Cube)		******	g/m2
LVIDd	4.90	4.90 (Last)	cm
LVIDs	3.56	3.56 (Last)	cm
EDV(Teich)		112.75	mL
ESV(Teich)		52.83	mL
EF(Teich)		53.14	%
SV(Teich)		59.92	mL
CO(Teich)		******	L/min
FS(Teich)		27.42	%
CI(Teich)		******	L/min/m2
SI(Teich)		******	mL/m2

Doppler Mode

Parameter	M1-M5	Value	Unit
RVSP			
TR Vmax	-245.26	-245.26 (Last)	cm/s
TR PGmax	24.06	24.06 (Last)	mmHg
RAP		******	mmHg
RVSP		******	mmHg
MV E Vel	46.34	46.34 (Last)	cm/s
MV E PG	0.86	0.86 (Last)	mmHg
MV A Vel	68.44	68.44 (Last)	cm/s

APPENDIX F: THE PYTHON CODE OF THE GENERATED RESULTS

```
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
import matplotlib
import seaborn as sns
import pandas as pd

import pandas as pd
df = pd.read_excel(r'C:\Users\lekatas\Documents\CPUT work staf 2021\2023 Statistical
Consultations\Maria Hartnick\Part2 Article\Corrected_data1.xlsx', sheet_name= 'Python')



df1 = df[[ "Visit", "Intervention","Visit_Intervention","Age",    "AO",   "LA",    "LA / AO Ratio",
          "IVSd","LVPWd",       "LVM", "LVIDd",          "LVIDs",          "EF (Teich)"]]
# drop rows with missing Reset index after drop
df1 = df1.dropna().reset_index(drop=True)
df1.to_excel("Visit_intervension.xlsx")

#scatter plot OA
sns.catplot(x="Intervention", y="AO", kind="swarm", hue = "Visit", data=df1)
plt.title("AO Size per visit & Intervention")
plt.ylabel("AO Size (cm)")
#scatter plot LA
sns.catplot(x="Intervention", y="LA", kind="swarm", hue = "Visit", data=df1)
plt.title("LA Size per visit & Intervention")
plt.ylabel("LA Size (cm)")
#scatter plot LA
sns.catplot(x="Intervention", y="LA / AO Ratio", kind="swarm", hue = "Visit", data=df1)
plt.title("LA / AO Ratio Size per visit & Intervention")
plt.ylabel("LA / AO Ratio")
#scatter plot LA
sns.catplot(x="Intervention", y="LVPWd", kind="swarm", hue = "Visit", data=df1)
plt.title("LVPWd Size per visit & Intervention")
```

```
plt.ylabel("LVPWd size (cm)")
#scatter plot LVM
sns.catplot(x="Intervention", y="LVM", kind="swarm", hue = "Visit", data=df1)
plt.title("LVM Size per visit & Intervention")
plt.ylabel("LVM size (cm)")
#scatter plot LVIDs
sns.catplot(x="Intervention", y="LVIDs", kind="swarm", hue = "Visit", data=df1)
plt.title("LVIDs Size per visit & Intervention")
plt.ylabel("LVIDs size (cm)")
#scatter plot LVIDd
sns.catplot(x="Intervention", y="EF (Teich)", kind="swarm", hue = "Visit", data=df1)
plt.title("EF (Teich)% per visit & Intervention")
plt.ylabel("EF (Teich)%")

#make pivot table - AO
pv1 = df1.pivot_table(values='AO', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("AO size - Visit & Intervention", fontsize=13)
plt.ylabel("AO size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. AO Size per intervention & Visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)

#make pivot table - LA
pv1 = df1.pivot_table(values='LA', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
```

```
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("LA size - Visit & Intervention", fontsize=13)
plt.ylabel("LA size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. LA size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)



#make pivot table - LA
pv1 = df1.pivot_table(values='LA / AO Ratio', index = 'Intervention',columns=
["Visit"],aggfunc= 'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("LA / AO Ratio - Visit & Intervention", fontsize=13)
plt.ylabel("LA / AO Ratio", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. LA / AO Ratio per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)

#
```

```
#make pivot table - IVSd
pv1 = df1.pivot_table(values='IVSd', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("IVSd size - Visit & Intervention", fontsize=13)
plt.ylabel("IVSd size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. IVSd size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)

#make pivot table - LVPWd
pv1 = df1.pivot_table(values='LVPWd', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("LVPWd size - Visit & Intervention", fontsize=13)
plt.ylabel("LVPWd size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. LVPWd size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
```

```
plt.xticks(fontsize=11,rotation=0)

#make pivot table - LVM
pv1 = df1.pivot_table(values='LVM', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("LVM size - Visit & Intervention", fontsize=13)
plt.ylabel("LVM size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. LVM size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)



#make pivot table - LVIDd
pv1 = df1.pivot_table(values='LVIDd', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("LVIDd size - Visit & Intervention", fontsize=13)
plt.ylabel("LVIDd size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
```

```
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. LVIDd size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)

#make pivot table - LVIDs
pv1 = df1.pivot_table(values='LVIDs', index = 'Intervention',columns= ["Visit"],aggfunc=
'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("LVIDs size - Visit & Intervention", fontsize=13)
plt.ylabel("LVIDs size(cm)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. LVIDs size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)

#make pivot table - EF (Teich)
pv1 = df1.pivot_table(values='EF (Teich)', index = 'Intervention',columns=
["Visit"],aggfunc= 'mean')
pv1.to_excel("test1.xlsx")
#plot pivot table
pv1.plot(kind='bar')
plt.xlabel("Intervention", fontsize=11)
plt.title("EF (Teich) - Visit & Intervention", fontsize=13)
plt.ylabel("EF (Teich)", fontsize=11)
plt.xticks(fontsize=11,rotation=90)
#make a heat map
map1 = sns.heatmap(pv1, annot=True,fmt=".3f", cmap="Reds") # add annot
```

```
#map1 = sns.heatmap(pv1, annot=True,fmt=".2f") # add annot
#map1.set_title("Heatmap - Average AO size")
plt.xlabel("Visits", fontsize=11)
plt.title("Ave. EF (Teich) size per intervention & visit", fontsize=13)
plt.ylabel("Intervention", fontsize=11)
plt.xticks(fontsize=11,rotation=0)



#BOX PLOTS
df1 = df[[ "Visit", "Intervention","Visit_Intervention","Age",    "AO",   "LA",    "LA / AO Ratio",
           "IVSd","LVPWd",       "LVM", "LVIDd",          "LVIDs",          "EF (Teich)"]]
df1=df1.dropna().reset_index(drop=True)

# BOX PLOTS OA
variable = df1["AO"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["AO"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["AO"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["AO"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["AO"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["AO"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["AO"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
```

```
box = plt.boxplot(data_1,labels=["All AO data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - AO per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)

# BOX PLOTS LA
variable = df1["LA"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["LA"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["LA"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["LA"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["LA"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["LA"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["LA"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All LA data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - LA per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)

# BOX PLOTS LA / AO Ratio
variable = df1["LA / AO Ratio"].to_numpy()
#Visit 1 Fermented Rooibos
```

```
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["LA / AO Ratio"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["LA / AO Ratio"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["LA / AO Ratio"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["LA / AO Ratio"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["LA / AO Ratio"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["LA / AO Ratio"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All LA/AO data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - LA/AO Ratio per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)



# BOX PLOTS IVSd
variable = df1["IVSd"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["IVSd"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["IVSd"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
```

```
v1_G = v1g["IVSd"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["IVSd"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["IVSd"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["IVSd"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All IVSd data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - IVSd per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)

# BOX PLOTS LVPWd
variable = df1["LVPWd"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["LVPWd"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["LVPWd"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["LVPWd"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["LVPWd"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["LVPWd"].to_numpy() # change to array
#Visit 2 Green Rooibos
```

```
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["LVPWd"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All LVPWd data","visit 1 FR","visit 1 P","visit 1 GR","visit
2 FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - LVPWd per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)



# BOX PLOTS LVM
variable = df1["LVM"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["LVM"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["LVM"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["LVM"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["LVM"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["LVM"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["LVM"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All LVM data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
```

```
plt.title(" Boxplots - LVM per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)



# BOX PLOTS LVM
variable = df1["LVIDd"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["LVIDd"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["LVIDd"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["LVIDd"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["LVIDd"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["LVIDd"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["LVIDd"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All LVIDd data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - LVIDd per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)



# BOX PLOTS LVIDs
variable = df1["LVIDs"].to_numpy()
#Visit 1 Fermented Rooibos
```

```
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["LVIDs"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["LVIDs"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
v1_G = v1g["LVIDs"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["LVIDs"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["LVIDs"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["LVIDs"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All LVIDs data","visit 1 FR","visit 1 P","visit 1 GR","visit 2
FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - LVIDs per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)



# BOX PLOTS EF (Teich)
variable = df1["EF (Teich)"].to_numpy()
#Visit 1 Fermented Rooibos
v1f = df1.loc[df1['Visit_Intervention'] == "Visit 1 Fermented Rooibos"]
v1_f = v1f["EF (Teich)"].to_numpy()
#Visit 1 Placebo
v1p = df1.loc[df1['Visit_Intervention'] == "Visit 1 Placebo "]
v1_P = v1p["EF (Teich)"].to_numpy() # change to array
#Visit 1 Green Rooibos
v1g = df1.loc[df1['Visit_Intervention'] == "Visit 1 Green Rooibos"]
```

```
v1_G = v1g["EF (Teich)"].to_numpy() # change to array
#Visit 2 Fermented Rooibos
v2f = df1.loc[df1['Visit_Intervention'] == "Visit 2 Fermented Rooibos"]
v2_f = v2f["EF (Teich)"].to_numpy()
#Visit 2 Placebo
v2p = df1.loc[df1['Visit_Intervention'] == "Visit 2 Placebo "]
v2_P = v2p["EF (Teich)"].to_numpy() # change to array
#Visit 2 Green Rooibos
v2g = df1.loc[df1['Visit_Intervention'] == "Visit 2 Green Rooibos"]
v2_G = v2g["EF (Teich)"].to_numpy() # change to array

data_1 = [variable,v1_f,v1_P,v1_G,v2_f,v2_P,v2_G]
#PLOT
box = plt.boxplot(data_1,labels=["All EF (Teich) data","visit 1 FR","visit 1 P","visit 1
GR","visit 2 FR","visis 2 P", "visit 2 GR"], notch=True)
plt.title(" Boxplots - EF (Teich) per visit & intervention", fontsize=12)
plt.xticks(rotation = 90)

Descriptive Stats table:
Min = df1['EF (Teich)'].min()
Max = df1['EF (Teich)'].max()
Q1 = df1['EF (Teich)'].quantile(q=0.25)
Q2 = df1['EF (Teich)'].quantile(q=0.50)
Q3 = df1['EF (Teich)'].quantile(q=0.75)
Mean= df1['EF (Teich)'].mean()
Median = df1['EF (Teich)'].median()
Var = df1['EF (Teich)'].var()
Stdv = df1['EF (Teich)'].std()
Skew = df1['EF (Teich)'].skew()
Kurt = df1['EF (Teich)'].kurt()

All_data = [Min,Max,Q1,Q2,Q3,Mean,Median,Var,Stdv,Skew,Kurt]

df2 = df1.loc[(df1["Visit_Intervention"] =="Visit 1 Fermented Rooibos")]
Min2 = df2['EF (Teich)'].min()
Max2 = df2['EF (Teich)'].max()
```

```
Q12 = df2['EF (Teich)'].quantile(q=0.25)
Q22 = df2['EF (Teich)'].quantile(q=0.50)
Q32 = df2['EF (Teich)'].quantile(q=0.75)
Mean2= df2['EF (Teich)'].mean()
Median2 = df2['EF (Teich)'].median()
Var2 = df2['EF (Teich)'].var()
Stdv2 = df2['EF (Teich)'].std()
Skew2 = df2['EF (Teich)'].skew()
Kurt2 = df2['EF (Teich)'].kurt()

V1FR = [Min2,Max2,Q12,Q22,Q32,Mean2,Median2,Var2,Stdv2,Skew2,Kurt2]

df3 = df1.loc[(df1["Visit_Intervention"] =="Visit 1 Placebo ")]
Min3 = df3['EF (Teich)'].min()
Max3 = df3['EF (Teich)'].max()
Q13 = df3['EF (Teich)'].quantile(q=0.25)
Q23 = df3['EF (Teich)'].quantile(q=0.50)
Q33 = df3['EF (Teich)'].quantile(q=0.75)
Mean3= df3['EF (Teich)'].mean()
Median3 = df3['EF (Teich)'].median()
Var3 = df3['EF (Teich)'].var()
Stdv3 = df3['EF (Teich)'].std()
Skew3 = df3['EF (Teich)'].skew()
Kurt3 = df3['EF (Teich)'].kurt()

V1P= [Min3,Max3,Q13,Q23,Q33,Mean3,Median3,Var3,Stdv3,Skew3,Kurt3]

df4 = df1.loc[(df1["Visit_Intervention"] =="Visit 1 Green Rooibos")]
Min4 = df4['EF (Teich)'].min()
Max4 = df4['EF (Teich)'].max()
Q14 = df4['EF (Teich)'].quantile(q=0.25)
Q24 = df4['EF (Teich)'].quantile(q=0.50)
Q34 = df4['EF (Teich)'].quantile(q=0.75)
Mean4= df4['EF (Teich)'].mean()
Median4 = df4['EF (Teich)'].median()
Var4 = df4['EF (Teich)'].var()
```

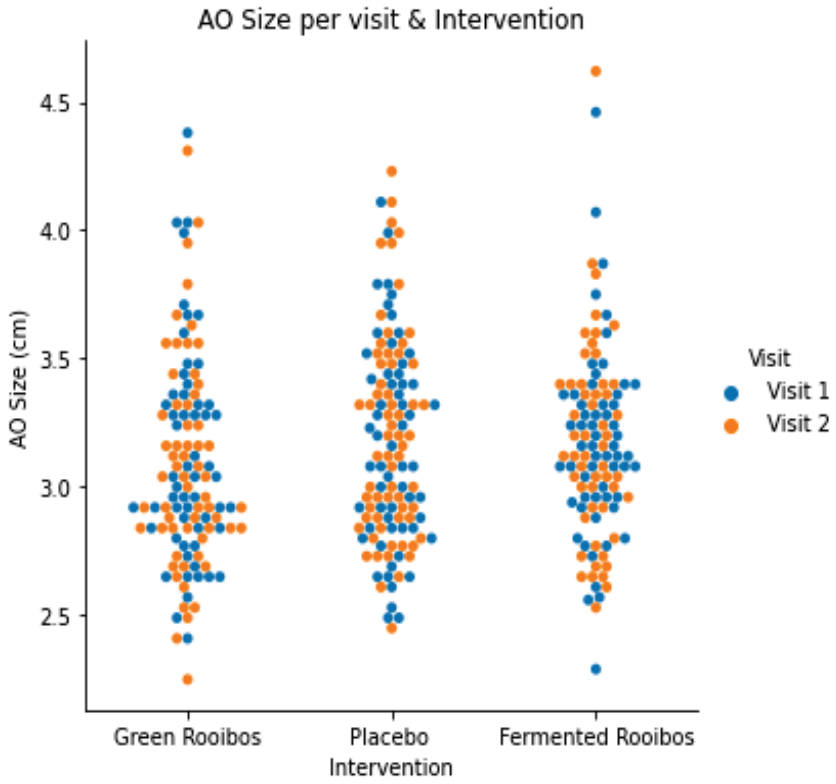
```
Stdv4 = df4['EF (Teich)'].std()
Skew4 = df4['EF (Teich)'].skew()
Kurt4 = df4['EF (Teich)'].kurt()

V1GR =[Min4,Max4,Q14,Q24,Q34,Mean4,Median4,Var4,Stdv4,Skew4,Kurt4]

df5 = df1.loc[(df1["Visit_Intervention"] =="Visit 2 Fermented Rooibos")]
Min5 = df5['EF (Teich)'].min()
Max5 = df5['EF (Teich)'].max()
Q15 = df5['EF (Teich)'].quantile(q=0.25)
Q25 = df5['EF (Teich)'].quantile(q=0.50)
Q35 = df5['EF (Teich)'].quantile(q=0.75)
Mean5= df5['EF (Teich)'].mean()
Median5 = df5['EF (Teich)'].median()
Var5 = df5['EF (Teich)'].var()
Stdv5 = df5['EF (Teich)'].std()
Skew5 = df5['EF (Teich)'].skew()
Kurt5 = df5['EF (Teich)'].kurt()

V2FR =[Min5,Max5,Q15,Q25,Q35,Mean5,Median5,Var5,Stdv5,Skew5,Kurt5]



#######5
df6 = df1.loc[(df1["Visit_Intervention"] =="Visit 2 Placebo ")]
Min6 = df6['EF (Teich)'].min()
Max6 = df6['EF (Teich)'].max()
Q16 = df6['EF (Teich)'].quantile(q=0.25)
Q26 = df6['EF (Teich)'].quantile(q=0.50)
Q36 = df6['EF (Teich)'].quantile(q=0.75)
Mean6= df6['EF (Teich)'].mean()
Median6 = df6['EF (Teich)'].median()
Var6 = df6['EF (Teich)'].var()
Stdv6 = df6['EF (Teich)'].std()
Skew6 = df6['EF (Teich)'].skew()
Kurt6 = df6['EF (Teich)'].kurt()
```

```
V2P =[Min6,Max6,Q16,Q26,Q36,Mean6,Median6,Var6,Stdv6,Skew6,Kurt6]

df7 = df1.loc[(df1["Visit_Intervention"] =="Visit 2 Green Rooibos")]
Min7 = df7['EF (Teich)'].min()
Max7 = df7['EF (Teich)'].max()
Q17 = df7['EF (Teich)'].quantile(q=0.25)
Q27 = df7['EF (Teich)'].quantile(q=0.50)
Q37 = df7['EF (Teich)'].quantile(q=0.75)
Mean7= df7['EF (Teich)'].mean()
Median7 = df7['EF (Teich)'].median()
Var7 = df7['EF (Teich)'].var()
Stdv7 = df7['EF (Teich)'].std()
Skew7 = df7['EF (Teich)'].skew()
Kurt7 = df7['EF (Teich)'].kurt()

V2GR =[Min7,Max7,Q17,Q27,Q37,Mean7,Median7,Var7,Stdv7,Skew7,Kurt7]

Statistic = ["Min","Max","Q1","Q2","Q3","Mean","Median","Var", "Stdv","Skew","Kurt"]

Descriptive_Stats_VO2 = pd.DataFrame(list(zip(Statistic,All_data,
V1FR,V1P,V1GR,V2FR,V2P,V2GR)),
            columns =["Statistic","All_data", "V1FR","V1P","V1GR","V2FR","V2P","V2GR"])

Descriptive_Stats_VO2.to_excel("mARIA_Stats.xlsx")
```

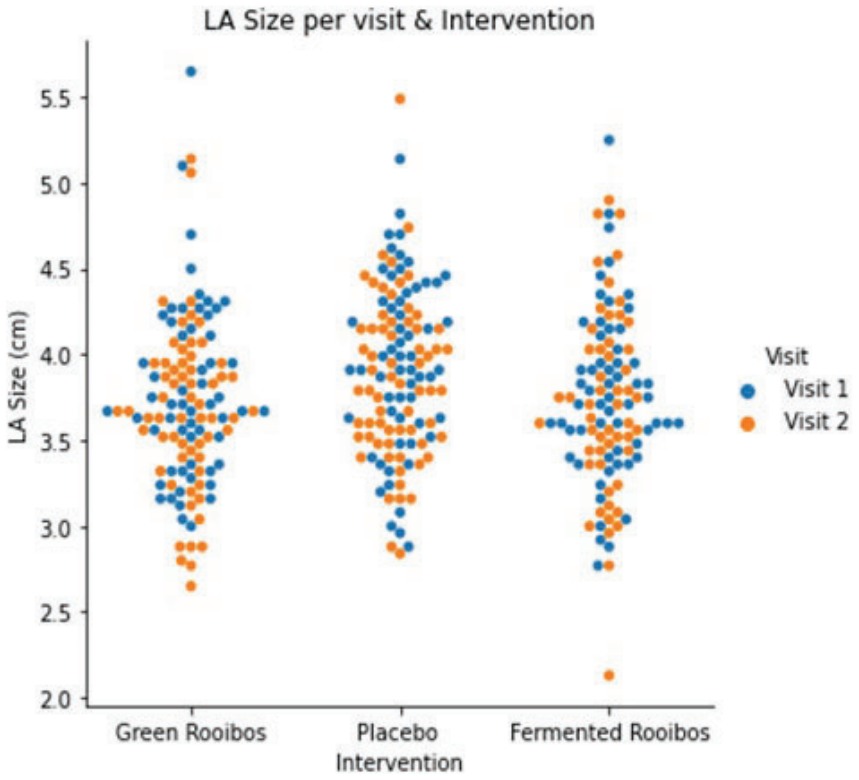
APPENDIX G: SCATTERPLOTS

Scatterplots Characteristics of the echocardiographic findings per Intervention for Each Visit

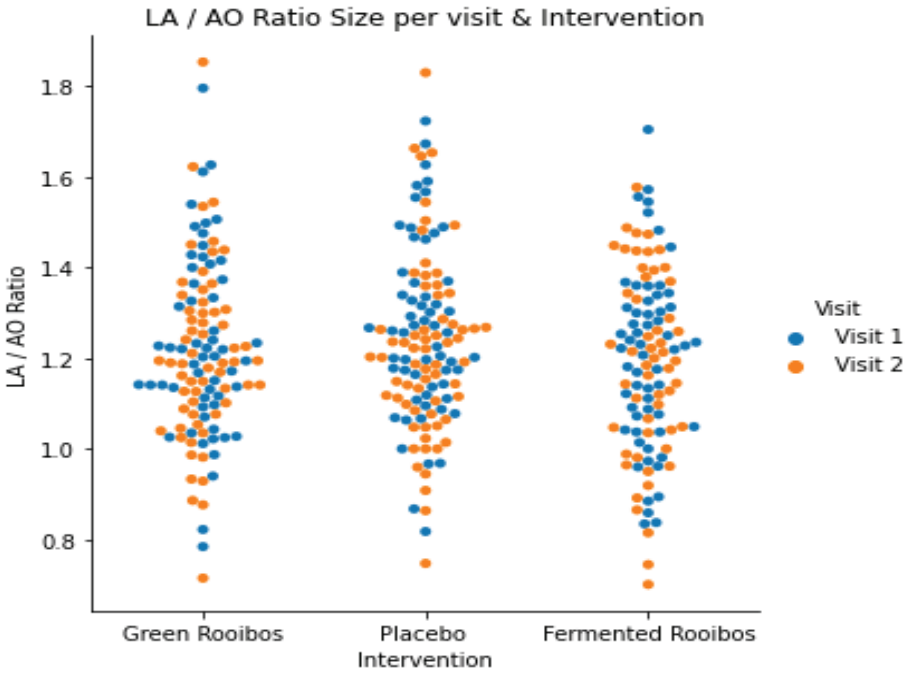
The scatter plots are visual demonstrations of the actual values in this data set. The blue colour indicates Visit 1, and the orange colour indicates Visit 2. According to the LA cm size per visit and intervention graph the Green Rooibos Intervention group demonstrates that the LA dimensions indicate that most observations were above size 3.0 cm and below 4.5 cm. However, it was noticed that there were some Visit 2 observations that were below 3.0 cm but above 2.5 cm. In addition, it also demonstrates that there were more Visit 1 observations that demonstrate above 4.5 cm dimensions compared to Visit 2 observations. This may account for a lower average for the Green rooibos tea Visit 2 intervention group compared to the Visit 1 Fermented Rooibos intervention group. With regards to Visit 2 Green Rooibos intervention for LVIDd, most observations were between 4.0 cm and 5.5 cm. Higher observations for the Green Rooibos group was seen in Visit 2 compared to Visit 1; hence there were no improvements in the Visit 2 Green Rooibos Intervention group. The Fermented Rooibos and the Green Rooibos groups did not perform well, as was indicated in the Descriptive statistics. The rest of the graphs can be interpreted in the same manner as above.



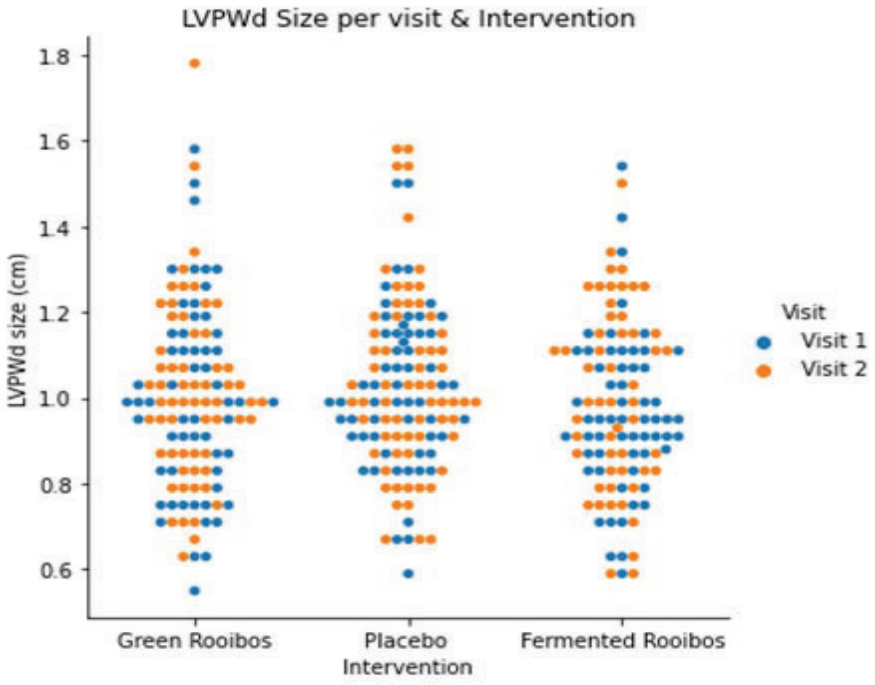
AO size per visit and intervention



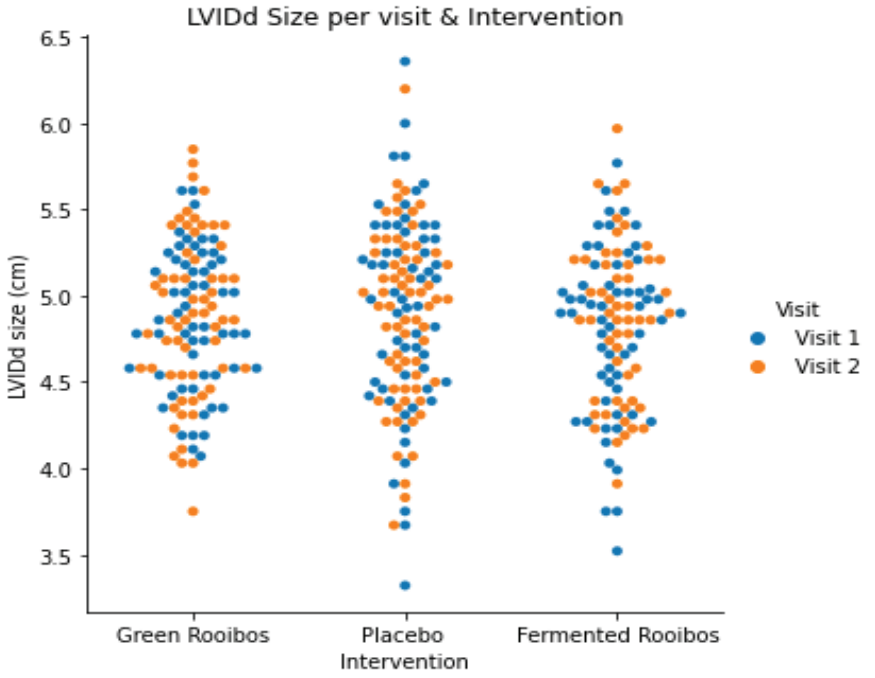
LA size per visit and intervention



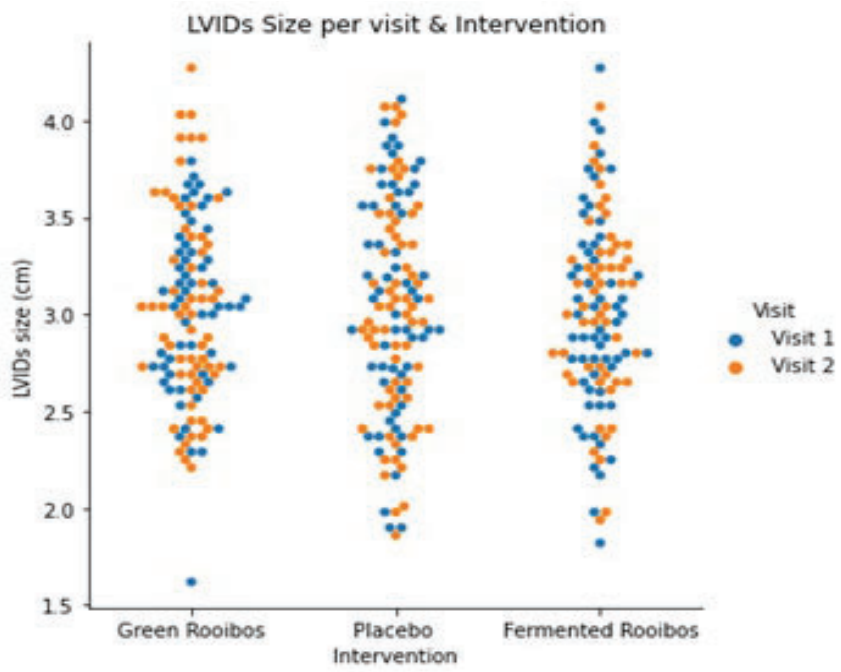
LA/AO ratio per visit and intervention



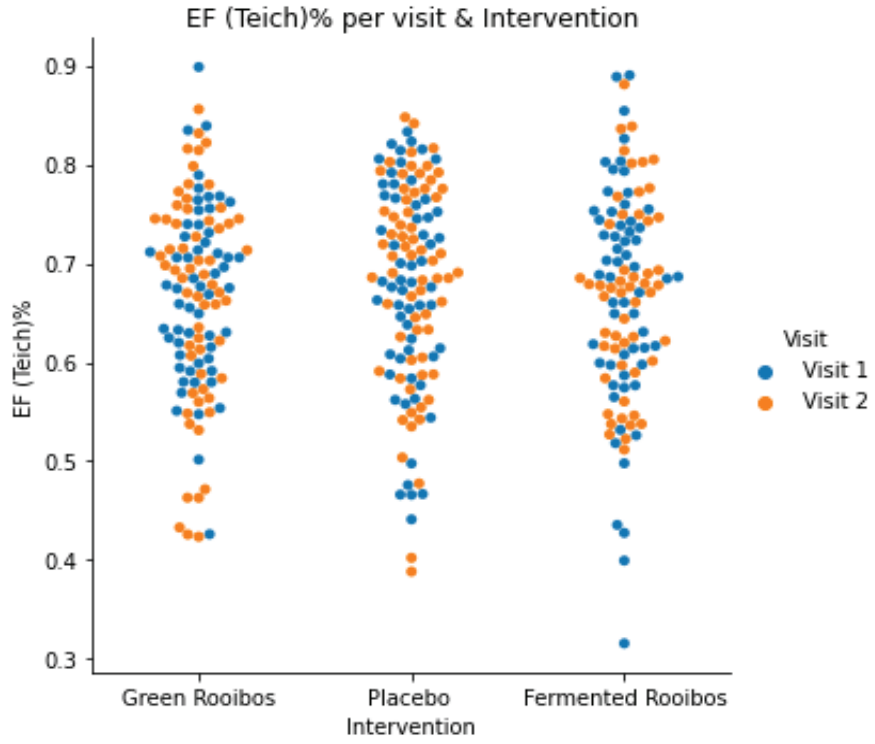
LVPWd per visit and intervention



LVIDd per visit and intervention



LVIDs per visit and intervention



EF% per visit and intervention

APPENDIX H: HEATMAPS

Heatmap characteristics of the echocardiographic findings per Intervention for each Visit

The heat maps are a visual representation of the echocardiography data per Intervention for each Visit and demonstrate the various values according to the different colour-coding schemes. The heatmaps for this research study, seen below, indicate that the higher the average size or dimension, the more concentrated the colour becomes, and the lower the average size or dimension, the less concentrated the colour becomes. Except for EF% where an increase suggests a positive indicator and desired.

The Echocardiography dimensions are demonstrated according to the different colour-coding schemes, in the following graphs. According to the graph, the Placebo Intervention group demonstrate the highest AO dimension; hence the concentration of the colour appears more intense. The same graph indicates that the Green Rooibos Intervention participant group demonstrated the lowest average AO dimension; hence the colour was less concentrated and therefore demonstrated that consuming Green Rooibos may result in a slight decrease in the AO size. Similarly, the Green rooibos Intervention participants, demonstrated a decrease in the LA size, IVSd, and LVM.

The Green Rooibos Intervention Echocardiography findings demonstrated a decrease in the above-mentioned variable sizes. Participants in the Traditional / fermented rooibos Intervention group demonstrated a slight improvement in LA size dimension; hence the colour demonstrated a slightly lighter concentration. With regards to the EF% in all the intervention groups, there was no deterioration or decrease in the systolic function because the % for both groups was within the normal limits at Visit 2. A slight improvement is noticeable in the Fermented Rooibos Intervention group, where the EF% demonstrate a slight improvement from 0.6677 to 0.6684.

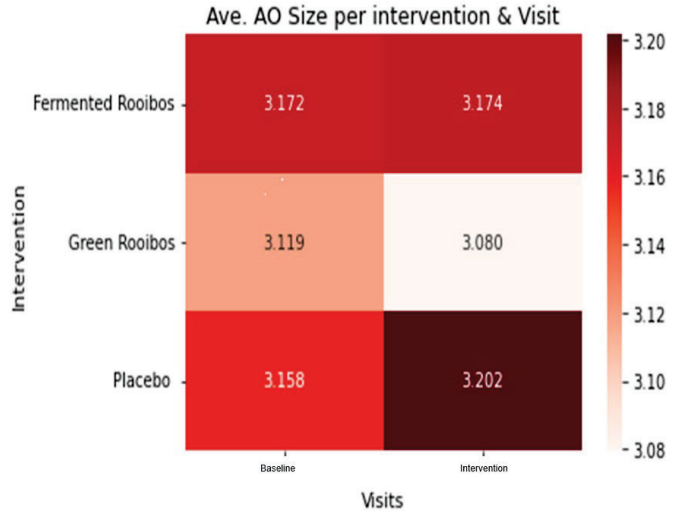


Figure 1: AO size per intervention and visit

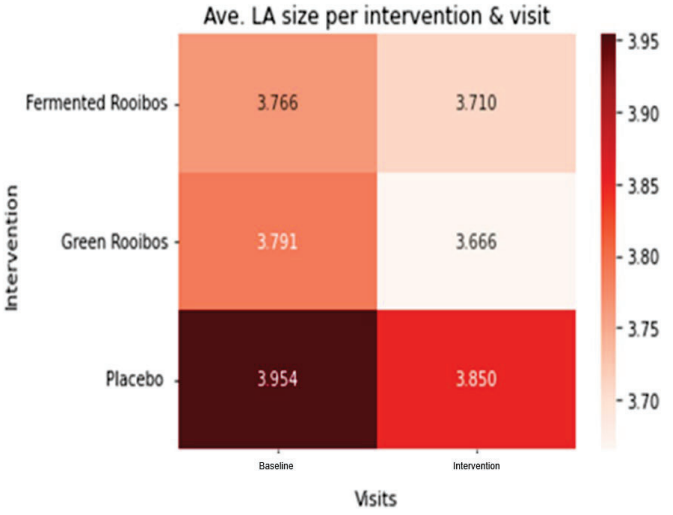


Figure 2: LA size per intervention and visit

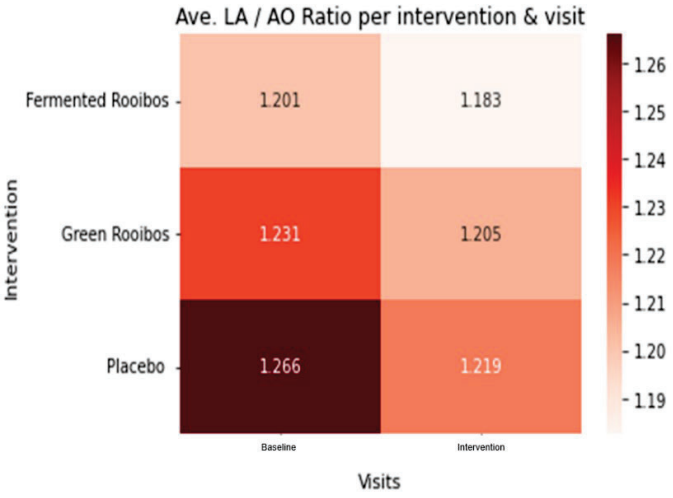


Figure 3: LA/AO ratio per intervention and visit

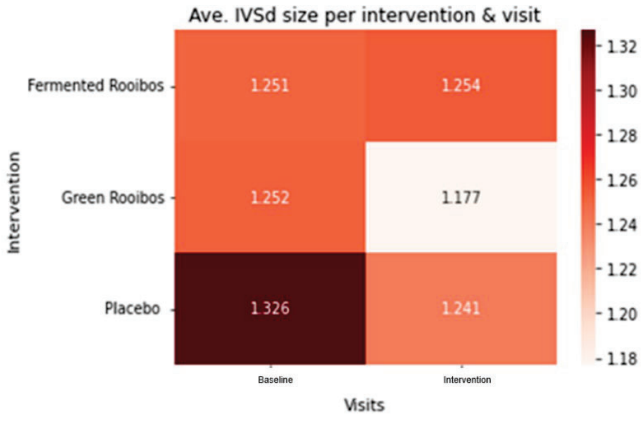


Figure 4: IVSd per intervention and visit

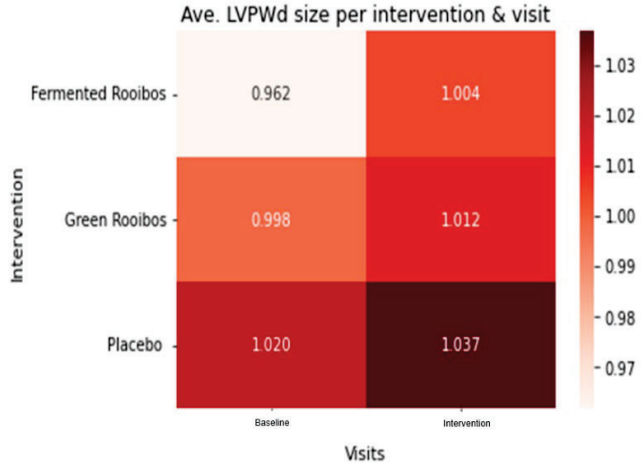


Figure 5: LVPWd per intervention and visit

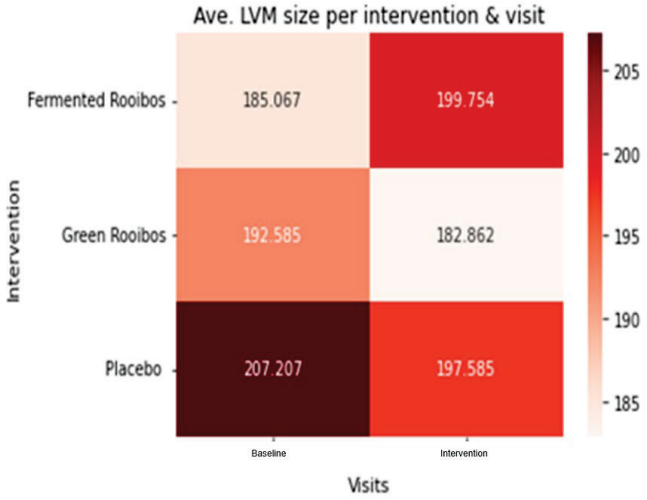


Figure 6: LVM per intervention and visit

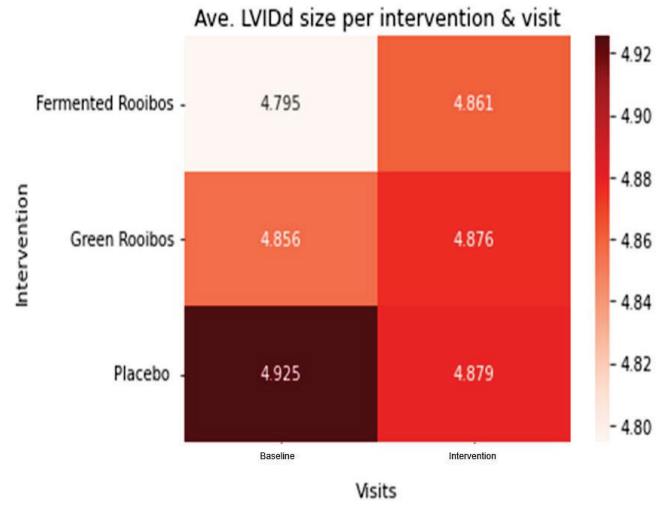


Figure 7: LVIDd per intervention and visit

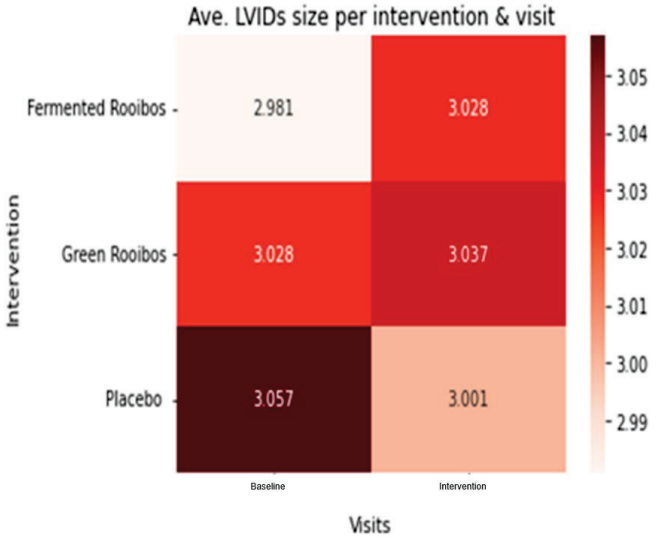


Figure 8: LVIDs per intervention and visit

In the following graph improvement is indicated by increase in colour

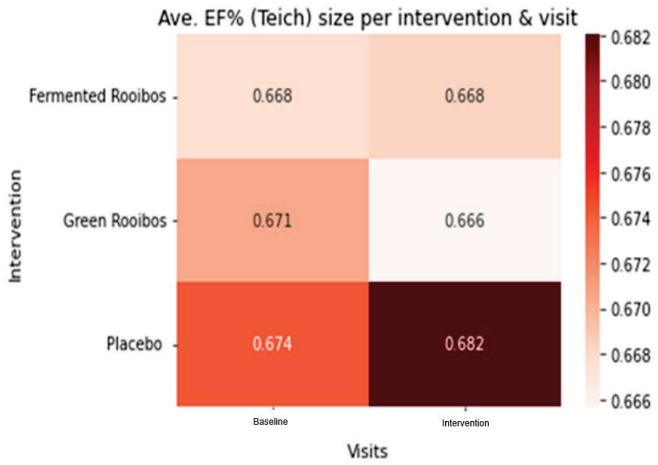


Figure 9: EF% per visit and intervention

Interesting to note that there is a significant improvement could be seen in the LVM in the placebo group, where the LVM decreased from 207.2068 g to 197.5851 g. This improvement could be attributed to the assumption that all participants consumed a healthy diet while part of this study.

APPENDIX I: GENERALISED ESTIMATING EQUATIONS APPLICATION

	AO (cm)			LA (cm)			LA / AO Ratio			IVSd (cm)			LVPWd (cm)			LVM(g)			LVIDd (cm)			LVIDs (cm)			EF (Teich) (%)			Diastolic Function		
	Type III			Type III			Type III			Type III			Type III			Type III			Type III			Type III			Type III			Type III		
Source	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value	Wald Chi-Square	df	p-value
(Intercept)	17332.537	1	0.000	19076.962	1	0.000	253.351	1	0.000	347.759	1	0.000	0.201	1	0.654	79450.977	1	0.000	67787.241	1	0.000	10436.959	1	0.000	1793.968	1	0.000			
Visit	0.574	1	0.449	7.275	1	0.007	5.359	1	0.021	14.273	1	0.000	2.656	1	0.103	0.035	1	0.852	1.340	1	0.247	2.193	1	0.139	0.900	1	0.343	27.853	1	<.001
Groups	1.584	2	0.453	4.903	2	0.086	1.035	2	0.596	2.600	2	0.273	1.191	2	0.551	2.301	2	0.317	1.215	2	0.545	0.384	2	0.825	0.758	2	0.685	3.882	2	.144
Gender	31.284	1	0.000	0.287	1	0.592	14.982	1	0.000	9.731	1	0.002	1.564	1	0.211	25.188	1	0.000	14.558	1	0.000	7.032	1	0.008	1.006	1	0.316	.218	1	.640
Visit*Groups	1.017	2	0.602	1.825	2	0.402	0.611	2	0.737	5.669	2	0.059	0.363	2	0.834	7.716	2	0.021	3.429	2	0.180	1.046	2	0.593	0.003	2	0.998	.128	2	.938

Figure 1 Association of all the echocardiography variables and the interventions

		AO (cm)																											
		Visit			Groups				Gender			Green			Placebo			Fermented											
<0.001		1	2	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2									
Mean		3.219	3.238		3.199	3.271	3.217	Gr.vs.P 0.688	3.394	3.071		3.206	3.191		3.245	3.296		3.205	3.228										
Std. Error		0.029	0.033	0.449	0.046	0.046	0.040	Gr.vs.F 1.000	0.054	0.025	0.000	0.050	0.053	0.755	0.047	0.056	0.254	0.041	0.049	0.575								
95% Wald Confidence Interval	Lower	3.162	3.173		3.111	3.181	3.139	P.vs F 1.000	3.290	3.023		3.109	3.090		3.155	3.189		3.127	3.134										
	Upper	3.277	3.305		3.289	3.362	3.296		3.501	3.121		3.306	3.296		3.338	3.407		3.286	3.325										

Figure 2 Association of AO dimension and the interventions

		LA (cm)																											
		Visit			Groups				Gender			Green			Placebo			Fermented											
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2									
Mean		3.845	3.751		3.753	3.893	3.749	Gr.vs.P 0.179	3.818	3.777		3.832	3.675		3.938	3.848		3.766	3.731										
Std. Error		0.040	0.041	0.007	0.062	0.055	0.055	Gr.vs.F 1.000	0.066	0.035	0.593	0.071	0.067	0.010	0.063	0.058	0.060	0.059	0.071	0.615								
95% Wald Confidence Interval	Lower	3.767	3.671		3.634	3.786	3.641	P.vs F 0.182	3.691	3.709		3.696	3.547		3.817	3.736		3.651	3.595										
	Upper	3.924	3.832		3.876	4.003	3.859		3.949	3.847		3.974	3.809		4.063	3.963		3.884	3.873										

Figure 3 Association of LA dimension and the interventions

		LA / AO Ratio																											
		Visit			Groups				Gender			Green			Placebo			Fermented											
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2									
Mean		1.206	1.173		1.186	1.205	1.178	Gr.vs.P 1.000	1.140	1.241		1.208	1.165		1.226	1.185		1.186	1.169										
Std. Error		0.014	0.015	0.020	0.019	0.021	0.021	Gr.vs.F 1.000	0.022	0.013	0.000	0.024	0.023	0.091	0.023	0.024	0.076	0.023	0.027	0.520								
95% Wald Confidence Interval	Lower	1.179	1.143		1.149	1.166	1.136	P.vs F 1.000	1.098	1.216		1.162	1.121		1.181	1.139		1.142	1.118										
	Upper	1.235	1.203		1.224	1.246	1.220		1.183	1.268		1.255	1.210		1.272	1.233		1.232	1.223										

Figure 4 Association of the LA/AO ratio and the interventions

		IVSd (cm)																											
		Visit			Groups				Gender			Green			Placebo			Fermented											
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2									
Mean		1.294	1.231		1.233	1.291	1.263	Gr.vs.P 0.322	1.312	1.214		1.282	1.186		1.334	1.250		1.267	1.259										
Std. Error		0.019	0.017	0.000	0.029	0.024	0.026	Gr.vs.F 1.000	0.027	0.017	0.002	0.036	0.029	0.002	0.030	0.025	0.002	0.028	0.031	0.784								
95% Wald Confidence Interval	Lower	1.257	1.199		1.178	1.245	1.213	P.vs F 1.000	1.260	1.181		1.213	1.131		1.277	1.202		1.213	1.200										
	Upper	1.331	1.264		1.290	1.339	1.315		1.366	1.248		1.355	1.244		1.394	1.300		1.323	1.321										

Figure 5: Association of the IVSd dimensions and the interventions

		LVPWd (cm)																			
		Visit			Groups				Gender			Green			Placebo			Fermented		
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2
Mean		0.992	1.020		1.006	1.021	0.991	Gr.vs.P 1.000	1.022	0.990		0.998	1.014		1.007	1.035		0.970	1.012	
Std. Error		0.015	0.016		0.024	0.019	0.020	Gr.vs.F 1.000	0.022	0.014		0.028	0.027		0.023	0.026		0.022	0.028	
95% Wald Confidence Interval	Lower	0.963	0.989	0.104	0.961	0.984	0.952	P.vs.F 0.832	0.980	0.963	0.214	0.944	0.962	0.566	0.963	0.984	0.383	0.927	0.958	0.181
	Upper	1.021	1.052		1.053	1.059	1.031		1.066	1.017		1.054	1.069		1.053	1.087		1.015	1.068	

Figure 6: Association of the LVPWd dimensions and the interventions

		LVM (g)																			
		Visit			Groups				Gender			Green			Placebo			Fermented		
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2
Mean		200.142	199.434		194.942	206.972	197.646	Gr.vs.P 0.473	219.487	181.857		198.841	191.120		210.659	203.350		191.394	204.102	
Std. Error		4.216	4.199		6.553	5.777	6.389	Gr.vs.F 1.000	6.751	3.911		7.636	7.379		7.029	6.503		6.707	7.102	
95% Wald Confidence Interval	Lower	192.046	191.371	0.852	182.512	195.953	185.512	P.vs.F 0.813	206.646	174.351	0.000	184.425	177.191	0.292	197.323	190.996	0.300	178.689	190.646	0.015
	Upper	208.579	207.836		208.218	218.610	210.574		233.126	189.685		214.385	206.144		224.896	216.502		205.002	218.508	

Figure 7: Association of the LVM weight and the interventions

		LVIDd (cm)																			
		Visit			Groups				Gender			Green			Placebo			Fermented		
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2
Mean		4.901	4.940		4.940	4.948	4.874	Gr.vs.P 1.000	5.038	4.806		4.913	4.967		4.970	4.925		4.819	4.929	
Std. Error		0.034	0.035		0.045	0.053	0.053	Gr.vs.F 1.000	0.051	0.034		0.049	0.058		0.064	0.059		0.061	0.059	
95% Wald Confidence Interval	Lower	4.834	4.872	0.247	4.852	4.844	4.772	P.vs.F 0.972	4.940	4.739	0.000	4.818	4.855	0.356	4.846	4.810	0.461	4.700	4.816	0.059
	Upper	4.968	5.009		5.029	5.053	4.978		5.139	4.873		5.010	5.082		5.098	5.042		4.941	5.046	

Figure 8: Association of the LVID dimensions and the interventions

		LVISd (cm)																			
		Visit			Groups				Gender			Green			Placebo			Fermented		
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2
Mean		3.050	3.105		3.102	3.074	3.056	Gr.vs.P 1.000	3.169	2.988		3.075	3.129		3.071	3.077		3.004	3.109	
Std. Error		0.039	0.038		0.053	0.057	0.056	Gr.vs.F 1.000	0.059	0.035		0.055	0.065		0.065	0.067		0.068	0.062	
95% Wald Confidence Interval	Lower	2.975	3.031	0.138	2.999	2.965	2.949	P.vs.F 1.000	3.055	2.919	0.009	2.970	3.004	0.335	2.946	2.950	0.924	2.874	2.989	0.127
	Upper	3.127	3.181		3.207	3.187	3.167		3.287	3.058		3.183	3.259		3.202	3.211		3.140	3.234	

Figure 9: Association of the LVIS dimensions and the interventions

		EF (Teich) (%)																			
		Visit			Groups				Gender			Green			Placebo			Fermented		
		1.000	2.000	p-value	Green	Placebo	Fermented	p-value	Male	Female	p-value	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2	Visit 1	Visit 2	Visit 1 vs Visit 2
Mean		0.669	0.661		0.662	0.672	0.662	Gr.vs.P 1.000	0.659	0.672		0.666	0.657		0.676	0.668		0.666	0.658	
Std. Error		0.008	0.007		0.009	0.010	0.011	Gr.vs.F 1.000	0.011	0.007		0.010	0.013		0.013	0.013		0.015	0.012	
95% Wald Confidence Interval	Lower	0.654	0.647	0.343	0.644	0.652	0.641	P.vs.F 1.000	0.638	0.659	0.314	0.646	0.633	0.549	0.652	0.643	0.563	0.637	0.635	0.634
	Upper	0.685	0.676		0.680	0.692	0.684		0.681	0.685		0.687	0.683		0.702	0.693		0.696	0.682	

Figure 10: Association of the EF percentage and the interventions

APPENDIX J: MALE AND FEMALE DESCRIPTIVE STATISTICS

Gender = Male								
AO (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	2.77	2.92	2.73	2.73	2.8	2.84
	Maximum	4.62	4.46	4.11	4.38	4.62	4.23	4.03
	Q1	2.88	3.18	3.29	3.05	3.04	2.92	2.96
	Q2	3.12	3.32	3.5	3.42	3.3	3.2	3.56
	Q3	3.4	3.52	3.78	3.67	3.58	3.52	3.73
	Mean	3.1509	3.3981	3.4919	3.4114	3.3325	3.3153	3.3938
	Variance	0.1553	0.1421	0.1196	0.2001	0.2011	0.2247	0.1773
	SD	0.3941	0.3770	0.3458	0.4473	0.4484	0.4741	0.4210
	Skew	0.6525	1.3288	-0.0270	0.5454	1.1748	0.9175	-0.0403
	Kurt	0.5859	2.2800	-0.5053	0.4750	2.2805	-0.3719	-1.3483

Figure 1: AO according to the Interventions

Gender = Male								
LA (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	3.24	2.88	3.04	2.77	3.16	2.65
	Maximum	4.62	4.74	4.7	5.65	4.82	4.74	5.14
	Q1	2.88	3.58	3.75	3.3	3.49	3.4	3.18
	Q2	3.12	3.75	3.99	3.635	3.83	3.91	3.4
	Q3	3.4	3.99	4.28	4.21	4.13	4.11	3.89
	Mean	3.1509	3.8271	3.9644	3.8014	3.8450	3.8013	3.5508
	Variance	0.1553	0.1551	0.1970	0.4564	0.2894	0.2216	0.4182
	SD	0.3941	0.3938	0.4439	0.6756	0.5380	0.4708	0.6467
	Skew	0.6525	0.8536	-0.7660	1.7067	0.1493	0.3380	1.2258
	Kurt	0.5859	0.3319	1.2881	3.5546	-0.1177	-0.6222	2.1072

Figure 2: LA according to the Interventions

Gender = Male								
LA/AO Ratio	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.2500	0.8372	0.8175	0.8219	0.7446	0.7470	0.7146
	Maximum	4.6200	1.4451	1.4760	1.5395	1.4877	1.6458	1.3013
	Q1	2.8800	1.0370	1.0669	1.0192	1.0088	1.0000	0.9309
	Q2	3.1200	1.1399	1.1373	1.1044	1.1694	1.2017	1.0763
	Q3	3.4000	1.2895	1.2482	1.2283	1.3595	1.2627	1.1549
	Mean	3.1509	1.1398	1.1472	1.1198	1.1719	1.1714	1.0505
	Variance	0.1553	0.0299	0.0334	0.0280	0.0455	0.0551	0.0242
	SD	0.3941	0.1728	0.1826	0.1674	0.2134	0.2347	0.1554
	Skew	0.6525	-0.1181	0.1511	0.8828	-0.2998	0.1013	-0.6301
	Kurt	0.5859	-0.7764	0.1124	2.4053	-0.5863	0.2034	0.5499

Figure 3: LA / AO ratio according to the Interventions

Gender = Male								
IVSd(cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	1.07	1.07	0.75	0.79	1.15	0.99
	Maximum	4.62	1.78	1.9	1.66	1.74	1.82	1.78
	Q1	2.88	1.13	1.26	1.1	1.13	1.19	1.13
	Q2	3.12	1.3	1.4	1.28	1.3	1.22	1.22
	Q3	3.4	1.58	1.65	1.47	1.5	1.3	1.38
	Mean	3.1509	1.3452	1.4313	1.2614	1.3135	1.3053	1.2677
	Variance	0.1553	0.0550	0.0587	0.0833	0.0576	0.0338	0.0429
	SD	0.3941	0.2346	0.2422	0.2886	0.2400	0.1838	0.2070
	Skew	0.6525	0.5526	0.2574	-0.2711	-0.1255	1.8774	1.2786
	Kurt	0.5859	-0.9439	-0.7263	-0.5140	-0.1412	3.6282	2.1338

Figure 4: IVSd according to the Interventions

Gender = Male								
LVPWd (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	0.71	0.67	0.63	0.75	0.79	0.71
	Maximum	4.62	1.22	1.3	1.5	1.3	1.54	1.78
	Q1	2.88	0.95	0.92	0.84	0.87	0.91	0.85
	Q2	3.12	1.07	1.01	1.01	1.09	0.95	0.99
	Q3	3.4	1.11	1.15	1.11	1.18	1.11	1.185
	Mean	3.1509	1.0276	1.0019	0.9950	1.0310	1.0387	1.0554
	Variance	0.1553	0.0140	0.0317	0.0492	0.0327	0.0452	0.0820
	SD	0.3941	0.1181	0.1782	0.2217	0.1809	0.2126	0.2863
	Skew	0.6525	-0.7842	-0.4405	0.4290	-0.1833	1.3073	1.3798
	Kurt	0.5859	1.0331	-0.0895	0.9864	-1.3582	1.2181	2.4855

Figure 5: LVPWd according to the Interventions

Gender = Male								
LVM (g)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	112.46	170.45	123.46	116.13	155.3	139.68
	Maximum	4.62	309.9	333	322.35	312.23	340.96	377.96
	Q1	2.88	178.96	179.915	172.7425	197.795	176.63	174.595
	Q2	3.12	215.09	236.505	207.165	223.135	199.53	189.4
	Q3	3.4	258.525	281.3125	244.2825	274.52	245.35	215.2
	Mean	3.1509	216.4995	235.8088	209.4907	226.6945	218.0860	209.7677
	Variance	0.1553	3,070.2680	2,555.7626	3,029.6546	3,043.8732	3,235.3073	4,063.3965
	SD	0.3941	55.4100	50.5546	55.0423	55.1713	56.8798	63.7448
	Skew	0.6525	- 0.1597	0.2821	0.1985	- 0.3702	1.1943	1.9331
	Kurt	0.5859	- 0.5910	- 0.8847	0.1661	- 0.3852	0.7652	3.7257

Figure 6: LVM according to the Interventions

Gender = Male								
LVIDd(cm)	**Statistic Description**	**All data**	**Baseline Fermented Rooibos**	**Baseline Placebo**	**Baseline Green Rooibos**	**Intervention Fermented Rooibos**	**Intervention Placebo**	**Intervention Green Rooibos**
	Minimum	2.25	4.03	4.39	4.35	4.23	4.27	4.39
	Maximum	4.62	5.61	6.36	5.61	5.65	6.2	5.77
	Q1	2.88	4.54	4.75	4.81	4.63	4.46	4.54
	Q2	3.12	4.98	5.175	5.1	5.18	4.94	4.86
	Q3	3.4	5.29	5.4	5.34	5.28	5.33	5.235
	Mean	3.1509	4.9262	5.1500	5.0964	5.0150	4.9773	4.9231
	Variance	0.1553	0.2027	0.2488	0.1169	0.1805	0.2631	0.1795
	SD	0.3941	0.4502	0.4988	0.3419	0.4249	0.5130	0.4236
	Skew	0.6525	-0.3200	0.7233	-0.5614	-0.4610	0.6815	0.5404
	Kurt	0.5859	-0.8772	1.0381	0.2702	-0.8041	0.7725	-0.4834

Figure 7: LVIDd according to the Interventions

Gender = Male								
LVISd (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	1.82	2.49	2.29	2.29	2.41	2.21
	Maximum	4.62	3.99	4.11	3.79	3.87	3.75	3.91
	Q1	2.88	2.785	2.97	2.7925	3.06	2.77	2.65
	Q2	3.12	3.04	3.435	3.18	3.24	3.12	2.73
	Q3	3.4	3.56	3.78	3.64	3.35	3.4	3.32
	Mean	3.1509	3.0605	3.3663	3.1650	3.1920	3.1260	2.9615
	Variance	0.1553	0.3498	0.2532	0.2473	0.1204	0.1881	0.2341
	SD	0.3941	0.5914	0.5032	0.4973	0.3470	0.4337	0.4838
	Skew	0.6525	-0.4285	-0.3295	-0.4630	-0.5590	-0.0329	0.6315
	Kurt	0.5859	-0.2919	-0.9746	-0.9366	1.8183	-0.9929	-0.1846

Figure 8: LVISd according to the Interventions

Gender = Male								
EF (Teich)(	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	0.3995	0.466	0.5509	0.5117	0.5422	0.4631
	Maximum	4.62	0.8902	0.7686	0.839	0.8139	0.7919	0.8143
	Q1	2.88	0.5542	0.513775	0.6112	0.592025	0.6048	0.64875
	Q2	3.12	0.6861	0.63885	0.6542	0.67345	0.659	0.7074
	Q3	3.4	0.75545	0.72635	0.736675	0.6888	0.7193	0.74255
	Mean	3.1509	0.6614	0.6263	0.6743	0.6520	0.6652	0.6907
	Variance	0.1553	0.0191	0.0117	0.0070	0.0060	0.0056	0.0077
	SD	0.3941	0.1381	0.1080	0.0838	0.0774	0.0747	0.0877
	Skew	0.6525	-0.1644	-0.3482	0.4780	-0.1243	0.0607	-1.3578
	Kurt	0.5859	-0.5762	-1.2672	-0.5458	-0.1380	-0.8039	3.1222

Figure 9: EF% according to the Interventions

APPENDIX K: FEMALE DESCRIPTIVE STATISTICS

Gender = Female								
AO (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	2.29	2.49	2.41	2.53	2.45	2.25
	Maximum	4.62	3.67	3.71	4.03	3.63	3.99	4.31
	Q1	2.88	2.91	2.8	2.77	2.805	2.84	2.7475
	Q2	3.12	3.08	3	2.92	3.08	3.12	2.92
	Q3	3.4	3.25	3.32	3.28	3.34	3.48	3.16
	Mean	3.1509	3.0468	3.0337	3.0233	3.0785	3.1651	2.9946
	Variance	0.1553	0.0819	0.1101	0.1311	0.0990	0.1468	0.1303
	SD	0.3941	0.2863	0.3318	0.3620	0.3147	0.3831	0.3610
	Skew	0.6525	-0.3069	0.2789	0.9474	-0.0294	0.3742	0.9180
	Kurt	0.5859	0.4007	-0.8315	1.0578	-0.9859	-0.6447	2.6012

Figure 1: AO according to the Interventions

Gender = Female								
LA (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	2.77	2.96	3	2.13	2.84	2.77
	Maximum	4.62	5.25	5.14	5.1	4.9	5.49	5.06
	Q1	2.88	3.4	3.52	3.36	3.22	3.54	3.52
	Q2	3.12	3.71	3.91	3.71	3.6	3.79	3.69
	Q3	3.4	4.12	4.39	4.15	3.95	4.17	3.95
	Mean	3.1509	3.7318	3.9498	3.7872	3.6285	3.8592	3.6973
	Variance	0.1553	0.2772	0.2784	0.2119	0.3043	0.2297	0.1823
	SD	0.3941	0.5265	0.5276	0.4603	0.5516	0.4793	0.4269
	Skew	0.6525	0.5267	0.0111	0.4742	-0.1116	0.5695	0.0343
	Kurt	0.5859	0.8229	-0.6589	0.2006	0.8295	1.6558	1.5702

Figure 2: LA according to the Interventions

Gender = Female								
LA / AO Ratio	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.2500	0.8343	0.9667	0.7841	0.7007	0.9444	0.8861
	Maximum	4.6200	1.7045	1.7233	1.7958	1.5771	1.8300	1.8535
	Q1	2.8800	1.0846	1.1969	1.1370	1.0444	1.1018	1.1352
	Q2	3.1200	1.2375	1.2830	1.2222	1.2131	1.2027	1.2241
	Q3	3.4000	1.3403	1.4623	1.4157	1.3866	1.3514	1.3481
	Mean	3.1509	1.2343	1.3102	1.2673	1.1892	1.2321	1.2472
	Variance	0.1553	0.0394	0.0335	0.0391	0.0428	0.0360	0.0328
	SD	0.3941	0.1986	0.1830	0.1978	0.2069	0.1897	0.1812
	Skew	0.6525	0.2022	0.4301	0.2790	-0.2273	1.0548	0.8280
	Kurt	0.5859	-0.0991	-0.3693	0.4180	-0.4499	1.2474	1.6191

Figure 3: LA/AO according to the Interventions

Gender = Female								
IVSd(cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	0.75	0.79	0.67	0.75	0.75	0.71
	Maximum	4.62	1.58	1.82	1.82	1.82	1.78	1.86
	Q1	2.88	1.05	1.07	0.99	1.07	1.11	1
	Q2	3.12	1.19	1.3	1.26	1.22	1.22	1.15
	Q3	3.4	1.36	1.46	1.46	1.36	1.34	1.33
	Mean	3.1509	1.1992	1.2874	1.2495	1.2185	1.2167	1.1525
	Variance	0.1553	0.0437	0.0608	0.0839	0.0529	0.0425	0.0560
	SD	0.3941	0.2092	0.2465	0.2896	0.2300	0.2062	0.2366
	Skew	0.6525	-0.0898	0.0487	0.0860	0.5939	0.1705	0.4252
	Kurt	0.5859	-0.7570	-0.5575	-0.7675	1.0456	0.4765	0.6132

Figure 4: IVSD according to the Interventions

Gender = Female								
LVPWd (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	0.59	0.59	0.55	0.59	0.67	0.63
	Maximum	4.62	1.54	1.5	1.58	1.5	1.58	1.54
	Q1	2.88	0.82	0.91	0.83	0.81	0.89	0.87
	Q2	3.12	0.91	0.99	0.99	0.99	1.03	0.99
	Q3	3.4	1	1.15	1.15	1.17	1.19	1.14
	Mean	3.1509	0.9253	1.0267	0.9991	0.9879	1.0369	1.0000
	Variance	0.1553	0.0397	0.0343	0.0525	0.0543	0.0481	0.0333
	SD	0.3941	0.1992	0.1851	0.2292	0.2330	0.2193	0.1825
	Skew	0.6525	1.1597	0.4199	0.3484	0.1544	0.5395	0.3058
	Kurt	0.5859	2.2261	0.7773	-0.2253	-0.6996	0.3404	0.4474

Figure 5: LVPWd according to the Interventions

Gender = Female								
LVM (g)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	105.8	89.66	87.03	102.28	91.96	49.75
	Maximum	4.62	294.93	342.36	356.23	302.96	348.03	325.62
	Q1	2.88	130.365	153.08	136.51	139.04	155.15	137.04
	Q2	3.12	164.875	195.54	163.38	182.34	185.04	169.635
	Q3	3.4	191.355	240.6	240.1	216.475	224.86	208.89
	Mean	3.1509	167.6958	196.5642	187.0814	183.4264	190.4671	175.5756
	Variance	0.1553	2,217.5560	3,501.3517	3,766.9457	2,469.1382	2,556.5164	3,074.9841
	SD	0.3941	47.0909	59.1722	61.3754	49.6904	50.5620	55.4525
	Skew	0.6525	0.9941	0.3627	0.7626	0.3862	0.5058	0.5098
	Kurt	0.5859	0.7387	- 0.1887	0.0254	- 0.0182	0.5590	0.2603

Figure 6: LVM according to the Interventions

Gender = Female								
LVIDd(cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	3.52	3.32	4.07	3.91	3.67	3.75
	Maximum	4.62	5.77	6	5.61	5.97	5.65	5.85
	Q1	2.88	4.3	4.42	4.46	4.35	4.48	4.55
	Q2	3.12	4.88	4.93	4.78	4.86	4.94	4.88
	Q3	3.4	5.02	5.25	5.14	5.02	5.195	5.24
	Mean	3.1509	4.7218	4.8419	4.7781	4.7673	4.8345	4.8635
	Variance	0.1553	0.2472	0.3755	0.1521	0.2075	0.2333	0.2385
	SD	0.3941	0.4972	0.6128	0.3901	0.4555	0.4830	0.4883
	Skew	0.6525	-0.5269	-0.4042	-0.0075	0.3907	-0.4614	-0.1933
	Kurt	0.5859	0.0833	-0.3032	-0.8936	0.3016	-0.3484	-0.5188

Figure 7: LVIDd according to the Interventions

Gender = Female								
LVISd (cm)	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	2.17	1.9	1.62	1.94	1.86	2.25
	Maximum	4.62	4.27	3.91	3.67	4.07	4.07	4.27
	Q1	2.88	2.6075	2.45	2.69	2.65	2.47	2.69
	Q2	3.12	2.86	2.92	3.04	2.88	2.96	3.02
	Q3	3.4	3.21	3.36	3.28	3.3	3.4	3.4
	Mean	3.1509	2.9371	2.9416	2.9800	2.9288	2.9522	3.0577
	Variance	0.1553	0.2210	0.3117	0.1753	0.2542	0.3340	0.2695
	SD	0.3941	0.4701	0.5583	0.4186	0.5042	0.5779	0.5191
	Skew	0.6525	0.7176	-0.0203	-0.6741	0.1899	0.1842	0.4295
	Kurt	0.5859	0.7292	-0.8468	1.2017	-0.1832	-0.6272	-0.5416

Figure 8: LVISd according to the Interventions

Gender = Female								
EF (Teich)(	Statistic Description	All data	Baseline Fermented Rooibos	Baseline Placebo	Baseline Green Rooibos	Intervention Fermented Rooibos	Intervention Placebo	Intervention Green Rooibos
	Minimum	2.25	0.3158	0.4412	0.4262	0.5225	0.3884	0.4236
	Maximum	4.62	0.8542	0.833	0.8985	0.881	0.8477	0.8558
	Q1	2.88	0.61245	0.6142	0.6037	0.5991	0.5966	0.5698
	Q2	3.12	0.69245	0.6831	0.6766	0.6752	0.7099	0.6818
	Q3	3.4	0.74595	0.7802	0.7271	0.7699	0.77545	0.74495
	Mean	3.1509	0.6713	0.6922	0.6693	0.6783	0.6876	0.6590
	Variance	0.1553	0.0112	0.0097	0.0080	0.0104	0.0126	0.0127
	SD	0.3941	0.1057	0.0986	0.0895	0.1021	0.1122	0.1127
	Skew	0.6525	-1.1815	-0.5236	-0.1315	0.1933	-0.8973	-0.4328
	Kurt	0.5859	2.4375	-0.2988	0.7097	-1.0195	0.2230	-0.5664

Figure 9: EF according to the Interventions

APPENDIX L: NOVA SINGLE FACTOR TEST DEMONSTRATING SIGNIFICANTS

ANOVA: Single Factor	**Age**			
SUMMARY				
Groups	Count	Sum	Average	Variance
Green Rooibos	73	3433	47.027	115.999
Placebo	78	3552	45.538	120.615
Fermented Rooibos	68	3121	45.897	101.736

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	89.7150	2	44.857	0.3962	0.6734	3.0377
Within Groups	24455.6092	216	113.220			
Total	24545.3242	218				

AOVA: Single Factor	**Weight**				
SUMMARY					
Groups	Count	Sum	Average	Variance	
Green Rooibos	73	6000	82.19726	476.043	
Placebo	78	6410	82.18077	393.555	
Fermented Rooibos	68	5256	77.28971	265.451	

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1125.296417	2	562.6482	1.47555	0.23095	3.03767
Within Groups	82364.0634	216	381.3151			
Total	83489.35982	218				

ANOVA: Single Factor	SBP			
SUMMARY				
Groups	Count	Sum	Average	Variance
Green Rooibos	73	9593	131.4064	343.689
Placebo	78	10329	132.4252	304.239
Fermented Rooibos	68	9073	133.4216	392.277

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	143.078679	2	71.53934	0.20754	0.81274	3.03767
Within Groups	74454.56135	216	344.697			
Total	74597.64003	218				

ANOVA: Single Factor	DBP			
SUMMARY				
Groups	Count	Sum	Average	Variance
Green Rooibos	73	6015	82.40183	121.966
Placebo	78	6663	85.42521	129.826
Fermented Rooibos	68	5751	84.57843	141.415

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	362.4145491	2	181.2073	1.38537	0.25245	3.03767
Within Groups	28252.94187	216	130.8007			
Total	28615.35642	218				

APPENDIX M: EFFECT SIZES CALCULATION

Effect sizes in repeated measures designs.
LA: effect size: d_{RM} = -.210 (Green) - adverse effect (a negative sign of correlation)
ISVd: effect size d_{RM} = -.224 (Placebo) - adverse effect (negative sign of correlation)
ISVd: effect size d_{RM} = 0.247 (Green) – Small effect
LVM: effect size: d_{RM} = .195 (Fermented) – Small effect

While steps 1 to 3 target at comparing independent groups, especially in intervention research, the results are usually based on intra-individual changes in test scores. Morris & DeShon (2002), suggest a procedure to estimate the effect size for single-group pretest-post-test designs by taking the correlation between the pre-and post-test into account:

$$\sigma D=\sigma \cdot 2 \cdot (1-\rho)$$

In case, the correlation is .5, the resulting effect size equals **1. Comparison of groups with equal size (Cohen's d and Glass Δ)**. Higher values lead to an increase in the effect size. Morris & DeShon (2002) suggest to use the standard deviation of the pre-test, as this value is not influenced by the intervention, thus resembling *Glass Δ*. It is referred to as $d_{Repeated\ Measures}$ (d_{RM}) in the following. The second effect size $d_{Repeated\ Measures,\ pooled}$ $(d_{RM,\ pool})$ is using the pooled standard deviation, controlling for the intercorrelation of both groups (see **Lakens, 2013, formula 8**).

Here, you can see the suggestions of Cohen (1988) and Hattie (2009 S. 97) for interpreting the magnitude of effect sizes. Hattie refers to real educational contexts and therefore uses a more benignant classification, compared to Cohen. We slightly adjusted the intervals, in case, the interpretation did not exactly match the categories of the original authors.

d	r*	η^2	Interpretation sensu Cohen (1988)	Interpretation sensu Hattie (2009)
< 0	< 0	-	Adverse Effect	
0.0	.00	.000	No Effect	Developmental effects
0.1	.05	.003		
0.2	.10	.010	Small Effect	Teacher effects
0.3	.15	.022		
0.4	.2	.039		Zone of desired effects
0.5	.24	.060	Intermediate Effect	
0.6	.29	.083		
0.7	.33	.110		
0.8	.37	.140	Large Effect	
0.9	.41	.168		
≥ 1.0	.45	.200		

* Cohen (1988) reports the following intervals for r: .1 to .3: small effect; .3 to .5: intermediate effect; .5 and higher: strong effect.

APPENDIX N: AN OVERVIEW OF THE RISK-OF-BIAS ASSESSMENT

This table provides a clear and concise overview of the risk-of-bias assessment for this study based on the Revised Cochrane Risk-of-Bias Tool for Randomised Trials (RoB 2) according to Eldridge et al. (2016).

Domain	Signalling Questions	Information	Response	Risk of Bias Judgment
1. Bias Arising from the Randomisation Process				**Low risk of bias**
1.1 Was the allocation sequence random?	The Randomisation was conducted by an external statistician using the Excel RAND function iteratively.	Yes	The randomisation process did not involve participant identification details, ensuring impartial and random allocation.	
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to the Rooibos interventions?	Allocation process was handled by an external statistician, not involving participant identification details.	Yes	The allocation was concealed from both the participants and the researcher throughout the study period.	
1.3 Did baseline differences between intervention groups suggest a problem with the randomisation process?	Random allocation was maintained, ensuring no significant baseline differences.	No	Random allocation was iteratively applied to avoid repetitions.	
2. Bias Due to Deviations from the Interventions				**Low risk of bias**
2.1 Were participants aware of their assigned intervention during the trial?	Double-blinded design; participants were not informed of their supplement assignment.	No	Blinding was maintained throughout the study.	
2.2 Were the study staff delivering the interventions aware of participants' assigned intervention during the trial?	Study staff remained blinded to the interventions.	No	Blinding was rigorously maintained to prevent performance bias.	
2.3 If there were deviations from the intended intervention, were they balanced between groups?	Compliance was monitored; non-compliant participants were removed.	Yes	Monitoring ensured balanced handling of deviations across groups.	
2.4 Was an appropriate analysis	Data analysis conducted with a qualified statistician.	Yes	Data were analysed in consultation with a	

used to estimate the effect of assignment to intervention?			statistician to ensure appropriate methods.	
3. Bias Due to Missing Outcome Data				**Low risk of bias**
3.1 Were data for this outcome available for all, or nearly all, participants randomised?	Compliance was checked; non-compliant participants were removed and documented.	Yes	Most data were available, and missing data were documented and accounted for.	
3.2 Were participants excluded due to missing outcome data?	Non-compliant participants were excluded.	Yes	Exclusions were based on predefined compliance criteria.	
3.3 Were missing data balanced across intervention groups?	Compliance issues were equally monitored across all groups.	Yes	Balanced handling of missing data.	
4. Bias in Measurement of the Outcome				**Low risk of bias**
4.1 Were outcome assessors aware of the intervention received by study participants?	Blood tests by blinded external nursing staff; echocardiography by blinded researcher.	No	Blinding was maintained during outcome assessments to prevent detection bias.	
4.2 Were measurement methods similar across intervention groups?	Standardised procedures were used for all measurements.	Yes	Uniform methods ensured consistency in outcome measurement.	
5. Bias in Selection of the Reported Result				**Low risk of bias**
5.1 Were the data analysed in accordance with a pre-specified plan that was finalized before unblinded outcome data were available for analysis?	Data were analysed as per a pre-specified plan, in consultation with a statistician.	Yes	Analysis followed the pre-specified plan, ensuring consistency and transparency.	
Overall Risk of Bias				**Low overall risk of bias**
		The study implemented rigorous measures to ensure internal validity and minimise bias. These included		

		external randomisation, blinding of participants and the study staff implemented standardised measurement methods, and thorough handling of missing data.		

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