DISSERTATION

THE RELATIONSHIP BETWEEN ALCOHOL CONSUMPTION AND METABOLIC SYNDROME AND ITS COMPONENTS AMONG ADULTS FROM DIKGALE HDSS LIMPOPO PROVINCE

by

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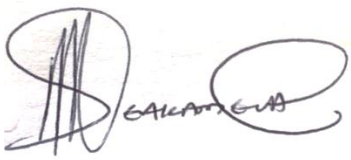
DECLARATION

I, Kagiso Peace Seakamela declare that the dissertation is hereby submitted to the University of Limpopo for the degree of Master of Science in Medical Sciences (Chemical Pathology) has not previously been submitted by me or any other person for any degree at this or any other University; and that it is my work in design and execution, and that all the sources that I have used or quoted have been indicated and acknowledged using complete references.

Signature:  Date: 10/10/2022

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DEDICATION

I dedicate my dissertation to my family who has always been there for me from the beginning of this study giving me moral support and their positivity which always re-energized and refreshed my views. Again, my deceased mom has always been the light and strength in my life.

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DEFINITION OF CONCEPTS

All concepts listed below were used as they are unless indicated otherwise.

Adult: an adult is a matured, fully developed human being who has reached the age where they are legally responsible for their actions (aged >18 years) (Centers for Disease Control and Prevention, 2011). In this study, an adult refers to men and women aged between 40 and 60 years.

Component: An essential building block of the secure and dependable system (Kreutz, Ramos and Verissimo, 2013). In this study, a component refers to any of the risk factors of cardiovascular diseases such as hypertension, type 2 diabetes mellitus, hypertriglyceridemia, hyperalphalipoproteinaemia, and abdominal obesity.

Diabetes mellitus: Is a systemic metabolic disorder characterized by a tendency to chronic hyperglycaemia (Marshall, Bangert and Lapsley, 2010).

Dyslipidaemia: is defined as Total Cholesterol, Low Density Lipoprotein Cholesterol (LDL-C) and triglycerides (TG) of > 5.2 mmol/L, >2.5 mmol/L and > 1.7 mmol/L, respectively and HDLC of < 1.03 mmol/L for men and <1.29 mmol/l for women (Dave et al., 2016).

Hypertension: Is defined as an average systolic BP (SBP) ≥140 mmHg or an average diastolic BP (DBP) ≥90 mmHg (Centers for Disease Control and Prevention, CDC, 2012).

Metabolic syndrome: Cluster of metabolic abnormalities that increase the risk of developing car diseases and Type 2 diabetes mellitus (Yki-Järvinen, 2014; Famuyiwa, Bitrus, Charles-Davies, et al, 2016).

Visceral Obesity: This is defined as a waist circumference of >102 cm for men and 88 cm for women (Zhao, Ford, Li, Tsai, and Dhingra, 2011).

ABBREVIATIONS

AWI-Gen- Africa Wits-INDEPTHdiovIN-DEPTH Partnership for Genomic Studies

CDC- Centers for Disease Control and Prevention

DHDSS- Dikgale Health and Demographic Surveillance system site

DM- Diabetes Mellitus

HDL-C- High-Density Lipoprotein Cholesterol

LDL- C- Low-Density Lipoprotein Cholesterol

MetS- Metabolic Syndrome

SPSS- Statistical Package for Social Sciences

TREC- Turfloop Research Ethics Committee

ABSTRACT

Title: The relationship between alcohol consumption and metabolic syndrome and its components amongst adults from Dikgale HDSS in Limpopo Province.

Background: Alcohol is the most consumed beverage worldwide and is linked to the prevalence of some risk factors of metabolic syndrome. However, the association between alcohol consumption and metabolic syndrome is insufficient and not well studied among rural Africans. This study aimed to determine the relationship between alcohol consumption and metabolic syndrome in adults (40-60 years) from a rural setting, in Limpopo Province.

Objective(s): To determine the prevalence of alcohol consumption, metabolic syndrome, and its risk factors and to assess these respective relationships in adults from Dikgale HDSS.

Methods: This study was correlational and retrospective, applying quantitative methods. Secondary data was collected under the Africa Wits-INDEPTH Partnership for Genomic research project. A sample size of 1398 individuals was collected. A student t-test, bivariate correlation, partial correlation, and logistic linear regression were used. The significance was set at a probability (p) of less than 0.05.

Results: Prevalence of alcohol consumption was 17%, of which (57%) were males. Hypertension (30%) and obesity (43%) were more prevalent. Non-alcohol consumers had significantly higher mean values for glucose and LDL-cholesterol with lower HDL cholesterol. The prevalence of metabolic syndrome was 24% and 22% whilst using two sets of criteria respectively. Non-alcohol consumers had a significantly higher prevalence of metabolic syndrome (24% and 24%) compared to alcohol consumers (12% and 14%). Logistic linear regression showed that alcohol consumption improves HDL cholesterol, hyperglycaemia, and metabolic syndrome. Univariate and multivariate regression shows that alcohol consumers are less likely to have low HDL cholesterol, visceral obesity and hypercholesterolaemia.

Conclusion: In conclusion, alcohol consumption may prevent the development of metabolic syndrome by altering some components of metabolic syndrome such as glucose levels and obesity, thus possibly offering some health benefits.

Keywords: alcohol consumption, metabolic syndrome, obesity, hypertension

CHAPTER 1

INTRODUCTION

Non-communicable diseases that include obesity, hypertension, and type 2 diabetes mellitus are on the increase worldwide (Bastien *et al.*, 2014). Globally, obesity is estimated to be at 35.7%; while hypertension and type 2 diabetes mellitus are at 31.1% and 9.5% in 2019 respectively (Shaw *et al*., 2010; Bastien *et al*., 2014; Mills et al., 2016; Saeedi *et al*., 2019).

In sub-Saharan Africa, obesity, hypertension and type 2 diabetes mellitus (DM) are estimated to be at 43%, 48%, and 16% respectively (Dalal *et al.*, 2011). According to previous studies in South Africa, the prevalence of cardiovascular diseases and type 2 diabetes mellitus were low in the black population 10% in the 1990s and 8.6% in 2005 respectively Yusuf *et al*., (2001) and Alberts *et al*., (2005). however, over the years, a high prevalence of non-communicable diseases has been observed both in urban van Zyl *et al*., (2012) and rural areas Ntuli et al., (2015). Recently, obesity, hypertension, diabetes mellitus and metabolic syndrome are reported to be 35%, 35%, 22% and 32% respectively in south Africa (Peer *et al.*, 2015; Berry *et al.*, 2017; Akokuwebe and Idemudia., 2021; Grundlingh *et al.*, 2022).

According to National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III criteria, metabolic syndrome is a cluster of any three non-communicable diseases viz hypertension, hyperglycaemia, central obesity, hypertriglyceridemia and hyperalphalipoproteinaemia (Bonomini *et al*., 2015). The causes of MetS are largely driven by lifestyle habits such as diet, smoking and lack of exercise (Vijay-kumar *et al*., 2010; Mafa *et al*., 2019). Alcohol consumption is one of the lifestyle habits which is being practised globally with a prevalence of 40.6% (Shield *et al.*, 2013). There are four types of alcoholic beverages cider, beer, spirit, and wine. The relationship between alcohol consumption and metabolic syndrome and components of metabolic syndrome has remained unclear and inconsistent (Alkerwi *et al.*, 2009).

In the study by Toffolo., (2012) light and moderate alcohol consumption of 30 g/day irrespective of the alcoholic beverage was shown to be protective against cardiovascular diseases by increasing HDL-cholesterol and Apolipoprotein A-I. In a study by Alcácera *et al*., (2008) consumption of spirits was associated with an increase in body mass index (BMI) whereas Sayon-Orea *et al*., (2011) reported no relationship between alcohol consumption and BMI. In a study by Shield *et al*., (2013) increased visceral obesity was observed in alcohol consumers compared to non-alcohol consumers. The above-mentioned studies were inconsistent on the impact of alcohol type and the amount consumed. For example, Alcácera *et al*., (2008) did not report on the quantity of alcohol consumed in their study. While, Sayon-Orea *et al*., (2011) and Shields *et al*., (2012) reported the amount of alcohol consumed but did not report the type of alcoholic beverage consumed. Bergmann *et al*., (2011) reported similar findings as Shield *et al*., (2013) the relationship was assessed using wine and beer without quantities as well.

Alcohol increased hepatic secretion of very-low-density lipoproteins (VLDLs) which resulted in increased circulating triglycerides in beer and spirit consumers who had 3-20 drinks/week (Klop *et al*., 2013). Whitfield *et al*., (2013) reported lower plasma triglycerides in participants who consumed alcohol moderately (1-2 drinks/day) as compared to non-alcohol consumers. The available literature on the association between alcohol consumption and triglycerides levels is inconsistent. Studies have reported on the effect of wine and beer without reporting on the amount of alcohol consumed (Arranz *et al.*, 2012).

The prevalence of metabolic syndrome varies demographically, and the practices of a population influence are reported to play a role (Bermúdez *et al.*, 2015; Kim and Cho, 2020; Manaf *et al.*, 2021). There are less data regarding the topic in our location, therefore is a need to contribute to the existing literature and debate using findings from our location.

CHAPTER 2

PROBLEM STATEMENT

In the past, the prevalence of cardiovascular diseases and type 2 diabetes mellitus were low in the black population. However, with urbanization and better living conditions, the events of the two are increasing. Metabolic syndrome (Met S) is considered an important risk factor for both type 2 diabetes mellitus and cardiovascular diseases. The role of modifiable risk factors, particularly alcohol consumption in driving MetS and its components has remained unclear.

Several studies have investigated the association between alcohol consumption and non-communicable diseases. These studies indicate that there seem to be contradictory findings on the association between alcohol and MetS and or its components. These controversies could have been caused by a sample size differences, differences in the type of alcohol consumed, the amount of alcohol consumed and ethnicity of the participants or place of residents. Little is known about the association of alcohol with MetS and or its components among Africans especially those residing in areas previously referred to as rural areas and which are in health transition. Therefore, the present study aims to determine the relationship between alcohol consumption, MetS and its components in adults residing in a rural setting, in Limpopo Province.

CHAPTER 3

LITERATURE REVIEW

This section reviews the available literature on alcohol consumption and metabolic syndrome and individual components of metabolic syndrome. Again, it extensively looks at the prevalence of all the components of metabolic syndrome.

3.1. Overview of alcohol and the risk of cardiovascular diseases

Alcohol is a type of drink or beverage produced by the process of fermentation and contains ethanol which is an organic compound that contains carbon hydrogen and oxygen (Monteiro Vieira *et al.*, 2018).

Production of African traditional beer involves several steps including malting, drying, milling, souring, boiling, mashing and alcoholic fermentation. The steps might differ based on location and community. The produced beer is enriched with nutrients including calories, B-vitamins, and essential amino acids. As compared to western beers, African traditional beers are less attractive because of their poor hygienic quality, organoleptic variations and shorter shelf life (Lyumugabe *et al.*, 2012).

In preparation of sorghum malt, grains are added into a sack and dipped into water for about a day or two. The swollen grains are then spread on the floor to dry up and left there for three days to germinate. The germinated grains are then spread on the floor, the malt is then crushed using grinding stones (Gadaga *et al.*, 1999).

Milling and souring can occur concurrently. Boiling and fermentation of this traditional beer take place in iron pots Choma *et al*., (2007). The boiling and fermentation processes are estimated to take 1 hour and three days respectively. Then, the debris is then removed using a wire sieve or cloth. Ethanol is the major alcohol at about 4% (Gadaga *et al.*, 1999).

Besides African traditional beverages, there are commercially brewed alcohol types viz. beer, wine and spirits which are consumed in the entire world (Chiva-Blanch *et al.*,

2013). The alcohol content of the above-mentioned beverages differs due to their manufacturing processes and ingredients. To increase shelf-life beer is heated at 60 degrees for 15 minutes and wine is treated with sulphur dioxide other than heat, to preserve flavour, taste, and colour (Buzrul., 2012).

The percentages of alcohol in beer and wines range from 5-21 per cent by volume, this is achieved by using different kinds of yeast strains in the fermentation process of these beverages. Spirits are produced with an added step called distillation to yield high alcohol volume (Luisa Alba-Lois., 2010).

Alcoholic beverages are amongst the highly consumed refreshments worldwide. In 2010 40.6% of the world population was recorded to be consuming alcohol (Shield *et al.*, 2013). The frequency of alcohol consumption is high in both developed and developing countries. In sub-Saharan countries, South Africa had the highest alcohol consumption recorded with a prevalence of 25% (Ramsoomar and Morojele., 2012). Maimela *et al*., (2016) reported alcohol consumption to be at 16.3% in Dikgale village, a rural area in Limpopo.

Alcohol is integral to most gatherings including funerals, and weddings. It is given as a form of hospitality, reward for achievement and sign of change in status at traditional ceremonies. In addition, alcohol is used as a form of payment and served in celebrations of a good harvest (Mcallister., 2003; Choma *et al*., 2007; Setlalentoa *et al*., 2010).

The amount of alcohol consumed determines either the beneficence or harmful effect of alcohol. Researchers have reported that there is a health benefits of low or moderate alcohol consumption (Herttua *et al*., 2011; Artero *et al*., 2015). Low alcohol intake even though the quantity and the type of drink were not stipulated was associated with a reduced risk of cardiovascular disease and mortality rate in a study by Gepner *et al.*, (2015).

Low-moderate alcohol consumption is reported to inhibit both gluconeogenesis and glycolysis. It was also reported to promote insulin sensitivity thus reducing the incidence

of diabetes mellitus. Alcohol consumers who had <20 and 20-60 g/day of alcohol improved compared to those who had >60 g/day were at a lower risk of acquiring diabetes mellitus
(Fernández-Solà., 2015). Excessive alcohol consumption irrespective of the drink and quantity has been reported to cause many diseases and health issues (Shield *et al.,* 2014). Some scholars have suggested that alcohol can directly or indirectly cause a disease. In terms of non-communicable diseases, alcohol consumption has been reported to be associated with the development of cancer, alcohol liver disease, diabetes mellitus, and cardiovascular diseases (Parry *et al*., 2011; Park and Kim., 2012).

Even though researchers reported that moderate alcohol use improves the cardiovascular system; Chikritzhs *et al*., (2015) have recommended against alcohol use for health benefits. Literature suggests that alcohol may lead to cardiovascular disease either directly, indirectly, or both. Alcohol has been reported to reduce myocardial contractility and induce arrhythmias and dilated cardiomyopathy. Excessive alcohol consumption of >60 g/day in men and >40 g/day in women may result in increased cardiovascular morbidity such as coronary and peripheral artery disease, heart failure, stroke, and hypertension (Molina *et al*., 2014; Fernández-Solà., 2015).

Alcohol is also known to be toxic to neurons, it can induce oxidative stress and damage blood vessels resulting in cardiovascular events (Das and Vasudevan., 2007; Lee *et al*., 2010; Shen *et al*., 2014). Literature suggests that alcohol can indirectly lead to cardiovascular diseases through the modulation of components of metabolic syndrome viz. chronic hyperglycaemia, visceral obesity, dyslipidaemia, and hypertension (Corrao., 2004; Mukamal *et al*., 2005; Sun *et al*., 2014). Type 2 Diabetes Mellitus and metabolic syndrome have been extensively associated with cardiovascular diseases and are regarded as independent risk factors for cardiovascular diseases (Bonomini *et al*., 2015; Matsuda and Shimomura., 2013; Fonseca., 2021). The next section describes the association between alcohol consumption and cardiovascular risk factors.

3.2. Association of alcohol consumption and cardiovascular risk factors

Alcohol consumption has been associated with metabolic syndrome and or components of metabolic syndrome. Metabolic syndrome is a known cardiovascular risk factor (Tune *et al*., 2017). It is affected by both genetic and epigenetic factors. The next section outlines how alcohol consumption affects individual components of metabolic syndrome.

3.2.1. Alcohol consumption and Hyperglycaemia

Hyperglycaemia is defined as fasting glucose levels of >7.0 mmol/L or non-fasting glucose of ≥11mmol/L (Alberti *et al*., 2009; van Zyl *et al*., 2012). Hyperglycaemia is a known clinical feature of diabetes mellitus which can be caused by insulin insensitivity in adults and no production of insulin in children. It has been classified as a cardiovascular risk factor (Van Dijk *et al.*, 2012). The distribution of the condition is skewed towards the elderly population with more elderly people acquiring diabetes mellitus (Geldsetzer *et al.*, 2018).

The worldwide trend of diabetes mellitus shows an exponential increase with 1.6 million Americans (<1%) in 1958, 30.2 million (12.2%) in 2015 and 48 million by 2050 *(*Chen *et al*., 2012; Stehouwer., 2018). The same increasing trend has been reported in South Africa. According to previous studies, the prevalence of cardiovascular diseases and type 2 diabetes mellitus were low in the black population 10% in the 1990s and 8.6% in 2005 respectively (Murray., 1996; Yusuf *et al*., 2001; Alberts *et al*., 2005). however, over the years the high prevalence of non-communicable diseases including diabetes mellitus has been observed both in urban and rural areas (van Zyl *et al*., 2012; Maimela *et al*., 2016).

There is no simple explanation for the growing trend in disease prevalence. It is a complex and multifactorial condition. The demographic, socio-economic and most importantly urbanization are the major contributors to the growing trend. With age the ability of the body to maintain itself deteriorates.

The liver is the principal metabolic regular of glucose and provides the body with 90-95% circulating glucose during a fasting state. The brain and muscles utilize most of the circulating glucose in the fasting state. Insulin levels are kept at levels relative to

circulating glucose to keep it within physiological ranges. In cases of health, the muscles can double their absorptive capability to cater for gross elevation in glucose. It has been documented that age decreases the ability of muscles to downregulate circulating glucose levels (He *et al.*, 2018).

The mechanism of reduced glucose absorptive capability with age has been reported to be a decrease in insulin sensitivity of the muscles. The increase in insulin insensitivity with age is promoted by decreased physical activity, muscle wasting and adipose tissue dysfunction (Chia *et al*., 2018).

Gender is an important factor in the ability of the body to regulate glucose. With given differences in the anatomy and physiological capabilities by gender, studies have reported a significant difference in the prevalence of hyperglycaemia between men and women. The sex hormones have an impact on the outcome of glucose tolerance and again the hormones affect the behavioural patterns between the two parties (Mauvais-Jarvis., 2018). Women are generally health-conscious, which makes them proactive and informed about health cases. The behavioural patterns by gender contribute to hyperglycaemia where men are more likely to be excessive smokers, and alcohol consumers (Chang *et al.*, 2010).

In low-income and developing countries, there seems to be a high gender inequality, where more men are employed compared to women. The types of jobs that most men in developing countries give them a protective effect against hyperglycaemia and cardiovascular risk. More women in developing countries are more likely to have chronic hyperglycaemia compared to men possibly due to the skewed gender inequality (Attanapola., 2004; Virtanen *et al*., 2013; Kivimäki and Kawachi., 2015; Huebschmann *et al*., 2019).

In South Africa, there is a shift in the type of meals Africans are currently consuming. Previously subsistence farming used to be the main source of food in most households in rural settings. Subsistence farming forces individuals to be physically active as they

work on the land. With urbanization rural dwellers have eased to produce their fruits and vegetables, they now depend on the food available on the market.

There is also a change in the diet where people are having more refined foods. The change in diet is driven by socio-economic status which comes with excess resources to purchase fast foods. The people in the working class do not have enough time on their hands to be able to exercise after work. Consumption of refined foods with high calories and reduced physical activity results in weight gain (Steyn *et al*., 2016; Mashinya *et a*l., 2018). The relationship between weight gain and insulin insensitivity is reported to be direct. Insulin resistance results in hyperglycaemia which is a cardiovascular risk factor (Wu and Ballantyne., 2017).

Light or moderate alcohol consumption is reported to improve insulin sensitivity. On the other hand, heavy alcohol consumption is reported to promote the development of diabetes mellitus which is a cardiovascular risk factor (Fernández-Solà., 2015; Steiner *et al*., 2015; Du *et al.*, 2017). A review by Ronksley *et al*., (2011) found a positive association between alcohol and diabetes mellitus in longitudinal studies. The findings from the study insinuate a possible protective impact of alcohol consumption on cardiovascular disease. The protection from alcohol consumption to cardiovascular disease is quantity dependent according to the reported literature.

The potential benefit of light-to-moderate alcohol consumption was reported in an experimental study by Steiner *et al*., (2015). In this study, cell cultures of cardiomyocytes were incubated in 50mM of alcohol for 30 minutes. There was a relocation of GLUT4 from the cytosol to the plasma membrane. The availability of GLUT4 in plasma reduces glucose concentrations thus reversing the cardiovascular risk effect of glucose. The time frame of this experiment could be too short to accept how alcohol consumption may prevent cardiovascular diseases.

Dose-response on alcohol consumption and incident of diabetes has shown a reduction in alcohol exposure in both men and women (Knott *et al*., 2015). Even though this study

managed to find balanced outcomes on gender, one should not ignore the potential effect of biological variance between the two groups. Again Sumida *et al*., (2007) reported inclined glucose levels in women than men even after the consumption of an equal amount of alcohol.

The mechanism through which hyperglycaemia results in cardiovascular diseases is reported to be through three pathways: the activation of protein kinase C, polyol and hexosamine. In the state of hyperglycaemia, glucose is metabolised via the polyol pathway. Glucose was converted to sorbitol by supplementation of nicotinamide adenine dinucleotide phosphate (NADPH) and aldolase.

In the presence of nicotinamide adenine dinucleotide (NAD+) and sorbitol dehydrogenase, sorbitol is further metabolised to fructose. This pathway causes NADH/NAD+ redox imbalance and these together with fructose produced are implicated in the pathogenesis of diabetes mellitus (Yan., 2018).

The hexosamine biosynthetic pathway metabolises fructose-6-phosphate produced from the glycolytic pathway. Under normal conditions the body does not use this pathway, it is only used during hyperglycaemic states. Glucosamine-fructose aminotransferase metabolizes fructose-6-phosphate to glucosamine-6-phosphate, which will then later be converted to Uridine-Diphosphate-N-acetylglucosamines. This pathway is known to produce transcription factors like transforming growth factor-alpha (TGF-α) and TGF-β which are responsible for the activation of collagen matrix and basement membrane thickening (Ighodaro., 2018).

Hyperglycaemia induces the production of reactive oxygen species (ROS), which increases endothelial oxidative stress. Hyperglycaemia-induced oxidative stress is a major cause of micro and macro-vascular diseases (Fiorentino *et al.*, 2013; Sluik *et al.*, 2016). People with type 2 diabetes mellitus and or metabolic syndrome are more likely to develop cardiovascular diseases which have increased to be amongst the highest causes of death worldwide (Panahi *et al.*, 2014).

3.2.2. Alcohol Consumption and Dyslipidaemia

Dyslipidaemia can be defined as Total Cholesterol, Low-Density Lipoprotein Cholesterol (LDL-C) and triglycerides (TG) of > 5.2 mmol/L, >2.5 mmol/L and > 1.7 mmol/L, respectively and high-density lipoprotein cholesterol (HDLC) of < 1.03 mmol/L for men and <1.29 mmol/L for women (James and Clee-man., 2001; Dave *et al*., 2016). These lipid irregularities are linked to the risk of developing atherosclerosis and cardiovascular diseases (Goedecke *et al.*, 2010).

Studies have associated dyslipidaemia with alcohol consumption. Consumption of heavy alcohol like wine and beer has been associated with elevated lipids (Arranz *et al.*, 2012; Shen *et al.*, 2014). The mechanism of how alcohol may lead to increased levels of atherogenic serum lipids has not been thoroughly explained. However, the proposed mechanism is that VLDL secretion is increased, impaired breakdown of fats and increased free fatty acid flow from adipose tissue to the liver (Klop *et al*., 2013). Researchers suggest that alcohol consumption increases the production of triglycerides and triglyceride-rich lipoprotein in the liver. Again, alcohol induces lipolysis in fatty tissues, which results in elevated fatty acids (Bessembinders *et al*., 2011).

In a study conducted by Maimela *et al*., (2016) in a rural black setting in South Africa, raised triglycerides were found to be 25.4%, with high total cholesterol at 32.6%. The prevalence of lipids in this population may predict the possibility of increased cardiovascular risk. The prevalence of alcohol consumption in this population is reported to be 16.3% with distribution skewed towards men with 28.8% to 8.8% for women.

Elevated lipoproteins can result in cardiovascular diseases in two ways, it can be through their homologic characteristics with plasminogen and plasmin. This makes them have prothrombotic functional effects without fibrinolytic activity. Again, they promote atherogenesis through intimal deposition of lipoprotein cholesterol (Nordestgaard *et al.*, 2010). The deposition of lipoprotein cholesterol in the intima results in the diameter of the vessels to decrease reducing blood flow. The formed plaque can shed off with increased blood pressure which might later cause blockage in the circulatory system.

HDL-C is regarded as a protector against cardiovascular diseases and thus it is referred to as anti-atherogenic lipoprotein. Low levels of HDL-C are associated with an increased risk of developing cardiovascular diseases (MacKey *et al.*, 2012). HDL-cholesterol is regarded as a cardio-protector due to its involvement in reverse cholesterol transport (Rothblat and Phillips., 2010; von Eckardstein and Kardassis., 2015).

Alcohol and Plasma lipoproteins

Alcohol consumption affects lipid metabolism in different ways, these pathways are complex, and some are not thoroughly evaluated. The next section examines how alcohol consumption affects the metabolism of lipids.

Alcohol and lipoprotein lipase (LPL) activity

An experimental study where an intravenous fat tolerance test (IFTT) was used to investigate the effect of alcohol found that alcohol inhibited the clearance rate of fat emulsion from the circulation. It was concluded that alcohol decreased the activity of Lipoprotein lipase which explains the elevation of triglycerides, VLDL-C and VLDL-C in the circulation (Zemánková *et al.*, 2015; Zhou *et al.*, 2016).

The effects of alcohol on lipid metabolism are diverse and more complex. Lipoprotein Lipase is the vital enzyme involved in lipid metabolism. Therefore, the activity of LPL is homeostatically controlled depending on the circulating plasma of TG-derived fatty acids and TG-rich lipoproteins (Kersten., 2014). The physiological function of LPL determines plasma TG clearance and tissue uptake of fatty acids.

Lipoprotein Lipase is involved in the clearance of TG-rich particles such as very-low-density lipoprotein (VLDL) and chylomicrons. Alcohol is known to negatively affect the activity of LPL in acute consumption thus resulting in hypertriglyceridemia (Kersten., 2014).

Acetate and G-protein-coupled receptor 43 (GPCR 43)

Researchers have stipulated that acetate the metabolite of alcohol is the component that makes alcohol have metabolic effects. G-protein-coupled receptor 43 which is the receptor for acetate, in adipose tissue results in inhibition of lipolysis and increased insulin sensitivity (Yeo *et al*., 2012; Kovář and Zemánková, 2015). In this study, alcohol reduced the serum triglycerides by inhibiting lipolysis which reduced the available free fatty acids for the synthesis of triglycerides and VLDL production in the liver (Kovář and Zemánková., 2015).

A study by Pownall *et al*., (2015) supported the above-mentioned findings, that GPCR 43 the receptor for acetate has lipolysis inhibitory effects. The GPCR 43 was also expressed in mice in response to acetate, which showed or confirmed that it does inhibit lipolysis at the cellular level. Alcohol inhibits the breakdown of lipids, which later results in decreased lipids in circulation. The mechanism how is explained above, the receptor responsible for the breakdown of lipids in the cell is inhibited by the component of alcohol known as acetate. The resultant inhibition of lipolysis improves dyslipidaemia, which is associated with cardiovascular diseases. There is a protective effect against cardiovascular disease from alcohol consumption.

Impact of alcohol on Redox Balance

Metabolism of ethanol to acetate results in 2 equivalents of reduced NADH every time ethanol is oxidised. This metabolism massively increases the NADH: NAD+ within the cell, which then favours the inhibition of fatty acid β-oxidation in the liver. The redox imbalance results in the accumulation of triglycerides in the hepatocytes (Shen *et al*., 2005; You and Arteel., 2019). Metabolism of alcohol to acetate indirectly results in raised lipid which then contributes to dyslipidaemia seen in alcohol consumers by disrupting the redox balance.

The studies about alcohol raise the hypothesis that the beneficial or negative effects of alcohol may be due to the compounds present in each alcohol type. This can be deduced from the sample quantity consumed, where the other alcohol type has harmful effects whereas the other produced the complete opposite. The same is seen in the lipid

profile, the next section will be looking at the components of alcohol called polyphenols which are said to be associated with lipid levels (Rada., 2017).

Alcohol and HDL-C, LDL-c, and Triglycerides

A review study by (Arranz *et al.*, 2012) reported that wine and beer have components called polyphenols which are the ones that bring about the protective effect of alcohol. Some compounds in alcohol make it an antioxidant which reduces LDL by inhibiting its oxidation, modification of cell signalling pathways and increasing the production of HDL-C.

In another review by Pownall *et al*., (2015) consumption of 30 g/day of alcohol increased HDL-C levels by a mean of 4 mg/dl irrespective of the kind of alcohol consumed. The findings in this study suggest that alcohol type has no impact on the outcomes of dyslipidaemia, but rather on the amount consumed. Therefore, alcohol is protective against cardiovascular risk when quantity is controlled. As interesting as these findings are, they raise the question as to which component of alcohol is active. Researchers have reported different components to be active viz. ethanol, acetate, and polyphenols.

Fernández-Solà., (2015) reported findings from a randomized beer feeding trial demonstrated that moderate alcohol consumption (30 g/day) increases serum lipids. It increased serum HDL-cholesterol by 5%, apolipoproteins A-I by 6%, and apolipoproteins A-II which provides indirect pathophysiological evidence. This is in agreement with the 25-35% protective effect of moderate alcohol use reported by Krenz and Korthuis., (2012) and Chiva-Blanch *et al*., (2015).

There is a general impression in this topic that 30 g/day of alcohol could be the borderline amount that brings about the protective effect of alcohol. This amount throughout the literature is regarded as moderate alcohol consumption. Alcohol type and amount bring about different outcomes even when both are kept constant, a different study design has been explored and all are at a chemical level. The next section explores the effect of alcohol on lipid profile at the molecular level using randomised mendelian study designs.

Mendelian randomization studies.

There is a single nucleotide polymorphism gene in the alcohol dehydrogenase gene 1B (ADH1B) which has been reported to code for the ADH1B enzyme (Li *et al*., 2011). This enzyme is primarily involved in the metabolism of alcohol. It was reported that carriers of the rs-1229984 A-allele in the study by Holmes *et al*., (2014) were moderate alcohol consumers as compared to non-carriers. They also showed to have lower levels of adiposity, and atherogenic lipids and are less likely to have cardiovascular diseases.

Mendelian studies in the East Asian region use the aldehyde dehydrogenase 2 (ALDH2) as the main variant of focus to study alcohol use. The ALDH2 gene is involved in the catabolism of acetal aldehyde which is a metabolite of alcohol. The rs671 single polymorphism is a characteristic of the gene in humans which makes those who are heterogenous for the single-A allele have reduced function of the ALDH2. Hence, they tend to consume less alcohol. Whereas people who are homogenous for the allele have a complete loss of function of the ALDH2 enzyme and most of them are alcohol abstainers (Taylor *et al.*, 2015).

Rs671 genotype was associated with higher levels of fasting blood glucose, HDL-C, triglycerides, and lower LDL cholesterol in alcohol consumers of 18.8 g/day for men and 1.3 g/day in women. This finding is supported by a study by Taylor *et al*., (2015) which reported an association of rs671 with higher levels of triglycerides. In a study by Cho *et al*., (2015) alcohol consumption increased the levels of triglycerides. Researchers suggested a possible impact of the ALDH2 rs671 variant on lipid profile without alcohol consumption.

These genes predispose people to alcohol consumption, and they determine the level of alcohol consumption, which directly or indirectly has an impact on the lipid profile. This could explain why people who consume alcohol might have different metabolic and phenotypic outcomes.

Researchers in a study by Vu *et al.*, (2016) have explained how alcohol improved HDL levels. Firstly, it was reported that alcohol increases the production in the liver and the transportation rate of ApoA-I and ApoA-II. Secondly, it increases cellular cholesterol efflux and plasma cholesterol esterification. Which later, increases muscle ATP-binding cassette ABCA1 that could be important in recycling natant HDL through reverse cholesterol transport. Utilization of the reverse cholesterol transport decreases available cholesteryl ester transfer protein CETP (Vu *et al*., 2016). Marshall *et al*, (2012) support this mechanism that shows that low CETP increases the HDL2-C levels which is a large HDL particle involved mostly in the reversal transport of cholesterol out of the circulation.

There are different LDL particles viz. LDL-C, which is big, and another is the smaller one (sdLDL). sdLDL promotes inflammation and plaque formation due to its susceptibility to oxidation and hence increases the chances of cardiovascular events (Hoogeveen *et al.*, 2014). This could mean that LDL results should be interpreted with caution, otherwise Vu *et al.*, (2016) suggested the use of total ApoB which could reflect the total atherogenic particles.

3.2.3. Alcohol consumption and hypertension

Vgontzas *et al*., (2009) defined hypertension as an average systolic BP (SBP) ≥140 mmHg or an average diastolic BP (DBP) ≥90 mmHg. Hypertension is an important risk factor for cardiovascular diseases according to Thayer *et al*., (2010).

Peltzer and Phaswana-Mafuya., (2013) reported alcohol consumption to be a risk for the development of hypertension. Results from a longitudinal study suggest that heavy drinking increases the odds of having hypertension compared to moderate alcohol consumption. Alcohol induces hypertension in different ways, by inducing endothelium and oxidative stress and the renin-angiotensin-aldosterone system. Alcohol increases angiotensin II levels, and again promotes the release of angiothelin 1 and 2. Both angiothelin 1 and 2 and angiotensin II are known for their vaso-constrictive characteristics (Husain *et al.,* 2014). The mechanism for how light and moderate alcohol consumption blood pressure is unclear and controversial (Briasoulis *et al.,* 2012).

Collart *et al*., (2015) suggest that alcohol consumption is responsible for 17% of reversible hypertension. The proposed mechanism was that alcohol increases the risk of cardiomyopathy and atrial fibrillation which leads to increased blood pressure causing hypertension. Collart *et al*., (2015) and Mancia., (2013) further demonstrated that patients were at a lower risk of hypertension after alcohol withdrawal and reduction in alcohol intake. A meta-analysis study by Kodama *et al*., (2011) also supports the above-mentioned studies that alcohol consumption leads to arterial fibrillation. They concluded that alcohol abstinence is the most favourable behaviour than moderate consumption. This suggests that alcohol could lead to cardiovascular diseases through the modulation of hypertension.

Ethanol alters plasma membrane permeability and increases intracellular calcium concentration. Increased influx of calcium in vascular smooth muscle cells causes disturbed muscle tone and resistance, which later leads to hypertension (Husain et al., 2014; Watson and Zibadi., 2017). Wang *et al*., (2016) elaborated on the increased calcium cellular influx, in their study they found that chronic alcohol consumption upregulates the expression of mitochondrial calcium uniporter (MCU) calcium channel and voltage-dependent anion channel (VDAC). Inhibition or suppression of the CsA-sensitive mitochondrial calcium efflux pathway results in calcium accumulation and retention in the mitochondrial matrix. Through the above-mentioned pathway, the cells swell resulting in increased blood pressure. Long *et al*., (2018) found comparable results to the two studies in China.

Again, Calcium is involved in muscular contraction, the more calcium the cell has the more excited it becomes. This will eventually lead to increased blood pressure if it occurs in vascular smooth muscles (Baek *et al.*, 2017).

Radical oxidative stress produced after alcohol consumption activates NADPH oxidase. NADPH oxidase introduces the NADP/NADPH ratio disturbances which eventually increases the oxidative stress (Husain *et al*., 2014). Larsen and Matchkov., (2016) found

an association between oxidative stress and hypertension which resulted in increased blood pressure.

30 g/day of ethanol has been linked with hypertension in women. Non-drinkers as compared to drinkers had an increased chance of hypertension, this shows a possible protective effect of alcohol on hypertension. By gender men and women who had 2-4 and 1-2 drinks respectively, showed a protective effect after consumption (Fisher *et al.,* 2018).

3.2.4. Alcohol consumption with metabolic syndrome

Alberti *et al.*, (2009) defined Metabolic syndrome (MetS) as a condition in which multiple interrelated CVD risk factors co-exist in an individual. National Cholesterol Education Program ATP III (NCEP ATP III) defines metabolic syndrome as the presence of any three of the risk factors and abdominal obesity. According to the International Diabetes Federation (IDF), metabolic syndrome is present if someone has abdominal obesity and any of three components of metS (Herath *et a*l., 2018).

According to Kingue *et al*., (2017) and Moore and Chaudhary., (2017) metabolic syndrome is a driving cause and a predictor of cardiovascular diseases, morbidity, and mortality. Internationally metabolic syndrome has a prevalence of 30%, and 39.4% in sub-Saharan Africa. In the South African black population, the prevalence of metabolic syndrome was reported to be 30.7% with women having higher components of metabolic syndrome as compared to men (Peer *et al.*, 2015).

Alcohol consumption, the most prevalent lifestyle practice which has been linked with metabolic syndrome and components of metabolic syndrome could be causing an incline in the prevalence of metabolic syndrome (Sun *et al.*, 2014). Zatu *et al*., (2015) reported a high prevalence of metabolic syndrome in heavy drinkers who had >20-30 g/day of alcohol and above as compared to light drinkers who only had 0-10 g/day.

Alcohol has a positive and negative impact on metabolic syndrome depending on the alcohol consumed. On contrary, Oh *et al*., (2018) reported that women who consumed

<5 drinks per session were at higher risk to develop metabolic syndrome. The study by Lee and Jang., (2021) reported <5 drinks per session as moderate alcohol consumption and it was reported to be associated with a reduced risk of metabolic syndrome. In another study conducted on a Korean population it was found that alcohol improves metabolic syndrome only when ≤5 g/day is consumed (Kim *et al.*, 2017; Suliga *et al.*, 2019; Chevli *et al.*, 2020).
Wakabayashi., (2010) reported that alcohol consumption of <20 g/day was associated with a lower prevalence of metS. The finding of the studies increases the uncertainty of the effect of alcohol quantity on metabolic syndrome (Kim *et al.,* 2013).

Cross-sectional evidence shows a positive relationship between alcohol and metabolic syndrome (Hu and Malik., 2010). Vieira *et al*., (2016) found a preventative effect of alcohol against metabolic syndrome in the ELSA-Brasil study. There was a j-shaped relationship between alcohol consumption and increased HDL-C levels, irrespective of alcohol type and timing.

A three-year follow-up by Kim *et al*., (2012) investigating the relationship between alcohol and metS found that alcohol consumption did not improve metabolic syndrome. Alcohol consumers compared to non-alcohol consumers had higher odds of developing metabolic syndrome (Park and Kim., 2012). The increase in alcohol quantity was also linked with the likelihood of developing metS even though researchers assessed only one type of alcohol that is consumed in the community which is a spirit.

In an experiment where rats were exposed to extracts of red wine, it was reported that red wine reduced the pro-inflammatory markers which are elevated in metabolic syndrome. The researcher reported that the polyphenols which are a component of red wine are the ones responsible for the protective effect of red wine towards metabolic syndrome (Janega *et al.*, 2014).

Socioeconomic factors also contribute to the prevalence of metabolic syndrome (Zhan *et al.*, 2012). Kim et al., (2013) and Alkerwi et al., (2009) associated socio-economic status

with higher odds ratio of having metabolic syndrome. Socioeconomic status and household income were associated with metabolic syndrome in women but not in men. Studies have also associated metabolic syndrome with education, where people who have lower education levels were more at risk of developing metabolic syndrome. The proposed mechanism is that people who have higher education are more informed of the impact of lifestyle behaviour on health. socioeconomic status has been reported to affect lifestyle choices like smoking and drinking (Zhan *et al.*, 2012).

Socioeconomic status affects lifestyle behaviour, this includes the type of alcohol consumed and how frequent people consume alcohol and exercise. Therefore, metabolic syndrome can be linked with lower socioeconomic status since the people in this category consume more alcohol, poor diet and exercise less (Lee and An., 2020).

Summary of literature:

Alcohol consumption has been implicated in the development of cardiovascular disease and metabolic syndrome. It results in cardiovascular diseases directly and indirectly. Also, Alcohol consumption results in metabolic syndrome by promoting the development of components of metabolic syndrome (Parry., 2011; Park et al., 2012; Molina *et al.*, 2014; Fernández-Solà, 2015; Jeong *et al.*, 2018).

Alcohol consumption is reported to contribute to the development of visceral obesity a component of metabolic syndrome (Shields *et al.*, 2012; Traversy and Chaput., 2015). Visceral obesity alone has been implicated in the development of metabolic syndrome by increasing its components viz. dyslipidaemia; hyperglycaemia and hypertension (Owolabi *et al.*, 2017). Studies have associated alcohol consumption with hypertension, dyslipidaemia, and hyperglycaemia (Erasmas *et al*.,2012; Peltzer and Phaswana-Mafuya, 2013; Shen *et al.*, 2014; Agongo *et al.*, 2019).

The amount and type of alcohol consumed have brought contradicting results in the available literature (Toffolo., 2012; Sun *et al*., 2014; Fisher *et al*., 2018). Studies have reported contradicting results regarding the impact of alcohol types. Some reported that

ethanol is an active component in alcoholic beverages, while others reported it to be polyphenol (Arranz *et al.*, 2012; Chiva-Blanch *et al.*, 2013; Janega *et al.*, 2014).

There is a gradual increase in the prevalence of cardiovascular risk factors with non-communicable diseases on the forefront (Alberts *et al.*, 2005; Van Zyl *et al.*, 2012; Ntuli *et al.*, 2015; Maimela *et al.*, 2016). The increase in the prevalence of the metabolic syndrome is reported to be a global burden including in South Africa (Motala *et al.*, 2011; Erasmus *et al.*, 2012; Saklayen., 2018; Monyeki *et al.*, 2020). socio-economic factors, age and gender are reported to be contributing factors to the prevalence of metabolic syndrome (Ramsoomar and Morojele., 2012; Mauvais-Jarvis., 2018; Lee and An., 2020)

previously, a high prevalence of cardiovascular risk factors was reported in an urban setting but Steyn *et al.*, (2016); Mashinya *et al.*, (2018); Hlangwani *et al.*, (2020) have reported a shift in lifestyle in rural areas. Few studies have been conducted in rural areas regarding the effect of alcohol consumption on metabolic syndrome in South Africa, including the study area in question.

CHAPTER 4

PURPOSE OF STUDY

4.1. Aim

To determine the relationship of alcohol consumption with Metabolic Syndrome and its components among adults aged 40-60years from Dikgale HDSS.

4.2. Objectives

- To determine the prevalence of alcohol consumption, metabolic syndrome, and individual components of metabolic syndrome in adults from Dikgale HDSS.
- To assess the relationship of alcohol consumption with metabolic syndrome and individual components of metabolic syndrome in adults from Dikgale HDSS.

4.3. Research question

What is the relationship between alcohol consumption with metabolic syndrome and its components among adults aged 40-60years from Dikgale HDSS?

CHAPTER 5

METHODOLOGY

5.1. Study Design

The present study was correlational and retrospective in design, applying quantitative methods. A correlational study is a scientific study in which a researcher investigates the relationship between variables (Mayer and Frantz., 2004). The present study was correlational since the association of alcohol consumption with metabolic syndrome and its components was assessed.

According to Hess., (2004) a retrospective study is a type of study that uses data that has been recorded for reasons other than present research interest. The study used secondary data collected under the Africa Wits-INDEPTH Partnership for Genomic (AWI-Gen) Research project and is securely stored. Quantitative research is a type of research that involves statistical analysis of the variables and is reported numerically (Creswell *et al.*, 2007). The quantitative method was used as numerical data such as the levels of lipids, blood pressure levels and glucose levels were analysed statistically.

The current study was retrospective in design, however, as a researcher, I was also involved in data collection:

a) Administering the questionnaire to study participants
b) Measurement of anthropometries
c) Entering data points into the online server (RedCap)

5.2. Study Site

The AWI-Gen data that was used in the present study was collected from participants residing at the Dikgale HDSS site. The site is located 40 km north of Polokwane, the capital of Limpopo Province and is comprised of 15 villages with a population size of 36000.

5.3. Study Population

The study population comprises mainly northern Sotho-speaking people aged 40-60 years.

5.4. Sampling and Sample Size

5.4.1. Sampling method

For the primary AWI-Gen study from which secondary data is derived, the sampling protocol was based on a total of 5479 individuals aged 40-60 years who were identified from the Dikgale HDSS census database. As this community was known to be highly mobile, trained field workers visited all households with adults aged 40-60 old who were permanent residents (an individual staying in the household for at least 6 months of the year, according to HDSS criteria) and invited them to participate in the study. A total of 1398 individuals who were willing to participate enrolled in the AWI-Gen population-based cross-sectional study that was conducted between November 2014 and August 2016. Since the present study used a secondary dataset, all the participants entered the dataset with complete records for the variables of interest (alcohol consumption, glucose, systolic and diastolic blood pressure, waist circumference, lipids) were part of the sample for the present study.

5.4.2. Inclusion criteria

All participants were included in the primary AWI-Gen study.

5.4.3. Exclusion criteria

The primary AWI-Gen study, from which the current secondary dataset is derived excluded pregnant women and individuals presenting with physical impairments that would prevent the measurement of anthropometric indices.

In the present study, participants with incomplete information on the data required for the present study and participants with any one of the variable values that are considered outliers were excluded.

5.5. Data Collection

The secondary data for use in the present study was primarily collected under the AWI-Gen project and the methods have been previously described (Ali *et al.*, 2018). The

secondary study extracted variables including Alcohol consumption, Anthropometries, Blood Pressure, Serum Lipids, and Glucose from the primary AWI-Gen study. In summary, the data was collected as follows:

5.5.1. Collection of Alcohol Consumption Data

The AWI-Gen developed questionnaire was used to capture information on socio-demography, lifestyle including alcohol consumption, diseases, anthropometry, and biomarkers. The questionnaire was first piloted on non-study participants to ensure its reliability and validity. See Appendix I for the questionnaire

5.5.2. Anthropometric measurements

(a) Weight (kg)

The body weight was measured to the nearest 0.1 kg with the participant wearing light clothes and without shoes using Omron Measuring Scale manufactured by Omron Healthcare (INC, CHINA).

(b) Height (m)

The body height was measured to the nearest 0.1 m using a stadiometer. They were standing vertically on the stadiometer without shoes.

(c) Calculation of BMI

Body mass index (kg/m^2) was calculated using measured weight and height, by the following equation:

$$\text{BMI} = \frac{\text{weight (kg)}}{[\text{height (m)}]^2}$$

(d) Waist Circumference

Waist circumference was measured using a measuring tape, the tape was placed around the participant's belly, around the belly button. And they were not allowed to take deep breaths during the session. Measurements were rounded off to the nearest 0.1 cm.

(e) Blood Pressure

Blood pressure was measured using an Omron blood pressure monitor (manufactured by Omron Healthcare INC, CHINA). The instrument uses three different cuff sizes depending on the arm i.e. small, medium, and large. Before measurement, all participants were allowed to relax for about five minutes and food intake, drink, talking, and smoking was not allowed during the assessment. The measurement was done three times per participant and recorded with three rests in between. Three readings were taken and the mean of the last two readings was used.

5.5.3. Laboratory methods

(a) Cholesterol determination

Cholesterol was determined using a Randox Plus clinical chemistry analyser (UK). The principle and method performance are explained in appendix ii.

(b) Triglycerides determination

Triglycerides were analysed using a Randox Plus clinical chemistry analyser (UK). The principle and method performance are explained in appendix ii.

(c) HDL-cholesterol determination

HDL-cholesterol was analysed using a Randox Plus clinical chemistry analyser (UK). The principle and method performance are explained in appendix ii.

(d) LDL cholesterol determination

According to the Heart UK – The Cholesterol Charity (2015) LDL cholesterol levels were calculated using the Friedewald equation:

LDL-cholesterol = Total cholesterol – HDL cholesterol – (Total triglyceride ÷ 2.19)

All measurements are in millimoles per litre (mmol/L)

(e) Glucose Determination

Glucose was determined using Randox Plus clinical chemistry analyser (UK). The principle and method performance are explained in appendix ii.

The reference ranges for the lipids and glucose are given in Appendix iii.

5.6. Data Cleaning

- Any participant with age below 40 and above 60 was excluded from the study
- Any participant with inadequate data were excluded (Alcohol history, Serum Lipids, Glucose, Anthropometries, and blood pressure)
- All participants should have the information required to address the aims and objectives of the study.
- Dataset was checked for any outliers and removed.

5.7. Data Analysis

5.7.1. Cut off values

(a) Obesity

Obesity was diagnosed when a BMI of ≥ 30kg/m^2 was observed (Zatu *et al.,* 2016)

(b) Central obesity

Waist Circumference is 102cm for men and 88 cm for women (Dalton *et al.,* 2003).

(c) Hyperglycaemia

Hyperglycaemia was diagnosed when fasting glucose was ≥ 7.0 mmol/l (Sardu *et al.*, 2020).

(d) Hypertension

Hypertension was diagnosed when diastolic and systolic blood pressure was ≥90 and ≥140 respectively (Vgontzas *et al.*, 2009).

(e) Hypertriglyceridemia

Hypertriglyceridemia was diagnosed when fasting triglycerides were >1.7mmol/l (Dave *et al.*, 2016).

(f) Hypercholesterolaemia

Hypercholesterolaemia was diagnosed when fasting total cholesterol was ≥ 5 mmol/l (Dave *et al.*, 2016).

(g) High low-density lipoprotein cholesterol (LDL-C)

High LDL-C was diagnosed when LDL-C was >3 mmol/l (Alberts *et al*., 2005)

(h) Low high-density lipoprotein cholesterol (HDL-C)

Low HDL-C was diagnosed when HDL-C was <1.2 mmol/l (Lone *et al*., 2017)

(i) Metabolic syndrome

The current study used both IDF and NCETP criteria to assess and diagnose metabolic syndrome. According to the IDF which diagnoses the presence of metabolic syndrome when there is a presence of visceral obesity two or more of the components of metabolic syndrome viz: hyperglycaemia, hypertension, low HDL-cholesterol, hypertriglyceridaemia (Herath *et al*., 2018) and according to NCEP ATP (III) which diagnosed metabolic syndrome when there's a presence of any three or more components of metabolic syndrome viz: hyperglycaemia, hypertension, visceral obesity, hypertriglyceridaemia (Alexander *et al.*, 2003; Ford., 2005; Lone *et al.*, 2017).

5.7.2. Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 software. The distribution of variables was determined and the variables which are normally distributed were expressed as the mean $\pm$ standard deviation. Variables that were not normally distributed are expressed as the median interquartile range (IQR) and were log-transformed to an approximately normal distribution, before applying the inferential statistical tests.

The following tests were used in the present study: Student t-test was used to compare means across the same variable between two groups, chi-square was used to assess means of continuous variables. A bivariate correlation was used to assess the association between alcohol consumption and components of metabolic syndrome, without controlling for possible confounders. Partial correlation was also used to assess the association between alcohol consumption and metabolic syndrome and components of metabolic syndrome after controlling for potential confounders such as age and gender. A logistic linear regression model that has metabolic syndrome and its components as the dependent variable and levels of alcohol consumption as the

independent variable was used to determine the odds of having metabolic syndrome among alcohol consumers. For all analyses, statistical significance was set at a probability (*p*) of less than 0.05.

5.8. Validity and Bias of the study

Reliability is the extent to which an experiment, test or any measuring procedure yields the same result when repeated whereas Validity is defined by the extent to which any measuring instrument measures what it is intended to measure (Thatcher., 2010). In the primary, AWI-Gen study validity was ensured by including controls in every test that was performed. The reliability and validity of each method are shown under method performance.

The following sources of bias were addressed as follows: -

- Sampling and gender bias could not be avoided as the study used data from primary research which used convenient sampling and more women than men participated.
- Methodological bias was addressed in the primary research, validated laboratory methods and questionnaires were used to collect data.
- Statistical bias was avoided by using appropriate statistical tests.
- Potential confounders of the association between alcohol consumption and metabolic syndromes such as age, and gender were controlled by using either or both linear and or logistic regression analysis.

5.9. Ethical Considerations

5.9.1. Approval

The study was approved by the Department of Pathology and Medical Sciences, School of Health Care Sciences, and the Faculty of Health Sciences. For ethical consideration, the study was submitted to the Turfloop Research Ethics Committee at the University of Limpopo and it was approved, the ethical approval letter and the study approval from the primary study are attached. The present study used secondary data and therefore no consent was needed from participants. Ethical approval number **TREC/181/2019:PG**

5.9.2. Anonymity and confidentiality

Confidentiality means not discussing information provided by an individual with others, whilst anonymity means presenting research findings in ways that ensure individuals cannot be identified (Crow *et al.*, 2006). Anonymity was ensured by using research identifiers. To ensure confidentiality the clinical and laboratory information of the participants is securely stored in the AWI-Gen study dedicated storage computer which is accessible to the principal investigator and fellow researcher of the study only.

5.9.3. Waste disposal

Not applicable in the present study, secondary data was used.

5.9.4. Harm

There was no harm to the participants since the present study used secondary data from the AWI-Gen study.

CHAPTER 6

RESULTS

This chapter entails the findings of the present study. The characteristics of the total study population are presented by gender; it provides the comparison of both anthropometric and biochemical measurements among individuals with metabolic syndrome, and those without. The relationship between alcohol consumption with metabolic syndrome and components of metabolic syndrome are also presented in this chapter.

6.1. Characteristics of the participants

The current study had 818 participants of which 671 were women and 147 were men.

Table 1: Characteristics of the participants by gender

Variable	**TP**	**F**	**M**	**P-value**
N	818	671	147	-
Age (years)	52.3±7.7	52.4±7.	52.3±7.7	0.905
BMI (kg/m²)	29.38± 7.97	30.94±7.75	22.23±4.15	<0.001
Obesity (%(n))	43.0 (352)	51.9 (348)	2.7 (4)	<0.001
Waist Circumference (cm)	92.3±16.0	94.4±16.0	82.8±12.0	<0.001
Visceral Obesity (%(n))	55.6(455)	66.0(443)	8.2(12)	<0.001
Waist hip ratio (cm)	0.87±0.09	0.86±0.09	0.91±0.07	<0.001
Visceral fat adipose tissue (cm)	6.75±2.23	6.81±2.26	6.45±1.96	0.071

Subcutaneous fat adipose tissue (cm)	2.05±1.08	2.28±1.03	1.00±0.54	<0.001
Diastolic blood pressure (mmHg)	81.51±12.71	81.83±12.58	80.09±13.23	0.134
Systolic blood pressure (mmHg)	126.77±20.74	126.70±21.04	127.06±19.33	0.850
Hypertension (%(n))	30.3(250)	31.1(211)	26.4(39)	0.253
Total cholesterol (mmol/l)	4.16±1.09	4.20±1.11	4.00±0.99	0.048
Hypercholesterolaemia (%(n))	15.9(130)	16.8(113)	11.6(17)	0.135
HDL-cholesterol (mmol/l)	1.17±0.37	1.16±0.34	1.25±0.48	0.008
Low HDL-Cholesterol (%(n))	58.0(479)	58.7(398)	54.7% (81)	0.375
LDL-Cholesterol(mmol/l)	2.50±0.8870	2.56±0.89	2.22±0.82	<0.001
High LDL-Cholesterol (%(n))	49.9(408)	53.1(356)	35.4(52)	<0.001
Triglycerides(mmol/l)	0.97(0.71-1.36)	0.95(0.71-1.36)	0.87(0.56-1.41)	0.288
Hypertriglyceridaemia (%(n))	13.3(109)	13.4(90)	12.9(19)	1.000
Glucose(mmol/l)	5.33±2.48	5.35±2.44	5.21±2.66	0.542
Hyperglycaemia (%(n))	12.7(104)	12.7(85)	12.9(19)	0.892

Insulin(µu/ml)	4.82(4.35-5.42)	7.37(4.51-11.45)	6.07(3.56-11.05)	0.157
Alcohol Consumption				
Non-drinkers (%(n))	83.5(683)	92.4(620)	42.9(63)	<0.001
Current drinkers (%(n))	16.5(135)	7.6(52)	57.1(84)	
Alcohol type:				
Beer (%(n))	38.4(72)	3.0(20)	35.4(52)	<0.001
Wine (%(n))	0.5(4)	0.3(2)	1.4(2)	0.150
Traditional beer (%(n))	4.2(34)	1.5(10)	16.3(24)	<0.001
Beer and Traditional beer (%(n))	2.9(24)	3.0(20)	2.7(4)	1.000
Beer and wine (%(n))	0.4(3)	0.1(1)	1.4(2)	0.085
Metabolic syndrome (NCEP (%(n))	23.8(197)	26.5(180)	11.5(17)	<0.001
Metabolic syndrome (IDF %(n))	22.2(183)	25.4(172)	7.4(11)	<0.001

p-value ≤0.05=significant, p-value≥0.05=insignificant, normally distributed data is presented as mean ± SD, Mean (IQR) for not normally distributed, and categorical data %(n)

Table 1 represents the baseline characteristic of the study population. The proportion of women was significantly higher than that of men 82% and 18 % respectively. The population has a mean age (years) and BMI (kg/m^2) of 52 and 29.98 respectively. Women had a significantly higher mean value of BMI as compared to men (p<0.001). The prevalence of obesity in the study population was 43%.

Women had significantly higher mean values of WC as compared to men ($p<0.001$). In the general population, 56% of participants had visceral obesity. Women had a significantly higher percentage of visceral obesity compared to men ($p<0.001$).

Women also had significantly higher mean values of subcutaneous tissue than men ($p<0.001$). There was no significant ($p=0.071$) difference in the levels of visceral adipose tissues between women and there was no significant ($p=0.134$ and $p=0.850$ respectively) difference in diastolic and systolic mean values between women and men. Overall, 30% of the participants were hypertensive.

Women showed significantly higher mean values for total cholesterol and LDL-cholesterol compared to men (p-0.048 and $p<0.001$ respectively). There was no significant difference in mean values for triglycerides and glucose ($p=0.288$ and $p=0.542$ respectively). In the overall population, 16% had hypercholesterolaemia, 14% had hypertriglyceridemia and 58% had low HDL-Cholesterol, and 13% were hyperglycaemic.

In the current study, 16% of the population were alcohol consumers. In the group of alcohol consumers, men constituted a significantly higher percentage of consumers as compared to women ($p<0.001$). There is also a significant difference in consumption of beer and traditional beer with men constituting higher percentages compared to women ($p<0.001$ and $p<0.001$ respectively). Amongst the alcohol type beer is the most consumed beverage.

Women showed a significantly higher percentage of metabolic syndrome as compared to men ($p<0.001$). The difference was also evident despite which Metabolic syndrome criterion was used (NCETP vs IDF criteria). The NCETP criteria yielded a higher percentage as compared to IDF across gender.

6.2. Comparison between alcohol consumers and non-alcohol consumers

Table 2. Comparison of biochemical and anthropometric measurements between alcohol consumers and non-alcohol consumers.

Variables	Non-alcohol consumers	Alcohol consumers	p-Value
N	683	135	
Age (years)	52.41±7.733	51.95±7.389	0.523
BMI (kg/m²)	29.52±8.21	28.61±6.54	< 0.001
Obesity %(n)	47.6(328)	20.4(28)	<0.001
Waist Circumference (cm)	93.62±15.76	85.82±15.81	< 0.001
Visceral Obesity (%(n))	61.2(422)	27.7(38)	< 0.001
Waist hip ratio (cm)	0.868 ±0.09	0.89±0.08	0.031
Visceral fat (cm)	6.80±2.25	6.54±2.03	0.213
Subcutaneous fat (cm)	2.17±1.06	1.44±0.99	< 0.001
Diastolic blood pressure (mmHg)	81.32±12.26	82.52±14.80	0.314
Systolic blood pressure (mmHg)	126.304±20.35	129.11±22.50	0.151
Hypertension (%(n))	29.8(205)	32.8(46)	0.472
Total cholesterol (mmol/l)	4.16±1.11	4.21±0.95	0.579
Hypercholesterolaemia (%(n))	13.9(95)	6.7(9)	0.373
HDL-cholesterol (mmol/l)	1.14±0.34	1.35±0.46	<0.001
Low HDL-Cholesterol (%(n))	61.3(419)	40.0(54)	< 0.001

LDL-Cholesterol(mmol/l)	2.53±0.90	2.33±0.81	0.015
High LDL-Cholesterol (≥3.0mmol/l), %	51.7(353)	40.7(55)	0.020
Triglycerides(mmol/l)	0.95(0.71-1.34)	1.05(0.73-1.48)	0.127
Hypertriglyceridaemia (%(n))	12.7(87)	16.3(22)	0.266
Glucose(mmol/l)	5.42±2.62	4.86±1.49	0.016
Hyperglycaemia (%(n))	13.9(95)	6.7(9)	0.021
Insulin(µu/ml)	3.37(1.33-5.93)	4.43(2.03-6.48)	0.277
Metabolic syndrome (NCEP (%(n)))	24.4(168)	11.7(16)	0.001
Metabolic syndrome (IDF (%(n)))	23.8(164)	13.9(19)	0.011

p-value ≤0.05=significant, p-value≥0.05=insignificant, normally distributed data is presented as mean ± SD, Mean (IQR) for not normally distributed, and categorical data %(n)

There was no significant difference in the mean age values between non-alcohol consumers and alcohol consumers (p=0.523). Obesity was significantly higher for non-alcohol consumers as compared to alcohol consumers, with a prevalence of 47.6% and 20.4% respectively (p<0.001). Non-alcohol consumers had a significantly higher prevalence of visceral obesity (61.2%) as compared to alcohol consumers (27.7%) (p<0.001). Non-alcohol consumers had significantly higher mean values for waist circumference and subcutaneous fat (p<0.001 and p<0.001 respectively). No significant difference was observed in the mean values for visceral fat between alcohol consumers and non-consumers (p=0.213).

There was no significant difference in the mean values for systolic and diastolic blood pressure between alcohol consumers and non-alcohol consumers (151 and p=0.314).

There was no significant difference in the prevalence of hypertension between alcohol consumers (32.8%) compared to non-alcohol consumers (29.8%) (p=0.472).

Alcohol consumers had significantly higher mean values for HDL cholesterol compared to non-alcohol consumers (p<0.001). Non-alcohol consumers had a significantly higher prevalence of low HDL-Cholesterol compared to alcohol consumers, 61% and 40% respectively (p<0.001).

There was no significant difference in mean values for total cholesterol, triglycerides, and insulin by alcohol consumption status (p=579, p=0.127 and p=0.277 respectively). Mean values for glucose among non-alcohol consumers were significantly higher as compared to alcohol consumers (p=0.016). Non-alcohol consumers had a significantly higher prevalence of hyperglycaemia compared to alcohol consumers (p=0.021).

Non-alcohol consumers showed a significantly higher prevalence of metabolic syndrome as compared to alcohol consumers using both NCETP and IDF criteria (p=0.001 and p=0.011 respectively). Using the NCETP criteria non-alcohol consumers had a prevalence of metabolic syndrome of 24.4% while alcohol consumers had a prevalence of 11.7%. meanwhile using the IDF guidelines non-alcohol consumers had a 25.4% prevalence of metabolic syndrome whereas alcohol consumers had 18.2%.

Table 3. The comparison of biochemical and anthropometric measurements between female alcohol consumers and non-alcohol consumers.

Variables	Non-alcohol consumers	Alcohol consumers	P-value
N	554	116	
Age (years)	52.27±7.63	52.89±7.934	0.429
BMI (kg/m^2)	31.17±8.02	29.84±6.06	0.093
Obesity %(n)	83.0(289)	17.0(59)	0.979
Waist Circumference (cm)	89.82±14.23	82.61±13.32	<0.001
Visceral Obesity (%(n))	89.9(313)	10.1(35)	<0.001
Waist hip ratio (cm)	0.86±0.09	0.85±0.12	0.005
Visceral fat (cm)	6.84±2.31	6.72±2.06	0.583
Subcutaneous fat (cm)	2.03±1.03	1.23±0.83	<0.001
Diastolic blood pressure (mmHg)	80.67±12.09	80.38±13.30	0.625
Systolic blood pressure (mmHg)	80.67±12.09	80.38±13.30	0.820
Hypertension (%(n))	30.4(190)	40.4(21)	0.133
Total cholesterol (mmol/l)	4.09±1.10	4.11±0.89	0.864
Hypercholesterolaemia (%(n))	87.4(83)	12.6(12)	0.183
HDL-cholesterol (mmol/l)	1.15±0.34	1.38±0.48	<0.001
Low HDL-Cholesterol (%(n))	59.9(375)	44.2(23)	0.027
LDL-Cholesterol(mmol/l)	2.48±0.89	2.26±0.77	0.015

High LDL-Cholesterol (%(n))	85.8(271)	14.2(45)	0.040
Triglycerides(mmol/l)	0.93(1.32-0.69)	0.99(1.33-0.71)	0.865
Hypertriglyceridaemia (%(n))	88.4(84)	11.6(11)	0.508
Glucose (mmol/l)	5.09±3.16	4.64±1.93	0.149
Hyperglycaemia (%(n))	88.9(72)	11.1(9)	0.110
Insulin(µu/ml)	3.18(5.97-1.33)	4.78(6.52-2.19)	0.045
Metabolic syndrome (NCEP (%(n)))	26.5(166)	26.9(14)	0.942
Metabolic syndrome (IDF (%(n)))	26.0(158)	26.7(14)	0.789

p-value ≤0.05=significant, p-value≥0.05=insignificant, normally distributed data is presented as mean ± SD, Mean (IQR) for not normally distributed, and categorical data %(n)

There was no significant difference in the mean age values between alcohol consumers and alcohol consumers (p=0.429). There was no significant difference in obesity (BMI ≥ 30), however, the prevalence of visceral obesity among non-alcohol consumers was significantly higher than that of non-alcohol consumers (83% vs 17%) (p=0.979 and p<0.001 respectively).

There was no significant difference in the mean values for systolic and diastolic blood pressure between female alcohol consumers and non-alcohol consumers (p=0.820 and p=0.625 respectively). Non-alcohol consumers had higher mean values for both systolic and diastolic blood pressure.

Alcohol consumers had significantly higher mean values for HDL cholesterol while non-alcohol consumers showed significantly higher mean values for LDL cholesterol (p<0.001 and p=0.015 respectively). There was no significant difference in mean values for total cholesterol, and triglycerides between alcohol consumers and non-alcohol consumers (p=864 and p=0.865 respectively). Alcohol consumers had significantly

higher mean values for insulin compared to non-alcohol consumers (p=0.045). There was no significant difference in the prevalence of hyperglycaemia between alcohol consumers and non-alcohol consumers (p=0.110).

There was no significant difference in the prevalence of metabolic syndrome for non-alcohol consumers and alcohol consumers by using both NCETP (26.5% and 26.9%) and IDF (26.0% and 26.7%) criteria (p=0.942 and p=0.789 respectively).

Comparison of biochemical and anthropometric measurements between alcohol consumers and non-alcohol consumers was repeated between men and women because the population of the current study consisted mainly of women and alcohol consumers were mainly men.

Table 4. The Comparison of biochemical and anthropometric measurements between male alcohol consumers and non-alcohol consumers.

Variables	**Non-alcohol consumers**	**Alcohol consumers**	**p-Value**
N	127	19	
Age (years)	51.92±7.86	54.79±6.21	0.131
BMI (kg/m²)	22.32±4.12	21.13±3.82	0.237
Obesity %(n)	3.2(2)	2.4(2)	0.770
Waist Circumference (cm)	109.56±13.85	105.42±16.03	0.236
Visceral Obesity (%(n))	91.3(94)	8.7(4)	0.008
Waist hip ratio (cm)	0.92 ±0.09	0.92±0.09	0.982
Visceral fat (cm)	6.45±2.00	6.41±1.65	0.942

Subcutaneous fat (cm)	2.77±0.96	2.76±0.89	0.983
Diastolic blood pressure (mmHg)	83.98±12.46	95.61±17.01	<0.001
Systolic blood pressure (mmHg)	128.77±21.01	144.03±31.69	0.007
Hypertension (Diastolic BP ≥90 mmHg, Systolic BP ≥140 mmHg)	23.8(15)	28.2(24)	0.586
Total cholesterol (mmol/l)	4.45±1.13	4.84±1.12	0.158
Hypercholesterolemia (%(n))	22.8(29)	30.0(6)	0.484
HDL-cholesterol (mmol/l)	1.11±0.30	1.19±0.24	0.279
Low HDL-Cholesterol (%(n))	76.2(48)	38.8(33)	<0.001
LDL-Cholesterol(mmol/l)	2.76±0.87	2.7±0.93	0.894
High LDL-Cholesterol (%(n))	89.0(10)	11.0(10)	0.238
Triglycerides(mmol/l)	1.05(1.48-0.76)	1.77(2.75-1.35)	0.022
Hypertriglyceridaemia (%(n))	83.5(106)	16.5(21)	0.001

Glucose(mmol/l)	4.78±2.01	5.35±1.44	0.245
Hyperglycaemia (%(n))	95.7(22)	4.3(1)	0.159
Insulin(µu/ml)	3.19(5.71-1.32)	2.17(5.51-1.09)	0.369
Metabolic syndrome (NCEP)	17.5(11)	7.1(6)	0.949
Metabolic syndrome (IDF)	9.5(6)	5.9(5)	0.404

p-value ≤0.05=significant, p-value≥0.05=insignificant, normally distributed data is presented as mean ± SD, Mean (IQR) for not normally distributed, and categorical data %(n).

There was no significant difference in the mean age values between non-alcohol consumers and alcohol consumers (p=0.131).

There was no significant difference in obesity (BMI ≥ 30) between alcohol consumers and non-alcohol consumers, with the prevalence of 3% and 2% for non-alcohol consumers and alcohol consumers, respectively (p=0.237). Non-alcohol consumers had a significantly higher prevalence of visceral obesity (91.3%) as compared to alcohol consumers (8.7%) (p=0.008). There was no significant difference in mean values for waist circumference and waist hip ratio between alcohol consumers and non-alcohol consumers (p=0.236 and p=0.982 respectively).

There was a significant difference in the mean values for systolic and diastolic blood pressure between alcohol consumers and non-alcohol consumers (p=0.007 and p<0.001 respectively). Alcohol consumers had higher mean values systolic and diastolic blood pressure compared to non-alcohol consumers. Alcohol consumers had a higher prevalence of hypertension (28.2%) as compared to non-alcohol consumers (24.8%).

Male alcohol consumers had significantly higher mean values of triglycerides compared to non-alcohol consumers (p=0.022). Non-alcohol consumers had a significantly higher

prevalence of low HDL cholesterol (76.2% and 38.8% respectively) and hypertriglyceridemia (83.5% and 16.5% respectively) as compared to alcohol consumers ($p<0.001$ and $p=0.001$ respectively).

There was no significant difference in the prevalence of metabolic syndrome between alcohol consumers and non-alcohol consumers using both NCETP and IDF criteria ($p=0.949$ and $p=0.404$ respectively).

6.2. Correlation and regression of alcohol consumption and metabolic syndrome and its components

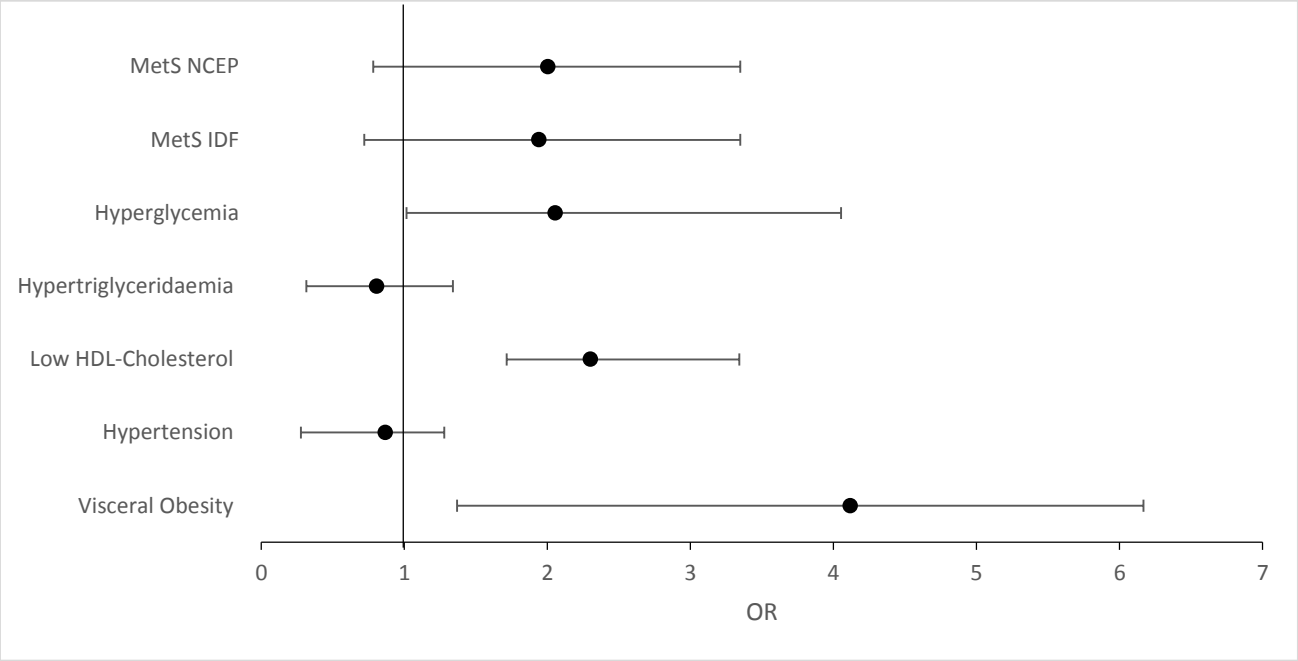

Diagram 1. Forest Plot illustrating the bivariate logistic regression for assessment of the association of alcohol consumption with metabolic syndrome and its components. Normal=1, OR>1=Positive relationship, OR<1=Negative relationship

The regression model constituted metabolic syndrome and components of metabolic syndrome as dependent variables and alcohol consumption as an independent variable.

In the current study population, alcohol consumers were significantly more likely to have visceral obesity, low HDL-Cholesterol, hyperglycaemia, and metabolic syndrome.

Table 6. Linear regression for assessment of the association of selected biochemical and anthropometric measurements with alcohol consumption in the study population

Variable	Univariate regression		Multi-variate regression (age and gender)	
	Beta	p-Value	Beta	p-Value
Waist Circumference (cm)	-7.488	<0.001**	-1.914	0.264
Systolic blood pressure (mmHg)			4.107	0.063
Diastolic blood pressure (mmHg)			3.085	0.025*
Triglycerides (mmol/l)			0.033	0.157
HDL-Cholesterol (mmol/l)	0.206	<0.001**	.218	<0.001**
Glucose (mmol/l)	-0.550	0.017*	-0.623	0.019*

***Significant at p-value ≤ 0.001; *Significant at p-value ≤ 0.05, Univariate regression the confounder was not controlled for in the model, in multi-regression confounders were controlled for. Beta <1= Negative relationship, Beta >1= positive relationship.*

Univariate regression consisted of metabolic syndrome and components of metabolic syndrome as the dependent variables while alcohol consumption was the independent variable.

Multi-variate regression consisted of metabolic syndrome and components of metabolic syndrome as dependent variables while alcohol consumption was the independent variable. For each analysis age, gender and all other components of metabolic syndrome were controlled for.

The univariate regression between alcohol consumption and visceral obesity shows that alcohol consumption is associated with low waist circumference. Multivariate regression analysis shows that alcohol consumption is associated with high blood pressure. Univariate and multi-variate regression analysis shows that alcohol consumption is associated with high HDL-cholesterol and low glucose levels.

CHAPTER 7
DISCUSSION

This chapter discusses the characteristics of the study population and the association between alcohol consumption, metabolic syndrome, and components of metabolic syndrome.

7. Characteristics of the population

7.2. Age

The current study is a secondary study from the AWI-Gen study, the primary objective of the AWI-Gen primary project was to determine the association of genomic and environmental risk factors for cardio-metabolic diseases (Mashinya *et al.*, 2020). The current study population has a mean age of 52 years. The age group was chosen because most cardiovascular diseases and cardiovascular disease risks present later in life (Keates *et al.*, 2017; Ruan *et al.*, 2018).

7.3. Gender

In the present study, there was a higher proportion of women as compared to men. This is in concord with previously reported studies in the same population (Choma *et al*., 2015; Maimela *et al.*, 2016). The same was also observed in a study by Gómez-Olivé *et al*., (2017) conducted in a rural area in Mpumalanga. According to Wang *et al*., (2013) the reason for this may be because women are readily accepting of health issues and more health conscious as compared to men. Another contributing factor could be that men have migrated to the big cities in search of income. Furthermore, the literature suggests that women live generally longer than men which might have reduced their availability during the recruitment process (Njwambe *et al*., 2019).

7.4. Obesity

The distribution of body mass index (BMI) suggests that most people 28.6 (23.1-34.1) in the population are classified between the overweight and obese category. The prevalence of obesity in the current study is higher than that of a previously reported

study in the neighbouring population (Sengwayo *et al.*, 2013). The age group in the Sengwayo *et al*., (2013) study was 18-65 years as compared to the current study which constituted most of the elderly pupils. The prevalence of obesity in the present study might be high due to the time at which the two studies were conducted. Sengwayo *et al*., (2013) sample collection were in 2010 whereas the AWI-Gen study which is the primary study was conducted in 2017. The prevalence of obesity in the current study is also higher than that reported in older previous studies conducted in the same area as the presents study Alberts *et al*., (2005) and Maimela *et al.,* (2016) but comparable to a more recent study by Mashinya *et al*., (2018). The global increase in obesity over the years as reported by Arroyo-Johnson and Mincey., (2016) could be the reason for the high obesity prevalence in the current study.

7.5. Hypertension

There was no significant difference in diastolic and systolic blood pressure between women and men. The prevalence of hypertension in the current study is higher than that of Alberts *et al*., (2005). The increase in obesity could have resulted in the rise in hypertension. The prevalence of hypertension in the current study is lower as compared to the previous study by Ntuli *et al*., (2015) which was conducted in the same area. The low prevalence of hypertension could be due to the difference in sample size. The current study only used a sample size of 818 as compared to the previous study which had 1281 participants and the use of different blood pressure monitoring equipment. Ntuli *et al* (2015) used a sphyngnometer which is less accurate as compared to the automated monitors used in the current study. The prevalence of hypertension in the current study is comparable compared to that reported by Maimela *et al*., (2016).

7.6. Biochemical results

There was no significant difference in total cholesterol between men and women in the current study. Means levels for total cholesterol in the current study are comparable with the means reported by Reiger *et al*., (2017). The prevalence of hypercholesterolaemia in the current study is lower than that of the Reiger *et al*., (2017) study conducted in a similar setting and population. The reason for the discrepancies in the prevalence of hypercholesterolaemia could be because the sample size for the previous study was

higher than that of the current study. This might have resulted in a higher prevalence of hypercholesterolaemia. Hypertension is more prevalent in urban areas compared to rural areas Van zyl *et al*., (2012) and hypertension is more prevalent amongst whites compared to blacks Kandala *et al*. (2021). The prevalence of hypercholesterolaemia for the current study is lower than that reported by Alberts *et al*., (2005) and Maimela *et al*., (2016).

There was no significant difference in triglycerides between men and women in the current study population. The prevalence of hypertriglyceridemia in the current study is lower than that reported by Maimela *et al*., (2016). In the previous and the current study, there was no confirmation of fasting, non-fasted individuals could cause an over-estimation of hypertriglyceridemia (Laufs *et al.*, 2020).

Men had significantly higher mean values for HDL cholesterol as compared to women. The HDL-cholesterol means in the current study is slightly lower than that of the previous study (Magwai., 2018). The study by Magwai., (2018) had a bigger sample size as compared to the current study, and the population was the same. The periods at which the samples were taken are not far apart enough for the population to develop the difference or show any difference in the lipid profile. The prevalence of low HDL cholesterol in the current study is higher than that reported by the INDEPTH study conducted in rural Agincourt (Reiger *et al.*, 2017). CardioCheck PA analyzer was used for the measurement of lipids in the study by Reiger *et al*., (2017). The analyzer was reported to underestimate lipids compared to published peer data.

Women had significantly higher LDL cholesterol as compared to men in the current study. In the study by Cook *et al*., (2021) there was no significant difference in the mean levels of LDL-Cholesterol between men and women. The study by Cook *et al*., (2021) had a smaller sample size as compared to the current study. This can make it difficult to detect the difference in parameters between males and females. The prevalence of high LDL-cholesterol for the present study population is lower compared to van Zyl et al., (2012). The study was conducted in a similar setting in Free State. The *van Zyl et al*., (2012) study also had a smaller sample size as compared to the current study. Which might be underestimating the prevalence of LDL cholesterol in their study. Again,

researchers reported sampling bias therefore their findings might not represent the general population.

There was no significant difference in glucose mean values between men and women in the current study population. Even though the mean values for glucose between the previous study and the current are comparable, the incline in hyperglycaemia is expected as diabetes has been rising over the years (Alberts et al., 2005; van Zyl *et al*., 2012).

A study by Peer *et.al*, (2015) which was conducted in a suburban area showed similar trends of hypertriglyceridemia, low HDL-C and hyperglycaemia. Due to the rapid urbanization that rural villages have gone through in the past 10 years, the comparison between urban and rural settings is yielding similar results (Wentzel-Viljoen *et al.*, 2018).

7.7. Alcohol consumption

The prevalence of alcohol consumption in the current study is comparable to that reported by Maimela *et al*., (2016) which was conducted in the same village. The study population for the current study and the previous study are the same and the data collection and study method are also the same. Which could explain the similarities in alcohol consumption prevalence. A study conducted in an urban setting in a Free State Province reported a higher prevalence of alcohol consumption as compared to the current study. The reason for this difference may be due to easy access to alcohol in urban areas (van Zyl *et al.*, 2020).

Socio-economic factors could also be playing a major role in the observed prevalence, very few people in the rural setting afford to purchase alcohol. There are also more male alcohol consumers as compared to women. The same was observed in a study by de Vlieg *et al*., (2021) which was conducted in rural Mpumalanga on Tsonga-speaking people.

The skewness in alcohol consumption by gender can also be explained by cultural practices and formalities. Given that in the northern Sotho-speaking communities men are readily accepted as alcohol consumers whereas it is taboo for women to consume

alcohol. Women are generally given more duties in their homes which gives them less opportunity to consume alcohol (Bobrova *et al*., 2010; Schulte., 2010; Mafa *et al*., 2019). If similar perceptions are still in place similar findings would be expected.

Similarly, a study by Trangenstein *et al*., (2018) found that more men consumed beer compared women. The prevalence of beer consumption in the current study is lower than that reported in Tshwane metropole Trangenstein *et al*., (2018). The Tshwane study had a larger sample size, and the mean age for the previous study was 36 years. This means that most of the participants were in the working class and can afford to buy alcohol. That could also be the reason for a higher prevalence of beer consumption as compared to the current study.

The prevalence of traditional beer consumption in the current study is lower than previously reported by Choma *et al*., (2007). The sample size for the current study is lower than that of the previous study. Over the years with urbanization and better living conditions, a vast of people have reduced their traditional beer intake for more commercialized alcoholic beverages (Hlangwani *et al.*, 2020).

The prevalence of wine in the current study is lower than that reported by Trangenstein *et al*., (2018). Due to the underreported prevalence of alcohol types in rural areas in the country, the researcher is unable to fairly compare the prevalence of wine consumption in a rural south setting.

There was no significant difference in the mean age values between alcohol consumers and non-alcohol consumers for the current study. The opposite was observed in a study by Geels *et al.*, (2013). The current study had elderly participants only, unlike Geels *et al.*, (2013) which had a range of participants from adolescents to the elderly of 97 years.

Several studies have reported a positive relationship between alcohol consumption and age, where they show an increase in alcohol intake with an increase in age. The comparison between age and alcohol consumption in the current study did not show any difference, this could be due to the small age gap in the current study population. Since the age gap is small the comparison did not show any trend between alcohol intake and age.

7.8. Metabolic syndrome

The prevalence of metabolic syndrome for the current study has been reported in two ways using both the NCETP and IDF guidelines. Using the IDF guidelines women had a higher prevalence of metabolic syndrome compared to men, the same outcome was observed in a study by Erasmus *et al*., (2012). The prevalence of metabolic syndrome in the current study is higher than that reported by Owolabi *et al*., (2017). Owolabi *et al*., (2017) study used the IDF criteria to classify metabolic syndrome, but they only assessed three out of five components of metabolic syndrome that are recommended by IDF. This might have resulted in an underestimation of metabolic syndrome in their study population.

Applying the NCETP guidelines, the prevalence of metabolic syndrome for women is also higher compared to males. The same distribution was observed in a study by Sengwayo *et al*., (2013). The prevalence of metabolic syndrome in the current study is lower compared to Peer *et al*., (2015). Peer *et al*., (2015) study were conducted in a semi-urban setting in Cape Town amongst black participants. The prevalence of MetS in the current study is comparable to a study by Motala *et al*., (2011) which was conducted in a rural setting in KwaZulu Natal. The discrepancies and agreement compared to the current study could be due to the study setting. People in a rural setting have fewer resources as compared to people in an urban or semi-urban setting.

7.9. Associations between alcohol consumption with metabolic syndrome and components of metabolic syndrome

Cardio-metabolic risk can be quantified based on the characteristics of the population, these characteristics are affected by many factors including geography and socio-economic factors (Schultz *et al.*, 2018). The next section explores the characteristics of the current study population in comparison with the previously conducted studies

7.9.1. The association between alcohol consumption and obesity.

According to Chakraborty., (2014) alcohol consumption contributes to weight gain and obesity. The proposed mechanism is that the calories in the alcohol content get stored

as adipose tissue which contributes to weight gain. When energy storage exceeds the energy expenditure there seems to be a positive increase in weight (Traversy and Chaput., 2015).

There was a significant difference in mean values for BMI between alcohol consumers and non-alcohol consumers without considering gender, the mean values for both groups are around the overweight classification.

In the current study, there was no significant difference in mean values for BMI and prevalence of obesity between alcohol consumers and non-alcohol consumers even after stratifying the population by gender.

Non-alcohol consumers had a higher prevalence of obesity and visceral obesity as compared to alcohol consumers for both genders. Traversy *et al*., (2015) and Koopmann *et al*., (2018) reported reduced levels of ghrelin which is an appetite-regulating hormone in alcohol consumers. This could mean that alcohol consumers are eating less as compared to non-alcohol consumers. The bivariate logistic regression revealed that alcohol consumers are more likely to have visceral obesity while the univariate regression showed that alcohol consumption is associated with lower waist circumference.

7.9.2. The association between alcohol consumption and hypertension

Previous studies in the same study area as the current study reported the prevalence of hypertension to be 47% (Maimela *et al.*, 2016). Peltzer and Phaswana-Mafuya., (2013) have reported an association between alcohol consumption and hypertension.

Controversial outcomes are reported from different study types, some reporting a positive effect whilst some report and negative effect Son *et al.*, (2011); Zatu *et al*., (2016); Roerecke *et al*., (2018). There are several mechanisms underlying the association between hypertension and alcohol consumption. Firstly, it is reported that alcohol increases the membrane permeability which causes an influx of calcium into the cells. That makes them depolarized and causes muscle resistance and hypertension (Husain *et al*., 2014). Secondly, Collart *et al*., (2015) reported that alcohol increases the risk of cardiomyopathy and atrial fibrillation which leads to increased blood pressure.

Lastly, Husain *et al*., (2014) reported that alcohol increases oxidative stress by disturbing the NADP/NADPH ratio.

In the current study, alcohol consumers had a higher prevalence of hypertension compared to non-alcohol consumers. Similar findings were reported in a study by Zatu et al., (2016) which was conducted in a rural black population. Another study by Peer *et al*., (2015) found similar results in an urban setting. This could support the published findings on alcohol consumption and hypertension. To exclude the possible bias, the population was stratified by gender and comparison by gender between alcohol consumers and non-alcohol consumers was done. There was no difference between gender, alcohol consumption and hypertension. The multi-variate analysis shows that alcohol consumption is associated with high blood pressure.

7.9.3. The association between alcohol consumption and other biochemical variables.

In the present study, non-alcohol consumers had a significantly higher mean value for glucose as compared to alcohol consumers in the current study. Logistic regression shows that alcohol consumers are more likely to have hyperglycaemia, whereas univariate and multi-variate shows that alcohol consumption is associated with low glucose levels. A review by Volaco and Ercolano., (2018) reported improved glycaemic status and increased glycaemic status with binge drinking. There is a U or V-shaped effect of alcohol consumption on glucose metabolism. Alcohol has been reported to inhibit gluconeogenesis and glycogenolysis in the liver which results in hypoglycaemia (Jang and Koh., 2012). And again other studies have reported improved insulin sensitivity which also improves glucose levels (Schrieks *et al.*, 2015).

Alcohol consumers had significantly higher mean values for HDL-cholesterol as compared to non-alcohol consumers. The same was also reported by Agongo *et al*., (2019) in the Black population from rural Ghana. The study by Agongo *et al*., (2019) did not exclude people who have withdrawn from consuming alcohol which could have overestimated the prevalence of alcohol and its association with increased HDL-C. Another study conducted in North West Province reported increased HDL-C levels with alcohol overuse (Zatu *et al.*, 2016).

HDL-cholesterol is an anti-atherogenic lipoprotein, it brings about protection against cardiovascular diseases by transporting cholesterol back to the liver. Low levels of HDL-cholesterol have been associated with cardiovascular disease (Mackey *et al*., 2012; Rosenson *et al.*, 2012). In the current study, non-alcohol consumers had a significantly higher prevalence of low HDL-cholesterol as compared to alcohol consumers. Non-alcohol consumers could be more at risk of cardiovascular disease in the current study population. Logistic regression reveals that alcohol consumers are more likely to have low HDL cholesterol, whereas univariate and multi-variate regression shows that alcohol consumption is associated with high HDL-cholesterol levels.

Non-alcohol consumers showed higher mean values for LDL-cholesterol as compared to alcohol consumers. Non-alcohol consumers had a significantly higher prevalence of high LDL cholesterol as compared to alcohol consumers. Ahaneku *et al*., (2014) reported a decrease in LDL-cholesterol, triglycerides and total cholesterol levels in alcohol consumers as compared to non-alcohol consumers. These findings could support and indicate a protective effect of alcohol consumption. After stratifying the current study population by gender, only women showed a significant difference in mean values for LDL-cholesterol with non-alcohol consumers having higher mean values for LDL-Cholesterol. The current study population consisted predominantly of more women than men, there were few men to show the difference in values for LDL-cholesterol between alcohol consumers and non-alcohol consumers.

G-protein-coupled receptor 43 which is the receptor for acetate, in adipose tissue results in inhibition of lipolysis and increased insulin sensitivity (Kovář and Zemánková, 2015). Inhibition of lipolysis reduces available free fatty acids which the liver uses to produce triglycerides and VLDL this inhibitory effect results in low plasma lipid levels.

There was no significant difference in mean values for triglycerides between alcohol consumers and non-alcohol consumers. Alcohol consumers had a higher prevalence of hypertriglyceridemia as compared to non-alcohol consumers, the same was observed in a study by Klop *et al*., (2013).

A comparison between women and men alcohol and non-alcohol consumers was done. There was no significant difference in mean values for triglyceride between women alcohol consumers and non-alcohol consumers. Women alcohol consumers had a lower prevalence of hypertriglyceridaemia compared to non-alcohol consumers. Male alcohol consumers had significantly higher mean values for triglycerides, while non-alcohol consumers had a significantly higher prevalence of hypertriglyceridaemia.

The reason for this difference could be that the study does not have enough women alcohol consumers in the population to show the difference in lipid profile. Again, the amount that women consume could be lower as compared to men for alcohol to have a significant impact on triglycerides.

There was no significant difference in mean values for total cholesterol between alcohol consumers and non-alcohol consumers. The findings of the current study contradict that of Rosoff *et al.*, (2019), where a dose-dependent increase in serum cholesterol was observed. The difference in these outcomes could be due to the difference in racial and geographical factors. Total cholesterol has been associated with cardiovascular diseases (Jeong *et al.*, 2018).

The mechanism by which alcohol affects total cholesterol is not clearly outlined in the literature. Dyslipidaemia has been associated with alcohol consumption. It has been reported to be another cause of morbidity and mortality as a component of non-communicable diseases. The proposed mechanism by which alcohol improves dyslipidaemia is through the alcohol content called polyphenols. Polyphenols are reported to inhibit β-oxidation of LDL, and initiate cell signalling pathways that increase the production of HDL-cholesterol (Arranz *et al.*, 2012).

7.9.4. The association between metabolic syndrome and alcohol consumption

Several studies have associated alcohol consumption with metabolic syndrome, some showing positive effects (Sun *et al*., 2014; Manolis et al., 2019). Whilst some are showing negative effects of alcohol on metabolic syndrome (Kim *et al.*, 2017).

The mechanism of how alcohol consumption results in metabolic syndrome are not clearly understood. The effect to which alcohol contributes to metabolic syndrome is multi-factorial and complex. some of the proposed mechanisms include oxidative damage, mitochondrial dysfunction, deposition of triglycerides, altered fatty acid extraction, decreased myofilament Ca2þ sensitivity, and impaired protein synthesis (Manolis *et al*., 2019).

In the current study, non-alcohol consumers had a higher prevalence of metabolic syndrome as compared to non-alcohol consumers. Alcohol consumers are more likely to have metabolic syndrome according to logistic regression. Using both IDF and NCEP-ATP III criteria non-alcohol consumers had a higher prevalence of metabolic syndrome as compared to alcohol consumers. The same was observed in a study by Owolabi *et al*., (2017) which used only the IDF criteria to classify metabolic syndrome.

Alcohol improves several components of metabolic syndrome viz. obesity, HDL-cholesterol and glucose as indicated above. Univariate and multi-variate regression shows that alcohol consumption is associated with low waist circumference, high HDL-cholesterol, and low glucose levels respectively. These findings could mean that alcohol has a protective effect against components of metabolic syndrome. Another comparison was done to assess the bias that could be caused by gender, and the results showed that there is no relationship between gender, alcohol consumption and metabolic syndrome.

CHAPTER 8
CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

a) Conclusion

The current study assessed the association between alcohol consumption and metabolic syndrome and components of metabolic syndrome among an adult black population residing in a rural area. The current study shows that non-alcohol consumers had a higher prevalence of metabolic syndrome as compared to non-alcohol consumers. Alcohol consumption is associated with low waist circumference, high HDL-cholesterol, and low glucose levels. Thus, there is a negative relationship between alcohol consumption and selected components of metabolic syndrome. Therefore, in the current study, it can be concluded that in an African population alcohol improves metabolic syndrome by modulating the components of metabolic syndrome namely central obesity, HDL-C and glucose levels.

Excessive alcohol consumption irrespective of the type of alcohol has been associated with the incidence of many diseases. Including the development of cancer, alcoholic liver disease, diabetes mellitus and cardiovascular diseases (Parry *et al*., 2011; Park and Kim., 2012; Shield et al., 2014). Alcohol consumption irrespective of alcohol type at low and moderate levels defined as ≤1 drink for females and ≤2 drinks for males is recommended. As it has been reported to reduce the risk of cardiovascular diseases (Hins and Rim., 2001; Pownall et al., 2015; Chiva-Blanch and Badimon., 2020).

b) Limitations

The results of the present study should be interpreted with caution because of the following limitations. Firstly, the primary study for the current study was cross-sectional in design. Due to the cross-sectional design of the study researchers cannot certainly conclude the causal effect of alcohol consumption on the development of metabolic syndrome (Gariepy *et al*., 2010; Islam *et al.*, 2021). Secondly, the primary study for the current study utilized a convenient sampling method. Since the primary study utilized a convenient sampling method there could be an under-estimation of the prevalence of components of metabolic syndrome (Elfil and Negida., 2019). Thirdly, alcohol consumption data were self-reported which could have caused an underestimation or

overestimation of alcohol consumption prevalence (Alling *et al.*, 2005; Gilligan *et al.*, 2019). Lastly, the quantity of alcohol consumed was not assessed therefore researchers could not investigate the effect of alcohol quantity on metabolic syndrome and its components.

c) Recommendations

One of the recommendations of the present study is that similar studies but longitudinal in nature, where random sampling of participants is employed and the quantity of alcohol consumed is measured, should be conducted to investigate the causal effect of alcohol consumption on metabolic syndrome in rural areas. Lastly, even though alcohol consumption showed improved effects on metabolic syndrome in the current study we still recommend the reinforcement of policies published to regulate alcohol consumption.

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APPENDICES

Appendix i: Questionnaire

AWI-Gen H3Africa

AWI-Gen H3Africa

Genomic and Environmental Risk Factors for Cardiovascular Disease in Africans

AWI-Gen Study Number: |__|__|__|__||__|	**Unique Site Identifier : |__|__|__|__||__|__|__|**

BARCODE STICKER
Please stick the barcode sticker here

1. GENERAL INFORMATION (Ditlabakelo ka kakaretšo)		
1.1	Data collection date (Letšatši la kgoboketšo ya tshedimošo)	| d | d | | m | m | m | | y | y | y | y |
1.2	Interviewer code (Nomoro ya mmotšiši)	_____
1.3	Start time (Nako ya go thoma)	| h | h |:| m | m | am / pm *[please circle appropriate time of day]* *[laetša nako ya letšatši ka ntikodiko]*

2. DEMOGRAPHIC INFORMATION				
2.1	First Name (Leina)			
2.2	Last Name (Sefane)			
2.3	Date of birth known? *[If no, skip to Q.2.5]* (A o tseba letšatši la gago la matswalo? *[ge o sa le tsebe fetela go Q.25]*)	Yes/Eng	No/Aowa	
2.4	Date of Birth *[eg. 27 SEP 1957]* (Letšatši la matswalo)	| d | d | | m | m | m | | y | y | y | y |		
2.5	Approximate year of birth (Kakanyo ya ngwaga wa pelego)	| 1 | 5 | | J | U | N| | y | y | y | y |		
2.6	Gender (Bong)	Female (Mosadi)	Male (Monna)	*[please tick the appropriate box]*

				[thaletša lepokisi la maleba]
2.7	Country (Naga)	South Africa (Afrika Borwa)		

PHENOTYPIC COLLECTION DATA

3. PREGNANCY (Go ima) [If male, please skip to the next section - Marital Status] [Ga e ba ke monna, fetela go tša nyalo]			
3.1	Are you pregnant? (A o mmeleng?)	Yes/Eng	No/Aowa
3.2	How many pregnancies have you had? (O ile wa ba mmeleng makgehlo a ma kae?)	|__|__|	
3.3	How many live births have you had? (O bile le dipelego tse kae tšeo di atlegilego?)	|__|__|	
3.4	Do you have regular (28-35 day) periods? (Aa lehlapo la gago le	Yes/Eng	No/Aowa

	ka tekanelo?)		
3.5	Date of last period (Letšatši-kgwedi la lehlapo la mafelelo)	| m | m | | y | y | y | y |	

4. SUBSTANCE USE (Tšhomišo ya ditagi)

4.1. Alcohol use (Tšhomišo ya dinotagi				
4.1.1	Do you drink alcohol? (A o nwa dinotagi?)	*[If no, skip to Q.9.3]* *[Ga ebake aowa fetela go Q 9.3]*	Yes/Eng	No/ Aowa
4.1.2	Over the past 30 days, on how many days did you drink one or more alcoholic beverages? (Matšatšing a masometharo ao a fitilego, ke matšatši a ma kae moo o nwelego dinotagi?)		|__|__| days/matšatši	
4.1.3	On the days that you drank alcoholic beverages, what was the average number of alcoholic beverages you had each day? (Go matšatši ao o nweleng dinotagi, ke dino tše kae tšeo o di nwelego letšatšing le tee?)		|__|__|	
4.1.4	What type of alcoholic beverage do you usually drink?		Beer Wine	

	(Ke mohuta o few a dinotagi oo o dulang o o nwa?)	Spirits Home brew Other (Specify/letša)_____

4.2. Tobacco use (Tšhomišo ya motsoko)				
4.2.1	Tobacco user? (O šomiša motsoko?)	*[If no, skip to Q.9.2]* *[Ga eba ke aowa, fetela go Q 9.2]*	Yes/Eng	No/ Aowa
4.2.2	Current smoker? (Ga bjwale o goga motsoko?)	*[If no, skip to Q.9.1.5]* *[ga eba ke aowa, fetela go Q 9.1.5]*	Yes/Eng	No/ Aowa
4.2.3	What do you smoke? (O goga motsoko o fe?)	*[please tick the appropriate box]* *[laetša lepokisi la maleba]*	Cigarettes/ Sekerete	Pipe/Peipi
4.2.4	How many do you smoke a day? (O goga tše kae letšatšing le le tee?)		|__|__|__|	
4.2.5	When did you stop smoking? (O lesitše o goga motsoko neng?)		| m | m | | y | y | y | y |	
4.2.6	For how long have you been smoking, or did you smoke? (Ke nako e kae o gago motsoko?)		|__|__| years/mengwaga	
4.2.7	Do you use snuff? (A o šomišaa seneifi?)	*[If no, skip to Q.9.1.10]* *[ga eba ke aowa, fetela go Q 9.1.10]*	Yes/Eng	No/ Aowa

4.2.8	How do you take snuff? (O tšea bjwang seneifi?)	Through your nose (Ka dinko) Through your mouth/on your lip (Ka molomo)

4.3. Drug use (Tšhomoša diokobatši)						
4.3.1		Do you, or have you ever taken marijuana, methamphetamines, cocaine or any other drugs?	Yes/Eng	No/Aowa	Don't know/Ga ke tsebe	Refuse to answer/ Oga an
4.2.10	Do you use chewing tobacco? (A o sohla motsoko?)	(A o ile wa/o goga pätše goba diokabatši?) *[If no, skip to Q.9.2]* *[ga eba ke aowa, fetela go Q 9.2]*	Yes/Eng		No/ Aowa	
4.2.11	How often do you use chewing tobacco? (a o sohla motsoko ga kangkang?)		Once a day/ ga tee letsatsing Twice a day/ ga bedi letsatsing Three times a day/ ga raro letsatsing More than three times a day/ go feta ga raro letsatsing			

					na o ar ab a

5. GENERAL HEALTH (Tša maphelo)

Please indicate if you have or had, any of the following illnesses

(Laetša ge mmago ana le, a ile a ba le malwetsi ao a latelago)

[Please tick the appropriate boxes]

[Thaletša ka lepokising la maleba]

5.1	Weight problem/obesity (Bothata bja boima)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba
5.2	High blood pressure (Madi a ma kgolo)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba
5.3	Heart problems (Bothata bja pelo)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba

5.4	High cholesterol (a lot of fat in your blood) (Makhura a mantši madding)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba
5.5	Breast cancer (Kankere ya letswele)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba
5.6	Cervical cancer	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba
5.7	Other cancers (Kankere tse dingwe)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba
5.8	Asthma or reactive air diseases (Asthma)	Yes/Eng	No/Aowa	Not sure/ Ga ke na bonnete	Refuse to answer/ O gana o araba

6. INFECTION HISTORY (Histori ya malwetši a phetetšo)

[Please tick the appropriate boxes]

[Thaletša ka lepokising la maleba]

6.1. HIV					
6.1.1	Have you been tested for HIV? *[If no, skip to Q.11.1.4]* (A o ile wa dira diteko tša HIV?) *[Ga eba ke aowa, fetela go Q 11.1.4]*	Yes/Eng	No/Aowa	Refuse to answer/ O gana o araba	
6.1.2	Are you HIV positive? (A ekaba o na le HIV?)	Yes/Eng	No/Aowa	Don't know/Ga a a tsebe	Refuse to answer/ O gana o araba
6.1.3	Do you use medication for it? (A o šomiša dihlare go okobatša HIV?)	Yes/Eng		No/Aowa	
6.1.4	Do you agree to have your blood sample tested for HIV? (O ka dumela gore madi a gago a dirwe diteko tša HIV?)	Yes/Eng		No/Aowa	

7. PHYSICAL ACTIVITY (Go itšhidulla)

7.1. Work and travel-related physical activity (Go itšhidulla oo o o amanang le mošomo)			
7.1.1	How many days do you work per week? (Bekeng ye tee o bereka matšatši a ma kae?)	|__| days/matšatši	
7.1.2	Do you work over the weekend? (A o bereka mafelelong a beke?)	Yes/Eng	No/Aowa
7.1.3	How long is your usual work day? *[Please indicate how many hours you work per day on average]* (O bereka nako ye kankang letšatšing le tee? *[laetša di iri tšeo o di šomang letšatšing la mošomo])*	|__|__| hours/di iri	
7.1.4	Does your work involve mostly sitting or standing still, or walking for very short periods? *[If yes, skip to Q. 15.1.11]* (A mošomo wag ago o o dira o dutše fase, o eme goba o tsamaya? *[Ge el eng, fetela go Q 15.1.11]*)	Yes/Eng	No/Aowa
7.1.5	Does your work involve vigorous activities (heavy lifting, manual labour)? *[If no, skip to Q.15.1.8]* (Aa mmereko wa gago ke wa boima? *[Ga ele aowa, fetela go Q 154.1.8]*)	Yes/Eng	No/Aowa

7.1.6	In a usual week, how many days are spent doing vigorous activities? (Mo bekeng, ke matšatši a ma kae moo o dirang mmereko wa boima?)	|__| days/matštši	
7.1.7	On a usual day of vigorous work, how many hours are spent doing these activities? (Mo letšatšing la mmereko wa boima, o fetša hora tše kae o dira mmereko wa boima?)	|__|__| hours/di iri	
7.1.8	Does your work involve moderate physical activity (brisk walking or carrying light loads)? *[If no, skip to Q.15.1.11]* (A mmereko wa gago ke wo o lekanetšego? *[Ga ele aowa, fetela go Q 15.1.11]*)	Yes/Eng	No/Aowa
7.1.9	In a usual week, how many days are spent doing moderate physical activities at work? (Mo bekeng, ke matšatši a ma kae moo o dirang mmereko wa go lekanela?)	|__| days/matštši	
7.1.10	On a usual work day, how many hours are spent doing moderately physical activities? (Mo letšatšing la mmereko wa boima, o fetša hora tše kae o dira mmereko wa go lekanela?)	|__|__| hours/di iri	
7.1.11	In a usual week, how many days do you walk or cycle? (Mo bekeng, ke matšatši a ma kae moo o	|__| days/matštši	

	tsamayang goba o namela paisekela?)		
7.1.12	Do you walk or use a bicycle (for at least 10 minutes at a time) to get to and from places? (A o a o tsamayang goba o namela paisekela tekano ya metsotso ye lesome go tloga lefolong le lengwe go ya go le lengwe?)	Yes/Eng	No/Aowa
7.1.13	On a usual day, how many hours do you spend walking or cycling? (Mo letšatšing, ke iri tše kae tšeo o difetšang o tsamaya goba o nametše paesekela?)	|__|__| hours/di iri	

7.2. Non-work related and leisure time physical activity (Go itšhidulla yo o sa amanego le mošomo)			
7.2.1	Do you engage in any physical activities in your spare time? *[If no, skip to Q. 15.3]* (A o a itšhidillo ge o sa dire selo? *[Ga ele aowa, fetela go Q 15.3]*)	Yes/Eng	No/Aowa
7.2.2	In your spare time do you do any vigorous activities like strenuous sport or exercise? *[If no, skip to Q.15.2.5]* (Mo nakong ye o sa direng selo o itšhidilla ka mokgwa wa go swana le dipapadi?	Yes/Eng	No/Aowa

	[Ga ele aowa, fetela go Q 15.2.5])		
7.2.3	In a usual week, how many days do you engage in vigorous activities? (Mo bekeng ke matšatši a ma kae moo o itšhidillago?)	|__| days/matštši	
7.2.4	In a normal day, how many leisure hours are spent doing vigorous activities? (Mo letšatšing le le tee, ke iri tše kae tšeo o di šomišago o itšhidolla?)	|__|__| hours/di iri	
7.2.5	In your spare time, do you engage in any moderately intense physical activities like walking or swimming? *[If no, skip to Q.15.3]* (Mo nakong ye o sa direng selo o itšhidilla ka mokgwa wa go swana le go sepela goba go rutha meetseng? *[Ga ele aowa, fetela go Q 15.3]*)	Yes/Eng	No/Aowa
7.2.6	In a normal week, how many days are spent engaging in moderately intense physical activity? (Mo bekeng ke matšatši a ma kae moo o itšhidillago?)	|__| days/matštši	
7.2.7	How many hours are spent doing these activities in a normal day? (Mo letšatšing le le tee, ke iri tše kae tšeo o di	|__|__| hours/di iri	

	šomišago o itšhidolla?)	

7.3. Sitting and reclining (Go dula le go kanama)		
7.3.1	Over the past 7 days, how many hours did you spend sitting or reclining per day (excluding sleep)? (Matšatšing a šupa a fitilego, o feditše iri tše kae o dutše fase mo letšatšing le tee?)	|__|__| hours/di iri
7.3.2	How many hours per day do you spend sitting, while you are at work? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase mmerekong?)	|__|__| hours/di iri
7.3.3	How many hours do you spend sitting watching TV per day, during the week? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o lebeletše thelebišene gare ga beke?)	|__|__| hours/di iri
7.3.4	How many hours per day, do you spend watching TV during the weekend? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o lebeletše thelebišene mafelelong a beke?)	|__|__| hours/di iri
7.3.5	How many hours per day, are spent sitting while using a computer outside of your normal working hours during the week?	|__|__| hours/di iri

	(Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o šomiša khomphuthara o se mmerekong gare gab beke?)	
7.3.6	How many hours per day are spent using a computer during the weekend? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o šomiša khomphuthara o se mmerekongmafelelong gab eke?)	|__|__| hours/di iri
7.3.7	How many hours per day do you spend sitting while travelling from place to place during the week? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o tloga felong le lengwe go ya go le lengwe gare ga beke?)	|__|__| hours/di iri
7.3.8	How many hours per day are spent sitting while travelling during the weekend? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o tloga felong le lengwe go ya go le lengwe mafelelong a beke?)	|__|__| hours/di iri
7.3.9	How many hours per day do you spend sitting while socializing during the week? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o bolela le batho gare ga beke?)	|__|__| hours/di iri

7.3.10	How many hours per day do you spend sitting while socialising over the weekend? (Mo letšatšing le tee, ke iri tše kae tšeo o difetšang o dutše fase o bolela le batho mafelelong a beke?)	|__|__| hours/di iri

8. Time at completion of questionnaire | h | h |:| m | m | am / pm

(Nako ya go fetša lenaneopotšišo)

Appendix ii: Method Principle

Glucose level estimation in plasma

Principle:

Plasma from the potassium oxalate tube was used to measure fasting glucose levels using a Randox Plus clinical chemistry analyser (UK). Glucose reagent category number GL8318 was used to analyse the samples. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide produced reacts with phenol and 4-aminophenazone to form a red/violet quinone imine dye as an indicator. The absorbance intensity measured at 505 nm is directly proportional to the glucose concentration. The assay uses an endpoint method and single-point calibration. The Randox Glucose assay has a wide measuring range of 0.36 – 35 mmol/l. The Clinical and Laboratory Standards Institute document EP15 was used for the verification of performance for precision and trueness of the Randox Plus, and a random selection of 150 samples was run in duplicate to ascertain the coefficient of variation of the laboratory technician, which was computed to be 2.3 %.

Method performance

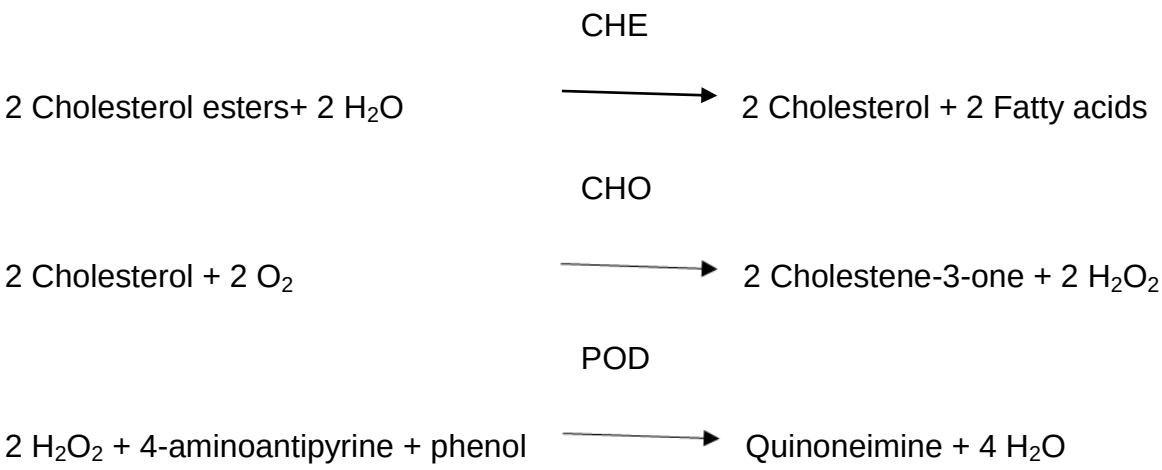
- The CV for the method within-run is 2.3%
- Sensitivity equals 0.04 mmol/L
- The method principle is interfered by ascorbate (<3% above 20mg/dL), icterus (<10% above 40 mg/dL), haemolysis (<3% above 5g/L) and lipaemia (10% above 40mg/dL).

Quality assurance

Control Cat. No. ODC0003 or ODC0004 will be used for quality assurance

Cholesterol level estimation in plasma

Principle:



The cholesterol reagent utilises an enzymatic method to measure cholesterol in human plasma. In this procedure, cholesterol ester in a sample is hydrolysed by cholesterol esterase (CHE). The free cholesterol produced is oxidised by cholesterol oxidase (CHO) to cholestene-3-one with the simultaneous production of hydrogen peroxide (H_2O_2), which oxidatively couples with 4-amino antipyrine and phenol in the presence of peroxidase (POD) to yield a chromophore. The red quinone imine dye formed can be measured spectrophotometrically at 540/600 nmol as an increase in absorbance. Reference Range 136 - 290 mg/dL.

Method performance

The CV for the method within-run is 0.91%

Sensitivity equals 0.07mmol/L

The method principle is interfered by ascorbate (10% above 3mg/dL), icterus (10% above 8mg/dL), haemolysis (10% above 5g/L) and lipaemia (3% above 1000mg/dL)

Quality assurance

Control Cat. No. ODC0003 or ODC0004 will be used for quality assurance

HDL-cholesterol level in plasma

Principle:

Anti-human – β – lipoprotein antibody

LDL, VLDL and Chylomicrons ⟶ Antigen-Antibody complexes

CHE and CHO

HDL-cholesterol + H_2O + O_2 ⟶ Cholest-4-en-3-one +Fatty acids + H_2O_2

POD

H_2O_2 + 4-AA + F-DAOS ⟶ Blue dye + F + $2H_2O$Anti-human-β-lipoprotein antibody in R1 binds to lipoproteins other than HDL (LDL, VLDL and chylomicrons).The antigen-antibody complex complexes formed block enzyme reactions when R2 id was added. HDL cholesterol is quantified by the presence of an enzyme chromogen system.

Method performance

The CV for the method within-run is 0.85%

Sensitivity equals 0.002 mmol/L

the method principle is interfered by ascorbate (3% above 20 mg/dL), icterus(3% above 40 mg/dL), haemolysis (3% above 5g/L) and lipaemia 10% above 900mg/dL)

Quality assurance

Control Cat. No. ODC0003 or ODC0004 will be used for quality assurance

Triglycerides level estimation in plasma

Principle

$$\text{Triglycerides} + 3\,H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\ \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK, Mg2+}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

$$H_2O_2 + \text{MADB} + \text{4AAP} \xrightarrow{\text{POD}} \text{Blue Dye} + OH^- + H_2O$$

This Triglyceride procedure is based on a series of coupled enzymatic reactions. The triglycerides in the sample are hydrolyzed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate is oxidized by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H2O2) and dihydroxyacetone phosphate. The formed H2O2 reacts with 4 aminophenazone and N, N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to produce a chromophore, which is read at 660/800nm. The increase in absorbance at 660/800 nm is proportional to the triglyceride content of the sample.

Method performance

The CV for the method within-run is 1.06%

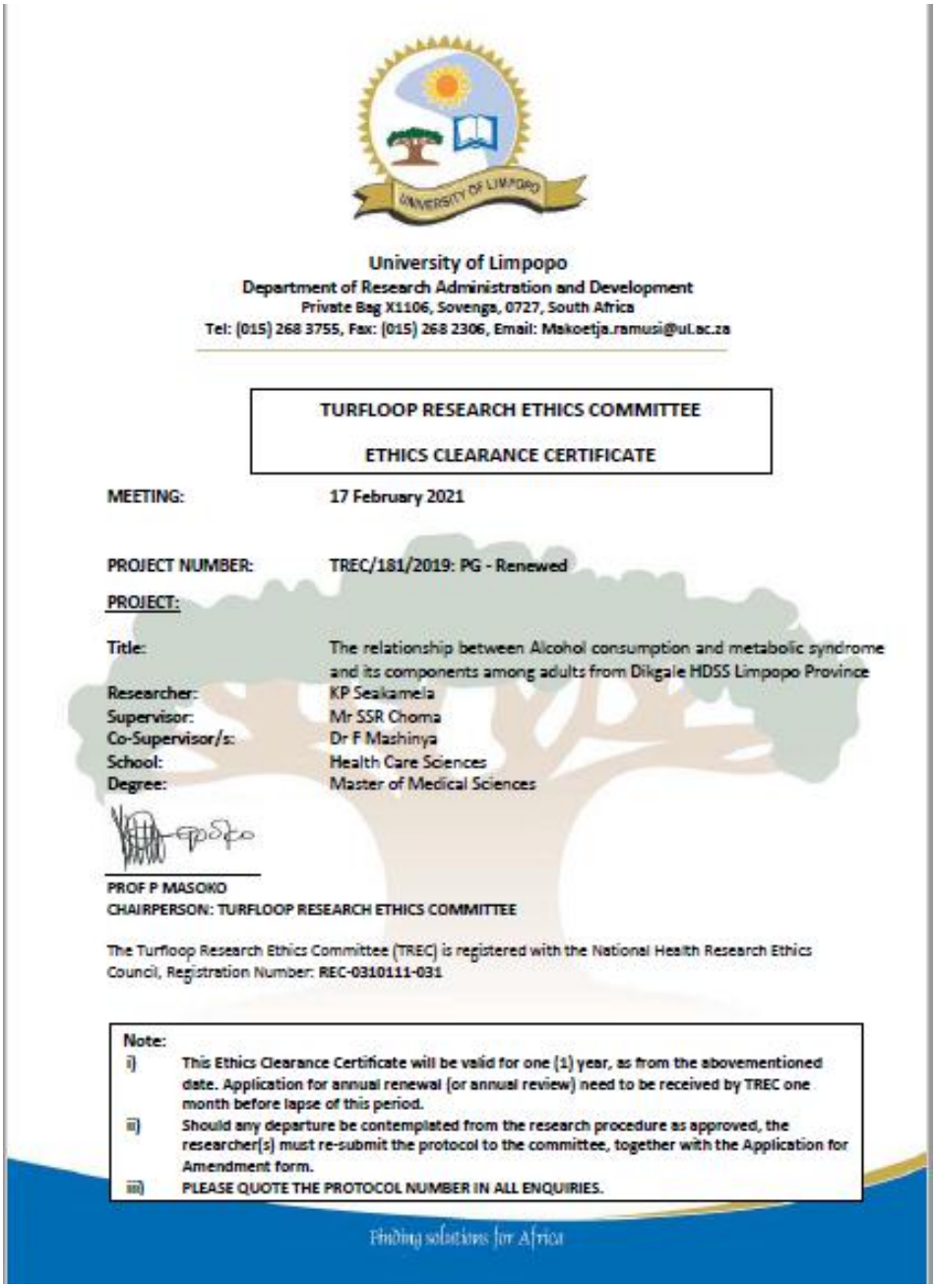
Sensitivity equals 0.01 mmol/L

The method principle is interfered by ascorbate (5% above 20 mg/dL), icterus (3% above 40 mg/dL) and heamolysis (3% above 5g/L)

Quality assurance

Control Cat. No. ODC0003 or ODC0004 will be used for quality assurance

Appendix iii: Ethical consideration



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TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING: 17 February 2021

PROJECT NUMBER: TREC/181/2019: PG - Renewed

PROJECT:

Title: The relationship between Alcohol consumption and metabolic syndrome and its components among adults from Dikgale HDSS Limpopo Province
Researcher: KP Seakamela
Supervisor: Mr SSR Choma
Co-Supervisor/s: Dr F Mashinya
School: Health Care Sciences
Degree: Master of Medical Sciences

PROF P MASOKO
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

Note:
i) This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.
ii) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.
iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Finding solutions for Africa

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To: Mr K Seakamela
Department of Pathology and Medical Sciences
University of Limpopo

From: Prof M Alberts
DIMAMO HDSS

Date: 22nd July 2019

Dear Mr Seakamela,

The data you require for your MSc project " The relationship between alcohol consumption and the Metabolic Syndrome and its components" will be extracted from the AWI-Gen database. When you are ready to receive the data please let us know. The data we will give you are the following: Alcohol consumption, weight and height, systolic and diastolic blood pressure, triglycerides, HDL-cholesterol and glucose.

Sincerely yours

M Alberts
DIMAMO HDSS